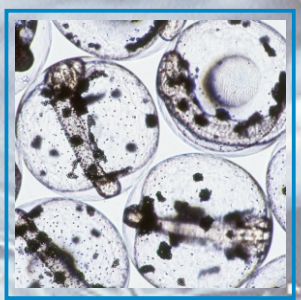
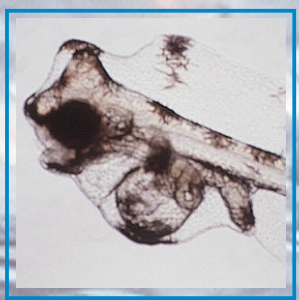
larvi 2001

september 3 - 6, 2001
ghent university, belgium



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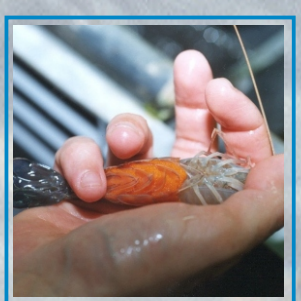

larvi 2001

3rd fish & shellfish larviculture symposium

gent, belgium, september 3-6, 2001

editors

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editorial

This book contains the mini-papers of the poster contributions and the abstracts of the oral papers, presented at the occasion of larvi 2001, the third symposium on fish & shellfish larviculture, organized on September 3-6, 2001 at the Ghent University, Belgium. As in the larvi ’91 and larvi ’95 issues, this book primarily provides the participants with detailed information on the scientific contents of the meeting, especially of the poster displays. Additionally, and in a broader sense, it can be used, both by participants and others, as a publication reflecting in a condensed form the present state of fish and shellfish larviculture.

The papers, included in this book, have been retained by the scientific committee in function of their relevance within the scope of the conference. Though not peer-reviewed, they have passed through a limited editing process in order to improve, where needed, compliance with the editors’ scientific and technical guidelines and uniformity of formatting.

A lot of support was received from the members of the scientific committee (Niall Bromage, John Castell, Jean Dhont, Marisol Izquierdo, Sachi Kaushik, Patrick Kestemont, Shunsuke Koshio, Frans Ollevier, Yngvar Olsen and Amos Tandler). We would like to express our sincere thanks to them.

Finally we would like to acknowledge the secretarial staff of the Laboratory of Aquaculture & Artemia Reference Center, Ghent University for their continuous help: Alex Pieters, Magda Vanhooren and Marc Verschraeghen; without them the realization of this book would not have been possible.

Gent, July 31, 2001

The Editors

TABLE OF CONTENTS

(in alphabetical order)

L. Asgari, C.R. Saad, A.R. Alimon, and M.S. Kamarudin
Protein and amino acid contents of broodstock tissues, eggs and newly hatched juvenile of red claw crayfish, *Cherax quadricarinatus*, under culture system

M.M. Babu and M.P. Marian
Developing bioencapsulated herbal products for maturation and quality larval production in *Penaeus monodon* (Linn.)

E. Bachère
Anti-infectious immune effectors in marine invertebrates as potential tools for disease control in larviculture

H.G.P. Bangi and M.A.R. Juinio-Meñez
The effects of adult nutrition and egg quality on larval development of the sea urchin *Tripneustes gratilla* (Echinodermata: Echinoidea)

Y. Barr, I. Rønnestad, and C.R. Rojas-Garcia
The digestive capacity for dietary protein in larval Atlantic halibut (*Hippoglossus hippoglossus*)

J.G. Bell, L.A. McEvoy, A. Estevez, R.J.Shields, and J.R. Sargent
Optimizing highly unsaturated fatty acid levels in first feeding marine fish larvae

I. Ben-Atia, S. Lutzky, Y. Barr, K. Gamsiz, Y. Shtupler, B. Koven, and A. Tandler
The effect of ozone treatment on egg and larvae performance in the gilthead seabream (*Sparus aurata*) and other marine species

L. Berg
Larval growth and juvenile quality and an attempt to describe food uptake in intensive rearing of halibut (*Hippoglossus hippoglossus*) larvae

Ø. Bergh, L. Torkildsen, S. Andersen, O.B. Samuelsen, A. Nylund, B.T. Lunestad, S. Mortensen, Ø. Strand, T. Magnesen, and C. Lambert
Pathogens in scallop, *Pecten maximus*, hatcheries and possible strategies to control them

G. Blanchard, A. Cuvier-Péres, and P. Kestemont
Temperature affects trypsin and amylase activities in Eurasian perch *Perca fluviatilis* three weeks after hatching

C. Boglione, E. Cataldi, M. de Francesco, M. Giganti, M. Gratani, C. Selmo, and S. Cataudella
Morphoecology and feeding behaviour in larval finfish: a new candidate species for aquaculture

J. Castell, T. Blair, S. Neil, K. Howes, S. Mercer, J. Reid, W. Young-Lai, B. Gullison, P. Dhert and P. Sorgeloos
The effect of different HUFA enrichment emulsions on the nutritional value of rotifers (*Brachionus plicatilis*) to larval haddock (*Melanogrammus aeglefinus*)

E. Castillo Corella, L. Ortiz Serrano, A. Saúco Roig, R. Rodríguez Ravelo, and M. Jover
Evaluation of three diets with different protein percents for juvenile *Cherax quadricarinatus*

B.T. Castro, M.G. Castro, A.R. De Lara, M.J. Castro, and S.A. Malpica
Perspectives of the development of *Artemia* culture in Mexico

M.G. Castro, B.T. Castro, L.M. Gallegos, M.J. Castro, S.A. Malpica, and A.R. De Lara
Incorporation of an antibiotic with *Artemia* metanauplii to inhibit the growth of *Pseudomonas aeruginosa*

R.O. Cavalli, F.M. Batista, P. Lavens, P. Sorgeloos, H.J. Nelis, and A.P. De Leenheer
The effects of dietary vitamin supplementation on maternal performance and larval quality of the prawn *Macrobrachium rosenbergii*

R.O. Cavalli, R. Hernández-Herrera, I.S. Racotta, P. Lavens, and P. Sorgeloos
The use of an ammonia stress test as a tool to evaluate larval quality

R. Chakrabarti and S. Kumar
Effect of feed protein manipulation on the survival, growth, and enzyme activities of *Cirrhinus mrigala* larvae

M.S. Chebanov and E.V. Galich
Ecological and morphological peculiarities of sturgeons reared under control of seasonal propagation of breeders

T. Chen, H. Chen, W. Gao, X. Xu, and X. Chen
Microcapsules containing mushroom polysaccharide and *Artemia* powder prove to enhance immunity of *Cambaroides*

W.C. Clarke and J.O.T. Jensen
Development rates for sablefish (*Anoplopoma fimbria*) and Pacific halibut (*Hippoglossus stenolepis*) incubated at 6ºC

J.M. Cobcroft, P.M. Pankhurst, and S.C. Battaglene
The visual field and feeding of cultured striped trumpeter (*Latris lineata*) larvae under differing light environments

A. Estévez, R. Carbó, J.I. Aguilar, and D. Furones
Culture of gilthead sea bream and dentex larvae in a closed recirculating system

J.O. Evjemo, K.I. Reitan, and Y. Olsen
Copepods as a food source in first feeding of marine fish larvae

I.-B. Falk-Petersen
Development of the digestive tract and associated organs in wolffishes (*Anarhichas lupus* L. and *A. minor*)

I.-B. Falk-Petersen
Influence of egg incubation temperature on yolk consumption, development, and survival of spotted wolffish embryos and larvae

A. Farías, J.G. Bell, I. Uriarte, R.J. Henderson, and J.R. Sargent
Effect of temperature and diet on growth and lipid composition of Chilean scallop *Argopecten purpuratus* (L.) larvae

D.F. Fegan
Commercial larviculture of penaeid shrimp

T.F. Galloway
Muscle growth in first feeding halibut larvae with different body growth rates

Y. García de Severeyn , H. Severeyn, W. Grant, and Y. Reverol
A model that simulates larval mortality of the bivalve mollusc *Tivela mactroides*

Y. García de Severeyn, H. Severeyn, and Y. Reverol
Larval mortality of the marine clam *Tivela mactroides* reared in the laboratory under different temperatures

A. García-Ortega, I. Abdo de la Parra, and C. Hernández
Weaning of Mexican bullseye puffer (*Sphoeroides annulatus*) larvae from *Artemia* nauplii to artificial microdiets

P. Gatland
Industrial larval culture of marine fish in the Mediterranean

I. Geurden
Methods to estimate food intake in fish larvae

B. Gomez-Gil, S. Soto-Rodríguez, A. García-Gasca, and A. Roque
Molecular characterization of vibrios isolated from penaeid shrimp larval culture

V. Grangier, J. Zambonino Infante, P. Quazuguel, and C. Cahu
Effect of phospholipid, protein hydrolysate, and vitamin content on formulated diets for marine fish larvae

A. Hagiwara, A. Akazawa1, Y. Sakakura, H. Chuda, K. Miyaki, and T. Arakawa
Food selectivity, growth, and survival of marine fish larvae fed different sizes of rotifers

L.C. Halfyard, K.S. Dwyer, C.C. Parrish, K. Jauncey, and J.A. Brown
Effects of dietary n-3 highly unsaturated fatty acids on growth, survival, and body composition of the common wolffish, *Anarhichas lupus*, and yellowtail flounder, *Limanda ferruginea*

K. Hamre, A. Øfsti, T. Næss, I. Opstad, and R. Nortvedt
Optimization of dietary macronutrient composition for Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae and juveniles

T. Harboe, A. Mangor-Jensen, J. Rainuzzo, K. Hamre and A. Kvåle
First feeding of halibut larvae on rotifers

R. Hernández-Herrera, C.I. Pérez-Rostro, F. Arcos, J.L. Ramírez, A.M. Ibarra, E. Palacios, and I.S. Racotta
Predictive criteria of shrimp larval quality: an experimental evaluation

W. Hong and Q. Zhang
Bred species and fry production of marine fish in China

C.H. Hsu, H.M. Su, and I.M. Chen
Effects of food types on the development and reproduction of *Apocyclops royi*

A.S. Jahangard, M.S. Kamarudin, A.A.Razak, C.R.Saad, and K. Sijam
Evaluation of *Panagrellus redivivus* from different culture media on survival and growth of silver barb *Puntius gonionotus* larvae

K.C.A Jalal, M.A. Ambak, T. Haji-Hassan, P. Sorgeloos, M. Said Mohd. Zaki, and C.R. Saad
Effects of *Spirulina* on the growth and carotenoid deposition in tropical sport fish *Tor tambroides* fry

L.M. Juárez
Shrimp hatcheries and the environment

B.G. Kamilov
Fish artificial reproduction for increasing fish productivity in water bodies in the Aral Sea basin under ecological crisis

S.F. Martínez-Díaz, M. Moreno-Legorreta, C.A. Alvarez-Gonzalez, R. Peña-Martínez, S. Dumas, and R. Vázquez-Juárez
Ecology of *Vibrio* and *Aeromonas* during the larval development of the spotted sand bass *Paralabrax maculatofasciatus*

S.F. Martínez-Díaz, M. Moreno-Legorreta, C.A. Álvarez-Gonzalez, S. Dumas, and R. Vázquez-Juárez
Elimination of associated microbial community and bioencapsulation of bacteria in the rotifer *Brachionus plicatilis*

C. Martins, R. Calado, M. Monteiro, O. Santos, and L. Narciso
Two main problems in *Artemia* utilization: biometry and fatty acid profile

M.C. Martins
Effects of temperature on the condition of *Loligo vulgaris* and *Loligo forbesi* (Mollusca: Cephalopoda) late embryos and paralarvae

E. Matus Nivón, M.S. Izquierdo López, H. Fernández Palacios, and C.M. Hernández Cruz
Lipid classes of *Pagrus pagrus* eggs and starved larvae from adults fed on three diets

L. Méndez, I.S. Racotta, B. Acosta, and G. Portillo-Clark
Effect of sediment on the growth and survival of *Penaeus stylirrostris* postlarvae

D. Mendiola, A. Martinez de Murguia, P. Álvarez, X. Guinda, and D.L. Herrero
Preliminary results on rearing experiments with mackerel larvae

C. Menezes, E. Dores, and P. Pousão-Ferreira
Control of bacterial flora of larvae, water and rotifer (*Brachionus plicatilis*) in larval rearing of white sea bream (*Diplodus sargus*, Linnaeus, 1758)

L. Mercier, J. de la Noüe, C. C. Parrish, and C. Audet
Acclimatization of the rotifer *Brachionus plicatilis* for rearing of winter flounder larvae

H. Migaud, J.N. Gardeur, L. Fordoxcel, P. Fontaine, and J. Brun-Bellut
Influence of the spawning time during the reproduction period on the larval quality of Eurasian perch, *Perca fluviatilis*

H. Migaud, Jean-Noël Gardeur, Patrick Kestemont, and Pascal Fontaine
Influence of temperature and photoperiod on the broodstock gonadogenesis, spawning and larvae quality of Eurasian perch, *Perca fluviatilis*

A.J. Ritar, B.J. Crear, and C.W. Thomas
Effects of *Artemia* feeding regimes on growth and survival of phyllosoma larvae of southern rock lobster (*Jasus edwardsii*)

R. Robert and M. Brown
The food value of microalgal concentrates for larvae and postlarvae of the pacific oyster, *Crassostrea gigas*

A. Roncarati, F. Gelli, F. Savorelli, L. Pregnolato, A.M. Cicero, G. Casazza, D. Palazzi, P.L. Trentini, and P. Melotti
Reproduction of sand smelt (*Atherina boyeri*, Risso), an autochthonous euryhaline species of the Adriatic sea, under laboratory conditions

I. Rønnestad, C.R. Rojas Garcia, Y. Kamisaka, W. Koven, Y. Barr, H.J. Fyhn, and L.E.C. Conceição
Ontogeny of digestive function of marine fish larvae

A. Roque, S. Soto, C. Cuenca, C. Bolan, and B. Gomez-Gil
The use of *Artemia franciscana* (Kellog 1906) in the research of diseases in aquatic organisms

C. Rougeot, P. Kestemont, and C. Mélard
Sex control in Eurasian perch, *Perca fluviatilis*

M. Rozihan, C.R. Saad, K.C.A. Jalal, A.R. Alimon, and M.H. Hatta
The utilization of plant-based protein for the growth and survival of *Macrobrachium rosenbergii* (de Mann)

E. Rurangwa, F. Volckaert, G. Huyskens, D.E. Kime, and F. Ollevier
CASA: a quantitative and rapid method for the assessment of sperm quality in fish

I. Salvesen, J. Skjermo, and O. Vadstein
Magic solutions to bacterial problems in early life stages: do they exist?

J.F. Samain, P. Sorgeloos, M. Caers, C. Van Ryckeghem, P. Soudant, O. Garcia, J. Espinosa, Y. Marty, M. Mathieu, C. Berthelin, C. Quere, J.R. Le Coz, C. Seguineau, and J. Moal
Nutrition and reproduction of the Pacific oyster *Crassostrea gigas*: main results of the European project giganuga (gigas nutrition and gametogenesis)

G.R. Schipp, Jérôme M. P. Bosmans, and Damon J. Gore
A semi-intensive larval rearing system for tropical marine fish

L.I. Semenenko, E. Styczynska-Jurewicz, and E.I. Myroshnichenko
Culture of the pacific mullet (*Mugil soiuy* basilewsky) larvae in the water bodies of various salinities

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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EGG QUALITY AND MUSCLE GROWTH IN ATLANTIC HALIBUT LARVAE

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Introduction

The Atlantic halibut (*Hippoglossus hippoglossus* L.) is the largest flatfish in the North Atlantic and is one of the most promising candidates for Norwegian marine aquaculture. Knowledge about muscle growth in this species is of great economical importance. This study was undertaken to see how egg quality evaluated by blastula morphology at the 4-8-cell stage affects larval growth in halibut. The effect of egg quality on muscle growth was evaluated by measurements of muscle fibre size and number in newly hatched larvae developed from good- and poor-quality eggs.

Materials and methods

The eggs were stripped from 5 females and fertilized with milt from 5 different males. Egg quality (blastula morphology) was evaluated at the 4-8-cell stage (Kjørsvik et al. 1990). The embryos were given a score from 0 to 5 (5 is best) (Braak, 1994; Shields et al., 1997), and they were then kept in individual compartments until hatching (n=144 per group). All eggs were incubated in stagnant filtered (17µm) seawater of 33ppt. Oxytetracycline (25ppm) was added to the water to reduce bacterial growth. The eggs were kept in darkness at 5-6°C until hatching, which occurred 79d° after fertilization. As a control 3×300 eggs from each group were incubated in 3-l glass bowls. These larvae were kept until 230d° (first feeding).

Ten larvae, 5 of good (score=5) and 5 of poor (score=0-3) quality, were sampled from the egg batches at hatching. The larvae were fixed in 2.5% formaldehyde and 2.5% glutaraldehyde in 0.08M cacodylate buffer, postfixed in 2% OsO4, and embedded in Epon. Standard length (SL), myotome height (MH), and yolk-sac volume were measured before embedding. Semithin (1.0µm) sections were made immediately posterior to the anus, stained with toluidine blue and basic fuchsin

(Hoffmann et al., 1983), and then viewed in a phase contrast microscope. Total muscle cross-sectional area, white muscle fibre number, and size was measured using a stereological software programme. Samples from the glass bowls were used to measure the dry weight (DW) of eggs, newly hatched larvae, and 230-d°-old larvae.

Results and discussion

The fertilization rate varied between the five egg batches (38-89%), but was not significantly correlated to the rate of normal blastula morphology. Survival during the egg stage was greatly affected by blastula morphology (*n*=10, r=0.81, *P*=0.02). Average hatching rate for good quality eggs was 53%, and 33% for poor quality eggs (*n*=10, r=0.66, *P*=0.02).

Larval DW at hatching was positively correlated with blastula morphology (*n*=54, r=0.52, *P*=0.00) and DW at 230d° (*n*=5, r=0.95, *P*=0.02). However, SL and MH at hatching were not affected by blastula morphology, and short and long newly hatched larvae grew to the same SL at 230d°. This was also the trend for the MH. Most marine fish larvae have allometric growth, and this will affect which part of the larva the energy will be allocated to (Osse et al., 1997). The head and tail regions are the most important for survival and feeding, so allometry is a possible explanation for why DW at hatching and first feeding were correlated while SL and MH at these stages were not.

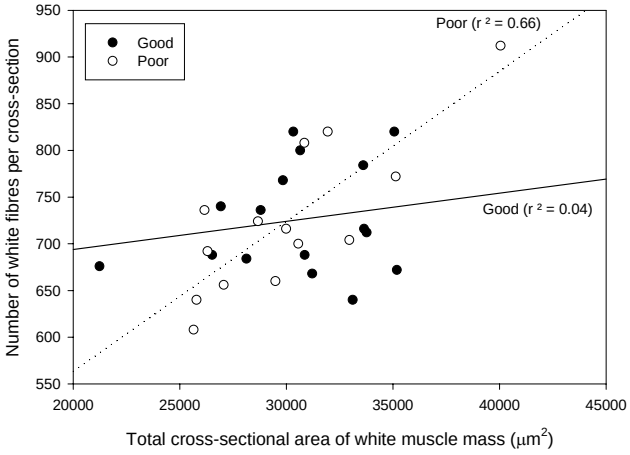

Fig. 1. Numbers of white fibres as a function of total cross-section area of white muscle fibres in newly hatched larvae from good and poor quality eggs obtained from five different females.

There were no significant differences in the average total cross-sectional area or number of white muscle fibres in a cross-section between larvae from good- and

poor-quality groups. However, in the larvae that were developed from poor-quality eggs, the number of white fibres increased significantly with increasing total cross-section area (n=14, r=0.81, P=0.00), but this was not the case for larvae developed from good-quality eggs (Fig. 1).

It therefore seems like hypertrophy of white muscle fibres had been a more dominating growth mechanism than hyperplasia during the embryonic stage of good-quality embryos. Further processing of the fibre size distribution data is needed before any conclusions can be made.

Muscle fibre growth patterns have been shown to vary between families of Atlantic salmon (*Salmo salar* L.) (Johnston and McLay, 1997). Although some differences were found in the present study between larvae from good- and poor-quality eggs within one egg batch, the variation between egg batches were often larger than the variation within an egg batch. Family relationships should therefore be taken into account when conclusions are made about muscle growth dynamics.

This study has focused on the growth of embryonic fibres. Seeing that fish grow by hyperplasia during a large part of adult life, the muscle-recruitment cells are important for further growth. A natural continuation of this study is therefore to look at the relationship between myoblasts/myosatellite cells and muscle fibre growth.

References

Hoffmann E.O., T.R. Flores, J. Coover, and H.B. Garrett II. 1983. Polychrome stains for high resolution light microscopy. Lab. Med. 14:779-781.

Johnston I.A. and H.A. McLay. 1997. Temperature and family effects on muscle cellularity at hatch and first feeding on Atlantic salmon (*Salmo salar* L.). Can. J. Zool. 75:64-74.

Kjørsvik E., A. Mangor-Jensen, and I. Holmefjord 1990. Egg quality in fishes. Adv. Mar. Biol. 26:71-113.

Osse J.W.M., J.G.M. van den Boogaart, G.M.J. van Snik, and L. van der Sluys. 1997. Priorities during early growth of fish larvae. Aquaculture 155:249-258.

Shields R.J., N.P. Brown, and N.R. Bromage. 1997. Blastomere morphology as a predictive measure of fish egg viability. Aquaculture 155:1-12.

van de Braak K. 1994. Morphological characteristics of early embryos in relation to later egg and larval development of cod (*Gadus morhua* L.). Student thesis, ERASMUS Programme ICP-94-NL-1139/01. Dept. Zoology, Norwegian University of Science and Technology. 40p.

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LARVAL REARING OF THE MEXICAN BULLSEYE PUFFER S*PHOEROIDES ANNULATUS* UNDER HATCHERY CONDITIONS.

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Introduction

In Mexico, marine aquaculture is an important and relatively new industry, however, marine aquaculture has remained restricted to the production of shrimp. Since 1984, the production of shrimp has increased from 7 tons to 23 749 tons in 1998, while in 1998, production of other marine species was negligible. Elsewhere, particularly in Asia, a more diverse production of species is making the aquaculture industry more stable to problems, such as disease outbreaks. For example, should the shrimp production fail, the second species could maintain the farm's cash flow. It would be a significant advantage for the marine aquaculture industry in Mexico if other species were identified for aquaculture production.

The bullseye puffer (*Sphoeroides annulatus*) is a hardy species, biologically similar to the successfully cultured tiger puffer in Japan and Korea (Kanazawa, 1991). The environments inhabited by the bullseye puffer are similar to those inhabited by shrimp, and it has been observed as a by-product in shrimp ponds. The apparent aquaculture potential of the bullseye, and similar environmental requirements as shrimp, suggest that the bullseye puffer would make a successful aquaculture species.

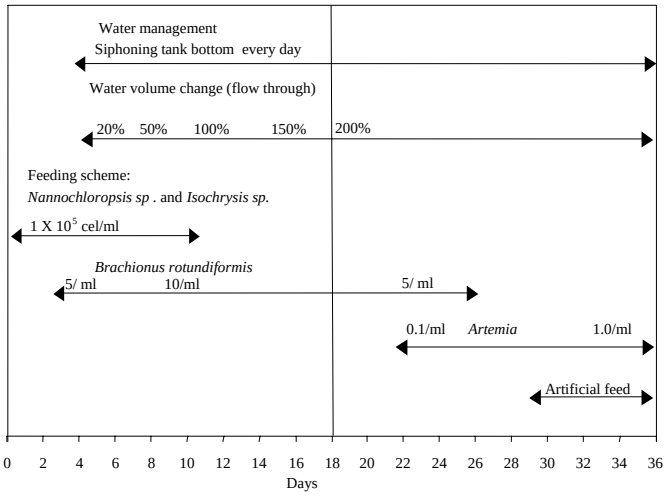
This paper describes the egg incubation and larval rearing of the bullseye puffer, and provides a basis for development of culture practices for this species.

Materials and methods

Eggs and sperm were obtained from wild mature puffer fish by applying gentle abdominal pressure to females and males, respectively. Fertilization was done by mixing eggs and sperm with filtered seawater. Fertilized eggs are strongly

adhesive and were attached to a glass slide and incubated in 70-l plastic tanks with flow-through filtered (5µm) and UV-disinfected seawater. Incubation temperature was 27.0±2°C and salinity was 35ppt. Eggs were inspected regularly to determine the time when they had begun to hatch.

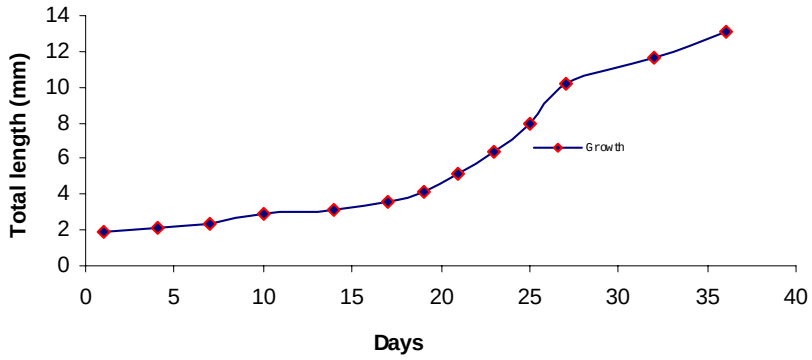
Newly hatched (Day 0) larvae were reared indoors in twelve 600-l circular black fiberglass tanks at temperatures ranging from 25.5-28.5°C, dissolved oxygen concentrations from 4.5-5.7ppm, and a salinity of 35ppt. Seawater used for larval rearing was passed through a sand filter and a 20-µm cartridge filter. The amount of daily water exchange was increased from 20% 3 days after hatching (DAH) to 200% 20 DAH onwards. The level of water in the tanks was increased from 300 l on 0 DAH to 800 l on 20 DAH. Aeration was progressively increased from minimal levels during the 2 first weeks to strong levels after the third week. The bottoms of the tanks were siphoned daily for organic residue and mortalities. From 1 to 11 DAH, *Isochrysis* sp. and *Nannochloropsis* sp. were added to the water to maintain a minimum microalgal concentration of 100 000 cells.ml^{-1}. Rotifers (*Brachionus rotundiformis*) enriched with *Nannochloropsis* sp. were added to the tanks at 5.ml^{-1} from 4 to 11 DAH, and 10.ml^{-1} from 11 DAH to 20 DAH. Algal and rotifer densities in the tanks were monitored daily at 8:00, 12:00, 16:00, and 20:00, and were adjusted to maintain the required density. The amount of rotifers was reduced to 5.ml^{-1} on 21 DAH when newly hatched *Artemia* nauplii were added at an initial concentration of 0.1.ml^{-1} and gradually increased to 1.ml^{-1} at 37 DAH. On 29 DAH larvae were gradually weaned onto artificial diets. The water and feeding management scheme is summarized in Fig. 1. Five larvae were collected from each tank every 3 days for length measurement.



Results

The egg fertilization rates were 97.61±2.65%. The incubation period was observed to vary with temperature. At 24.5°C, the eggs initiated hatching at 96 hours after fertilization (AF) and at 27.5°C, the eggs initiated hatching at 72 hours (AF). The eggs were spherical, 0.7±0.03mm in diameter, demersal, strongly adhesive, and transparent, thus the embryo and its developmental stages could be seen through the egg membrane. The yolk contained a cluster of small oil globules. Dead eggs were white and opaque.

Newly hatched larvae measured 1.92-2.08mm total length (TL) and had a mean dry weight of 24.8±1.05µg. The larvae were characterized by a huge yolk sac which was approximately half the total length, the larval body was curved around the yolk sac with only the tail section not coming into contact with it. After hatching the larvae floated in the water column without significant movement, except for sporadic tail thrusts. The mouth opened at 1 DAH, but absorption of the yolk was completed 4 to 5 DAH, by which time the larvae had attained a mean 2.09± 0.02mm TL. Growth of larvae (TL) over time is shown in Fig. 2.



Discussion

These results describe the early growth and development of the eggs and larvae of the bullseye puffer and constitute a protocol that was successfully used to grow eggs and larvae through to the juvenile stage. Similar results have been obtained for several species of pufferfish such as *Canthigaster valentini*, *C. rivulata*, *C. rostrata*, *Takifugu exascurus*, *T. porphyreus*, *T. nipholes* and *T. rubipres* (Arai and Fujita, 1988; Stroud et al., 1989; Sikkel, 1990; Fujita and Honma, 1991; Fujita and Abe, 1992): eggs are spherical, demersal, adhesive,

and 0.7-1.28mm in diameter, the newly hatched larvae measured 2.0-3.1mm TL, and absorption of the yolk sac occurred 4-5 days after hatch. The present work represents an important step in the investigation of culture techniques for the bullseye puffer, illustrating its suitability as a promising species for aquaculture.

Acknowledgements

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References

Arai A. and S. Fujita. 1988. Spawning behavior and early life history of the sharpnose puffer *Canthigaster rivulata* in the aquarium. Jap. J. Ichthyol. 35(2):194-200.

Fujita S. and Y. Honma. 1991. Induction of ovarian maturation and development of eggs, larva and juveniles of the puffer, *Takifugu exascurus*, reared in the laboratory. Jap. J. Ichthyol. 38(2):211-218.

Fujita S. and T. Abe. 1992. Induction of ovarian maturation and development of eggs, larvae, and juveniles of the purple puffer Takifugu porphyreus reared in the laboratory. Nippon Suisan Gakkaishi. 58(9):1627-1632.

Kanazawa A. 1991. Puffer fish *Fugu rubripes*. p.123-129 In: Wilson, P. 1991. Handbook of nutrient requeriments of finfish. CRC Press USA.

Sikkel P.C. 1990. Social organization and spawing in the Atlantic shrapnose puffer, *Canthigaster rostrata* (Tetraodontidae). Environ. Biol. Fishes 27:243-254.

Stroud G.J., B. Goldman, and W. Glandstone. 1989. Larval development, growth and age determination in the sharpnose pufferfish *Canthigaster valentini* (Teleostei: Tetradontidae). Jap. J. Ichthyol. 36(3):327-337.

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EFFECT OF THE DIETARY AA/EPA RATIO ON THE n-3 HUFA NEEDS OF GILTHEAD SEA BREAM (*SPARUS AURATA*) LARVAE FED MICRODIETS

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Introduction

Recent studies have demonstrated the importance of arachidonic acid (20:4n-6, AA) in fish larvae, as well as the importance of simultaneously considering the requirement of AA, eicosapentaenoic (20:5n-3, EPA), and docosahexaenoic (22:6n-3, DHA) acids (Bessonart, 1997; Sargent et al., 1999). Thus, besides the well documented importance of DHA for survival, growth, and development of several marine fish larvae, the elevation of dietary AA in gilthead sea bream larvae has been found to improve growth, survival (Bessonart et al., 1999), and resistance to handling stress (Koven et al., 2001).

In 15-day-old gilthead sea bream (*Sparus aurata*) larvae, n-3 highly unsaturated fatty acid (n-3 HUFA) requirement has been found to be variable, depending on factors such as the dietary DHA/EPA ratio (Bessonart, 1997) or the polar lipid content and n-3 HUFA content of polar lipids (Salhi et al., 1999).

The present work was conducted in order to study the effect of the AA/EPA ratio on the n-3 HUFA requirement of 16-day-old gilthead sea bream larvae fed microdiets.

Materials and methods

Nineteen-day-old gilthead sea bream larvae (5.63mm in total length) were fed for 20 days on two experimental microdiets differing in the total n-3 HUFA, EPA, and AA content, but containing the same amount of DHA (Table I) .

Microdiets were based on freeze-dried squid powder (Rieber and Son, Bergen, Norway) and lipid-extracted (73.5g·100g^{-1}diet), and contained 5.3g·100g^{-1}diet of vitamin mixture, 4.5g·100g^{-1} diet of mineral mixture, 3.0g·100g^{-1}diet of attractant, 3.0g·100g^{-1}diet of soybean lecithin, and free arachidonic acid (Sigma, St. Louis, USA), oleic acid (Merk, Darmstadt, Germany), and fish oils EPA28 and DHA27 (Nippai Co., Ltd, Tokyo, Japan). Dietary n-3 HUFA content was lower in Diet II due to a lower EPA level. This EPA reduction was compensated by increasing the amount of AA in Diet II (Table I).

Table I. Protein, lipid, and essential fatty acid content (% dry weight) of the experimental microdiets.

	Diet I	Diet II
Crude protein	79.3	79.7
Total lipids	15.1	12.8
Σ n-3 HUFA	1.91	1.49
DHA	1.08	1.00
EPA	0.82	0.46
AA	0.36	0.60
DHA / EPA	1.32	2.17
AA / EPA	0.44	1.30

Larvae were divided into groups of 2000 fish and distributed into six 100-l tanks (3 tanks per diet), where they were fed 1.0-2.0g·tank^{-1}·day^{-1} of the experimental microdiets using automatic feeders. Larvae were also fed rotifers (3.5×10^5 per tank twice a day) cultured on baker's yeast, which contained only trace amounts of n-3 HUFA. No microalgae were added to the rearing tanks. During the trial, temperature ranged from 19.5-20.0°C, and a photoperiod of 12h artificial light was maintained.

Larval growth and survival were assessed by measuring the total length of 30 fish per tank and individual counting of live fish at the end of the trial. The methodology used for lipid and fatty acid analyses is described in Salhi et al. (1999). A Student test was used for statistical comparison of means.

Results and discussion

After 20 days of feeding the experimental diets, larval growth (total length) and survival were not significantly affected ($P<0.05$) by the reduction of dietary n-3 HUFA from 1.91 % (Diet I) to 1.49% (Diet II) based on a reduction of the EPA content from 0.82 to 0.46% (Table II). In a previous study (Salhi, 1997), an experiment conducted under similar conditions – feeding 10-day-old gilthead sea

bream larvae with the same kind of microdiets – showed that a dietary n-3 HUFA reduction from 2.1 to 1.6%, based on a reduction of both EPA (from 0.73 to 0.57%) and DHA (from 1.27 to 0.94%) and maintaining a DHA/EPA ratio of ~1.7, resulted in a lower larval growth (larval total length and dry body weight).

Table II. Growth (mm total length), survival (%), and total, neutral, and polar lipid content (% dry weight) of the larvae.

	Initial	Diet I	Diet II
Total length	5.63 ± 0.61	8.64[a] ± 1.09	8.52[a] ± 0.66
Survival	–	28.8[a] ± 6.5	23.6[a] ± 7.6
Total lipids	17.37	15.79 [a] ± 1.19	16.04[a] ± 0.31
Neutral lipids	7.72	6.45[a] ± 0.84	6.58[a] ± 1.56
Polar lipids	9.45	9.34[a] ± 1.34	9.38[a] ± 1.53

Values having the same letter within a row were not significantly different ($P<0.05$)

In the present study, dietary DHA was similar in both diets and the reduction of EPA was accompanied by an increase of AA in Diet II. Thus, it seems that a combination of increasing dietary AA and decreasing dietary EPA could result in an improved larval performance, reflected in this study in a lower need of n-3 HUFA in microdiets. In this regard, Bessonart et al. (1999) found that feeding 17-day-old gilthead sea bream larvae on microdiets containing ~2% n-3 HUFA and 0.7-0.8% EPA, the dietary inclusion of 1% AA resulted in an improved larval growth, while survival was improved by including 1.8% AA in the diet. Besides the importance of dietary AA as an essential fatty acid for these fish, an importance of the dietary AA/EPA ratio comes from the competitive interactions between these fatty acids – AA and EPA compete for the cyclo-oxygenase and lipoxygenases that produce eicosanoids from these fatty acids; the eicosanoids produced from AA are generally more biologically active than those produced from EPA, and the respective eicosanoids compete for the same cell membrane receptors (Sargent el al., 1999). Moreover, a competition between these 20C HUFA in phospholipid biosynthesis has also been observed in gilthead sea bream larvae fed microdiets with different AA/EPA ratios (Bessonart, 1997).

Total, polar, and neutral lipid content of the larvae (% dry basis) was not affected by the different diets. However, compared to the initial larvae, a slight reduction of larval total lipids mainly due to a reduction in neutral lipid content was observed, reflecting the low lipid content of the experimental microdiets (Table I).

References

Bessonart M. 1997. Efecto de la relaciones EPA/DHA y EPA/AA en le cultivo larvario de la dorada (*Sparus aurata*). Ph.D. Thesis, Universidad de Las Palmas de Gran Canaria, Spain.

Bessonart M., M.S. Izquierdo, M. Salhi, C.M. Hernández-Cruz, M.M. González, and H. Fernández-Palacios. 1999. Effect of dietary arachidonic acid levels on growth and survival of gilthead sea bream (*Sparus aurata* L.) larvae. Aquaculture 179:265-275.

Koven W., Y. Barr, S. Lutzky, I. Ben-Atia, R. Weiss, M. Harel, P. Behrens, and A. Tandler. 2001. The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead sea bream (*Sparus aurata*) larvae. Aquaculture 193:107-122.

Sargent J., L. McEvoy, A. Estévez, G. Bell, M. Bell, J. Henderson, and D. Tocher. 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217-229.

Salhi M. 1997. Estudio de los requerimientos lipídicos de larvas de dorada (*Sparus aurata*) alimentadas con microdietas. Ph.D. Thesis, Universidad de Las Palmas de Gran Canaria, Spain.

Salhi M., C.M. Hernández-Cruz, M. Bessonart, M.S. Izquierdo, and H. Fernández-Palacios. 1999. Effect of different dietary polar lipid levels and different n-3 HUFA content in polar lipids on gut and liver histological structure of gilthead sea bream (*Sparus aurata*) larvae. Aquaculture 179:253-263.

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EFFECTS OF TEMPERATURE AND RETENTION TIME OF OVULATED EGGS ON THE QUALITY OF EMBRYOS AND ON THE OCCURRENCE OF TRIPLOID FRY IN RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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Introduction

The quality of fish eggs regarding developmental ability is known to exhibit large variations in aquaculture as well as in wild stocks (Kjorsvik et al., 1990). Egg quality has generally been assessed through empirical morphological criteria (egg shape, oil globules distribution, etc.) or through developmental criteria (survival and morphological characteristics at different stages). However, not much attention has been paid to the possibility that more or less discreet non-lethal chromosomal anomalies can escape to superficial observations and therefore become a genetic burden. The present experiment was designed in order to explore such a possibility in rainbow trout, *Oncorhynchus mykiss*, by testing two important factors for aquaculture: temperature during maturation and ovulation, and aging of ovulated eggs in the body cavity.

Materials and methods

Two to four weeks before expected spontaneous ovulation time, 39 rainbow trout of a spring-spawning strain were obtained from a hatchery in Brittany and held in a recirculated water unit.

Males and 12 females were held at 12°C, whereas the 12 other females were held at 17°C.

Twice a week, females were sedated with 0.15ml.l^{-1} phenoxy-2-ethanol and checked for ovulation by manual pressure on the abdomen. 400ml of ovulated eggs and an aliquot of coelomic fluid were sampled at the time of detected ovulation and 1, 2, and 3 weeks thereafter.

The coelomic fluid was discarded from ovulated eggs and each sample was inseminated with 15µl of a pool of semen obtained from five males presenting the highest sperm motility. Egg batches were transferred to individual incubators at 10°C. Unfertilized and fertilized eggs, eyed embryos, hatchlings, and swim-up fry were counted. The ploidy level of resorbed fry was determined by flow cytometry (Lecommandeur et al., 1994).

Each coelomic fluid sample was centrifuged at 4000×g for 10min. pH, osmolality, and protein concentration (Sedmak and Grossberg, 1977) were measured.

Results and discussion

Eyeing, hatching, and swim-up rates significantly decreased after two weeks of egg retention at 12°C. This is in agreement with previous work by Sakai et al. (1975) and Springate et al. (1984). In contrast, the morphological abnormality rate of fingerlings significantly increased after only one week of egg retention. Thus, the malformation rate appears as an important quality estimator that should be used in addition to eyeing rate to fully estimate egg quality.

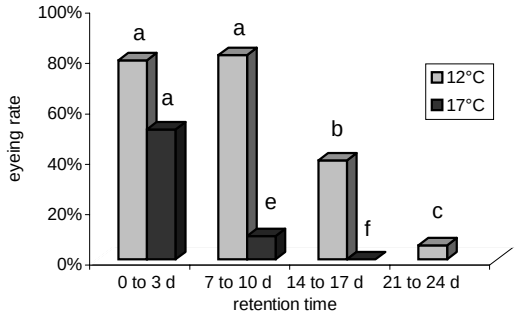

Fig. 1. Influence of egg retention time on the rate of eyed embryos.

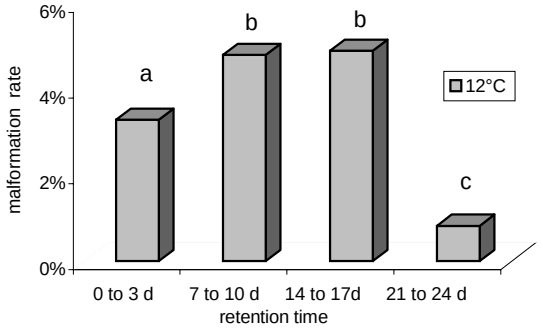

Fig. 2. Influence of egg retention time on morphological abnormalities at swim-up.

Triploid fry were observed after one week of retention at 12°C, sometimes at high proportion (up to 50% 7-10 d after detected ovulation). In contrast to previous reports presenting natural triploidy as an isolated phenomenon (Thorgaard et al., 1982; Miller et al., 1984), this experiment shows that such a phenomenon could occur in usual farming practices, particularly when ovulations are checked only once a week.

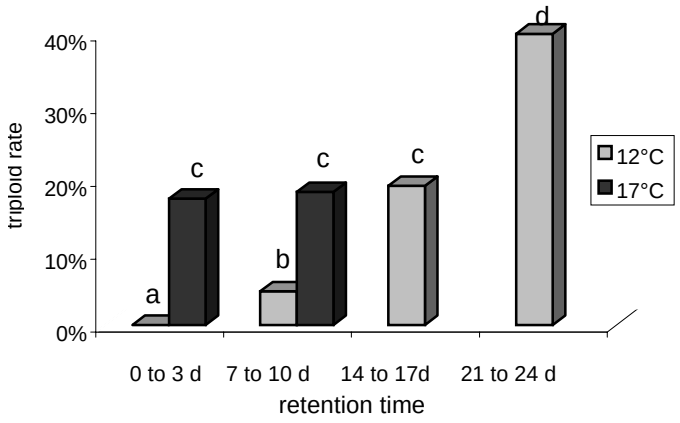

Fig. 3. Influence of egg retention time on the rate of triploid fry.

The effect of overripeness on egg quality was highly variable among individuals and this variability increased with egg retention time.

The eggs from different females spawning exhibited a different sensitivity to aging, which confirms results obtained by Escaffre et al. (1976).

Temperature amplified the effect of overripening on egg quality. Developmental rates decreased much more rapidly at 17°C than at 12°C, and the triploid rate was significantly higher at high temperature for all retention times.

The pH and osmolality of coelomic fluid showed a significant decrease during egg aging, whereas protein concentration significantly increased. Therefore, those parameters can be used as helpful egg quality predictors.

Conclusion

This study demonstrates that unadapted broodstock management, which could favour chromosomal abnormalities not necessarily linked to detectable malformations, can damage egg viability.

References

Escaffre A.M., J. Petit, and R.Billard. 1976. Evolution de la fécondabilité des ovules de truite arc en ciel laissés dans la cavité coelomique après ovulation. Rev. Trav. Inst. Pêches marit 40(3-4):561-562.

Kjorsvik E, A. Mangor-Jensen, and I. Homefjord. 1990. Egg quality in fishes. Adv. Mar. Biol. 26:71-113

Lecommandeur D., P. Haffray, and L. Philippe. 1994. Rapid flow cytometry method for ploidy determination in salmonid eggs. Aquacult. Fish. Manage. 25:345-350.

Miller G.D, J.E. Seeb, B.G. Bue, and S.Sharr. 1994. Saltwater exposure at fertilization induces ploidy alterations, including mosaicism, in salmonids. Can. J. Fish. Aquat. Sci 51(1):42-49.

Sakai K , M. Nomura, and H. Oto. 1975. The over-ripening phenomenon of rainbow trout-II, Changes in the percentage of eyed eggs, hatching rate and incidence of abnormal alevins during the process of over-ripening. Bull. Jap. Soc. Sci. Fish. 41(8):855-860.

Sedmak J and S.E. Grossberg. 1977. A rapid, sensitive, and versatile assay for protein using coomassie brillant blue G250. Anal. Biochem. 79:544-552.

Thorgaard G.H , P.S. Rabinovitch, M.W. Shen, A.E. Gall, J. Propp, and F.M. Utter. 1982. Triploid rainbow trout identified by flow cytometry. Aquaculture 29:305-30.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECTS OF ANTIBACTERIAL AGENTS ON THE HATCHING PERCENTAGE AND BACTERIAL LOAD IN THE HATCHING MEDIUM OF *ARTEMIA URMIANA* CYSTS

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Introduction

The usefulness of brine shrimp *Artemia* in the feeding of larvae of fishes and crustaceans has been well documented in the past few decades (Shelbourne, 1964; Gilmour et al., 1975; Sorgeloos, 1980). It is also known that the larvae of marine fish and shrimp are highly susceptible to microbial infections (Lavens and Sorgeloos, 1996). It is believed that the live food, including *Artemia*, can be an important source of potentially pathogenic bacteria. It has been observed that under high cyst-density hatching conditions, bacterial concentrations in the hatching solution may reach 10^8 colony forming units (CFU).ml^{-1} in less than 20 hours (Gilmour, 1975). Contamination of *Artemia* cysts with different pathogenic agents, especially with *Vibrio* sp., reduces the hatching percentage of the cysts on one hand and causes transmission of the pathogenic bacteria to the growing fish and shrimp larvae on the other hand, which can cause disease/mortality outbreaks in the larval rearing. Therefore, quality control of the cysts and cleaning them from probable infecting agents is very important.

Material and methods

Low hatching *A. urmiana* cysts were used for this experiment. Different disinfecting and cleaning agents used in the experiment include antibiotics, UV irradiation, heat shock, decapsulation, and hypochlorite. The experimental cysts were divided into 6 groups: group one (1×4) acted as control which were hatched according to the standard procedure (Sorgeloos et al., 1986); group 2 cysts (1×4) were disinfected using 200ppm hypochlorite for 20 minutes (Lavens and Sorgeloos, 1996); group 3 cysts (1×4) were decapsulated according to (Lavens and Sorgeloos, 1996) before the hatching procedure; and group 4 cysts (1×4)×4 were treated with UV irradiation for 1, 3, 6, and 12 hours before hatching. For this purpose, dry cysts were spread uniformly in one layer in

different Petri dishes and kept inside the UV box for the above-mentioned periods, shaking them in intervals. Group 5 cysts (1×4)×2 were subjected to heat shock (100°C for 1 sec and 73°C for 5 sec) before hatching, and the last group of cysts (1×4)×4 were hatched with the hatching medium containing 60ppm Penicilline-Streptomycin, Chloramphenicol, Oxytetracyclin, and Tetracyclin antibiotics (modified from Coleman et al., 1987). Hatching water was filtered/sterilized and all air tubes were fitted with anti-bacterial filters in order to prevent bacterial contamination through aeration. Hatching percentage was calculated after 24 hours incubation in all trials and replicates according to Sorgeloos et al. (1986). Immediately after sampling for hatching percentage, loopfuls from the hatching solutions were streaked on Difco Marine Agar and cultured at ambient temperature for 24 hours and bacteria were counted using standard bacterial techniques.

Results and discussion

Results showed that hatching of the cysts increased considerably when cysts were decapsulated, disinfected, or treated with antibiotics. Hatching percentage of the cysts increased by 20.8%, 15.6%, 10.7%, 8.2%, 7.3%, and 6.5% as a result of decapsulation, disinfection by hypochlorite, Penicillin-Streptomycin, Chloramphenicol , Oxytetracyclin, and Tetracyclin antibiotics, respectively. But heat shock seemd to kill the *Artemia* embryos, as no hatching was observed, and UV irradiation reduced hatching percentage, especially at prolonged exposures. It was observed that untreated cysts were populated by a sparse number of bacteria, totaling 1×10^{7} bacteria.ml^{-1} of the hatching medium, and turning it turbid. Contrarily, all antibiotics, especially Penicillin-Streptomycin (1×10^{4}CFU.ml^{-1}) and Chloramphenicol (2×10^{4}CFU.ml^{-1}), reduced the quantity of bacteria in the hatching solution considerably, producing very clear hatching water. No bacterial growth was observed in heat shock treatments, indicating complete disinfection of the cysts. Slight reduction in the bacterial quantity was observed due to UV irradiation (3.1×10^{6}), whereas decapsulation (1.4×10^{5}) and disinfection by hypochlorite (1.4×10^{5}) resulted in a considerable reduction in the bacterial load in the hatching medium. Therefore, it could be concluded that the routine decapsulation and disinfection of cysts by hypochlorite is more convenient and better for disinfecting *Artemia* cysts rather than these other techniques.

References

Austin B. and D.A. Allen. 1981. Microbiology of Laboratory hatched brine shrimp (*Artemia*). Aquaculture 26:369-383.

Coleman D.E., L.K. Nakagawa, R.M. Nakamura, and E. Chang. 1987. p.153-157. In: The Brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in

Aquaculture. G. Persoone, P. Sorgeloos, O.A. Roels, and E. Jaspers (Eds.), Universa Press, Wetteren (Belgium).

Gilmour A., M.F. McCallum, and M.C. Allen. 1975. Antibiotic Sensitivity of Bacteria isolated from the canned eggs of the Californian brine shrimp (*Artemia salina*). Aquaculture 6:221-231.

Lavens P. and P. Sorgeloos. 1996. Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper 361, FAO, Rome (Italy). 375p.

Shelbourne J.E. 1964. The artificial propagation of marine fish. p.1-38. In: Advances in Marine Biology, Vol.2. F.S. Russel (Ed.), Academic Press, New York.

Sorgeloos P. 1980. The use of the brine shrimp *Artemia* in Aquaculture. p.25-46. In: The Brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. G. Persoone, P. Sorgeloos, O. A. Roels, and E. Jaspers (Eds.), Universa Press, Wetteren (Belgium).

Sorgeloos P., P. Lavens, P. Leger, W. Tackaert, and D. Versichele. 1986. Manual for the culture and use of brine shrimp *Artemia* in aquaculture. State University of Gent, Belgium. 319p.

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THE STUDY ON THE TECHNOLOGY OF PRODUCING SEA BASS (*DICENTRARCHUS LABRAX* L. 1758) LARVAE IN HATCHERIES IN TURKEY

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Introduction

Marine fish cultivation within the Turkish mariculture industry has shown a rapid improvement in the last 15 years. The number of hatcheries dealing with larval production has increased up to 19, and the number of enterprises dealing with cultivation up to 243. In these hatcheries, production of sea bass and sea bream (*Sparus aurata*) is being periodically continued apart from the pre-studies of other species such as red sea bream (*Pagrus major*), common dentex (*Dentex dentex*), sharp-snout bream (*Puntazzo puntazzo*), striped bream (*Lithognathus mormyrus*) and turbot (*Psetta maxima*). The finfish mariculture industry comprises approximately 490 production units operating in about 11 countries of the Mediterranean basin. Particular reference is made to sea bream and sea bass culture (Stephanis, 1995).

Jones (1989) reported that there were many firms cultivating marine fish like sea bream, sea bass and turbot, of which the production capacity of hatchery units was around several million larvae. Improvement strategies and planning administrations in Asian countries, which are the leading aquaculture countries in the world, produce 83% of the total production. Garvey and Bennett (1991), in the their studies dealing with fish cultivation and the legal improvements in Western Ireland, emphasized that aquaculture could be seen as an important potential source for the development of the country and could be considered as a strong development factor in local, regional, national and even EU standards. In Israel, marine culture has shown an improvement in cultivation of sea bream and sea bass in intensive pool culture and cage rearing for the last ten years, although the studies on other species are in different stages of their improvement (Kissil, 1996). In Portugal, because the market demand has increased for sea bream and

sea bass since 1992 and the techniques of rearing have developed, cultivation of these species has rapidly increased.

Materials and methods

In this study, hatcheries for marine fish in Turkey have been chosen as research subjects. In 2001, the number of hatcheries rearing marine fish is nineteen. During the period of this study (September 2000–January 2001), thirteen hatcheries were active, three were at the stage of establishment, two were inactive and one was dealing with only egg production and marketing. In this study, full counting method was used; ten hatcheries were interviewed and three refused to participate. Five of these ten hatcheries interviewed are in the province of ⁄zmir country, four in the province of Mu□la country and one is in the province of Adana country. By their request, the names of the firms were kept hidden.

A survey was conducted among all active businesses to determine the systems used for sea bass larvae rearing, and the questions prepared for the poll were answered by authorities of the firms themselves. Then, after gathering the necessary data, and collecting later data, we evaluated them by comparing this information with international studies.

Results

In hatcheries concerned with the prelarval stage, it has been calculated that stocking densities were 100-150 larvae.l^{-1}. Larvae were kept in tanks with 10% water exchange with a temperature of 15-16°C in total darkness.

In 40% of hatcheries concerned with the post-larval stage, stock density was 100-150 larvae.l^{-1} and, in general, water exchange was 10% at the beginning of that stage and 40-50% at the end of that stage. Sixty percent of the hatcheries decreased the salinity level down to 24‰ and the other 40% down to 26‰. A seawater inlet was applied from the surface with desaturation column. For the illumination of larvae tanks artificial light was used with light intensity adjusted by a rheostat. Eighty percent of hatcheries concerned with the larval stage used rotifer (*Brachionus* sp.) for the first feeding, and 60% of them did not know the origin of the rotifer they use, while 13% did not know the size of it. In general, all of the hatcheries use algae and Selco in rotifer enrichment.

In all hatcheries observed, it was noticed that INVE-originated *Artemia* were used and the size of the first *Artemia* given were between 400-450µm. Ninety percent of the hatcheries use INVE-originated microparticulate food with *Artemia*. It has been reported that in 70% of the hatcheries, digestive tract

fullness rate of larvae was checked and found to be 80% and above. Seventy percent of the hatcheries involved in this study classified larval performance though growth measurement via a measuring box at least three times during production.

Almost all the hatcheries faced the problem of air swallowing, and applied the darkness method as treatment. Eighty percent of the hatcheries involved in this study sorted the larvae with swimbladders from those without and 38% used the method of density. Forty three percent of the hatcheries performed this sorting process during days 80-90, and most of them eliminate the fish without swimbladders. Although 90% of the hatcheries observed swimbladder hypertrophy, 44% face hypertrophy in each cultivation period. Sixty seven percent of the hatcheries declared a swimbladder hypertrophy rate below 25%, and 67% used high illumination to treat it, while 56% used high illumination when faced with a swimbladder hypertrophy rate of 10-20%.

Discussion

In order to be able to provide the best vitelline absorption of hatched larvae, it has been reported that they should be kept in a dark place. In view of this fact, 90% of the hatcheries use darkness in pre-larval stage. During larval production period, seawater flow should be 0-30% during days 1-30 and 30-50% during days 30-50 (Bromage and Roberts, 1995). Seventy percent of the hatcheries in the pre-larval stage, and 60% at the beginning of post-larval stage used 10% seawater flow. However, 80% of them used 40-50% seawater flow at the end of post-larval stage. The results we have obtained from hatcheries agree with previous research (Katavic, 1986).

Enriched or not, *Artemia* is an important source of live food to for cultivating sea bass larvae (Barnabe and Guissi, 1993). *Artemia* enriched with unsaturated fatty acids (20:5n-3; 22:6n-3) increase larval survival rates. All of the hatcheries participated in this study use Selco products as *Artemia* enrichment. Artificial food given in the weaning stage from live food to artificial food must be determined according to the feeding needs of larvae (Barnabe, 1991).

As a result, cultivation of sea bass larvae in hatcheries in our country has been affected by French technique. It has also been found that hatcheries did not perform any microscopic studies at critical points in cultivation. For instance, many firms did not check the digestive tract fullness rate of larvae at the first feeding or weaning stage, which is quite important for solving the problems faced during cultivation. Among the firms that answered this poll, 60% that used rotifer as the first food did not know the origin of rotifer they used, and this causes a lack of information about the food content and ingredients of the live

food given to larvae. Aside from this, the studies performed to assess swimbladder formation, either in the country or abroad, exhibit parallels, because the eye method used for sorting is not productive; fish without swimbladders can cause a lot of economical loss for the hatcheries. As well, nearly 100% of *Artemia* or microparticulate food used by hatcheries in our country is provided by INVE. In the light of these data, it has been established that the hatcheries in our country apply improvement techniques as much as possible, but as mentioned above, they do not completely use some of the engineering knowledge.

References

Barnabe G. 1991. La cria de lubina y de dorada. p.573-612. In: Acuicultra, Omega, S.A., Barcelona, Spain.

Barnabe G. and A. Guissi. 1993. Combined effects of diet salinity on European sea bass larvae *Dicentrarchus labrax*. J. World Aquacult. Soc. 24(4):439-450.

Bromage N.R. and R.J. Roberts. 1995. Broodstock management and egg and larval quality. Blackweel Sciences, p.138-168.

Garveys S. and J. Bennett. 1991, Aquaculture and rural development in the West Ireland, EAS Spec. Publ. 14:149-163.

Jones A. 1989. Recent development in marine fish culture. p.179-185. In: Aquaculture Europe 89, Bordeaux 24 Oct. 1989, Bordeaux.

Katavic I. 1986. Diet involvement in mass mortality on the development of the sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 58:45-54.

Kissil G.W. 1996. Aquaculture in Israel. World Aquacult. 27(1):25-30.

Stephaniz J. 1995. Mediterranean aquaculture industry trends in production trends in production, markets and marketing. Marketing of aquaculture products. Proceedings of the seminar of the seminar of the CIHEAM network on socio-economic and legal aspects of aquaculture in the Mediterranean (Selam), Thessaloniki (Greece), 11-13 Oct. 1995, p.93-102, Cah. Options Mediterr. Vol. 17.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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DEVELOPMENT OF SOME DIGESTIVE ENZYMES IN SPOTTED SAND BASS *PARALABRAX MACULATOFASCIATUS* LARVAE

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Introduction

The spotted sand bass (*Paralabrax maculatofasciatus*) is a marine species with a good potential for culture in Mexico. In marine fish with indirect ontogeny, such as spotted sand bass, the digestive capacity is limited, since the digestive tract is undifferentiated in the early stages (Peña-Martínez, 2000). Studies of the larval digestive capacity in this species can help improve survival and growth. Moreover, it can help in the development of an artificial diet. Assessment of enzymatic capacity in fish larvae is more difficult than in adults since their digestive tract is not completely developed. Nevertheless, some authors have determined this capacity by using *in vitro* biochemical detection and electrophoretic techniques. It has been proposed that the digestive capacity of the marine fish larvae is not related to the exogenous enzymes from live food (Moyano et al., 1996). The objective of the present study is to determine the activity of some digestive enzymes in starved larval spotted sand bass using biochemical techniques.

Materials and methods

Eggs were obtained from spotted sand bass broodstock at CICIMAR-IPN. Fertilized eggs were maintained at 24°C and 35ppt salinity. Eggs, yolk-sac larvae of 12 and 24h after hatching (HAH), and larvae of 48, 72, and 96HAH were sampled. Samples were rinsed with distilled water, freeze-dried, and stored at -50°C until biochemical analysis. Frozen eggs (n = 1480), whole yolk-sac larvae (n = 200-300), and larvae (n = 150-300) were weighed and homogenized in 4-8 volumes of Tris-HCl (50mM, pH 7.5). The suspensions were centrifuged (12 000G for 15min, 5°C) and the supernatants were kept at -50°C. Soluble protein content was determined by the Bradford procedure (Bradford, 1976) using bovine albumin as standard. Enzyme activities are expressed as specific activity mU.larvae^{-1}. The enzymatic activities were

determined by triplicates using different biochemical techniques: alkaline protease (Hernández-Cortés, 1993), acid protease (Anson, 1938), non-specific esterase (Versaw et al., 1989), and amylase (Vega-Villasante et al., 1993). Trypsin and chymotrypsin hydrolyses were measured using specific substrates – TAME and SAAPNA, respectively (Hummel, 1959, modified). The units used to express enzymatic activities were the quantity of enzyme required to increase 0.01 units of absorbance per minute at 25°C. Electrophoresis (PAGE) in 7.5% acrylamide gels under native conditions were carried out according to Laemmli (1970). PAGE and zymograms were made with the same concentration of protein. Zymograms for non-specific esterase and amylase were conducted using β-naphtyl capryolate and starch, respectively. Electrophoresis were performed at a constant voltage of 120V for 1h at 5°C. One-way ANOVA was applied to larval weights enzymatic activities, followed by a LSD test when significant differences (P<0.05) were detected.

Results

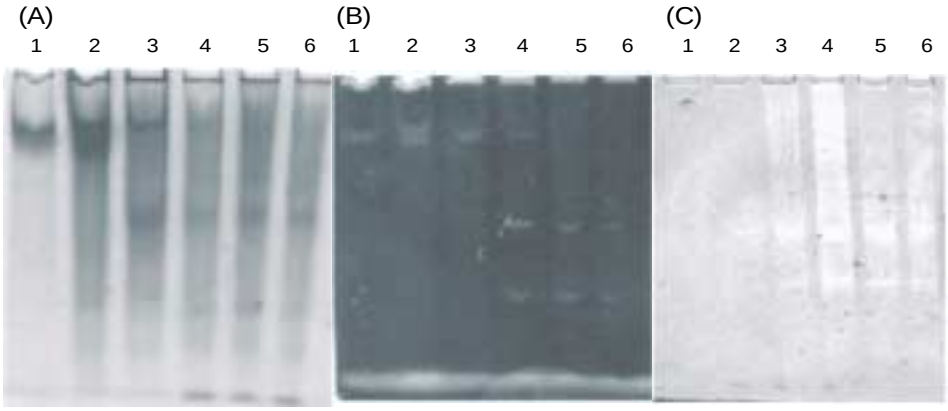
Table I. Values of dry weight, protein extract concentration, amylase, alkaline protease, lipase, and hydrolysis of chymotrypsin and trypsin (mean ± SD, n = 3) in starved spotted sand bass larvae. (*) Mouth opening.

	Time (HAH)					
	0	12	24	48*	72	96
Dry weight ($\mu g.ind^{-1}$)	3.4 ± 0.1^{a}	2.0 ± 0.2^{b}	1.9 ± 0.4^{b}	1.9 ± 0.2^{b}	1.6 ± 0.1^{c}	1.5 ± 0.1^{c}
Protein extract concentration ($mg.ml^{-1}$)	3.8 ± 1.5^{c}	5.2 ± 1.9^{b}	7.6 ± 0.7^{a}	7.7 ± 0.6^{a}	3.5 ± 1.1^{bc}	2.6 ± 1.4^{c}
	Activity ($mU.larvae^{-1}$)					
Amylase	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	4.6 ± 1.1^{b}	47.9 ± 2.4^{a}	6.7 ± 1.2^{b}	7.9 ± 4.8^{b}
Alkaline protease	0.0 ± 0.0^{b}	0.3 ± 0.2^{a}	0.3 ± 0.1^{a}	0.3 ± 0.2^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
Lipase	0.4 ± 0.3^{e}	1.8 ± 0.7^{d}	6.3 ± 1.4^{b}	12.0 ± 0.7^{a}	3.4 ± 0.6^{c}	1.2 ± 0.5^{de}
	Hydrolysis of specific activity ($mU.larvae^{-1}.min^{1}$)					
Chymotrypsin	222.5 ± 53.4^{a}	46.1 ± 15.4^{b}	22.9 ± 3.5^{b}	5.4 ± 1.3^{c}	8.5 ± 2.3^{c}	1.1 ± 0.2^{d}
Trypsin	133.8 ± 36.6^{a}	$33.3\pm8.2b^{c}$	62.6 ± 10.2^{b}	14.9 ± 1.4^{c}	26.5 ± 3.8^{c}	12.6 ± 3.0^{c}

Eggs showed the highest weight compared to the other developmental stages. Larval weight decreased during the experimental period. Significant differences were detected between the different sampling hours for protein extract concentration, alkaline protease hydrolysis, and lipase and amylase activities. Significantly higher protein extract concentrations were obtained at 24 and 48HAH. Significantly higher amylase and lipase activities were observed at 48HAH, a time at which the absorption of yolk-sac was completed. A marked decrease of protein concentration and all activities was observed at 72HAH. No pepsin-like activity was detected at any time. Trypsin and chymotrypsin hydrolysis showed significant differences among sampling hours; the highest

values were observed at time 0 (eggs) and constantly decreased over the experimental period (Table I).

In PAGE, the protein extract showed at least seven different bands (Fig 1A). A progressive increase in the number of bands was observed according to time. However, the intensity of some of these bands also decreased at 48HAH. Zymograms of amylase and lipase activities showed different band. Some of these correspond to those observed in the PAGE. Two groups of bands of amylase activity were observed through time. The first group presented a strong activity from time 0-24HAH, and then disappeared at 72 HAH. The second group appeared at 48HAH and was still present at 96HAH (Fig. 1B). The lipase activity showed two different bands that were very clear at 48HAH, after which they were more diffused (Fig. 1C).



Discussion

Our results confirm the presence of enzymes in the absence of exogenous food. This has also been observed in *Dicentrarchus labrax* and *Sparus aurata*, where the secretion of digestive enzymes is detected before the first exogenous feeding (Zambonino-Infante and Cahu, 1994; Moyano et al., 1996). In these studies, activities of various enzymes increase with age. However, in starved larvae these activities decreased after 48h, probably due to the degeneration of secretory tissues. Our study showed that the highest trypsin and chymotrypsin activities were detected in the egg. This could be due to the process of absorption of the yolk-sac, since it contains a great quantity of biomolecules related to metabolism (Munilla-Morán et al., 1990). On the other hand, the pepsin-like enzyme activity

was not detected. The digestive system of spotted sand bass is not completely differentiated until 16DAH (Peña-Martínez, 2000) Therefore, its enzymatic capacity is limited.

Conclusions

We conclude that the spotted sand bass larvae present, before the introduction of live food, rudimentary enzymatic equipment which seem to be involved in the absorption of the yolk-sac and the digestion of exogenous food.

References

Anson M.L. 1938. The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. J. Gen. Physiol. 22:79-89.

Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Hernández-Cortés M.P. 1993. Proteinasas con actividad quimotripsina y colagenasa en la langostilla *Pleuroncodes planiceps* (Decapoda). Bachelor's thesis. UABCS. Mexico. 109p.

Hummel B.C.W. 1959. A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. Can. J. Biochem. Physiol. 37:1393-1399.

Laemmli U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Moyano F.J., M. Díaz, F.J. Alarcón, and M.C. Sarasquete. 1996. Characterization of digestive enzymes activity during larval development of gilthead sea bream (*Sparus aurata*). Fish Physiol. Biochem. 15(2):121-130.

Munilla-Morán R., J.R. Stark, and A. Barbour. 1990. The role of exogenous enzymes in digestion in culture of turbot larvae (*Scophthalmus maximus* L.). Aquaculture 88:337-350.

Peña-Martínez R. 2000. Histología e histoquímica del desarrollo ontogenético del tubo digestivo de larvas de la cabrilla arenera (*Paralabrax maculatofasciatus*) (Percoidei: Serranidae). Master's thesis, CICIMAR-IPN. 113p.

Vega-Villasante F., H. Nolasco-Soria, and R. Civera-Cerecedo. 1993. The digestive enzymes of the Pacific brown shrimp (*Penaeus californiensis*) I. Properties of amylase activity on digestive tract. Comp. Biochem. Physiol. 112B(1):123-129.

Versaw W., S.L. Cuppett, D.D. Winters, and L.E. Williams. 1989. An improved colorimetric assay for bacterial lipase in nonfat dry milk. J. Food Sci. 54(5):232-254.

Zambonino-Infante J.L. and C. Cahu. 1994. Development and response to a diet change of some digestive enzymes in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiol. Biochem. 12(5):399-408.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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A GLUTAMATE DEHYDROGENASE TRANSCRIPT SPECIFIES THE EARLY DEVELOPMENT OF PRONEPHROS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Introduction

Glutamate dehydrogenase (GDH) catalyses the reversible reaction of glutamate synthesis and oxidation. This later function is part of the transdeamination reaction, which is the general pathway for amino acid oxidation occurring in the liver. GDH is thus a key enzyme for protein as well as for energy metabolisms. Because fish oxidize largely dietary amino acids for energetic purposes, glutamate dehydrogenase activity has been mostly studied in the liver of those species. But this enzyme is present in many different tissues (Mommsen et al., 1980). Recently, a cDNA coding for GDH has been cloned from a rainbow trout liver library. In adults, we have shown that this transcript (*gdh*1) was expressed more strongly in the kidneys than in the liver when fish were fasted, although its expression seems to vary with the nature of the diet (Mambrini et al., 2000). Because the kidneys are assumed to have a primordial role in the absorption of nutrient from the yolk sac, the objective of the present study was to investigate the expression of *gdh*1 during the embryonic development of rainbow trout by whole-mount *in situ* hybridization.

Material and methods

Ovules of spring spawning rainbow trout (*Oncorynchus mykiss*) were artificially fertilized and incubated at 10°C. Eggs were sampled at different stages of development (21 to 29, according to Vernier, 1969). They were fixed in 4% paraformaldehyde for 1h, embryos were dechorionated with fine forceps, dehydrated and stored in methanol at -20°C.
Whole mount *in situ* hybridizations were done as described for medaka embryos (*Oryzias latipes*; Joly et al., 1997). A correct permeabilization of the rainbow trout embryos was ensured by increasing the duration of proteinase K treatment (Sigma, 50µg.ml^{-1}, 45min and 1 hour, for stages 21-25 and 26-29, respectively).

Sense and antisense DIG labeled probes corresponding to the 3' untranslated region of *gdh*1 were used. Some hybridized embryos were wax embedded and sectioned at 8μm in the sagittal plane. Embryos were observed with a stereomicroscope (Leica M10) and sections with a microscope (Leica, Leitz DMRB).

Results and discussion

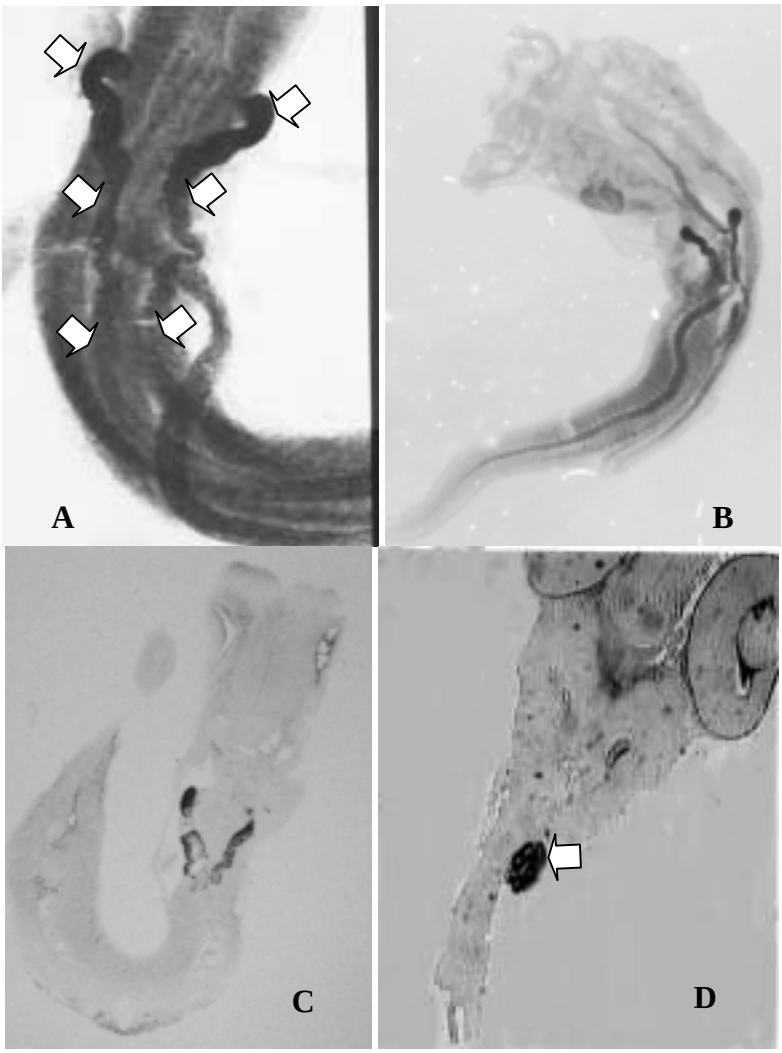

Fig. 1. Localization of *gdh*1 expression in rainbow trout embryos, stage 21 (A), 22 (B), 23 (C) and 27 (D), sagittal sections photographed with a photomicroscope (×10).

By whole-mount *in situ* hybridization, we followed *gdh1* expression from stage 21, when kidneys differentiate (Vernier, 1969) to hatching (stage 29). No signal was observed with the control sense riboprobe. The first signal detected with the anti-sense riboprobe is observed at stage 21 all over two paired organs resembling pronephric kidneys (Fig. 1A). From stage 22 onwards, the two organs merge at the level of the convoluted segments (Fig. 1B). After stage 23, the label is more intense in the proximal part of the organ (Fig. 1C), and reveals structures elongated and irregular in shape forming tubules with varying lumen diameters. At stage 24, when the intestinal enlarges (Vernier, 1969), *gdh*1 is expressed in the developing intestine (Fig. 1D). After stage 28, the labeling was undetectable, either because the tissues were imperfectly permeabilized at these stages, or because *gdh*1 expression was too low.

The pattern of *gdh*1 expression reveals, that the early pronephros consists of two distinct segments merging later on, as proposed by Tytler (1988). The time course of *gdh* expression has been studied in higher vertebrates, but mostly in the liver (Notemboom et al., 1997). A previous study showed that GDH activity is important in chicken mesonephros as soon as this organ differentiates (Wang, 1972). In fish, the kidneys differentiate before the liver, and are supposed to play a major role in the resorption of the yolk nutriments; GDH may thus have an important role for the energy supply in the early stages of development.

References

Joly J.S., F. Bourrat, V. Nguyen, and D. Chourrout. 1997. Ol-Prx 3, a member of an additional class of homeobox genes is unimodally expressed in several domains of the developing and adult central nervous system of the medaka (Oryzias latipes). Proc. Natl. Acad. Sci. 94:12987-12992.

Mambrini M., L. André, S. Panserat, E. Perrot, A. Guyonvarch, and D. Chourrout. 2000. Expression différentielle de deux transcrits codant la glutamate déshydrogénase chez la truite arc-en-ciel. Nutr. Clin. Métabol. 14(suppl. 2):160s

Mommsen T.P., C.J. French, and P.W. Hochachka. 1980. Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. Can. J. Zool. 58:1785-1799.

Notenboom R.G., A.F. Moorman, and W.H. Lamers. 1997. Developmental appearance of ammonia-metabolizing enzymes in prenatal murine liver. Microsc. Res. Tech. 39:413-23.

Tytler P. 1988. Morphology of the pronephros of the juvenile brown trout, Salmo trutta. J. Morph. 195:189-204.

Vernier J.M. 1969. Table chronologique du développement embryonnaire de la truite arc-en-ciel, *Salmo gairdneri Rich*. 1836. Ann. Embryol. Morphol. 2:495-520.

Wang KM. 1972. Changes of enzymic activity in mesonephros as a function of age. Comp. Biochem. Physiol. 42B:143-155.

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RECENT IMPROVEMENTS IN BROODSTOCK MANAGEMENT AND LARVICULTURE IN MARINE SPECIES IN POLYNESIA AND NEW CALEDONIA: GENETIC AND HEALTH APPROACHES

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Aquaculture production in the French Overseas Territories in the south-Pacific (French Polynesia and New-Caledonia) is based on 3 main species*:* the shrimp *Penaeus stylirostris*, the black-lip pearl oyster *Pinctada margaritifera*, and the fish *Lates calcarifer*. The fish and shrimp farming industries rely on enclosed broodstock, whereas the pearl industry still remains dependent upon the natural reproductive cycle. Most of the control factors of gametogenesis, temperature, hormones, and nutrition are known in shrimp and fish and are used to control reproduction. In the pearl oyster, gametogenesis is also subjected to these factors, but nutritional needs remain largely unknown. Since in the wild, the annual temperature variation differs according to the latitude of the Islands, the influence of this factor on gametogenesis is under investigation. The zootechnical level reached allows planning of crossbreeding at an individual level in shrimp and fish, but the individual spawning remains inadequately controlled in pearl oyster. Shrimp and fish hatchery technology now includes the routine use of micro-particulate diets as partial substitution for algae and *Artemia* nauplii, as well as antibiotics and fungicide for shrimp larval rearing. Recently, an alternative method to antibiotherapy in larval shrimp has been tested in disease control using probiotic bacteria. These results could find also an application in pearl oyster larval rearing.

The optimization of genetic resources is a real challenge for the fish and shrimp industries as the species were introduced into the 2 territories. Refrigeration of the shrimp sperm now permits an interesting way to re-introduce variability at a low sanitary risk level as molecular markers have proven the loss of variability of the Tahitian strain of *P. stylirostris* after more than 10 generations of domestication without genetic caution. The use of the same markers may improve the genetic management by allowing paternity controls. Similarly, molecular biology may help to re-build the genealogy of the Tahitian broodstock of *L. calcarifer*, which has only 3 generations of domestication, and give key elements to determine the best crosses to limit future inbreeding. As a local

species, the Polynesian pearl oyster industry has been developed up to now thanks to the natural genetically wild spat of *P. margaritifera* caught in islands or atolls of French Polynesia. The genetic differentiation of the populations of French Polynesia has already been studied and is under deeper investigation to elaborate conservation strategies. Genetic improvement of shrimp through selective breeding is underway in Tahiti, and the efficiency of early grading in the genetic selection for growth is presently under assessment on a pilot scale in New-Caledonia. The fish farming industry is too young and too small to start with genetic selection. The long-term development of the pearl industry relies on the development of hatcheries which could produce improved spat for different traits, either through polyploidy or through selection – 2 hatcheries have already started to produce spat, which is the first step towards domestication.

French Polynesia and New Caledonia are not affected by the viral outbreaks affecting shrimp elsewhere in the world (White spot and Taura viruses) and pearl oysters in Japan (Akoya virus). This situation is mainly due to their geographical isolation from the affected areas. Furthermore, to maintain the sanitary status importation of aquatic living animals are regulated. In this context, the IFREMER lab in Tahiti is equipped with a quarantine device to introduce new genetic resources. To protect the pearl oyster industry in French Polynesia, a zoo sanitary watching network of pearl oyster stocks is coming up in order to detect the emergence of any pathogens and be able to take the appropriate measures to avoid the spread of disease.

Aquaculture productions in the French Pacific Overseas Territories (French Polynesia and New Caledonia) are good cases to study how research and production interact. The determination of the scientific priorities for a sustainable development is related to socio-economics and legal aspects.

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AMINO ACID PROFILES OF SEA BREAM (*SPARUS AURATA*) AND SOLE (*SOLEA SENEGALENSIS*) DURING FIRST FEEDING: ARE CURRENTLY USED DIETS IMBALANCED IN AMINO ACIDS?

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Introduction

Since growth is essentially protein deposition (Houlihan et al., 1993), it is important that dietary amino acid profiles meet larval amino acid requirements, in order to optimize growth. The amino acid profile of fish larvae is considered a good indicator of its essential amino acid requirements (Watanabe and Kiron, 1994). This amino acid profile can be corrected for the bioavailability of individual amino acids in order to have a better estimate of the amino acid requirements.

Early cultivation of fish larvae relies largely on feeding strategies based on live food. Sea bream (*Sparus aurata*) and sole (*Solea senegalensis*) are no exceptions to this rule. The objective of this work is to compare the amino acid profiles of larvae and food during larval first feeding and identify possible imbalances.

Materials and methods

S. aurata larvae were stocked in two cylindroconical 70-l white tanks, in a closed system. Rearing water was maintained at 18.1±0.3°C and 36.8±0.9ppt. From mouth opening (3 days after hatching) onwards, larvae were fed rotifers enriched for 24h in DHA Protein Selco (INVE Aquaculture, Belgium). Samples were taken at 4 and 7DAH for proteinic amino acid analyses and dry weight determinations.

Newly hatched *S. senegalensis* larvae were stocked in two cylindroconical 100-l white tanks, in a closed system. Rearing water was maintained at 19.0±0.7°C and 35.4±0.6ppt. Larvae were fed rotifers from mouth opening (2DAH) until 5DAH, with *Artemia* nauplii (BE 480, INVE, Belgium) being introduced at 3DAH. Rotifers were enriched for 24h in 50% *Tetraselmis suecica var.chuii* and

50% *Isochrysis galbana*. Samples were taken at 2 and 5DAH for proteinic amino acids analyses and dry weight determinations.

Rotifers from both experiments were sampled for proteinic amino acid analyses. Proteinic amino acids were obtained after hydrolysis of proteins from larvae and rotifers in 6M HCl at 110°C over 24h in nitrogen-flushed glass vials. Amino acids were analyzed using a Gilson HPLC System. α-amino-butyric acid was used as internal standard.

Essential amino acid profiles of larvae were corrected for the bioavailabilities of each individual amino acid due to selective absorption and/or catabolism. Bioavailabilities were estimated using diets enriched in ^{13}C and comparing specific activities in the individual amino acids in larvae and rotifers. Specific activities were obtained by high-resolution ^{13}C-NMR spectroscopy together with HPLC analysis (Conceição et al., unpublished data).

Results and discussion

Fig. 1 compares the essential amino acid profile of sea bream larvae, corrected for the bioavailabilities for each amino acid, with the amino acid profile of rotifers enriched in DHA Protein Selco. Methionine, tyrosine, isoleucine, and valine in the rotifers do not seem to meet the amino acid requirements of sea bream larvae 4DAH. Similar results (not shown) were obtained at 7DAH. Methionine is probably the limiting amino acid during this period. Dry weight was 38±3μg for larvae aged 4DAH and 37±4μg for 7DAH.

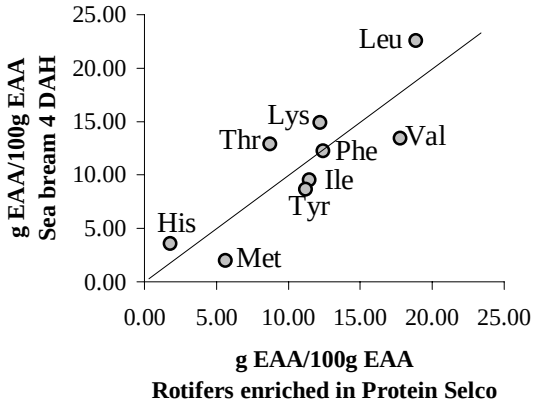

Fig. 1. Essential amino acid profile (excluding arginine and tryptophan) of sea bream larvae aged 4 days after hatching, corrected for the bioavailabilities for each amino acid, versus essential amino acid profile of rotifers enriched in DHA Protein Selco.

Comparison between the essential amino acid profile of sole larvae (corrected for the bioavailabilities for each amino acid) with the amino acid profile of rotifers enriched in *Tetraselmis suecica var.chuii* and *Isochrysis galbana* is represented in Fig. 2. Rotifers seem to be deficient in methionine, tyrosine, isoleucine, and valine to sole larvae at 2DAH. Similar results (not shown) were obtained at 5DAH. Methionine is probably the limiting amino acid at first feeding. Dry weight was 35±1μg at 2DAH and 40±4μg at 5DAH.

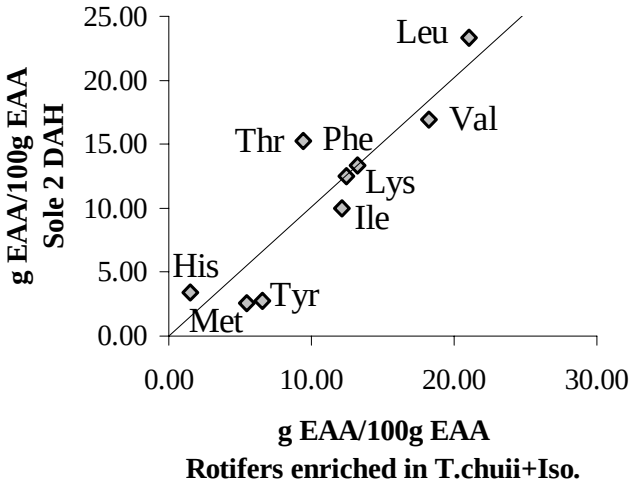

Fig. 2. Essential amino acid profile (excluding arginine and tryptophan) of sole larvae aged 2 days after hatching, corrected for the bioavailabilities for each amino acid, versus essential amino acid profile of rotifers enriched in *Tetraselmis suecica var.chuii* and *Isochrysis galbana*.

Dietary imbalances in the amino acid profile were also suggested for turbot (Conceição et al., 1997) and African catfish (Conceição et al., 1998) larvae.

Conclusions

Live food usually given to sea bream and sole at first feeding do not seem to meet the larval amino acid requirements. Manipulation of the dietary amino acid composition may be necessary. Further studies should be done on the effects of these imbalances in terms of growth, survival, and metabolism of fish larvae.

Acknowledgements

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References

Conceição L.E.C., T. van der Meeren, J.A.J. Verreth, M.S. Evjen, D.F. Houlihan, and H.J. Fyhn. 1997. Amino acid metabolism and protein turnover in larval turbot (*Scophthalmus maximus*) fed natural zooplankton or *Artemia*. Mar. Biol. 129:255-265.

Conceição L.E.C., R. Ozório, E.A. Suurd, and J.A.J. Verreth. 1998. Amino acid profiles and amino acid utilisation in larval African catfish (*Clarias gariepinus*): effects of ontogeny and temperature. Fish Physiol. Biochem. 19:43-57.

Houlihan D.F., C.J. Carter, and I.D. McCarthy. 1993. Biochemical correlates of growth rate in fish. p.45-71. In: Fish Ecophysiology. J.C. Rankins and F.B. Jensen (Eds.). Chapman & Hall, London.

Watanabe T. and V. Kiron. 1994. Prospects in larval dietetics. Aquaculture 124:223-251.

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PROTEIN AND AMINO ACID CONTENTS OF BROODSTOCK TISSUES, EGGS, AND NEWLY HATCHED JUVENILE OF RED CLAW CRAYFISH, *CHERAX QUADRICARINATUS*, UNDER CULTURE SYSTEM

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Introduction

Crayfish are commercially important in the USA, Western Europe, China, and Australia. The total production of freshwater crayfish is approximately 7000-10 000 metric tons (MT) per annum (Hurnes, 1989). Red claw crayfish, *Cherax quadricarinatus*, is one of the most acceptable and commercial crayfish in the world aquaculture industry. This species has been recently introduced in some parts of Malaysia. There is considerable interest in the development of hatchery, nursery, and grow out technology for this crayfish in Malaysia.

Literature review reveals that there is no notable scientific work on the body protein level and protein nutritional requirement of the crayfish broodstock under indoor culture system. Moreover, there is a lack of information on amino acid content of broodstock tissues, eggs, and newly hatched juveniles of red claw crayfish. This study was thus carried out to provide some information on protein and amino acid levels of muscle, gonads, eggs, and newly hatched juveniles of this commercially important crayfish. The information can be used as a guideline in developing high quality diet for crayfish broodstock in order to produce healthy juveniles for grow out phase.

Materials and methods

Female *Cherax quadricarinatus* were selected randomly from broodstock tanks in a culture system at the University Putra Malaysia hatchery Complex. Samples from gonads, eggs, and newly hatched juveniles were subjected to protein analysis according to Bradford (1976) via the micro-method of the Bio-Rad protein assay kit (Bio-Rad laboratory) after pretreatment with 0.5N NaOH at 100ºC for 20 minutes and followed by neutralization with HCl (Meyer &

Walther 1988). Bovine serum albumin was used as standard. All samples for amino acid analysis were hydrolyzed in duplicate with 6N HCl at 110ºC for 24h, and derivatized with phenylisothiocyanate (PITC) before chromatographic separation using a Pico-Tag reversed phase 3.9×150mm HPLC column (Water, Division of Millipore, Milford, MA). The amino acid analysis was performed on the Water Pico-Tag amino acid analysis System which consisted of two M510 solvent delivery systems controlled by a Water 840 System and a Water 490 multiwavelengh detector (240µm/0.2AUFS) following a modified procedure of Hagen et al. (1989). A Water M712 WISP automatic injector was used to inject samples into the system. Chromatographic peaks were integrated, identified, and quantified with a Water Expert chromatography (version 6.2) software package by comparing it to known standards (amino acid standard H, Pierce Rockford, IL). Data for gonadal and embryonic development were arcsine-transformed and subjected to one-way analyses of variance, appropriately followed by Duncan's test.

Results

The protein level of three stages of female ovarian development as identified by Jones (1995) is shown in Table I.

Table I. Percent protein in ovarian stages of *Cherax quadricarinatus*. Means within column and within the same superscript are not significantly different ($P<0.05$).

Characteristics	Ovary stage	% Protein ± SD
1. No ovary discernible	Immature	10.48±0.68 [a]
2. Ovary visible, individual ova not discernible	Maturing	12.98±0.54 [b]
3. Ovary clearly visible (olive green), individual ova apparent	Mature	36.34±0.99 [c]

SD = standard deviation of the mean

Table II presents the protein content of different embryonic stages as identified by Jones (1995) and newly hatched juveniles.

Table II. Morphological characteristics and % protein for various stages of *Cherax quadricarinatus* embryonic development. Means lettered in the same superscript are not significantly different ($P<0.05$).

Egg stage	Morphological characteristics of egg	% Protein ± SD
1	Olive green, elongated	48.71 ± 6.79[a]
2	Dark brown, rounded	59.38 ± 3.89[a]
3	Yellow/orange	62.50 ± 4.98[b]
4	Red, eyes not visible	63.52 ± 2.02[b]
5	Red, eye visible	64.23 ± 9.20[b]
6	Red, eyes, and pereiopods visible	65.29 ± 5.59[b]
7	Egg hatched but juvenile still attached to pleopod	54.64 ± 3.69[a]

SD = standard deviation of the mean

Protein level in female gonad and embryo increased from lowest in first stage of gonad to highest in stage 6 (Fig. 1).

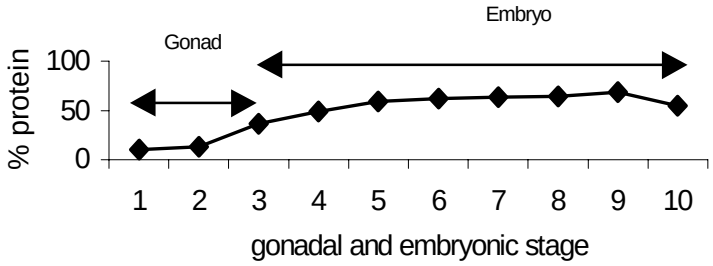

Fig. 1. Protein content of various stages of gonads and embryo

Obvious differences were observed in the amino acid pattern of crayfish gonads, eggs, and newly hatched juveniles (Table III). Crayfish gonads had significantly higher concentrations of most EAA followed by newly hatched juveniles. The lowest content of EAA was found in the egg among all tissues (Fig. 2).

Table III. The essential amino acid composition of gonad, egg and newly hatched juvenile of red claw crayfish (g.100g^{-1} sample).

EAA	Gonad	Egg	Newly Hatched Juvenile ± SD
Arginine	4.33±0.07^{c}	3.8±0.02 ^{a}	4.09±0.07^{b}
Cystine	0.28±0.02^{a}	0.38±0.19^{a}	0.35±0.05^{a}
Histidine	0.33±0.00 ^{c}	0.71±0.04^{a}	1.28±0.19^{b}
Isoleucine	2.29±0.04^{c}	1.83±0.04^{a}	2.07±0.02^{b}
Leucine	3.84±0.03^{c}	2.99±0.09^{a}	3.44±0.04^{b}
Lysine	2.87±0.00 ^{a}	2.63±0.28 ^{a}	2.74±0.03 ^{a}
Methionine	1.07±0.02 ^{b}	0.88±0.05^{a}	0.98±0.04^{ba}
Phenylalanine	3.65±0.02 ^{c}	3.18±0.08 ^{a}	3.46±0.03 ^{b}
Threonine	2.31±0.03^{c}	2.08±0.02^{a}	2.43±0.03^{b}
Valine	2.71±0.07^{c}	2.31±0.00 ^{a}	2.46±0.03^{b}

Threonine, methionine, cystine, and lysine were not significantly different (P>0.05), among the various tissues. The lowest histidine content was recorded in gonad comparing to eggs and newly hatched juveniles.

Discussion

An increase in ovarian and embryo protein content with maturation (see Tables I and II, and Fig. 1) emphasizes the importance of protein in the synthesis of VG (egg yolk vitellogenin) and LV (egg yolk lipoprotein, or lipovitellin). Read and Caulton (1980) have reported similar increases in ovarian development of marine shrimp *Penaeus indicus*. Since reproduction and gonadal maturation are

periods of intense biosynthesis, it may be expected that protein requirements during maturation are higher than for non-reproducing adults.

The differences observed in the EAA composition of the various tissues (Table III) were probably due to different types or ratios of protein. Declining EAA concentration in embryonic stages (fertilized eggs) may be partly due to the dependency of the embryo on amino acids derived from the egg yolk for developing organ systems and metabolism. The amino acid composition of various tissues of gonad, eggs, and newly hatched juveniles might shed some light on red claw crayfish broodstock nutrition, due to the important role it plays in the formation of egg protein for newly hatched juvenile.

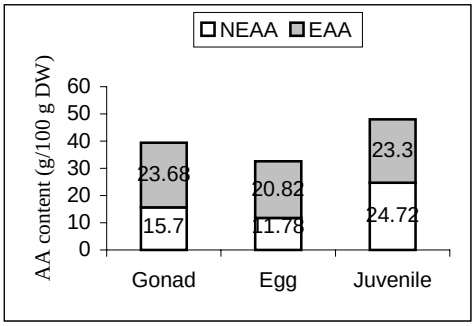

Fig. 2. Contents of essential and non-essential amino acid of gonad, egg, and newly hatched juvenile of red claw crayfish.

References

Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254

Hagen S.R., B. Frost, and J. Augustine. 1989. Procolumn phenylisothiocyanate derivatization and liquid chromatography of amino acids in food. J. Assoc. Off. Anal. Chem. 72:912-9

Hubbard D., E. Robinson, P. Brown, and W. Daniels. 1986. Optimum ratio of dietary protein to energy for red claw crayfish (*Procambarus clarkii*). Prog. Fish-Cult. 48:233-237

Huner J.V. 1989. Overview of international and domestic freshwater crawfish production. J. Shellfish Res. 8(1):259-265

Jones C.M. 1995. Production of juvenile red claw crayfish *Cherax quadricarinatus* (Von Martens) (Decapod, Parastacidae). 1. Development of hatchery and nursery procedures. Aquaculture 138:221-238

Meyer E. and A. Walther. 1988. Methods for the estimation of protein, lipid, carbohydrate and chitin levels in freshwater invertebrates. Arch. Hydrobiol. 113:161-177

Read G.H.L. and M.S. Caulton. 1980. Changes in mass and chemical composition during the molt cycle and ovarian development in immature and mature *Penaeus indicus* Milne Edwards. Comp. Biochem. Physiol. 66A:431-437.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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DEVELOPING BIOENCAPSULATED HERBAL PRODUCTS FOR MATURATION AND QUALITY LARVAL PRODUCTION IN *PENAEUS MONODON* (L.)

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Abstract

In India, the annual brooder shrimp requirement is about 300 000 and some times the cost of a single brooder goes up to US$1000. Since the eyestalk ablated shrimp produced poor quality larvae, improvement of the larval quality by applying herbal biomedicinal products along with products inducing reproduction (aphrodisiac characteristics) have been undertaken. Three herbal biomedicinal products having aphrodisiac characteristics such as *Withania somnifera*, *Ferula asafoetida*, and *Mucuna pruriens* along with an appetizer *Piper longum* were selected and four different combinations were made. They were fed to the uni-eyestalk-ablated shrimp, *Penaeus monodon* through adult *Artemia*. In the biomedicinal herbal product fed group, the number of eggs released was 8.0, 5.9, and 3.6×10^5 in the first, second and third spawning, respectively. The increase of eggs was significantly ($P<0.05$) influenced by the second combination than the other three combinations. In the control group, the egg release was 4.8×10^5 in the first spawning, 3.2×10^5 in the second spawning and 2.8×10^5 in third spawning. The animals fed with the herbal biomedicinal products were tested for the larval qualities such as survival, embryo and larval abnormalities, size variation and stress tolerance. They resulted in 85.35% survival in PL20, total abnormalities of embryos and larvae 17.31 and 51.58% respectively, size variation 19.65% in PL20 and stress tolerance, i.e., 75.20% survival in PL20 stage.

Introduction

A steady supply of spawners is essential for the efficient programming of hatchery operations (Babu, 1999). All the hatcheries in operation today rely on brood stock collected from the wild. With rapid increase in the number of hatcheries, operators today are experiencing an apparent shortage of quality wild spawners. Moreover, the spent spawners are rarely used because of its poor performance in the

subsequent spawning. Larval quality is one of the key factors affecting the culture result of the shrimps. Attempts to establish a system to produce healthy larval shrimp have been initiated by Chen et al. (1988). Approximately 65-75% of culture ponds obtained successful results with a production of more than 5mt.ha^{-1} when healthy larvae of giant tiger shrimp were cultured. Preliminary studies carried out by researchers to investigate the effect of herbal products on growth, stress resistance and reproduction in crustaceans revealed that herbal product combinations improved the survival, growth, and fecundity in anostracans like *Artemia* (Hilda, 1992). As the above studies were encouraging, influencing reproduction in the crustacean-brine shrimp, the herbal products have been tested in the shrimp, *Penaeus monodon* to influence reproduction and produce quality shrimp larvae.

Materials and methods

Disease free and healthy spawners of *P. monodon* (150±20g) were collected and transported with less stress following the method of Babu and Marian (1998). The acclimatized animals were treated for the pathogenic microorganisms (Babu, 1999). After the treatment they were allowed for spawning. The spent *P. monodon* were selected for the present experiment. They were maintained in a density of 5.m^{-2} in a maturation tank in the male:female ratio of 1:2. In addition to treatment feed (i.e., herbal-bioencapsulated *Artemia*), squid and crab feed were given at a quantity of 15% of the body weight of the animal. Feed was given two times a day, 8 a.m. in the morning soon after the water exchange (50-100 %)and then in the evening at 6 p.m. Unused feeds and the molt debris were removed regularly at the time of water exchange. Four combinations (1:2:3:4; 4:3:2:1; 2:1:4:3, and 3:4:1:2) of herbal products were prepared by mixing the herbal micronized (<50µm) powder and then enriched into adult *Artemia*. The enriched *Artemia* were fed to the ablated spawners twice a day at the rate of 2g per spawner. On maturation, the treated animals were transferred into the spawning tank. The control group, were also fed with unherbal-enriched adult *Artemia* . The fecundity of spawners was determined by counting the eggs in the spawning tank and the calculation was made by random sampling. The percentage of larval abnormalities was observed with the help of standard references and the size variations were calculated by taking mean size of the population. For stress the study, the post larvae were subjected to 100ppm formalin for 1 hour and for all other early stages of larvae they were exposed only to 50ppm formalin for 1 hour. After one hour, the survival percentage was calculated.

Results

Table I shows the fecundity of *P. monodon* spawner when fed with the 2nd

combination of herbal product enriched *Artemia*. In the experimental animals, the eggs released during the first, second and third spawnings were 8.04, 5.98, and 3.68×10^5 respectively. In the control animals, the highest egg release of 4.86×10^5 was obtained only in the first spawning, followed by 3.24 and 2.80×10^5 in the second and third spawning, respectively. The survival was higher in the herbal product enriched *Artemia* fed shrimp compared to the control (Table 1).

Table I. Effect of herbal product-enriched *Artemia* on fecundity and larval survival.

Time of spawning	Fecundity (Lakhs)		Stages of larvae	Larval survival (%)	
	with herbal	without herbal		with herbal	Without herbal
I	8.04 ± 1.06	4.86 ± 1.17	Nauplii	94.54 ± 2.60	80.32 ± 2.14
II	5.98 ± 1.30	3.24 ± 1.01	Zoea	93.01 ± 1.47	74.15 ± 2.45
III	3.68 ± 0.46	2.80 ± 0.57	Mysis	90.3 ± 3.28	60.38 ± 3.50
			Postlarvae	85.35 ± 0.61	57.7 ± 3.80

The size variation was less in the herbal treated group than the control (Fig. 1).

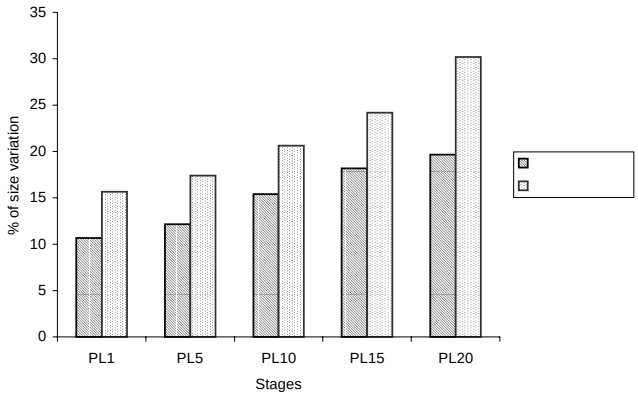

Fig. 1. Effect of herbal product-enriched *Artemia* on size variation of *P. monodon* postlarvae

Table II. Reduction in the abnormal development of embryo and larvae when fed on the second combination of herbal-enriched *Artemia*.

Time of spawning	% of abnormal embryos		Stages of larvae	% of abnormal larvae	
	with herbal	without herbal		with herbal	Without herbal
I	3.76 ± 1.64	7.68 ± 1.60	Nauplii	12.76 ± 1.50	17.35 ± 2.04
II	5.30 ± 2.67	10.43 ± 1.52	Zoea	14.82 ± 1.78	18.40 ± 2.13
III	8.25 ± 1.30	14.50 ± 1.40	Mysis	16.40 ± 2.70	22.64 ± 1.61
			Postlarvae	7.60 ± 1.70	13.22 ± 1.28

Table III. Influence of herbal product-enriched *Artemia* on stress resistance in *P. monodon* larvae.

Stages	Formalin stage	
	with herbal	without herbal
Nauplii (II)	60.12 ± 2.34	40.34 ± 2.60
Zoea (II)	70.36 ± 3.46	42.75 ± 3.40
Mysis (II)	62.48 ± 2.14	50.1 ± 2.61
Postlarvae (II)	75.2 ± 2.72	55.3 ± 3.62

Discussion

The continuous supply of cholesterol and PUFA derivatives is required principally for biomembrane formation after every molt. Significant replenishment of the same helps to curb larval abnormalities and Runt deformities (Arellano, 1990). As the phytosterols in the herbal products are precursors of cholesterol, optimal incorporation of those ingredients could have compensated for the adequate cholesterol levels in the larval production (Akiyama et al., 1992). Correlating the stress sensitivity indices with different larval qualities the primary effects of the ayurvedic herbal product reach an index, expressing onto secondary, tertiary improvements stress resistance when being challenged with formalin stress. Stress test in the experimental groups is the typical example helping for studying diet quality. It is possible to establish a relationship between the result obtained during larval and postlarval culture as a function of feeding regimes of the herbal product also.

References

Akiyama D.M., W.G. Doming, and A.L. Lawrence. 1992. Penaeid shrimp nutrition. p.535-568. In: Marine shrimp culture – Principles and practices. A.W. Fastand and L.J. Lester (Eds.) Elsevier Science.

Arellano E. 1990. Fatty acid composition of wild and cultured *Penaeus vannamei* as a method to evaluate post larval quality. Abstract. 21st Annual WAS Conf..

Babu M.M and M. P. Marian. 1998. Live transport of gravid *Penaeus monodon* using mesocarp dust. Aquacult. Eng, 18:149-155.

Babu M.M. 1999. Developing bioencapsulated Ayurvedic products for maturation and quality larval production in *Penaeus monodon*. Ph.D. Thesis, M.S. University, Tirunelveli, India.

Chen J.C., P.C. Liu, Y.T. Lin, and C.K. Lee. 1988. Superintensive culture of red tail shrimp, *Penaeus penicillatus*. J. World. Aqua. Soc. 19:127-131.

Hilda K.M. 1992. Performance evaluation of reprostim on the survival, growth and reproduction of *Artemia franciscana*. M.Sc. thesis, Madurai Kamaraj University, India.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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ANTI-INFECTIOUS IMMUNE EFFECTORS IN MARINE INVERTEBRATES AS POTENTIAL TOOLS FOR DISEASE CONTROL IN LARVICULTURE

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The aquaculture of molluscs and crustaceans is a global concern, and all producing countries are concerned by the repeated appearance of new infectious diseases that affect cultured species and so threaten aquaculture's sustainability. The development of infectious diseases in aquaculture partly came from an intensification of production. Among the causative agents encountered in marine aquaculture, viral and bacterial problems as well as fungal diseases, dominate the technology of larval production. Most often, larval mortalities have been associated with bacterial contamination favoured by the rearing conditions. Different bacterial genera are highlighted – *Pseudomonas*, *Alteromonas*, *Aeromonas*, *Bacillus* – but the Gram-negative *Vibrionacea* bacteria indubitably represents the most harmful pathogenic bacteria for both mollusc and crustacean larvae and juveniles.

Difficulties of disease control in marine aquaculture come mainly from the differences in susceptibility of animals according to their developmental stages (from larvae to adults) and from the etiological diversity of pathogens that affect them during these developmental stages. To avoid bacterial disease in larval production, zootechnical progresses have been made. So far, antibiotics have been intensively used as preventive and curative measures, but such practices are now questioned because regular use of antibiotics has led to the appearance of drug-resistant bacteria and can also result in environmental imbalances. Methods have been proposed for the control of disease in aquaculture, such as the use of probiotic bacteria, or the improvement of the larval quality with nutrition. However, alternative treatments as well as animal health monitoring could also be established as preventive measures. In the long term, the most effective way for sustainable aquaculture production will certainly rely on the production of selected animals for resistance to diseases, but until now, few papers can be found on this topic. Growth traits are most often selected in farmed animals. So far, in marine invertebrates, selection programs have been based on criteria of resistance to one specific pathogen in pressure selection or natural selection as done, for example, in oysters facing protozoan diseases. However, in such a strategy, the risk of

development of new pathogens on selected strains is not eliminated. Consequently, immune criteria as enhancement of non-specific defense responses are beginning to be considered, to further knowledge of marine invertebrate immunity and ontogeny of the immune system. Moreover, it will also be necessary to progress in the genetic characterization of the cultured species.

The innate immune response of molluscs and crustaceans relies upon the production of antimicrobial peptides that are active against a large range of pathogens. Antimicrobial peptides share common structural features: they have often a low molecular weight (below 10kDa) and are cationic molecules (positively charged at a physiological pH). Because of structural features such as a high content in proline residues or the presence of cysteine residues forming internal disulfide bridges, their resistance to proteases is rather high in sera. As factors of innate immunity, two main advantages of the antimicrobial peptides are that they can function without high specificity and that they are nontoxic to eukaryotic cells. They are also rapidly synthesized at low metabolic cost. Whereas host defense mechanisms have only been recently investigated in cultured marine invertebrates, to date, more than 400 antimicrobial peptides have been isolated mainly (about 50%) from insects, but also from plants and vertebrates. In the mussels *Mytilus edulis* and *M. galloprovincialis*, numerous antimicrobial peptides have been characterized, all cysteine-rich, belonging to three different groups: defensin-like peptides, mytilins, and myticins. These peptides have a broad range of antimicrobial activity against Gram-positive and Gram-negative bacteria, and against filamentous fungi. In crustaceans, a family of antimicrobial peptides named penaeidins have been fully characterized and cloned from the hemocytes of *Penaeus vannamei*, and they have also been found in other species. Data have been obtained for the first time in crustaceans on their properties, the localization and regulation of gene expression, as well as on their expression during shrimp development. The penaeidins display both antifungal and anti-Gram-positive bacterial activities, and a chitin-binding property that could provide the shrimp protection during molting stages. Altogether, these data provided clues to understanding the role of antimicrobial peptides in mollusc and shrimp immune responses to microbial infections; they also gave evidence that the two phyla exhibit similarities of anti-infectious processes mediated by peptides that also appear closely related to those described for chelicerate and vertebrate peptides.

Investigation of the innate immune systems of marine invertebrates may give new insights into the management and control of infectious diseases in aquaculture. In particular, anti-infectious effectors remain a vast domain to be explored, which presents various application possibilities by their use as therapeutic agents, as tools for monitoring the health status of cultured animals, and by the use of encoding genes as selection markers for improving resistance to infections. Moreover, potential applications for disease control could be provided to all stages of production channels of both molluscs and shrimp, from larviculture to juvenile rearing.

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THE EFFECTS OF ADULT NUTRITION AND EGG QUALITY ON LARVAL DEVELOPMENT OF THE SEA URCHIN *TRIPNEUSTES GRATILLA* (ECHINODERMATA: ECHINOIDEA)

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Abstract
The percentage of abnormal larvae and survivorship of metamorphosed larvae produced by adults in the four feeding treatments were not significantly different. However, larvae produced by starved adults had the highest percentage of abnormalities and the lowest survivorship of metamorphosed larvae. Larval survivorship (up to presettlement stage) was significantly lower in starved compared to larvae from fed animals. No statistically significant variations were observed in the larval development rates with respect to the different feeding treatments. However, results suggest that starved adults and animals fed a low ration of *Sargassum* produced eggs with high lipid concentrations that had shorter larval duration.

Introduction

Extensive studies have been done on the feeding and nutritional ecology of sea urchins. Very limited studies include the effect of adult nutrition and egg quality on subsequent larval development. Practically no information is available for *Tripneustes gratilla*, despite the fact that this species has high culture potentials because of its high growth rates and early attainment of sexual maturity. The present study bears on the existing hypothesis that the differences during oogenesis affect embryogenesis and larval ecology (Giese and Pearse, 1974). Moreover, adult food determines egg quality since food has the most direct effect in terms of organic matter contribution to the eggs (e.g., Emlet et al., 1987). This study was conducted to determine and compare larval survivorship and development to metamorphosis in *T. gratilla* in relation to egg quality and adult feeding history.

Materials and methods

Three independent batches of juvenile *T. gratilla* (38.55±0.12mm) were grown to sexual maturity in cages (50 urchins.m^{-3}) in the reef flat of Santiago Island in Bolinao, Pangasinan, northwestern Philippines. The sea urchins were either

starved or fed *Thalassia* seagrass (high ration) and *Sargassum* seaweed (i.e., high and low ration) for 6 months. After the grow-out period, a total of 27 females per treatment (i.e., 9 from each batch) were induced to spawn in the laboratory to determine egg quality parameters (i.e., egg size, egg number, percent fertilization, and biochemical concentrations). The same set of eggs produced by the three batches of adults were used to produce and rear larvae, and determine survivorship and development rates. The larvae were fed daily with pure microalgae *Isochrysis galbana* and *Chaetoceros gracilis* 20 000 cells.ml^{-1} to 40 000 cells.ml^{-1} depending on the stage of the larvae. The development rates and survivorship of the different stages of the larvae from the different treatments were monitored every 2 days until the time of settlement. The number of abnormal larvae was recorded. The development rates of the different stages of the larvae were determined based on the time when more than 50% of the larvae sampled were at a specific stage (e.g., 2-arm, 4-arm larval stage, etc.). At the end of the larval monitoring period, the presettlement larvae from each culture jar were induced to settle and metamorphose on benthic diatom (*Navicula ramossissima*) films and the number of metamorphosed larvae was recorded after 2 weeks.

Results

Significant variations were observed in the larval survivorship of the three batches of *T. gratilla* with respect to adult feeding treatments. The overall average percent survivorship was lowest in larvae from starved parents, and significantly differed with survivorship of larvae from “fed” parents (Table I). Although there was no significant difference in the overall average percent survivorship of larvae from “fed” parents, larvae from adults fed a high ration of *Sargassum* had relatively higher survivorship compared to larvae from adults fed a high-ration *Thalassia* and low-ration *Sargassum*. No significant variations were observed in the larval development rates with respect to the adult feeding history. Although variations observed were not statistically significant, starved and low ration *Sargassum*-fed parents produced larvae with relatively faster development rates to presettlement stage compared to larvae produced by parents fed a high ration of *Thalassia* and high ration *Sargassum*. The overall mean percentage of abnormal larvae and the survivorship of metamorphosed larvae were not significantly different among the four adult feeding treatments. However, the average percentage of abnormal larvae was found lowest in larvae of high ration *Sargassum*-fed parents and highest in larvae of starved parents. Likewise, the average survivorship of metamorphosed larvae was found highest from high ration seagrass-fed parents and lowest from starved parents.

Discussion and conclusions

Results of this study clearly demonstrate the significant effects of parental nutrition and egg quality on subsequent larval survivorship in *T. gratilla*. The seaweed *Sargassum* is a better food for *T. gratilla* than seagrass *Thalassia* with respect to egg quality and larval survivorship and development (i.e., percentage of abnormal and metamorphosed larvae). Adults fed a high ration of *Sargassum* that produced the highest number of eggs of the highest quality (i.e., fertilization success and egg carbohydrate, protein and lipid concentrations, Bangi and Juinio-Meñez, unpublished data) produced larvae with the relatively highest survivorship and lowest percentage of abnormality.

Table I. Various egg and larval quality parameters of *T. gratilla* reared under four feeding treatments in field cages for 6 months.

Treatment	EGG					
	Mean Size (µm)	Average Number per female	Average % Fertilization	Mean Concentration ($mg.ml^{-1}$)		
				Carbohydrate	Protein	Lipid
Thalassia (High)	78.54	503 262	58	18.43	73.44	52.05
S.E.	0.04	15 280	0.25	2.87	4.46	11.94
Sargassum (High)	78.53	2 911 481	82	41.15	85.87	124.92
S.E.	0.05	89 664	0.9	2.56	3.37	23.31
Sargassum (Low)	79.42	417 918	62	16.86	69.12	123.64
S.E.	0.01	8334	0.85	0.45	2.31	15.98
Starved	78.16	12 770	14	8.11	56.34	98.66
S.E.	0.03	1286	0.45	0.59	3.82	18.99

	LARVAE			
	Mean % survivorship	Average larval duration	Average % abnormality	Average % metamorphosis
Thalassia (High)	58.89	31.2	4.03	9.12
S.E.	2.89		0.22	2.52
Sargassum (High)	67.61	31.1	3.73	7.92
S.E.	2.13		0.27	2.4
Sargassum (Low)	61.92	25.2	4.54	6.79
S.E.	2.76		0.23	2.4
Starved	50.85	21.5	5	6.44
S.E.	3.59		0.7	4.5

Egg number and quality (fertilization success and egg carbohydrate and protein concentrations) and subsequent larval survivorship and development of animals fed *Thalassia* (high ration) are comparable to those fed a low ration of *Sargassum*. Algae have higher proportion of digestible, and therefore available, energy and nutrients than seagrass (Duarte, 1990). In addition, absorption efficiency of food in sea urchins is 70-80% for seaweeds and only 50% for seagrasses (Mukai and Nojima, 1985). It is likely that *T. gratilla* in the present study was more efficient in

absorbing nutrients from *Sargassum* than *Thalassia* such that there is higher concentration of carbohydrate, protein, and lipid in the eggs and subsequently higher survivorship of the larvae. The quantity of food is also important. Adults fed a high ration of *Sargassum* produced higher number and quality of eggs and subsequent larval survivorship compared to adults fed a low ration of *Sargassum*. Similarly, in the seastar *Luidia clathrata* and sea urchin *Arbacia lixula*, a decrease in food ration led to a decrease in egg quality (i.e., % fertilization; George et al., 1991). Survival rates of the sea urchin *A. lixula* larvae were higher from parents fed a high food level than in larvae from parents fed a low food level.

Results indicate that larval development and percentage metamorphosis in *T. gratilla* are not significantly affected by parental nutrition. This differs from the study by George (1996) on other echinoderms, where the differences observed in larval growth and development were significantly related to adult nutritional state. Thus, larval nutrition in *T. gratilla* may be more important in controlling larval development and metamorphosis.

In the present study, starved parents that produced the poorest quality of eggs (fertilization success and egg carbohydrate and protein concentrations) produced larvae with the lowest survivorship, highest percentage of abnormality, and lowest survivorship during metamorphosis, but appeared to compensate by producing eggs of high lipid concentration and larvae with relatively faster development rate. Likewise, adults fed a low ration of *Sargassum* also produced poorer quality eggs and larvae but appeared to compensate by producing the largest eggs with high lipid concentration and larvae with relatively faster rate of development. These results indicate that *T. gratilla* have a compensatory mechanism in egg quality and larval development in response to adult starvation and low food supply.

References

Duarte C.M. 1990. Seagrass nutrient content. Mar. Ecol. Progr. Ser. 67:201-207.

Emlet R.B., L.R. McEdward, and R.R. Strathmann. 1987. Echinoderm larval ecology viewed from the egg. p.55-136. In: Echinoderm Studies. Vol. 2. Jangoux, M. and Lawrence, J.M. (Eds.). Balkema, Rotterdam.

George S.B. 1996. Echinoderm egg and larval quality as a function of adult nutritional state. Oceanologica Acta. 19:297-308.

Giese A.C. and J.S. Pearse. 1974. Introduction: General Principles. p.1-49. In: Reproduction of Marine Invertebrates. Vol. I. A.C. Giese and J.S. Pearse (eds.). New York Academic Press.

Mukai H. and S. Nojima. 1985. A preliminary study on grazing and defecation rates of a seagrass grazer, *Tripneustes gratilla* (Echinodermata: Echinoidea) in a Papua New Guinean seagrass bed. Spec. Pub. Mukaishima Mar. Biol. Stn.:185-192.

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THE DIGESTIVE CAPACITY FOR DIETARY PROTEIN IN LARVAL ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*)

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Introduction

An artificial diet that could replace or reduce the dependency of fish larvae on live food organisms would be a major advancement for the commercial production. One of the differences between artificial food particles and live food is the high concentration of protein in microdiets. This leads to the question of the fish larval capacity to digest and assimilate dietary proteins. There are several direct and indirect data that support low utilization of proteins in the early larval stages (Rust, 1995; Rønnestad et al., 1999; 2000).

This study is a first step towards quantification of the capacity of the halibut larvae to digest, absorb, and assimilate dietary proteins.

Material and methods

Atlantic halibut (*Hippoglossus hippoglossus*) larvae were reared at AKVAFORSK Sundalsøra, Norway (see Holmefjord et al., 1993). The larvae – 633 degree days post hatch (body mass 43.2-106.7mg wet weight), 33 days after first feeding – have not yet developed a functional stomach (Bæverfjord and Lein, unpublished). Twenty-four hours prior to the experiment, larvae were transferred to an aquarium with clean seawater to evacuate the digestive system before experimentation.

Four protein solutions of bovine serum albumin (BSA, Sigma Chemicals, St. Louis, MO) and ^{14}C-methylated BSA (DuPont NEN, Boston, MA) were prepared in phosphate buffer 0.01M containing 7, 28, 112, and 448ng BSA.nl^{-1}. The four solutions were approximately iso-labeled at 4.26cpm.nl^{-1}. Equal volumes of ca. 100nl of the four protein solutions were tube-fed into the lumen of the primordial stomach to six larvae in each treatment as described by Rønnestad et al. (2001). After tube-feeding, the larvae quickly recovered during a 3-min rinse period before being incubated in individual wells (5ml seawater).

After 8 hours of incubation, larvae were sampled. The larval bodies were fractionated by a 6% TCA extraction (Body-TCA-solubles, including the pool of free amino acids, FAA, as well as Body-TCA-precipitates, including proteins). In order to distinguish between unabsorbed ^{14}C-BSA evacuated from the gut and ^{14}C-CO_2 originating from catabolism of the absorbed ^{14}C-BSA, the water pH was decreased in order to release, transfer, and entrap metabolically produced ^{14}C-CO_2 as described by Rønnestad et al. (2001). All samples were counted by liquid scintillation.

Results

With increasing amounts of protein tube-fed into the digestive system, the amount of protein absorbed increased (Fig. 1). Thus, in fish fed 0.6µg BSA, 0.5µg was absorbed from the gut (sum of Body-TCA-precipitate, Body-TCA soluble, and CO_2), while in fish fed 55µg BSA, 33µg was absorbed from the gut.

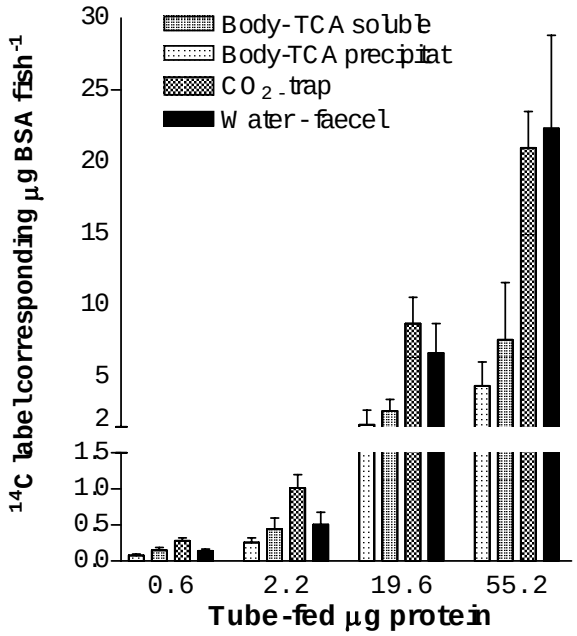

Fig. 1. Tube-fed Atlantic halibut larvae: Effect of increased intestinal loading with 14C-labeled dietary protein (Bovine serum albumin) on the label distribution in various compartments.

The efficiency of protein absorption decreased with increased intestinal loading of BSA (Fig. 2). When the results were weight normalized (Fig. 2), the efficiency of the protein utilization start to decrease sharply around 0.2-0.3µg dietary BSA mg^{-1} wet weight larvae.

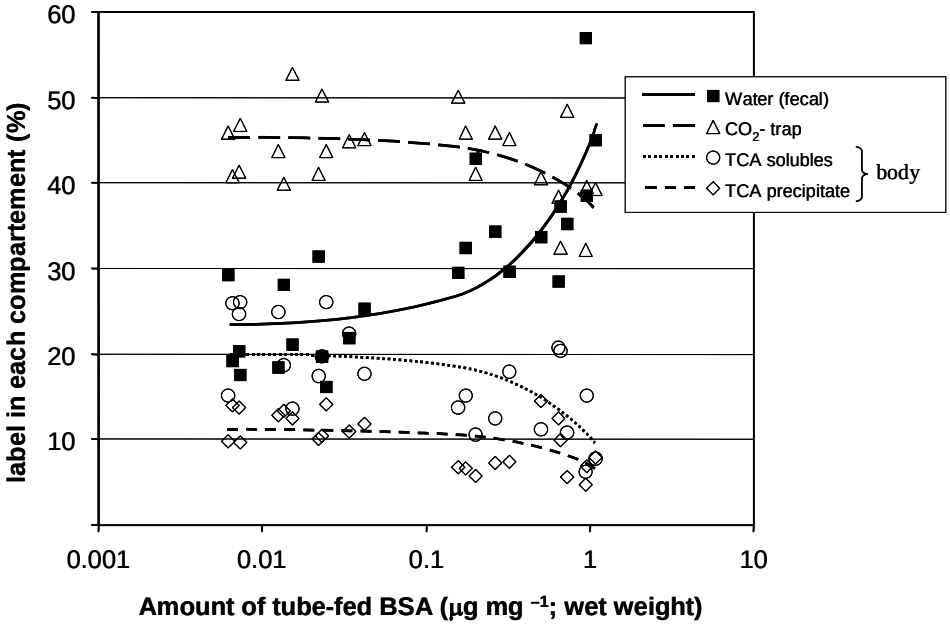

Fig. 2. Weight-specific compartmental distribution of 14C-labeled bovine serum albumin (BSA) 8h after tube-feeding it to Atlantic halibut larvae.

Discussion

The study shows the potential of this method for rapid assessment of the digestibility of diets or diet components. Thus, the method may be a practical tool in larval diet development as well as for physiological and nutritional studies.

The percentage of the dietary protein that was assimilated into the body at the end of the 8-h incubation following tube feeding of a single pulse was low, around 30% and 20% for the lowest and highest amount of BSA delivered, respectively. The amount assimilated into the larval tissues did not increase in proportion to the amount of the protein deposited into the fish digestive system. This shows that the protein utilization efficiency decreased, suggesting a saturation response of either the digestion or the absorption of the protein. However, we did not reach the maximum values with the amounts used. A saturation-like trend of the digestive and absorptive capacity could be expected, although the saturation level can be expected to improve if more adequate proteins are used. A further improvement is expected if some of the protein is replaced by free amino acids or peptides as found in a study on sea bass, *Dicentrachus labrax*, where partial substitution of di-and tri-peptides for native

proteins in diet improved the larval development (Zambonino Infante et al., 1997).
Further studies of the effects of different protein quality, free amino acids, and peptides on fish larval digestion, assimilation, and other physiological aspects are ongoing in our group.

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References

Holmefjord I., J. Gulbrandsen, I. Lein, T. Refstie, P. Leger, T. Harboe, I. Huse, P. Sorgeloos, S. Bolla, Y. Olsen, K.I. Reitan, O. Vadstein, G. Øie, and A. Danielsberg. 1993. An intensive approach to Atlantic Halibut fry production. J. World Aqua. Soc. 24(2):275-284.

Rønnestad I., L.E.C. Conceição, C. Aragão, and M.T. Dinis. 2000. Free amino acids are absorbed faster and assimilated more efficiently than protein in post-larval Senegal sole (*Solea senegalensis*). J. Nutr. 130:2809-2812.

Rønnestad I., C.R. Rojas-García, S. Tonheim, and L.E.C. Conceição. 2001. *In vivo* studies of digestion and nutrient assimilation in marine fish larvae. Aquaculture (In press)

Rønnestad I., A. Thorsen, and R.N. Finn. 1999. Fish larval nutrition: Recent advances in amino acid metabolism. Aquaculture 177:201-216

Rust M.B., R.W. Hardy, and R.R. Stickney. 1993. A new method for force-feeding larval fish. Aquaculture 116:341-352.

Zambonino Infante J.L, C.L. Cahu, and A. Peres. 1997. Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrachus labrax* larval development. J. Nutr. 127:608-614.

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OPTIMIZING HIGHLY UNSATURATED FATTY ACID LEVELS IN FIRST FEEDING MARINE FISH LARVAE

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Introduction

Although culture of marine fish has become established throughout the world and involves a diverse range of species, low larval survival, coupled with poor larval and juvenile quality, particularly with respect to dorsal pigmentation in flatfish, remains a major problem for producers. While the use of calanoid copepods has proved beneficial in improving dorsal pigmentation, eye migration, and retinal development in halibut, problems with providing sufficient copepods, as well as increased disease risk, have limited the use of copepods in marine fish culture. Therefore, the majority of hatcheries have relied on readily available cultures of rotifers (*Brachionus plicatilis)* and *Artemia* nauplii as first-feeding organisms.

The dietary essentiality of the n-3 highly unsaturated fatty acids (HUFA) docosahexaenoic acid (22:6n-3; DHA) and eicosapentaenoic acid (20:5n-3; EPA) for normal growth and development of marine fish larvae is well documented (Sargent et al., 1999). In addition, as marine fish are also unable to synthesize arachidonic acid (20:4n-6; AA), the requirement for this HUFA, must also be considered. This paper describes the importance of the ratio of these three essential HUFA in larval live-prey diets, including the chemical form of HUFA delivery and the subsequent effects on dorsal pigmentation and metamorphosis.

HUFA delivery via live prey

As described above, the three main methods of HUFA delivery to first-feeding marine fish larvae are via copepods, rotifers, and *Artemia* nauplii, with the latter two requiring supplementary enrichment to augment a natural paucity of HUFA. Fish, and in particular fish eggs, are naturally enriched in 22:6n-3 and 20:5n-3, usually in a ratio of >2 (Tocher and Sargent, 1984). It is noteworthy that Reitan et al. (1994) were able to eliminate malpigmentation in turbot larvae when the DHA/EPA of the live prey was >2. The ability to achieve this ratio in different live

prey, either natural copepods or enriched rotifers and *Artemia*, is shown in Table I. The data shows the effect of using a DHA-rich fish oil, such as tuna orbital oil (TOO), compared to an EPA-rich oil, such as Marinol (MO), on the HUFA composition of rotifers and *Artemia.* Unless a DHA-rich fish oil is used, it is not possible to get a DHA/EPA ratio of >2, as with copepods, or even rotifers. In *Artemia*, the situation is worse in that, even by enriching with TOO, the DHA/EPA ratio is only 1.3 compared to 0.3 in nauplii enriched with Marinol. The inability to enrich *Artemia* nauplii with DHA is a major drawback in their suitability as first-feeding prey for marine fish larvae. This effect in *Artemia* can be explained by (a) their naturally high lipid content prior to enrichment and, in particular, the presence of fatty acids of poor nutritional value in unenriched nauplii; (b) a relatively high EPA content in unenriched nauplii; and (c) the rapid rate of retroconversion of DHA to EPA in enriched *Artemia* nauplii (Navarro et al., 1999).

Table I. HUFA compositions (weight % of total lipid) of rotifers and *Artemia* nauplii, enriched[1] with either tuna orbital oil (TOO) or Marinol[2] (MO) and *Eurytemora velox* and *Tisbe furcata* copepods.

Fatty acid	Rotifer		*Artemia*		Copepod	
	TOO	MO	TOO	MO	*E. velox*	*T. furcata*
20:4n-6	1.1	0.8	1.8	1.2	1.8	1.7
20:5n-3	4.6	13.1	7.6	11.6	10.8	11.2
22:6n-3	12.7	6.5	10.0	3.0	21.8	24.7
DHA/EPA	2.8	0.5	1.3	0.3	2.0	2.2
EPA/AA	4.2	16.7	4.2	9.5	6.0	6.6

[1]All enrichment oils contained 12% w/w soya PC. [2]Predominantly sardine, anchovy, and pilchard oils.

The importance of type of lipid enrichment

The ability of dietary phospholipid to enhance growth and development of larval fish is now well established and may in part be due to their ease of digestion in comparison to triglycerides. However, there is considerable evidence that early developing marine fish larvae have an absolute requirement for pre-formed phospholipids, due to their inability to synthesize phospholipids *de novo*. It is known that the acyl chain in the *sn*-2 position of dietary triglycerides is preserved in fish storage lipids. The action of gut lipases remove the acyl chain from the *sn*-1 position, of both triglycerides and phospholipids, allowing absorption of *sn*-2 lyso-lipids into the gut lumen followed by reacylation on the *sn*-1 position, thereby generating a phospholipid that retains the original 2-acyl fatty acid. In *Artemia*, the *sn*-2 position on phospholipids is predominantly 18:3n-3 or 18:2n-6, while DHA is only a minor component. This is confirmed in Table II, which shows the HUFA content of the polar lipid fraction from enriched *Artemia* and rotifers as well as the calanoid copepod, *Calanus finmarchicus*. These data suggest that phospholipids derived from rotifers and copepods can be incorporated directly into cell membranes of rapidly growing larvae and will contain a profile of bioactive HUFA, especially DHA, which will be beneficial for larval growth and

development. The values for enriched *Artemia* show that even when a DHA-rich enrichment is used, the concentration of DHA in the polar lipid fraction is very low with subsequently very low DHA/EPA ratios.

Table II. HUFA compositions (weight %) in the total polar lipid fraction of rotifers and *Artemia* nauplii enriched with either TOO/herring roe phospholipid (TOO; 88/12 w/w) or Super Selco™ (SS), and *Calanus finmarchicus* copepods.

Fatty acid	Rotifer		*Artemia*		*C. finmarchicus*
	TOO	SS	TOO	SS	
20:4n-6	2.0	1.4	2.4	1.6	0.6
20:5n-3	8.1	11.1	11.5	14.9	24.0
22:6n-3	12.4	9.8	2.4	0.6	40.6
DHA/EPA	1.5	0.9	0.2	0.0	1.7
EPA/AA	4.1	7.9	4.8	9.2	40.0

Effects of HUFA on pigmentation and eye migration in larval flatfish

Abnormal patterns of pigment distribution are commonly encountered in the production of flatfish, including turbot, halibut, and Japanese flounder, and appear to be improved by optimizing DHA levels in feed (Reitan et al., 1994; McEvoy et al., 1998). However, Japanese flounder showed improved pigmentation success in diets supplemented with AA. From these studies, it is difficult to decide whether malpigmentation is due to a lack of DHA or an excess of EPA. Given that most triglyceride oils used for enrichment contain all three essential HUFA, any change in DHA will unavoidably affect EPA and AA levels, as well as their respective ratios. To investigate the effect of variation in the DHA/EPA/AA ratio on pigmentation success, we conducted trials with turbot larvae fed rotifers followed by *Artemia*, enriched with blends of TOO and oils rich in EPA or AA. In this way, DHA levels were maintained while the EPA/AA ratio was varied. Similar trials were conducted with halibut, however in this case, rotifer feeding was not possible. The results of pigmentation success in the turbot and halibut larvae fed rotifers and/or *Artemia* enriched with different oils are shown in Table III. The results show a significant negative correlation between dietary AA content (and thereby brain AA content) and pigmentation, and a weaker positive correlation between EPA and pigmentation, in turbot. A similar relationship exists in halibut, although the % with perfect pigmentation was lower than in turbot. The difference between halibut and turbot may be explained by the absence of rotifer feeding in halibut and the subsequent benefits of supplying pre-formed phospholipids rich in DHA. In addition, the halibut were not fed the experimental enrichments from first feeding, having been weaned initially onto a control diet of Algamac™/SuperSelco™. These and subsequent experiments have demonstrated that the pre-570-day° feeding stage is critical in determining pigmentation success in halibut. Nonetheless, the apparent competitive effect between AA and EPA in determining pigmentation success strongly suggests that eicosanoids are involved in pigmentation. This theory is supported by the improved pigmentation observed in halibut enriched with fish oil and evening primrose oil (EPO) (Table III) when compared to the control enrichment. EPO contains 18:3n-6 which can be

elongated to 20:3n-6, a potent competitor of AA, as is EPA, in terms of eicosanoid production.

Table III. Pigmentation (% of larvae showing normal ocular pigmentation) in turbot and halibut fed diets with varying 20:5n-3, 20:4n-6, and 18:3n-6 content.

Fish	Diet	Pigmentation (%)
Turbot	EPA 20[1]	86.7 ± 5.8
	EPA 30[1]	82.1 ± 10.7
	AA 3[2]	78.8 ± 13.5
	AA 7[2]	32.6 ± 17.4
	AA 15[2]	0.9 ± 0.9
Halibut	EPA 20	23.0 ± 11.3
	EPA 30	27.8 ± 12.5
	AA 3	11.7 ± 1.5
	AA 7	9.6 ± 2.9
	AA 15	1.0 ± 1.7
	EPO 30[3]	17.3 ± 16.3
	Control[4]	2.6 ± 3.0

[1,2]Value following EPA or AA describes that HUFA's content in the enrichment emulsion. [3]30% evening primrose oil concentrate/50% mackerel-sprat-herring oil. [4]Algamac 2000™/Super Selco™ (1:1 w/w).

References

Estevez A., L.A. McEvoy, J.G. Bell, and J.R. Sargent 1999. Growth ,survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus)* larvae fed live-prey enriched in arachidonic and eicosapentaenoic acids. Aquaculture 180:321-343.

McEvoy L.A., T. Naess, J.G. Bell, and Ø. Lie 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus)* fed enriched *Artemia*: a comparison with fry fed wild copepods. Aquaculture 163:237-250.

Navarro J.C., R.J. Henderson, L.A. McEvoy, M.V. Bell, and F. Amat. 1999. Lipid conversions during enrichment of *Artemia.* Aquaculture 174:155-166.

Reitan K.I., J.R. Rainuzzo and Y. Olsen 1994. Influence of live feed on growth, survival and pigmentation of turbot larvae. Aquacult. Int. 2:33-48.

Sargent J.R., L.A. McEvoy, A. Estevez, J.G. Bell, M.V. Bell, R.J. Henderson, and D.R. Tocher. 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217-229.

Tocher D.R. and J.R. Sargent. 1984. Analyses of lipids and fatty acids in ripe roes from some northwest European marine fish. Lipids 19:492-499.

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THE EFFECT OF OZONE TREATMENT ON EGG AND LARVAE PERFORMANCE IN THE GILTHEAD SEABREAM (*SPARUS AURATA*) AND OTHER MARINE SPECIES

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Abstract

Infectious diseases are often the reason for the shortage of fry of farmed species. These diseases are frequently transferred from infected broodstock to the eggs. Consequently, the use of ozonated seawater to disinfect fish eggs before stocking could result in improved egg and larval performance, and reduce the risk of transferring disease from broodstock to their offspring. The aim of the present study was to determine the optimal exposure time of seabream eggs to ozonated seawater ($0.3mg.l^{-1}$) to obtain improved egg and larval performance. In the present study, groups of eggs of gilthead seabream (*Sparus aurata*) were washed for different periods of time in seawater containing dissolved ozone ($0.3mg.l^{-1}$) and compared to eggs rinsed with non-ozonated seawater or unwashed eggs (control). The ozone treatments, measured in CT units (ozone concentration in $mg.l^{-1}$ × exposure time in min), were evaluated by their effect on egg mortality and hatching, as well as larval survival, swim bladder inflation, and incidence of deformed larvae. Bacterial levels on the egg surface were effectively reduced to zero when measured 144h after exposure to CT values ranging from 0.6-4.8. The percent hatching was consistently high (83.7-89.5%) in eggs exposed to CT levels from 0-1.2 but decreased to 36.5 and 20.4% in eggs exposed to higher CT levels of 2.4 and 4.8, respectively ($P<0.05$). Three days after hatching, a CT value of 0.6 gave the highest larval survival (82±4.5%, $P<0.05$) compared to CT 0 (68.4±2%) and CT 4.8 (69.5±5%) and continued to perform best 13 days after hatching (survival of 80±4%, $P<0.05$). Larvae originating from eggs exposed to a CT value of 0.6-4.8 demonstrated significantly higher swim bladder inflation (95-99%) than the 0 CT (76%) and control (75±3%) treatments. Moreover, the incidence of deformed larvae was markedly lower ($P<0.05$) in the 0.6-4.8 CT treatments (5-3%) compared to the control and 0 CT treatments (12-11%). On the other hand, the incidence of

deformed larvae at hatching was higher (P<0.05) in eggs exposed to CT values of 2.4 (5±1%) and 4.8 (8±1.5%) compared to the control and the 0-1.2 CT treatments (1-2.5%), suggesting the potentially deleterious effect of overexposure of dissolved ozone in seawater.

No significant (P>0.05) increase in egg mortality was found 25h after ozone exposure. However, hatching rate and larval survival improved markedly if the CT level did not exceed 1.2. This suggests that the egg chorion reduces the impact of this strong oxidant on the fish embryo and ozonated seawater may be a useful approach to disinfect the external surface of the fresh spawn of farmed teleosts such as the gilthead seabream (*Sparus aurata*). Exposure to ozonated seawater of other marine fish species such as European sea bass (*Dicentrarchus labrax*), red drum (*Sciaenaps ocellatum*), and barramundi (*Lates calcarifer*) eggs was effective in improving the larval performance. In addition we showed that there is a direct relationship between egg diameter and its sensitivity to the ozone treatment. Moreover, this relationship will be used as a predictive tool for the optimal ozone treatment dose applied in the future on eggs of other fish species.

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LARVAL GROWTH AND JUVENILE QUALITY AND AN ATTEMPT TO DESCRIBE FOOD UPTAKE IN INTENSIVE REARING OF HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*) LARVAE

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Introduction

In halibut rearing, larval growth and juvenile quality are still variable. Suboptimal *Artemia* nutritional content or suboptimal food uptake and poor larval feeding conditions may be underlying causes, partly confirmed by the fact that differences in the amount of gut contents are quite frequently seen within a 24-h cycle and between days. To be able to distinguish between the effect of quantitative and qualitative food availability, it is of fundamental importance to know the actual quantitative food uptake. In a closed system, this can be defined as the difference between the amount of food supplied and the food density at a certain time (van der Meeren, 1995). To define food uptake in a commercial rearing tank is, however, much more difficult due to a significant loss of food items through the water exchange. This paper describes a preliminary attempt to define the food uptake in commercial rearing of halibut larvae, together with larval growth and juvenile quality. The final purpose is to investigate irregularity in food uptake and suboptimal larval feeding conditions.

Materials and methods

Larval numbers and survival were roughly estimated by visual and experiential evaluation at the transfer of larvae between production units at different stages. Growth is by wet-weight and calculated as:

$$\frac{(\ln w_2 - \ln w_1)}{(t_2 - t_1)} \times 100$$

Larvae were fed *Artemia* (GSL) enriched on DC DNA Selco (INVE) for 24h, with the *Artemia* further stored and enriched on a mixed diet of DC DNA Selco and Anglo Norse®Micro Feed (Norwegian Herring Oil and Meal Industry Research Institute) during 24- feeding periods.

A continuously flow (5-10% of total flow-through) was siphoned from the tank outlet through a filter, and the total amount of *Artemia* in the outlet water (=total loss) was determined on the basis of subsampling the concentrate, usually once a day. The amount of *Artemia* supplied to the tanks (=amount of food) was likewise determined by subsampling, and food uptake was calculated as the difference between the amount of food and total loss in number of *Artemia* per tank during 24h.

Results and discussion

Food uptake (Fig. 1) has possibly been affected by the quite unknown pattern of larval mortality, and there may be too much uncertainty in estimates of loss and food supply to evaluate the apparently great difference in food uptake between certain days. Still, the pattern of food uptake seemed to be much the same in all tanks, and in weeks 2-4 there seemed to be no increase in food uptake, strongly indicating a limited quantity of food available, which could hardly be an effect of mortality which mainly occurs the first two weeks. Corcobado Oñate et al. (1991) have earlier described a fairly great difference in gut contents during a 24-h period in turbot larvae, without claiming that this should cause a difference between days. Variable and relatively low growth in two tanks (Fig. 2) further indicates limited food availability, and given the fairly good growth in one tank, this does not indicate that *Artemia* is the main limiting factor of growth.

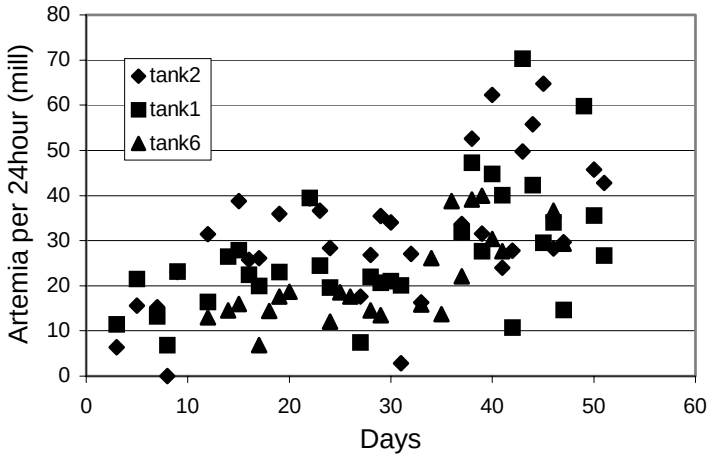

Fig. 1. Total food uptake in three different tanks during days of start-feeding.

If the low food uptake at day 8 is not a sampling artifact, (Fig. 1, tanks 1 and 2), it may be related to extinguishing the UV light at day 7, in accordance to other

reports (Naas et al., 1994; Browman et al., 1994). This may in turn lend evidence supporting the effect of light on behaviour and food uptake of halibut larvae.

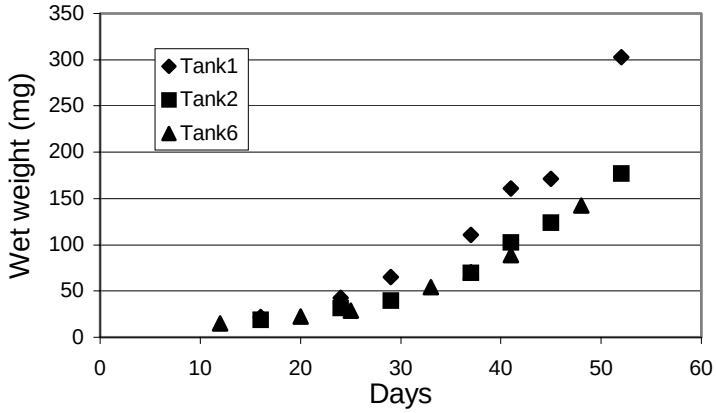

Fig. 2. Larval growth during days of start-feeding.

There was a fairly low proportion of normal juveniles (Table I), although almost 100% eye migration. This is often seen in halibut reared on *Artemia* diets and is considered an effect of inadequate quality of food items (Shields et al., 1999). Although the highest growth and best quality occurred in the same tank (Table I), it is not possible to confirm any connection between growth and quality. Increased number of normal juveniles has been seen earlier (Table II) despite fairly low growth rates (result not shown here) using the same enrichment diet. The growth pattern is possibly more important to juvenile quality than growth rate.

Table I. Larval survival and growth and juvenile quality in different tanks. For explanation see Table II. Eye migration was 100%, except for 2 individuals in tank 6 not included in table.

	number in startfeeding		survival %	growth rate to day 41	% quality		
	start	end			normal	½W	1W
Tank 2	35000	12000	34	6.7	15	3	82
Tank 1	40000	8000	20	8.0	31	3	66
Tank 6	38000	8000	21	6.1	25	6	69

While Table I shows high indices of eye migration and only two main categories of quality (normally pigmented or both sides white), the result in Table II is slightly more variegated and may bee more similar to what has been reported by Shields et al. (1999).

Table II. Juvenile quality (%) of halibut fed *Artemia* (year 2000). nP=normal pigmented (dorsal pigmentation), ½W =dorsal side half white, 1W=both sides completely white, 2P=both sides pigmented, nor=normal=nP+normal eye migration.

Tank	Normal eye migration				Partially eye migration				No eye migration				Total
	nP	½W	1W	2P	nP	½W	1W	2P	nP	½W	1W	2P	
99	49	20	21	1	5	0	1	0	0	0	2	2	100
94	53	21	16	1	4	0	1	0	3	1	0	1	100
92	52	10	25	0	4	1	1	0	2	0	0	5	100
93	41	19	28	0	1	0	0	0	1	0	1	9	100
99	43	6	23	0	3	2	0	0	2	3	1	17	100
Total	48	15	23	0	3	0	1	0	2	1	0	6	100

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References

Browman H.I., I. Novales-Flamarique, and C.W. Hawryshyn. 1994. Ultraviolet photoreception contributes to prey search behaviour in two species of zooplanktivorous fishes. J. Exp. Biol. 186:187-198.

Corcobado Oñate F., A. Coo, R. Arnaiz, F. Amoedo, and N. Rua. 1991. Daily ration of turbot larvae, *Scophthalmus maximus* in intensive culture. p.119-121. In: Larvi'91. P. Lavens, P. Sorgeloos, E. Jaspers, F. Ollevier (Eds). EAS Spec. Publ. 15.

Naas K.E., T. Harboe, Y. Attramadal, A. Mangor-Jensen, and I. Huse. 1995. Effect of ultraviolet light on distribution and feeding of halibut larvae (*Hippoglossus hippoglossus* L.). p.377-380. In: Larvi'95. P. Lavens, E. Jaspers, and I. Roelants (Eds). EAS Spec. Publ. 24.

Shields R.J., J. Gordon Bell, Frederic S. Luizi, B. Gara, N.R. Bromage, and J.R. Sargent. 1999. Natural copepods are superior to enriched artemia nauplii as feed for halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. J. Nutr. 129:1186-1194.

van der Meeren T. 1995. Feed consumption and gut evacuation in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. p.381-384. In: Larvi'95. P. Lavens, E. Jaspers, and I. Roelants (Eds). EAS Spec. Publ. 24.

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PATHOGENS IN SCALLOP, *PECTEN MAXIMUS*, HATCHERIES AND POSSIBLE STRATEGIES TO CONTROL THEM

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Introduction

The commercial production of scallop juveniles in Norway in 2000 was approximately two million, satisfying the demand of a small but growing industry. Predictable and sustainable production of juveniles is a prerequisite for the industrialization of shellfish aquaculture. Scallop juvenile production is often associated with high mortality, with a suspected bacterial etiology (Nicolas et al., 1996). The mechanisms causing mortalities have been poorly understood, apart from the isolation and characterization of the scallop larval pathogen *Vibrio pectenicida* in France (Lambert et al., 1998). The antibacterial agent routinely used in European hatcheries has so far been chloramphenicol, an agent which is now banned for use with animals destined for human consumption in the EU and EEA countries (Torkildsen et al., 2000). This leads to a demand for alternative prophylaxis suitable for larval and juvenile scallop culture, and a need for greater understanding and control of factors leading to mortalities. The purpose of this paper is to provide a brief overview of results from several research projects, with the overall aim of increasing survival of scallop larvae and juveniles.

Materials and methods

Egg groups were obtained from a commercial scallop hatchery (Scalpro AS, Rong Norway) where the broodstock, originating from either the Hordaland or Sør-Trøndelag county, were conditioned. Spawning, fertilization, and incubation

in egg and larval incubators were performed as described by Torkildsen et al. (2000), with the exception of trials with large scale incubators where eggs or veligers were transferred to the large (4700-l) upwelling "silo" incubators with semi-continuous addition of food at Austevoll Aquaculture Research Station, and reared as described by Andersen et al. (2000).

Bacterial samplings from scallop larvae and incubator water were performed as described by Torkildsen et al. (2000) and Andersen et al., (2000). A multidish system was developed as a challenge model. In each 2-ml well, 10-15 larvae were reared for 48h without addition of food. The system was used for screening a large number of bacterial isolates for positive or negative impact on larval scallop survival. The isolates were from larval rearing trials with high or low mortality, and from algal cultures.

Apart from application of probiotics, other strategies for microbial control include application of matured water and ozonation of inlet water with subsequent detoxification by thiosulfate.

Ongrowing facilities were either in hanging cultures near the hatchery using natural phytoplankton for food, or at a land-based nursery using phytoplankton from a landlocked heliothermic basin at Tysnes, Norway, traditionally used for oyster cultivation (Strand, 1996). A monitoring program was established at these facilities (Mortensen et al., 2000).

Results and discussion

None of the strains associated with mortality of scallop larvae at the commercial hatchery resembled *V. pectenicida*. A similar conclusion could be drawn from strains isolated from Austevoll Aquaculture Research Station. However, by means of the challenge experiments, it was possible to identify two different groups of Gram negative bacterial isolates as opportunistic pathogens to the larval stages of scallop. Both strains were representative of isolates from larval cultures with high mortality. Further characterization and taxonomic description of the strains is now in progress (Torkildsen et al., unpublished results).

In the challenge experiments, approximately 5% of the bacterial strains that were tested had a significant positive effect on larval survival compared to the unchallenged control groups. No indication of production of bactericidal or bacteriostatic compounds from these strains was found (Lambert et al., unpublished results). Thus, the nature of the probiotic effect remains unknown. Studies with application of probiotics to cultivation systems for of scallop larvae are now in progress.

The large silo systems showed promising results (Andersen et al., 2000), with typical yields of 2-7 million pediveligers per silo (Andersen and Bergh, unpublished results). Flow-through systems are assumed to allow organic materials to be removed from the tanks at a constant rate. Together with the nearly continuous addition of food, this results in the removal of the shifts in organic matter concentration characterizing the traditional stagnant cultivation systems.

However, flow-through systems are dependent on a reliable water quality, which is dependent on the season and water source. Generally, during spring blooms, mortalities have been experienced in flow-through systems as well as stagnant systems. Several explanations have been put forward. Increased bacterial growth is experienced due to increased organic matter content of the inlet water following the collapse of algal blooms, possibly favoring conditions for opportunistic pathogens. In addition to the organic matter content, collapse of algal cells implies increased amounts of algal toxins in hatchery inlet water. Trials with ozonation of inlet water, degrading algal toxins, followed by thiosulfate detoxification of the compounds generated by the ozonation is thus being evaluated.

Application of matured water has been tested on a number of egg batches. This method is principally related to the use of flow-through systems, as it gives rise to a slow-growing microbiota, possibly with a lower number of opportunists. As a prophylactic strategy, it is limited to bacteria, however, and little or no protection against viruses or other harmful biological or chemical contamination takes place. Although giving rise to better results than the conventional batch system, the matured water method has not proved sufficiently reliable, possibly due to effects of algal toxins in inlet water. Probably, application of ozonated water detoxified with thiosulfate treatment, in combination with probiotics, will be a system with higher degree of control. It could be added that disinfection by ozonation has proved far superior to aldehydes or other commonly used disinfectants with respect to the effect against marine viruses.

No diseases have been recorded from scallops in Norway (Mortensen, 2000). Ongrowth in sea hanging culture has been carried out without problems due to parasites. However, predation by seastars has occasionally caused significant losses. In the nursery and heliothermic basin, however, significant problems with parasites did occur. Extensive hydroid (*Obelia*) and polychaete (*Polydora* sp.) fouling caused massive mortality during the 1997 season (Mortensen et al., 2000), leading to a loss of one third of Norway's production of scallop juveniles that year. This system was subsequently abandoned as an ongrowth facility for scallops. Landlocked heliothermic basins are still considered useful for production of scallop (*Crassostrea gigas*) spat and oyster (*Ostrea edulis*) larvae

and juveniles. Land-based nurseries based on cultivated algae with a higher degree of microbial control will probably be more used in the future.

References

Andersen S., G. Burnell, and Ø. Bergh. 2000. Flow-through systems for culturing great scallop larvae. Aquacult. Int. 8:249-257

Lambert C., J.L. Nicolas, V. Cilia, and S. Corre. 1998. *Vibrio pectenicida* sp. nov., a pathogen of scallop *(Pecten maximus)* larvae. Int. J. Syst. Bacteriol. 48:481-487.

Mortensen S. 2000. Scallop introductions and transfers, from an animal health point of view. Aquacult. Int. 8:123-138.

Mortensen S., T. van der Meeren, A. Fosshagen, I. Hernar, L. Harkestad, L. Torkildsen, and Ø. Bergh. 2000. Mortality of scallop spat in cultivation, infested with tube dwelling bristle worms, *Polydora* sp. Aquacult. Int. 8:267-271.

Nicolas J.L., S. Corre, G. Gauthier, R. Robert, and D. Ansquer, 1996. Bacterial problems associated with scallop *Pecten maximus* larval culture. Dis. Aquat. Org. 27:67-76.

Strand Ø. 1996. Enhancement of bivalve production capacity in a landlocked heliothermic marine basin. Aquacult. Res. 27:355-373

Torkildsen L., O.B. Samuelsen, B.T. Lunestad, and Ø. Bergh. 2000. Minimum inhibitory concentrations of chloramphemicol, florfenicol, trimethoprim/sulfadiazine and flumequine in seawater of bacteria associated with scallops (*Pecten maximus*) larvae. Aquaculture 185:1-12.

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TEMPERATURE AFFECTS TRYPSIN AND AMYLASE ACTIVITIES IN EURASIAN PERCH *PERCA FLUVIATILIS* THREE WEEKS AFTER HATCHING

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Introduction

It is well established that temperature affects both digestion and metabolism in poïkilotherms (Jobling 1994). The changes that occur during thermal acclimation must involve a series of adaptations at the enzymatic level, and there have been a number of studies carried out in order to examine the effects of temperature change on rates of enzymatic activity in fish liver and muscle tissues. In larvae, the digestive system is rudimentary, and a progressive specialization takes place (Alliot, 1979). In this paper, the hypothesis that temperature must influence enzyme secretions in the digestive tract of larvae was challenged.

Materials and methods

Larval rearing was conducted in 25-l tanks in recirculating system during 21 days (400 larvae per tank). During the 5 days after hatching, perch larvae were reared at 15°C. After this date, triplicate groups of tanks were assigned to differing temperature treatments: 20°C (high temperature: HT), 15°C (medium temperature: MT), and 12°C (low temperature: LT). Larvae were fed newly hatched *Artemia* nauplii from day 2-15 and one-day-old *Artemia* metanauplii from day 16 to the end of the experiment. The food was distributed slightly in excess throughout the photophase.

At the end of the experiment, survival rates were determined by counting all individuals in each tank. In order to monitor growth, 10 larvae per tank were sampled on day 7, 14, and 21 from each group and kept at -20°C prior to weighing. About 60 larvae were collected from each tank on day 7 and day 14, and 40 larvae were collected on day 21, in order to detect the first enzyme expression. Fish were sampled in the morning, before feeding began, and were immediately stored at -80°C for subsequent dissection and assays. Larvae were

dissected on a glass maintained at 0°C under a binocular microscope. The heads and tails were removed and their digestive tract isolated. The enzymatic assays were performed as described by Cahu and Zambonino Infante (1994). Samples were homogenized in 5 volumes (v/w) of ice-cold distilled water. Trypsin and amylase activities were assayed according to Tseng et al. (1984) and Metais and Bieth (1968), respectively. Enzyme activities are expressed as specific activity (i.e., U mg.protein^{-1}). Total protein content was determined by the Bradford procedure (Bradford 1976).

Results are given as mean ± SD. Data were compared by one-way analysis of variance followed by Student's test when significant differences were found at a 0.05 level.

Results and discussion

At the end of the experiment (day 21), weights were 2.9±1.27 mg, 3.1±0.96 mg and 11.3±4.67 mg for LT, MT, and HT, respectively. Temperature, which is known to influence both ingestion and metabolism, also affected growth rates. The growth of Eurasian perch larvae reared at 20°C was comparable to that reported previously under similar feeding conditions (Kestemont et al., 1996). Below this temperature, growth was highly and significantly (P>0.05) reduced. Specific growth rate (SGR = 100 (ln FBW - ln IBW.Δt^{-1}), with IBW, FBW: initial and final body weight of fish (mg), respectively, and Δt: time interval (days)), averaged 6.20%.d^{-1}, 6.54%.d^{-1} and 12.63%.d^{-1} for LT, MT, and HT larvae, respectively. On the other hand, rearing temperature did not significantly affect the survival rates, ranging from 32-36.8% (Table I).

Table I. Comparison of growth performances in relation to rearing temperature in post-hatch larvae of *Perca fluviatilis*.

Groups	LT	MT	HT
Temperature (°C)	12	15	20
IBW (mg)	0.8	0.8	0.8
FBW (mg) ± SD	2.94^{a} ± 1.27	3.16^{a} ± 0.96	11.35^{b} ± 4.67
Growth (mg.j^{-1})	0.102^{a}	0.112^{a}	0.502^{b}
SGR (%.j^{-1})	6.2^{a}	6.54^{a}	12.63^{b}
Survival (%) ± SD	36.83^{a} ± 6.33	34.58^{a} ± 5.80	32^{a} ± 0

For the pancreatic enzymes investigated, perch exhibited a specific activity since the early stages of development (Figs. 1 and 2). The presence of pancreatic trypsin and amylase specific activity during the first developmental days in perch larvae has already been described (Cuvier-Péres and Kestemont, 2001). For both enzymes, thermal acclimation induced increased activities in

cold-acclimated fish (LT). This is in agreement with previous studies indicating that, in fish liver cells or muscle tissues, some enzymes activities tend to increase under low-temperature conditions, and decrease under high-temperature conditions (Jobling, 1994).

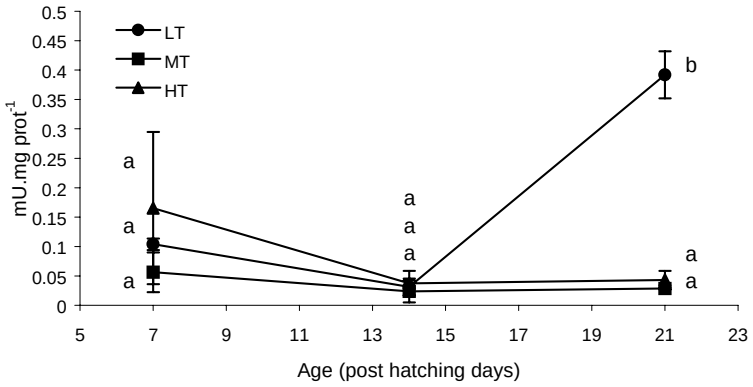

Fig.1. Specific activity of trypsin in post-hatching larvae of *Perca fluviatilis*. Means ± SD with differing superscripts are significantly different (P<0.05).

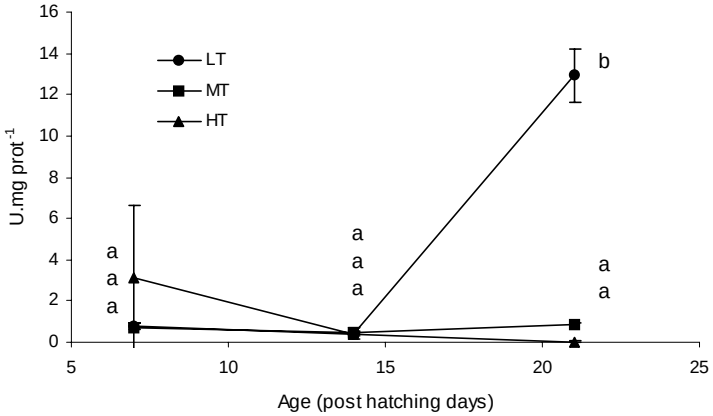

Fig.2. Specific activity of amylase in post-hatching larvae of *Perca fluviatilis*. Means ± SD with differing superscripts are significantly different (P<0.05).

Because a change in temperature may have marked influences on the catalytic properties of enzymes, an increase in enzyme concentration may not always have the effects of offsetting the reduction in catalytic activity caused by low temperature. A change in temperature would result in shifts in the relative proportions of two or more enzyme isoforms such that overall enzyme activity remained closely matched to the prevailing temperature conditions

(Rungruangsak-Torrissen et al., 1998). It is known that trypsin and amylase are polymorphic in several fish, but nothing is known in Eurasian perch. It could thus be interesting to investigate to what extent enzyme polymorphism contributes to the changes in activity observed during thermal acclimation.

References

Alliot E. 1979. Enzymologie digestive. III Evolution de quelques activités digestives au cours du développement larvaire des téléostéens. p.79-87. In: Nutrition des poissons, Actes de colloque. Fontaine M. CNERMA-Paris.

Bradford M.M. 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Cahu C.L. and J.L. Zambonino Infante 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: effect on digestive enzymes. Comp. Biochem. Physiol. 109A:213-222.

Cuvier-Péres A. and P. Kestemont. 2001. Development of some digestive enzymes in Eurasian perch larvae *Perca fluviatilis*. Fish Physiol. Biochem., submitted.

Jobling M. 1994. Fish Bioenergetics. Chapman & Hall, Fish and Fisheries Series 13, London. 309p.

Kestemont P., C. Mélard, E. Fiogbé, R. Vlavonou, and G. Masson. 1996. Nutritional and animal husbrandry aspect of rearing early stages of Eurasian perch *Perca fluviatilis*. J. Appl. Ichthyol. 12:157-165.

Métais P. and J. Bieth. 1968. Détermination de l'α-amylase par une microtechnique. Ann. Biol. Clin. 26:133-142.

Rungruangsak-Torrissen K., G.M. Pringle, R. Moss, and D.F. Houlihan. 1998. Effects of varying rearing temperatures on expression of different trypsin isozymes, feed conversion efficiency and growth in Atlantic *salmon (Salmo salar* L.). Fish Physiol. Biochem. 19:247-255.

Tseng H.C., J.H. Grendell, and S.S. Rothman. 1982. Food, duodenal extracts, and enzyme secretion by the pancreas. Am. J. Physiol. 243:G304-G312.

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MORPHOECOLOGY AND FEEDING BEHAVIOUR IN LARVAL FINFISH: A NEW CANDIDATE SPECIES FOR AQUACULTURE

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Introduction

The need to diversify aquaculture production to resolve the market saturation for sea bass and sea bream means that more knowledge concerning the feeding behaviour during the larval and juvenile stages of new candidate species is required. Unfortunately, the available literature often does not offer such information, making it difficult to set up the proper rearing techniques and avoid expensive and prolonged experimental trials when setting up the right feeding protocols for innovative species. However, it is possible to, at least indirectly, acquire some information on trophic behavior by means of studies on sensory equipment development during larval ontogenesis, where the progressive differentiation of sense organs involved in perception and selection of food items gradually modulates the larval trophic behavior.

To this aim, this study offers some preliminary information on the sensory and digestive systems during ontogenesis in *Diplodus puntazzo* and *Seriola dumerilii* obtained through out scanning electronic microscopy (SEM) and histological observations of wild and hatchery-reared larvae and juveniles.

Materials and Methods

A total of 130 individuals of *D. puntazzo* and *S. dumerilii* were observed using a scanning electron microscope (SEM) and a light microscope (LM) (Table I).

D. puntazzo hatchery specimens were sampled during two different reproduction events, carried out in the Ittica Ugento S.p.A. hatchery (southern Italy, November 1996). Two- to twenty-day-old larvae were sampled from the first event, while the others came from the second event. The wild *D. puntazzo* were caught along the Gulf of Tigullio coast (west-northern Italy) by purse seine. *S. dumerilii* larvae and juveniles came from an experimental reproduction carried

out in Acquacoltura Lampedusa hatchery (insular Italy) in 1998. Histological examinations were performed both on paraffin and resin (Spurr) inclusions.

Table I. Characteristics of sampling. *: days from hatching; #: number of observed individuals.

Species	Origin	Age*	# SEM exam.	# histological exam.	Range (mm)
	Hatchery	2-66	55	0	3.1- 28.6
D. puntazzo	Wild	?	6	8	14.2 –62.9
	Total		61	8	3.1-62.9
S. dumerilii	Hatchery	1-151	35	26	1.15-270

Results

D. puntazzo. Whilst the digestive apparatus is already differentiated in 14.2-mm larvae, the dentition of *D. puntazzo* is still differentiating. Postlarvae (14.2-58mm), in fact, showed two irregular rows of canine-like teeth, lined along the pre-maxillary and dental, while juveniles which were longer than 58mm had 4 medial pairs of incisiform teeth on each lip, rare residual caniniforms (which tend to disappear during growth), and transitional teeth toward the molariform definitive form, laterally lined in a row. On the dorsal and ventral pharynx, specialized areas are progressively differentiated as development occurs and are characterized by differently shaped teeth and chemoreceptive areas (Fig. 1). The digestive tract appears to be completely differentiated in 62.9-mm juveniles. The

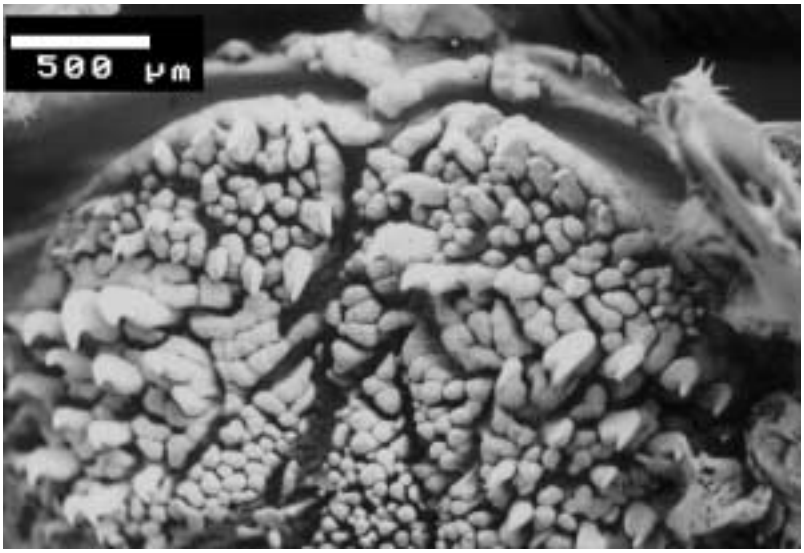

Fig. 1. SEM micrograph of dorsal pharynx of a wild *D. puntazzo* (58mm TL).

first appearance of inner taste buds was not registered before the 48th day from hatching; the outer ones not before the 58th day. The mechano-receptors of the lateral line system are canalized starting from the 66th day. The olfactory organ completes the deepening and nares are formed on the 58th day.

S. dumerilii. A canalized lateral line system, as well as vision capacity, is already achieved at the 30th day after hatching (mean TL = 13.12mm). Inner taste bud differentiation is very precocious (5th day), while the outer ones differentiate afterwards (57th day). Canine-like teeth appear at first in 10-day-old larvae on pre-maxillary, at the 18th day on dental and pharynx (Fig. 2), and at the 35th on the branchial arches.

Fig. 2. SEM micrograph of dorsal pharynx of 152 days-old *S. dumerilii*.

Digestive functionality changes with age: at the 18th day after hatching, gastric glands are not differentiated and the presence of intestinal mucosa vacuolizations denotes an endocellular digestion, which compensates the yet-to-be-achieved gastric one. 35-day-old larvae have a stomach with rare gastric glands and an extra cellular digestion (adult-like).

Conclusions

The rearing of *D. puntazzo* should take into consideration the organoleptic properties of administered prey because the post-larvae precociously achieve (11.4-13mm TL) the differentiation of the inner taste buds, which give to the larvae the possibility to accept or reject the ingested item on an organoleptic basis. Olfaction, vision, and mechano-reception are involved in prey selection.

As far as *S. dumerilii* is concerned, our results show that the ram feeding is present also during the juvenile phase and not only in adults, as has been previously described in literature. This is probably an integration to predatory activity. The observed precocious sensory differentiation makes this species very sensitive to dimensional, behavioural, morphological, and organoleptic characteristics of administered food items.

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SKELETAL DESCRIPTORS FOR THE QUALITY ASSESSMENT OF TWO INNOVATIVE SPECIES OF MEDITERRANEAN AQUACULTURE: SHARPSNOUT SEA BREAM AND PANDORA

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During the last decade, anomalies of shape and/or number of skeletal elements have been utilized to compare sea bass (*Dicentrarchus labrax*) or sea bream (*Sparus aurata*) lots from different hatcheries with wild specimens (Boglione et al., 1993; 1994; 2000; 2001; Sola et al., 1998). These skeletal descriptors were used to assess the morphological quality of reared fish, with wild lots utilized as a quality reference. Such quality assessment made it possible to quantify the qualitative characteristics of different lots, to produce a hierarchy among the different farm productions, and to discriminate between fish reared with different technologies. This approach was aimed to identify which commercial farms were able to produce wild-like fish, so valorizing aquaculture production economically (no discrimination on behalf of consumers towards farmed fish) and making it possible to utilize these fish for seeding in confined coastal areas (lagoons, 'valli', extensive ponds). The recent market saturation for sea bass and sea bream production, in fact, provoked a reduction in market prices, with a resulting necessity to improve the quality of farm fish and/or to rear other, innovative, species. Furthermore, the wild-like phenotype has been demonstrated (Boglione et al., 2001) to be obtainable by rearing fish with semi-intensive, low impact, methodologies, able to simulate natural conditions, as far as the hatchery conditions allow. Consequently, the fish with wild-like morphology often show wild-like behaviour, so assuring satisfactory survival rates when seeded in confined environments. The identification of low impact hatcheries which produce high quality fish, which can be used for sea-ranching or extensive purposes instead of wild fry, satisfies the recommendations stipulated in Article 9 of the Code of Conduct for a Responsible Fishery (FAO, 1995).

The present study refers to the utilization of such skeletal descriptors to compare wild and reared post-larvae and juveniles of sharpsnout sea bream (*Diplodus puntazzo*) and pandora (*Pagellus erythrinus*) to assess the actual production of such innovative species.

A total of 1016 wild and reared post-larvae and juveniles of sharpsnout sea bream and pandora were inspected for skeletal abnormalities. Reared sharpsnout sea bream (DPr) were sampled in two different commercial hatcheries, both from southern Italy (Ittica Mediterraneo, Sicily, 522 individuals from two different egg batches; Ittica Ugento, Puglia, 102 individuals). The wild lot (DPw) was caught in Sicily (Licata). Reared pandora (PEr) samples came from an experimental reproduction program carried out in 1997 (COISPA, Puglia); the wild ones (PEw) were caught along the Lazio coast (central Tyrrhenian Sea) in 1998.

All samples were fixed in buffered formaldehyde (10%) and *in toto* stained with alizarin red for calcified structures and alcian blue for cartilage (Park and Kim, 1984; Taylor and Van Dike, 1985). Meristic counts and skeletal and developmental anomaly surveys were carried out according to the methodology described in Boglione et al. (2001). The resulting data was used to create frequency histograms for each descriptor and lot.

Data related to subsets of reared individuals at different ages were also analysed and the matrix with the overall data was submitted to Multi-Response Permutation Procedure (MRPP) and to Indicator Species Analysis (Dufrene and Legendre, 1997).

The results evidenced differences in meristic counts, and in skeletal anomaly patterns and frequencies between wild and reared individuals, in both species.

Individuals with at least one anomaly were particularly frequent in reared lots, more so than in the wild ones, with the DPr lot showing the highest values. The DPr and PEr lots produced similar values when only severe anomalies (kyphosis, lordosis, scoliosis, deformation or fusion of vertebrae, cephalic anomalies, abnormal swim bladder, calculi in the urinary ducts) were taken into consideration. Severe anomalies were completely absent in wild lots of both species, with the only exception being four sharpsnout sea breams, which showed a very slight operculum curvature.

MRPP was applied to the anomalies data matrix to test the hypothesis of no difference between two or more groups of entities. The results showed that the mean intra-group distance for wild and reared sharpsnout sea bream was significantly lower than expected when both groups were taken from a single population.

Differences in terms of quality were noticeable between the two lots of farmed sharpsnout sea breams.

The quality assessment approach used in this study made it possible to identify consistent differences among the morphologies of wild and reared fish, with different levels of disturbance generated by the different captive conditions. The results indicate the necessity to improve rearing techniques for these two innovative species.

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References

Boglione C., G. Marino, B. Bertolini, A. Rossi, F. Ferreri, and S. Cataudella. 1993. Larval and post larval monitoring in sea bass: morphological approach to evaluate finfish seed quality. p.189-204. In: Production, Environment and Quality. Bordeaux Aquaculture '92. G. Barnabé and P. Kestemont (Eds). EAS Spec. Publ. No. 18, Ghent, Belgium.

Boglione C., G. Marino, F. Ferreri, M.G. Finoia, M. Scardi, E. Fresi, and S. Cataudella. 1994. Anatomical aspects for seed quality assessment in sea bass (*Dicentrarchus labrax*): hatchery and wild populations. Actes du colloque "Measures for success: Metrology and instrumentation in aquaculture management", Bordeaux Aquaculture 1994, Mars 23-25, 1994, P. Kestemont, J. Muir, P. Sévila, and P. Williot (Eds), CEMAGREF-Edition, p.191-197.

Boglione C., M. Cecchetti, C. Costa, M. De Francesco, S. De Innocentiis, M. Dell'Aquila, M. Fantozzi, G. Ferzini, F. Gagliardi, A. Loy, F. Mugnai, C. Selmo, M. Scardi, A. Spanò, and S. Cataudella. 2000. Seed quality in wild and reared Mediterranean finfish. Cah. Options Méditerr. 47:89-95.

Boglione C., F. Gagliardi, M. Scardi, and S. Cataudella. 2001. Skeletal descriptors and quality assessment in larvae and post-larvae of wild and reared gilthead Sea Bream (*Sparus aurata*, L. 1758). Aquaculture 192:1-22.

Dufrêne M. and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345-366.

Park E.H. and D.S. Kim. 1984. A procedure for staining cartilage and bone of whole vertebrate larvae while rendering all other tissues transparent. Stain Technol. 59(5):269-272.

Sola L., S. De Innocentiis, A.R. Rossi, D. Crosetti, M. Scardi, C. Boglione, and S. Cataudella. 1998. Genetic variability and fingerling quality in wild and reared stocks of European Sea Bass. Cah. Options Méditerr. 34:273-280.

Taylor W.R. and G. Van Dike. 1985. An enzyme method of clearing and staining small vertebrates. Proc. U.S. Natl. Mus. 122:1-17.

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IMPROVEMENT OF THE NUTRITIONAL VALUE OF ROTIFERS FED ALGAE-BASED DIETS

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Introduction

Fatty acid composition of live food has been shown to be one of the main factors in nutrition of larval marine fish and it is very important to strictly meet the requirements of the highly unsaturated fatty acids (HUFA) such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Those requirements are related to both quantitative presence into the diets and their relative ratio, especially DHA/EPA and EPA/AA. Many studies have been carried out towards the optimisation of the ratio of these nutrients for larvae and several techniques have been tested for the enrichment of live food i.e. rotifer *Brachionus plicatilis* and *Artemia* spp. Recently Sargent et al. (1999) suggested the following dietary ratio for European sea bass (*Dicentrarchus labrax*) larvae: DHA/EPA = 2/1 and EPA/AA = 1/1. Also, Mourente et al. (1999) studied the essential fatty acids requirements of common dentex (*Dentex dentex*) and found to be optimal a level of 3.97% n-3 HUFA on a dry weight basis. In order to achieve a higher HUFA content of live food, which is the first food for many fish species, there are now some new HUFA sources. Those products are based on phototrophic or heterotrophic organisms such as the algae *Criptecodinium* sp. or the fungus *Morteriella alpina*. The former is characterized by a high DHA content while the latter is rich in AA. The aim of the present study is to test the effectiveness of enriching rotifers with these products in view of the fatty acid composition, with a particular attention of essential fatty acids, their ratio and their overall level.

Materials and Methods

Four different treatments were fed rotifers (*Brachionus plicatilis* – L strain): DHA Protein Selco (PS) (INVE, Belgium) was used as a control diet and three different blends of the following ingredients: AquaGrow Advantage (Martek Biosciences Corporation, USA) – a product based on the heterotrophically grown dinoflagellate *Criptecodinium* sp.; AquaGrow AA (Martek Biosciences Corporation, USA) – based on the heterotrophically grown fungus *Morteriella*

alpina; and the concentrated microalgal paste *Nannochloropsis* IA (Reed Mariculture Inc., USA). These ingredients were combined to obtain different DHA/EPA and EPA/AA ratios: 2.3 and 1.8 (Diet A), 3.6 and 1.0 (Diet B), 6.3 and 1.0 (Diet C), respectively (Table I).

Table I. Composition of diets.

%DW/Diet	PS	A	B	C
Protein (N × 6.25)	27.2	28.6	24.2	25.0
Fat	25.4	22.4	25.1	22.6
Ash	11.3	23.5	26.4	31.4
	Fatty acid (% total FA)			
Total saturates	32.8	30.7	30.8	30.2
Total monoenes	15.4	19.0	16.3	14.5
Total (n-6)	11.1	8.4	10.6	8.3
Total (n-3)	32.0	36.9	37.1	44.2
20:4 n6	0.9	6.0	8.0	6.3
20:5 n3	8.0	10.8	7.9	6.0
22:6 n3	19.2	25.0	28.2	37.4
DHA/EPA	2.4	2.3	3.6	6.3
EPA/AA	8.5	1.8	1.0	1.0

Rotifers were inoculated at a density of 500 individuals.ml^{-1} in 40-l polyethylene cylindrical tanks. The culture water consisted of seawater (25‰ salinity) kept at 28±1°C. Rotifer cultures undergoing enrichment were fed the equivalent of 0.6 g dry weight per 10^6 rotifers applied twice ($0.3g.10^{-6}$ rotifers per aliquot) over 24 hours. Three tank replicates were set up for each treatment. At the end of the enrichment procedure, rotifers were harvested and thoroughly washed with deionized water until salt and food were removed and finally freeze-dried before analysis. Lipids were extracted using the method of Folch et al. (1957). Fatty acid methyl esters (FAME) of total lipid were prepared by acidified methylation with 1% sulphuric acid in methanol (v/v). Fatty acid composition either of diets (Table I) and rotifers (Table II) was determined in a GC Varian 3380 01 gas chromatograph fitted with a 30m×0.25µm×0.325mm capillary column (DB-23 J&W Scientific). Nitrogen was used as a carrier gas. Individual FAME was identified by comparison with known standards (Sigma-Aldrich) and a well characterized oil (Supelco). Crude protein (Kjeldhal, nitrogen X 6.25) and ash content (at 600° for 3 hours) were also determined both for diets and rotifers. Results were checked using Grubb's test in order to reject outliers and then subjected to a one-way ANOVA. Differences between means were compared using the Newmann-Keuls multiple comparison test (GraphPad Prism 3.00; Graph Pad Software, San Diego, CA).

Results and discussion

In terms of percentages, total n-3 content in rotifers was decreased compared with the corresponding treatment. Rotifers fed the three blends are characterized by a high content in total n-3 ranging from 17.1% to 20.1% in comparison with the treatment PS (11.5%). Total n-6 ranged from 9.0% to 11.0% and substantially reflected the enrichment preparations. The DHA level were highest in treatment C (12.6%) compared with treatments B (10.3%), A (8.3%), and PS (4.3%). EPA levels ranged from 5.6% (B) to 3.8% (C) while the level of AA in treatments A, B, and C was elevated, reflecting the high levels of this fatty acid in the enrichment blends. PS treatment was characterized by a low level of AA (0.9%). This resulted in the PS treatment rotifers having significantly higher (P<0.001) EPA/AA ratio (5.3) than in A (1.4), B (0.9), and C (0.9) treatments. The DHA/EPA ratio also showed differences between the treatments ranging from 3.0 (A) to 1.2 (PS). In conclusion, the products used in this trial seem to be very promising in order to obtain enriched live food with a very high content of n-3. Furthermore, even though further investigation are necessary to standardized the enrichment procedures, it seems possible to obtain specific contents and ratios of essential fatty acids by means of different percentages of these products in the diet.

Table II. Composition of rotifers fed diets.

%DW/Diet	PS	A	B	C
Protein (N × 6.25)	62.3 ± 0.9	61.8 ± 1.1	59.0 ± 1.7	58.7 ± 0.9
Lipid	13.9 ± 0.4	16.0 ± 1.2	14.7 ± 0.5	15.1 ± 0.6
Ash	5.5 ± 1.0	4.4 ± 0.5	5.6 ± 0.5	5.4 ± 2.1
		Fatty acid (% tot FA)		
Total saturates	22.4 ± 0.8^{b}	29.0 ± 0.4^{a}	26.6 ± 1.1^{a}	29.5 ± 0.0^{a}
Total monoenes	48.1 ± 0.7^{a}	38.9 ± 1.0^{b}	36.6 ± 0.4^{bc}	35.2 ± 0.4^{c}
Total (n-6)	9.0 ± 0.5^{a}	9.1 ± 0.1^{a}	11.0 ± 0.2^{a}	10.2 ± 0.0^{a}
Total (n-3)	11.5 ± 0.2^{b}	17.1 ± 0.6^{a}	19.4 ± 1.1^{a}	20.1 ± 0.9^{a}
20:4 (n-6)	0.7 ± 0.1^{d}	4.0 ± 0.0^{c}	5.7 ± 0.3^{a}	4.9 ± 0.3^{b}
20:5 (n-3)	3.8 ± 0.3^{b}	5.6 ± 0.0^{a}	5.0 ± 0.4^{ab}	4.2 ± 0.2^{b}
22:6 (n-3)	4.6 ± 0.3^{b}	8.3 ± 0.3^{b}	10.3 ± 0.7^{b}	12.6 ± 0.5^{b}
DHA/EPA	1.2 ± 0.1^{a}	1.5 ± 0.1^{a}	2.1 ± 0.0^{a}	3.0 ± 0.1^{b}
EPA/AA	5.3 ± 0.0^{a}	1.4 ± 0.0^{b}	0.9 ± 0.0^{b}	0.9 ± 0.0^{b}

Different letters indicate statistical differences (P<0.001).

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References

Folch J, N. Lees, and G.H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226:487-509.

Mourente G., D. Tocher, E. Diaz-Salvago, A. Grau, and E. Pastor. 1999. Aquaculture 179:291-307.

Sargent J., G. Bell, L. McEvoy, D. Tocher, and A. Estevez. 1999. Aquaculture 177:191-199.

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DOMESTICATION OF THE BARRAMUNDI COD, *CROMILEPTES ALTIVELIS* (VALENCIENNES): INVESTIGATION OF THE REPRODUCTION

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Introduction

Barramundi cod, *Cromileptes altivelis*, also known as humpback grouper or pantherfish, is one of Asia's most highly-prized food fish, with a potential to reach up to US$130-150 per kilogram (live) on the Hong-Kong and southern Chinese markets (Rimmer, 2000). This tasty fish grows to 1kg in 24 months and can easily reach a weight of 6-12kg. It is found throughout South East Asia, as well as in northern Australia (Northern Territory, Queensland).

At the Darwin Aquaculture Centre, we have been holding barramundi cod broodstock since October 1998. During this time we have been focusing on broodstock management of this hard-to-keep and hard-to-breed species. To date, the main difficulties have been keeping the broodfish disease-free and obtaining reliable quantities of high quality fertilized eggs.

Materials and Methods

Broodstock husbandry. The fish were initially held in two 20 000-l indoor fiberglass tanks with 300-400% water exchange per day from October 1998 to April 2000. The fish were subjected to a photoperiod of 13h L/11h D (artificial light). The water temperature and salinity ranged from 24.6-30.6ºC and 30-36ppt, respectively.

Since April 2000, the fish have been held in an outside 45 000-l tank. The fish receive a photoperiod ranging from 11.4-12.8h L (natural light). The water temperature and the salinity fluctuate seasonally from 23.8-31.4ºC and 30-36ppt, respectively.

The fish were originally fed *ad libitum* a mixture of squid, mullet, and coral prawns supplemented with vitamin premix four times per week (2-6% of

biomass). Prawns were a non-preferred food by the fish and were later eliminated from the diet.

Monitoring of the gonadal development was generally conducted once a month by catheterization. The maturation of males was assessed on the milting condition or on the presence of sperm and testis in the samples, and females were assessed on oocyte development stage.

Results

Maturity of female. Barramundi cod are protogynous hermaphrodites, changing sex from female to male.

Females mature from 1-4.5kg. Mature females with ripe oocytes were available throughout the year (15-91% mature). Photoperiod, salinity, or water temperature did not appear to affect the percentage of mature females. However, the maturity condition of each female (percentage of ripe oocytes) was significantly improved once the fish were transferred outside. Mature females also had a higher percentage of ripe oocytes when water temperature was in the range 24-28ºC.

Maturity of male. At the Darwin Aquaculture Centre, females changed sex to males at a weight of 4.5-5.0kg.

Most of our males were mature at capture and for the first month in captivity. They have since not re-matured. All the "supposed males" weighing over 5.0kg and coming from captive sex-changed females have not matured.

Recent treatment of males with 17α-methyltestosterone, either by cholesterol- or silastic-based pellets, has resulted in an improvement in their reproductive condition characterized by the presence of sperm in the samples and the occurrence of 2 running ripe males.

Artificial spawning. From January 1999 to March 2000, several attempts at induced spawning resulted in one release of unfertilized eggs. Females and males were induced with either Ovaprim® (LHRHa + dopamine antagonist) at $0.5ml.kg^{-1}$ or LHRHa (50µg.kg-1) cholesterol pellets. No natural spawning was observed over this period.

Natural spawning. Once the broodstock were transferred to the outdoor tank (April 2000), natural spawning of unfertilized eggs occurred within two weeks. From this date, the natural spawning of unfertilized eggs appears to be sporadic, unpredictable, and does not follow any obvious pattern (moon phase, temperature, salinity, etc).

Disease. In the first 18 months of the project, the broodstock were affected by a serious pre-existent and chronic parasitic pathogen – the larvae of the nematode Anisakidae, *Terranova* type 2 sp. This parasite is mainly present in the digestive system and the peritoneal cavity of the fish. Infection was associated with vibriosis, peritonitis, and septicaemia and was responsible for the death of 50% (17) of our broodstock over this period. This parasite was a major problem in our research and prevented us from undertaking a number of reproduction experiments.

All broodstock were treated with the broad-spectrum antiparasitic agent Ivermectin with 2-4 injections at a dose rate of 200µg.kg^{-1}, 4-5 weeks apart. During this treatment, we noted that special care should be given to the fish and any other treatments (formalin, fresh water bath, anaesthesia) should be avoided as the fish were extremely sensitive and easily stressed. There have been no mortalities in the last 9 months.

We also observed that fish kept indoors developed ulcers or skin/fin erosion, but they quickly healed after the fish were moved outside. The fish kept outside have not developed any ulcers or skin erosion.

Discussion

Throughout this project, we have been grateful for information received from the Fisheries Research Station of Gondol, Bali (Indonesia) – especially Dr. Ketut Sugama. Dr. Sugama's team has successfully bred barramundi cod, and their information combined with our experience leads us to the following observations and recommendations for holding this species at our Centre:

Observations

- The combination of a natural light regime and a large tank triggered natural spawning and improved the maturity of the females.
- The use of a large outdoor tank improved the appetite, general health, and condition of the fish.
- Constant and medium-low tropical temperatures (24-28°C) improve the maturity of the females and general condition of the fish.
- Barramundi cod are extremely sensitive to water quality. At the Darwin Aquaculture Centre, we are pumping water from close to a mangrove area. As a result of this location the water contains a heavy load of fine particles (less than 10µm) which pass through the sand filter system. The water quality deteriorates particularly during the spring tides (up to 7-m tides). At these periods, our barramundi cod lose appetite and they become less active.

Recommendations

We are currently upgrading our broodstock tanks to help improve the maturity of the fish and to have better control of natural spawning events:

- Tanks larger than 60 000 l (2m deep × 7m diameter).
- Improve water quality by partially recirculating the tank water through a sand filter, protein fractionator, and UV system. New water should first be treated in a settling tank before going through the recirculated system of each tank.
- All tanks provided with a temperature control system to maintain a water temperature within 24-28ºC all year round.
- Increase the number of broodstock (currently 18) up to 40-50 fish, to allow more replicates for each experimental treatment.
- Maintain a strict disease protocol and treat any new broodstock with ivermectin.

References

Rimmer M. 2000. Potential for Reef Fish Aquaculture in Queensland. Aquaculture Symposium, Reef Fish Aquaculture - Potential, Constraints and Status. Mercure hotel Harbourside, 26 July 2000, Cairns.

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OPTIMIZING BROODSTOCK PERFORMANCE: MATURATION, FECUNDITY, AND GAMETE QUALITY

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Abstract

Supply problems of egg and larvae, often known collectively as seed, together with the quality of the seed in terms of survival and performance, contribute some of the most important constraints to current and future aquacultural developments. Improvements in our understanding of the appropriate culture conditions, feed requirements, and management procedures for broodfish are essential if we are to optimize broodstock performance and program reproductive development to reliably produce the numbers of eggs and larvae at the times required by grow-out farms. Only by optimizing this production will the industry be able to produce the year-round supplies of product of consistent size and quality required by retail markets.

In this review, we will identify some of the problems of broodstock performance, mentioning ways in which these constraints might be addressed and seed production improved. This will include data on the hormonal and environmental manipulation of maturation and spawning and the effects of nutritional status on broodstock fecundity, performance, and egg quality.

Acknowledgements

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LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wile and P. Sorgeloos (Eds)
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DEVELOPING LARVICULTURE PROTOCOLS FOR COLD-WATER MARINE FINFISH

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Abstract

With the dramatic and devastating collapse of many sectors of the wild fishery off the east coast of Canada, efforts have increased to expand opportunities for aquaculture. One effort has been on diversifying the aquaculture base in the region by developing new marine finfish species. This is often problematic, as we do not have a good understanding of the ecological conditions to which these marine larvae are adapted, which leads to the use of suboptimal hatchery or rearing protocols. Over the last five (5) years we have conducted research to improve the production of juveniles in two marine species of interest in Atlantic Canada: Atlantic cod (*Gadus morhua*) and yellowtail flounder (*Limanda ferruginea*).

Atlantic cod is the reason the island of Newfoundland was settled and, until the early 1990's, was the basis of a large industrial fishery. In the mid 1980's, the ongrowing of small cod caught inshore was started, but the moratorium curtailed that activity and signaled the start of efforts to farm cod from hatchery production. Around the same time, the fishery for yellowtail flounder was stopped and efforts to produce this small flounder began. Experiments designed to address problems with larval survival were initiated and efforts on these two species proceeded in parallel.

Atlantic cod

We began intensive production experiments on Atlantic cod in the early 1990's prior to the closure of the wild fishery. Early experiments focused on prey and light requirements for maximal production of larvae. Further experiments have been conducted to improve the start-feeding protocols in relation to flow rate, prey

density, and prey type so that a more refined schedule of start-feeding was achieved. In addition, we have compared different stocks of cod from the North Atlantic coast of the USA and Canada and found that larvae from some stocks perform better then others in culture. Using the information from the above experiments, we expect a good production of cod juveniles from efforts to be undertaken in the summer of 2001. We will present these protocols and production figures in this talk and review production protocols from other efforts on cod in the US, UK, and Norway.

Yellowtail flounder

As mentioned, we began experiments on the intensive production of yellowtail flounder around the same time as for Atlantic cod. Given that the production problems are similar among marine fish species, we focused on prey and light requirements for optimal production of yellowtail larvae. Slight differences were found regarding prey density requirements compared to cod. However, high light levels were found to improve production, which is similar to cod. In addition, experiments were conducted to refine feeding frequency of live feed to larvae and results suggested that feeding live feed twice per day was sufficient. Over the five (5) years of work, larval and juvenile growth and survival were significantly improved.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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ALTERNATIVES TO LIVE MICROALGAE AS HATCHERY FEEDS: THRAUSTOCHYTRIDS AND ALGAL CONCENTRATES

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Introduction

Microalgae have been the traditional food for many larval and juvenile animals in aquaculture. However, their mass cultivation can be up to 50% of a hatchery's operating costs (Coutteau and Sorgeloos, 1992). Therefore, there is a need for cost-effective alternatives to live algal production (Robert and Trintignac, 1997). Dried thraustochytrids and microalgal concentrates both show promise as alternative 'off-the-shelf' diets. Thraustochytrids – microorganisms possibly related to red and brown algae – have attracted interest because they are rich in the essential polyunsaturated fatty acid docosahexaenoic acid (DHA) and can be grown to high biomass at low cost (Lewis et al., 1999). On the other hand, algal concentrates produced by centrifugation can be used effectively as feed for oyster larvae and spat (Heasman et al., 2000). In this paper, we first report on two living thraustochytrids isolated from Australian waters as feeds for juvenile Pacific oysters (*Crassostrea gigas*). We also report on the efficacy of concentrates prepared from the microalga *Chaetoceros calcitrans*, using a novel flocculation procedure, as feed for juvenile scallop (*Pecten fumatus*).

Materials and methods

Thraustochytrids for juvenile oysters. Two thraustochytrids isolated from Australian waters (code names: THBI02 and THBI03) were grown at 25ºC in a seawater media containing yeast extract, peptone, glucose, and agar, and harvested at late logarithmic phase. They were assessed as part of a mixed diet for juvenile (1.5mm) oyster *Crassostrea gigas*. One ml of the oysters was placed into each of 20 chambers suspended in individual 10-l buckets (Brown et al., 1998). Buckets were filled each day with 1µm-filtered seawater (13.5°C) and the

temperature allowed to gradually increase to 19.5°C by the following day. Seawater was mixed by an aquarium pump. Oysters were fed a base 20% diet component of the microalga *Tetraselmis suecica* (2mg day^{-1}). The remaining 80% (8mg day^{-1}) comprised of additional *T. suecica*, the commercially available thraustochytrid AlgaMac 2000 (Aquamarine Biofauna), or the live thraustochytrids THBI02 or THBI03. There was an additional treatment where no extra feed was given (control). Growth of the oysters was assessed as the change in their dry weight (DW) after 21 days.

Algal concentrates for juvenile scallops. Concentrates were prepared by flocculation of 20-l cultures of *C. calcitrans* (Knuckey, 1998). This procedure is detailed elsewhere (Robert and Brown, this issue), but in brief involved the addition of an anionic polyacrylamide solution to the culture at pH 10.2. The flocculate sedimented from solution, and was collected to give 1:200 concentration. The concentrated algae was stored at 4ºC in 200-ml glass bottles (tops loose to allow some gas exchange) either in the dark, or under 20µmol photon $m^{-2}s^{-1}$.

Culture systems for the feeding experiment were identical to those described for oysters. The water temperature was 19±1ºC. Scallops (0.8ml initial volume, 700µm size) were fed one of 6 treatments (4 replicates) daily. All scallops received 1.5mg of live *C. calcitrans* (basal diet). One treatment received no extra food; other treatments were supplemented with 6mg additional live *C. calcitrans*, or one of four concentrates. The concentrates were either "fresh" (6±3 days) or "old" (20±4 days), and stored in the dark, or in light. Growth of the scallops was assessed as the change in their DW after 15 days.

Results

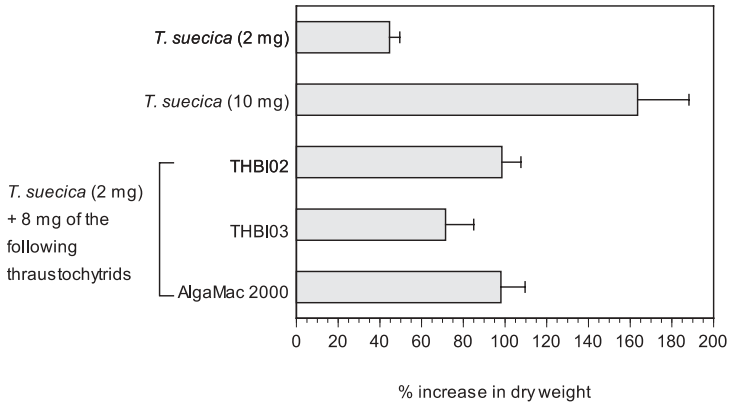

Fig. 1. Growth (% increase in dry weight) of juvenile Pacific oysters *C. gigas*, initial size 1.5mm, fed microalgal and thraustochytrid diets over 21 days. Values are mean ± S.D.

For oysters, the basal 2-mg ration of *T. suecica* produced the smallest increase in DW (45%), and the 10-mg ration of *T. suecica* the largest (164%) (Fig. 1). Supplementation of 2mg *T. suecica* with the thraustochytrids improved oyster growth, with THBI02 and AlgaMac 2000 (98-99% increase in oyster DW) being more effective than THBI03 (72% increase) (P<0.05). Survival was not significantly different between treatment groups (>95%; P<0.05%).

For scallops, the basal 1.5-mg ration of *C. calcitrans* produced a 228% increase in DW (Fig. 2). Supplementation with additional live *C. calcitrans* or concentrates of *C. calcitrans* improved growth (384-462% in DW). There were no significant differences in the growth of the scallops fed the supplementary *C. calcitrans*, except that live *C. calcitrans* outperformed old concentrates that were stored in the dark (P<0.05). The survival of scallops was not different between treatment groups (51-62%; P<0.05).

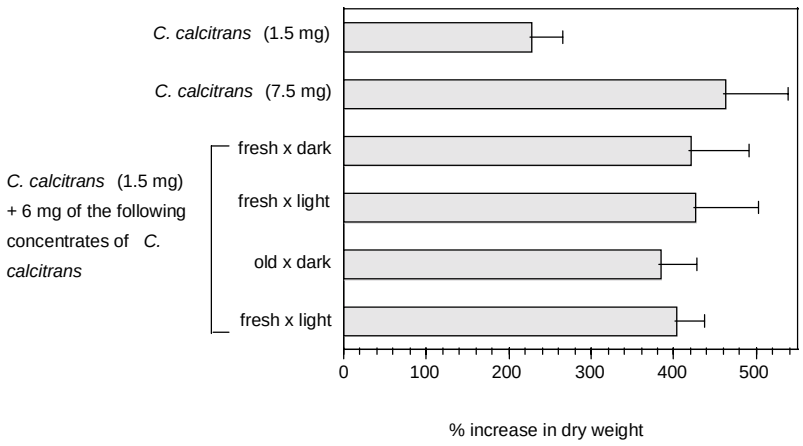

Fig. 2. Growth (% increase in dry weight) of juvenile scallops *P. fumatus*, initial size 700µm, fed live and flocculated concentrates of *C. calcitrans* over 15 days. Fresh = concentrates aged 6±3 days; old = concentrates aged 20±4 days; dark = concentrates stored in dark; light = concentrated stored in light. Values are mean ± S.D.

Discussion and conclusions

The two live thraustochytrids that we assessed as feed for oysters were chosen from other candidate strains on the basis of good cell division rates, high content of DHA (both contain >15% of total fatty acids as DHA; Peter Mansour, unpublished data), and suitable size range for ingestion by animals (<20 microns). Both strains were effectively ingested and assimilated, as demonstrated by improvements in oysters' growth rate compared to the basal

diet. This finding was positive; however, growth rates were no better than the commercial dried thraustochytrid preparation, AlgaMac 2000. These Australian strains may therefore be of limited commercial value as mollusc feeds, though manipulation of their culture conditions (e.g., to increase DHA) could potentially improve their nutritional value.

Algal concentrates appear to have more potential as 'off-the-shelf' replacements of live microalgae. For juvenile scallops, live microalgae could be substituted by 80% with concentrates prepared by flocculation and stored for up to 20 days, without major reductions in scallops' growth. Similarly, flocculated concentrates of *C. calcitrans* forma *pumilum* are equally effective as the live microalga, within mixed diets for larval and juvenile *C. gigas* (Robert and Brown, this issue). Advantages of the flocculation method are that it is simple and inexpensive (chemical costs <US$1 per 1000 l culture; Knuckey, 1998) and is a volume-independent process. The process could be readily adapted by hatcheries on-site to prepare their own concentrates, thereby allowing them to streamline and improve their efficiency of algal production. Under a different scenario, large algal production facilities – with economies of scale – could produce algal concentrates and sell to hatcheries at significantly lower costs than live microalgal production within standard hatcheries.

References

Brown M.R., M.A. McCausland, and K. Kowalski. 1998. The nutritional value of four Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat. Aquaculture 165:281-293.

Coutteau P. and P. Sorgeloos. 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. J. Shellfish Res. 11(2):467-476.

Heasman M., J. Diemar, W. O'Connor, T. Sushames, and L. Foulkes. 2000. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs – a summary. Aquacult. Res. 31:637-659.

Knuckey R. 1998. Australian microalgae and microalgal concentrates for use as aquaculture feeds. Unpublished Ph.D. thesis, University of Tasmania. 254 p.

Lewis T., P. Nichols, and T. McMeekin. 1999. The biotechnological potential of thraustochytrids. Mar. Biotechnol. 1:580-587.

Robert R. and M. Brown. The food value of microalgae concentrates for larvae and postlarvae of the Pacific oyster *Crassostrea gigas*. Larvi '01– this issue.

Robert R. and P. Trintignac. 1997. Substitutes for live microalgae in mariculture: a review. Aquat. Living Resour. 10:315-327.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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NUTRIENTS AFFECTING QUALITY IN MARINE FISH LARVAL DEVELOPMENT

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Abstract

Marine fish larvae undergo major functional and morphological changes during their developmental stages. For example, the digestive tract develops and matures during the first week of life, and larvae acquire their juvenile morphology and pigmentation after metamorphosis. Several factors can interfere with the normal development of larvae and affect larval quality in the hatchery. Malformations are mainly noted for jaw, gill and spinal cord (scoliosis, lordosis, and coiled vertebral column). Pigmentation defects can be also considered malformations.

It has been demonstrated that genetic or environmental parameters impair skeletal formation. The influence of diet on larval formation has been investigated in freshwater species such as carp, Ayu fish, and, more recently, in marine species. Some data on the effect of some lipid-soluble nutrients have been obtained by using live prey as a diet. In particular, the effect of polyunsaturated fatty acids on pigmentation has been extensively studied in flatfish, such as Atlantic halibut and Japanese flounder. Nevertheless, though authors generally believe that n-6 (arachidonic and docosapentaenoic acids) and n-3 (eicosapentaenoic and docosahexaenoic acids) highly unsaturated fatty acid, as well their ratios, are important for pigmentation, no accurate data on optimal supply have been determined until now.

In recent years, experimental compound diets have been formulated and allow more accurate studies on the effect of different nutrients on development. Diets inducing harmonious growth in juveniles cannot be directly used for larvae, since they result in impaired larval development: poor growth, high mortality, and malformation rate. These effects can be due to nutrients whose concentration and/or nature would be inadequate for early developmental stages. Lipidic, lipid-soluble components, as well as peptidic components, have been shown to affect larval quality.

Studies conducted on sea bass reported that high dietary phospholipid (PL) levels induce a beneficial effect in marine fish larvae and juveniles. The increase in PL concentration, from 3 to 12% in a diet specially formulated for larvae, led to a drastic fall in the larval malformation rate, from 35% to 2%. It has been shown in freshwater species that among phospholipids, phosphatidylinositol is crucial in preventing skeletal deformities. The assay of brush border membrane enzymes of enterocytes revealed that PL also play an important role in enterocyte maturation. Retinoic acid (active form of vitamin A) is a nutrient whose role in development has been clearly established in vertebrates, including zebrafish and Japanese flounder. Too low or too high a level in retinoic acid perturbs the expression of homeobox genes, which control vertebral axis formation. We recently investigated the functions of retinoic acid receptors (RAR/RXR) in cranio-facial development during the post-embryonic stage of larval flounder by using different RAR/RXR agonists. It was demonstrated that the use of retinoid agonists resulted in lower induce malformations of lower jaw; each retinoic acid receptor has a different role in lower jaw development during the post-embryonic stage.

Concerning peptidic components, it has been shown that protein hydrolysate also has a beneficial effect on larval development, when it does not improve or, in some cases, depresses juvenile growth. The dietary incorporation of commercial hydrolysate, such as CPSP (Concentré Protéique Soluble de Poisson, oligopeptides with 20 amino acid chain length) or experimental hydrolysate, such as short peptides (di- and tripeptides), led to a strong decline in malformed larvae. The replacement of 50% dietary proteins by hydrolysate induced a malformation rate four times lower than a diet incorporating native protein. The beneficial effect of hydrolysate has been related to the specific capacity of young stages to digest and absorb short peptides. These dietary peptides improve enterocyte differentiation in developing animals. We can assume that this positive effect on enterocyte differentiation also concerns other cells involved in organogenesis, in particular, those involved in antero-posterior axis formation.

Another important observation on improved larval quality concerns dietary supplementation of taurine. Japanese flounder, red sea bream, yellowtail, and puffer fish fed more than 1% taurine supplemented diet (containing 1.5-2% taurine in diet) showed high growth rate and feed efficiency; and in particular, the behavioural characteristics of Japanese flounder resembled wild fish.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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LARVAL DEVELOPMENT OF THE MEDITERRANEAN CLEANER SHRIMP *LYSMATA SETICAUDATA* (RISSO, 1816) (CARIDEA: HIPPOLYTIDAE) FED ON DIFFERENT DIETS – COSTS AND BENEFITS OF MARK-TIME MOLTING

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Introduction

Marine ornamental shrimps of the *Lysmata* spp. are among the most popular species in the aquarium trade industry due to their striking colours and delicacy. Most of these shrimp are collected in the wild, and the ecological impacts of this capture have not been properly estimated. Although biological and zootechnical studies on the rearing of these species have been conducted (Zhang et al., 1998), its culture is not able to fulfill the increasing demand and minimize the ecological impacts.

To satisfy the commercial demand of ornamental shrimps, it is important to evaluate the rearing potential of some temperate Eastern Atlantic and Mediterranean shrimps. The Mediterranean cleaner shrimp (*Lysmata seticaudata*) is one of the species that fulfills most of the requirements of ornamental species. Besides its beautiful coloration and associative cleaning behaviour with several fish species, it presents a certain tolerance to the higher temperatures typical of tropical aquaria. Nevertheless, there is always an aspect that must be carefully analyzed in the culture of any marine species: larval rearing. One of the problems associated with certain caridean shrimp larvae is mark-time molting. This type of molting can be briefly described as "a sequence of molts in which very little change in morphology takes place" (Gore, 1985). Although this problem is commonly explained by unsuitable rearing conditions, more and more evidence seems to indicate that this type of ecdysis also exists under natural conditions. Nonetheless, this type of molting can be a serious bottleneck to commercial larval culture.

The objective of the present work was to study the effect of different live diets (decapsulated *Artemia* cysts and metanauplii enriched with Algamac 2000®) on larval stage duration and larval survival. Larval stage duration of larvae from

different females, but fed on the same diet (metanauplii enriched with Algamac 2000®), is also analyzed.

Materials and methods

Ovigerous females caught with hand nets at a depth of 0.5-2m were brought to the laboratory and placed in the dark until hatching (35±1‰ salinity, 12±1ºC, and with moderate aeration). Larvae from three females were isolated from each other in three different batches (batch A, B, and C). Thirty randomly selected larvae from batch A and B were reared individually in small plastic containers (20ml each) and were fed on metanauplii enriched with Algamac 2000®(Aquafauna - Biomarine Inc). Salinity was maintained at 35±1‰, the photoperiod was 8h L:16h D, and temperature was kept at 17±2ºC. Sixty randomly selected larvae from batch C were reared individually in the same conditions as previously described, except for 30 of those larvae which were fed on decapsulated *Artemia* cysts until the fifth zoeal stage was reached, and subsequently fed on enriched metanauplii. The rearing containers were checked daily for waste and dead larvae, which were always removed in order to prevent bacterial proliferation. The water from each container was renewed daily.

Artemia franciscana (Kellogg, 1906) cysts from Aqua-Elite® strain (lot 84110) were hatched and decapsulated under standard conditions (Sorgeloos et al., 1986). The enrichment was conducted during 16h in 1-l beakers, with strong aeration and a maximum density of 50 nauplii.ml^{-1} (approx. 0.1g of product in 1 l). Food was supplied daily, at a density of 5000 prey.l^{-1}.

Analysis of variance (ANOVA) was used and differences between treatments means were determined using the Tukey-Kramer's test. All results were considered statistically significant at the $P<0.05$ probability level.

Results and discussion

The larvae fed initially on decapsulated cysts (batch C) presented the highest survival rate (26.7%), although the differences were not statistically significant from the survival rates of larvae fed on enriched metanauplii reared individually (batch A and B; 21.3% and 22.1%, respectively). All larvae from batch C fed on enriched metanauplii from the start died in the ninth zoeal stage, before metamorphosing, probably due to some unknown bacteriological contamination.

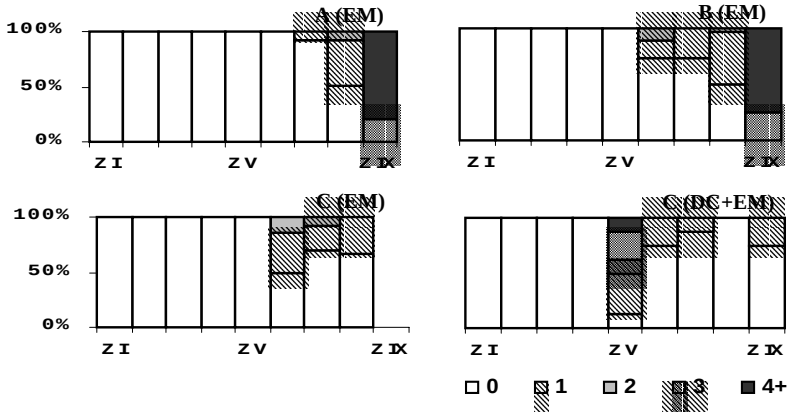
In general, the larvae from batch C fed initially on decapsulated cysts presented a significantly longer larval stage duration in the first five zoeal stages, than the larvae from the same batch fed on enriched metanauplii (Table I). These differences were particularly clear in the fifth larval stage ($P<0.0001$), when the

larvae suffered a series of mark-time molts and were not able to advance to the next larval stage. Nevertheless, the use of enriched metanauplii from zoea V onwards enabled the larvae to reach the post-larval stage. These results probably suggest that decapsulated cysts are not a suitable diet for advanced larval stages, and if an appropriate diet is not supplied, the larvae will undergo a series of mark-time molts and eventually die. The larvae from the three different batches fed on enriched metanauplii presented a slightly different larval stage duration in the first six zoeal stages. However, those differences disappeared in the last larval stages, which presented similar durations (Table I). This suggests that some broodstock variability may exist in earlier larval stages. However, a suitable diet can minimize these differences, ensuring that most larvae are in the same larval stage, and thus allowing the mixing of different larval batches for communal rearing.

Table I. Average larval stage duration (days ± standard deviation) of larvae from different batches (A, B, and C), fed on enriched metanauplii (EM) and decapsulated cysts and enriched metanauplii (DC+EM).

	Batch A (EM)	Batch B (EM)	Batch C (EM)	Batch C (DC+EM)
Zoea I	2 ± 0.1	3 ± 0.3	4 ± 0.5	4 ± 1.4
Zoea II	3 ± 0.4	4 ± 0.5	3 ± 0.5	6 ± 1.4
Zoea III	3 ± 0.6	3 ± 0.8	4 ± 0.4	4 ± 0.7
Zoea IV	2 ± 0.6	4 ± 1.0	4 ± 0.4	6 ± 1.6
Zoea V	3 ± 0.2	3 ± 0.7	4 ± 1.7	22 ± 11
Zoea VI	3 ± 0.5	5 ± 2.7	8 ± 5.4	7 ± 4.3
Zoea VII	5 ± 1.4	6 ± 2.3	6 ± 2.3	5 ± 2.3
Zoea VIII	8 ± 1.4	5 ± 4.1	6 ± 2.4	5 ± 2.3
Zoea IX	48 ± 15.9	45 ± 18.5	–	8 ± 5.2

Though all batches displayed mark-time molting, the larvae fed initially on decapsulated cysts (batch C) were the only ones exhibiting this pattern in the fifth zoeal stage. As it as been already described, the replacement of the diet by enriched metanauplii in the zoea V stage allowed the larvae to continue their larval development and reduced the number of mark-time molts in the next larval stages. The occurrence of mark-time molting is even more significant when the number of terminally additive molts (mark-time molts occurring in the last larval stage) is analyzed (Fig. 1). Although decapsulated cysts are not a suitable diet for late larval stages, probably due to lack of predatory stimulation, its high nutritional profile appears to be appropriate for the first zoeal stages of *Lysmata* larvae. In fact, this may be the main reason why larvae initially fed on this diet showed a reduced number of terminally additive molts. This is of



particular interest since it is generally accepted that a high number of terminally additive molts generally leads to the death of the reared larvae.

Fig. 1. Number of mark-time molts in each zoeal stage of larvae from different batches (A, B and C), fed on enriched metanauplii (EM) and decapsulated cysts and enriched metanauplii (DC+EM).

Conclusions

Mark-time molting is certainly an excellent adaptation for larval development in the natural environment, when food may not always be available. But when the main goal is to mass-rear larvae, this “natural” behaviour may become a serious constraint. Therefore, in future studies, different diets should be tested in order to minimize this type of molting and optimize juvenile production.

Acknowledgements

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References

Gore R. 1985. Molting and growth in decapod larvae. p. 1-65. In: Crustacean issues - Larval growth. AA Balkema Publishers edition. Vol. 2. Rotherdam, Natherlands: Wenner, A. 236p.

Sorgeloos P., Ph. Lavens, P. Léger, W. Tackaert, and D. Versichele. 1986. Production of *Artemia* nauplii and decapsulated cysts for use as food source in the hatchery production of fish and crustaceans. p. 69-117. In: FAO Manual for the Culture of Brine Shrimp *Artemia* in Aquaculture. University of Ghent, Ghent, Belgium. 319p.

Zhang D., Lin J., and Creswell R. 1998. Ingestion rate and feeding behaviour of the peppermint shrimp *Lysmata wurdemanni* on *Artemia* nauplii. J. World Aquacult. Soc. 29(1):97-103.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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ARACHIDONIC ACID: A BIOLOGICAL INDICATOR OF PHYSIOLOGICAL STRESS IN THE BRINE SHRIMP *ARTEMIA*?

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Introduction

The genus *Artemia* (Crustacea; Anostraca) is a complex of sibling species and superspecies defined by the criterion of reproductive isolation. Two bisexual species are represented in the New World: *Artemia persimilis* and *Artemia franciscana*. *Artemia franciscana* is found in the State of Rio Grande do Norte (RN) in Northeastern Brazil as a result of inoculations made in a very large saltwork in Macau (RN) in April 1977 with cysts from a San Francisco Bay (California, U. S. A.) stock (Camara and Castro, 1983).

Literature data on the composition of polyunsaturated fatty acids (PUFA) of different strains of *Artemia franciscana* show a high variability. Nonetheless, most strains contain linoleic acid (LA, 18:2n-6) and some arachidonic acid (AA, 20:4n-6) as principal n-6 PUFA. Furthermore, when linolenic acid (LNA, 18:3n-3) is present in high amounts, eicosapentaenoic acid (EPA, 20:5n-3) is generally absent or present in very low amounts (Léger et al., 1986; Navarro et al., 1993).

In this paper, we discuss the biometrical characteristics and the atypical composition of fatty acid methylesters (FAME) of *Artemia franciscana* cysts collected in two saltworks in the State of RN, Northeastern Brazil.

Materials and methods

Cysts were harvested from two saltworks in the State of RN, NE-Brazil: Salina Diamante Branco (SDB) in Galinhos (05º05'S; 36º16'W) and Salina Coqueiros (COQ) in Grossos (04º58'S; 37º09'W). Cysts from SDB were collected in September 1998 following a massive reinoculation program carried out with *Artemia franciscana* from a San Francisco Bay (California, USA) stock. Cysts from COQ were harvested in November 1998. Cyst samples from both saltworks were processed, hydrated, and measured according to Sorgeloos et al. (1986). The composition of fatty acid methylesters (FAME) was determined at the

Artemia Reference Center (University of Ghent, Belgium) following the ICES (International Council for Exploration of the Sea) standard procedure (Coutteau and Sorgeloos, 1995).

Results and discussion

The results of mean cyst diameter (μm) were 252.30±10.21 and 242.36±9.36 in the SDB and COQ samples, respectively. The size of the cysts from both samples is comparable to San Francisco Bay *Artemia franciscana* (Sorgeloos et al., 1986). In fact, recent electrophoretic evidence (similarity of samples of Macau, NE-Brazil, and San Francisco Bay, USA) confirmed the proposed origin, from San Francisco Bay cysts, of the RN population (Gajardo et al., 1995).

Table I. Principal fatty acid composition (% of total fatty acids and $mg.g^{-1}$ dry weight) of *Artemia franciscana* cysts collected in Galinhos (SDB) and Grossos (COQ) saltworks, NE-Brazil.

Fatty acid	Galinhos (SDB)		Grossos (COQ)	
	(%)	($mg.g^{-1}$ DW)	(%)	($mg.g^{-1}$ DW)
14:00	1.4	1.7	1.9	2.8
16:0	13.4	17.0	13.9	20.8
16:1n-7	11.0	13.9	12.2	18.3
17:0	1.8	2.3	1.9	2.8
18:0	3.9	4.9	3.8	5.8
18:1n-9	20.5	25.9	18.6	27.9
18:1n-7	13.0	16.4	11.5	17.2
18:2n-6	6.6	8.3	7.1	10.7
18:3n-3	4.2	5.3	2.3	3.4
20:4n-6	4.1	5.2	6.1	9.2
20:5n-3	6.2	7.8	6.9	10.3
22:6n-3	0.1	0.2	0.1	0.2
n-6 PUFA	11.3	14.3	14.2	21.2
n-3 PUFA	11.2	14.2	9.9	14.9
n-3 HUFA[1]	6.6	8.3	7.2	10.7

[1]Sum of n-3 ≥ 20:3n-3.

The FAME results are presented in Table I. In general, the FAME profiles from SDB and COQ conform to values seen in other strains and/or batches of marine-type *Artemia franciscana* (Léger et al., 1986; Navarro et al., 1993). However, SDB and COQ cyst samples had substantial amounts of both linolenic acid (LNA, 18:3n-3) and eicosapentaenoic acid (EPA, 20:5n-3). Furthermore, linoleic acid (LA, 18:2n-6) was present in relatively higher amounts in both SDB ($8.3 mg.g^{-1}$ DW) and COQ ($10.7 mg.g^{-1}$ DW) cyst samples than anticipated. Accordingly, the levels of

arachidonic acid (AA, 20:4n-6) in the SDB (5.2mg.g^{-1} DW) and COQ (9.2mg.g^{-1} DW) cyst samples are probably the highest reported in literature for this fatty acid.

Polyunsaturated fatty acids such EPA and AA are required by all animals for providing metabolic energy, the synthesis of cell membranes, and as a source of precursors for eicosanoids. AA is the major precursor of eicosanoids in aquatic animals, with eicosanoids formed from EPA being less biologically active than those formed from AA (Sargent et al., 1999). The eicosanoids are a range of highly active C20 compounds formed in small amounts by virtually every tissue in the body and involved in a great variety of physiological functions. In broad terms, the eicosanoids are produced in response to stressful situations, both at a cellular and whole body level (Sargent et al., 1999). Elevated levels of AA have been linked to the stressful migration from freshwater to seawater following smoltification in salmon, and to experimentally (dietary) induced stress in turbot larvae (Sargent et al., 1999).

The brine shrimp *Artemia* may reproduce by oviparity (producing cysts) or by ovoviviparity (producing live offspring). Although the factors influencing cyst formation in *Artemia* are not fully understood, oviparity generally prevails under unfavorable environmental conditions (Sorgeloos et al., 1986). In the present study, *Artemia franciscana* populations from SDB and COQ were most likely under environmental stress. Although SDB is a large (>2500ha) salt operation comprised of thirteen large evaporation ponds (average depth of 1.0m), cysts from SDB were collected after a massive reinoculation program (with *Artemia franciscana* from a San Francisco Bay stock) that led to a higher incidence of oviparity. On the other hand, COQ is a small (<5ha) family operation consisting of shallow (average depth of 0.4m) ponds of less than 0.5ha in size that are highly susceptible to environmental changes. Thus, aside from dietary factors, the extremely high levels of AA found in the present study might be a response to the ongoing adaptation of the reinoculated strain of *Artemia franciscana* in SDB and to the fluctuating environmental conditions typically found in COQ.

References

Camara M.R. and E.V. Castro. 1983. *Artemia salina* L. (Anostraca): uma opção para a aqüicultura do nordeste do Brasil. Rev. Bras. Zool. 1:145-147.

Coutteau P. and P. Sorgeloos. 1995. Intercalibration exercise on the qualitative and quantitative analysis of fatty acids in *Artemia* and marine samples used in mariculture. ICES Coop. Res. Rep. 211:30.

Gajardo G., M. da Conceição, L. Weber, and J.A. Beardmore. 1995. Genetic variability and interpopulational differences in *Artemia* strains from South America. Hydrobiologia 302:21-29.

Léger Ph., D.A. Bengtson, K.L. Simpson, and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Ann. Rev. 24:521-623.

Navarro J.C., F. Amat, and J.R. Sargent. 1993. The lipids of the cysts of freshwater- and marine-type *Artemia*. Aquaculture 109:327-336.

Sargent J., G. Bell, L. McEvoy, D. Tocher, and A. Estevez. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177:191-199.

Sorgeloos P., P. Lavens, Ph. Léger, W. Tackaert, and D. Versichele. 1986. Manual for the culture and use of Brine Shrimp *Artemia* in Aquaculture. State University of Ghent, Belgium. 319p.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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DIFFERENT NUTRITION MIXES OF MICROALGAE AS FOOD SHRIMP LARVAE OF *LITOPENAEUS STYLIROSTRIS*

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Introduction

High-quality microalgae in shrimp larviculture constitutes a primary requirement for good nutrition in the initial stages of development. It has been recognized that lipids, as fatty acids, are essential for the larvae of marine organisms, since they provide both metabolic energy and the building blocks for cell membrane synthesis (Kontara et al., 1997). However, the quality of essential nutrient components of microalgae varies according to culturing conditions and species composition. To evaluate those differences, we analyzed the gross chemical and fatty acids compositions in the microalgae administered as mixes in the culture of larvae of *Litopenaeus stylirostris.*

Materials and methods

This study was conducted in Bahia de Kino, Sonora, Mexico, in a commercial laboratory. Nauplii VI of blue shrimp were placed in 300-l cylindrical tanks (200 nauplii.l^{-1}) and fed the larvae of the microalgae mixes for twelve days. Larval conditions were controlled for temperature, pH, salinity, and oxygen at 29^{o}C, 7.8, 35‰, and 5.8mg.l^{-1}, respectively. Larvae were sampled at random for growth promotion analysis.

The microalgae in the mixes were species currently used in commercial systems. Additionally, we tested *Nannochloropsis oculata* because of its demonstrated high fatty acid content. The mixes used in our experiments were: (A) *Chaetoceros muelleri, N. oculata,* and *Dunaliella* sp. with supplementary food; (B) *Ch. muelleri, Isochrysis* sp., and *Dunaliella* sp., without supplementary food; and (C) *Ch. muelleri, Isochrysis* sp., and *Dunaliella* sp., with supplementary food. Protocol feeding regimen followed that of the commercial laboratory.

The microalgae were cultured in containers at volumes of 3000-8000 l, except for *N. oculata* that was cultured in 300-1 tanks. Culture conditions were as follows: indoor culture 25°C with continuous illumination; Solarium 20-32°C; and outdoor 19-35°C. Prior to daily feeding of larvae, the microalgae were sampled in volumes of 15-250ml, depending on the analyzed nutrient. Samples were vacuum-filtered on glass microfibre filters, which were stored at -60°C for chemical analysis. Gross chemical analysis was done using micro-methods; fatty acids were analyzed according to AOCS-Ce 2-66 (AOCS, 1993).

Results and discussion

Nutrient compositions of the different mixes are shown in Fig. 1. These values did not show significant differences for the three mixes, Kruskal-Wallis test, $P>0.05$.

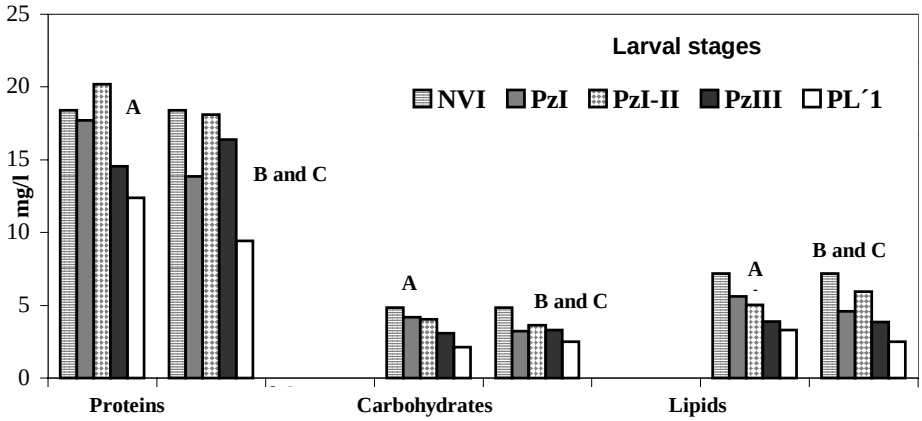

Fig. 1. Gross chemical composition of the microalgae mixes used to feed larval shrimp.

N. oculata has not been used as shrimp larvae feed in production systems. However, Castell et al. (1989) reported that if the species is fed to rotifers, which in turn are fed to fish larvae, results are excellent because of the high content of HUFA in *N. oculata*. This species could be an alternative for feeding shrimp larvae considering its culture conditions, similar to those of the other species.

In our study *N. oculata* showed a high concentration of 20:5(n-3), with a mean value of 4.4mg per 100mg of sample in the Solarium and a mean value of 1.1mg per 100mg indoors. For *Ch. Muelleri*, 20:5(n-3) was 7.17mg per 100mg indoors and 0.7mg per 100mg in the Solarium. For *Isochrysi*s sp., values were 0.14 and 8.6mg per 100mg indoors and outdoors, respectively. Tamaru et al. (1993)

reported values lower than 1mg per 100mg in *N. oculata*, similar to our findings for indoors (Table I).

We have shown that there is variability in the fatty acid profile, depending on chemical and physical factors, as well as harvesting time and cultured species. All of these factors have an influence on fatty acid concentration (Renaud et al., 1994), and should be considered for feeding larvae of cultured organisms.

Table I. Fatty acid composition of the microalgae fed to larvae of *Litopenaeus stylirostris* (mg per 100mg of lipids).

Fatty Acids	Culture conditions								
	Solarium			Indoor			Outdoor		
	CH*	Nan*	Tiso*	Tiso*	CH*	Nan*	CH*	Tiso*	Dun*
Total	47.61	80.69	79.86	65.32	73.95	32.26	51.18	54.70	55.10
Sat.	60.14	91.09		66.61		46.87			
Total	14.48	0.61	7.36	25.98	7.13	39.05	23.95	3.52	32.55
Monounsat.	44.08	11.86		28.20		63.32			
Polyunsaturated									
18:2(n-6)	0.83	0.14	1.17	0.12	0.11	0.08	0.48	0.53	5.13
	1.63	0.31		0.64		11.32			
18:3(n-3)	Nd	nd	1.20	nd	nd	nd	0.71	nd	nd
Total:	0.83	0.14	2.36	0.12	0.11	0.08	1.19	0.53	5.13
	1.63	0.31		0.64		11.32			
Highly Unsaturated									
18:4(n-3)	0.78	0.10	0.30	1.35	0.30	0.05	nd	nd	nd
	1.06			1.85					
20:4(n-6)	0.28	0.29	3.28	0.53	1.49	0.17	5.29	1.10	3.97
	2.46	2.15		1.39		0.32			
20:4(n-3)	0.53	0.13	1.94	0.08	2.09	0.89	2.16	3.25	1.21
	3.50			1.69					
20:5(n-3)	0.54	4.31	0.51	0.14	7.17	1.14	6.18	8.60	0.65
	0.87	4.48		0.15		1.16			
21:5(n-3)	Nd	nd	nd	nd	1.29	nd	2.92	nd	nd
22:5(n-6)	0.58	0.30	0.87	nd	0.24	0.05	4.03	19.10	nd
	1.52					0.23			
22:6(n-3)	0.23	0.13	1.89	0.50	0.20	0.06	1.73	7.37	0.43
	1.57	1.86		1.14					
Total	3.25	6.70	8.79	4.23	12.78	1.68	22.30	39.42	6.26
	15.83	7.17		4.63		2.41			
Not identified	3.40	0.12	1.61	2.10	6.02	0.20	0.33	1.19	0.80
	7.41	1.26		2.10		2.64			

* CH = *Chaetoceros muelleri*; Nano = *Nannochloropsis oculata*; Tiso = *Isochrysis* sp.; Dun = *Dunaliella sp*.; Two analysis determinations

nd = not detected.

In growth promotion analysis, feeding of mix A to Mysis I resulted in an average length of 3.61mm; feeding mix B, 3.36mm; and feeding mix C, 3.58mm. Covariance analysis showed no significant differences in growth promotion between the mixes (P>0.05). Size was greater in our study than findings by Kitani (1986). Covariance analysis showed no significant difference for the growth equation slopes (P>0.05).

Nauplii VI to MI survival was 90.2%, 81.2%, and 76.5% for mixes A, B, and C, respectively. There were no significant differences in larval survival for the three mixes (P>0.05). Survival is comparable to that in mass production systems.

Conclusions

A microalgal mix fed to larvae must meet the quality they need for growth. Mix A used in our study is an alternative for feeding *Litopenaeus stylirostris* at different stages of development. Eliminating additional feed in this experiment did not affect growth and survival of the larvae during the time of culture.

References

AOCS. 1993. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. AOCS Inc. USA.

Castell J.D., J.C. Kean, L.R. D´Abramo, and D.E. Conklin. 1989. A standard reference diet for crustacean nutrition research. I. Evaluation of two formulations. J. World Aquacult. Soc. 20(3):93-99.

Kitani H. 1986. Larval Development of the blue shrimp *L. stylirostris* Stimpson reared in the Laboratory. Bull. Jap. Soc. Sci. Fish. 52(7):1121-1130.

Kontara E.K.M., P. Coutteau, and P. Sorgeloos. 1997. Effect of dietary phospholipids and requirements for and in corporation of n-3 highly unsaturated fatty acids in postlarval *L. stylirostris* Bate. Aquaculture 158:305-320.

Renaud S. M., L.V. Thinh, and D.L. Parry.1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture 170:147-159.

Tamaru C.S., R. Murashige, C.S. Lee, H. Ako, and V. Sato. 1993. Rotifers fed various diets of backer´s yeast and/or *N. oculata* and their effect on the growth and survival of striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos*) larvae. Aquaculture 110:361-372.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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IMPORTANCE OF SOLUBILITY AND HYDROLYSIS ON UTILIZATION OF DIETARY PROTEIN BY CARP (*CYPRINUS CARPIO*) LARVAE

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Introduction

Previous investigations of dietary protein utilization in carp larvae showed that casein hydrolysate resulted in lower larval survival and growth than casein in diets which contained also fish meal hydrolysate as a protein source (Carvalho et al., 1997). In contrast, in other experiments with cyprinid larvae, the partial replacement of the native casein by either casein hydrolysate (Szlaminska et al., 1993) or sodium caseinate (non-hydrolyzed soluble casein) was found to improve larval performance (Radünz-Neto et al., 1993). Since both casein hydrolysate and sodium caseinate are soluble (contrary to native casein) the relative importance of solubility and hydrolysis on the observed effects remained unclear. The present work aimed at evaluating the relative importance of solubility and hydrolysis of dietary casein on the utilization of this protein by first-feeding carp larvae.

Materials and methods

Carp (*Cyprinus carpio*) larvae were reared from the start of exogenous feeding in similar conditions as described by Carvalho et al. (1997). Insoluble casein (native casein) and two forms of soluble casein (S) – non-hydrolyzed (sodium caseinate) and hydrolyzed (casein hydrolysate, H) – were used as dietary protein sources. Diets (isonitrogenous and isoenergetic) differed with respect to the amount of total soluble (hydrolyzed and non-hydrolyzed) protein and to the relative proportion of the two forms of soluble protein. Diet S0 included only the native casein as the protein source. In the other diets, 25, 50, 75, and 100% of the native casein was replaced by soluble casein. In diets with 25% soluble casein, the proportions of casein hydrolysate relatively to total soluble protein were 0, 25, 50, and 100% (in diets S25, S25H25, S25H50, and S25H100, respectively). In diets with 50 and 75% soluble casein, these proportions were 0, 50, and 100% (in diets S50 and S75, S50H50 and S75H50, S50H100 and

S75H100, respectively). The diet with 100% soluble casein contained only sodium caseinate without casein hydrolysate (diet S100). All diets were tested in duplicate and a duplicate group was kept starved as a negative control.

Survival was checked daily. Samples of 10 individuals were taken every week from each tank, anaesthetized, and measured. At the end of the experiment, larvae were starved for 24h and then final weight was taken of the whole tank population.

Results and discussion

Total mortality of the unfed group occurred at day 8 confirming the absence of dissolved organic matter in circulation, which might be used as food by larvae. From the first week onwards survival stabilized in groups fed diets S25, S25H25, S25H50, S50, S75, and S100. In groups fed diets with high levels of hydrolysates survival decreased throughout the experimental period. No larvae survived by the end of the second week in the group fed with the highest level of hydrolysate (diet S75H100). The best final survival was obtained with diet S25H25, but not significantly higher than that registered with diets S25, S25H50, S50, S75, and S100. Growth differences were already observed by day 7. At the end of the trial, larvae fed diet S25H25 showed the best growth performance, although not statistically different from that of larvae fed diet S25. Those larvae fed diets with the highest levels of hydrolysates (S50H100 and S75H50) presented a very significant reduced growth compared to all the others.

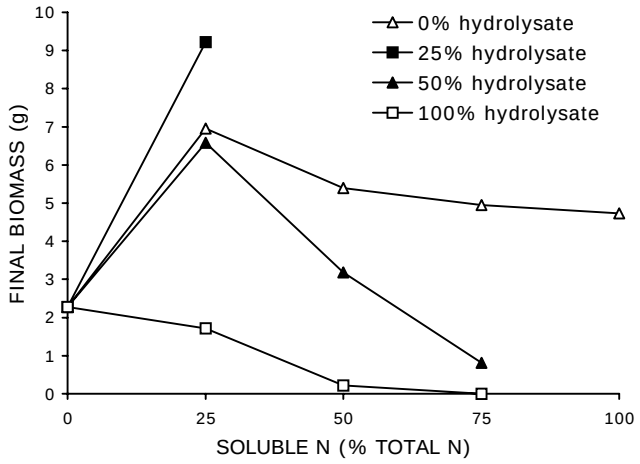

Fig. 1. Effect of the dietary level of soluble nitrogen (N) and content of hydrolysate on the theoretical final biomass (weight × survival).

Results evidenced the importance of the form of dietary protein for fish larvae (Fig. 1). The incorporation of sodium caseinate in the diets, particularly at a

level corresponding to a substitution of 25% of the native casein, effectively improved larval performance. It seems probable that the addition of soluble casein would facilitate the degradation of diets by larval enzymes. Accordingly, Szlaminska et al. (1993) suggested that, despite the digestive potential of larvae, the hard texture of native casein would limit its utilization by preventing the penetration of proteolytic enzymes. On the other hand, the addition of casein hydrolysate was clearly beneficial only if soluble nitrogen did not exceeded 25% of total nitrogen and when the hydrolysate did not contribute to the entire solubility (Fig. 1). In fact, whatever the dietary percentage of soluble nitrogen, if it was entirely provided by the hydrolysate (case of diets S25H100, S50H100, and S75H100), the results were always inferior to those obtained with the diet containing the native casein as the only nitrogen source.

As conclusion, it was confirmed the poor feeding utilization of native casein by carp larvae, contrarily to juveniles that seem to use this protein more efficiently (Sen et al., 1978). A balanced incorporation of alternative soluble forms of casein in diets will enhance its utilization by larvae. Finally, particular attention should be paid to protein hydrolysates since excessive dietary amounts of this soluble form led to significant negative effects on larval performance, as previously noticed in carp and sea bass larvae (Carvalho et al., 1997; Zambonino Infante et al., 1997; Cahu et al., 1999).

References

Cahu C., J.L. Zambonino Infante, P. Quazuguel, and M.M. Le Gall. 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. Aquaculture 171:109-119.

Carvalho A.P., A.M.Escaffre, A. Oliva-Teles, and P. Bergot. 1997. First feeding of common carp larvae on diets with high levels of protein hydrolysates. Aquacult. Int. 5:361-367.

Radünz-Neto J., N. Charlon, A.M. Escaffre, G. Corraze, and P. Bergot. 1993. Effet de l'huile de foie de morue sur la survie et la croissance des larves de cyprinidés (*Carassius auratus* L., *Cyprinus carpio* L.). p. 541-550. In: Fish Nutrition in Practice (Ed. Kaushik, S.J. and Luquet, P.). INRA, Paris (Les colloques no. 61). 972 p.

Sen P.R., N.G.S. Rao, S.R. Ghosh, and M. Rout. 1978. Observations on the protein and carbohydrate requirements of carps. Aquaculture 13:245-255.

Szlaminska M., A.M. Escaffre, N. Charlon, and P. Bergot. 1993. Preliminary data on semisynthetic diets for goldfish (*Carassius auratus* L.) larvae. p.607-612. In: Fish Nutrition in Practice. S.J. Kaushik and P. Luquet (Eds.). INRA, Paris (Les Colloques no. 61). 972p.

Zambonino Infante J.L., C.L. Cahu, and A. Péres. 1997. Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. J. Nutr. 127:608-614.

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THE EFFECT OF DIFFERENT HUFA ENRICHMENT EMULSIONS ON THE NUTRITIONAL VALUE OF ROTIFERS (*BRACHIONUS PLICATILIS*) TO LARVAL HADDOCK (*MELANOGRAMMUS AEGLEFINUS*)

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Introduction

Haddock (*Melanogrammus aeglefinus*) has been identified as a candidate for commercial culture in Atlantic Canada (Henry, 1997; Litvak, 1998). The principle obstacle to haddock culture is poor survival (average ~2%, range 0-33%) through the first-feeding larval stage, which requires live food organisms, to weaning and metamorphosis. The essential fatty acid (EFA) requirements of first-feeding haddock larvae have not been identified, however, two species of cod (gadids similar to haddock) require live food organisms enriched with docosahexaenoic acid (DHA) (*Gadus macrocephalus*, Takeuchi et al., 1994; *G. morhua*, Galloway et al., 1998). Rotifers (*Brachionus plicatilis*) have been found deficient in n-3 HUFA (particularly 20:5n-3 [EPA] and 22:6n-3 [DHA]) when used as the live food for cool-water marine fish larvae (Rodríguez et al., 1996; Rainuzzo et al., 1997). Recent research has shown that the n-6 HUFA, arachidonic acid (20:4n-6, AA) is also important (Koven et al., 2001). This study was designed to determine the effect of different rotifer enrichments during the first 15d of larval haddock feeding on growth, survival, and fatty acid composition.

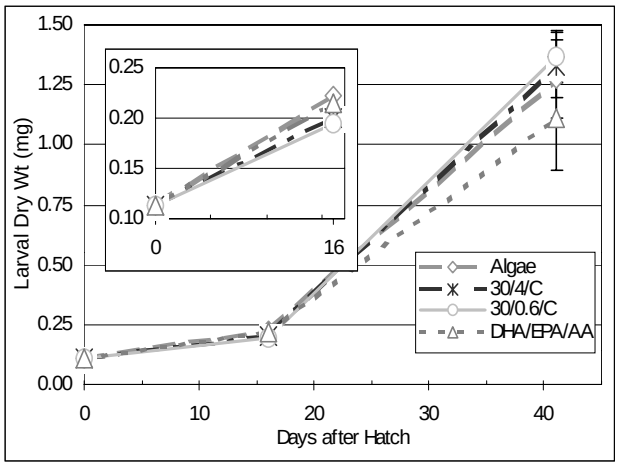
Materials and methods

Mass production of rotifers (Aquafarms FL, USA) was carried out by batch culture in 1.4-m^3 fiberglass tanks. Rotifers were maintained on a mixed algal diet of *Isochrysis galbana*, *Tetraselmis suecia*, *Pavlova lutheria*, and *Nannochloropsis* sp. in 1m^3 seawater (24°C) at 200-250 rotifers.ml^{-1} under constant light. Harvested rotifers were enriched in 25-l glass carboys using the following ICES emulsions (ICES, 1997): ICES 30/4/C (DHA); ICES 30/0.6/C (DHA:EPA); ICES

DHA/EPA/AA; and a mixed-algae control. Rotifers were acclimated to larval culture temperature over a period of ~16h prior to being fed to the larvae. A batch of haddock eggs from captive broodstock was incubated at 6°C in 250-l upwelling conical-bottom incubators. Hatched larvae were transferred to 20×50-l plastic tanks (5 tanks per treatment) and stocked at 20 larvae.l^{-1}. Larval tanks were supplied with 1-µm-filtered seawater (0.2 $l.min^{-1}$, 6-12°C, salinity 29ppt), 24h light (240lux), and 500ml algae 2× daily. The enriched rotifer treatments were fed to the larvae 3× daily (0900, 1500, and 2000) from 2dph (days post hatch) to 15dph, then all larvae were fed mixed-algae rotifers until weaning onto ICES Standard Reference Weaning Diet (Coutteau et al., 1995) at 25dph. Larval samples at 16 and 41dph were analyzed for dry matter and fatty acid composition. Survival (%) was determined at 41dph. Statistical analysis was performed using SYSTAT 10 (ANOVA and Tukeys HSD).

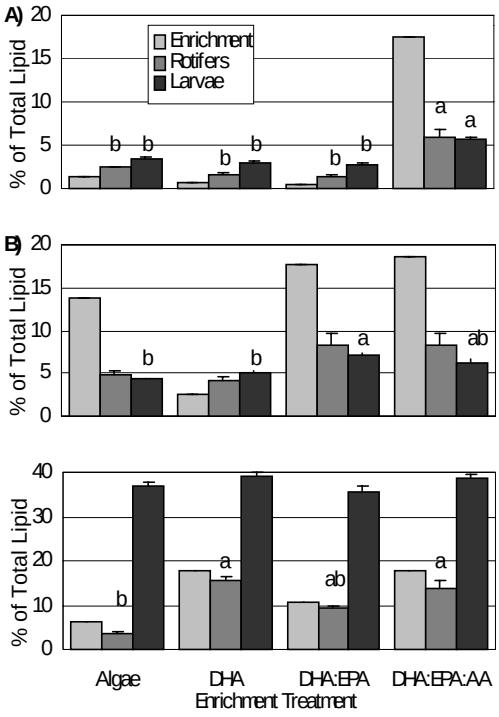
Results

At 16dph, larvae fed mixed-algae rotifers had the greatest dry weight (mean±SD; 0.22±0.08mg); followed by the DHA:EPA:AA treatment (0.21±0.07), and the DHA and DHA:EPA treatments (0.20±0.08), but there were no significant differences (P<0.05) in mean dry weights after 16 or 41d (Fig. 1). One tank fed mixed-algae rotifers and one fed DHA:EPA:AA rotifers had 100% mortality by 41dph. In the other tanks, survival ranged from 0.4-11.9%. Due to variations among replicates, there were no significant differences among treatments. The survival rates (mean±SE) of larvae by treatment were 5.9±2.4% (DHA:EPA), 4.7±1.8% (mixed-algae), 3.1±1.1 (DHA), and 3.1±1.9% (DHA:EPA:AA).



The enrichments significantly effected the fatty acid composition of the rotifers and larvae. The AA content of the rotifers enriched with DHA:EPA:AA (5.9±2.1%) was significantly higher (P<0.005) than all other rotifers. The mixed-algae rotifers had 2.4±0.1% AA. Rotifers fed the other low AA enrichments had greater than 1% AA, which was likely retained from algae they were fed before

enrichment. All larvae had AA levels higher than the rotifers they were fed, except those DHA:EPA:AA enriched (Fig. 2A). The mean percent of EPA in the rotifers tended to reflect that of their enrichments, but due to variation within treatments, the differences among treatments were not significant (P<0.005) (Fig 2B). Larvae fed the mixed-algae and the DHA rotifers were significantly lower in EPA than the DHA:EPA treatment (P<0.005). Except for the DHA treatment, the percentages of EPA decreased from enrichment to rotifer to larvae, suggesting that in 3 treatments, the dietary level of EPA exceeded the requirement. The DHA content of the rotifers in all treatments was lower than the enrichment (Fig. 2C). The mixed-algae rotifers had significantly lower levels of DHA than those enriched with DHA:EPA:AA or DHA. The content of DHA in all larvae remained high after 15 days of feeding and there was no significant effect of treatment.



Discussion

DHA dominated the HUFA of the larvae and the n-3/n-6 ranged from 19.6 in newly hatched larvae to 3.7 and 3.9 in the mixed algae and DHA:EPA:AA treatments, respectively. Either there is a strong specific retention of dietary DHA, or the larvae highly conserve the maternal DHA provided in the egg yolk. While the content of AA (0.9±0.3%) and EPA (10.0±0.1%) in the larvae were almost the same as levels in haddock eggs (1.1±0.1 and 11.8±1.0, respectively), there was a significant increase in the percentage of DHA in newly hatched haddock larvae (37.4±1.3%) compared with the level in the haddock egg lipid (19.4±3.4%). EPA levels decreased in all treatment groups during the first 15 days feeding, while the DHA remained the same or increased slightly and the level of AA increased. Since the AA levels in the larvae were higher than in the rotifers (except for larvae fed the DHA:EPA:AA enriched rotifers), and the AA was higher than in newly hatched larvae, it is probable that this fatty acid is also important in early larval development and that a level between 3-5% of the total fatty acids will prove to be

optimal. Sargent et al. (1999) stated that both the amount and proportions of DHA, EPA, and AA are important in marine fish nutrition and suggested that the optimum ratios may vary with specific species but would be in the range of 10:5:1 for DHA:EPA:AA. If one supposed that an increase or decrease in the proportion of one of these HUFA in the larvae relative to the live food indicated a relative deficiency or excess of that particular HUFA, we might suggest that the optimal levels of these fatty acids in the diet would be 40:5:4 for larval haddock. However, since the larvae can specifically retain DHA and AA at higher concentrations than provided in the diet, the required levels for these fatty acids in the diet is obviously lower. In the case of our study, it appears that rotifers grown on a mixed-algae diet of *I. galbana, T. suecia, Nannochloropsis* sp., and *P. lutheria* were able to satisfy the EFA requirements of haddock larvae, since none of the enrichment emulsions provided any significant improvement in growth or survival.

References

Coutteau P., G. Van Stappen, and P. Sorgeloos. 1995. A standard experimental diet for the study of fatty acid requirements of weaning and first ongrowing stages of the European sea bass (*Dicentrarchus labrax* L.): selection of the basal diet. ICES Mar. Sci. Symp. 201:130-137.

Galloway T.F., H. Kryvi, K. Hoehne, and E. Kjørsvik. 1998. Muscle growth in cod larvae in response to dietary HUFA ratios. VIII International Symposium on Nutrition and Feeding of fish. Recent Advances in Finfish & Crustacean Nutrition. Las Palmas de Gran Canaria, Spain June 1-4, 1998. p.187.

Henry R. 1997. Overview of recent developments in haddock culture in New Brunswick. Aquacult. Assoc. Canada Spec. Publ. 2:37-40.

ICES. 1997. Report of the Working Group on Marine Fish Culture for the International Council for the Exploration of the Sea. Murcia, Spain 23-26 June 1997. ICES CM 1997/F:7

Koven W., Y. Barr, S. Lutzky, I. Ben-Atia, R. Weiss, M. Harel, P. Behrens, and A. Tandler. 2001. The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. Aquaculture 193:107-122.

Litvak M. 1998. The development of haddock culture in Atlantic Canada. Bull. Aquacult. Assoc. Canada 98:30-33.

Rainuzzo J.R., K.I. Reitan, and Y. Olsen. 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155:103-115.

Rodríguez C., J.A. Pérez, M.S. Izquierdo, J.R. Cejas, A. Bolanos, and A. Lorenzo. 1996. Improvement of the nutritional value of rotifers by varying the type and concentration of oil and the enrichment period. Aquaculture 147:93-105.

Sargent J., L. McEvoy, A. Estevez, G. Bell, M. Bell, J. Henderson, and D. Tocher. 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217-229.

Takeuchi T., F. Zheng, K. Yosheda, J. Hirokawa, and T. Watanabe. 1994. Nutritive value of DHA-enriched rotifer for larval cod. Nippon Suisan Gakkaishi 60:641-652.

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EVALUATION OF THREE DIETS WITH DIFFERENT PROTEIN PERCENTS FOR JUVENILE *CHERAX QUADRICARINATUS*

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Abstract

The objective of the present study was to determine the influence of three types of diets with different protein contents on the survival and growth of juvenile *Cherax quadricarinatus*. 6000 organisms (50%) were selected from a population of 12 000. They were place in three 2.5-m^3 canaletas, with 2000 organisms per canaleta. Food was given twice a day (7:00 a.m. and 4:00 p.m.), in a period of two months, at 3% of the biomass. In order to determine the weight gain in the animals, weekly samples were taken. It is recommended that the best protein level in the diet of *Cherax quadricarinatus* must be of at least 31%. Concerning weight gain and survival, it was observed that Diet I (25% protein) presented the worst of both, while in Diets II (31% protein) and III (43% protein), survival did not decrease.

Introduction

One of the biggest challenges facing aquaculture today is undoubtedly how to achieve higher yields in food production to satisfy the needs of the increasing global population. By the year 2025, the world population will have grown by approximately 2.5 billion. Assuming that the current consumption per capita remains the same, the total demand of fish products must then increase to 165 million tons. If, moreover, marine fishing continues to land 85 million tons per year, the production guaranteed by aquaculture will have to be duplicated in 25 years and reach 80 million tons.

In Cuba, the freshwater finfish culture industry deals with an important task: to compensate for the shortage brought about by the dwindling wild fishery (High Fleet) and to eliminate the need to import fish.

Considering that food is one of the most expensive factors in aquaculture, and that it is essential in meeting the nutritional requirements of cultured species (Jones, 1990; 1995), the objective of this study was to determine the effects of three diets with different protein contents on the survival and growth of juvenile *Cherax quadricarinatus*.

Materials and methods

The research was carried out at the juvenile facility at Guantánamo from January 5 to February 5, 2001. 6 000 (50%) were selected out of a total population of 12 000, and were placed in three 2.5-m^3 canaletas, with approximately 2 000 organism per canal.

Diets I and II were made to a raw protein level (25% and 31%, respectively) while Diet III (43% protein) was supplied by state company (Table I). This food was given twice a day (7:00 a.m. and 4:00 p.m.) for two months, at 3% of the biomass. Water quality parameters are presented in Table II. In order to determine the weight gain in the animals, weekly samples were taken, and a randomized design was used.

Table I. Composition of the diets.

Diet I (20% protein)		Diet II (31% protein)		Diet III (40% protein)	
Ingredient	%	Ingredient	%	Ingredient	%
Oil of fish	3	Soya flour	21	Enterprise food	43
Shrimp flour	25	Fish flour	20.3		
Fish flour	10	Shrimp flour	20.0		
Soya flour	5	Milled corn	17.3		
Mashed rice	25.5	Flour wheat	20		
Wheat flour	1.0	Leave yodada	0.4		
Saved of rice	30.5	Micro ingredients	1.0		

Results and discussion

The results of the weight gains of the different diets are shown in Table III. The best was observed in Diet II, with 99g at the end of the experiment. Similar results for this species were observed by Cortés (2000) with 25, 35, and 45% protein contents. However, those obtained by Webster et al. (1994) differ from Atimo-Pérez (1999). Jones (1990) points out that cultivation systems for redclaw as well as in other freshwater crabs require supplementary diets to increase

weight gains and survival. On the other hand, Ye and Rouse (1994) and Anson and Rouse (1996) reported the importance of a nutritionally complete diet for the cultivation of this species, especially in less intensive cultivation. Kondos (1990) stated that after vitamins, protein is one of the most expensive ingredients for the formulation of diets. We supported Webster et al. (1994), who formulated practical diets with varying protein levels from 23% to 55%, and concluded that the most adequate seems to be 33%.

Table II. Water quality parameters of this experiment compared to those of Jones (1990).

Parameters	Jones (1990)	Present Study
Temperature (ºC)	24-32	26
Dissolved oxygen ($mg.l^{-1}$)	> 5	8.84
Total hardness ($mg.l^{-1}$)	> 50	150
Chlorides ($mg.l^{-1}$)	> 50	53
Total alkalinity ($mg.l^{-1}$)	> 100 and < 300	372
pH	7.5-8.0	7.57
Ammonia ($mg.l^{-1}$)	< 0.5	0.0525
Saltpeters ($mg.l^{-1}$)	< 0.3	0.002
Nitrates ($mg.l^{-1}$)	< 0.5	0.0
Turbidity (cm)	40-60	36

Nonetheless, we did not coincide with Anson and Rouse (1996) who compared many commercial diets for aquaculture with a 32-40% protein range, leading to the assertion that with a 40% protein content the best weight gains were achieved.

Table III. Weight gain compared between the three diets.

Diets	Initial weight (g)	Final weight (g)	Daily gain (g)
Diet I (25% protein)	0.02	8.0^{a}	0.133
Diet II (31% protein)	0.02	9.9^{b}	0.164
Diet III (43% protein)	0.02	9.2^{c}	0.153

Lowercase letters indicate significant differences.

Survival was not altered by the treatments with Diets II and III (65%; Table IV), but was with Diet I (less then 50%), thus illustrating that the protein needs in this stage are very high. The percentage recommended for this species (Operational procedures of Work for the Cultivation of the Lobster of Sweet Water, 1999) is 50%, similar to those reported by Cortés (2000), who obtained 68% of survival in diets with 20-43% protein level. On the other hand, the Secretary of Fishing, Mexico (1994) points out that the protein is one of the nutrients that influences this indicator.

Table IV. Percent survival at completion of the experiment.

Diets	Survival (%)
Diet I (25% protein)	45.2
Diet II (31% protein)	66.8
Diet III (43% protein)	65.6

Conclusions

1. The optimum protein level of the evaluated diets is 31%.
2. Survival did not suffer any alterations with diets II and III.
3. Diet I resulted in the worst weight gain and survival.

References

Anson K. and D.B. Rouse. 1996. Evaluation of several commercially available feeds and to crustacean reference diet for juvenile Australian net claw crayfish, *Cherax quadricarinatus*. J. Appl. Aquacult. 6:65-76.

Alison M.K. and D.B. Rouse. 1999. Protein requirements for juvenile Australian Red Claw Crayfish. (In press) Freshwater crayfish 12:000-000.

Cut E. 2000. Evaluation of diets with different contained proteico for juvenile of lobster of sweet water. V International Symposium of Nutrición Acuícola. Mexico, 2000.

Jones C.M. 1990. The Biology and Aquaculture Potential of Tropical the Freshwater Crayfish *Cherax quadricarinatus*. Queensland Department of Primary Industries Information series QI90028 109p.

Jones C.M. 1995. Evaluation of six diets fed to redclaw, *Cherax quadricarinatus* (von Martens), held in pond enclosures. Freshwater Crayfish 10:469-479.

Kondos A. 1990. Supplementary feed essential for crayfish. Aust. Fish. 49(11):28-32

Secretary of Fishing, Mexico. 1994. Technological scientific manual for the cultivation of the Lobster of Water Dulce (*Cherax quadricarinatus*).

Operational procedures of Work for the Cultivation of the Lobster of Sweet Water, MIP, 1999.

Webster C.D. 1994. Evaluation of practical feed formulations with different protein levels for juvenile net claw crayfish (*Cherax quadricarinatus*). Trans. Kentucky Acad. Sci. 55:108-112.

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PERSPECTIVES OF THE DEVELOPMENT OF *ARTEMIA* CULTURE IN MEXICO

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Introduction

In Mexico, most of the territory is located in tropical and subtropical areas, which is the most adequate for the cultivation of most species of shrimp. Since 1973, the cultivation of shrimp has been in development. The most recent statistics show that 372 shrimp farms and 63 laboratories of postlarvae are operating, with a production of 2 090 455 001 of postlarvae per year (SEMARNAT 2001).

The greatest number of laboratories of shrimp postlarvae are found in the northern states, specifically Sonora and Sinaloa, which also have the greatest number of shrimps farms. This is the reason why work is underway to establish cultivation of *Artemia* in order to satisfy the needs of these productions centers. At present, the first Mexican effort to cultivate *Artemia* is in operation with a surface area of 300ha, but by now, only 20ha are in production. This farm is located near the coastal lagoon of Ohuira, in the State of Sinaloa, and has a production of 50 tons per month.

The demand of *Artemia* nauplii required by each shrimp postlarva, from protozoea III to postlarvae PL5 stages, is 723 (Sánchez, cited in Abreu, 1987); with these data, is possible to calculate the demand of cysts in the production of postlarval laboratories in Mexico. For the 63 laboratories of postlarvae of shrimp currently operating in Mexico, it is estimated that the consumption of *Artemia* cysts is 6747.3kg per year.

With the estimated laboratory demand of cysts, it is certain that new efforts will be required for cultivation of *Artemia*. This year, two new aquaculture projects are beginning: one in the State of Sonora with 150ha, and one in the State of

Oaxaca, which at the moment is a saltworks and which will initially develop 40ha for the cultivation of *Artemia*.

References

Abreu-Grobois A. 1987. El uso de la *Artemia* en criaderos de larvas de Camarón. Informe Técnico. Estación “Mazatlán”, Instituto de Ciencias del Mar y Limnología, UNAM. 9p.

SEMARNAT. 2001. Sistema de Información Estadístico de la Dirección General de Acuacultura. Secretaría del Medio Ambiente y Recursos Naturales. México.

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INCORPORATION OF AN ANTIBIOTIC WITH *ARTEMIA* METANAUPLII TO INHIBIT THE GROWTH OF *PSEUDOMONAS AERUGINOSA*

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Introduction

Traditionally, for the treatment of illnesses of aquatic organisms, chemical products are used which are added directly to the water or administrated through food. With these two forms of administration, there is a disadvantage that the medicine may be lost in the water and cause pollution problems, in addition to problems of inadequate doses reaching the organisms. For this reason, it is necessary to find alternate ways of administering medicines used in aquaculture. The aim of this study was to develop a simple and economical methodology, which incorporates a medicine in the metanauplii of *Artemia*, which may be absorbed by the organisms requiring treatment.

Materials and methods

The materials employed were: *Artemia* cysts from Ohuira, Sinaloa, Mexico; a strain of *Pseudomonas aeruginosa* (CFQ-B021 ATTCC 27853), and an antibiotic in the form of 400-mg tablets of pefloxacin (Peflacina®). The antibiotic was administrated in two doses: one at 1200mg (3 tablets) and the other at 1600mg (4 tablets). Each tablet was converted into powder and placed in 500ml of sterilize water, salinity 40g.l^{-1}. This mixture was dissolved in a domestic blender at maximum velocity for one minute, and subsequently filtered through a 53-µm-mesh sieve.

The metanauplii obtain from the hatching of 1g of cysts were added to the mixture of the medicine, and were kept during 4h, enough time to fill the digestive tract.

In order to determine the zone of inhibition, 100×17mm Petri dishes were used with 21ml of Müller-Hinton agar (Bioxon Lab), containing 1ml of *P. aeruginosa* in suspension at 10^8CFU.ml^{-1}, which corresponds to one half of the tube of McFarland. Metanauplii with food in the digestive tract were selected in groups of 10, 25, 50, 75, 100, 500, and 1000. These were triturated in sterilized mortar and 47µl of each were place in each stainless steel penicilinder (7mm diameter, 10mm high). Six penicilinders were place in a circle in each Petri dish with a radius of separation of 2.8cm; five penicilinders contained organisms with medicine and one was the control. A duplicate experiment was carried out for each concentration of medicine. The dishes were place in a incubator at 35±2°C for 48h and the zones of inhibition were measured.

Results and discussion

In the 1200-mg dose with 500 and 1000 organisms, the zone of inhibition grew 7mm and 9mm, respectively, and in the groups with few metanauplii, this zone did not appear. In a dose of 1600mg, the growth of the inhibition zone appeared in the groups of 100, 500, and 1000 with an average of 16mm, 18mm, and 19mm, respectively. No zone of inhibition was observed around the penicilinders in the control (Fig. 1).

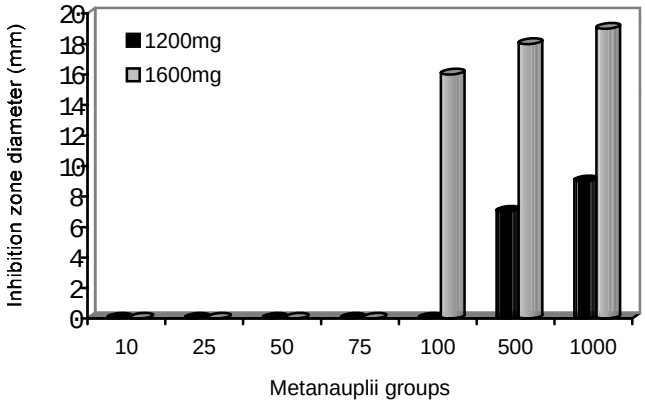

Fig. 1. Zone of inhibition diameter in different groups of *Artemia* metanauplii with 1200mg and 1600mg of pefloxacin.

The results show that using the antibiotic pefloxacin at dose of 1600mg in 100 metanauplii chosen with full digestive tracts and exposed to the antibiotic for 4h is sufficient to inhibit the bacterial grow of *P. aeruginosa*, causing a zone of inhibition measuring 16mm. Monhney et al. (1990) showed that with 100 metanauplii, an inhibition zone of 17mm was achieved using the medicine

sulfonamide (Romet-30®), and this concentration is recommended to treat bacterial infections in fish and shrimp in the mysis stage.

This study showed with the simple technique of incorporating the medicine such as the antibiotic pefloxacin into metanauplii of *Artemia* could be of great value for aquaculturists. It also showed that *Artemia,* aside from being an excellent food source, is a good vector for medicines, already established by Chérel and Nin (1992) and Chair et al. (1995). Moreover, using this crustacean for therapeutic and prophylactic treatment, the cost of medical/antibiotic applications can be reduced, as a great quantity is lost in the water and environmental problems can occur during direct administration.

References

Chair M., M. Dehasque, and P. Sorgeloos. 1995. Live food mediated drug delivery as a tool for disease treatment in larviculture: A case study with turbot *Scophthalmus maximus*. J. World Aquacult. Soc. 26(2):217-219.

Chérel P. and F. Nin. 1992. Antibiotherapy using biocarries (*Artemia salina*) in hatcheries. p.389-393. In: Chemotherapy in Aquaculture: from theory to reality. C. Michel and D.J. Alderman (Eds.). Office International Des Epizooties. Paris, France. 567 p.

Mohney L.L., D.V. Lightner, and R. Williams. 1990. Bioencapsulation of therapeutic quantities of the antibacterial Romet-30 in nauplii of the brine shrimp *Artemia* and in the nematode *Panagrellus redivivus*. J. World Aquacult. Soc. 1(3):186-191.

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THE EFFECTS OF DIETARY VITAMIN SUPPLEMENTATION ON MATERNAL PERFORMANCE AND LARVAL QUALITY OF THE PRAWN *MACROBRACHIUM ROSENBERGII*

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Abstract

The effects of vitamin C (ascorbic acid – AA) and vitamin E (tocopherol) on the maternal performance and offspring quality of the freshwater prawn *Macrobrachium rosenbergii* were investigated. Three diets containing increasing levels of 2-ascorbyl-L-polyphosphate, and a fourth one containing comparatively higher α-tocopherol acetate (α-TA) levels, were fed to prawn females during 155 days.

Higher dietary levels of AA and α-TA did not affect moulting, growth, or mortality rates of the broodstock. Also, breeding frequency and fecundity were not related to the dietary treatments. However, the contents of AA and α-tocopherol in the midgut gland, ovary, eggs, and newly hatched larvae increased along with higher dietary levels of these vitamins. The present results suggest that broodstock diets containing 60µg AA.g^{-1} DW and 300µg α-TA.g^{-1} DW are sufficient to ensure proper reproduction and offspring viability. However, feeding *M. rosenbergii* females higher dietary levels of both AA and α-TA (each around 900 µg.g^{-1} DW) might increase larval quality, as demonstrated in this study by the higher tolerance to the exposure to ammonia.

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THE USE OF AN AMMONIA STRESS TEST AS A TOOL TO EVALUATE LARVAL QUALITY

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Introduction

Although current hatchery practices enable the supply of sufficient numbers of fry, larval quality has become a major concern. Available methods to estimate larval quality involve the exposure of the animals to a short but extreme environmental stress (usually salinity) or the use of morphometric and/or behavioural indicators (Fegan, 1992). Nevertheless, none of these criteria are considered sensitive enough to provide a reliable assessment of larval quality. Acute ammonia bioassays are a routine practice in toxicology, hence a similar procedure could also be of value in the assessment of larval quality. In this study, the feasibility of an ammonia stress test as a tool in the evaluation of larval quality was examined in a series of experiments.

Materials and methods

Prawn (*Macrobrachium rosenbergii*) and penaeid shrimp (*Penaeus vannamei*) larvae obtained from various sources and different zootechnical backgrounds were exposed to increasing concentrations of total ammonia (TAN; $NH_4^+ + NH_3$) and a control (no ammonia added) according to the standardized procedures for ammonia toxicity tests (Greenberg et al., 1992). TAN concentrations were based on preliminary results and varied according to the species and larval stage. After 24h of exposure, larvae presenting no movement of appendages and not responding to mechanical stimuli were considered dead. Tolerance to TAN was estimated as the mean lethal concentrations for 50% of the population (24h LC_{50}) through Probit analysis. Differences on the LC_{50} values were graphically determined with polynomial regressions. Alternatively, tolerance to ammonia was also

estimated as the survival after 24h exposure to a single TAN concentration. In this case, data were normalized (arcsine) and subjected to ANOVA ($P<0.05$).

In experiment 1, newly hatched prawn larvae from the same spawn were divided in two groups and fed either enriched (n-3 HUFA + 20% ascorbyl palmitate) *Artemia* nauplii or 24h-starved *Artemia* nauplii. Prey density was kept at 10-15 nauplii.ml^{-1} up to postlarvae. Temperature, salinity, and photoperiod were maintained at 28°C, 12g.l^{-1}, and 12h light (respectively), while pH varied from 7.8-8.2. Larvae from both treatments were periodically sampled for the estimation of the 24h LC_{50}, total length, and staging. The larval stage index (LSI) was calculated as $LSI = \Sigma S_i / N$, where S_i is the larval stage ($i = 1$ to 12) and N is number of larvae examined (Maddox and Manzi, 1976). To assess the reproducibility of the results, 3 trials were repeated in time.

In experiment 2, prawn larvae were obtained from captive females fed diets with increasing ascorbic acid (AA) levels (59, 122, and 919µg AA.g^{-1}) during 155 days. Tolerance to ammonia (24h LC_{50}) was determined in newly hatched and 8-day-old larvae from various spawns of these females.

In experiment 3, three replicate groups of protozoea II larvae obtained from wild and domesticated stocks of *P. vannamei* were exposed to 30mg.l^{-1} TAN for 21, 24, and 27h. The results of ammonia tolerance were then related to their survival to postlarvae (PL), which were obtained as described in a previous work (Palacios et al., 1999).

Finally, in experiment 4, a similar methodology applied in experiment 3 was adopted. Thirty individual spawns obtained as nauplii from a commercial *P. vannamei* hatchery were reared to postlarvae. The tolerance of protozoea II larvae to ammonia was estimated and correlated to their survival to PL.

Results and discussion

In experiment 1, larval tolerance to ammonia could be distinguished as early as LSI 4 (Fig. 1), and this trend was more apparent as development proceeded. The LC_{50} values were consistently higher for larvae fed enriched *Artemia* in all 3 trials, which demonstrates the reproducibility of the results. More importantly, it indicates not only the superior physiological condition of larvae fed enriched *Artemia*, but also the possibility of differentiating larval quality in terms of ammonia tolerance.

Higher levels of AA in the broodstock diets resulted in an increased tolerance to ammonia of both newly hatched and 8-day-old larvae (Table I). These results confirm that ammonia tolerance may be used to distinguish larvae originating

from females with different nutritional backgrounds. Similar results were obtained in a previous study where larvae from females fed higher levels of polyunsaturated fatty acids had a higher tolerance to ammonia (Cavalli et al., 1999).

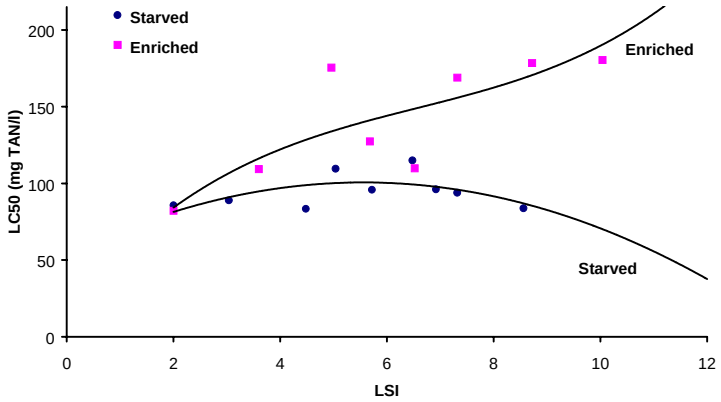

Fig. 1. Polynomial regression of the 24h LC_{50} for total ammonia (mg $TAN.l^{-1}$) in relation to the larval stage index (LSI) of prawn larvae fed enriched or starved *Artemia* nauplii.

In experiment 3, larvae obtained from the domesticated *P. vannamei* stock presented a higher survival to the ammonia stress, and this was related to their final survival to PL (Table II). Similarly, in experiment 4, a significant correlation (r=0.59, $P<0.001$) between the survival of protozoea II larvae to the exposure to ammonia and the subsequent survival to PL was found. Furthermore, it is also worth noting that the ammonia stress test was sensitive enough to detect differences in the early larval stages, i.e., as early as LSI 4 (experiment 1), newly hatched prawn larvae (experiment 2), and penaeid protozoea II larvae (experiments 3 and 4).

Table I. Tolerance to total ammonia (24h LC_{50}) of newly hatched and 8-day-old prawn larvae from females fed diets with ascorbic acid (AA) levels of 59, 122, and 919µg.g^{-1}.

	59µg AA.g^{-1}	122µg AA.g^{-1}	919µg AA.g^{-1}
Newly hatched larvae	42.4 ± 6.0^{b}	$45.0 \pm 6{,}9^{ab}$	52.3 ± 5.2^{a}
8-day-old larvae	132.9 ± 12.8^{b}	135.2 ± 13.5^{b}	155.1 ± 10.9^{a}

It is therefore concluded that the ammonia stress test is a reproducible and sensitive criterion for the evaluation of larval quality, even for early larval stages. Present results also indicate the possibility of applying this methodology as a predictive indicator of larval viability. Nevertheless, further work is

warranted to assess the precise relationship between larval quality, as evaluated by the ammonia stress test, and the subsequent performance during grow-out.

Table II. Survival of protozoea II larvae after exposure to total ammonia (30mg TAN.l^{-1}) and subsequent survival to postlarvae (PL) originated from wild and domesticated broodstocks of *Penaeus vannamei*.

	Duration (h)	Wild stock	Domesticated stock	ANOVA
Survival to TAN	21	50.5 ± 3.7	62.8 ± 10.1	$P = 0.13$
	24	27.9 ± 10.9	46.4 ± 6.4	$P = 0.06$
	27	16.4 ± 6.8	29.1 ± 3.8	$P < 0.05$
Survival to PL (%)		54	86	

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References

Cavalli R.O., P. Lavens, and P. Sorgeloos. 1999. Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. Aquaculture 179:387-402.

Fegan D. 1992. Recent developments and issues in the penaeid shrimp hatchery industry. p. 55-70. In: Proceedings of the Special Session on Shrimp Farming. Wyban. J. (Ed.). World Aquaculture Society Meeting. Baton Rouge, World Aquaculture Society.

Greenberg A.E., L.S. Clescerim, and A.D. Eaton. 1992. Standard methods for the examination of water and wastewater (18th Ed.). Washington, USA: American Public Health Association, American Water Works Association and Water Pollution Control Federation.

Maddox M.B. and J.J. Manzi. 1976. The effects of algal supplements on static system culture of *Macrobrachium rosenbergii* (de Man) larvae. Proc. World Maricult. Soc. 7:677-698.

Palacios E., C.I. Perez-Rostro, J.L. Ramírez, A.M. Ibarra, and I.S. Racotta. 1999. Reproductive exhaustion in shrimp (*Penaeus vannamei*) reflected in larval biochemical composition, survival and growth. Aquaculture 171:309-321.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECT OF FEED PROTEIN MANIPULATION ON THE SURVIVAL, GROWTH, AND ENZYME ACTIVITIES OF *CIRRHINUS MRIGALA* LARVAE

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Introduction

The intensification of an aquaculture system is associated with the enrichment of water with dissolved inorganic nutrients, especially nitrogenous compounds (NH_{4+} and NO_{2-}) and phosphorous. Nutrition and feeding have a central role in the sustainable development of animal production. Feed is the biggest source of nutrition loading in the environment in fish production, though it is not fish that pollute, but rather feed and feeding. Feeding of fish with high-protein feeds resulted into accumulation of inorganic nitrogen. Ammonia excretion is higher when fish are fed a diet based on soluble fish protein concentrate than when fed diets containing fishmeal as the protein source. Thus, reducing environmental effects of aquaculture through improvements in nutrient utilization by fish and capture of waste products is critical for aquaculture production to increase (Hardy, 1999). Rodehutscord et al. (1994) suggest that a surplus of protein in diets can be avoided if fishmeal is replaced by wheat gluten, which also reduces the nitrogen excretion. The adjustment of the C/N ratio in the feed is an effective means to control the pond water quality (Rudacille and Kohler, 1998). The use of biofilters also reduces ammonia and nitrite to less harmful nitrate species. The Indian major carp *Cirrhinus mrigala* (mrigal) is an important commercial fish species in India. Although several on-farm feeds are in use, commercial fish feeds manufactured on the basis of a proper understanding of dietary nutritional requirements are not available (Seenappa and Devraj, 1995). The present experiment was conducted to determine the effect of three different levels of protein on growth, food utilization, and digestive enzyme activities of *Cirrhinus mrigala* (mrigal) and on the maintenance of water quality in the recirculating system.

Materials and methods

Feed ingredients – fishmeal, soybean meal, wheat flour, and maize flour – used in the present study were finely ground, and proximate analysis was performed.

Based on this analysis, three practical diets were formulated to contain increasing levels of protein – 30, 40, and 50% (Table I). The finely ground ingredients were thoroughly blended with cod liver oil using a food mixer for 15min. Vitamin and mineral mixes were then added with continuous mixing. Distilled water (27°C) was slowly added to the mash to attain the desired consistency for pelleting. Each diet was then extruded in a meat grinder using a 1-mm die and dried for 4h below 45°C, and subsequently air-dried overnight to a moisture content less than 10%. Dried feeds were chopped into pellets in a blender and passed through sieves to ensure a homogenous particle size (0.5-1.0mm) of sinking pellets. Feeds were then stored at -8°C until use.

Table I. Composition of test diets (g $100g^{-1}$dry weight).

	30% protein	40% protein	50% protein
Fish meal	13.94	21.32	29.00
Soybean meal	27.87	42.64	58.03
Wheat flour	24.30	13.26	1.69
Maize flour	24.30	13.26	1.69
Cod liver oil	9.00	9.00	9.00
Vitamin premix	0.30	0.30	0.30
Mineral premix	0.25	0.25	0.25

Cirrhinus mrigala larvae (223±2mg) were stocked into 15-l glass aquaria operated as recirculating systems. The stocking density was 6 larvae per aquarium ($400.m^{-3}$). Each feed was offered once daily at 2% of body weight. Three replicates were used for each feeding scheme. Water quality parameters were monitored at weekly intervals throughout the experimental period. The water temperature ranged from 24.8-25.1°C. Ammonia, nitrite, and phosphate were measured according to standard methods. All fish were harvested after 110d of culture, and survival and growth rates were determined. Food conversion ratios (FCR) were calculated as dry weight of food per body weight increase. For the study of amylase and proteolytic enzyme activities, fish were sampled at 0800h, before morning feeding. Proteolytic enzyme activity was measured and amylase activity was assayed. Differences in fish growth, survival and water quality parameters were evaluated by analysis of variance and DMR test. The level of significance was established at $P<0.05$.

Results and discussion

Growth of mrigal in weight increased as the protein level of the diet increased from 30% to 40% and decreased as the protein level increased further to 50% (Table II). A similar result was also obtained in bighead (*Arstichthys nobilis*); when the protein level was increased from 20 to 30%, the growth was increased, and as the protein level increased further, the growth decreased (Santiago and

Reyes, 1991). Seenappa and Devraj (1995) observed that the fingerlings of catla (*Catla catla*) performed better in diets with 30 and 35% protein when fat and carbohydrate levels were 4% and 35%, respectively. In rohu (*Labeo rohita*), a 40%-dextrin and 30%-crude protein diet showed the maximum utilization of protein for growth (Erfanullah and Jafri, 1995). The weight gain of red drum (*Sciaenops ocellatus*) increased accordingly with increased dietary protein and energy content (Thoman et al., 1999). There was no difference in the survival rate of mrigal among three treatments. In catla, poor survival was found in fish fed on diets containing less protein (25%) and high fat (12%), and better survival occurred in diets with increased protein and carbohydrate levels (Seenappa and Devaraj, 1995). Food was most efficiently utilized in the fish fed 40% protein, evident from the significantly lower (P<0.01) FCR value. FCR was highest in fish fed 50% protein diet. But the studies on catla (Seenappa and Devraj, 1995) and rohu (Erfanullah and Jafri, 1995) showed that food conversion was not significantly affected by the major nutrients. Specific proteolytic enzyme activity explained the reason of better performance of mrigal in the 40% protein diet. Enzyme activity was significantly higher in this group than others. Amylase activity showed the opposite trend – it was maximum in the fish fed 50% protein diet, followed by 30% protein diet and 40% protein diet.

Table II. Means (± SE) of average harvest weight, survival, FCR, proteolytic enzyme activity and amylase activity of mrigal fed diets with varying levels of protein. Means followed by different letters are significantly different (P<0.05).

	30% protein	40% protein	50% protein
Average weight (mg)	364 ± 18.8^{b}	510.07 ± 7.57^{a}	338.4 ± 7.21^{c}
Survival (%)	100^{a}	100^{a}	100^{a}
FCR	4.53 ± 0.7^{b}	2.65 ± 0.07^{c}	5.87 ± 0.38^{a}
Proteolytic enzyme (μg tyrosine mg^{-1} protein h^{-1})	76.09 ± 13.87^{c}	107 ± 14.7^{a}	81.6 ± 12.78^{b}
Amylase activity (μg of maltose mg^{-1} protein h^{-1})	13.75 ± 1.62^{b}	7.88 ± 1.55^{c}	15.98 ± 1.55^{a}

The pH of water ranged from 7.29-8.38, 7.58-8.42, and 7.63-8.38 in 30, 40, and 50% protein diets, respectively. Dissolved oxygen level was maintained above 5mg.l^{-1} with the help of aerators. It was at a maximum in the 30% protein diet (7.67±0.34mg.l^{-1}), followed by 40% protein diet (7.57±0.34 mg.l^{-1}), and 50% protein diet (7.47±0.31mg.l^{-1}). Ammonia levels increased with the increase of dietary protein level in this study. It was significantly higher (P<0.05) in the 50%-protein diet treatment (8.17±1.4mg.l^{-1}) than others. It was minimized (7.19±1.22mg.l^{-1}) in the 30%-protein treatment. Nitrite levels were 80.87±17mg.l^{-1}, 46.20±19.90mg.l^{-1}, and 26.70±14mg.l^{-1} in 30, 50, and 40% protein-containing diets, respectively. As the amount of nitrogen excreted is dependent on the quality and quantity of dietary protein, the manipulation of protein levels in the present study resulted in various levels of inorganic nitrogen levels in the culture systems. Better performance of fish in the 40% diet-fed group was due to prevalence of lower levels of ammonia and nitrite than the

other two systems. Avnimelech et al. (1994) observed better growth of tilapia in 20% protein treatment due to lower concentrations of toxic inorganic nitrogen species. The protein content of diets of rainbow trout *Oncorhynchus mykiss* can be reduced from 46 to 38% in dry matter, and nitrogen excretion per kg gain can be reduced by 23-43% (Rodehutscord et al., 1994). Significantly higher (P<0.05) levels of phosphate were observed in the 40% protein-fed group (21.47±3.4mg.l^{-1}). The results of the present study indicate that maximum growth and feed conversion efficiency of mrigal were observed in fish fed a diet containing 40% protein. This also helps in the maintenance of better water quality in the recirculating culture system. This information will help in the development of better rearing conditions of mrigal.

References

Avenemelech Y., M. Kochva, and S. Diab. 1994. Development of control intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Bamidgeh 46: 119-131.

Erfanullah and A.K. Jafri. K. 1995. Protein – sparing effect of dietary carbohydrate in diets for fingerling *Labeo rohita.* Aquaculture 136:331-339

Hardy R.W. 1999. Collaborative opportunities between fish nutrition and other disciplines in aquaculture : an overview. Aquaculture 177:217-230.

Rodehutscord M., S. Mandel, and E. Pfeffer. 1994. Reduced protein content and use of wheat gluten in diets for rainbow trout : effects on water loading with N and P. J. Appl. Ichthyol. 10:271-273.

Rudacille J.B. and C.C. Kohler. 1998. Dietary protein requirement of juvenile white bass *Morone chrysops.* Aquaculture 1998 Book of Abstracts, Las Vegas, World Aquaculture Society, pp. 457

Santiago C.B. and O.S. Reyes. 1991. Optimum dietary protein level for growth of bighead carp (*Aristichthys nobilis*) fry in a static water system. Aquaculture 1993:155-165.

Seenappa D. and K.V. Devaraj. 1995. Effect of different levels of protein, fat and carbohydrate on growth, feed utilization and body carcass composition of fingerlings in *Catla catla* (Ham.). Aquaculture 129:243-249.

Thoman E.S., D.A. Davis, and C.R. Arnold. 1999. Evaluation of growout diets with varying protein and energy levels for red drum (*Scianops ocellatus*). Aquaculture 176:343-353.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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ECOLOGICAL AND MORPHOLOGICAL PECULIARITIES OF STURGEONS REARED UNDER CONTROL OF SEASONAL PROPAGATION OF BREEDERS

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Introduction

The sturgeon stock in Sea of Azov is presently maintained by artificial reproduction. Currently, the long-term management strategy of the sturgeon population is based on a new concept of enhancement and genetic monitoring. There have been biological bases and ecologically technological principles of artificial reproduction developed with intraspecific diversity conservation. They include the methods of seasonal maturity control to obtain the best-quality hatch all year round and to use breeders of different spawning run periods, which have multilocus polymorphism.

The use of ecological and hormonal methods to control seasonal propagation of stellate sturgeon (*Acipenser stellatus*), Russian sturgeon (*A. gueldenstaedtii*), beluga (*Huso huso*), and sterlet (*A. ruthenus*) makes it possible to stagger the sexual cycle of breeders from the various biological groups for early (5 months) or later (6 months) spawning (Chebanov, 1998). The program of complex ecological-morphological monitoring of fry reared at sturgeon hatcheries involves the estimation of length and weight variation as well as the physiological-biochemical indices, teratological analysis, thermal stability, salt resistance, oxygen deficiency resistance, and melanophore adaptive response ("background") as criteria of their physiological fitness (Galich, 2000).

Materials and methods

These experiments were performed in 1998 at Adygei sturgeon hatchery, the largest in the Sea of Azov basin.

The comparative analysis was performed in three experimental groups of eggs, prelarvae, larvae, and fingerlings of Russian and stellate sturgeons:

I. with the use of conventional biotechniques
II. with non-durably delayed completion of the reproduction cycle (10-25 days) kept at 6°C temperature
III. following a durable (up to 3 months) shift in the cycle

To reveal the adaptability of experimental fingerlings to perform pigmental reactions to the change of background, 20- to 25-day-old Russian and stellate sturgeon larvae were selected. The melanophore index (mi) values were estimated by the five-point system scale proposed by K.D. Krasnodembskaya (1994).

The neuro-pharmacological testing of fingerlings by reaction to the effect of neurotropic substances was performed at the different concentrations (50 and 75 $mg.l^{-1}$) of tricaine-methansulfonate anesthetic (MS-222).

Results and discussion

The teratologic analysis of experimental fish development displayed the lack of notable differences in frequency of occurrence of the typical morphological anomalies relating to the fingerlings, obtained with the use of conventional biotechniques. The defects observed most frequently are as follows: the anomaly of pectoral fins (up to 20%) and olfactory organs (up to 10%). The process of development enjoyed a 17-fold decrease of the rate of morphological disorders in the living sturgeons. The anomalies shared by the stellate sturgeons (defects of head form and olfactory organs, underdevelopment of gill covers) rapidly fell from 69 to 13% following transition to exogenous feeding.

The ecological and physiological estimation of the fingerlings showed the adequate and timely adaptive reaction of melanophores towards dark and light background. The more distinct adaptive type of “background” is noted in the dark vessels with gross and fine fingerlings (90%). The inadequate reaction in white vessels was established only in the sturgeons with delays in growth. There was a close feedback between melanophore index and the fingerlings’ weight and length in light vessels.

The fry expressed high ecological flexibility for extreme values of ecological factors – i.e., temperature (32°C), salinity (12‰), and oxygen deficiency.

The highest salt resistance for sturgeons was established: 87% of individuals survived during 24 hours in saline. The viability of stellate sturgeons under extreme salt load was considerably lower (30-37°C), but appeared age-specific. The high resistance to oxygen deficiency ($2.4mg.l^{-1}$) revealed in experimental Russian sturgeons is characteristic for older individuals. This resistance in stellate sturgeons was much higher ($1.7\ mg.l^{-1}$).

50mg.l^{-1} of MS-222 completely suppressed the motility only in 70-80% of the fry and fingerlings of various species and mass towards the end of narcotization (30 minutes). In this case, the fry of Russian sturgeon showed the highest resistance to this concentration of tranquilizer, and sterlet fry showed the lowest resistance (80% of fry "fell asleep" after 15 minutes). The restoration of motility in stellate sturgeon fry was more rapid (3 minutes) than those of Russian sturgeon.

Conclusions

The experiment illustrated early euryhaline character, thermal stability, and oxyresistance of sturgeon fry and fingerlings under controlled seasonality in their reproduction. This could be representative of adaptive abilities to survive in natural conditions, though normal developmental morphology and physiology is not guaranteed.

The analysis of fingerling viability calls for more broad criteria determination in parallel with its ecological and morphological estimate.

References

Chebanov M.S. 1998. Conservation of sturgeon Genetic Diversity: enhancement and Living Gene Bank. p.163-173. In: Action before Extinction Proceedings of the International workshop. Vancouver, Canada.

Galich E.V. 2000. Ecologo-morphological characteristics and neuro-pharmacological express-testing of juveniles of various sturgeon species grown in pools. p. 227-229. In: The International Conference "Sturgeons on the Threshold of the XXIst century". Book of Abstracts. Astrakhan, September 11-15, 2000. KaspNIRKH, Astrakhan.

Krasnodembskaya K.D. 1995. Sturgeon larvae adaptation in the context of sturgeon propagation. p.268-278. In: International Symposium on Sturgeons. Proceedings. September 6-13, 1993. Moscow-Kostroma-Moscow Russia. VNIRO Publishing, Moscow.

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MICROCAPSULES CONTAINING MUSHROOM POLYSACCHARIDE AND *ARTEMIA* POWDER PROVE TO ENHANCE IMMUNITY OF *CAMBAROIDES*

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Introduction

Antibiotics are presently widely used in aquaculture to treat aquatic animals and increase their survival. A downside of this is the possible induction of drug tolerance. β-1,3D polysaccharide, derived from mushrooms, can enhance the immunity of both humans and animals – including aquatic animals such as larvae, fish, etc. (Soderhall and Cerenius, 1992). However, it is water soluble, therefore it is difficult to use it as a feed for aquatic animals.

Artemia is a very nutritious food source for aquatic larvae. However, it cannot survive in fresh water, and it is too large for aquatic larvae to ingest. Therefore, during high yield season of *Artemia*, much of this precious source is wasted. Microencapsulation is a widely used technique with many advantages, e.g., the ease to change aggregate condition, improve particle size, protect nutritional composition of contents, control drug release, etc. (Dianzhou, 1999).

In this experiment, mushroom polysaccharide and *Artemia* nutritional components are microencapsulated and subsequently fed to *Cambaroides* in order to enhance their immunity.

Materials and methods

Mushroom polysaccharide is produced at very low costs according to a patented technique developed in our laboratory. *Artemia* is taken from salt ponds in Lianyunggang, Jiangsu Province, China.

Microencapsulation. 1.5g of glutin and 1.5g of Arabic gum are dissolved in 50ml

water. 1.2g of polysaccharide and *Artemia* powder in the proportion 1:0.2 are dissolved in water, 20ml of Arabic gum solution is added, and then mixed evenly. The glutin solution is regulated to pH 7-8 by adding 20% NaOH, and then mixed with the above solution. The subsequent steps for microencapsulation are the same as in Dianzhou (1999). The size and shape of microcapsules are measured using a microscope.

Assay of immune activity and growth activity. Forty *Cambaroides* larvae are randomly divided into control and experimental groups. The control group is fed flour, while the other is fed with microcapsules containing mushroom β-1,3D polysaccharide and *Artemia* powder for 7 days. The methods for detecting the immune activity induced by microcapsules are described in Boman (1974) and Hultmark (1980). This activity is calculated according to the following formula:

$$U_\alpha=[(A_0 - A)/A]^{1/2}$$

U_α: immune activity of *Cambaroides*; A_0 and A: represent the values of OD_{570nm} of the serum of the *Cambaroides* in control and test groups, respectively.

The growth rates are also calculated by measuring the lengths of the two groups of *Cambaroides*.

Results

Microcapsules. The microcapsules are stable, round, smooth, and measure 75-100μm in size.

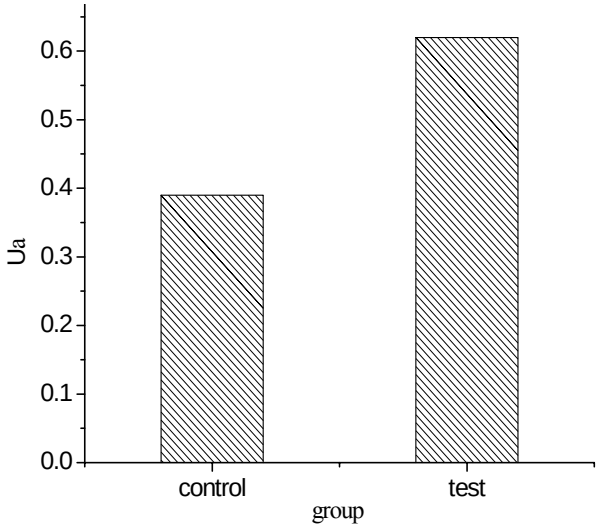

Fig.1 Immune activitv

Immune activity. The immune activity of the experimental group is 50% higher than that of the control group (Fig. 1).

Growth rate. The experimental group grows very well and shows much more activity than the control group. Moreover, they are 20% longer than the control group (Fig. 2).

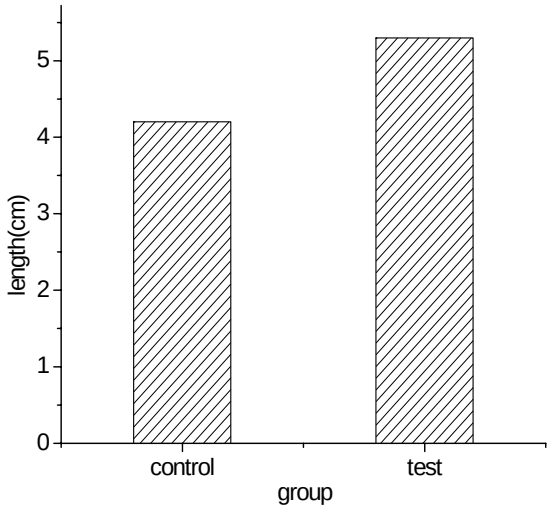

Fig.2 Growth rate

Discussion

Microcapsules containing mushroom β-1,3D polysaccharide and *Artemia* powder are successfully made in our laboratory and show good biological activity, however, this activity should be tested in the natural environment. It should also be considered to what extent the use of antibiotics might be reduced by using this technique, and whether it is possible to use other cheaper materials for microcapsulations.

References

Boman H.G. 1974. Insect immunity. I. Characteristics of an inducible cell-free antibacterial reaction in hemolymph of *Samia cynthia* pupae. Infect. Immun. 10(1):136-45.

Dianzhou B. 1999. Drugs (4th version). People Health Press, Beijing.

Hultmark D. 1980. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of *Hyalophora cecropia*. Eur. J. Biochem.106(1):7-16.

Soderhall K. and L. Cerenius. 1992. Crustacean immunity. Ann. Rev. Fish Dis. 2:3-23.

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DEVELOPMENT RATES FOR SABLEFISH (*ANOPLOPOMA FIMBRIA*) AND PACIFIC HALIBUT (*HIPPOGLOSSUS STENOLEPIS*) INCUBATED AT 6ºC

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Introduction

Measurement of yolk absorption rate is important because it is a critical component in determining the optimum time for start-feeding of larvae. The sablefish (*Anoplopoma fimbria*) and Pacific halibut (*Hippoglossus stenolepis*) are candidates for introduction to intensive aquaculture. Alderdice et al. (1988) measured larval growth and yolk absorption rates from two groups of sablefish eggs incubated at 5.6°C. Similarly, McFarlane et al. (1991) measured larval growth and yolk absorption rates from one group of Pacific halibut eggs incubated 6°C. To improve the understanding of larval development for these two species, we require more measurements from more larvae over a longer period. Hence, this study presents data for larval and yolk sac measurements obtained from progeny of the sablefish and Pacific halibut broodstock held at the Pacific Biological Station.

Materials and methods

Sablefish larvae were sampled from 18 spawnings of 12 females and incubated in upwelling incubators at 6°C. Samples of 10 larvae were collected from these spawnings at intervals ranging from 10-49 days (i.e., 60-294°C-days) from fertilisation. The number of larval samples obtained from each ovulation ranged from 2 to 7. The total number of larval samples was 48.

Pacific halibut larvae were obtained from 10 spawnings of 3 females and incubated in upwelling incubators at 6°C. Samples of 10 larvae were collected at intervals ranging from 16-61 days (i.e., 96-366°C-days) from fertilisation. The number of larval samples from each spawning ranged from 1-13. The total number of larval samples was 83.

Measurements were made using a Wild-Lietz dissecting microscope at 12X and 6X magnification. Total length (TL in mm) was measured from tip of snout to end of caudal fold and standard length (SL in mm) from tip of the snout to end of the notochord. Yolk volume was determined by measuring the length and width of the yolk and using the equation for a prolate spheroid (i.e., volume = $4/3 \bullet \pi \bullet$[length semi-axis]$\bullet$[width semi-axis]^2).

The length and yolk volume data were then plotted against development time (i.e., °C-days). Regression equations were calculated for total and standard length and yolk volume for both sablefish and Pacific halibut. Total and standard length data of larvae were fitted to a simple curvilinear equation (i.e., $y=a+b \bullet x^{0.5}$). The curvilinear equation ($y=a+b \bullet x^{-1}$) was fitted to the yolk volume data.

Results

The regression equations were highly significant with R^2 values (adjusted for degrees of freedom) ranging from 0.82 to 0.96. The equations for sablefish larval growth (mm) are:

$$y(\mathrm{TL}) = 0.61216 + 0.54120 \bullet x(^{\circ}\mathrm{C} - \mathrm{days})^{0.5} \quad R^2=0.84,\ n=48 \text{ (Equation 1)}$$

$$y(\mathrm{SL}) = 0.79992 + 0.50249 \bullet x(^{\circ}\mathrm{C} - \mathrm{days})^{0.5} \quad R^2=0.82,\ n=48 \text{ (Equation 2)}$$

The corresponding equations for Pacific halibut larval growth are:

$$y(\mathrm{TL}) = 1.00316 + 0.72564 \bullet x(^{\circ}\mathrm{C} - \mathrm{days})^{0.5} \quad R^2=0.96\ n=83 \text{ (Equation 3)}$$

$$y(\mathrm{SL}) = 1.01759 + 0.70187 \bullet x(^{\circ}\mathrm{C} - \mathrm{days})^{0.5} \quad R^2=0.96,\ n=83 \text{ (Equation 4)}$$

The equation for sablefish yolk volume (YV, mm^3) is:

$$y(\mathrm{YV}) = -1.5795 + 384.1072 \bullet x^{-1}(^{\circ}\mathrm{C} - \mathrm{days}) \quad R^2=0.95,\ n=48 \text{ (Equation 5)}$$

The regression line with 95% confidence limits for sablefish yolk volume is shown in Fig. 1. The arrow indicates that the lower 95% confidence limit crosses $0.1mm^3$ at 220°C-days from fertilisation (i.e., 37 days at 6ºC). The corresponding Pacific halibut yolk volume equation is:

$$y(\mathrm{YV}) = -4.53990 + 1937.1940 \bullet x^{-1}(^{\circ}\mathrm{C} - \mathrm{days}) \quad R^2=0.82,\ n=83 \text{ (Equation 6)}$$

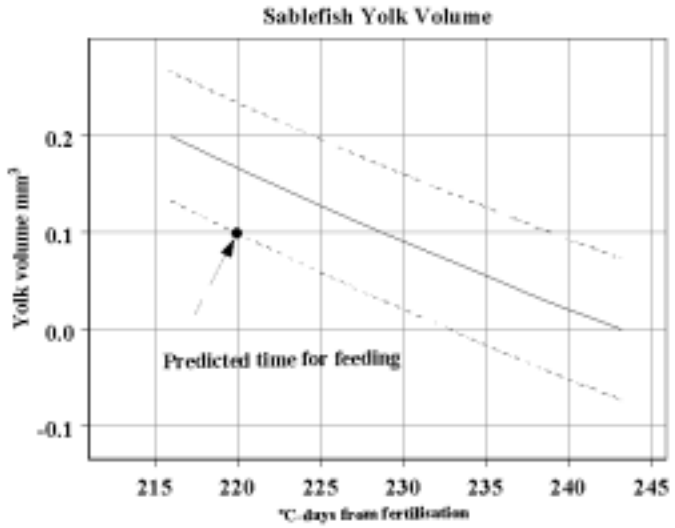

Fig. 1. Regression with 95% CL for sablefish yolk volume.

The regression line with 95% confidence limits (CL) for Pacific halibut yolk volume is shown in Fig. 2. The arrow indicates that the lower 95% CL reaches 0.1 mm^3 at 363°C-days from fertilisation (i.e., 60 days at 6ºC).

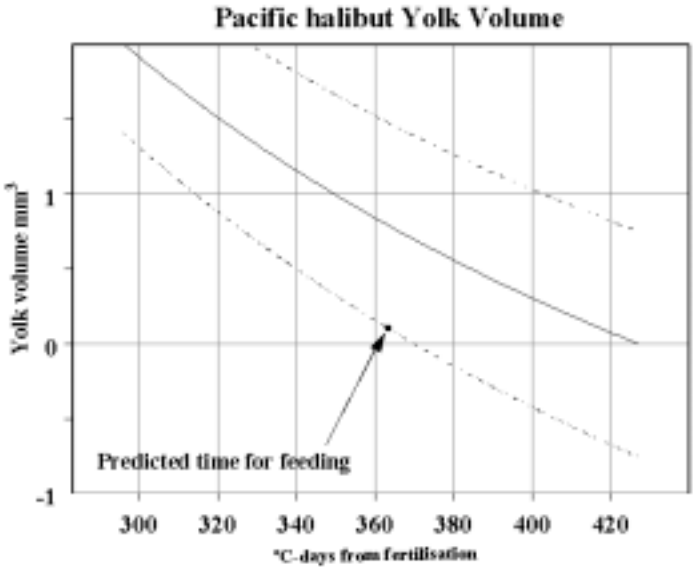

Fig. 2. Regression with 95% CL for Pacific halibut yolk volume.

Discussion

The regression equations for sablefish larval growth were highly significant, with R^2 values (adjusted for degrees of freedom) of 0.84 for TL and 0.82 for SL. Equation 1 predicted values for sablefish TL similar to those predicted by the model of Alderdice et al. (1988). During the period of 112-224°C-days from fertilisation, differences in TL predicted by the two models ranged from 0.0-0.5mm.

Regression equations for Pacific halibut larval growth were highly significant, with R^2 values for both TL and SL of 0.96. The TL model for Pacific halibut (Equation 3) also predicted values similar to those reported by McFarlane et al. (1991). However, the predicted lengths diverged at later development times, with a difference of 1.7mm at 360°C-days from fertilisation.

The objective of this study was to determine larval growth rates and develop a model to predict when yolk absorption of sablefish and Pacific halibut larvae has progressed sufficiently to allow introduction of exogenous food. On the basis of our analysis, we estimate that sablefish larvae will have $0.1mm^3$ yolk at 220°C-days from fertilisation. For Pacific halibut, the corresponding time is 363°C-days from fertilisation. We expect that a yolk volume of $0.1mm^3$ will be adequate for larvae to adapt to feeding but this needs to be verified in future experiments.

Acknowledgements

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References

Alderdice D.F., J.O.T. Jensen, and F.P.J. Velsen. 1988. Incubation of sablefish (*Anoplopoma fimbria*) in a system designed for culture of fragile marine teleost eggs. Aquaculture 71:271-283.

McFarlane G.A., J.O.T. Jensen, W.T. Andrews, and E.P. Groot. 1991. Egg and yolk sac larval development of Pacific halibut (*Hippoglossus stenolepis*). Int. Pac. Halibut Comm. Tech. Rep. 9:22p.

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THE VISUAL FIELD AND FEEDING OF CULTURED STRIPED TRUMPETER (*LATRIS LINEATA*) LARVAE UNDER DIFFERING LIGHT ENVIRONMENTS.

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Introduction

Striped trumpeter *Latris lineata* have been under investigation for the last 12 years as a potential candidate for temperate marine culture. Significant progress has been achieved in the areas of broodstock management and year-round supply of gametes (Morehead et al., 2000), however larviculture has remained problematic (Cobcroft et al., 2001; Trotter et al., 2001). The feeding performance of finfish larvae plays a major role in their survival and growth in culture. Most finfish larvae, including striped trumpeter (Pankhurst and Hilder, 1998), are primarily visual feeders (Blaxter, 1986). As a result, the determination of visual functional capabilities and the optimal light regimes for larviculture are important aspects of developing successful production techniques. This study describes the development of the eyes, determines the functional visual field of feeding larvae, and investigates feeding performance under a range of environmental light conditions.

Materials and methods

The development of the eyes in cultured striped trumpeter larvae was examined through histology and scanning electron microscopy. Video cinematography was used to determine the reactive distance and the visual field of striped trumpeter larvae feeding in culture tanks. Short-term feeding trials were used to determine the optimal light intensity and the effect of microalgal cell density (greenwater/turbidity) on larval feeding performance.

Results and discussion

The structure of the retina and lens, and the presence of the optic nerve, indicated the eye was functional, coincident with first feeding on day 7 post-hatching. The structure of the photoreceptors in different regions of the retina of the larvae suggested the region specialized for the most acute image formation was the dorso-temporal retina. This corresponds to a theoretical visual field in the anterio-ventral environment, relative to a larva's horizontal orientation.

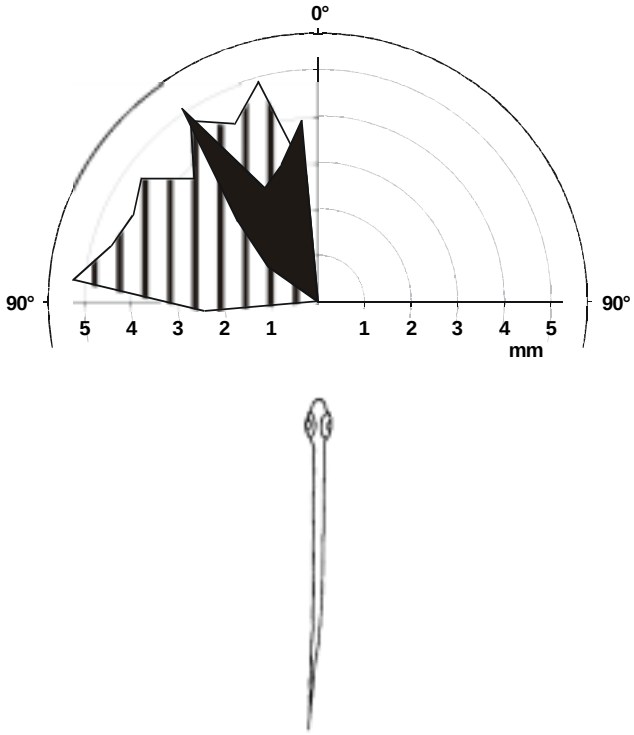

Fig. 1. The visual field of striped trumpeter larvae on day 13 (black fill) and day 17 (striped fill) post-hatching determined from maximum reactive distances in each of the 10° divisions of reactive angle from pooled left and right-hand-side reactions to rotifer prey.

Analysis of video cinematography of feeding larvae confirmed a functional visual field in the frontal plane, which increased in area with larval ontogeny (Fig. 1). The maximum reactive distance of the larvae to their prey was less than a body length, and was similar to that observed in other teleost larvae (Blaxter 1986). Small reactive distances limit the volume of water in which larvae can search for prey. These results highlight the importance of supplying prey densities in culture that provide larvae with adequate energy for growth while

minimizing the energetic costs involved in searching for prey (Hunter, 1980; Blaxter, 1986).

In feeding trials, larvae fed equally well in clearwater in a light intensity range of 1-10µmol.s^{-1}.m^{-2} when evaluated in terms of both the proportions of larvae feeding and larval feeding intensity. An ontogenetic improvement in visual sensitivity of larvae was indicated by improved feeding in a one-hour feeding time at 0.1µmol.s^{-1}.m^{-2}, from 26% of larvae feeding and a mean of 1.6 rotifers consumed per feeding larva on day 8, to 96% and 13.4 rotifers consumed per larva on day 23 post-hatching. An improvement in the visual sensitivity of finfish larvae has been demonstrated in other species and is associated with ontogenetic changes in the eye (Blaxter, 1968). Algae-induced turbidity (greenwater) had different effects on larval feeding response, dependent upon the previous visual environment of the larvae. Young larvae, day 9 post-hatching, and reared in clearwater showed decreased feeding capabilities with increasing turbidity (algal cell density), while older clearwater-reared larvae fed well at all turbidities tested. Likewise, greenwater-reared larvae had increased feeding capabilities in the highest algal cell densities tested (32 and 66 nephelometric turbidity units, NTU) compared with those in low algal cell density (6 NTU), and clearwater (0.7 NTU) to which they were naive. In other studies, turbidity induced by microalgae or by inert particles has improved larval feeding performance (Naas et al., 1992), had no effect on feeding (Gulbrandsen et al., 1996), or has decreased larval feeding (Breitberg, 1988). However, the present study demonstrates that the previous visual environment of larvae can affect subsequent feeding responses, and as such, should be considered when interpreting the results of feeding trials and when altering culture conditions in hatcheries.

Future culture trials with striped trumpeter larvae will incorporate the light regimes determined in this study and research will focus on determining the nutritional requirements of larvae.

References

Blaxter J.H.S. 1968. Visual thresholds and spectral sensitivity of herring larvae. J. Exp. Biol. 48:39-53.

Blaxter J.H.S. 1986. Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. Trans. Amer. Fish. Soc. 115:98-114.

Breitburg D.L. 1988. Effects of turbidity on prey consumption by striped bass larvae. Trans. Amer. Fish. Soc. 117:72-77.

Cobcroft J.M., P.M. Pankhurst, J. Sadler, and P.R. Hart. 2001. Jaw development and malformation in cultured striped trumpeter *Latris lineata*. Aquaculture, in press.

Gulbrandsen J., I. Lein, and I. Holmefjord. 1996. Effects of light administration and algae on first feeding of Atlantic halibut larvae, *Hippoglossus hippoglossus* (L.). Aquacult. Res. 27:101-106.

Hunter J.R. 1980. The feeding behaviour and ecology of marine fish larvae. p.287-330. In: Fish behaviour and its use in the capture and culture of fishes. Bardach J.E., J.J. Magnuson, R.C. May and J.M. Reinhart (Eds.). International Center for Living Aquatic Resources Management, Manila, Philippines. 512p.

Morehead D.T., A.J. Ritar, and N.W. Pankhurst. 2000. Effect of consecutive 9- or 12-month photothermal cycles and handling on sex steroid levels, oocyte development, and reproductive performance in female striped trumpeter *Latris lineata* (Latrididae). Aquaculture 189:293-305.

Naas K.E., T. Næss, and T. Harboe. 1992. Enhanced first feeding of halibut larvae (*Hippoglossus hippoglossus* L.) in green water. Aquaculture 105:143-156.

Pankhurst P.M., and P.E. Hilder. 1998. Effect of light intensity on feeding of striped trumpeter *Latris lineata* larvae. Mar. Fresh. Res. 49:363-368.

Trotter A.J., P.M. Pankhurst, and P.R. Hart. 2001. Swim bladder malformation in hatchery-reared striped trumpeter *Latris lineata* (Latridae). Aquaculture 198:41-54.

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AMINO ACID METABOLISM AND AMINO ACID REQUIREMENTS IN FISH LARVAE AND POSTLARVAE

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Abstract

In comparison to mammals, fish – in particular, young stages – are thought to have a lower adaptability of amino acid (AA) metabolism and higher AA requirements. Little work has been done on AA requirements of fish larvae, largely due to difficulties in applying traditional methodologies to such small animals. This is particularly true during the early life stages when fish are fed exclusively on live food. This paper reviews recent findings in amino acid metabolism that contribute to understand the qualitative AA requirements of fish larvae.

A new method has been developed to estimate metabolic budgets for different AA, through tube-feeding of a mix of AA containing a ^{14}C-labelled AA. This method allows estimation of AA digestive absorption, AA conversion into lipidic molecules, AA oxidation, and conservation of AA in the free AA and in the protein pools. Results with herring (*Clupea harengus*) larvae and sole (*Solea senegalensis*) post-larvae show that fish have the capacity to spare indispensable AA at the expenses of dispensable AA at early life stages. This suggests that fish larvae may have a better capacity of regulating AA catabolism than previously believed. Thereby, the AA requirements for energy production can probably be met largely at the expenses of oxidation of dispensable AA.

Another new method has been established to estimate simultaneously the bioavailability of several individual AA in fish larvae. This method combines the use of ^{13}C-labeled live food and ^{13}C-NMR spectroscopy. The bioavailabilities of the various AA have been shown to differ considerably in larval sea bream (*Sparus aurata*). Histidine, tyrosine, methionine, and lysine have higher bioavailabilities than threonine and leucine. Information on the bioavailability of individual AA together with the AA profile of the larval protein should allow defining of the ideal dietary AA profile for a given species.

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ARE THERE ADVANCES BEING MADE IN UNDERSTANDING DIETARY NITROGEN UTILIZATION FOR GROWTH IN LARVAL FISH?

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Abstract

The growth effect of dietary sources of amino acids – free, peptide, or protein-bound – is most likely related to fish species, ontogenetic development of digestive tract, and/or nature of the protein. With respect to adult fish (with differentiated digestive tract), a time lag difference between absorption of free amino acids and absorption of protein-bound amino acids was associated with "flooding" in the former, and resulted in negative efficiency in amino acid utilization for protein synthesis and, consequently, growth. It has been demonstrated that dietary free amino acids, presented as amino acid mixture diets, were not metabolized following ingestion, and were largely excreted. The finding that an increased meal frequency enhanced utilization of free-amino-acid-mixture diets corroborates the latter conclusion.

This information contrasts with numerous claims in the literature that larval fish utilize free amino acids more efficiently than proteins. Is there a reason that larval fish would respond differently than juveniles to a diet formulated on the basis of free amino acids? The high protein synthesis rate and growth rate in larval fish in comparison to juveniles and adults requires enhanced availability and faster amino acid absorption. Simultaneously, an *in vitro* study suggested that amino acids presented as peptides are better absorbed than free amino acids. However, peptide carriers and hydrolysis by brush border membrane enzymes in the fish intestine are poorly described and no explanation is given for up to 4-fold differences in permeability among different peptides.

The experiment to be described in this presentation compares diets formulated based on free, peptide, and protein-bound amino acids fed to rainbow trout alevins.

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THE NUTRITIONAL QUALITIES OF *PANAGRELLUS REDIVIVUS* WHEN CULTIVATED IN OATS ENRICHED WITH *SPIRULINA*

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Introduction

The microworm *Panagrellus redivivus* is a free-living nematode that is found in soil and also in the fermentation of meals. It measures 250μm at birth and reaches a length of up to 2.0mm as an adult. It can tolerate a salinity of 14g.l^{-1} and wide temperature variations, although its growth is most favoured by a temperature of between 22-28°C. This study presents an analysis of the amino acid and essential fatty acid content present in *P. redivivus* cultivated in commercial oats enriched with *Spirulina*. In addition, an analysis was made of their calcium and phosphorus contents when cultivated in oats with *Spirulina* and eggshells. The aim of this study was to compare this nematode with the nauplii of *Artemia* as a possible alternative food source for the larval stages of commercially important fish and crustaceans.

Materials and methods

The cultivation of the nematodes was carried out in triplicate in plastic containers measuring 16cm^2 and 6cm deep. Each one was filled with 150g of oats flakes, 200ml of tap water, and 10g (wet weight) of microworms. To another group of three containers with the same contents, 5g of *Spirulina* was added at a temperature of between 14-24°C. The samples were maintained at room temperature for six weeks, at the end of which specimens were collected using a sieves with a mesh of 0.54 and 0.74μm.

High-pressure liquid chromatography was used for the analysis of the amino acids. The technique used was cation exchanged resin, diluted with buffers on a pH gradient from 3.1-5.6, and a range of citrates from 0.2M-2.0M (Beckman, 1985). An automatic analyzer Beckman System Gold 6300 was employed.

In the case of the fatty acids, total lipids were extracted three times using three different solvents (hexane, chloroform, and acetone). The fatty acids were esterified by means of 6% sulfuric acid in methanol. This was heated to a temperature of 85-90°C with the addition of 1.5ml of distilled water and 2.0ml of hexane. A microliter of the supernatant was extracted and injected into a Perkin Elmer Autosystem 9000 gas chromatographer which equipped with a FID detector and a Restek Stabilwax 30m×0.25mm×0.2µm polar column with a polyethyleneglycol phase.

The analysis of the calcium was carried out using titulation with EDTA. The oats were enriched: one with 5g of eggshells, one with 5g of *Spirulina*, and the other with 2.5g of eggshells and 2.5g of *Spirulina*. Phosphorus was detected by spectrophotometry with an absorbance of 400nm.

Results

The results obtained are presented in Tables I and II, where they are compared with the data reported for *Artemia*.

Table I. Amino acid content of *Panagrellus* and *Artemia* (% of protein).

Amino acid	Oats	Oats + *Spirulina*	*Artemia**
Aspartic acid	14.49	22.58	
Threonine	9.55	10.68	2.83
Serine	13.01	15.75	
Glutamic acid	34.9	61.09	
Glycine	18.08	22.59	
Alanine	23.73	21.23	
Cystine	3.22	4.55	0.23
Valine	8.47	14.68	1.69
Methionine		0.22	1.87
Isoleucine	5.03	9.28	3.91
Leucine	13.65	21.26	4.02
Tyrosine	3.5	4.27	2.63
Phenylalanine	6.85	9.78	2.92
Histidine	2.81	4.61	2.22
Lysine	3.14	5.72	4.28
Arginine	6.83	10.83	4.61

* Adults fed with *Spirulina* (Castro, 1993)

Table II. Fatty acid content of *Panagrellus* and *Artemia* (% total lipids).

Essential fatty acid	Chemical structure	Oats	Oats+*Spirulina*	*Artemia*
Palmitic acid	C16:0	16.3	19.26	15.5
Palmitoleic acid	C16:1	0.67	2.12	13.3
Stearic acid	C18:0	2.44	3.47	6.5
Oleic acid	C18:1	34.55	35.04	14.3
Linoleic acid	C18:2	24.46	22.92	28.3
Linolenic acid	C18:3w6	0.11	0.35	4
Eicosenoic acid	C20:1	2.12	1.68	
Eicosadienoic acid	C20:2	0.27	0.61	
Eicosatrienoic acid	C20:3	1.3	0.67	
Arachidonic acid	C20:4	0.81	0.04	0.7
Eicosapentanoic acid	C20:5	0.67	0.06	1.4
Heneicosanoic acid	C21:0	0.14	0.32	
Behenic acid	C22:0	0.04	0.1	
Erucic acid	C22:1		0.07	
Docosadieenoic acid	C22:2	0.3		
Docosahexaenoic acid	C22:6	0.8	0.12	
Total number of analyses		21	25	

In Table III, the quantities of calcium and phosphorus in the experimental mixtures are shown.

Table III. Calcium and phosphorus content of *Panagrellus.*

Experimental mixture	meq $Ca.100^{-1}$	Calcium (%)	meq $P.ml^{-1}$	Phosphorus (%)
Oats	0.29	0.29	0.0024	0.6
Oats+egg shell	0.5	0.5	0.0021	0.52
Oats+*Spirulina*	0.46	0.46	0.0016	0.4
Oats+eggshell+*Spirulina*	0.54	0.54	0.0024	0.57

Discussion

The essential amino acids (threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine) were found to be present both in the organisms cultivated only with oats and those that obtained oats enriched with *Spirulina*. However, in the organisms fed with this alga, a greater percentage of each amino acid could be seen. Moreover, methionine was not found in the organisms obtained only by oats. In the case of the essential fatty acids (linoleic and linolenic acids) required for aquatic animals, they occurred in both cases. It should be noted that other fatty acids important in the diet of crustaceans and fish such as palmitic, oleic, arachidonic, and docosahexaenoic

acids were found to be present (Watanabe et al., 1978; 1980; 1983; Léger et al., 1986; Navarro, 1992),

Although these acids are not present in great quantities, taking them one by one, it is important to note that this is probably due to the fact that the analysis carried out was qualitative, so that the percentage was diluted in order to detect a greater number of fatty acids. Comparing them with *Artemia*, the microworms have similar quantities of some of the amino acids and essential fatty acids. This is especially the case when they have been enriched with *Spirulina*, for which reason they should be considered as an option for the feeding of the larval stages of fish and crustaceans. They could be used either as a complementary food source or as a substitute one when *Artemia* is found to be too costly. The calcium and phosphorus content is sufficient to cover the needs of fish, but is inadequate for those of the crustaceans (Davis and Lawrence, 1997).

References

Beckman. 1985. The System 6300 Series High performance amino-acids analysers. Instruccion Manual. Spinco División of Beckman, Division Instrument. Palo Alto, Cal. 294p.

Castro B.T. 1993. Biología y cultivo de *Artemia franciscana* en el Ex_Lago de Texcoco, Ecatepec, Estado de México. (Ph.D. Thesis). Facultad de Ciencias. UNAM. México, D.F. 72p.

Davis D.A. and A.L. Lawrence. 1997. Minerals. 150-163. In: Crustacean Nutrition. L.R. D'Abramo, D.E. Conklin, and D.M. Akiyama (Eds.). The World Aquaculture Society. Louisiana. 587p.

Leger P. D.A. Bengtson, K.I. Simpson, and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as food source. Oceanogr. Mar. Biol. Ann. Rev.521-623.

Navarro J.C., F. Amat, and J.R. Sargent. 1992. Lipid composition of cysts of the brine shrimp, *Artemia* sp. from Spanish populations. J. Exp. Mar Biol. Ecol. 155:123-131.

Watanabe T. F. Oowa, C. Kitajima, and S. Fujita. 1978. Nutritional quality of brine shrimp *Artemia salina* as a living feed from the viewpoint of essential fatty acids for fish. Bull. Jap. Soc. Sci Fish. 44:1115-1121.

Watanabe T. F. Oowa, C. Kitajima, and S. Fujita 1980. Relationship between dietary value of brine shrimp *Artemia* and their content of ω3 highly unsaturated fatty acids. Bull. Jap. Soc. Sci Fish. 46:35-41.

Watanabe T., T. Tamiya, A. Oka, M. Hirata, C. Kitajima, and S. Fujita. 1983. Improvement of dietary value of live foods for fish larvae by feeding them on ω3 highly unsaturated fatty acids and fat soluble vitamins. Bull. Jap. Soc. Sci Fish. 49:471-479.

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PROLONGED *ARTEMIA* ENRICHMENT: AN EASY TOOL TO REDUCE THE *ARTEMIA* CONSUMPTION IN MARINE FINFISH HATCHERIES

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Introduction

Due to high market prices of *Artemia* cysts caused by the high demand and high variability in availability, the need has grown to use these resources in a more efficient way. Although many efforts are being made in the development of live food replacement diets, *Artemia* is still indispensable for optimal performance of marine fish larvae.

Prolonged *Artemia* enrichment using PROLON® (Inve Aquaculture NV, Belgium) results in a 25-30% nauplius biomass increase, allowing a 30% lower *Artemia* feeding compared to standard practices. In this paper, production-scale test results on sea bass and sea bream are presented.

Materials and methods

The trials were carried out in two different Mediterranean hatcheries using sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) larvae. Culture volumes were 6000 l in hatchery 1 and 20 000 l and 10 000 l in hatchery 2 for the sea bass and sea bream trial, respectively. The trials with prolonged *Artemia* enrichment were performed in duplicate and compared to control tanks (standard enriched *Artemia*).

The control treatments were fed with standard enriched *Artemia* (GSL *Artemia* cysts, Inve Aquaculture NV, Belgium). Larvae in the Prolon treatments were fed with prolonged enriched *Artemia*. Prolonged enrichment is an additional 24h enrichment, performed after the standard enrichment. Prolonged enrichment was carried out at a nauplius density of 200-250.ml^{-1}. The temperature during enrichment was kept at 28°C. The PROLON® was added in 3 ratios spread over 24h. The first enrichment dose was 400ppm, and the second and third doses were 200ppm each.

The feeding regime for the larvae in the control treatments was the standard feeding regime used in the two hatcheries. The feeding regime for the Prolon treatments consisted of a 30% *Artemia* reduction (in number of individuals) compared to the control feeding regime from days 30 or 38 post-hatch onwards, for sea bass and sea bream.

The fry were weaned around day 57 post-hatch. Final evaluation of the trials was performed at the first grading (around day 70). Parameters taken into consideration for evaluation of the trials were survival, *Artemia* consumption, larval growth, deformity levels, and resistance to induced stress (salinity shock, after Dhert et al., 1992).

Results and discussion

Hatchery 1, Sea bream trial. An overview of the results obtained in this trial is given in Table I.

Table I. Results of the sea bream experiment in hatchery 1 (6000-l tanks).

Criteria	Control 1	Prolon 1	Control 2	Prolon 2
Biomass at day 70 (g)	7880	9380	7200	7245
Individual wet weight at day 70 (mg)				
Big	213	204	99.4	90
Small	123	132	52.2	38
Individual wet weight at day 85 (mg)	400	416	149	129
% Big/small at 1st grading	74/26	68/32	25/75	17/83
Mortality during weaning (%)	0.4	0.4	5.6	3.5
Quality check at day 85 (%)				
Lordosis	9.5	5.2	4.7	2.3
Incomplete operculum unilateral	10.0	2.8	15.5	9.0
Incomplete operculum bilateral	1.0	1.2	0	0
Normal fish	81.5	92.0	79.8	88.7
Cumulative Stress Index				
At day 40	93	80	127	78
At day 70	22	20	131	95
Artemia consumption (kg.million^{-1} fry produced)	86	61	70	42

The biomass output was comparable for all tanks, with the exception of the Prolon tank in the first replicate. The weaned fry obtained in the Prolon treatments were smaller compared to the fry in the control treatment, but the

difference became less obvious at the second grading (day 85). In the control treatment, a higher incidence of lordosis was obtained compared to the Prolon treatment and this again for both replicates. The percentage of fry having a partial lack of operculum was also lower for the Prolon treatment. Another positive effect obtained through the Prolon feeding was the higher resistance during the salinity stress test. The fact that the prolonged enriched metanauplii showed a higher Σ(n-3)HUFA level (results: not shown), could have had a contribution to the increased stress resistance of the larvae. Mortality during weaning was low for both treatments in the two replicates. Regarding the *Artemia* consumption, a considerable reduction was obtained for the Prolon treatments in both replicates. A decrease of 30% was shown in the first replicate and 40% in the second replicate, bringing the *Artemia* consumption down to 61 and 42kg.million^{-1} fry produced, respectively.

Hatchery 2, Sea bream trial. The evaluation of this trial was performed around day 60 (end weaning) and an overview of the results is given in Table II.

Table II. Results of the sea bream experiment in hatchery 2 (10 000-l tanks).

Criteria	Prolon 1	Prolon 2	Control
Individual Wet Weight at day 60 (mg)			
Big	96	69	97
Small	39	35	57
% Big/small at 1st grading	70/30	53/47	67/33
Mortality during weaning (%)	0.16	0.09	0.14
Quality check at day 100 (%)			
Lordosis	0	0	0
Incomplete operculum	9.4	6.0	12
Head deformities	1.3	2.5	5
Artemia consumption (kg.million^{-1} fry produced)	46	59	84

The mortality observed during the weaning was negligible in all tanks. Also in hatchery 2 the individual wet weights were lower for the fry in the Prolon treatment at day 60, confirming the results obtained in hatchery 1. The deformity levels were again lower for the Prolon treatment. Also in this trial, the *Artemia* consumption was reduced considerably with 46% for Prolon 1 and 30% for Prolon 2, compared to the control tank.

Hatchery 2, Sea bass trial. Results from the large-scale trial on sea bass are presented in Table III. The first replicate was evaluated at day 65, the second replicate at day 75.

In the sea bass trial, the fry that received the prolonged enriched *Artemia* were not smaller compared to the fry in the control tanks. This could be due to the fact that the *Artemia* reduction was lower than the foreseen reduction: in the first replicate 16% less *Artemia* was fed compared to the control and in the second replicate the reduction was only 7%. This can be explained by the fact that the *Artemia* consumption in the feeding regime for the Prolon treatment was too high, caused by an overestimation of the number of larvae present in the tank. Deformity levels for the two treatments were comparable.

Table III. Results of the sea bass experiment in hatchery 2 (20 000-l tanks).

Criteria	Prolon 1	Control 1	Prolon 2	Control 2
Individual wet weight (mg)	224	191	243	238
Mortality during weaning (%)	0.70	0.55	0.65	0.68
Quality check at day 100 (%)				
Lordosis	1.3	8.4	7.9	3.6
Incomplete operculum	3.9	0	1.1	5.4
Head deformities	3.9	0	1.1	5.4
Normal fish	90.0	91.6	89.9	85.6
Artemia consumption (kg.million^{-1} fry produced)	79.3	93.9	53.4	57.4

Conclusions

These production scale trials showed that Prolon could easily be adapted in commercial sea bream and sea bass hatcheries. The performance of the Prolon tanks, fed with prolonged enriched *Artemia* showed to be equal or superior to the control treatment in terms of survival, deformity levels and resistance to stress conditions (not verified for sea bass). Only the size of the sea bream fry around day 70 was smaller in the Prolon treatment compared to the control treatment. This phenomenon was however not observed in the Sea bass trial.

As a conclusion it can be said that Prolon is an easy to apply solution for marine finfish hatcheries helping to reduce their *Artemia* consumption.

Reference

Dhert P., P. Lavens, and P. Sorgeloos. 1992. A simple test for the evaluation of cultured fry of marine fish. Med. Fac. Landbouw Univ. Gent. 57:2135-2141.

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PROGRESS AT PRODUCTION SCALE WITH VARIOUS *ARTEMIA* SUBSTITUTES AND SUPPLEMENTS FOR MARINE FISH LARVAE

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Abstract

Over the last few years, *Artemia* sources have been available in variable quantities, forcing marine hatcheries to adjust their rearing methodology in function of this natural resource. Different approaches have been investigated such as improved weaning strategies, the use of high quality early weaning diets, and the optimization of the *Artemia* consumption. All these approaches were verified at production scale in European sea bream and sea bass hatcheries. *Artemia* consumption was reduced by appropriate weaning strategies based on revised feeding regimes. Using Inve's existing high-quality weaning diets, *Artemia* consumptions for the production of European sea bass and sea bream were decreased from 150kg to 90kg and from 120kg to 70kg cysts per million fry produced, respectively.

New high-quality early weaning diets, developed through modern feed processing technology including live food substitution components, could further reduce the *Artemia* consumption by another 30-40% without impairing larval survival and quality. A more efficient use of *Artemia* was achieved by the introduction of a prolonged *Artemia* enrichment technique that allowed a 25-30% nauplius biomass increase. Adapting the feeding regime in terms of this biomass increase provided a further decrease in *Artemia* cysts consumption. Besides the economical benefit, an improved quality was observed for the fry fed with prolonged enriched *Artemia* metanauplii.

Above-mentioned strategies showed that the *Artemia* consumption for sea bass and sea bream could be reduced considerably, although the lower limit to which the *Artemia* consumption can be decreased has not been determined until now. The effect of a drastic *Artemia* reduction without compromising larval survival and quality in terms of deformity levels still needs to be investigated.

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IDENTIFICATION OF INTESTINAL ANAEROBIC BACTERIA OF CULTURED FOODFISH AND SHRIMP FOR POTENTIAL USE AS PROBIOTICS

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Introduction

The normal intestinal anaerobic bacterial flora of fish and shrimp, for the most part, remains unknown. The roles of these anaerobic bacteria in disease, digestion, nutrition, and the immune response have never been thoroughly investigated in lower animals. The purpose of this ongoing research is to isolate and to identify the anaerobic intestinal flora of cultured fish and shrimp. To date, the following species have been sampled: rainbow trout (*Oncorhynchus mykiss*), southern flounder (*Paralichthys lethostigma*), tilapia (*Oreochromis* spp.), channel catfish (*Ictalurus punctatus*), ornamental fish *(Astronotus ocellatus* and *Pterophyllum scalare*), and shrimp (*Litopenaeus vannamei*). Several anaerobic genera, including *Bacteroides*, *Clostridium*, *Fusobacterium*, and *Peptostreptococcus*, were identified from fish and shrimp intestines. Enzyme analyses of bacteria from shrimp were found to produce esterase, galactosidase, and fucosidase. Enzymatic profiles of bacteria from fish were found to produce trypsin, lipase, esterases, phosphohydrolase, leucine arylamidase, acid and alkaline phosphatases, and α- and β-glucuronidases**.**

The important role of the normal anaerobic intestinal flora has been elucidated in man and domesticated animals such as ruminants, pigs, and chickens. It is now recognized that the indigenous intestinal anaerobic microflora play a substantial role in maintaining individual health. The anaerobic gut flora of humans and other animals are known to be involved in a variety of functions, including enzymatic digestion of food residues, synthesis of vitamins, suppression of reactions which result in the generation of carcinogenic metabolites, and detoxification of potentially toxic substances (Hentges, 1992; Rowland, 1992). The presence of anaerobes in lower vertebrates and invertebrates, let alone their importance, is largely unknown and unstudied (Sugita et al., 1982). Identifying the diversity of the microbial populations in the intestinal ecosystem may

favorably impact the propagation of species in aquaculture. Once identified, understanding the physiological interactions of the normal flora and host may have implications for the future. For example, new dietary formulations utilizing anaerobic flora as probiotics may effectively combine enhanced nutrition with disease prophylaxis.

Materials and methods

Intestines of fingerling rainbow trout (*Oncorhynchus mykisss*), juvenile tilapia (*Oreochromis* spp), channel catfish (*Ictalurus punctatus*), southern flounder (*Paralichthys lethostigma*), angelfish (*Pterophyllum scalare*), oscar (*Astronotus ocellatus*), and adult shrimp (*Litopenaeus. vannamei*) were removed for sampling of anaerobic bacteria. Sampling for obligate intestinal anaerobes was carried out in a Bactron II anaerobe chamber containing a mixture of 5% H_2, 5% CO_2, and 90% N_2 gases. Bacterial samples were plated onto pre-reduced anaerobically sterilized media (PRAS) to eliminate oxygen stress associated with other anaerobic culture methods. Both nonselective media such as Brucella blood agar and media selective for clostridia and bacteroids were used for isolation. Plates were incubated at 25-28°C for a minimum of 48h. Aerotolerance testing was performed on chocolate agar in a 5%-CO_2 atmosphere. Gram reaction, colony morphology, and aerotolerance identified isolated anaerobes. Biochemical reactions were used to identify isolates to genus. Isolates also were inoculated onto An-Ident and Minitek multitest systems (Summanen et al., 1993). Fatty acid profiles of selected isolates were determined by gas chromatography on the MIDI system (Jang and Hirsh, 1991). To determine the effect of antimicrobial drugs on the normal anaerobic gut flora, minimum inhibitory concentrations (MIC) to tetracycline and metronidazole were performed for selected isolates using the Etest (Sanchez and Jones, 1992). The resistance to kanamycin (1000ug), vancomycin (5ug), and colistin (10ug) was determined for all isolates (Dixon, 1997; Quinn, 1996).

To facilitate identification of bacterial enzymes, the API ZYME system, containing a panel of 19 substrates, was used.

Results and discussion

Preliminary data indicate that the diversity of anaerobic flora varies with host species. To date, several anaerobic genera, including *Bacteroides*, *Clostridium*, and *Fusobacterium*, have been identified from the intestinal tracts of ornamental fish and channel catfish. *Clostridium* and *Peptostreptococcus* were isolated from tilapia. Only species of *Clostridium* were isolated from the guts of rainbow trout and southern flounder. Based on these limited results, it appears that the anaerobic gut flora of warmwater fish is more abundant and diverse than that of coldwater fish. Several genera of anaerobic bacteria were also identified from

shrimp. Two Gram-positive genera – *Clostridium* and *Peptostreptococcus* – and two Gram-negative genera – *Bacteroides* and *Fusobacterium* – were present in the gut of sampled shrimp. However, most isolates from shrimp and fish could not be identified beyond the genus level, indicating that new, previously undescribed species may be present. Most of the isolates did not identify with any of the code numbers in either the An-Ident or Minitek systems. Identification was not expected because these multitest systems contain very few environmental isolates within their databases. Because very little data is available on the normal anaerobic intestinal flora of not only the species in aquaculture, but also those in the wild, much more research is needed to provide both biochemical and genetic databases for the identification of this group of bacteria.

Enzyme analyses of *Bacteroides* from shrimp were found to produce esterase, alpha-galactosidase, and alpha-fucosidase. Enzymatic profiles for isolates of *Clostridium* sp., *Fusobacterium* sp., and *Peptostreptococcus* sp. from shrimp intestinal tracts were found to produce trypsin, lipase, alpha and β-glucuronidase**.** Enzymatic profiles of bacteria from fish were found to produce trypsin, lipase, esterases, phosphohydrolase, leucine arylamidase, acid and alkaline phosphatases, and α- and β-glucuronidase. The bacterial enzyme profiles from ornamental fish were more extensive than those of southern flounder and trout.

Antibiotic treatment may also effect normal anaerobic flora. During this study, hatchery rainbow trout fingerlings were placed on oxytetracycline therapy. Prior to antibiotic treatment, only one organism isolated from trout was resistant to tetracycline, but following the initiation of treatment, twelve isolates were resistant to tetracycline. Bacterial resistance to metronidazole was less common than that evidenced to tetracycline, with only four isolates from rainbow trout showing resistance. Metronidazole is considered the universal drug of choice for anaerobic infections, and only works under anaerobic conditions. Although its use is limited in aquaculture, metronidazole is heavily utilized in the production of ornamental fishes to control infections with intestinal flagellates like *Spironucleus* and *Hexamita*. The MIC values for bacteria isolated from ornamental fish were higher than those for bacteria isolated from trout and southern flounder. The effect of other antibiotics used in aquaculture on the normal anaerobic gut flora of fish and shrimp remains unknown.

Standard techniques used in clinical laboratories were utilized for the isolation and identification of environmental bacteria. While these techniques are routinely applied to clinical samples, they are rarely utilized for environmental isolates. This research indicates that commercially available media for anaerobic growth and cultivation can be successfully utilized for processing environmental samples. Using this technology, the normal anaerobic intestinal flora of cultured shellfish and foodfish can be isolated and partially identified. The role of

anaerobic flora in such metabolic processes as digestion, absorption, and host defense can further be delineated. Understanding the interactions of the normal flora may have implications for future development of diets. New dietary formulations utilizing anaerobic flora as probiotics may effectively combine enhanced nutrition with disease prophylaxis.

References

Dixon B.A. 1997. Identification of intestinal anaerobic bacteria of cultured foodfish and shrimp for potential use as probiotics. Abstract. IV Congresso Ecuatoriano de Acuicultura. Guayaquil, Ecuador, October 1997.

Jang S.S. and D.C. Hirsh. 1991. Identity of *Bacteroides* isolates and previously named *Bacteroides* spp. in clinical specimens of animal origin. Am. J. Vet. Res. 52:738-741.

Quinn H. 1996. Anaerobic bacteria of the gastrointestinal tract of the rainbow trout (*Oncorhynchus mykiss*) and angelfish (*Pterophyllum scalare*). Masters Thesis. Department of Biological Sciences, California State University, Hayward, CA

Sanchez M.L. and R.N. Jones. 1992. Etest, an antimicrobial susceptibility testing method with broad clinical and epidemiological application. The Antimicrobial Newsletter: 8 (#1).

Sugita H., A. Enomoto, and Y. Deguchi. 1982. Intestinal microflora in the fry of *Tilapia mossambica*. Bull. Jap. Soc. Sci. Fish. 48:6.

Summanen P., E. Baron, D. Citron, H. Wexler, and S. Finegold. 1993. Wadsworth Anaerobic Bacteriology Manual, 5th ed. Star Publishing.

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REPRODUCTIVE PERFORMANCE AND OFFSPRING QUALITY IN MUD CRAB (*SCYLLA PARAMAMOSAINE*) BROODSTOCK FED DIFFERENT DIETS

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Introduction

Studies on crustacean broodstock nutrition began concertedly during the last decade with the growing demand for controlled reproduction in commercial facilities. However, reliable data on the nutrient requirements specific to maturation, reproduction and embryogenesis in crustaceans are scant and fragmentary (Harrison, 1990). At present, the only available data on broodstock maturation diets for mud crab is from research conducted by Millamena and Quinito (2000). Therefore to improve the understanding of the important role of nutrition in maturation of mud crab, a series of studies was conducted with the aim of establishing standard techniques for mass production of berried females producing high quality larvae. In the present study, the nutritional value of diet composed of a mixture of fresh food items was compared with two types of artificial diets.

Material and methods

The experiment was conducted in concrete tanks of 2.0×5.0×0.8m using mud (at a thickness of 20cm) as tank substrate. The tanks were filled with filtered seawater to a depth of 60cm and aerated. Salinity was maintained at 30±1ppt and temperature at 28±1°C. The water in each tank was replaced 100% daily with fresh seawater.

Before test initiation, mud crab females with an average individual wet weight of 254±51g were acclimated and reared together in one experimental tank. They were fed with a mixture of fresh food (stingray, squid, shrimp, and trash fish) until they spawned. After spawning, the crabs were weighed, measured (width and length of carapace), labeled, and eyestalk ablated. The spent spawners were randomly divided over three tanks and subjected to the dietary treatments. A

mixture of fresh food containing 20% *Artemia* biomass was used as the control diet. The control diet was compared to two types of artificial diets, diet A1, based on Marsden et al. (1997) and diet A2, based on an experimental formulation for *Liptopenaeus vannamei* broodstock. The crabs were fed the experimental diets twice a day with the daily amount calculated as 20% of total body weight.
Gonadal maturation was first checked one week after ablation. Thereafter, survival and reproductive output were observed daily and every three days, respectively. Crabs that spawned were transferred to 2-m^3 tanks for egg incubation and hatching. Fecundity was estimated from the total egg mass weight and the number of eggs in triplicate egg samples, then the total number. The total number of zoea produced per female was calculated by counting triplicate 1-l samples from the hatching tank. Offspring quality was evaluated through starvation test.

Statistical analysis was performed through analysis of variance, followed by Tukey's multiple range test (Sokal and Rohlf, 1995).

Result and discussion

The results presented in Table I showed that the crabs fed diet A2 had a significantly better hatching rate (95.3%) compared to the control diet (89.2%). No significant difference of hatching rate was observed for the crab fed diet A1 (90.9%) compared to diet A2 (95.3%). Survival of larvae under starvation conditions (Table II) was however significantly better when they originated from broodstock fed the control diet (66.0% at 60h, 52.5% at 72h, and 45.0% at 84h) compared to those fed diet A1 (49.0% at 60h, 20.3% at 72h, and 0% at 82h) and diet A2 (45.5% at 60h, 18.0% at 72h, and 0% at 82h).

Table I. Reproductive performance of mud crab broodstock fed various diets.

Parameter	Type of food		
	Control	Diet A1	Diet A2
Maturity (% of total females)	100	100	100
Spawning (% of total females)	100	100	100
Hatching (% of total females)	100	100	100
Latency period (d)	21.8±1.0	22.8±4.0	21.3±2.8
Incubation time (d)	9.0±0.8	8.6±0.9	8.8±1.0
Fecundity (eggs.female^{-1} $\times 10^6$)	2.33±0.61	2.10±0.31	2.28±0.35
Fertilization rate (%)	71.1±12.1	82.1±11.4	83.7±13.3
Hatching rate (%)	89.2±2.2^{b}	90.9±3.8^{ab}	95.3±1.7^{a}
Zoea production (larvae.female^{-1} $\times 10^6$)	2.07±0.55	1.91±0.31	2.18±0.38
Phototactive larvae (% of total)	99.5±0.3	96.8±3.0	97.5±1.5

Research on nutrient requirements for broodstock maturation relies greatly on formulated diets. These artificial diets offer after all many advantages compared to fresh feed, including a reliable supply, minimal preparation time and known nutrient content. Moreover, they offer the opportunity to orally administer drugs such as hormones or supplementary vitamins (Marsden et al., 1997). Millamena and Quinito (2000) reported that provision of a formulated diet in combination with natural food results in improved consistency in reproductive performance of mud crab broodstock. The results of this study suggest that the sole use of the artificial feeds tested here could not improve reproductive performance.

Table II. Survival (%) of mud crab larvae under starvation conditions.

Exposure (h)	Type of broodstock diet		
	Control	Diet A1	Diet A2
0	100	100	100
12	100	100	100
24	95.0±1.2	95.0±6.0	93.5±1.9
36	91.0±2.6[a]	87.0±6.0[b]	87.5±1.9[ab]
48	77.3±5.6	66.5±9.0	67.5±3.4
60	66.0±6.7[a]	49.0±2.6[b]	45.5±2.6[b]
72	52.5±5.3[a]	20.3±2.1[b]	18.0±3.7[b]
84	45.0±3.0	0	0
96	30.5±7.9	0	0
108	25.5±7.5	0	0
120	18.5±2.5	0	0

In literature, much effort has been directed to study lipid requirements during broodstock maturation. It has been reported that dietary highly unsaturated fatty acids (HUFA) and the n-3/n-6 ratio could improve the reproductive performance and hatchability of eggs in tiger shrimp (Millamena, 1989). Contrasting results were however reported by Marsden et al. (1997). The results of our study, however, showed no significant effect of the different levels of n-3 HUFA in the diets on reproductive performance of mud crab broodstock. Despite the essential fatty acid value in fresh food (control diet) being lower than that of artificial diets (Table III), broodstock fed fresh food produced stronger larvae (as determined by the starvation test). This might be due to the fact that the fresh food contained higher levels of other essential nutrients (e.g., protein level, 66.75% for the control versus 40.43-43.3% for the other diets), which apparently played a more crucial role during starvation. It has been proven that the different

protein levels in the broodstock diets resulted in a different protein levels of larvae produced (Table III), and this could apparently affect larval survival under starvation condition.

Table III. Biochemical composition of fresh food (FF) and formulated diets (A1 and A2) used, and mud crab larvae.

Nutrient	Type of broodstock diet			Mud crab larvae		
	FF	A1	A2	FF	A1	A2
Protein (%)	66.75	40.43	43.33	38.12	30.14	31.06
20:5n-3 (EPA, $mg.g^{-1}$)	1.62	5.51	4.90	3.97	4.58	4.11
22:6n-3 (DHA, $mg.g^{-1}$)	5.20	12.35	8.17	4.08	4.98	4.32
Σ n-3 HUFA ($mg.g^{-1}$)	7.08	19.79	14.32	8.85	11.46	9.72

References

Harrison K.E. 1990. The role of nutrition in maturation, reproduction, and embryonic development of decapod crustaceans: A review. J. Shellfish Res. 9(1):1-28.

Marsden G.E., J.J. Guren, S.W. Hansford, and M.J. Burke. 1997. A moist artificial diet for prawn broodstock : its effect on the variable reproductive performance of wild caught *Penaeus monodon*. Aquaculture 149:145-156.

Millamena O.M. 1989. Effect of fatty acid composition of broodstock diet on tissue fatty acid patterns and egg fertilization and hatching in pond reared *Penaeus monodon* Fabricius broodstock. Asian Fish. Sci. 22:127-134.

Millamena O.M. and E. Quinitio. 2000.The effects of diets on reproductive performance of eyestalk ablated and intact mud crab *Scylla serrata*. Aquaculture 181:81-90.

Sokal R.R. and F.J. Rohlf. 1995. Biometry. The principles and practice of statistics in biological research. W.H. Freeman and company, San Francisco. 887p.

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INFECTION BY ECTOPARASITES IN CULTURED *PANGASIUS* CATFISH FINGERLINGS IN THE MEKONG DELTA

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Introduction

The freshwater Asian catfish, *Pangasius hypophthalmus*, is an indigenous fish species within the Mekong Delta. Successful artificial production *of P. hypophthalmus* in the late 1980's in Vietnam provided a year-round supply of fish seed (Cacot, 1999), and the number of families raising this fish has increased. Culture occurs in both cages and pond systems with cage culture being a more intensive production system compared with the polyculture practices, which are found in rurally located ponds. The increased intensification has enhanced fish larval mortalities predominantly due to ectoparasites. Many protozoan infections have been identified in cultured *P. hypophthalmus* where the main cause of parasite loading has been from *Microsporidia* species. Studies on the various parasite fauna related to the larval fish mortalities and the chemical treatments applied are described in this manuscript.

Materials and methods

Fingerlings were between 25 to 45 days old and collected from ponds and cages located in An Giang, Dong Thap and CanTho Provinces, Vietnam from July to December 2000. In total, 123 *P. hypophthalmus* and 32 *P. boucouti* fingerlings were collected. Samples for parasitological analyses were taken according to the method of Dogiel et al. (1933) cited by Ha Ky (1968). The mucus on the skin, fins and gills were taken and smeared onto clean microscope slides and ecto- and endoparasites observed by light microscopy at 10× and 40× objective. White-coloured cysts found in the muscle, liver, spleen and kidney also were taken and examined microscopically at the same objective. Both the infection rate (number of infected fish per total number of fish sampled and presented as percentage) and the intensity of the infection (average number of parasite per magnification at 10×) were calculated from the observational studies.

Diseased *P. hypophthalmus* fingerlings infected with species of *Trichodina* and *Dactylogyrus* or *Microsporidia* and *Mixobolus* sp and obtained from nursing ponds in Omon district in CanTho province. Experimental fish were randomly placed into 24 groups with 60 fish per group. Each group was maintained in an aquarium (70 l) containing 40 l of water. Oxygen was supplied by air-stone during the experiment. Dead fish were removed and counted before being checked for parasites. For each treatment applied the infection rate was calculated and observations were made on fish behavior throughout the experimental time (10 days).

Commercial Formalin (laboratory-grade, labeled as 37% formaldehyde) and Neguvon (96% Trichlorphon) were purchased from Bayer company, Vietnam. Trichlorphon. is also commercially recognized as Metrifonate, Masoten and Chlorophos. The fish groups received 20, 30, or 40ppm formalin or 0.5, 1.0, or 1.5 ppm Neguvon by bath with the chemicals for 6 hours every second day for a total of 6 days. The control fish groups received no chemical treatments and all studies performed in triplicate.

Results

The highest percentage infection in *P. hypophthalmus* fingerlings was found with *Trichodina* species and the lowest percentage infection was with *Dactylogyrus* species (Table I). The parasite species identified were *Microsporidia, Mixobolus, Henneguya, Trichodina, Chilodonella*, and *Dactylogyrus*. All species were found in *P. hypophthalmus* fingerlings. Only *Microsporidia* and *Dactylogyrus* were found throughout the experimental sampling period.

Table I. Baseline data on percentage infection and intensity of infection on *P. hypophthalmus* fingerlings.

Parasite Species	Infection rate (%)	Intensity of infection
Trichodina	93.33	5-27
Dactylogyrus	33.33	2-7
Microsporidia	46.67	0-4
Mixobolus	60.00	1-3

Administration of formalin had little effect on the level of infection for *Microsporidia* and *Mixobolus* species (Table II). No infection by *Trichodina* or *Dactylogyrus* species occurred when formalin was administered at 40ppm only. The percentage mortality in the formalin-treated fingerling groups increased as the dose applied extended from 20 to 40ppm. However, the treatments did reduce the percentage mortality when compared with the control group that had the highest mortality.

No infection with *Trichodina* was found in any of the Neguvon dose groups compared with the control, which had 100% infection rate with this parasite (Table II). Neguvon also reduced infection by *Dactylogyrus* species but only at 1 and 1.5ppm. The highest dose of Neguvon applied lowered the infection rate by *Microsporidia* and *Mixobolus* parasites slightly compared with the control fingerling groups. The highest percentage mortality was found in the control group compared with any of the Neguvon-treated groups.

Table II. Effect of formalin (F) and Neguvon (N) treatments on infection rate, intensity of infection, and total percentage mortality.

Treatment (ppm)		Parasite species	Infection rate (%)		Intensity		Total mortality rate (%)	
F	N		F	N	F	N	F	N
20	0.5	*Trichodina*	13	0	2-13	0	8±1	11±3
		Dactylogyrus	17	10	1-3	0-3		
		Microsporidia	42	47	0-5	0-4		
		Mixobolus	63	62	0-3	1-3		
30	1.0	*Trichodina*	0	0	0	0	11±2	11±5
		Dactylogyrus	3	0	0-2	0		
		Microsporidia	47	47	0-4	0-4		
		Mixobolus	58	58	1-3	1-2		
40	1.5	*Trichodina*	0	0	0	0	18±7	14±7
		Dactylogyrus	0	0	0	0		
		Microsporidia	38	38	0-3	0-4		
		Mixobolus	62	55	1-2	0-3		
Control		*Trichodina*	97	100	5-51	5-37	40±2	41±1
		Dactylogyrus	43	42	2-9	2-7		
		Microsporidia	43	43	0-4	0-4		
		Mixobolus	65	65	0-3	0-4		

Mortality rate is average ± standard deviation.

Discussion

The classification system devised by Ha (1968) to identify the parasite fauna of freshwater fish in Northern Vietnam was used in this experiment with infected *Pangasius* species. The parasite species identified included *Microsporidia, Mixobolus, Henneguya, Trichodina, Chilodonella*, and *Dactylogyrus*. These are common species found in freshwater systems and have been found in many other cultured fish species in the Mekong Delta (Te, 1998).

The results from this study showed that both formalin and Neguvon treatments reduced the infection rate of *Trichodina* and *Dactylogyrus* species in *P. hypophthalmus* fingerlings. It was interesting to observe that neither treatment at any dose applied appeared to affect the infection rate of *P. hypophthalmus* fingerlings to *Microsporidia* and *Mixobolus* species when compared with the infection rate in the control fish that had received no treatments.

The highest percentage mortality was found in the control groups for both treatments. These fingerlings had received no chemical treatments and had died from parasite related diseases. Relatively lower percentage mortalities were found in the formalin treated fingerling group at 20 and 30ppm compared with the highest dose administered at 40ppm. Similar percentage mortality was observed in all of the dose groups given Neguvon. However, all these parasitic diseases caused high mortalities in *P. hypophthalmus* and *P. bocourti* during last two years in the Mekong Delta. The results from this study would suggest that application of formalin or Neguvon may be of beneficial to families culturing *P. hypophthalmus* fingerlings by reducing parasite-related mortalities, particularly from infection by *Trichodina* or *Dactylogyrus* species and lowering stress levels thus enhancing fish health status and survival.

References

Cacot P. 1999. Study of the sexual cycle and controlled reproduction of *Pangasius bocourti* (Sauvage, 1880) and *Pangasius hypophthalmus* (Sauvage, 1878) in the Mekong delta in Vietnam. Institut National Agronomique Paris-Grignon (France). p.262. (In French, abstract in English).

Dung T.T., G. Jenny, D.T.H. Oanh, Z. Jeney, and N.A. Tuan. 1998. Results of the Baseline Survey on Fish Health Management in Freshwater Aquaculture of the Mekong Delta of Vietnam. International Conference on Fisheries and Food Security beyond the Year 2000.

Dung T.T. and N.T.N. Ngoc. 2000. Study on the Ecto-Parasite Infection in *Pangasius hypophthalmus* Fingerling. The International Workshop of the "Catfish Asia" project in Bogor (Indonesia).

Ha K. 1968. Parasite fauna of freshwater fish in North of Vietnam some measures for prevention and therapy of fish disease PhD Thesis. 253p. (in Russian with English abstract)

Kanchanakarn S. 1986. Toxicity of Dipterex to Striped Snakehead (*Channa striatus* Fowler) Sliver Barb (*Puntius gonionotus* bleeker) and Common Carp (Cyprinus carpio Linn.) M.Sc Thesis Kasetsart University, Thailand. 66p.

Kulow H., W. Schaperclaus, and K. Schreckenbach. 1992. Prophylaxis and therapy of fish diseases. Fish Dis. 1:177-201.

Readon I.S. and R.M. Harrell. 1990. Acute toxicity of formalin and coper sulfate to striped bass fingerlings held in varying salinities. Aquaculture 87:255-270.

Te B.Q. 1998. Parasitic Fauna of the Freshwater Fish of the Mekong Delta (Lower Mekong river Delta - Methods for Prevention and Treatment. The AAHRI Newsletter, Volume 7 Number 1, July 1998.

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UTILIZATION OF FIVE MARINE MICROALGAL SPECIES IN LARVAL FEEDING OF SHRIMP, *PENAEUS JAPONICUS* – I. MONOALGAL SPECIES

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Abstract

A larval feeding experiment was conducted to study the utilization of five algal species in larval feeding of *Penaeus japonicus*. Five species of algae were used: diatoms (*Chaetoceros calcitrans*, and *Skeletonema costatum*), green flagellated algae (*Tetraselmis chuii*), brown flagellated algae (*Isochrysis galbana*), and non-flagellated green algae (*Chlorella vulgaris*). Results showed that shrimp larvae of *P. japonicus* fed diatom species of *Chaetoceros* and *Skeletonema* were superior to survival rate and percent metamorphosis, followed by those fed flagellated green algae, *Tetraselmis*,. However, poor results were obtained when larvae fed non-flagellated green algae (*Chlorella*).

Introduction

The success of any shrimp farming operation depends upon the availability of a ready supply of larvae or seeds for growing to market size (Lavens and Sorgeloos, 1996). Larval feeding is a more critical factor affecting postlarval shrimp production especially in the early larval stages. Algae are widely used as food in commercial shrimp hatcheries. Coutteau (1996) reported that more than 40 different species of microalgae, isolated in different parts of the world, are cultured in pure strains in intensive culture and used in larviculture feeds. Algae in shrimp hatchery consisted of two groups "flagellate" and "diatoms". *Tetraselmis* and *Isochrysis* are flagellated algae which can be moved by action of one or more flagella. However, *Chaetoceros* and *S. costatum* are diatoms having an outer shell composed of silica, but they can remain buoyant. The most frequently used species in commercial mariculture operations are the diatoms *S. costatum*, *C. gracilis*, and *C. calcitrans*. Culture of *Chaetoceros* and *Tetraselmis* can both be easily contaminated by other

phytoplankton or zooplankton species, while *Skeletonema* is difficult to maintain during summer, especially when water temperature may rise above 30°C, but are not available year-round (Laing, 1991). The present work aims to study the utilization of five algal species – *C. calcitrans, S. costatum, T. chuii, Isochrysis galbana*, and *Chlorella vulgaris* – as food in larval nutrition of *P. japonicus*.

Materials and methods

Starter cultures of algae was obtained from SEAFDEC, Philippine. Culture of algae and preparation of *Artemia* cysts used was performed as described by Lavens and Sorgeloos (1996). Larvae were fed algae *ad libitum*. Larvae were stocked in 20 glass beakers (1-l capacity) according to Smith et al. (1991), with four beakers per treatment. Water exchange was 30-100% daily. Seawater was UV-irradiated and filtered by 5 and 10-µm cotton cartridge filters. Water temperature, salinity, pH, DO, and photoperiod for rearing were 28±1°C, 33±2 ppt, 8.5±0.2, and 7±1ppm and 12 D/L,, respectively. Shrimp larvae were counted daily, measured, and observed under a binocular microscope. About 10-30 larvae from each replicate in all experiments were dried on a glass slide at 60°C for 48h. Analysis of variance was carried out according to Snedecor and Cochran (1982) using a complete randomized design.

Results and discussion

Diatom species (*Skeletonema* and *Chaetoceros*) followed by *T. chuii*, were better in survival rate, metamorphosis, final length, and dry weight than *Isochrysis* (Table I). The poorest results were obtained with *Chlorella*. Survival of *P. japonicus* larvae fed *Skeletonema* during N_6-M_1 and N_6-PL_1 was 97.5 and 96%, respectively. These results are in agreement with results of Kuban et al. (1985) who found survival rate was 95-99% for *P. stylirostis, P. vannamei*, and *P. aztecus* reared in 1-l Imhoff cones. Feeding with *T. chuii* and *Isochrysis* during N_6-M_1 and N_6-PL_1 resulted in an 88.5-79.7% and 64.25-56 % survival, respectively (Table I). Previous results ranged between 77-96% for different species (Smith and Lawrence, 1988; Talley et al., 1988). Metamorphosis is the most important criterion for choosing a suitable algal species, since reduction of the time for metamorphosis to PL_1 and a higher percent metamorphosis is preferable. In the present study, there was a significantly higher (P>0.05) time to metamorphosis between tested algal species. *Skeletonema* and *Chaetoceros* spent seven days metamorphosing, with 100% metamorphosis to postlarvae. However *Tetraselmis* and *Isochrysis* spent eight days with 95.2%, and nine days with 40%, respectively. On the other hand, *Chlorella* spent the highest number of days (12), with the lowest percent (20%) to metamorphose (Table I). Final length was higher for larvae fed diatoms than green and brown algal species.

Dry weight of larva fed *Skeletonema*, *Chaetoceros*, *Tetraselmis*, and *Isochrysis* were 74, 70.5, 52, and 50 µg.PL^{-1}, respectively (Table I). These findings were lower than results of Kuban et al. (1985) for *P. aztecus*, *P. setiferus*, *P. stylirostris*, and *P. setiferus*. These differences may be due to the use of different species and experimental conditions. Superiority of the diatom species may be due to their small size (4-5µm.cell^{-1}) and higher contents of silica that are needed for larval molting. *Isochrysis* is rich in 22:6ω_3 content but it is not suitable for early stages (nauplius and zoea) because they are too large.

Table I. Survival, metamorphosis, final length, and dry weight of larvae fed monoalgal species.

Species of algae*	*Chaetoceros*	*Skeletonema*	*Tetraselmis*	*Isochrysis*	*Chlorella*
Cell size (µm.cell^{-1})	7	4	7	11	3
Survival % N_6-M_1	97.00^{b}	97.50^{a}	88.50^{c}	64.25^{d}	47.50^{e}
Survival % N_6- PL_1	92.00^{b}	96.0^{a}	79.75^{c}	56.30^{d}	27.00^{e}
Metamorphosis** (days)	7.00^{a}	7.00^{a}	8.00^{b}	9.00^{c}	12.00^{d}
Metamorphosis %	100^{a}	100^{a}	95.20^{b}	40.00^{c}	20.00^{d}
Final length (mm)	6.24^{a}	6.29^{a}	5.50^{b}	4.71^{c}	3.00^{0d}
DW (µg.PL^{-1})	70.5^{b}	74.0^{a}	52.0^{c}	50.00^{0d}	22.00^{e}

*Values in column having the same superscript are not significant at 0.05 level.

** Metamorphosis means period with days which spent from N_6 to PL_1

Reitan et al. (1997) stated that the fatty acids compositions of the algal species showed taxonomic similarities, with the diatoms exhibiting a high relative content of 14:0, 16:0, 16:1, and 20:5, while the green flagellated algae showed somewhat higher intraspecific variation. The content of HUFA – in particular 20:5ω_3, 20:4ω_6, and 22:6ω_3 – is of major importance in the evaluation of the nutritional composition of an algal species to be used in larval feeding. Coutteau (1996) found that significant concentrations of 20:5ω_3 are present in the diatom species and high concentrations of 22:6ω_3 are found in *I. galbana*.

References

Coutteau P. 1996. Micro-algae. p.7-48 In: Manual on the production and use of the live food for aquaculture. P. Lavens and P. Sorgeloos (Eds.), FAO Fish. Tech. Pap. 360.

Kuban F.D., A.L. Lawrence, and J.S. Wilkenfeled. 1985. Survival, metamorphosis

and growth of larvae from four species fed six food combinations. Aquaculture 47:151-162.
Laing I. 1991. Cultivation of marine unicellular algae. MAFF Laboratory Leaflet No. 67. Directorate of Fisheries Research Lowestoft, UK, 31p.
Lavens P. and P. Sorgeloos. 1996. Manual on the production and use of the live food for aquaculture. FAO, Fisheries Technical paper 360, No. 361.
Reitan K.I., J.R. Rainuzzo, G. Oie, and Y. Olsen. 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155:207-221.
Smith L., T.M. Samocha, J.M. Biedenbach, and A.L. Lawrence. 1991. Use of one–liter Imhoff cones in larviculture production. p.287-300. In: Marine shrimp culture: principles and practices. A.W. Fast and L.J. Lester (Eds.) Dev. Aquacult. Fish. Sci. 23.
Smith L.L.and A.L.Lawrence. 1988. The importance of fatty acids in a dry feed used as a complete replacement for *Artemia*. J. World Aquacult. Soc. 19:65A.
Snedecor G.W. and W.G. Cochran. 1982. Statistical methods 6th ed. Iowa State University. Press, Ames. Iowa.
Talley S.E, L.L. Smith, and A.L. Lawrence. 1988. The effect of various commercial artificial seasalts on the growth of *P. vannamei*. J. World Aquacult. Soc. 19:69A.

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THE USE OF ROTIFERS CULTURED ON DIFFERENT MICROALGAL SPECIES IN LARVAL FEEDING OF SEABASS, *DICENTRARCHUS LABRAX*

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Introduction

Production and rearing of fish larvae have been identified as major constraints to many aquaculture processes. Availability of a reliable and highly nutritional larval food is one of the crucial demands at this production stage. Rotifers, *Brachionus plicatilis*, have been widely used in marine hatcheries due to their ideal size, quick reproductive rate, and ability to feed on different algae, formulated diets, and baker's yeast. However, rotifers fed diets and yeast are transferred to high algal concentrations for a short time to improve their nutritional quality prior to use as feed for fish larvae. The chemical composition of rotifers is similar to that of the algae upon which they fed (Caric et al., 1993). Therefore, enrichment of rotifer with n-3 HUFA by feeding them on rich sources of n-3 can be accomplished through feeding on different microalgae (Koven et al., 1989). The majority of marine fish larvae require unsaturated fatty acids such as eicosapentaenoic acid (EPA – 20:5n-3) and docosahexaenoic acid (DHA – 22:6n-3). High levels of EPA may affect pigmentation. Kanazawa et al. (1982) reported that EPA was preferentially incorporated into the gall bladder, swim bladder, and alimentary tract, and secondly into the liver and gill tissue. Adding microalgae to rotifers as a long-term enrichment technique (combining growth and n-3 HUFA enrichment during the production phase of rotifers) seems to be very efficient for obtaining a high n-3 HUFA content in rotifers, while maintaining a normal lipid content. The aim of this study was to investigate the effect of dietary EPA levels, supplied in the rotifer cultured on different micro-algae (*Chlorella vulgaris*, *Tetraselmis chuii*, and a mixture of both) on survival and growth of European seabass (*D. labrax*) larvae to 24 days after hatching.

Materials and methods

Larvae used in this study were obtained from the Maturation and Spawning Division of the Mariculture Research Center hatchery (Suez Canal University, El-

Arish, Egypt). Broodstock were collected from Bradawil Lagoon. Photoperiod manipulation was used to induce ovarian maturation. After hatching, 1500 larvae were stocked into nine 60-l glass aquaria, three aquaria per treatment. A long-time enrichment technique of rotifers was done by rearing three different populations of the rotifers on different algal species – *Tetraselmis chuii* (diet A), *Chlorella vulgaris* (diet C), and a mixture of both – at ratio of 1:1 by volume (diet B). Rotifers were cultured in eight 300-l fiberglass tanks and maintained at 20±1°C, 20ppt salinity, and 800lux light intensity with a 12L/12D daily photoperiod regime. Rotifers were harvested in a 40-µm-mesh plankton bucket and rinsed with clear seawater before being given to experimental larvae. The starter inoculations of test algal species were obtained from SEAFDEC (Iloilo, Philippines). Algae were cultured in seawater supplemented with a culture medium (Walne, 1966) and maintained in chamber with controlled conditions (20±2°C, 20ppt salinity, and illumination of 1000 lux for 12h L/12h D daily. Mass culture was conducted in large fiberglass tanks (2.0-m^3 capacity) and used to feed rotifers. *Artemia* cysts of GSL (Argenta, USA) were treated with sodium hypochlorite for decapsulation and incubated in seawater to hatch.

Aquaria were provided with filtered seawater and fine aeration. The experiment was carried out under natural lighting. Water quality in the larval aquaria was maintained through partial exchange day 2 onwards – about 30-100% of the water volume was replaced daily by new filtered seawater. Dissolved oxygen levels were maintained by an air blower through air stones. Larvae were reared at ambient salinity (35ppt) and temperature (16±2°C). The experiment lasted 24 days. Dead larvae were counted and removed daily. Rotifers were added to the larvae from day 3-17 and kept at a density of 3-10.ml^{-1} of rearing water. However, feeding with *Artemia* was started at day 10 with a density of 0.5-3.ml^{-1}. Total lipids were extracted according to Folch et al. (1957). Fatty acids were determined by using gas liquid chromatography (GLC) in methyl ester form as described by Nelson et al. (1969). Total length was determined by measuring 20 larvae per aquaria weekly using an ocular micrometer and the number of larvae was counted. Analysis of variance was carried out using a complete randomized design. Differences were subjected to Duncan' s Multiple Range test.

Results and discussion

Table I shows the fatty acid profile of marine microalgae and rotifers used in the present study. The results show that *Chlorella* had higher percentage of 16:0, 18:1n-3, and 18:2n-3 than *Tetraselmis*. However, *Tetraselmis* contained more 16:1n-3, 17:0, 18:3n-3, 20:4n-3, 20:5n-3 than *Chlorella*. The fatty acids 15:0, 16:2n-3, 20:2n-3, 20:5n-3, and 22:6n-3 were absent in *Chlorella*. *Tetraselmis* did not have 15:0, 20:2n-3, 20:3n-3, and 22:6n-3. EPA was found to be 5.11, 2.31, and 0.58% of total lipids in rotifers fed diets A, B, and C, respectively. In addition, DHA was absent in both rotifers and their diets. Whyte and Nagata (1990)

reported that fatty acid profiles of rotifers and corresponding diets fed to the rotifers indicated transferal and storage of major fatty acid constituents in the feeding process. They also suggested an endogenous synthesis of these acids and/or assimilation and concentration of minor amounts that were not evident in the profiles of the diets. Successful rearing of marine fish depends on a good correspondence between the specific dietary requirements and the biochemical composition of the fish prey. The level of n-3 HUFA in the prey is considered a major factor for dietary value, particularly arachidonic acid (ARA – 20:4n-3), EPA, and DHA. Rotifers fed *Chlorella* were low in these fatty acids. However, rotifers fed *Tetraselmis* had more EPA than those fed *Chlorella* (Table I). EPA content was found to be 5.11, 2.31, and 0.58% of total lipids in tissues of rotifers fed diets A, B, and C, respectively. Reitan et al. (1997) found that the use of *B. plicatilis* as feed for marine fish larvae could be enhanced through enrichment with various diets containing different n-3 HUFA levels, accomplished by feeding with algae rich in essential fatty acids for either a short time (24h prior to feeding fish larvae) or a long time (throughout the rotifer culture period).

Table I. Fatty acid profiles of rotifers and their algal feeds (% of total lipids).

Fatty acid	*Tetraselmis*		*Tetraselmis+Chlorella*		*Chlorella*	
	Algae	Rotifers	Algae	Rotifers	Algae	Rotifers
14:0	0.37	1.15	0.42	2.00	0.29	3.65
14:1n-3	0.31	0.71	0.35	0.81	0.38	0.60
15:0	–	0.25	–	0.11	–	0.49
16:0	13.8	13.51	26.9	15.21	43.6	20.8
16:1n-3	6.01	1.00	3.71	1.21	1.32	0.91
16:2n-6	0.39	0.43	0.23	0.32	–	0.18
17:0	2.31	1.56	1.10	1.36	0.37	–
18:0	0.22	3.77	5.01	8.07	0.69	2.98
18:1n-9	2.90	–	3.21	3.96	4.61	–
18:2n-6	10.08	7.79	14.11	10.88	20.1	13.32
18:3n-3	12.09	15.65	7.12	9.13	2.69	0.02
20:2n-6	–	0.41	–	0.59	–	0.79
20:3n-6	–	0.81	0.27	4.10	0.41	7.00
20:4n-6	1.11	3.11	0.59	5.09	0.25	4.97
20:5n-3	6.15	5.11	3.16	2.31	–	0.58
Lipids* %	12.29	13.11	9.91	10.42	8.68	9.78

In the present study, results show that survival rate is consistently increased when larvae are fed diet A, followed by those fed diet B. The poorest larval survival rate was obtained when larvae were fed diet C. The same trend was found for final length and growth. In addition, marine fish larvae have been reported to be unable to synthesize *de novo* either n-6 or n-3 polyunsaturated fatty acids. On the other hand, rotifers have been found to synthesize only minor amounts of n-3 polyunsaturated fatty acids. Therefore, these acids must be provided by the rotifer diet to meet larval fish requirements. Results of Epifanio et al. (1976) showed that far better growth is obtained with mixtures of two or more species of algae than with single species.

The present study used three treatments to achieve 5.11, 2.31, and 0.58% in total lipid of EPA, corresponding to diets A, B, and C, respectively. Results indicate that survival and growth of *D. labrax* larvae are markedly increased as EPA levels increase. Similar results were found by Watanabe et al. (1989) who found low growth and survival rates when red sea bream larvae were fed EPA-deficient diets, and were effectively improved by elevation of dietary EPA. Léger et al. (1987) found a linear relationship between the EPA content of *Artemia* and the biomass of *Mysidopsis bahia* to which freshly hatched *Artemia* were fed. Thus, EPA is one of the most important n-3 HUFA that should be found in larval feeds.

References

Caric M., J. Sanko-Njire, and B. Skaramuca. 1993. Dietary effects of different feeds on the biochemical composition of the rotifer (*Brachionus plicatilis* Müller) Aquaculture 110:141-150.

Epifanio C.E., L.C. Mootz, and Ch. Turk. 1976. Growth of oysters in a recirculating maricultural system. Proc. Natl. Shellfish Assoc. 66:32-37.

Folch J., M. Lees, and G.H.S. Sloane. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Kanazawa A., S. Teshima, and N. Imatanaka. 1982. Tissue uptake of radioactive eicosapentaenoic acid in the red sea bream. Bull. Jap. Soc. Sci. Fish. 48(10):1441-1444.

Koven W.M., G.W. Kissil, and A. Tandler. 1989. Lipid and n-3 requirement of *Sparus aurata* larvae during starvation and feeding. Aquaculture 79:185-191.

Léger P., P. Bengtson, P. Sorgeloos, K.L. Simpson, and A.D. Beck. 1987. The nutritional value of *Artemia*: a review. p.357-372. In: *Artemia* research and its applications. Volume 3. P. Sorgeloos, D.A. Bengtson, W.Decleir and E. Jaspers (Eds.), Uyniversa Press, Wetteren, Belgium.

Nelson J.P., A.J. Milum, and H.D. Fister. 1969. Gas chromotographic determination of tocopherols and sterol in soya sludges and residues, an improved method. J. Amer. Oil Chem. Soc. 47:259-261.

Reitan K.I., J.R. Rainuzzo, G. Oie, and Y. Olsen. 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155:207-221.

Walne P.R. 1966. Experiments in the large scale culture of the larvae of *Ostrea edulis*. Fishery Invest., Lond. Ser. II, 25:53p.

Watanabe T., M.S. Izquierdo, T. Takeuchi, S. Satoh, and C. Kitajima. 1989. Comparison between eicosapentaenoic and docosahexaenoic acids in terms of essential fatty acid efficiency in larval red sea bream. Nippon Suisan Gakkaishi 55:1635-1640.

Whyte J.N.C. and W.D. Nagata. 1990. Carbohydrate and fatty acid composition of rotifer, *Brachionus plicatilis*, fed monospecific diets of yeast or phytoplankton. Aquaculture 89:263-272.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECT OF PRE-WEANING FEEDING REGIME ON WEANING SUCCESS OF *SOLEA SENEGALENSIS*

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Introduction

Both in juvenile and larval fish, it is generally believed that a higher feeding frequency will maximize growth (e.g., Holm et al., 1990; Haylor 1993). This has particular emphasis in the pre-weaning stages, which are characterized by a high potential for larval growth (Conceição, 1997). Therefore commercial hatcheries producing marine fish species generally supply food to the larvae several times during the day or even continuously.

The sole, *Solea senegalensis*, is a species with a high potential for aquaculture in Southern Europe (Dinis et al., 1999). However, differing from other species sole larvae settle in the bottom of the tanks well before weaning can commence and display then a peculiar feeding behaviour. Sole larvae do not react readily to food supply, rather grazing *Artemia* at the bottom of the tanks (Dinis et al., 2000). Therefore, the aim of this study was to test different feeding frequencies during the pre-weaning period and verify if these would affect the weaning success of sole, *S. senegalensis*. Growth and survival of sole larvae were compared in a 43-day experiment with two pre-weaning feeding regimes, continuous feeding 12 hours a day and feeding twice daily.

Materials and methods

Eggs were obtained from natural spawn of sole broodstock maintained in the LEOA laboratory at the University of Algarve. The fertilized eggs were placed in a 100-l conical cylindrical fiberglass tank in a closed recirculation system. Newly hatched larvae were transferred to a 200-l conical cylindrical fiberglass tank closed recirculation system. A photoperiod of 12L:12D was used.

Temperature, salinity and dissolved oxygen were measured daily. Temperature and salinity averaged, respectively, 20.9±0.6°C and 32.0±1.0‰. Dissolved oxygen in water was around 6.45±0.7mg.l^{-1}.

Larvae were fed at 3 days after hatching (DAH) with rotifers (*Brachionus plicatilis*) enriched with microalgae, *Isochrysis galbana* and *Tetraselmis suecica*. At 5DAH larvae were fed also with *Artemia* sp. nauplii (Be 480 strain, INVE Aquaculture, Belgium). Rotifers were gradually removed until the 8DAH. *Artemia* sp. metanauplii enriched with *I. galbana* and *T. suecica* were provided to the larvae after 10DAH.

At 20DAH, larvae were transferred to 50-l white flat-bottom plastic tanks in a closed recirculation system. Larvae were put at the density of 3000.m^{-2}, with three replicate tanks per treatment. *Artemia* sp. (RH strain, INVE Aquaculture, Belgium) metanauplii enriched with Super SELCO (INVE Aquaculture, Belgium) were supplied to the larvae until the weaning started. Throughout the whole experiment it was attempted to feed fish close to satiation, based on predicted maximum growth. Both treatments received always the same daily ration per fish.

The pre-weaning experiment started at 26DAH, with the larvae being continuously fed 12h per day (using a peristaltic pump) or fed twice daily (morning and late afternoon). *Artemia* supply was gradually changed from live to frozen *Artemia* metanauplii between 30 and 37 DAH. The weaning of the larvae started at 40DAH, after one day fasting, and the experiment was ended at 69DAH. The weaning diet used was AgloNorse (Norsildmel Innovation AS, Bergen, Norway) no.2 (0.6-1.0mm) initially, and after 63DAH, AgloNorse no.3 (1.0-1.6mm). The inert diet was supplied to the larvae with automatic feeders every hour during 18h a day.

Results and discussion

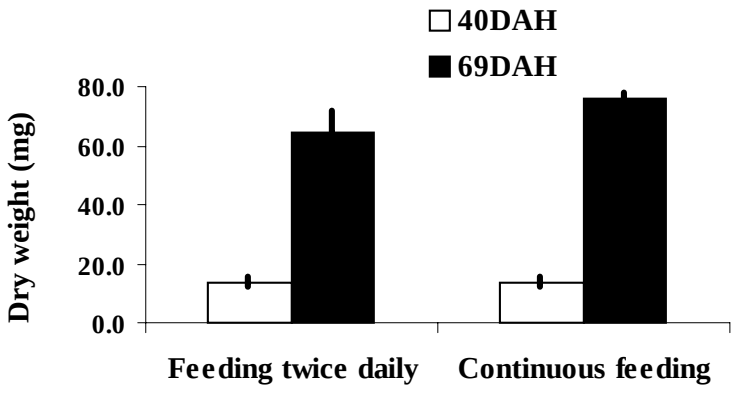

Fig. 1. Dry weight of sole at 40DAH and 69DAH (means±SD).

At 40DAH there were no significant differences in growth between treatments (Fig. 1), but the coefficient of variation in the continuous feeding regime (29.4%) was higher than in feeding twice daily regime (21.8%). These results are probably due to a higher competition for food in the continuous feeding regime. This was likely caused by a lower prey concentration in the tanks with the higher feeding frequency. The increased competition between fishes in the same tank led to a high growth heterogeneity in the continuous feeding regime. No noticeable mortality was verified until 40DAH. These results are in apparent contradiction with common notion that increased competition between fishes in the same tank leads to a lower growth heterogeneity (e.g., van der Meer et al., 1997). However, it should be noted that in the present experiment both treatments received an identical number of prey per fish.

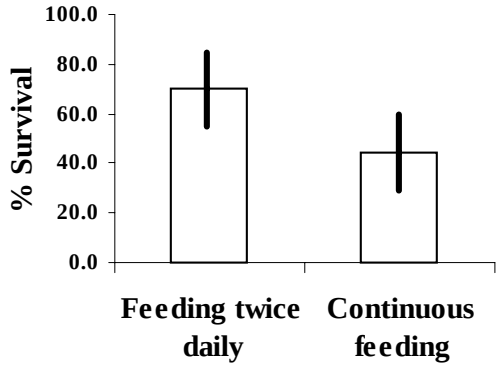

Fig. 2. Survival of sole at the end of the experiment (69DAH) (means±SD).

At 69DAH, dry weight (Fig. 1) and relative growth rate (RGR) were higher (P<0.05) in the continuous feeding regime (DW= 76.2±2.1mg and RGR= 6.3±0.7) than at feeding twice daily (DW= 64.1±7.7mg and RGR= 5.6±0.7). These values indicate that sole fed twice daily were smaller and grew less than sole fed continuously. Survival (Fig. 2) and food conversion rate (FCR) were higher (P<0.05) in the fish fed twice daily (69.8±14.8% survival and FCR= 1.7±0.2) than at continuous feeding regime (44.3±15.2% survival and FCR= 1.3±0.2). In summary, results of the experiment indicate that once fish were weaned continuous feeding regime produced fewer bigger sole, while feeding twice daily lead to smaller fish and a higher survival. This suggests that mortality during the weaning period was selective towards smaller fish in the continuous feeding regime.

The results of this experiment demonstrate that the pre-weaning regime affects the weaning success of sole.

References

Conceição L.E.C. 1997. Growth in early life stages of fishes: an explanatory model. Ph.D. Thesis, Wageningen University, The Netherlands. 209p.

Dinis M.T., L. Ribeiro, L.E.C. Conceição, and C. Aragão. 2000. Larvae digestion and new weaning experiments in *Solea senegalensis*. Cah. Opt. Méditerr. 47:193-204.

Dinis M.T., L. Ribeiro, F. Soares, and C. Sarasquete. 1999. Cultivation potential of sole *Solea senegalensis* in Portugal and Spain. Aquaculture 176:27-38.

Haylor G.S. 1993. Controlled hatchery production of *Clarias gariepinus* (Burchell 1822): an estimate of maximum daily feed intake of *C. gariepinus* larvae. Aquacult. Fish. Manage. 24:473-482.

Holm J.C., T. Refstie, and S. Boe. 1990. The effect of fish density and feeding regimes on individual growth rate and mortality in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 89:225-232.

Van der Meer M.B., H. Van Herwaarden, and M.C.J. Verdegem. 1997. The effect of number of meals and frequency of feeding on voluntary feed uptake of *Colossoma macropomum* (Cuvier). Aquacult. Res. 28:419-432.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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DISINFECTION OF *SPARUS AURATA* EGGS WITH GLUTARALDEHYDE

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Introduction

Fish eggs are disinfected in fish hatcheries in order to prevent diseases. Harboe et al. (1994) observed that the treatment of halibut eggs with glutaraldehyde resulted in improved larval survival rate and size at the first-feeding stage. Salvesen and Vadstein (1995) compared chloramine T, glutaraldehyde, iodine, and sodium hypochlorite for egg treatment in plaice, cod, and halibut, and concluded that glutaraldehyde was the most promising agent. According to Salvesen et al. (1997), the glutaraldehyde treatment must be adapted for each species. The present study aims to determine the conditions in which glutaraldehyde can be used for disinfecting the eggs of sea bream (*Sparus aurata*).

Materials and methods

Eggs originating from the natural spawning of *Sparus aurata* broodstock in the Ferme Marine de Douhet (France) were sent to the laboratory in containers with or without addition of TRIS buffer and tetracycline. Only buoyant eggs were retained. Trials were performed in filtered and UV-irradiated recirculated seawater at 18°C. Eggs were treated at 4 developmental stages: 4-8 cells, morula, blastopore closure (end of epiboly), and heart-beating. Three glutaraldehyde concentrations (C) of 200, 300, and 400ppm, and several times of contact (T) from 2-10min, were tested. Eggs were immersed in 500ml of seawater in which glutaraldehyde was added and were agitated gently to ensure that the entire surface was exposed to glutaraldehyde. After treatment, eggs were rinsed and placed in incubators (two groups of 1149±461 eggs for each treatment). Control groups (without treatment and agitation) were included in all trials. Other control groups (without treatment and with agitation for 4min) were present in the first trial. The results of the trial were evaluated 1 or 2 days after hatching using three criteria: hatching rate (H, % of initial eggs), normal larvae (nL, % total hatched larvae) and imprisoned larvae (larvae inside the chorion; pL, % of initial eggs).

The bactericidal effect of glutaraldehyde was checked in egg samples following treatment by 200ppm for 2-6min. Eggs were shaken vigorously in a sterile Letheen solution for 1min. After 1h, 1ml of this solution was added to 10ml of sterile water (17ppt) and inoculated to Petrifilm SM plates. Bacteria were counted after 4 days incubation at a mean temperature of 21°C.

Percentages (after angular transformation) were compared by analysis of variance, followed by Newmann-Keuls test when significant differences were found at the 0.05 level.

Results and discussion

Trial 1 showed that hatching rate was significantly decreased following egg manipulation and glutaraldehyde treatment (200ppm for 4min) at stage 4-8 cells and stage morula (Table I). In contrast, it was unaffected by manipulation and the same glutaraldehyde treatment at a later stage (blastopore closure). A significant but very small effect was found for the heart-beating stage. No significant effect was found for nL. In the second spawn, glutaraldehyde treatment had a small significant effect on pL.

Table I. Percentage of hatched (H), normal (nL) and imprisoned (pL) larvae following egg treatment at different stages (treatment: 200ppm during 4min, CxT = 800) in Trial 1.

Stage	Spawn		Control	0*	800	800 t**
4-8 cells	1	H	-	-	49.0	-
	1	nL	-	-	91.6	-
	1	pL	-	-	0.1	-
Morula	1	H	84.6[a]	-	70.8[b]	-
	1	nL	87.5	-	86.5	-
	1	pL	0.0	-	1.4	-
Blastopore closure	1	H	98.0	98.5	97.9	98.1
	1	nL	93.5	93.0	94.3	92.0
	1	pL	0. 0	0.0	0.04	0.04
Blastopore closure	2	H	99.1	-	96.0	-
	2	nL	92.2	-	93.0	-
	2	pL	0.0[b]	-	0.3[a]	-
Heart-beating	2	H	98.5[a]	-	96.9[b]	-
	2	nL	89.8	-	89.1	-
	2	pL	0.0	-	0.7	-

* groups without treatment but agitated gently during 4min

** eggs transported in seawater with TRIS buffer and tetracycline

Values within the same line not sharing a common superscript are significantly different ($P<0.05$)

The influence of the duration of a treatment with glutaraldehyde 200ppm was examined in trials 2 and 3 (Table II). Treatments during 6 or 7min or longer significantly decreased the hatching rates. The percentages of nL and pL were significantly affected by 8 or 9min and more exposure times. The differences observed between the two trials for identical treatments suggest that the toxicity of glutaraldehyde might depend on initial egg quality.

Table II. Percentage of hatched (H), normal (nL) and imprisoned (pL) larvae in relation with the exposure time (T) to glutaraldehyde (200ppm) in trials 2 and 3.

T	CxT	Trial 2			Trial 3		
		Blastopore closure			Heart-beating		
		H	nL	pL	H	nL	pL
Control		98.2^{a}	90.0^{ab}	0.0^{c}	96.9^{a}	80.6^{ab}	0.7^{d}
4 min	800	94.8^{ab}	93.3^{a}	0.2^{c}	92.6^{ab}	84.1^{a}	1.1^{d}
5 min	1000	92.9^{ab}	91.6^{ab}	0.4^{c}	-	-	-
6 min	1200	91.9^{ab}	90.1^{ab}	2.1^{bc}	88.1^{b}	76.2^{ab}	3.3^{d}
7 min	1400	83.9^{b}	87.4^{ab}	4.3^{bc}	-	-	-
8 min	1600	83.7^{b}	87.1^{ab}	3.3^{bc}	78.2^{c}	75.2^{ab}	10.7^{c}
8.5 min	1700	-	-	-	62.7^{d}	74.5^{ab}	12.2^{bc}
9 min	1800	67.9^{c}	78.8^{bc}	14.1^{a}	61.4^{d}	68.5^{bc}	19.8^{a}
9.5 min	1900	-	-	-	63.6^{d}	61.1^{c}	18.1^{ab}
10 min	2000	69.2^{c}	70.7^{c}	7.4^{ab}	56.1^{d}	62.7^{c}	24.3^{a}

Values within the same column not sharing a common superscript are significantly different ($P<0.05$).

The effects of combinations of C and T resulting in three values of the products CxT (800-900, 1200 and 1500-1600, respectively) were compared in trials 4 and 5 (Table III).

The lowest value of the CxT combinations (800-900) appeared harmless, since it resulted in the same percentages of hatched larvae, normal larvae and imprisoned larvae as the untreated control. In contrast, significant differences were observed between treated and control eggs for CxT=1200 and 1600, with negative effects more marked for 400 than for 300ppm.

Besides, treatment with 200ppm glutaraldehyde during 4min appeared efficient since no development of bacterial colony was observed after Petrifilm inoculation.

In conclusion, results showed that sea bream eggs are very sensitive to manipulation and treatment during the first stages of development. Glutaraldehyde appeared suitable for disinfecting sea bream eggs if the

treatment is applied at the stage of blastopore closure or heart-beating and if the value of the CxT product is less than 1000. Beyond this threshold, established for a temperature of 18°C, toxic effects of glutaraldehyde were observed, especially in poor quality eggs.

Table III. Percentage of hatched (H), normal (nL), and imprisoned (pL) larvae following egg treatment with different exposure times and different glutaraldehyde concentration in trials 4 and 5.

			Trial 4			Trial 5		
	T	CxT	blastophore closure			Heart-beating		
			H	nL	pL	H	nL	pL
Control			99.1^{a}	92.2^{a}	0.00^{c}	98.6^{a}	96.5	0.3^{b}
200 ppm	4 min	800	96.0^{ab}	93.0^{a}	0.3^{bc}	97.9^{a}	90.9	0.7^{b}
	6 min	1200	97.2^{ab}	85.3^{ab}	0.2^{bc}	95.0^{a}	87.9	1.5^{b}
	8 min	1600	93.8^{b}	88.7^{a}	1.8^{a}	96.7^{a}	82.5	2.2^{b}
300 ppm	3 min	900	96.8^{ab}	89.3^{a}	0.2^{bc}	99.2^{a}	95.6	0.1^{b}
	4 min	1200	96.3^{ab}	90.7^{a}	0.2^{bc}	95.5^{a}	96.0	2.8^{b}
	5 min	1500	92.9^{b}	85.2^{ab}	1.7^{a}	87.5^{b}	86.5	10.6^{a}
400 ppm	2 min	800	97.4^{ab}	92.3^{a}	0.1^{bc}	98.2^{a}	86.3	1.1^{b}
	3 min	1200	92.9^{b}	78.4^{b}	2.3^{a}	97.0^{a}	92.0	1.7^{b}
	4 min	1600	92.2^{b}	77.4^{b}	0.9^{ab}	81.5^{b}	96.8	15.0^{a}

Values within the same column not sharing a common superscript are significantly different (P<0.05).

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References

Harboe T., I. Huse, and G. Øie. 1994. Effects of egg disinfection on yolk sac and first feeding stages of halibut (*Hippoglossus hippoglossus* L.) larvae. Aquaculture 119:157-165.

Salvesen I. and O. Vadstein. 1995. Surface disinfection of eggs from marine fish: evaluation of four chemicals. Aquacult. Int. 3:155-171.

Salvesen I., G. Øie, and O. Vadstein. 1997. Surface disinfection of Atlantic halibut and turbot eggs with glutaraldehyde: evaluation of concentrations and contact times. Aquacult. Int. 5:249-258.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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CULTURE OF GILTHEAD SEA BREAM AND DENTEX LARVAE IN A CLOSED RECIRCULATING SYSTEM

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Introduction

Most of the hatcheries and ongrowing farms in Spain are land-based facilities that operate by flow-through systems, where nutrients in the wastewater are discharged directly to the sea. However, pumping and heating-cooling operations of these farms are very costly. Over the last few years, a growing interest for recirculation technology has been detected not only in Spain, but in other countries (Libey and Timmons, 1998). Because of its location on the Ebro River delta and being surrounded by several thousand rice fields that produce large amounts of agricultural sewage, the Centre d'Aquicultura–IRTA required a recirculation system to ensure good quality water for fish larval rearing. A convenient portable device has been designed for recirculation, being used for the first time for seabream and dentex larval rearing.

Description of the recirculation units

Broodstock. Two 4000-m^3 tanks were used. Water for recirculation was taken from a standpipe located in the center of the tanks that was perforated toward the top. During the spawning season, 50-l collector tanks were placed outside the broodstock tanks with their outlet pipes connected. Water to be recirculated was taken from these collector tanks after passing through a 500-μm mesh egg-collector. Solids (faeces and feed) were siphoned daily from the bottom of the tanks. This relatively particle-free water was then recirculated through (1) a sand filter, (2) a biofilter, and (3) a battery of 4 screening cartridges down to 10μm, before being pumped into the broodstock tanks. All the filters (sand, biofilter, cartridges, and UV), pumps, and measuring electrodes (flow rate, oxygen, temperature, and salinity) were assembled on a portable pallet easily transportable within the hatchery. To avoid gas supersaturation, degassing columns filled with corrugated plastic cylinders were placed in the inlets. Biofilm carriers in the biofilter were the same corrugated plastic cylinders used in the degassing columns. Nitrifying microorganisms were added to the biofilters using a commercial product (Glassclear, 1ml per 100 l water for 7 days) together with fish pellets 8 weeks (18ºC) before transferring the fish. Nitrites and ammonia concentrations were measured weekly, and the fish were transferred

when both levels reached zero (Fig. 1). For seabream broodstock, temperature was maintained at 18ºC using a 8hL:16hD photoperiod and a completely closed recirculation system. Fish were fed with a Proaqua seabream feed at the beginning of the spawning season and home-made pellets (40% squid and trash fish, 40% Proaqua fish meal, 15% tuna orbital oil, 0.2% vitamin mixture) at the end.

Larvae. Eight 500-l tanks were used for larviculture. In this case, water to be recycled was taken from an outlet pipe placed in the side wall near the surface of the tanks, and covered with a nylon mesh which size varied depending on the food used (150µm during rotifer feeding, 300µm for *Artemia*, and 500µm for cofeeding *Artemia* and weaning diets). Recycled water was filtered up to 1µm and disinfected using an UV unit. Faeces, dead prey, and dead larvae were siphoned daily from the bottom of the tank. Temperature was kept at 19ºC, salinity at 34.5‰, and pH between 7.5-8.6. A semi-closed recirculation system was used for larvae, with less than 10% of the water renewed. Before entering the fish tanks, the recycled water together with new inlet water was filtered to 1µm and treated by UV light. Seabream larvae were fed enriched rotifers from day 2-20, enriched *Artemia* nauplii from day 15-45, and weaned from day 35 post hatching. Enrichment was carried out according to Estevez et al. (1999)

Weaning. Six 1500-l tanks were used. Water was recycled after passing through a pipe covered with a 1000-µm nylon mesh placed in the center of the tank. Recycled water used for weaned fish was 19-20ºC, 34.5‰ salinity, and pH 7.5-8.6, and treated as in the larval tanks. INVE weaning diets Proton 1 and Proton 4 were used for feeding the fish.

Results and discussion

Performance of these recirculation units can be measured in terms of fish production. In the case of broodstock (Table I), spawnings were obtained everyday from January 8 (one week after placing the broodstock in the rearing tanks) until March 20, with an average egg production of 2-4 million eggs per female and high fertilization rates. Fish health was very good and no symptoms of parasites or infection were detected during the spawning season. Egg production varied from 20-400ml eggs.day^{-1} (1608 eggs.ml^{-1}) and quality, measured as transparency and presence of more than 1 oil globule, was very good. Hatching rates (Table II) varied between 30-84% in the spawnings used for larval rearing at the beginning of the spawning season (Fig. 2). Survival rates were lower than expected (0.8-7.3%), with most of the mortality due to stress (high density of larvae) and cannibalism. No toxicity or infection symptoms were observed. Swimbladder inflation was nearly 100% and opercular deformity was observed in less than 5% of the fish.

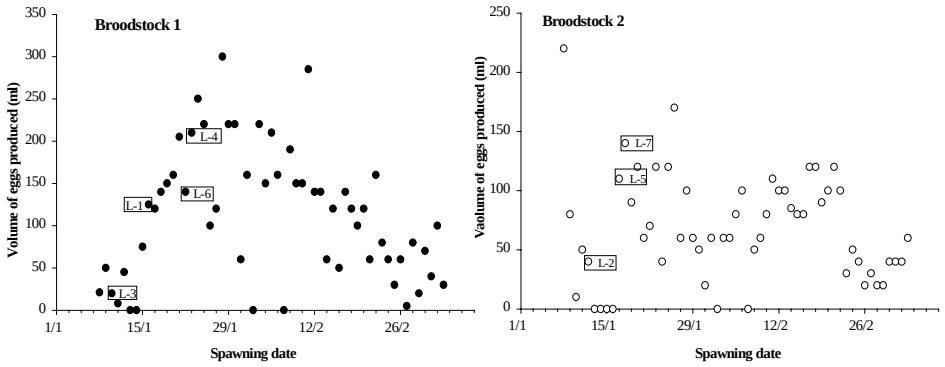

Fig. 2. Spawnings of gilthead seabream maintained in recirculation conditions used for

Table I. Broodstock performance using recycled water during 2001 spawning season.

	Broodstock 1	Broodstock 2
Nº Females	3	3
Nº Males	7	8
Total fecundity (# eggs per fish)	4 034 800	2 438 300
Spawning frequency (# spawns per fish)	78	73
Date of spawnings	8 Jan to 20 Mar	8 Jan to 20 Mar
Daily food intake (% fish weight/day)	0.8-1	0.8-1
% Fertilized eggs produced (mean±SD)	96.9±4.4	84.6±19.4

Table II. Hatching and survival (after weaning, day 70) rates (%) of seabream larvae cultured using recycled water.

Spawning date	Broodstock	Hatching rate	Larval tank	Survival rate
12 January	1	40.0	L-3	7.3
12 January	2	62.2	L-2	2.5
16 January	1	84.0	L-1	0.9
17 January	2	65.0	L-5	0.8
18 January	2	30.0	L-7	2.0
22 January	1	64.8	L-6	1.7
23 January	1	74.6	L-4	0.8

Until now, most of the farms working with recirculated water are fish ongrowing facilities (Lygren, 1993; Eikegrokk and Ulgenes, 1998; Honda, 1998), measuring performance in terms of fish growth and health. These systems can also operate in hatcheries if an especial control of the microbial flora is taken. Data presented here are only preliminary and further research is needed, especially in terms of microbial growth and methods to reduce larval stress. Results on dentex rearing using these recirculation units will be presented during the Conference.

References

Eikebrokk B. and Y. Ulgenes. 1998. Recirculation technologies in Norwegian aquaculture. p.129-137. In: Proceedings of the 2nd international conference on recirculating aquaculture. G.S. Libey and M.B. Timmons (Eds) Virginia July 16-19, 1998. 409p.

Estevez A., L.A. McEvoy, J.G. Bell, and J.R. Sargent. 1999. Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live-prey enriched in arachidonic and eicosapentaenoic acids. Aquaculture 180:321-343

Honda H. 1998. Recirculating aquaculture systems in Japan. p.124-128. In: Proceedings of the 2nd international conference on recirculating aquaculture. G.S. Libey and M.B. Timmons (Eds). Virginia July 16-19, 1998. 409p.

Libey G.S. and M.B. Timmons. (Eds.) 1998. Proceedings of the 2nd international conference on recirculating aquaculture. Virginia July 16-19, 1998. 409p.

Lygren E. 1993. Recirculation technique for hatcheries and ongrowing farms. p.351-359. In: Fish Farming Technology. Reinertsen, Dahle, Jorgensen, and Tvinnereim (Eds.). Blakema, Rotterdam.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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COPEPODS AS A FOOD SOURCE IN FIRST FEEDING OF MARINE FISH LARVAE

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Abstract

In Norway, collection of zooplankton has been used for semi-intensive production of larvae of the coldwater marine fish species Atlantic halibut (*Hippoglossus hippoglossus*) and cod (*Gadus morhua*). From natural marine lagoons, copepods, commonly represented by the genus *Temora* sp., *Eurytemora* sp., *Acartia* sp. and *Centropages* sp. (size fraction between 300 and 1200µm), are used as live food organisms for the fish larvae. The copepods are collected from the water by a pump-induced water flow, size-fractionated using wheel filters, and distributed to the larval tanks. Within this semi-intensive rearing system, the growth and survival of the fish larvae show high variation between individual rearing units. This could be related to the variability of the zooplankton species composition, and the fact that parasites and diseases are transferred to the larvae from the marine zooplankton. Larval pigmentation is normal when copepods are used, and the fatty acid composition of the live food is considered to be important in this respect.

Copepods are often used in combination with enriched *Artemia* sp. or their larger metanauplius stages. Frozen copepods (mainly copepodid stages of *C. finmarchicus*) have also been used as food for halibut and cod fry, in combination with formulated diets in the weaning period, or in combination with *Artemia* sp. and/or live copepods.

It is generally believed that copepods can meet the nutritional requirements of the fish larvae, particularly with regard to the requirements of n-3 HUFA and DHA. The % content of n-3 HUFA is almost two times higher in copepods than in cultivated live food organisms like enriched *Artemia* sp. The two dominant fatty acids of copepods, DHA and EPA, might constitute 55-60% of the total fatty acids. The protein content is also relatively high in copepods, ranging between 53-58% of dry weight in four different species examined. It may be

assumed that an ideal live food organism for halibut and cod larvae would have similar lipid and fatty acid content as the natural diet, represented by copepods. Copepods should therefore be used as a main reference during efforts to define the lipid content in *Artemia* sp. and rotifers post-enrichment.

First feeding experiments with halibut larvae have shown that the fish maintain the initial high DHA content during the first feeding period if the live food organisms have a high DHA content, like copepods. Usually this fish shows normal pigmentation and development. The fish larvae show more or less the same response if well-enriched *Artemia* sp. is used (high HUFA content). However, the growth rates often increase when *Artemia* sp. rather than copepods are fed to the halibut larvae. This is related to the higher individual dry weight and energy content of *Artemia* sp. relative to most copepods. Malpigmented and poorly developed larvae with low survival are often seen when *Artemia* sp. containing low levels of n-3 HUFA and DHA are fed to the halibut larvae.

The relationship between the DHA content of the live food organisms and the DHA content in fish larvae shows the importance of considering the nutritional content of natural prey organisms of fish larvae, and use this as a reference to further improve and develop the enrichment techniques for rotifers and *Artemia* sp. Feasible whole-year production of fish fry must rather rely on intensively cultivated live food organisms, rather than copepods. This is related to the fact that copepods are available only at certain periods of the year, and the highly variable concentrations of these organisms in the seawater. As a supplement to *Artemia* sp. and rotifers, copepods can be used during the early phase of the first feeding period for both cod and halibut, if the location of the farm allow harvesting of zooplankton.

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DEVELOPMENT OF THE DIGESTIVE TRACT AND ASSOCIATED ORGANS IN WOLFFISHES (*ANARHICHAS LUPUS* L. AND *A. MINOR*)

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Introduction

Detailed morphological and histological studies of embryos and larvae are important components of describing the biological peculiarities of various species. Even if the basic mechanisms of development are similar in all teleost species, differences exist with regard to the relative time of development of specialized tissues and organs (Govoni, 1980; O´Connell, 1981; Pedersen and Falk-Petersen, 1992). Genetic differences, yolk content of the eggs, as well as the environment influence size and developmental stage at hatching (Blaxter, 1988). The typical marine larvae hatching from a small pelagic egg is 3-5mm long, transparent with scattered pigment spots, has a relatively large yolk sac and head, pigmented or unpigmented eyes, a simple straight gut, closed mouth, and a larval finfold. In these larvae, the digestive tract is relatively straight and underdeveloped at hatching. During the relatively fast larval yolk absorption period, the digestive system and eyes become functional. Some species hatch at underdeveloped stages, e.g., halibut (Pittman, 1991). A stomach is absent in all larval teleosts and develops during metamorphosis in two distinct parts in many species: the corpus, with its mucous surface epthelium and tubular glands, arises first and immediately afterwards, the pylorus is formed.

Wolffishes (*Anarhichas lupus and A. minor*), salmonids, and several other species having large, yolk-rich eggs and long incubation times, and hatch as large "larvae" (or alevins) with particularly well-developed anatomies, blood circulation, sense organs, and skeletons (Ballard, 1973; Wallace et al., 1989; Falk-Petersen and Hansen, 1999; Falk-Petersen et al., 1999). The eggs of wolffishes belong to the largest of marine teleosts and have long incubation times – 800-1000 daydegrees, depending on temperature (Falk-Petersen et al., 1999; Hansen and Falk-Petersen, 2001). In nature, the pelagic larvae feed for many weeks before seeking bottom habitat.

Materials and methods

Eggs from wolffishes *(A. lupus* and *A. minor*) were stripped and fertilized after ovulation and incubated in upstream incubation units (Falk-Petersen et al., 1999). Embryos, larvae, and juveniles were fixed in 2.5% glutardialdehyde and 2.5% paraformaldehyde in 0.05N cacodylate buffer (modified Karnovsky fixation medium) at different developmental stages and embedded in either Paraplast for general histological preparations or Epon epoxy resin for ultrastructural studies in the electron microscope. The material was collected from egg batches incubated at constant 6 or 8°C. Paraplast- and epoxy-embedded macro-sections were photographed in a Leica Wild M10 stereomicroscope with a Leica photoautomat (Wild MPS45).

Results

In wolffishes, the intestinal tube is visible in the 5-mm-long embryo at about 176d° (22d incubation at 8°C). A liver rudiment with undifferentiated hepatocytes is also noted at this point. The intestinal tube has a very narrow lumen and the mucosa cells are cylindrical. After 240d° (30d at 8°C), the mouth opening is established in the now 8-mm-long embryo. The digestive tract is still relatively straight, but with folded mucosa in the oesophagus and intestine and a particularly expanded hind part with sphincter muscle. Small pigment spots are noted dorsally, and a urinary bladder is visible.

At 350d° (44d at 8°C), the 11-mm-long embryo has a stomach and folded intestine with ahighly expanded lumen, a large liver, green gallbladder, and pancreatic tissue with zymogen granules.

At 416d° (52d at 8°C), the 13-mm embryos have numerous mucous cells in the oesophageal epithelium, an expanding stomach, strong pigmentation in the intestinal region with dark particles also observed within the lumen. At 500d° (63d at 8°C), the 15-mm embryos have frequent peristaltic movements in the expanded intestine and a visible anal opening. Numerous teeth buds are present in the jaws and buccal cavity in 17-mm-long embryos (576d°, 72d at 8°C).

Pigmentation density increased with embryo development, and was particularly intense in the abdominal region in the spotted wolffish (*A..minor)*. In the 18- to 19-mm-long embryos, the digestive tract is twisted and well differentiated and at hatching between 800 and 970d° (21- to 23-mm-long embryos) the mouth has numerous teeth and the whole digestive apparatus appears well advanced and functional. Numerous gastric glands are noted in the stomach mucosa and pinocytotic activity is visible in the intestine.

Wolffish larvae hatch with apparently fully formed and functional digestive and associated organs, and can be fed directly on formulated feed. The only obvious larval characters remaining in the newly hatched individuals are the small yolk sac and lack of ossification of the cartilaginous skeletal elements. Remnants of the yolk sac remain for several weeks, and dissappear latest in the individuals that for some reason never start feeding. The non-feeding larvae die when the yolk reserves are exhausted after three to five weeks. The alimentary tract, as well as other tissues and organs, slowly degenerate during this period.

Discussion

Although the basic mechanisms of development are similar in fish species, considerable differences exist with respect to the relative time of development of the different organs or organ systems in relation to each other. Also, later in development, through the influence of ambient temperature, fish larvae can show both diverging body lengths and states of organ development at the same absolute age (Blaxter, 1986; 1988; Hansen and Falk-Petersen, 2001; Falk-Petersen, unpublished data). The tissues and organs of wolffishes are well differentiated at hatching. The digestive tract in fact appears to be fully functional long before hatching. The advanced development of the alimentary system as well as other associated and vital organs are characteristic for wolffishes, and explains the successful start-feeding of wolffish larvae on formulated feeds immediately after hatching. In salmonids for example, which appear to have a functional stomach before changing from endogenous to external food, gastric glands are found several days before swim up but not at hatching, and pyloric caeca were distinguished 21 days after hatching (at 10°C) (Wallace et al., 1989).

Comparing organogenesis and functionality of the various organs at early life stages are difficult, not only because much of the available information is fragmentary, but also because the formation of an organ does not always prove its functionality (Kjørsvik and Reiersen, 1992). Combined histomorphological and biochemical studies are particularly useful with regard to proving the onset of functionality of various larval processes. A better understanding of the individual details and developmental sequences may improve incubation, start-feeding, and weaning of marine fish larvae. Morphological and functional differences between fish species and during ontogenesis may explain why some are easier to cultivate than others are. Comparisons should also be carried out with larvae in their natural environments.

References

Ballard W.W. 1973. Normal embryonic stages for Salmonid fishes, based on Salmo gairdneri Richardson and Salvelinus fontinalis (Mitchill). J. Exp. Zool. 184:7-26.

Blaxter J.H.S. 1986. Development of sense organs and behaviour in teleost larvae with special reference to feeding and predator avoidance. Trans. Amer. Fish. Soc. 115:98-114.

Blaxter J.H.S. 1988. Pattern and variety in development. p.1-58 In: Fish physiology XI. The physiology of developing fish. Part A. Eggs and larva. W.S. Hoar & D.J. Randall, (eds). Academic Press, N.Y.

Falk-Petersen et al. 1999. Cultivation of the spotted wolffish (*Anarhichas minor* Olafsen) – A new candidate for coldwater fish farming. Aquacult. Res. 30:711-718.

Govoni J.J. 1980. Morphological, histological, and functional aspects of alimentary canal and associ-ated organ development in larval *Leiostomus xanthurus*. Rev. Can. Biol. 39:69-80.

Hansen T.K. and I.B. Falk-Petersen 2001. The influence of rearing temperature on early development and growth of spotted wolffish *Anarhichas minor* (Olafsen). Aquacult. Res. 32:1-10.

Kjørsvik E. and A.L.Reiersen 1992. Histomorphology of the early yolk-sac larvae of the Atlantic halibut *(Hippoglossus hippoglossus L.*) – an indication of the timing of functionality. J. Fish Biol. 41:1-19.

O'Connell C.P. 1981. Development of organ systems in the Northern anchovy, *Engraulis mordax,* and other teleosts. Amer. Zool. 21:429-446.

Pedersen T. and I.B. Falk-Petersen 1992. Morphological changes during metamorphosis in cod (*Gadus morhua L.*), with particular reference to the development of the stomach and pyloric caeca. J. Fish Biol. 41:449-461.

Pittman K. 1991. Aspects of the early life history of the Atlantic halibut (*Hippoglossus hippoglossus L.*):Embryonic and larval development and the effects of temperature. Doctoral Thesis, University of Bergen.

Wallace J.C., A.B. Amin, and A.M. Gudmundsson 1989. The histology and physiology of the transfer from yolk absorption to exogenous feeding in Arctic charr (*Salvelinus alpinus* (L.)). Rapp. P.-v. Reun. CIEM:191:482.

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INFLUENCE OF EGG-INCUBATION TEMPERATURE ON YOLK CONSUMPTION, DEVELOPMENT, AND SURVIVAL OF SPOTTED WOLFFISH EMBRYOS AND LARVAE

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Introduction

Temperature is a major factor influencing the developmental rate and survival of fish (Rosenthal and Alderdice, 1976; Blaxter, 1992; Kamler, 1992) and optimal environmental conditions are important for successful cultivation of species in aquaculture. Yolk is generally utilized faster at higher temperatures, and the efficiency with which yolk is transformed into body tissue and the effect of temperature on utilization are likely important determinants of early survival. The spotted wolffish (*Anarhichas minor* Olafsen) is an arctic-boreal species and one of the new candidates for cold water aquaculture (Falk-Petersen et al., 1999). When introducing new species for cultivation, it is particularly important to determine the temperature window within which development proceeds normally and the survival of viable offspring is maximized (Hansen and Falk-Petersen, 2001).

Materials and methods

Eggs from various spotted wolffish females were stripped and fertilized (Falk-Petersen et al., 1999). The fertilized eggs were transferred to up-stream incubation units in triplicates at selected temperature regimes from 4-8°C, and mortality, yolk utilization, development, hatching success, larval size, start-feeding success, and growth of larvae were recorded.

Results

The survival of spotted wolffish eggs during the incubation period was significantly different between the three incubation temperatures, and best in batches incubated at a constant 6°C. Highest mortality was generally noted during the period of cell cleavage, early gastrulation, and organogenesis, as well as immediately prior to hatching.

The incubation time decreased with increasing temperature, but the number of daydegrees until hatching increased with increasing temperature. Morphological differences reflecting variations in differentiation were noted among larvae originating from the different temperature groups. Transfer of yolk to embryonic tissue appeared to be most efficient at 4°C. The weights of normal hatched larvae were otherwise positively correlated with the initial egg sizes in all temperature groups, but incubation temperature also affected the weights of newly hatched larvae. The largest larvae hatched from eggs incubated at 4°C, and these also showed highest growth rates when start-fed at 8°C. Survival was highest among the offspring from eggs incubated at 6°C.

Conclusions

Incubation temperature affected developmental rate, yolk consumption, incubation time, egg survival, and final size and survival of spotted wolffish larvae. The highest frequency of survival during incubation, hatching, and start-feeding was registered in egg batches incubated at constant 6°C. The size of newly hatched spotted wolffish larvae was positively correlated with initial egg size, and the largest larvae hatched from eggs incubated at 4°C. The best larval growth rates during start-feeding were observed when the temperature at first feeding exceeded the egg incubation temperature.

References

Blaxter J.H.S. 1992. The efffect of temperature on larval fishes. Neth. J. Zool. 42:336-357.

Falk-Petersen I.B., T.K. Hansen, R. Fieler, and L.M. Sunde. 1999. Cultivation of the spotted wolffish (*Anarhichas minor* Olafsen) – A new candidate for coldwater fish farming. Aquacult. Res. 30:711-718.

Hansen T.K. and I.B. Falk-Petersen. 2001. The influence of rearing temperature on early development and growth of spotted wolffish *Anarhichas minor* (Olafsen). Aquacult. Res. 32:1-10.

Kamler E. 1992. Early life history of fish. An energetics approach. Chapman & Hall. 267 p.

Rosenthal H. and D.F. Alderdice. 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J. Fish. Res. Bd. Canada 33:2047-2065.

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EFFECT OF TEMPERATURE AND DIET ON GROWTH AND LIPID COMPOSITION OF CHILEAN SCALLOP *ARGOPECTEN PURPURATUS* (L.) LARVAE

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Introduction

The Chilean scallop (*Argopecten purpuratus*) is an excellent candidate to diversify aquaculture in the south of Chile, currently dominated by salmon farming. However, mass production is concentrated in the north of Chile (24°S), where the natural settlement of this species occurs. In southern Chile (42°S), the mass production of juveniles is in hatcheries with an ambient temperature of about 19°C and the 2-mm spat must survive through ongrowing until they reach 8cm in the cold waters between 10-15°C. In this study, we examined the lipid classes and highly unsaturated fatty acids (HUFA) in larvae cultured at different temperatures and investigated the nutritional efficacy of different microalgal diets during thermal adaptation.

Materials and methods

Experiments. Two larval experiments were conducted at two different temperatures, 11 and 19°C, using different algal diets. Diets in the first experiment were the flagellate *Isochrysis galbana* grown at 5 and 25°C, and an optimal mixture of *Chaetoceros neogracile* (high-protein) and *Isochrysis aff galbana* (clone T-*Iso*; high-protein) (Uriarte and Farías, 1999). Diets in the second experiment were *I. galbana* grown at 5°C, *C. neogracile*, and a mixture of both microalgae. Eighteen 20-l tanks stocked with 5 larvae.ml^{-1} were used for each experiment. The first experiment was run in winter and the second in early spring.

Lipid analysis. Samples of microalgal diets were stored in chloroform:methanol (2:1) containing 0.01% butylated hydroxytoluene (BHT). Lipid extraction, separation of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), and fatty acid methylation and quantification were performed as described by Bell et

al. (1993). The lipid classes were separated and quantified by high performance thin layer chromatography (HPTLC).

Statistical analysis. The data were analyzed by factorial ANOVA. Data expressed in percent were arcsine transformed. The comparisons between means of pairs of factors (i.e., temperature-diet) were run by Tukey´s *a posteriori* multiple tests. Where variance was not normal, data were analyzed by the non-parametric test of Kruskal Wallis. Values were regarded as significant when $P<0.05$.

Results

Lipid composition of microalgal diets. Regardless of growth temperature, triacylglycerols (TAG) were the principal lipid class present, accounting for between 12.4-25.7% of the total lipid. Monogalactosyldiacylglycerols (MGDG) accounted for between 10.3-19.9% of lipid with the lowest values being obtained in *I. galbana* grown at 5ºC ($P=0.013$). (Table I) The only effect of growth temperature on the content of highly unsaturated fatty acids (HUFA) was observed on the ratio of arachidonic acid (AA) and eicosapentaenoic acid (EPA. The percentages of AA and EPA were significantly higher in diets containing *C. neogracile* compared to those containing *I. galbana*. DHA was significantly higher in the total lipids of *I. galbana* diets compared to those containing *C. neogracile.*

Table I. Lipid class compositions of different microalgal diets used in experiments 1 and 2. Values are % of total lipids.

Exp.	Diet	PC	PE/SQDG	DGDG	MGDG	FFA	TAG
1	*I. galbana*, 25°C	8.5±1.1	4.3±2.2	6.1±3.2	18.0±2.9^{ab}	4.4±0.5	15.7±5.1
1	*I. galbana*, 5°C	7.6±1.5	4.0±0.8	6.0±3.6	10.3±1.5^{a}	4.0±0.8	24.3±5.6
1	*C. neo.* H and T-*Iso*, 25°C	7.6±0.8	8.5±1.1	7.5±3.7	19.9±0.6^{b}	5.2±0.2	15.8±1.6
2	*C. neogracile*, 25°C	6.2±0.3	2.3±2.0	9.8±1.2	18.7±1.1^{b}	7.3±1.2^{b}	12.4±3.8
2	*I. galbana*, 5°C	6.9±2.2	4.6±1.8	6.6±2.5	10.9±1.7^{a}	2.3±0.3^{a}	25.7±1.8
2	*I. galbana*, 5°C, *C. neogracile*, 25°C	5.3±2.4	7.9±1.4	4.6±0.8	15.5±0.8^{ab}	5.5±0.2^{ab}	21.7±6.6

Values are mean ± SE, $n=3$. Values in the same column, within each experiment, having different superscript letters are significantly different ($P<0.05$). H indicates high protein strain of T-*Iso* and *C. neogracile.*

Growth and larval survival. The growth rate, in terms of length increase per day, was a mean of 1.25µm.day^{-1} in experiment 1 while this reached only

0.69µm.day^{-1} in experiment 2. There was lower mean survival in experiment 2 (22.2%) compared to experiment 1 (56.5%).

Lipid class of larvae. The first experiment showed that larval PC decreased with cold acclimation while the cholesterol increased (P=0.001 and P=0.02, respectively). PE was not significantly affected by temperature in this experiment. TAG was affected significantly by diet but not by temperature. The highest value of sterol esters (SE) was obtained in larvae reared at 11°C fed with *I. Galbana* grown at 5°C. However, in experiment 2, larvae cultured at low temperature had the lowest ratio of PC/PE (Table I).

Fatty acid composition of PC. AA, DHA, and total HUFA in PC varied significantly with temperature in experiment 1, with the lowest values in larvae reared at 11°C (for AA, P=0.03; for DHA, P=0.01; for total HUFA, P=0.008). EPA levels were affected by temperature, with highest values found in larvae cultured at 19°C, and were also affected by diet with maximum values in larvae fed a mixture containing *C. neogracile*. (Table II).

Table II. Fatty acid compositions of phosphatidylcholine (PC) in *A. purpuratus* larvae in experiment 1. Values are weight % of total fatty acids.

Temp.	Diet	AA	EPA	DHA	Total HUFA
11°C	*I.galbana*,5°C	1.6±0.2^{a}	6.0±0.7^{a}	18.0±1.1^{a}	31.9±1.9^{ab}
11°C	*I.galbana*, 25°C	1.2±0.1^{a}	4.7±0.4^{a}	13.3±1.8^{a}	25.9±2.3^{a}
11°C	*C.neogracile* H and T-*Iso* H	1.7±0.1^{a}	6.7±0.7^{a}	13.5±1.8^{a}	26.7±3.2^{a}
19°C	*I.galbana*,5°C	2.5±0.8^{b}	7.2±1.8^{ab}	23.0±1.6^{b}	39.3±0.4^{b}
19°C	*I.galbana*,25°C	1.7±0.4^{a}	5.1±0.8^{a}	19.0±1.9^{b}	33.4±3.7^{ab}
19°C	*C.neogracile* H and T-*Iso* H	3.3±0.7^{b}	10.8±0.5^{b}	17.6±2.2^{b}	36.7±2.3^{b}

Values are mean ± SE, n=3. Values in the same column with different superscript letter are significantly different (P<0.05). H indicates high protein strains of T-*Iso* and *C. gracilis.*

Fatty acid composition in PE. DHA was the only essential fatty acid that changed in PE being affected significantly by temperature. The lowest values were observed at low temperature (P=0.018).

Fatty acid composition in TL. Both temperature and diet affected percentages of AA and EPA in total lipids (TL) (P=0.01). AA was significantly increased in larvae grown at 19°C and fed microalgal mixture, while larvae fed with both types of *I. galbana* and cultured at 11°C showed the lowest values. The level of EPA was significantly higher in larvae cultured at 19°C and fed microalgal mixture, intermediate in larvae reared at 19°C and fed *I. galbana*-grown at 5°C,

and lower in the remaining combinations. The major HUFA in total fatty acids was DHA, which was significantly affected only by temperature.

Discussion

When *I. galbana* was grown at a low temperature, there was a trend towards increased HUFA, in particular DHA, but the results were not significant. The lipid analysis and growth-survival resultssuggest that AA is not an essential fatty acid in larval scallops. The highest growth and survival obtained with *I. galbana* alone in both experiments could be an indicator that EPA is less important than DHA in larval nutrition of *A. purpuratus*.

PC decreased significantly with cold acclimation of scallop larvae, while cholesterol increased. Larvae showed a decreasing PC/PE ratio as a result of cold acclimation. The low TAG observed in larvae fed with the mixture T-*Iso* H + *C. gracilis* H may be due to feeding high-protein (low-lipid) microalgae containing 3.2% less lipid than normal protein mixture (Uriarte and Farías, 1999). The better survival and growth rate of larvae in experiment 1 compared to experiment 2 may be explained by the higher values of PC, TAG, and the PC/PE ratio in the first experiment. Increased TAG levels correlate with well-fed, healthy larvae in finfish.

The increasing level of unsaturated fatty acids in phospholipids during cold acclimation as a mechanism for conserving membrane fluidity appears to be a common feature of membrane systems (Hochachka and Somero, 1984). However, in the HUFA compositions of larval scallop lipids, we observed the opposite, possibly due to a poor capacity for thermal acclimation of *A. purpuratus* larvae.

The fatty acid composition of TAG largely reflects the diet composition, but membrane phospholipids can also alter their ratio of EPA/DHA as a function of diet in juvenile scallops (Coutteau et al., 1996). In scallop larvae, fatty acid compositions in PC changed as a result of feeding different microalgal diets while HUFA in PE were lessaffected by diet.

References

Bell J.G., J.R. Dick, and J.R. Sargent. 1993. Effects of diets rich in linoleic or linolenic acid on phospholipid fatty acid composition and eicosanoid production in Atlantic salmon (*Salmo salar*). Lipids 28:819-826.

Coutteau P., J.D. Castell, R.G. Ackman, and P. Sorgeloos. 1996. The use of lipid emulsions as carriers for essential fatty acids in bivalves: a test case with juvenile *Placopecten magellanicus*. J. Shellfish Res. 15:259-264.

Hochachka P.W. and G.N. Somero. 1984. Biochemical adaptation. Princeton University Press. 540p.

Uriarte I. and A. Farías. 1999. The effect of dietary protein content on growth and biochemical composition of Chilean scallop *Argopecten purpuratus* (L.) postlarvae and spat. Aquaculture 180:119-127.

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COMMERCIAL LARVICULTURE OF PENAEID SHRIMP

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In the years since Larvi' 95, the commercial shrimp hatchery industry has been confronted by several major issues, principal among these being disease concerns, the availability and quality of larval feeds, particularly *Artemia*, and postlarval quality issues. The impact of these factors on the hatchery business has been profound. At the same time, the trend towards environmental purchasing policies, "best management practices", certification and "chain of custody" controls in the global shrimp market is likely to have an increasing impact in the commercial larviculture sector over the next ten years.

The possible role of hatcheries and broodstock on the transmission of shrimp virus diseases came to the fore with the outbreak of White Spot Syndrome Virus (WSSV), first in Asia and then in the Americas. The available evidence appears to point towards the movement of live shrimp broodstock, larvae and postlarvae as one of the major factors contributing to the spread of the disease both nationally and internationally. This has resulted in considerable pressure on hatcheries to place greater emphasis on biosecurity and health management protocols as a means of preventing the vertical transmission of disease. In Thailand, for example, hatcheries are frequently required to submit samples for testing before sale or transfer to farmers' ponds to ensure that they are not infected by WSSV. In some cases, hatcheries or groups of hatchery operators have installed the necessary equipment to carry out these tests themselves to avoid later problems through rejection of infected batches of shrimp.

Wide differences in availability and prices of broodstock, larvae and postlarvae between countries have also increased the demand for international movement of shrimp stocks, both legally and illegally. In Asia, broodstock and postlarvae are regularly sold across national boundaries and the international trade in nauplii and postlarvae of shrimp in the Americas was severely impacted by the outbreak of WSSV in the late 1990's. The development of guidelines and methods to ensure that such transfers are carried out safely is lagging behind the development of the international trade. Recent initiatives by several groups have addressed these issues at the international level with the aim of controlling and reducing the risk of disease transfer as a result of this increasing trade.

Artemia prices have skyrocketed in the past 5 years as its availability continues to fall. Such a drastic increase in the cost of one of the key components of the larval diet has caused hatcheries to drastically alter their strategies with respect to *Artemia* use. Increased use of alternative diets and live feeds, reduced dependence on live *Artemia*, enrichment strategies, and the use of *Artemia* biomass have all been adopted by commercial hatcheries to overcome the problems associated with reduced *Artemia* supply.

This brief presentation will review some of these key issues and their influence on commercial penaeid larviculture practices and their likely influence on future developments.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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MUSCLE GROWTH IN FIRST FEEDING HALIBUT LARVAE WITH DIFFERENT BODY GROWTH RATES

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Introduction

The swimming musculature is the largest and fastest growing tissue in fish larvae, and it has a great impact on the larvae's ability to catch prey and avoid predators. During the 50 day long yolk sac phase of halibut larvae (*Hippoglossus hippoglossus* L.) this tissue grows predominantly by an increase in size of the muscle fibres that were present at hatching, while recruitment of new fibres becomes increasingly important as a growth mechanism towards the end of the yolk sac phase (Galloway et al., 1999). Nothing is known about muscle growth in first feeding halibut larvae. The present study describes muscle growth patterns in first feeding halibut with different body growth rates.

Materials and methods

Halibut eggs and yolk-sac larvae from a single spawning batch were incubated according to the procedures at Akvaforsk, Norway. At approximately 270d° (day 0) the larvae were transferred to a 1200-l tank with newly hatched *Artemia* nauplii and dim overhead light. The temperature was then gradually increased from 7°C to 13°C. Ten days after the onset of first feeding the larvae were transferred to 20-l rearing units at a density of $20.l^{-1}$. The larvae were fed *Artemia* sp. nauplii with 3 different enrichments, in order to induce different growth rates.

Daily weight increase (%) was calculated from dry weight measurements on day 0 and 62. For histological analyses 5-6 larvae from each treatment were sampled on day 0 and 62, fixed in 2.5% paraformaldehyde and 2.5% glutaraldehyde in cacodylate buffer and embedded in methacrylate resin (Historesin). Standard length and myotome height was measured before embedding. Sections 3µm thick were made immediately posterior to the body cavity and stained with toluidine blue and basic fuchsin. Assuming bilateral and dorso-ventral symmetry, the number of white fibres in one epaxial quadrant of the myotome was counted and multiplied by 4, in order to get an estimate of the total muscle fibre number in a

myotomal cross section. From this the daily increase in fibre number was calculated.

Results and discussion

At the onset of first feeding the mean larval dry weight, length and myotome height were 1.4±0.3mg, 13.7±0.4mm, and 0.9±0.0mm, respectively. Over the next 62 days the larval growth varied between different *Artemia* enrichments fed to the larvae (Table I), so that the mean dry weight of larvae from treatment 1 was more than 4 times that of larvae from treatment 3. Metamorphosis had started in most larvae at day 62.

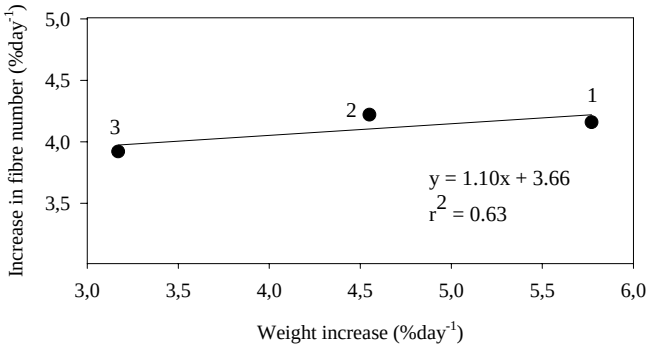

Table I: Growth data at day 62 after the onset of first feeding.

Treatment	Standard length (mm)	Myotome height (mm)	Dry weight (mg)	Weight increase (%.day^{-1})
1	26.5 ± 3.1	6.9 ± 0.8	45.4 ± 9.6	5.8
2	21.6 ± 3.0	5.6 ± 1.0	22.0 ± 2.0	4.6
3	19.5 ± 3.1	4.6 ± 1.1	9.7 ± 1.8	3.2

In all treatments the number of white muscle fibres per cross section increased from 1600 at the onset of first feeding to approximately 20 000 at 20mm length, after which the muscle fibre recruitment levelled off. In the sparse literature available on larval muscle growth (Akster et al., 1995; Nathanailides et al., 1995; Hanel et al., 1996; Alami-Durante et al., 1997; Johnston et al., 1998), increased body growth rates are generally associated with an increased white muscle fibre recruitment, irrespective of the cause of the different growth rates. Such a correlation has also been found for larval cod (Galloway, 1999), but does not seem to be valid for first feeding halibut (Fig. 1), where the muscle fibre recruitment

rate was relatively constant with increasing body growth rate. The different body growth rates therefore seem to be best explained by differences in hypertrophy throughout the first feeding stage in halibut larvae. However, the muscle fibre size distribution data remain to be analysed.

Muscle fibres cannot multiply by simple mitosis since each fibre contains many nuclei and a large proportion of contractile proteins. In fish, new muscle fibres are formed when mononucleated cells located between mature muscle fibres – the myosatellite cells – divide and fuse (Koumans and Akster, 1995). Myosatellite cells also provide nuclei for fibres that are growing in volume. The number of myosatellite cells present in the early life stages is believed to have a profound effect on the future growth potential of the fish (Johnston et al., 1998). An ongoing continuation of the present study is therefore to investigate myosatellite cell dynamics in halibut larvae, by immunohistochemical and molecular biological methods.

Acknowledgements

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References

Akster H.A., J.A.J. Verreth, I.L.Y. Spierts, T. Berbner, M. Schmidbauer, and J.M.W. Osse. 1995. Muscle growth and swimming in larvae of *Clarias gariepinus* (Burchell). ICES Mar. Sci. Symp. 201:45-50.

Alami-Durante H., B. Fauconneau, M. Rouel, A. Escaffre, and P. Bergot. 1997. Growth and multiplication of white skeletal muscle fibres in carp larvae in relation to somatic growth rate. J. Fish Biol. 50:1285-1302.

Galloway T.F. 1999. Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and halibut (*Hippoglossus hippoglossus* L.). PhD thesis, Norwegian University of Science and Technology, Norway.

Hanel R., J. Karjalainen, and W. Wieser. 1996. Growth of swimming muscles and its metabolic cost in larvae of whitefish at different temperatures. J. Fish Biol. 48:937-951.

Johnston I.A., N.J. Cole, M. Abercromby, and V.L.A. Vieira. 1998. Embryonic temperature modulates muscle growth characteristics in larval and juvenile herring. J. Exp. Biol. 201:623-646.

Koumans J.T.M. and H.A. Akster. 1995. Myogenic cells in development and growth of fish. Comp. Biochem. Physiol. 110A(1):3-20.

Nathanailides C., O. Lopez-Albors, and N.C. Stickland. 1995. Influence of prehatch temperature on the development of muscle cellularity in posthatch Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 52:675-680.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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A MODEL THAT SIMULATES LARVAL MORTALITY OF THE BIVALVE MOLLUSC *TIVELA MACTROIDES*

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Abstract

Laboratory data from culture of the marine bivalve *Tivela mactroides* was used to develop a simulation model that simulates larval mortality at different water temperatures and use this model to simulate mortality at more extreme temperatures than those observed in the lab. The model represents a cohort of larvae introduced as trochophores and subsequently pass through straight-hinge veliger, umbo, and pediveliger stages before becoming juveniles. Larval mortality of *T. mactroides* at 22, 25, and 28°C decreased with increasing water temperature, and there was no significant difference (P>0.05) between simulated and observed data. Simulated larvae failed to develop beyond the pediveliger stage at 20 and 18°C, and failed to develop beyond the umbo stage at 16°C. Although the current model predicts less mortality at temperatures up to 34°C, excessive bacterial growth and reduction of feeding rate may occur as temperatures approach this upper lethal limit.

Introduction

Tivela mactroides, a bivalve that has sustained a local fishery in western Venezuela for decades, has recently been essentially eliminated after a brief period of intense commercial exploitation (1993-1995), a huge oil spill (1997), and excessive rain (1999) that dropped natural salinity to almost freshwater conditions. Current efforts to aid recovery of natural stocks of *T. mactroides* in Venezuelan waters focus on establishment of fisheries regulations and development of protocols to raise seed (pre-juvenile and juvenile stages) under controlled laboratory conditions (Severeyn et al., 1996). An effective re-population program will require production of massive amounts of seed in specialized spawning and larval rearing centres. At present, the primary obstacle

to producing *T. mactroides* seeds in commercial quantities in nurseries is identification of appropriate physico-chemical conditions, which may in fact lie outside of the range of the animal's natural reproductive conditions. Successful production of bivalves requires continuous monitoring and control of environmental variables – in particular, the ability to predict effects of temperature on larval mortality has become a fundamental issue in contemporary shellfish aquaculture (Barnabe, 1994). In this paper, we describe, based on laboratory experiments (Reverol et al., 1998), a simulation model that emulates the larval mortality of *T. mactroides* at different water temperatures, and use this model to predict the behavior of this parameter at temperatures more extreme than those studied in the laboratory.

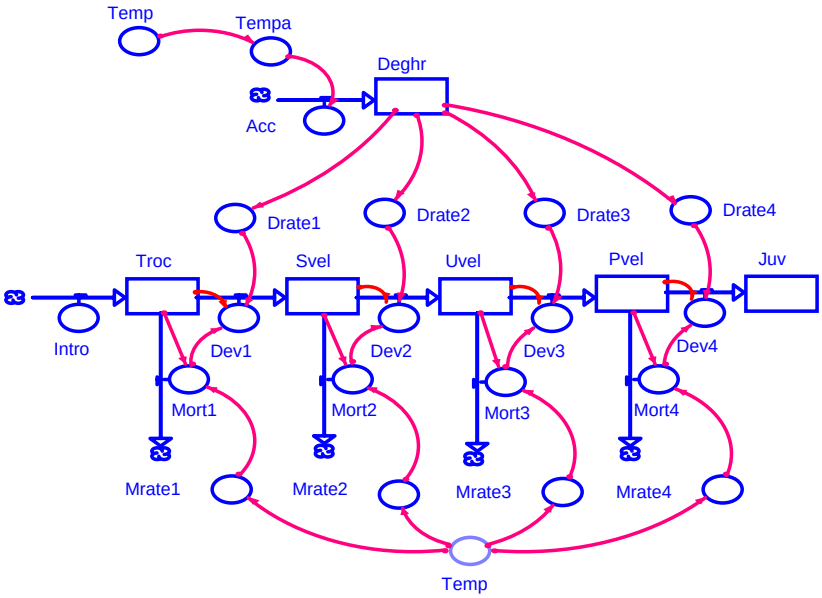

Fig. 1. Model representing the development (Dev) and mortality (Mort) of *T. mactroides* larvae introduced (Intro) as trochophores (Troc) and subsequently passing through straight-hinge veliger (Svel), umbo (Uvel), and pediveliger (Pvel) stages before becoming juveniles (Juv). Development rates (Drate) are controlled by accumulation of degree hours (Deghr) of "adjusted" water temperature (Tempa) and mortality rates (Mrate) are controlled by water current temperature (Temp).

Materials and methods

Data of larval *T. mactroides* mortality was obtained from Reverol et al. (1998). The model (Fig. 1), programmed in STELLA® (High Performance Systems, Inc., 1997) using difference equations with a time step of 1h, represents the development (Dev) and mortality (Mort) of *T. mactroides* larvae in relation to water temperature (Temp, °C). A cohort of larvae are introduced (Intro) as

trochophores (Troc) and subsequently pass through the straight-hinge veliger (Svel), umbo (Uvel), and pediveliger (Pvel) stages before becoming juveniles (Juv). Development rate of each stage is controlled by the accumulation of degree hours (Deghr) of "adjusted" water temperature (Tempa). Adjusted water temperature was calculated as:

$$Tempa = (-0.66667 + 0.06667 \times Temp) \times Temp$$

Developmental rate equations representing the proportion of individuals passing from one stage to the next were estimated by relating experimental data of larval development to water temperature, hypothesizing that development time is controlled by an accumulation of degree-hours of water temperature, and estimating the number of degree-hours that had accumulated at 25°C in the laboratory before the first and last individual "arrived" at each developmental stage. Mortality rate equations representing the proportion of individuals dying in the trochophore (Mrate1), straight-hinge veliger (Mrate2), umbo (Mrate3), and pediveliger (Mrate4) stages each hour are controlled by current water temperature (Temp). These equations were obtained by regressing the proportion of individuals dying during the indicated stage in the laboratory on the water temperature at which the experiment was conducted. We evaluated the model by simulating development and mortality of *T. mactroides* larvae at water temperatures of 22, 25, and 28°C, and comparing model predictions with laboratory data. We ran ten replicate simulations at each temperature and introduced variation in rates of development and mortality commensurate with the variation observed in the laboratory.

Results and discussion

There was no statistically significant difference between simulated and observed median mortalities ($F=1.47$, $df=54$, $P=0.23$). The mean proportion of animals dying during each larval stage decreased with increasing water temperature, both in the laboratory and in the simulations. Simulation of larval mortality at water temperatures ranging from 16 (near the lethal minimum) to 34°C (near the lethal maximum) indicates that larvae passed from trochophore to straight-hinge veliger, straight-hinge veliger to umbo, umbo to pediveliger, and pediveliger to juvenile after approximately 5, 10, 95, and 180h (respectively) at 34°C, whereas approximately 13, 30, 300, and 550h (respectively) were required at 22°C. Larvae failed to develop beyond the pediveliger stage at 20 and 18°C, and failed to develop beyond the umbo stage at 16°C.

Temperature plays an important role in the culture of bivalves (Stickney, 1994), especially during embryonic stages when small temperature variations may markedly affect development. Although much literature exists concerning the influence of temperature on growth of mussels and oysters (see listing in

Albentosa et al., 1994), there are comparatively few studies on clams. Likewise, although there are a variety of models simulating effects of water temperature on cultured organisms (see Jorgensen et al., 1996, for a review), relatively few have represented temperature as one of the main factors controlling mortality rates of larval stages. Temperature models dealing with this issue have been reported for organisms of agricultural importance, such as insects (Linardi and Botelho, 1997), but we are unaware of other models directly representing the effect of water temperature on larval mortality of bivalve molluscs in culture systems.

Results of both our laboratory and simulated experiments confirm that low temperatures increase larval mortality in tropical bivalves. At low temperatures, metabolism decreases, so larvae grow less (Albentosa et al., 1994), in part because they are less able to capture food. In our simulations, *T. mactroides* developed successfully at 22°C or above, but failed to complete the pediveliger stage at 20°C and failed to complete the umbo stage at 16°C. With regard to the optimal temperature for rearing *T. mactroides*, the current model predicts less mortality at temperatures up to 34°C. However, excessive bacterial growth and reduction of feeding rate may occur as temperatures approach the upper lethal limit in *T. mactroides* (Reverol et al., 1998). Thus, although the optimal temperature may be somewhat warmer than 28°C, we suspect that negative effects of large bacterial populations, which currently are not included in the model, will become a limiting factor before the upper lethal temperature is reached. More detailed laboratory investigations of survival near the upper lethal temperature should indicate the amount of physiological detail that will need to be added to the model to simulate system dynamics at these higher temperatures.

References

Albentosa M., R. Beiras, and A. Perez Camacho. 1994. Determination of optimal thermal conditions for growth of clam (*Venerupis pullastra*) seed. Aquaculture 126:315-328.

Barnabé G. 1994. Aquaculture: Biology and Ecology of Cultured Species. Ellis Horwood Series in Aquaculture and Fisheries Support. 657p.

Jorgensen S.E., S.B. Halling, and S.N. Nielsen. 1996. Environmental and ecological modeling. Lewis Publishers, New York, USA. 672p.

Linardi P. and M. Botelho. 1997. Effects of larval nutrition on the postembryonic development of *Ctenocephalides felis felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 34:494-497.

Reverol Y., Y. García de Severeyn, H. Severeyn, and J. Delgado. 1998. Efecto de la temperatura sobre el desarrollo larvario del guacuco *Tivela mactroides*. Boletin del Centro de Investigaciones Biológicas-LUZ 32:167-177.

Severeyn H., F. Morales, A. Godoy, and J. Delgado. 1996. Dinámica poblacional de la almeja marina *Tivela mactroides* en la playa de Caño Sagua. Edo. Zulia, Venezuela. Investigaciones Científicas 1:27-37.

Stickney R. 1994. Principles of Aquaculture. John Wiley & Sons, Inc., New York, USA, 502p.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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LARVAL MORTALITY OF THE MARINE CLAM *TIVELA MACTROIDES* REARED IN THE LABORATORY UNDER DIFFERENT TEMPERATURES

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Abstract

In this paper we present results of laboratory experiments on the effect of water temperature on larval mortality of the commercially important bivalve mollusc *Tivela mactroides*. We cultivated 6000 fertilized eggs in the laboratory at constant water temperatures of 22, 25, and 28°C, and monitored larval development from the trochophore to the pediveliger stage, to estimated mortality (%) at each temperature. In the laboratory, mean accumulated mortality from fertilized egg to Pediveliger was 82.4 at 22°C; 63 at 25°C, and 55.4 at 28°C. Results suggest that water temperature is inversely related to larval mortality in such a way that as temperature increases, larval mortality decreases, between the range of temperatures studied.

Introduction

Within marine aquaculture, molluscs represent one of the most important groups due to their low cost of production compared with other zoological groups (Bautista, 1989). Within molluscs, clams are important for aquaculture in the Northern Hemisphere because they are easily fed and have a high market demand (Ruiz and Ruffini, 1981). However, the culture of bivalves, which has become an important economic activity, demands continuous monitoring and control of environmental variables to reduce costs and increase profits. The ability to predict the effect of factors such as temperature on larval survival has become a fundamental issue in contemporary shellfish aquaculture. *Tivela mactroides* is a bivalve that has been exploited commercially in western areas of Venezuela but now is depleted. Present studies of *T. mactroides* are focusing on its culture to produce seeds that may be planted in the Gulf of Venezuela to repopulate the natural stock. In this paper we present results of laboratory

experiments in which larval development of *T. mactroides* was monitored at different water temperatures to calculate larval mortality.

Materials and methods

Specimens for this investigation were taken from Caño Sagua Beach, Gulf of Venezuela (71°56'21.5"W, 11°21'8.5"N). Once in the laboratory, animals were acclimated to laboratory conditions (24-48h) and put in 9l aquaria with filtered and sterilized water (UV irradiation) of the sampled station. Despite the acclimation process to avoid stress, most animals spawned before ending this process. Once male and female gametes were obtained, 1ml of sperm solution was mixed with 5ml of eggs (500 eggs.ml^{-1}) (García de Severeyn et al., 1994) in aquaria of 200ml. After fertilization occurred, five replicates consisting of 200 fertilized eggs for each temperature tested, were separated into 120ml aquaria. Sets of replicates were hold at three temperatures (22, 25, and 28°C) and the experiment performed twice for a total of 6000 larvae monitored (two experiments, three temperatures, five replicates, 200 larvae per replicate). Just after the first larval stage appeared, we monitored larval development from the trochophore to the pediveliger stage recording the amount of death from one stage to the next. These data were collected for the three temperatures studied.

Results and discussion

Laboratory results indicate that the mean accumulated mortality (%) from fertilized egg to trochophore, from trochophore to straight-hinge veliger, from straight-hinge veliger to umbo, and from umbo to pediveliger, were 28.8, 37.6, 66.0, 82.4, respectively, at 22°C; 27.0, 33.8, 52.2 and 63.0 at 25°C; and 25.0, 30.4, 42.8 and 55.4, respectively, at 28°C. (Table I). At trochophore and straight-hinge veliger there was not significant differences of larval mortality among temperatures. Absolute larval mortality among temperatures within each stage followed the same trend with higher mortality associated with fertilized egg evolving toward trochophore. The lower absolute larval mortality occurred between trochophore and straight-hinge veliger. These overall results suggest a clear effect of temperature on the larval mortality within the tested range: as temperature increases the mean larval mortality of each stage becomes smaller. This is consistent with the expected effect of higher temperatures, that increasing the metabolic rates makes growth faster.

Temperature plays an important role in the culture of bivalves, especially during embryonic stages when small temperature variations may affect development markedly (Bautista, 1989). Results in our laboratory confirm that low temperatures increase mortality of larval stages of the studied clam and agree with previous studies (Loosanoff and Davis, 1963; Davis and Calabresse, 1969);

at low temperatures metabolism decreases so larvae grow less (Albentosa et al., 1994), in part because they are less able to capture food (Walne, 1985). Interestingly, this is not the case for some temperate species such as *Mya arenaria*, which exhibit faster larval development and less mortality at lower temperatures (Sticney, 1964). Results are also consistent with those of Loosanoff and Davis (1963) and (Walne, 1985), who successfully reared larvae of *Mercenaria mercenaria* from fertilized egg to pediveliger at temperatures between 18 and 30°C, but noted that no larvae developed normally to the straight-hinge veliger stage at 15°C.

Table I. Accumulated mean mortality for larval stages of *Tivela mactroides* reared at different temperatures

Stages	Temperatures		
	28°C	25°C	22°C
Trocophore	28.8	27.0	25.0
SHV	37.6	33.8	30.4
Umbo	66.0	52.2	42.8
Pediveliger	82.4	63.0	55.4

SHV=Straight-hinge veliger

With regard to the optimal temperature for rearing *T. mactroides*, present data predicts less mortality as temperatures up to the upper lethal limit of 35. However, excessive bacterial growth and reduction of feeding rate may occur as temperatures approach the upper lethal limit in *T. mactroides* (Reverol, 1998) and other bivalves such as *Pteria sterna* (Salas and Espinosa, 1990), *Mercenaria* and *Venerupis* (Walne, 1985) and *Ostrea edulis* (Utting, 1988). Thus, although the optimal temperature may be somewhat warmer than 28°C, we suspect that the negative effects of large bacterial populations, will become the limiting factor before the upper lethal temperature is reached.

The present experimental results show that there is an inverse relationship between temperature and larval mortality. This relationship confirms what has been reported for other species of bivalve molluscs. Larvae of bivalve molluscs living in nerithic habitats, such as the area from where *Tivela mactroides* was collected, are exposed to a wide range of environmental changes, including temperature, that affect their mortality. What we have seen in the laboratory is an image of what really happens in nature. Thus, larvae normally develop at different rates depending on temperatures. In this manner, the larval cycle development time will change as a function of temperature changes so larval mortality will change consequently. In this case, even though temperatures as low as 22°C are unusual in the habitat where it normally lives, the present results

show that larvae of *Tivela mactroides* are not able to complete the whole cycle because they die before reaching the required time for development.

References

Albentosa M., R. Beiras, and A. Perez Camacho. 1994. Determination of optimal thermal conditions for growth of clam (*Venerupis pullastra*) seed. Aquaculture 126:315-328.

Bautista C. 1989. Moluscos. Tecnología de Cultivo. De. Mundi-Prensa. 167p.

Davis H. and A. Calabrese. 1969. Survival and Growth of larvae of the European oyster (*Ostrea edulis* L.) at different temperature. Biol. Bull. 136:193-199.

García de Severeyn Y., H. Severeyn, and J. Ewald., 1994. Early development of the estuarine mollusks *Polymesoda solida* (Philippi, 1946) (Bivalvia:Corbiculidae) in Lake Maracaibo, Venezuela. Amer. Malacol. Bull. 11(1):51-56.

Loosanoff V.L and H. Davis. 1963. Rearing of Bivalve mollusks. Adv. Mar. Biol. 1-136.

Reverol Y., Y. García de Severeyn, H. Severeyn, and J. Delgado. 1998. Efecto de la temperatura sobre el desarrollo larvario del guacuco *Tivela mactroides*. Boletin del Centro de Investigaciones Biológicas-LUZ 32:167-177.

Ruiz M.E. and E. Ruffini 1981. Obtención de larvas de bivalvos en condiciones controladas del laboratorio. Universidad de Oriente. Departamento de Ciencias Marinas 14p.

Salas L and E. Espinoza. 1990. Crecimiento y sobrevivencia de larval de la ostra concha nácar *Pteria sterna* en condiciones del laboratorio. Ciencias Marinas 16(4):29-41

Sticney A.P. 1964. Salinity, temperature and food requirement of soft-shell clam larvae in laboratory culture. Ecology 45(2):283-291

Utting S.D. 1988. The growth and survival of hatchery-reared *Ostrea edulis* (L) spat in relation to environmental conditions. Aquaculture 69:(1-2):27-38

Walne P.R. 1985. Cultivo de los moluscos bivalvos. Editorial Acribia, Zaragosa, España, 436p.

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WEANING OF MEXICAN BULLSEYE PUFFER (*SPHOEROIDES ANNULATUS*) LARVAE FROM *ARTEMIA* NAUPLII TO ARTIFICIAL MICRODIETS

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Introduction

The bullseye puffer (*Sphoeroides annulatus*) is a potential marine species for aquaculture in the Pacific coast of Mexico. At present, there is no published information on weaning for this fish. In species evaluation for commercial culture, it is necessary to formulate and test suitable artificial diets for weaning and ongrowing. High mortalities can occur during weaning if fish larvae present low ingestion rates or poor food digestion (Watanabe and Kiron, 1994). The assimilation efficiency for artificial diets in marine fish larvae can be improved if an easy-to-digest diet is provided. The digestibility of microbound diets for fish larvae can be improved by the use of decapsulated cysts of *Artemia* as the protein source (García-Ortega et al., 2000). This protein source was tested in weaning experiments with larvae of bullseye puffer.

In this study, two experiments were carried out to test the suitability of experimental microdiets for feeding hatchery-produced bullseye puffer larvae, to test a weaning protocol for the species, and to determine an early weaning time.

Materials and methods

Larvae of bullseye puffer were obtained by artificial spawning of wild broodstock. Before weaning, the larvae were progressively reared with microalgae (*Nannochloropsis* and *Isochrysis* spp.), rotifers (*Brachionus rotundiformis*), and *Artemia* nauplii. In a first weaning experiment, two microbound diets were tested with 60-day-old fish (initial weight 0.69g; length 3.0cm). The diets were prepared with different protein sources: diet 1 was prepared with decapsulated cysts of *Artemia* (74%, all percentages in dry weight), fishmeal (15.7%), fish oil (2.6%), mineral premix (1%), vitamin premix (1.7%), and carboxy-methyl-cellulose (5%); diet 2 was made with fishmeal (27.5%), squid (20.1%), tuna fish gonad (19.7%), shrimp meal (18.8%), fish oil

(2.1%), lecithin (1%), dextrin (2.1%), mineral premix (2%), vitamin premix (3.2%), BHT (0.5%), and alginate (3%). Larvae were reared in a seawater flow-through system with nine 600-l tanks at a density of 50 larvae per tank. Water temperature during the experiment fluctuated from 29.4-30.6°C and salinity from 28-32ppt. Three replicate tanks were used for each dietary treatment and the control with live *Artemia* nauplii. The fish larvae were weaned over a period of five days in which the *Artemia* nauplii were completely replaced by the artificial diet from a density of one nauplii per ml to zero at a daily rate of 20%. The particle size of the microbound diets was 420-700µm. During weaning, the first feeding of the day was done with the microdiets to allow maximal acceptance of the diet, and the first of two daily nauplii feedings was gradually postponed 30 minutes per day. The microdiets were given *ad libitum* five times daily at 09:00, 12:00, 15:00, 18:00, and 20:00 during 11 weeks. The growth (wet weight and length) was measured in 10 fish per tank every week, with the exception of week 10.

In a second experiment, 29-day-old larvae (initial weight 38.4mg; length 11.1mm) were stocked in four 600-l tanks with flow-through at a density of 150 fish per tank. Diet 1 was used to test three different fish ages for the initiation of weaning – 29, 34, and 39 days after hatching. One tank was fed exclusively live *Artemia* nauplii as a control. Weaning and feeding was done as indicated for the first experiment, except that a smaller particle size was used 190-420µm, *Artemia* density was 0.5 nauplii per ml, and the water temperature and salinity fluctuated from 29.7-30.8°C and from 32-34ppt, respectively. The weight and length of 10 larvae per tank was measured every three days, and the experimental period lasted 23 days. In both experiments, survival was determined at the end of the experimental period.

Results and discussion

In the first experiment, small differences were found in growth between the two diets. However, higher final weight (7.0g; Fig. 1), length (6.3cm), and survival (92%) were achieved with diet 1. Visual verification of larval feeding and food presence in the gut showed that both diets were well accepted by the fish. Growth results were similar as those obtained for the larvae of puffer fish *Takifugu rubripes* of the same age (Han and Yoshimatsu, 1997). Once it was determined that larvae of the bullseye puffer could be adequately reared with artificial diets, earlier times for weaning were investigated to reduce the period of feeding with live *Artemia* nauplii.

In the second experiment, no differences in mean growth (Fig. 2) and survival were found among treatments. The final survival rates were 49%, 46%, and 45% for fish weaned at days 29, 34, and 39 post-hatch, respectively. The control larvae obtained higher final weight and survival (63%). In early weaning trials with marine fish, a lower growth is obtained with the artificial diet treatments compared to the live food control, which is an indication of a delay in the maturation of some digestive processes in fish larvae (Cahu and Zambonino

Infante, 1994). Nevertheless, the results in the present study indicate that bullseye puffer larvae can be weaned at day 29 post-hatch (38.4mg), and possibly earlier without considerable reduction of growth or survival. This represents an attractive aspect of the species to take into consideration for its culture at a commercial scale.

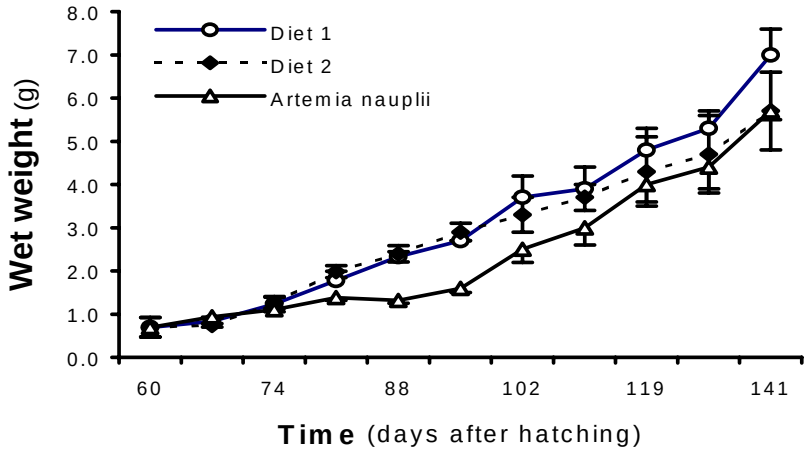

Fig. 1. Growth of bullseye puffer larvae during and after weaning with experimental microbound diets.

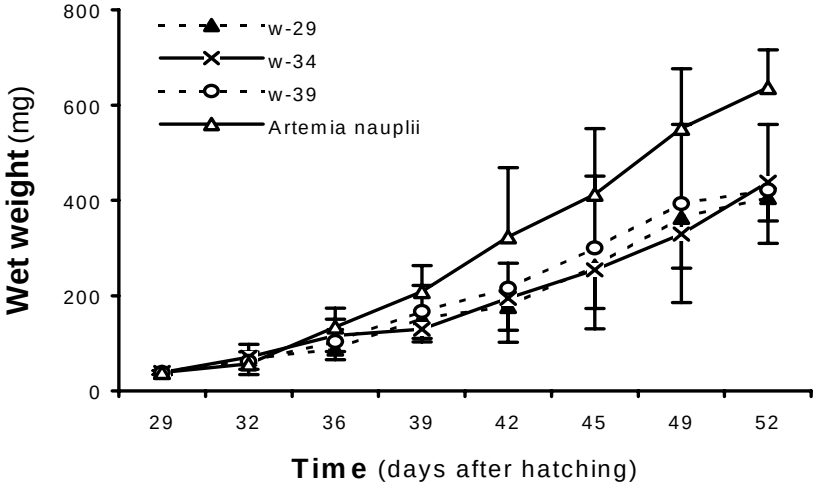

Fig. 2. Growth of bullseye puffer larvae weaned at 29, 34, and 39 days after hatching (w-29. w-34 and w-39 respectively).

Acknowledgements

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References

Cahu C.L. and J.L. Zambonino Infante. 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: effect on digestive enzymes. Comp. Biochem. Physiol. 109A:213-222.

García-Ortega A., A. Koussoulaki, H. Boer, and J. Verreth. 2000. *In vitro* protein digestibility of *Artemia* decapsulated cysts and nauplii, and of microbound diets for larval fish. Aquacult. Res. 31:475-477.

Han K. and T. Yoshimatsu. 1997. Early developmental stages in laboratory-reared puffer fish, *Takifugu rubripes*. Sci. Bull. Fac. Agr. Kyushu Univ. 51:157-165.

Watanabe T. and V. Kiron. 1994. Prospects in larval fish dietetics. Aquaculture 124:223-251.

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INDUSTRIAL LARVAL CULTURE OF MARINE FISH IN THE MEDITERRANEAN

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The precursor to the development of the Mediterranean aquaculture industry has been the availability of seed stock from hatcheries. Has this been a success or continuing struggle? Looking over the past evolution of marine finfish production in the Mediterranean, which has seen growth from 5620 tons and 25 million juveniles in 1990 to 109 866 tons and 483.5 million in 2000, a substantial industry has been created. Average ex-farm prices (bass and bream) fell from around €14 per kilo in 1989 to €6 in 1994, but have now stabilized at this level and are bumping along between €5.3 and €6.4 per kilo. The price of the final product determines what hatcheries are likely to receive for their juveniles, and during the months when fish prices are low, on-growers are reluctant to purchase juveniles. They may, however, switch their juvenile purchases to a species that has a higher market value during this period.

Continuous pressures in the market to reduce fry costs, yet also to maintain margins for the producer, have been facilitated by improvements in production technology. These pressures will continue and as the industry consolidates with production from both the smaller and larger hatcheries both increasing, attention has to be given to ensuring complete customer satisfaction to enable the successful producer to sell its juveniles at optimum prices.

The characteristics of juvenile or fry quality include their ability to grow in the ongrowing facilities, their disease resistance, and their appearance. Most juvenile producers pay great attention to morphological deformities such as missing opercula, bent spines, and head, tail, or jaw deformities, as these are easy to spot and often indicate problems elsewhere with the fry. As important to the grower, if not more so, is fry growth and disease-resistance, which can be difficult to determine before the juveniles are purchased. The reputation of the hatchery now becomes very important and its history of past juvenile performance will enable it to sell its juveniles during periods of abundant fry availability.

Hatcheries can improve juvenile performance by careful monitoring of growth, disease incidence, and morphological deformities. Nature and nurture are both important factors for fry quality and both require extensive research input. A breeding program can prevent inbreeding and ensure good performance in growth and disease resistance, but the results will take 3-4 years before they become apparent over the noise of environmental factors which effect juvenile quality.

Technologies such as vaccination, ozone sterilization, and seawater recirculation have the ability to improve disease resistance and growth potential in the short term. The development of disease-resistant strains from selective breeding and prudent stock management in the long term will also contribute to this goal.

The challenges for future juvenile production in the Mediterranean are:

- diversification of species suitable for industrial production
- development of technologies which reduce input costs
- sustainability by adopting environmentally friendly production systems
- orientation towards ensuring customer satisfaction

These will be achieved by detailed and continuing research on both nutrition and environmental requirements for species already cultured and for new ones. Development of technology such as the use of recirculation has advanced rapidly over the last five years, but has not been widely adopted. This type of technology promises to improve the quality of juveniles whilst reducing input costs and the impact of juvenile production on the environment. Adopting a professional approach to juvenile marketing by providing back-up and monitoring services to customers will reap rewards. Investment in training and attention to detail by responsible producers will ensure the exciting history of Mediterranean fry production continues to thrive into the 21st century and does not become a struggle.

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METHODS TO ESTIMATE FOOD INTAKE IN FISH LARVAE

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Introduction

Fish larvae are known to eat more and grow faster than larger fish when expressing food intake (FI) and weight gain per unit of body weight. Accurate knowledge on FI in larviculture is essential in order to adjust the daily feeding levels according to the objective of obtaining either maximum growth or optimal food efficiency. Present paper describes methods reported in literature to estimate FI in freshwater and marine fish larvae (<50mg body weight).

Gut content-transit time model

Gut content and food transit are two parameters often used to predict FI in fish. In larvae, gut contents can be estimated directly by counting the food particles in the transparent animals or by weighing the material found in the dissected gut, or indirectly, by analysing the whole larva for a marker, which may be naturally present in the diet (e.g., ascorbic acid sulfate in *Artemia* cysts or fluorescent pigments in algae) or added to the diet (e.g., chromic oxide, specific stains, opaque or radioactive substances). Numerous models have been developed to describe gut evacuation in fish. For larvae, the exponential evacuation models (e.g., Elliot and Persson, 1978) are generally preferred as they approximate well the transit of multiple small-sized food organisms. With the assumption that larvae feed until the digestive tract is filled

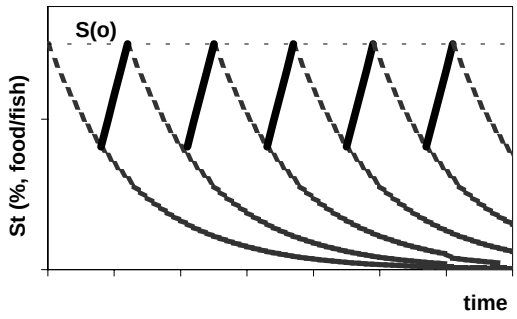

Fig. 1. Theoretical variation of the gut content in function of time: exponential gut evacuation (dashed) and gut filling (solid line).

(Fig. 1), the model calculates FI_t at time t as the difference between gut capacity (S_0) and gut content at time t (S_t) where $S_t = S_0e^{-Rt}$ (R: evacuation rate, t^{-1}). This gives $FI_t=S_0(1-e^{-Rt})$ and daily $FI=(24/t)FI_t$ with 24/t the feeding interval.
Reported transit times of food through the digestive tract in larvae (of <50mg) are generally of the order of magnitude of 1h, but can vary from as little as a few minutes to over 15h (Kaushik and Dabrowski, 1983; Haylor, 1993; Yufera et al., 1993; 1995; Day et al., 1996, Szlaminska et al., 1998). Several factors are known to influence food transit (e.g., temperature, food density, previous duration of food deprivation). Most studies indicate an increase in food transit time with increasing larval age. Relative intestinal length (% body length) of *Cyprinus carpio* larvae increases from 40% to 120% during the first 3 weeks after hatching (Kamler, 1992). Average gut contents (%, ww content/ww fish) were estimated at 20 % in catfish larvae in the range of 5-50mg ww (Haylor, 1993) but can vary greatly between species.

Examples of daily FI (%, dw food.dw^{-1} fish) based on the exponential gut content-transit model and assuming 24h continuous feeding are about 45% in 1.1mg dw *Clarias gariepinus* fed hydrated *Artemia* cysts (Haylor, 1993), 85% and 280% in 65µg dw *Sparus aurata* when fed rotifers or microcapsules (Yufera et al., 1993; 1995), and 130 and 77 % for *Solea solea* larvae of 107 and 165µg dw, respectively, fed 18h.d^{-1} on *Artemia* nauplii, with 1.9µg dw per nauplii (Day et al., 1996).

Potential errors in the model may be introduced by inaccurate estimations of both parameters. By this way, FI may be underestimated (i.e., gut evacuation time overestimated) when using, for instance, a partially absorbed marker or a marker with a proportionally slower evacuation, a single instead of multiple meal feeding protocol, a lower temperature, a linear instead of exponential evacuation model, etc.

Bioenergetic approach

Bioenergetic models provide information on the energy partitioning in growing animals. FI is here estimated by the summation of growth and a series of metabolic and digestive costs and losses: FI (J per day per unit BW)=G+M+E, in which each of the parameters, i.e., growth (G), metabolic losses (M), excretion, and egestion (E), is converted into energy units. Metabolism, active or basal, can be calculated from the uptake of O_2 in the feeding or constant-weight resting larvae. Active metabolism may be approximated at 2.5 times the basal metabolism, which in turn may be estimated by the allometric relationship between metabolism and body mass, adapted for each larval species. Only a few studies measured larval excretion products. In a study on carp larvae, postprandial and basal ammonia-N excretions were proposed for predicting FI (Kaushik and Dabrowski, 1983). Due to the difficulties for quantifying excretion

and in particular faeces in fish larvae, others (e.g., van der Meeren, 1991) proposed to replace the 'E' component of the formula by an empirical coefficient that accounts for the digestibility of the food (proportional to larval size) and corrects for the fraction of absorbed food fraction lost in metabolism.
The accuracy of the FI estimates by the bioenergetic approach depends, aside from the accuracy of the measurements of metabolic parameters, on the reliability of the many specific caloric conversion factors and on several untested assumptions (e.g., fish activity) or external factors (e.g., stress).

Growth at graded levels of food supply

A more simple approach, common in fish nutrition studies, estimates FI by comparing growth at graded levels of food supply over a period of a few days. This method allows the determination of the maximal FI, yielding the maximal growth and of the optimal FI resulting in the best food conversion efficiency). Bryant and Matty (1980) estimated maximal daily FI (%, dw food.dw^{-1} fish) at 200-250% for 1-10mg ww *C. carpio* larvae (T: 24°C, SGR: 50%) fed *Artemia* nauplii and to 100-150% for 10-50mg ww larvae (SGR=35%). High values of maximal FI (150% d^{-1}) were also found for 2-50mg ww *C. gariepinus* (T: 30°C, SGR: 35-40%) fed dry *Artemia* cysts (Verreth and den Bieman, 1987). This laboratory method is also being used to evaluate the extent to which larval growth may be influenced by food densities in the field.

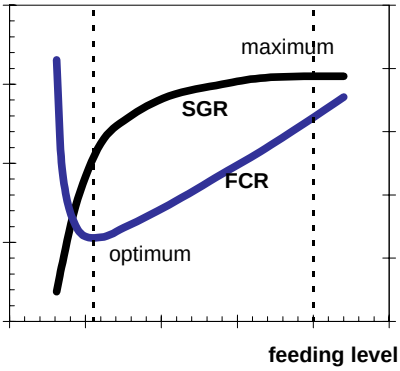

Fig. 2. Variation of daily specific growth rate and food conversion ratio as a function of feeding level.

Other methods

Other methods measure the difference between the amount of food offered and that remaining after a certain feeding time or obtain daily FI data by multiplying the quantity of food consumed during the first meal by the number of meals given per day. In both cases there is a risk of overestimating FI since, for the first method, samples are mostly taken in the water column, not considering the dead prey residing at the bottom of the tank, whereas, for the second method, daily FI is mostly extrapolated from the consumption in the first meal which is given after a night starvation.

There also exists a large variety of other methods that appreciate food consumption for studying feeding behaviour, food preference or food acceptability. They use single stomach content analyses, video recording, microscopic fluorescence observations, etc. These methods allow comparative studies but their results are difficult to convert into quantitative estimations of FI.

Conclusions

The two most common methods for measuring FI in big fish (i.e., visual estimation of satiation following hand feeding of a meal and recording of fish demand in "self-feeder" devices) do not apply for small larvae so that other methods are required. These methods necessitate however specific cautions in the case of larvae since the experimental errors inherent to each technique may be more important than in slow-growing larger fish.

References

Bryant P.L. and A.J. Matty. 1980. Optimisation of *Artemia* feeding rate for carp larvae (*Cyprinus carpio* L.). Aquaculture 21:203-212.

Day O.J., D.A. Jones, and B.R. Howell. 1996. Food consumption, growth and respiration of sole, *Solea solea*, during early ontogeny in a hatchery environment. Aquacult. Res. 27:831-839.

Elliot J.M. and L. Persson. 1978. The estimates of daily rates of food consumption for fish. J. Anim. Ecol. 47:977-991.

Haylor G.S. 1993. Controlled hatchery production of *Clarias gariepinus*: an estimate of maximum daily feed intake of *C. gariepinus.* Aquacult. Fish. Manage. 24:483-493.

Kamler E. 1992. Early Life History of Fish. An Energetics Approach. Chapman & Hall, London, 267p.

Kaushik S.J. and K. Dabrowski. 1983. Postprandial metabolic changes in larval and juvenile carp (*Cyprinus carpio*). Reprod. Nutr. Develop. 23:223-234.

Szlaminska M., T. Weglenska, J. Hamackova, J. Kouril, P. Kozak, and I. Adamkova. 1998. Passage time of *Artemia* nauplii through the gut of tench (Tinca tinca L.) larvae at 22°C. Czech J. Anim. Sci. 43:521-523.

van der Meeren T. 1991. Selective feeding and prediction of food consumption in turbot larvae (*Scophthalmus maximus* L.) reared on the rotifer *Brachionus plicatilis* and natural zooplankton. Aquaculture 93:35-55.

Verreth J. and H. den Bieman. 1987. Quantitative feed requirements of African catfish (*Clarias gariepinus*) larvae fed with decapsulated cysts of *Artemia*. I. The effect of temperature and feeding level. Aquaculture 63:251-267.

Yufera M., A. Polo, and E. Pascual. 1993. First results on feeding rates of *Sparus aurata* L. p.160-166. In: Fish Larval Physiology and Biochemistry. B.T. Walther and H.J. Fyhn (Eds), University Bergen, Bergen.

Yufera M., C. Fernandez-Diaz, and E. Pascual. 1995. Feeding rates of gilthead seabream (*Sparus aurata*) larvae on microcapsules. Aquaculture 134:257-268.

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MOLECULAR CHARACTERIZATION OF VIBRIOS ISOLATED FROM PENAEID SHRIMP LARVAL CULTURE

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Introduction

The *Vibrio* genus has been identified as the principal cause of vibriosis both at larval culture and grow-out operations. The main *Vibrio* species isolated from diseased shrimps (where a true association with a pathogenic process has been demonstrated) are *V. harveyi* (Abraham et al., 1997) for larvae and *V. penaeicida* (Ishimaru et al., 1995) for juveniles and adults. For all other species, a clear reproduction of the infectious process has not been demonstrated.

The taxonomy of *Vibrio* is in the process of revision due to the increasing results obtained by molecular techniques. Special emphasis has been given to the ribosomal gene 16S, but unfortunately, the 16S rDNA is unable to resolve closely related species, such as *V. harveyi* and *V. carchariae.* Moreover, it has been suggested that *V. carchariae* is a junior synonym of *V. harveyi* (Pedersen et al., 1998).

Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) has been especially useful because it interrogates the whole genome. Repetitive non-coding DNA segments such as ERIC, have been proven to be able, not only to distinguish between species, but to differentiate strains of the same species, e.g., *V. cholerae* (Rivera et al., 1995) among others.

The present investigation aims to differentiate between strains identified as *V. harveyi* or *V. carchariae* isolated from penaeid larval culture systems employing ERIC-PCR.

Material and methods

Bacterial strains were isolated from penaeid shrimp larvae culture systems from several locations and others were kindly donated from places where a vibriosis epizootic has been recorded (Table I). DNA of each strain was extracted using

the Promega Wizard® Genomic DNA Purification kit (A1120, Madison, USA) according to the manufacturer instructions.

The PCR reaction mix contained 16.96µl of water, 1.50µl of 25-mM $MgCl_2$, 1.32µl of a 2.5-mM dNTP mix, 2.50µl of 10X PCR buffer, 0.26µl of each primer (250 $\mu g.ml^{-1}$), 0.2µl of Taq polymerase (5 $units.\mu l^{-1}$), and 2.0µl of DNA for final volume of 25µl. The PCR reagents employed were Promega PCR core system I (M7660). ERIC-PCR was performed with primers ERIC1R 5'-ATGTAAGCTCCTGGGGATTCAC-3' and ERICC2 5'-AAGTAAGTGACTGGGGTGAGCG-3'. The amplification protocol was 94°C for 5min followed by 35 cycles of 94°C for 1min, 52°C for 1min, and 72°C for 5min, with a final extension of 72°C for 10min. The amplification products were resolved in a 2.0% agarose gel in TAE buffer, 6µl of a gel-loading dye were mixed with 25µl of the reaction and 10µl of the mixture were loaded into the gel. 4µl of the PCR molecular weight marker (Promega G316A) were added in a separate well. The gel was electrophoresed at 4-8°C for 2h at 70V and photographed with Polaroid Polapan 667 film.

Results and discussion

A slight difference was observed in the banding pattern produced with ERIC-PCR by *V. harveyi* and *V. carchariae* type strains (Table I and Fig. 1C), only one extra band was recorded in *V. harveyi*. Strains Z_2 and Z_3 produced almost the same banding pattern (Fig. 1D, some bands merely visible), therefore they are considered the same strain or a very close one, even so because they were isolated from the same broodstock tank. Strains ML and 2Mz also showed a similar banding pattern (Fig. 1A), but strain ML was isolated in La Paz, Baja California and strain 2Mz in Mazatlán, Sinaloa, both at the entrance of the Gulf of California. No other matching patterns were observed among the strains tested as can be appreciated in some examples presented in Fig. 1B, therefore they are considered individual strains.

ERIC-PCR was useful to differentiate between isolates of *Bartonella henselae* that produced relative simple banding patterns (Sander et al. 1998). Similar results were observed in this investigation where isolates could be easily distinguished. In the case of vibrios, this technique was useful to differentiate between toxigenic and nontoxigenic strains of *V. cholerae* serogroups from a variety of sources (Rivera et al. 1995). Although ERIC comprehends only Enterobacteriaceae in its name, it is not restricted to this group since these repeated sequences are present in several Gram-negative bacteria.

ERIC-PCR can be employed in epidemiological studies in aquaculture facilities either in the same area or in different locations, thus providing a very strong tool

to understand basic pathogenic trends in aquaculture. Samples can be rapidly analyzed, even in the same day, if the DNA extraction is performed by boiling the bacterial growth in a buffer for a few seconds and employing it as the PCR template.

Table I. List of bacterial isolates identified as *V. harveyi*-*V. carchariae* from penaeid larval systems and type strains. Molecular weight of bands amplified with ERIC-PCR from these isolates.

Strain	Source	Location	Molecular weights (bp)
ATCC14126	*V. harveyi* type strain. Dead amphipod *Talorchestia* sp.	Woods Hole, MA, USA	750, 704, 649, 563, 513, 463, 354, 330
LMG7890^T	*V. carchariae* type strain. Kidney of brown shark	Baltimore, MD, USA.	750, 707, 642, 567, 523, 467, 332
1A	Near shore seawater	Santa Bárbara Bay, Son., México (*L. stylirostris*)	1264, 954, 781, 128
ML	Larvae	Hatchery in La Paz, BCS, México (*L. vannamei*)	2227, 1510, 1234, 1106, 432
2 MZ	Seawater from a broodstock tank	Hatchery in Mazatlán, Sin., México (*L. stylirostris*)	2227, 1510, 1224, 1106, 428
10 MZ	Nauplii	"	1057, 955, 764, 592, 387
11Mz	"	"	2976, 1151, 1020, 818, 659, 444
STD3-131	Diseased postlarvae	Ecuador (*L. vannamei*)	2750, 1518, 1261, 1087, 771
STD3-1002	Diseased postlarvae	China	2201, 1226, 1067, 750, 683
Ea	Hatching system	Hatchery in Santa Clara Gulf, Son, México (*L. stylirostris*)	2292,1504, 1333, 1198, 1076, 954, 835, 463 119
Na	Nauplii	"	1957, 1169, 806
Z_1	Seawater from a broodstock tank	"	1587, 1169, 783
Z2	"	"	2655, 1789, 1693, 1554, 1165, 1076, 773
Z3	"	"	2679, 1767, ?, 1583, 1183, 1083, 773
M1	"	"	2298, 1224, 1106, 780, 119
PL96-11-6	Diseased postlarvae	Philippines (*P. monodon*)	1202, 1103, 783
IPL8	Postlarvae with luminescent vibriosis	"	2282, 1246, 1103

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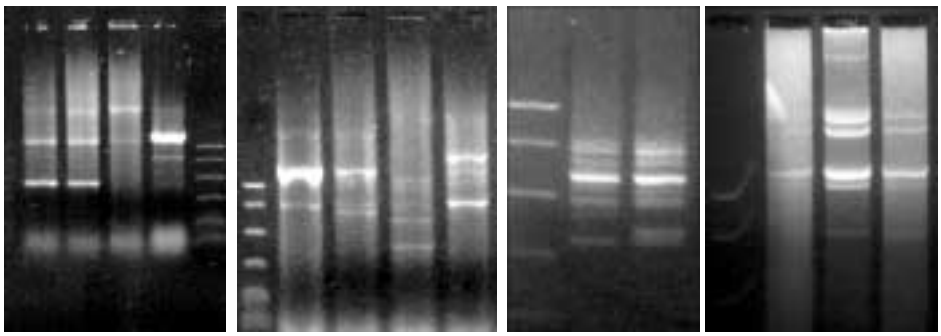
Dr. Leonardo Lizárraga, Dr. Celia Lavilla-Pitogo and Prof. Jean Swings for the donation of some strains. This investigation was funded by CONACyT project J-28344.



A B C D

Fig. 1. ERIC-PCR of selected strains. **A**. line 1, ML; 2, 2Mz; 3, IPL8; 4, M1; and 5, marker. **B**. line 1, marker; 2, M1; 3, STD3-1002; 4, 10Mz; and 5, STD3-131. **C**. line 1, marker; 2, *V. carcahriae*; and 3, *V. harveyi*. **D**. line 1, marker (2 upper bands); 2, Z1; 3, Z2; and 4, Z3. PCR molecular weight marker = 1000, 750, 500, 350, 150, and 50bp.

References

Abraham T.J., R. Manley, R. Palaniappan, and K. Dhevendaran. 1997. Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimp. J. Aquacult. Trop. 12:1-8.

Ishimaru K., M. Akagawa-Matsushita, and K. Muroga. 1995. *Vibrio penaeicida* sp. nov., a pathogen of kuruma prawns (*Penaeus japonicus*). Int. J. System. Bacteriol. 45:134-138.

Pedersen K., L. Verdonck, B. Austin, D.A. Austin, A.R. Blanch, P.A.D. Grimont, J. Jofre, S. Koblavi, J.L. Larsen, T. Tiainen, M. Vigneulle, and J. Swings. 1998. Taxonomic evidence that *Vibrio carchariae* Grimes *et al*. 1985 is a junior synonym of *Vibrio harveyi* (Johnson and Shunk 1936) Baumann *et al*. 1981. Int. J. System. Bacteriol. 48:749-758.

Rivera I.G., M.A. Chowdhury, A. Huq, D. Jacobs, M.T. Martins, and R.R. Colwell. 1995. Enterobacterial repetitive intergenic consensus sequences and the PCR to generate fingerprints of genomic DNAs from *Vibrio cholerae* O1, O139, and non-O1 strains. Appl. Environ. Microbiol. 61:2898-2904.

Sander A., M. Ruess, S. Bereswill, M. Schuppler, and B. Steinbrueckner. 1998. Comparison of different DNA fingerprinting techniques for molecular typing of *Bartonella henselae* isolates. J. Clin. Microbiol. 36:2973-2981.

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EFFECT OF PHOSPHOLIPID, PROTEIN HYDROLYSATE, AND VITAMIN CONTENT ON FORMULATED DIETS FOR MARINE FISH LARVAE

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Introduction

Several studies conducted on sea bass larval nutrition have led to the formulation of diets inducing good growth and survival (Zambonino Infante and Cahu, 1999). Nevertheless, the optimal concentration of some ingredients, such as lipid, phospholipid, and a vitamin/mineral mixture, still needed to be improved. In the same way, the efficiency of medium chain protein hydrolysate compared to short peptides remained to be tested.

Materials and method

Dicentrarchus labrax larval rearing was conducted in 35-l conical tanks and lasted 40 days. Initial larval density was 60 larvae.l^{-1}. Larvae were fed from mouth opening (day 5) exclusively with microparticulated compound diets. Four diets were tested (Table I). Diets B, C, and D were derived from diet A (patent WO0064273). In diet B, commercial protein hydrolysate was replaced by di- and tri-peptides, diet C contained 50% more phospholipid, and diet D contained only one third of the vitamin and mineral supply compared to diet A. At day 40, thirty larvae per tank were sampled for weight and deformity evaluation. Sampling, dissection, and purification of intestinal brush border membranes were conducted as reported in Cahu and Zambonino Infante (1994). Two intestinal enzymes, markers of enterocyte compartments, were assayed: alkaline phosphatase in brush border membrane, and leu-ala peptidase in cytosol. Data were compared using ANOVA, followed by Newman-Keuls when significant differences were found at the 0.05 level.

Results and discussion

Best growth and survival were obtained in groups fed A and C diets, mainly differing by their phospholipid content (Table I). This suggests that 11%

phospholipid incorporation in diet is sufficient to sustain larval development. Growth and survival were lower in the group fed diet B, in which protein hydrolysate (20 amino acid peptide) was replaced by di and tri-peptide. Groups fed A, B, and C exhibited low deformity percentages (<5%). In these diets, vitamin and mineral content was high compared to the diet formulated for juveniles. Nevertheless, a decrease in vitamin and mineral content induced significantly lower rearing performances: depressed growth and survival and especially high percentages of deformed larvae. The involvement of vitamin C and A in larval deformities has been reported in fish (Dabrowski 1988, Dedi et al. 1995). But the optimal level of each vitamin still needs to be determined.

Table I. Rearing performances of the four experimental groups at day 40. Means ± S.D. (n=4) with different superscript letters are significantly different (P<0.05).

	Group A	Group B	Group C	Group D
Survival (%)	56 ± 2.1^{a}	38 ± 5.1^{b}	53 ± 1.3^{a}	27 ± 2.9^{c}
Wet weight (mg)	83 ± 1.6^{a}	50 ± 1.9^{c}	76 ± 2.5^{b}	30 ± 0.67^{d}
Deformities (%)	2.7 ± 0.19^{b}	2.2 ± 1.93^{b}	4.4 ± 3.8^{b}	14.6 ± 6.44^{a}

Analysis of activities in brush border membranes and cytosol in enterocytes indicates the maturation level of larvae digestive tract. Alkaline phosphatase activity at day 25 and 40 was significantly lower in the group fed diet D compared to the other three groups, suggesting a delay in enterocyte maturation (Table II). This observation was confirmed by the ratio of brush border enzyme activity versus cytosolic enzyme activity (Table III).

Table II. Specific activity of alkaline phosphatase (mUI.mg^{-1} protein) assayed in purified brush border membranes. Means ± S.D. (n=4) with different superscript letters are significantly different (P<0.05).

	Group A	Group B	Group C	Group D
At day 25	722 ± 165.0^{a}	530 ± 94.0^{ab}	683 ± 103.1^{a}	361 ± 87.0^{b}
At day 40	1590 ± 283.0^{b}	2459 ± 201^{a}	1796 ± 58.0^{b}	796 ± 67.7^{c}

The lipid analysis in diets and in whole-body larvae at day 40 (Fig. 1) led to the following comments: the lipid level in larvae, and particularly neutral lipid, changes with dietary lipid level. It seems that fat deposition can equally occur in larvae as in juveniles. The phospholipid level in larvae was not influenced by the dietary phospholipid level.

Table III. Ratio segmental activity of alkaline phosphatase/segmental activity of leu-ala. Means ± S.D. (n=4) with different superscript letters are significantly different (P<0.05).

	Group A	Group B	Group C	Group D
At day 25	75 ± 2.2^{a}	46 ± 10.8^{b}	48 ± 2.4^{b}	27 ± 4.9^{c}
At day 40	199 ± 43.4^{a}	186 ± 26.3^{a}	194 ± 49.0^{a}	77 ± 37.8^{b}

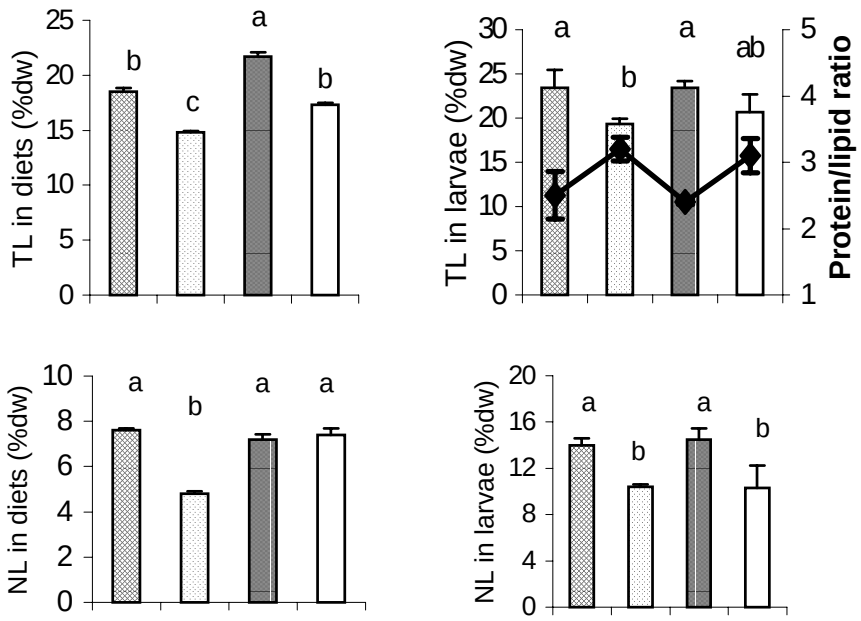

Fig. 1. Total (TL) and neutral lipid (NL) content (%dw) in the 4 experimental diets and in the larvae fed these diets. Protein/lipid ratio in whole-body larvae in bold. Means ± SD (n=4) with different superscript letters are significantly different (P<0.05).

Protein content was also analyzed in whole-body larvae. The two groups showing the best growth also exhibited a protein/lipid ratio of around 2.4. This ratio was around 3 in groups having the lowest growth. This ratio seems to reflect the physiological status of larvae.

Conclusion

This experiment showed that 11% phospholipid is sufficient for ensuring good larval development. With the neutral lipid/phospholipid proportions used in this

study, it appears that 14.8% total lipid in diet (diet B) is limiting for sea bass larval growth.

References

Cahu C. and J.L. Zambonino Infante. 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: effect on digestive enzymes. Comp. Biochem. Physiol. 109:213-222.

Dabrowski K., S. Hinterleitner, C. Sturmbauer, N. El-Fiky, and W. Wieser. 1988. Do carp larvae require vitamin C? Aquaculture 72:295-306.

Dedi J., T. Takeushi, S. T. Seikai, and T. Watanabe. 1995. Hypervitaminosis and safe levels of vitamin A for larval flounder (*Paralichthys olivaceus*) fed *Artemia* nauplii. Aquaculture 132:135-146.

Zambonino Infante J.L. and C. Cahu. 1999. High dietary lipid levels enhance digestive tract maturation and improve *Dicentrarchus labrax* larval development. J. Nutr. 129:1195-1200.

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FOOD SELECTIVITY, GROWTH, AND SURVIVAL OF MARINE FISH LARVAE FED DIFFERENT SIZES OF ROTIFERS

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Introduction

The euryhaline rotifers *Brachionus plicatilis* and *B. rotundiformis* show strain specificity in size and population growth. Only limited information has been available about how fish larvae utilize food resources containing different sizes of rotifers (Oozeki et al., 1992). Such features may be utilized in the feeding regimes for rearing various species of marine fish larvae.

Materials and methods

We conducted larval rearing of *Seriola quinqueradiata*, *Verasper variegatus*, and *Platycephalus* sp. from day 0 to day 15-20 posthatch by feeding two strains of *B. plicatilis* (designated as LL and L strains) and one strain of *B. rotundiformis* (S strain). The average lorica lengths of LL, L, and S strains were 248, 193, and 147μm, respectively. In order to see the effect of the type and size of rotifers, each rotifer strain was either singly fed or two rotifer strains were mixed (LL strain + L strain, L strain + S strain) and fed to fish larvae. Fish larvae reared in 2-l tanks were transferred to 30-l tanks, and rotifers were fed to larvae at 5-10ind.ml^{-1} for the LL and L strains and 5-30ind.ml^{-1} for the S strain. After 30min, 20 larvae were sampled and their gut was dissected to determine the rotifer type, rotifer number, and lorica length of rotifers ingested. Measured rotifers were divided into groups with different lorica size at 10μm interval. Food size selectivity of fish larvae was evaluated using Ivlev's selectivity index (Ivlev, 1965).

Under the same feeding conditions, survival and growth of larvae in 1-l tanks was monitored at 3-day intervals. The rotifer excretion rate and daily feeding amount of larvae were determined every 3 days from the mouth opening until the beginning of notochord flexion. From these data, the total number of rotifers

ingested by a larva was estimated using the formula reported by Yamashita and Bailey (1989).

Results and discussion

The mouth size (upper jaw length × $2^{0.5}$) of three fish larvae at the onset of feeding was 510, 280, and 260µm for *V. variegatus*, *S. quinqueradiata*, and *Platycephalus* sp., respectively. For all fish species, larger (developed) larvae selectively ingested large-sized rotifers (when single feeding) and strains (when mixed feeding) according to their growth. But the trend was strongest with *Platycephalus* sp., which has the smallest mouth size among three species. The feeding selectivity of larvae against different sized rotifers is not dependent on the mouth size of larvae.

The larval notochord flexion began on day 20, 15, and 20 with *V. variegatus*, *S. quinqueradiata*, and *Platycephalus* sp., respectively. At the end of the experiment, growth and survival of larvae fed with S strain was 7.7mm (total length) and 15.1% with *V. variegatus* and 6.3mm and 25.8% with *Platycephalus* sp. Better larval survival and growth were obtained when larvae were fed L strain. These were 8.8mm and 33.3% with *V. variegatus* and 7.3mm and 42.9% with *Platycephalus* sp. With *S. quinqueradiata*, survival and growth did not differ between larvae fed with different sized rotifer strains, but better survival and growth were obtained when larvae were fed higher density of rotifers at 30ind.ml^{-1} (total length = 6.9mm, survival = 35.8%) than at 10ind.ml^{-1} (total length = 6.7mm, survival = 17.2%). The total number of ingested rotifers (LL strain) was largest with *V. variegatus* (60 100 rotifers), and followed by *Platycephalus* sp. (18 600 rotifers) and *S. quinqueradiata* (8500 rotifers).

References

Ivlev V.S. 1965 Experimental ecology of the feeding of fishes. Yale University Press, New Haven, CT, 302p.

Oozeki Y., A. Hagiwara, H. Eda,and C.-S. Lee. 1992. Developmental changes of food selectivity of striped mullet *Mugil cephalus* during the larval stage. Nippon Suisan Gakkaishi 58:1381.

Yamashita Y. and K.M. Bailey. 1989. A laboratory study of the bioenergetics of larval walleye Pollock, *Theragra chalacogramma*. Fish. Bull. 87:525-536.

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EFFECTS OF DIETARY n-3 HIGHLY UNSATURATED FATTY ACIDS ON GROWTH, SURVIVAL AND BODY COMPOSITION OF THE COMMON WOLFFISH, *ANARHICHAS LUPUS*, AND YELLOWTAIL F9LOUNDER, *LIMANDA FERRUGINEA*

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Introduction

In recent years, the common wolffish, *Anarhichas lupus*, and yellowtail flounder, *Limanda ferruginea*, have been researched as promising cold-water candidates for aquaculture. However, knowledge of the feeding strategies and nutritional requirements of these species is limited, thus two parallel studies were conducted using the International Council for the Exploration of the Sea (ICES) reference diets to investigate *n-3* highly unsaturated fatty acid (HUFA) requirements. Quantitative n-3 HUFA requirements for various marine fish have been found to range from approximately 0.5-5.0% of the dry diet (Tucker, 1998). EFAs play a vital role in the metabolic functions of fish, especially in poikilothermic animals, which must tolerate changing temperatures. As cold-water species, it is expected that first feeding wolffish and juvenile yellowtail flounder will require high levels of n-3 HUFA in their diets to have good growth and survival.

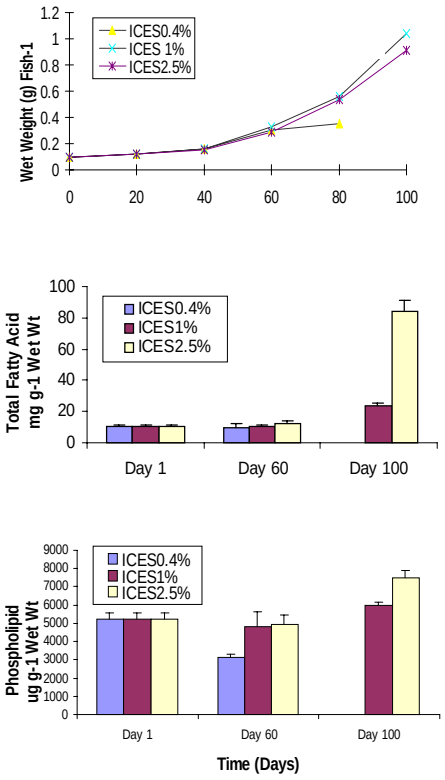
Materials and methods

First Feeding Wolffish. Newly hatched wolffish were stocked at 90 fish.l^{-1} (initially ~0.1g.fish^{-1}) in 9 shallow-water white mini-tanks. Rearing parameters included: 1-l water volume, 70-90ml.min^{-1} water flow, 6±0.5°C water temperature, 33±1‰ salinity, 200-270 lux light intensity at an 18L:6D photoperiod, and aeration. Feeding conditions consisted of simultaneously offering SuperSelco enriched *Artemia* (1000.l^{-1}) and a dry ICES diet (0.4%, 1%, or 2.5% HUFA to excess) during the first 30 days, weaning to day 40 and only providing a dry diet from day 40-100; 3 feedings per day were provided and each diet was tested in triplicate. Fish were sampled at 20-day intervals for growth (weight, length), lipid and amino acid analysis, and daily removal of mortalities for cumulative calculation of survival rates.

Juvenile Yellowtail Flounder. 0+ yellowtail flounder (initially ~1.9g $fish^{-1}$) were stocked at 15 $fish.tank^{-1}$ in 9 black tanks. Rearing parameters included: 13-l water volume, 500-800 $ml.min^{-1}$ water flow, 7±0.1°C water temperature, 32±1‰ salinity, 600±100 lux light intensity at an 18L:6D photoperiod, aeration. Feeding conditions consisted of a dry ICES diet (0.4%, 1% or 2.5% HUFA to excess) during the 72 day trial, with 3 feedings per day and each diet tested in triplicate. Fish were sampled every two weeks for growth and by dissection at the end of the experiment for liver and muscle tissue.

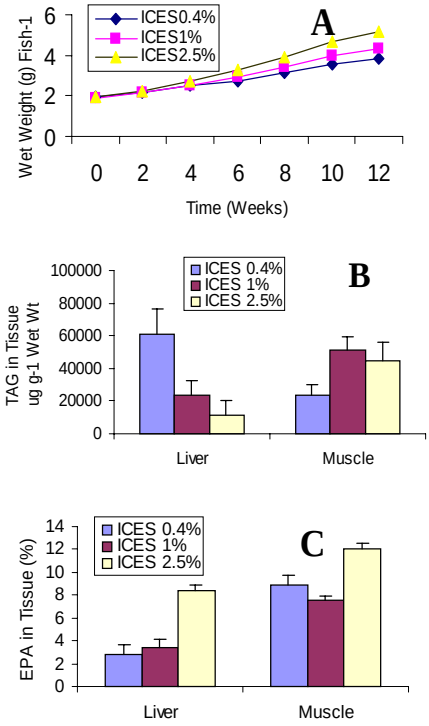
ICES Diets. Proximate composition of the ICES Standard Weaning Diets were 94.6% dry matter, 13.4% total lipid, 63.0% protein and 6.6% ash (ARC, 1996). Of the total lipid fraction 0.4% HUFA was the background total, with plant and fish oil portions modified to make up the 1 and 2.5% HUFA diets.

Biochemical Analysis. Diets and fish tissue samples were analyzed for proximate composition, lipid profiles and amino acids. Fatty acids were determined using a 2:1 chloroform and methanol extraction, a C17:0 internal standard, derivatization to fatty acid methyl esters with H_2SO_4 and measurement using a GC with a 30-µm DB225 (J&W Scientific) column. Lipid classes were determined using an internal standard of 3-Hexadecanone and a Mark V Iatroscan. Amino acids (only for wolffish) were determined using an AEC internal standard, deproteinization, the buffer lithium method and a Beckman 121MB Amino Acid Analyzer. The amounts of fatty acid and amino acid were calculated based on the weight of fish tissue samples or relative percentage.



Results

Survival for the first feeding wolffish was over 85% for the 1 and 2.5% diets but 100% mortality occurred by day 80 for the 0.4% diet. Symptoms of fainting and shock syndrome were



noted between days 50-80 followed quickly by death. However, in the juvenile yellowtail flounder, no mortality was observed over the duration of the trial.

Growth for wolffish fed diets 1 and 2.5% HUFA was not significantly different (Fig. 1A) but total fatty acid content was highest in fish fed the 2.5% diet and lowest for 0.4% (Fig. 1B)(P<0.05). This total fatty acid content corresponded to eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) patterns. Triacylglycerol (TAG) was not significantly different but did show an increasing trend in relation to low HUFA content. Phospholipids (PL) were significantly different with higher levels being deposited in fish fed higher levels of HUFA (Fig. 1C). The amino acid taurine was significantly higher in fish fed 2.5% PUFA and least for those eating the 0.4% diet.

Growth was greatest in yellowtail flounder fed the 2.5% diet and was poorest at 0.4% (Fig. 2A) (P<0.05). The proportion of triacylglycerol found in the liver was significantly higher in fish fed 0.4% HUFA than those fed the 2.5% diet (Fig. 2B). There were no significant differences in amount of EFAs in the liver or muscle tissue of fish fed 0.4 or 1% HUFA, but in the liver fish fed the 2.5% diet had significantly higher levels of EPA and AA than fish fed the other diets (Fig. 2C) (P<0.05).

Discussion

Within the first 60-80 days, wolffish fed the 0.4% HUFA diet showed deficiency symptoms of poor survival, growth and the fainting / shock syndrome, which has also been noted in other marine finfish (Sargent et al., 1989; Tucker, 1998). Although there was no significant difference in growth for the 1 and 2.5% HUFA fed wolffish, significant differences in total fatty acid content suggest that the

2.5% diet resulted in more effective tissue deposition. Halfyard et al. (1998) noted a similar pattern of deposition of phospholipids, acyl lipids (fatty acid containing lipids), and taurine, a non-essential amino acid, in fish fed better performance diets. The particular importance of DHA and PL for the larval development stage has also been discussed in other studies (Koven et al., 1993; Tucker, 1998).

The post-metamorphic yellowtail flounder did not exhibit any of the chronic EFA deficiencies observed in the first feeding wolffish and other marine finfish. However, fish fed the lowest level of n-3 HUFA showed poor growth and significantly higher levels of TAG in their liver. The accumulation of neutral lipid which occurred in the livers of yellowtail flounder has been observed in other animals, including rats and EFA-deficient fish (Fukuzawa, 1971). This accumulation is suggested to be the result of an impairment in lipoprotein synthesis, preventing lipids from being transported out of the liver (Sargent et al., 1989). The ratio of dietary DHA/EPA is thought to have an effect on growth, increased tolerance to stress and other parameters in fish (Koven et al., 1993; Ibeas et al., 1997). There was preferential conservation of HUFA in polar portions of the lipid fraction in fish fed 0.4% HUFA but in the neutral lipid fraction the DHA / EPA ratio decreased indicating a depletion of selected essential fatty acids.

Based on growth and retention of fatty acids, as well as the suggestion of EFA deficiencies in fish fed the lower HUFA diets, it is recommended that post-metamorphic yellowtail flounder be fed a diet containing 2.5% HUFA 10% lipid diet. The survival and growth requirements for first feeding wolffish appear to be met by either the 1 or 2.5% HUFA diets.

References

ARC (Artemia Reference Centre). 1996. ICES Standard Weaning Diets. http://allserv.rug.ac.be/~booghe/james/arc/ices/ices7.htm

Fukuzawa T., O.S. Privett, and Y. Takahashi. 1971. Effect of fatty acid deficiency on lipid transport from liver. Lipids 6:388-393.

Halfyard L.C, D. Drover, C.C. Parrish, and K. Jauncey. 1998. Growth, survival, lipid and amino acid composition in striped wolffish, *Anarhichas lupus*, fed commercial marine starter diets. Bull. Aquacult. Assoc. Canada 98-2:41-43.

Ibeas C., J.R. Cejas, R. Fores, P. Badía, T. Gómez, and A. Lorenzo Hernández. 1997. Influence of eicosapentanoic and docosahexanoic acid ratio (EPA/DHA) of dietary lipids on growth and fatty acid composition of gilthead seabream (*Sparus aurata*) juveniles. Aquaculture 150:91-102.

Koven W.M., A. Tandler, D. Sklan, and G.W. Kissil. 1993. The association of eicosapentaenoic and docosahexaenoic acids in the main phospholipids of different-age *Sparus aurata* larvae with growth. Aquaculture 116:71-82.

Sargent J., R.J. Henderson, and D.R. Tocher. 1989. The lipids. p.153-218. In: Fish Nutrition. Halver J.E. (Ed.). Academic Press, New York, NY, USA. 798p.

Tucker J.W. 1998. Marine Fish Culture. Kluwer Academic Publishers, Boston, USA. 750p.

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OPTIMIZATION OF DIETARY MACRONUTRIENT COMPOSITION FOR ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*, L.) LARVAE AND JUVENILES

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The optimal dietary macronutrient composition for Atlantic halibut at the weaning stage has yet to be investigated. Very young juveniles may have different requirements than larger fish. The data are important for the formulation of weaning diets and can, with caution, be used for optimizing live feed for the larval stage. A three-component mixture design was used to investigate the optimum composition of macronutrients for Atlantic halibut juveniles (0.5g start weight). Protein, lipid, and carbohydrate were varied between 530-830, 50-300 and 0-150g.kg^{-1} dry wt., respectively. Twenty of the twenty-one diets produced were fed to fish in single tanks, while one diet was fed to fish in four replicate tanks to estimate inter tank variation. The experiment lasted for two months.

Carbohydrate levels above 50g.kg^{-1} were suboptimal for halibut, resulting in lowered specific growth rates, increased hepatosomatic indices, and accumulation of carbohydrate in the liver. At low carbohydrate levels (0 and 50g.kg^{-1}), the specific growth rates appeared similar with lipid levels between 50-250g.kg^{-1}, while 300g lipid.kg^{-1} resulted in depressed growth. At higher carbohydrate levels, fish growth was reduced with lipid levels increasing above 100g.kg^{-1}. It can be concluded that Atlantic halibut juveniles have low tolerance to dietary carbohydrate, whereas dietary lipid can be varied over a wide range without affecting growth performance. The protein requirement appears to be in the range of 630-680g.kg^{-1}.

The macronutrient composition of copepods agrees well with the optimal composition for Atlantic halibut juveniles, with lipid varying between 9-25%, protein contents around 60-70%, and negligible carbohydrate. On the other hand, enriched *Artemia* may have fat levels around 30%, protein down to 40% and glycogen levels around 7%. An experiment was conducted to compare zooplankton and enriched *Artemia* as feed for start-feeding Atlantic halibut larvae. Results from this experiment will be discussed in relation to the results listed above.

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FIRST FEEDING OF HALIBUT LARVAE ON ROTIFERS

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Introduction

Experiments on first feeding of halibut larvae on rotifers were conducted in the early 1990s. In those experiments, *Artemia* were used after the rotifer period before the fry were weaned to a dry diet. However, comparable results of growth and survival of halibut larvae were obtained with *Artemia* alone. Due to costs with both rotifer and *Artemia* production, rotifers were skipped. Later, availability and prices of *Artemia* became undesirable. In addition, *Artemia* substitutes (formulated feeds) have improved. The use of rotifers is therefore once again actualized. In this experiment, rotifers and *Artemia* were tested as first feed on a large scale.

Material and methods

Halibut larvae, in triplicate tanks, were fed either rotifers or *Artemia* for 26 days. The rotifers were grown in a continuous culture with 10-15 daily exchange, and fed ROTIMAC. The *Artemia* were short-time enriched on DHA Selco. From day 27 onwards, both larval groups were fed *Artemia* until fry quality could be examined. Larval growth, mortality, pigmentation, and eye migration were assessed. The prey organisms and larvae were analyzed with regard to chemical composition.

Results and discussion

Halibut larvae fed rotifers performed best with regard to growth until day 13. At this age, the mean weight of the rotifer-fed larvae was 1.65mg DW and 1.10mg DW for the *Artemia*-fed larvae. However, this situation changed during the next 10 days, when the *Artemia*-fed larvae grew best. At day 26, the mean weight of the rotifer-fed larvae was 3.32mg and 4.50mg for the *Artemia*-fed larvae. Larval mortality was highest in the rotifer group in the first 10 days, and the mortality declined thereafter to nearly zero in this group. In the tanks fed *Artemia*, the

mortality varied between replicates and this can be related with a nodavirus outbreak in one of the tanks.

A higher proportion of halibut fry fed *Artemia* enriched on DHA Selco were mal pigmented compared to the fry fed rotifers. In the *Artemia* group, 62% of the fry were albino, compared to 26% in the rotifer-fed group. The eye migration was relatively low in both groups, and best eye migration was found in the *Artemia* group.

Short-time enriched *Artemia* contained more lipid than the rotifers. The presence of the fatty acids arachidonic acid, EPA, and DHA were, however, not significantly different (measured as % of total fatty acids). In the larvae however, the level of DHA was nearly twice as high in the larvae fed rotifers compared to larvae fed *Artemia*.

Lipid oxidation products (TBARS) were measured in both *Artemia* and rotifers. High levels (429) were observed in rotifers in the last period of the experiment.

Conclusions

- Halibut larvae had higher growth when fed rotifers compared to *Artemia* the first period of start feeding. Thereafter, larvae fed *Artemia* performed best. This could be due to lipid oxidation in rotifers, or that rotifers are too small a feed particle.

- Halibut larvae fed rotifers had, in general, better pigmentation compared to larvae fed *Artemia*.

- Eye migration was low in both groups.

- The DHA level in larvae fed rotifers was significant higher than larvae fed *Artemia*.

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PREDICTIVE CRITERIA OF SHRIMP LARVAL QUALITY: AN EXPERIMENTAL EVALUATION

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Introduction

Shrimp larval quality is a major concern for production purposes and research is often directed to improve this larval quality. Several criteria are actually in use (for review, see Bray and Lawrence, 1992) although it is not always clear if they really reflect an overall condition of larvae. In the present work, several criteria were evaluated in terms of the probability to perform better under culture conditions.

Methods

Three larviculture sets were used for the present study. Each consisted of individual spawns reared to the postlarval stage (PL) under general conditions described in a previous work (Palacios et al., 1999).

The variables recorded in the three sets are summarized in Table I.

Table I. Variables analyzed.

Stage	Biochemical composition	Production variables*	Morphometric traits	Stress tests
Egg	TL, TG, CHO, G, TP	Number, fertilization rate	Diameter	
Nauplii	TL, TG, CHO, G, TP	Number, hatching rate	Length	
Zoea		Survival	Length	High Ammonia
Mysis		Survival, metamorphosis	Length	
Postlarvae (PL)		Survival, metamorphosis, survival PL2-20	Length	Low salinity in PL2 and PL20

*Survival to mysis and PL1 was calculated from nauplii, whereas metamorphosis to those larval stages were calculated on the basis of the previous larval stage (zoea and mysis, respectively). For zoea stage, survival = metamorphosis because it is calculated on the basis of stocked nauplii V stage. TL = Total lipids, TG = triglycerides, CHO = carbohydrates, G = glucose, TP = total protein.

Biochemical composition was analyzed as described in Palacios et al. (1999): triglycerides and glucose by enzymatic-colorimetric methods, carbohydrates by anthrone, proteins by Comassie-blue, and lipids by sulpho-phosphovanillin. Stress tests were done in triplicate for each larviculture tank, which corresponds to an individual spawning. For ammonia stress, larvae in zoea II stage were exposed to 20 $mg.l^{-1}$ total ammonia concentration for 24h, based on previous standardization of 24h LC_{50} of 19.4 $mg.l^{-1}$ in closed flasks with aeration. For salinity stress tests, 18ppt and tap water were used to expose PL2 or PL20, respectively, during 30 minutes.

Some differences exist between the three sets due to their particular conditions:

1) Spawns (*n*=52) were obtained from the egg stage which allowed recordings of fecundity, fertilization and hatching rates data. In this set, it was possible to divide spawns into three categories: without hatching (until eggs or embryos = group "E") , with hatching but no further development to PL(until nauplii = group "N"), and with successful development to PL2 (group "PL").
2) This set starts at nauplii stage from individual spawnings (*n*=43) reared to PL20 stage although some stress tests were not done (high ammonia and low salinity in PL2). In contrast to the first larviculture set, no recordings of fecundity, fertilization and hatching rates were possible.
3) Same as above (*n*=38), but high ammonia and low salinity stress test in PL2 were done in this larviculture set.

Results and discussion

Data obtained from the first larviculture set are reported in Table II. Higher levels of total carbohydrates and lipids were observed for eggs that successfully developed into PL. The well-known importance of lipids for early larval development is further supported in the present study. The importance of carbohydrates could be related to active exoskeleton formation through nauplii stages.

Table II. Biochemical composition and morphological traits of eggs in three groups of spawns obtained in the first larviculture set (see methods).

	E (*n*=20)	N (*n*=13)	PL (*n*=19)	ANOVA
Triglycerides ($mg.g^{-1}$)	25.1 ± 1.37	23.0 ± 1.40	27.5 ± 1.50	n.s.
Glucose ($mg.g^{-1}$)	3.87 ± 0.16	3.54 ± 0.19	4.39 ± 0.40	n.s.
Carbohydrates ($mg.g^{-1}$)	8.92 ± 0.54[a]	8.37 ± 0.54[a]	10.8 ± 0.77[b]	$P<0.03$
Protein ($mg.g^{-1}$)	65.6 ± 3.84	64.8 ± 5.94	64.8 ± 3.57	n.s.
Total lipids ($mg.g^{-1}$)	41.5 ± 2.27[a]	37.7 ± 1.92[a]	63.3 ± 2.16[b]	$P<0.001$
Egg diameter (µm)	261 ± 1.0	262 ± 7.0	261 ± 0.6	n.s.

Data are reported as mean. One-way ANOVA results are reported in last column. Means not sharing the same superscript are significantly different, as determined by Tukey test.

Correlation analyses were done between the different variables analyzed for the 19 spawnings with successful development to PL. Survival to mysis and to PL1 were positively correlated with survival to zoea (r=0.84, and r=0.78, $P<0.001$, respectively). This could be useful to propose a minimum survival to zoea value as a criterion to continue with larval culture. Number of nauplii and hatching rates were also correlated to survival to PL1 (r=0.64 and r=0.5, $P<0.05$, respectively). These criteria could be useful as predictive criteria to decide if nauplii should be stocked for larval culture. Among the biochemical variables, only lipid content in eggs was correlated to metamorphosis to mysis (r=0.59, $P<0.05$), reinforcing the results mentioned in Table II. No correlation was found between survival to ammonia, salinity stress test, or any other variable. Morphological traits of larvae were not correlated to survival during culture.

As occurred in the first larviculture set, survival to mysis and PL were significantly correlated to survival to zoea in the second and third larviculture sets. No information about nauplii number and hatching rate was obtained for these sets and thus the results of the first set could not be verified for these variables. Biochemical variables in nauplii were not correlated or were negatively correlated to survival during culture. As indicated in a previous study (Palacios et al., 1999) and from results of the first larviculture set, biochemical composition of eggs seems to be more importantly related to performance during culture than nauplii biochemical composition. As in the first larviculture set, morphological traits of larvae were not related to larvae survival.

Correlation coefficients between stress tests and larvae survival or metamorphosis obtained from the third larviculture set are shown in Table III. In contrast to the first larviculture set, a positive correlation was found between the survival to 20mg.l^{-1} total ammonia and survival to several larval stages. Although the 20mg.l^{-1} concentration was used based on LC_{50} determination (see Methods), average survival to ammonia exposure was higher in the first larviculture set (81%) than in the third one (16.3%). A too-high survival could have masked the relation, although a too-low survival can also skew the results. The use of LC_{50} (Cavalli et al., 2000) rather than a single ammonia concentration could be more useful to establish the relation with larval quality, although it would be impractical if many larviculture tanks (19-43 in the present study) are to be tested. The marked differences in ammonia survival between the first and the third larviculture sets are difficult to explain, but they are not related to the general performance during culture, because average survival in the first set (53.7% to PL1) was similar to the average survival of the third set (57.7%).

Regarding survival to a low salinity stress test, it is interesting to note that when applied to PL2 stage, a significant correlation with survival to further stages (PL20) was obtained. The salinity stress test is generally used in more advanced

PL stages to assess if PL are suitable for stocking in nursery or growout conditions, or as a tool in nutritional studies (Bray and Lawrence, 1992). The present study indicates that it could be a predictive indicator of PL performance during culture. Survival to low salinity in PL2 was not significantly correlated to the same stress test applied in PL20. Osmoregulatory capacity in penaeid shrimp is considered to be fully developed in PL10, whereas PL2 present an intermediate strategy between osmoconformation and osmoregulation (Charmantier et al., 1988). Survival to low salinity stress in PL2 probably reflects the general condition of the organisms, whereas in PL20 it also depends on their osmoregulatory capacity. Surprisingly, a significant negative correlation was obtained between salinity stress test in PL20 and survival during early larval culture. This result is difficult to explain in the light of the present data and further studies are needed to examine this relation more thoroughly.

Table III. Correlation coefficient between production variables and survival to stress test. (* $P<0.05$)

	Survival to zoea	Survival to mysis	Survival PL1	Survival PL1-PL20	Meta-morphosis to mysis	Meta-morphosis to PL1
Low salinity PL2	-0.229	-0.320	-0.256	0.451*	-0.296	0.297
Low salinity PL20	-0.436*	-0.641*	-0.593*	0.248	-0.504*	-0.045
High ammonia	0.865*	0.682*	0.594*	-0.168	0.339	-0.163

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References

Bray W.A. and A.L. Lawrence. 1992. Reproduction of *Penaeus* species in captivity. p.93-170. In: Marine Shrimp Culture: Principles and Practices. Fast A.W. and J.L. Lester (Eds.) Elsevier, Amsterdam.

Cavalli R.O., E. Vanden Berghe, P. Lavens, T.T.N. Thuy, M. Wille, and P. Sorgeloos. 2000. Ammonia toxicity as a criterion for the evaluation of larval quality in the prawn *Macrobrachium rosenbergii*. Comp. Biochem. Physiol. 125C:333-343.

Charmantier G., M. Charmantier-Daures, N. Bouaricha, P. Thuet, D.E. Aiken, and J.P. Trilles. 1988. Ontogeny of osmoregulation and salinity tolerance in two Decapod Crustaceans: *Homarus americanus* and *Penaeus japonicus*. Biol. Bull. 175:102-110.

Palacios E., C.I. Pérez-Rostro, J.L. Ramírez, A.M. Ibarra, and I.S. Racotta. 1999. Reproductive exhaustion in shrimp (*Penaeus vannamei)* reflected in larval biochemical composition, survival, and growth. Aquaculture 171:209-221.

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BRED SPECIES AND FRY PRODUCTION OF MARINE FISH IN CHINA

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Abstract

Artificial breeding of marine fish in China has been developing for 50 years. By the year 2000, at least 51 species in 23 families of marine fish have been successfully bred. Large quantities of artificial fry can meet the needs of both marine cage and pond culture in some species.

Introduction

Early studies on artificial propagation of marine fish in China were conducted in the species in the family Mugilidae in the late 1950s (Lei et al., 1965). In the 1980s, China began to carry out the studies on the artificial propagation and breeding techniques in the species of red seabream, Japanese flounder, black porgy, large yellow croaker, etc. The successful techniques of artificial breeding have been established and millions of fry have been bred in these species (Li et al., 1990; Lin et al., 1994; Su et al., 1997). Since the 1990s, artificial propagation and breeding techniques of marine fish in China have been developed by increasing species and fry numbers, with special stress laid on valuable species. Fry breeding has entered a period of sustainable development (Zhang and Hong, 2000).

Bred species and fry production

The bred species and their annual artificial fry production in the past 50 years are shown in Table I. The species in the family of Sciaenidae are the major fish in the artificial propagation and breeding (Cai, 1997; Lei, 1998; Zhang and Hong, 2000). So far, the entire artificial cultured species in China are: *Pseudosciaena crocea*, *Sciaenops ocellatus*, *Lateolabrax japonicus*, *Liza haematocheila*, *Miichthys miiuy*, *Pagrosomus major*, *Paralichthys olivaceus*, *Sparus macrocephalus*, *Sparus latus*, *Scophthalmus maximus*, and *Mugil cephalus*.

Main techniques of larval rearing

Broodfish. In earlier practices, the broodfish were usually obtained from the wild

and held captive for a short period before induction of maturation and ovulation with hormones. The fertilized eggs were obtained through artificial fertilization. Since the 1980s, wild broodfish have been cultivated either in outdoor earth ponds or seawater net cages. It is shown that cultivation of broodfish in net cages is effective in significantly improving gonadal development for most breeding species. However, for a small number of species (e.g., grey mullet, Chinese black sleeper, and blue spotted mudskipper), the broodfish are suitable for cultivation in earth ponds. Since the 1990s, most broodfish have been initially cultivated from artificial fry to the adult either in net cages or earth ponds, indicating that the marine fish culture in China has developed towards the entire artificial culture stage.

Table I. Bred species and fry production of marine fish in China.

Family	Species	# Fry (millions)
Sciaenidae	Large yellow croaker (*Pseudosciaena crocea*)	1300
	Red drum (*Sciaenops ocellatus*)	10
	Cuneate drum (*Nibea miichthioides*)	10
	Miiuy croaker (*Miichthys miiuy*)	1-10
	Dusky roncador (*Megalonibea fusca*)	0.1-1
	Chu's drum (*Nibea chui*)	0.1-1
	Yellow drum (*Nibea albiflora*)	0.1-1
Sparidae	Red seabream (*Pagrosomus major*)	10
	Black porgy (*Sparus macrocephalus*)	1-10
	Yellowfin porgy (*Sparus latus*)	0.1-1
	Stumpnose seabream (*Rhabdosargus sarba*)	0.1-1
Mugilidae	Redeye mullet (*Liza haematocheila*)	10
	Grey mullet (*Mugil cephalus*)	0.1-1
Serranidae	Japanese sea perch (*Lateolabrax japonicus*)	10
	Banded grouper (*Epinephelus awoara*)	< 0.1
	Red spotted grouper (*Epinephelus akaara*)	< 0.1
	Blacksaddled grouper (*Epinephelus fario*)	< 0.1
	Malabar grouper (*Epinephelus malabaricus*)	0.1-1
	Giant grouper (*Epinephelus tauvina*)	< 0.1
	Hunchbacked grouper (*Cromileptes altivelis*)	< 0.1
Pomadasyidae	Three banded sweetlip (*Plectorhynchus cinctus*)	10
	Skewband grunt (*Hapalogenys nitens*)	1-10
	Javelin grunt (*Pomadasys hasta*)	1-10
	Threeline grunt (*Parapristipoma trilineatus*)	0.1-1
Paralichthyidae	Japanese flounder (*Paralichthys olivaceus*)	1-10
Latidae	Silver sea perch (*Lates calcarifer*)	1-10
Tetraodontidae	Tiger puffer (*Takifugu rubripes*)	0.1-1
	Puffer (*Takifugu pseudommus*)	0.1-1

Table I (cont.). Bred species and fry production of marine fish in China.

Family	Species	# Fry (millions)
	Obscure puffer (*Takifugu obscurus*)	< 0.1
Lutjanidae	Russell's snapper (*Lutjanus russelli*)	1-10
	Red fin snapper (*Lutjanus erythropterus*)	0.1-1
	Purplish red snapper (*Lutjanus entimaculatus*)	< 0.1
Scorpaenidae	Schlegel's rockfish (*Sebastes schlegeli*)	0.1-1
	Dusky stingfish (*Sebastiscus marmoratus*)	< 0.1
Carangidae	Dumeril's amberjack (*Seriola dumerili*)	0.1-1
	Ovate pompano (*Trachinotus ovatus*)	0.1-1
Eleotridae	Chinese black sleeper (*Bostrichthys sinensis*)	1-10
Bothidae	Turbot (*Scophthalmus maximus*)	0.1-1
Periophthalmidae	Blue spotted mudskipper (*Bleophthalmus pectonirostris*)	< 0.1
Synanceiidae	Devil stinger (*Inimicus japonicus*)	< 0.1
Hexagrammidae	Fat greenling (*Hexagrammos otakii*)	< 0.1
Syngnathidae	Three-spotted seahorse (*Hippocampus trimaculatus*)	< 0.1
	Kellogg's seahorse (*Hippocamous kelloggi*)	< 0.1
	Japanese seahorse (*Hippocamous japonicus*)	< 0.1
Pleuronectidae	Stone flounder (*Kareius bicoloratus*)	< 0.1
	Marbled sole (*Limanda yokohamae*)	< 0.1
Clupeidae	Gizzard shad (*Clupanodon punctatus*)	0.1-1
Rachycentridae	Cobia (*Rachycentron canadum*)	< 0.1
Exocoetidae	Flying fish (*Prognichthys agoo*)	< 0.1
Platycephalidae	Indian flathead (*Platycephalus indicus*)	0.1-1
Acipenseridae	Chinese sturgeon (*Acipenser sinensis*)	< 0.1

Induced spawning. Most broodfish cultivated in either net cages or earth ponds can easily spawn in either indoor cement pools or the net cages with exogenous hormonal treatment, whereas a small portion of species (e.g., red spotted grouper, banded grouper, red seabream and Japanese flounder) can spawn spontaneously without any hormonal treatment (Wang, 1996). Originally, fish pituitary gland and HCG were applied to induce maturation and ovulation. In the middle of the 1970s, LHRH-$_A$ was added, and since 1987, DOM has also been applied. The combination of LHRH-$_A$ and DOM has been proved to be effective in the induction of maturation and ovulation in marine fish.

Diet-series for the larvae. The diet-series provided for marine fish larvae are usually bivalve fertilized eggs and trochophores, rotifers (*Brachionus plicatilis*), *Artemia* (*Artemia spp.*) nauplii, copepods, cladocerans, adults of *Artemia*, and fish or shrimp meat, etc. In order to increase the quantities of DHA and EPA in foods, which are necessary for the larval development, food organisms are usually enriched with certain kinds of nutrients before feeding. For example, rotifers fed on bread yeast should be cultivated with highly dense *Chlorella* and the *Artemia*

nauplii should be fed on fish oil or cuttlefish liver oil prior to being provided to the fish larvae. On the basis of necessary nutrients for the marine fish larvae, artificial diets (containing n-3 PUFA, phospholipids, vitamins, and microelements) have been manufactured successfully to replace a portion of *Artemia* nauplii or the total quantities of adult *Artemia* and fish meat.

Ways of larval breeding. In China, the breeding of most species is usually conducted in the spring, whereas only a small number of fish are bred in the autumn and winter. Two ways of larval breeding are employed: indoor cement pools and outdoor earth ponds. Larvae bred in indoor cement pools have a higher survival rate than in outdoor earth ponds because culture water is steadier in indoor cement pools. However, in outdoor earth ponds there are a variety of food organisms that can provide suitable foods for the larvae at different developmental stages. Hence, larvae grow more rapidly and stronger, suffer less disease, and can avoid cannibalism when bred in outdoor earth ponds. Outdoor earth pond breeding also requires less investment and can yield larger sized fry on a large scale. The combination of indoor cement pool breeding at earlier developmental stages and outdoor pond breeding at later developmental stage would be a better practical way for the marine fish breeding in south China.

References

Cai G.X. 1997. On the development and trends of artificial propagation techniques of marine fish in China. S. China Sea Res. Dev. 1:51-56 (in Chinese).

Lei J.L., H.C. Wang, and C.C. Wang. 1965. The fry-rearing experiments of the sea mullet (*Mugil so-iuy*). Mar. Fish. Res. 1:23-24 (in Chinese with English abstract).

Lei J.L. 1998. Overview on marine industrialized fish farming in China. Modern Fish. Inf. 13:4-10 (in Chinese).

Li J. E., Y.J. Ou, and B.T. Xu. 1990. Studies on the biology and fry mass production of black porgy, *Sparus macrocephalus* (Basilewsky). S. China Sea Res. 2:36-44 (in Chinese with English abstract).

Lin J.Z., Y.Z. Zhang, and J.B. Zheng. 1994. Studies on the fry rearing techniques of the autumn-winter spawning groups of red porgy, *Pagrosomus major* (Temminck et Schlegel). J. Xiamen Fish. Coll. 16:1-9 (in Chinese with English abstract).

Su Y.Z., Z.Y. Zheng, and L. You. 1997. A study on techniques of artificial propagation and breeding of large yellow croaker, *Pseudosciaena crocea* (Richardson). Modern Fish. Inf. 12:21-27 (in Chinese).

Wang H.S. 1996. Studies on the artificial propagation of *Epinephalus akaara*. Natural spawning of red spotted grouper (*E.akaara*) under laboratory condition. Mar. Sci. 6:4-8 (in Chinese).

Zhang Q. and W. Hong. 2000. Status and prospects of artificial propagation and breeding technique of marine fish in China. Modern Fish. Inf. 15:3-6 (in Chinese).

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EFFECTS OF FOOD TYPES ON THE DEVELOPMENT AND REPRODUCTION OF *APOCYCLOPS ROYI*

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Introduction

For many marine fish species, fry production is uneconomical or impossible using rotifers or *Artemia* as larval diets. This is mostly a result of poor survival at the first feeding stage. Problems associated with rotifer and brine shrimp include nutritional deficiencies and inappropriate prey size. Copepods constitute the principal trophic link between microbial systems and higher trophic levels, and are the major food items of larval fish in the sea. They can synthesize essential fatty acids, especially 22:6ω-3, which makes them very attractive larval feeds (Nanton and Castell, 1999). The provision of copepod nauplii in early larval diets often increases survival of fish.

Apocyclops royi is a newly recorded cyclopoid copepod species found in the Tan-Shui River estuary and brackishwater shrimp ponds in I-Lan and Tainan, Taiwan (Chang et al., 1991). The genus broods its eggs in two sacs. It is also one of the most used copepods for grouper hatcheries in Taiwan (Su et al., 1997). We have cultured it in the laboratory for over 30 months using algal foods so that it is attractive for aquaculture due to easy access to the stock culture. Before developing an economical mass culture system, we examined the effects of food types on growth and reproduction.

Materials and methods

The nauplii of *Apocyclops royi* were picked under a microscope from the stock-culture hold in Tungkang Marine Laboratory, TFRI, located at south of Taiwan. Each newly hatched nauplius (less than 8h after hatch) was put into one of 24 wells in a culture vessel. In one well, 2.5ml culture medium containing various food types was added. The food included four microalgal and seven commercial feeds. Microalgae were harvested at the late exponential growth stage and used with fixed concentration, that is *Tetraselmis chui* (TET,

30×10^4cells.ml^{-1}), *Chaetoceros muelleri* (CHE, 150×10^4cells.ml^{-1}), *Isochrysis galbana* (ISO, 300×10^4cells.ml^{-1}), and *Nannochloropsis oculata* (NAN, 1200×10^4cells.ml^{-1}). Artificial feeds were BP-1000E (ESP Taiwan Co.), GSP-500 (GSP Taiwan Co.), Micro-Macω30 (W30 USA Co.), Algamac-2000 (ALG USA Co.), DHA-Protein Selco (DPS Belgium Co.), Culture Selco (CSE Belgium Co.), and Baker's yeast (BYE Taiwan Co.), all using 100ppm. The culture conditions were 25°C, 25‰, 40μE.m^{-2}.s, and a 16L:8D photoperiod. Every day the growth of *A. royi* was observed under microscope, and transferred to new culture vessels with new culture medium. When the nauplii had developed to the copepodite stage, the culture container was changed. Every 12 individuals were moved into one 100-ml beaker containing 30ml of culture medium, thus, if matured, they could mate at once. Nauplii produced were picked, fixed, and counted daily. The rearing experiments ended when all copepods died. Each food type had 36 duplicates in the nauplii stage and triplicates in the copepodite stage. The survival rate, developmental time, nauplius production, and fecundity were used to assess the copepod responses. The data was analyzed by a computer commercial program (SPSS 7.0).

Results and discussion

The results showed that growth and reproduction of copepods fed with algae, exclusive of *N. oculata* (undigestable thick cell wall), were better than those fed with artificial diets. The copepods fed *T. chui* grew fastest, matured (8.7 days after hatching) early (Table I), and produced the most abundant offspring (average 250 nauplii per female, Fig. 1) and fecundity (13.5 nauplii per female per day, Fig. 1).

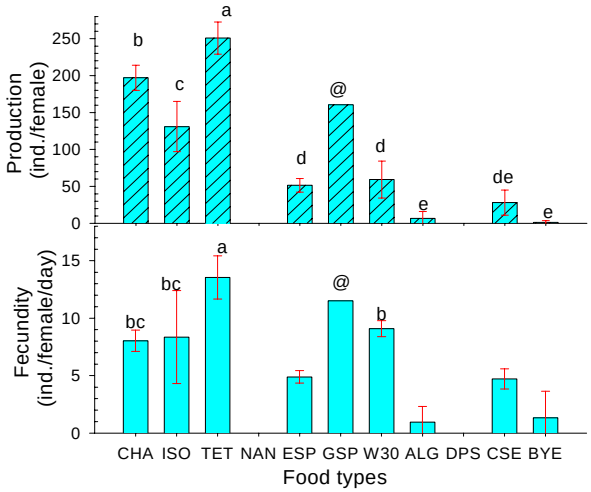

Fig. 1. The nauplius production per female (above) and fecundity per female per day (below) of one female *Apocyclops royi* fed on various food types. (Vertical bar = ±SD, @ = not include in statistics analysis, due to just only one surviving female).

Among artificial diets, copepods fed with Culture Selco had the highest survival, 70.8%, which is somewhat comparable to those fed with algae, but took a longer time (17.5 days on average) to become mature. Those fed with Micro-Macω30 had the highest fecundity, but only 9.1 nauplii per female per day. The food containing higher protein or DHA contents did not assure better survival, growth, or reproduction, while *T. Chui* containing low EPA and no DHA (Su et al., 1988) showed the best nutritional value for *A. royi*. Some special nutritional constituent might be the key factor affected (Jónasdóttir, 1994). Further investigation is needed to clarify this. The shorter generation time, from hatch to mature, of *A. royi* (8.7days) compared to *Acartia* sp. (10 days, Ohno and Okamura, 1988) and *Tisbe furcata* (12-22days, Abu-Rezq et al., 1997) indicated that the estuarine copepod *A. royi* is a potential food organism for mass culture.

Table I. The survival and development time of *Apocyclops royi* fed on various food type (Mean±SE, NI=nauplius stage I, CI=copepodite stage I, M=first nauplii laid).

Food type		Survival rate (%)		Average development time (days)	
		NI ~CI	NI ~ M	NI ~ CI	NI ~ M
Algal species	CHA	89.6 ± 8.6^{a}	84.7 ± 9.7^{AB}	5.3 ± 0.6^{b}	10.7 ± 0.8^{B}
	ISO	95.8 ± 4.5^{a}	89.3 ± 6.3^{A}	5.2 ± 1.0^{b}	11.3 ± 1.1^{C}
	TET	91.7 ± 6.8^{a}	77.8 ± 9.6^{B}	4.0 ± 0.3^{a}	8.7 ± 0.6^{A}
	NAN	0.0 ± 0.0^{b}	N. A.	N. A.	N. A.
Artificial diets	ESP	47.2 ± 4.8^{b}	25.0 ± 8.3^{B}	9.4 ± 2.7^{bc}	23.0 ± 1.7^{AB}
	GSP	18.8 ± 8.0^{d}	22.1 ± 5.1^{B}	5.7 ± 0.7^{a}	25.3 ± 4.0^{AB}
	W30	79.2 ± 8.3^{a}	36.1 ± 19.2^{B}	5.6 ± 0.9^{a}	38.0 ± 16.5^{C}
	ALG	39.6 ± 18.5^{bc}	25.0 ± 22.0^{B}	11.3 ± 2.5^{d}	30.7 ± 4.6^{BC}
	DPS	5.6 ± 4.8^{d}	N.A.	9.5 ± 0.7^{bc}	N.A.
	CSE	87.5 ± 10.8^{a}	70.8 ± 10.8^{A}	8.1 ± 1.4^{b}	17.5 ± 0.6^{A}
	BYE	25.0 ± 13.6^{cd}	25.0 ± 16.7^{B}	9.9 ± 1.4^{cd}	30.0 ± 3.5^{BC}

N.A. = Not applicable.

References

Abu-Rezq T.S., A.B. Yule, and S.K. Teng. 1997. Ingestion, fecundity, growth rates and culture of the harpacticoid copepod, *Tisbe furcata*, in the laboratory. Hydrbiologia 347:109-118.

Chang W.B., F.L. Wu, K.S. Wu, and C.H. Lei. 1991. Study on the zooplankton of shrimp ponds in Taiwan. COA Fisheries Series No. 28:53-76. (in Chinese)

Jónasdóttir S.H. 1994. Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica:* laboratory observations. Mar. Biol. 121:67-81.

Klein Breteler W.C.M. and N. Schogt. 1994. Development of *Acartia clausi* (Copepoda, Calanoida) cultured at different conditions of temperature and food. Hydrobiologia 292/293:467-479.

Nanton D.A. and. J.D. Castell. 1999. The effects of temperature and dietary fatty acids on the fatty acid composition of harpacticoid copepods, for use as a live food for marine fish larvae. Aquaculture 175:167–181.

Ohno A. and Y. Okamura. 1988. Propagation of calanoid copepod, *Acartia suensis*, in outdoor tanks. Aquaculture 70:39-51.

Su H.M., C.H. Lei, and I.C. Liao. 1988. The effect of environmental factors on the fatty acid composition of *Skeletonema costatum*, *Chaetoceros muelleri* and *Tetraselmis chui*. J. Fish. Soc. Taiwan 15:21-34. (in Chinese)

Su H.M., M.S. Su, and I.C. Liao. 1997. Collection and culture of live foods for aquaculture in Taiwan. Hydrobiologia 358:37-40.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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EVALUATION OF *PANAGRELLUS REDIVIVUS* FROM DIFFERENT CULTURE MEDIA ON SURVIVAL AND GROWTH OF SILVER BARB *PUNTIUS GONIONOTUS LARVAE*

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Introduction

The free-living nematode *Panagrellus redivivus* has shown great potential as an alternative live food for different commercial cultured shrimp and fish species (Kahan, 1975; 1980; Fontain et al., 1982). They are easily and inexpensively cultured on simple and cheap media (Rouse et al., 1992). Very recently, this nematode has been introduced to Malaysia and successfully tested on some commercial local fish. Thus, this study was carried out to evaluate the effect of feeding nematode *P. redivivus* from various local cultured media sources on survival, growth, and some biochemical compositions of silver barb *Puntius gonionotus* larvae.

Materials and methods

The three cultured media evaluated for nematode culture were oatmeal, sweet potato, and unpolished rice powder, all enriched by cod liver oil. Five-day-old larvae (3.54mm length and 0.075mg weight) were stocked in triplicate 10-l aquaria for each treatment at 10 larvae.l^{-1}. Harvested nematodes were fed to larvae on *ad libitum* two times daily for 16 days. The control group was reared on *Artemi*a nauplii during the course of experiment. Sampling for length and weight was occurred every four days. Mortality was recorded every day.

Total protein and lipid were determined according to Bradford (1976) by micro method of the Bio-Rad protein assay kit (Bio-Rad laboratory) and Holland and Hannat (1973) respectively. Results were given as means (n=3) and data were compared using one-way ANOVA followed by Duncan test when significant differences were found at 0.05 level.

Results

Results of survival rate, specific growth rate, mean length and weight for all treatments are summarized in Figs. 1 and 2, and Table I.

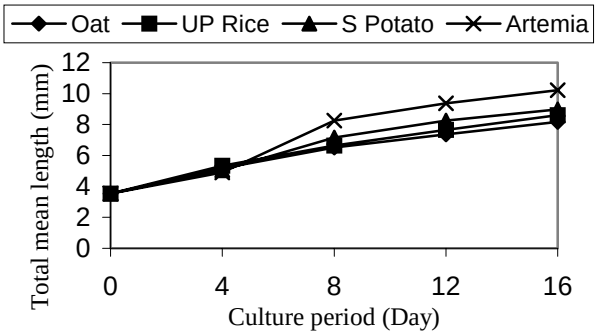

Fig. 1. Changes in growth of silver barb *P. gonionotus* fed on enriched (cod liver oil) nematodes from different media for 16 days.

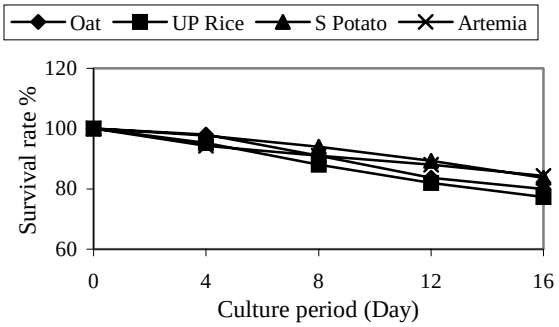

Fig. 2. Survival rate of *Puntius gonionotus* larvae fed on different enriched (Cod liver oil) media source of nematodes for 16 days.

Table I. Growth and survival rate of *Puntius gonionotus* larvae fed on different media source nematode

Diet	Survival rate (%)	SGR (% day)	Total length (mm)	Total weight (mg)
Artemia	85±6.93 [a]	25.91±0.48 [a]	10.22±0.24 [a]	4.63±0.29 [a]
Nematode (S Potato)	83.7±9.24 [a]	24.35±0.25 [b]	8.97±0.13 [b]	3.64±0.15 [b]
Nematode (UP Rice)	77.33±9 [a]	23.61±0.32 [b]	8.6±0.15 [b]	3.24±0.17 [b]
Nematode (Oat)	80±9.64 [a]	22.58±0.75 [c]	8.17±0.32 [c]	2.67±0.35 [c]

Survival rate was generally high ranging from 77.3-85% but were not significantly different (P>0.05) among various nematodes source. Specific growth rate was higher for larvae fed on *Artemia* followed by sweet potato and unpolished rice powder nematodes. Larvae fed on oatmeal-cultured nematodes exhibited lower specific growth rate.

The effect of different media culture on the major body component of nematodes and fish fed on those nematodes sources are presented in Tables II and III, respectively. The highest protein content was observed among fish at *Artemia* fed larvae followed by larvae fed on unpolished rice powder nematodes but it was not significantly different (P>0.05) from those of other treatments. Total lipid was generally high and was not significantly different among all treatments. Highest lipid content was recorded in fish fed on *Artemia* and unpolished rice powder and lowest in sweet potato nematodes larvae. Nematodes grew on sweet potato revealed highest protein and lipid among all treatments. Lowest lipid content was recorded in nematodes grew on oatmeal.

Table II. Biochemical composition of nematode *Panagrellus redivivus* from different media sources.

Diet	*Artemia*	Nematode Oat	Nematode S Potato	Nematode UP Rice
Protein (%DW)	59.19±3.06 [a]	63.32±3.66 [a]	64.58±2.44 [a]	63.18±5.22 [a]
Lipid (%DW)	17.36±2.17 [a]	14.57±0.55 [a]	17.98±1.39 [a]	16.64±1.82 [a]

Table III. Biochemical composition of larvae fed on nematodes from different media sources.

Diet	*Artemia*	Nematode Oat	Nematode S Potato	Nematode UP Rice
Protein (%DW)	64.83±4.27 [a]	56.58±5.93 [a]	60.70 ±4.29 [a]	62.22±4.1 [a]
Lipid (%DW)	17.7±2.52 [a]	16.08±2.21 [a]	14.36 ±1.5 [a]	16.33±0.02 [a]

Dissolved oxygen ranged from 4.9-6.1mg.l^{-1} and temperature, pH, and ammonia remained at satisfactory levels for fish.

Discussion

Differences in growth of *P. gonionotus* larvae fed on different source of nematodes were probably due to variation in nutrition quality. Based on survival and growth performance, sweet potato nematodes were better utilized by *P. gonionotus* larvae than unpolished rice powder and oatmeal nematodes. This could be attributed to higher protein and lipid contents of sweet potato

nematodes among all nematodes sources. Oatmeal nematodes fed larvae, exhibited poorer growth, among all treatments. This is probably related to lower lipid content of oatmeal nematodes. It has been emphasized that lipids play an important role in metabolism and cell membrane structure (Rainuzzo et al., 1997). Furthermore, higher n-3 PUFA (unpublished data) in oatmeal nematodes may be considered as another reason for lower growth of this freshwater fish compared with others nematodes. It has been established that n-6 PUFA are more important in freshwater fish than n-3 PUFA (Watanabe, 1982). This study has shown that the media can alter the nutritional quality of nematodes on which they are cultured.

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References

Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Fontaine C.T., D.B. Revera, H.M. Morales, and C.R. Mock. 1982. Observation on the mass propagation of the common soil nematode *Panagrellus redivivus* for use as foodstuff in penaeid shrimp hatcheries. NOAA Technical Report, National Marine Fisheries Service, Galveston Laboratory, Galveston, Texas, USA.

Holland D.L. and P.J. Hannant. 1973. Addendum to a micro-analytical scheme for biochemical analysis of marine invertebrate larvae. J. Mar. Biol. Assoc. UK 53:833-838.

Kahan D. and Z. Appel. 1975. The value of *Pangrellus sp*. (Nematode) as food for fish. 10th European Symposium on marine biology, Ostend, Belgium, Sept.17-3, 1975. 1:243-253.

Kahan D., T. Barel, Y. Brandestien, M Rigbi. and B. Oland. 1980. Free living Nematodes as a dietary supplement in the rearing of fish fry and hatcheries. GFCM Stud. Rev. 57:67-68.

Reinuzzo J.R., K.I. Reitan, and Y. Olsen. Y. 1997. The significant of lipids at early stages of marine fish: a review. Aquaculture 155:103-115.

Rouse D.A., C.D. Webster, and I.A. Radwin. 1992. Enhancement of the fatty acid composition of the nematode *Panagrellus redivivus* using three different media. J. World Aquacult. Soc. 23:89-95.

Watanabe T. 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. 73B:3-15.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECTS OF *SPIRULINA* ON THE GROWTH AND CAROTENOID DEPOSITION IN TROPICAL SPORT FISH *TOR TAMBROIDES* FRY

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Abstract

Tor tambroides fry with a mean of 8.0±0.72mm standard length (SL) and weighing (W) 0.06±0.01g were stocked at the rate of fifty (50) individuals in each of the fifteen 150-l rectangular fiberglass tanks for a period of 5 weeks. 45% protein diet without additive *Spirulina* was treated as control and denoted as S_0. While diets refer to S_1, S_2, and S_3 had respective *Spirulina* percentages of 0.05, 0.10, and 0.20 Respectively. The experimental fish were collected both at the beginning and the end of the experiment to determine the growth and carotenoid pigments deposited in *T. tambroides* fry. Fish fry fed on diet with 0.10% *Spirulina* showed significantly higher growth ($P<0.05$) and carotenoid deposition in flesh and skin than those of other diets. This study indicates that β-carotene was a dominant carotene in fish flesh compared to other carotenoids. Furthermore, it can be concluded that the utilization of β-carotene depends on the optimum level beyond of which would give the lower efficiency.

Introduction

The determination of quantity, form and digestibility of pigmenting carotenoids in raw materials are of fundamental importance in fish feed industry. Fish indeed unlike other animal species, donot synthesize carotenoids or to make them from other compounds. The utilization of algae proves particularly interesting because not only algae can be incorporated into the food as feed additive without any specific technological processing but they also favour fish growth (Choubert, 1979; Reed et al, 1976). *Tor tambroides* fish is a potential for aquaculture as it

currently fetches high market prices in Malaysia as well as in Southeast Asian countries.

Materials and Methods

The experiment was done in the fresh water hatchery at Universiti Putra Malaysia, Terengganu for a period of 5 weeks. *T. tambroides* fry with a mean of 8.0±0.72mm standard length (SL) and weighing (W) 0.06±0.01g were stocked at the rate of fifty (50) individuals in each of the fifteen 150-l rectangular fibre glass tanks. A complete randomised design was employed in this experiment consisted of five treatments with three replicates. The experimental fish fry were collected at the beginning and the end of the experiment for carotenoid analysis and their diet was prepared as well (Jalal et al., 1999). The powdered micro algae *Spirulina* (OSI *Spirulina platensis* – King's Brand, USA) was incorporated at the rate of 0, 0.05, 0.10, and 0.20% respectively which were referred to as diets S_0, S_1, S_2, S_3. Diet without *Spirulina* (S0) was treated as control.

The fish fry were fed to satiation five times daily from 1st day of stocking to 35th day. Ten fish from each tank were randomly taken at 7, 14, 21, 28, and 35 days (respectively) to determine the development at different days. The growth performances of the experimental fish fry were monitored in terms of absolute growth and specific growth rate were measured at the beginning and the end of the experiment. The carotenoid was extracted from 5-g sub-samples and total carotenoid concentration was determined at 480 nm with a UV spectrophotometer (Model: Shimaozu Double Beam UV- 180) following the method given by Liao et al. (1993). The acetone in carotenoid solution was evaporated in vacuum (desiccator) and β-carotene and other carotenoids were identified with the aid of thin-layer chromatography (TLC). The elution solvent was 20% acetone in petroleum ether. The data was analysis with ANOVA (Duncan's Multiple Range Test).

Results and Discussion

T. tambroides fry fed with diets S_2 and S_3 showed the better absolute and specific growth, which were significantly higher (P<0.05) than those of diets S_0 and S_1 (Fig. 1)**.** The growth increased with respect to increased carotenoid in flesh. Figure 1 showed strong relation of carotenoid content with weight gain of *T. tambroides* fry during the addition of *Spirulina* diet. The total carotenoid contents of flesh and skin were presented in Table I. The various feeding treatments showed significant differences (P<0.05) in growth and carotenoid deposition in flesh. Fish fry showed increase in flesh carotenoids concentration in diets S_2 and S_3 than those of S_0 and S_1.

Fish fry fed with 0.10% *Spirulina* diet showed significantly higher (P<0.05) amount of carotenoid in flesh than those of other diets during 35 days of feeding.

While fish fry fed with 0.05% *Spirulina* showed a negligible amount of carotenoid in their flesh. The amount of β-carotene showed similar trends of deposition like total carotenoids (Table II). An interesting feature is that the amount of total carotenoids and β-carotene were higher in flesh compared to skin. TLC results indicted that pigments of the β-carotene in the flesh of fish fry are more prominent in diet S_2 and diet S_3.

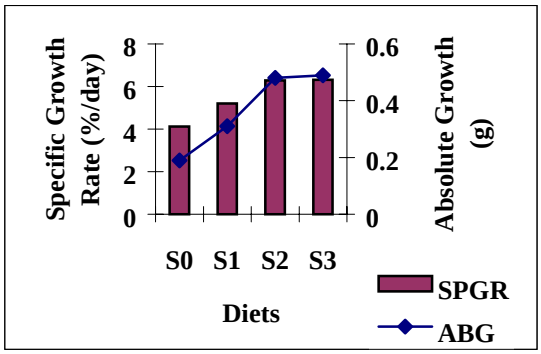

Fig. 1. Absolute growth and specific growth rate of *T. tambroides* fry.

Table I. Total carotenoid content (μg.g^{-1}) of *T. tambroides* fry fed on different diets.

Diets	Flesh (μg/g)	Flesh (%)	Skin (μg/g)	Skin (%)	Total Carotenoid (μg/g)
Initial	0.24±0.03	80.89	0.06±0.02	19.11	0.30±0.02
S_0	0.42±0.04^{a}	85.03^{a}	0.07±0.02^{a}	14.97^{a}	0.49±0.05^{a}
S_1	4.39±0.03^{b}	96.62^{b}	0.15±0.03^{b}	3.38^{b}	4.54±0.04^{b}
S_2	6.86±0.05^{c}	96.94^{c}	0.22±0.01^{c}	3.06^{c}	7.08±0.06^{c}
S_3	6.18±0.02^{d}	96.87^{d}	0.20±0.03^{d}	3.13^{d}	6.38±0.03^{a}

Note: Different superscripts in the same column show significant difference (P<0.05).

Table II. Total β-Carotenoid content (μg/g) of *T. tambroides* fry fed on different diets.

Diets	Flesh (μg/g)	Flesh (%)	Skin (μg/g)	Skin (%)	Total Carotenoid (μg/g)
Initial	cd	Cd	cd	Cd	cd
S_0	cd	Cd	cd	Cd	cd
S_1	1.67±0.15^{a}	96.90^{a}	0.05±0.02^{a}	3.10^{a}	1.72±0.16^{a}
S_2	3.81±0.05^{b}	97.94^{b}	0.08±0.01^{b}	2.06^{b}	3.88±0.05^{b}
S_3	3.18±0.02^{c}	97.85^{c}	0.07±0.01^{c}	2.15^{c}	3.25±0.02^{c}

Note: Different superscripts in the same column show significant difference (P<0.05) cd = could not detected.

The significant differences among the treatments demonstrated that β-carotene could affect the flesh carotenoids levels in the flesh and skin of the fish. Diet without S*pirulina* additive was the lowest among the diets followed by 0.05% *Spirulina* diet. This may be due to the absence or lower dose of β-carotene, which enhancing the growth of *T. tambroides* fry. This is agrees with the findings of Liao et al. (1993). According to them, body weight of shrimp fed with *Spirulina* diet surpassing compared to diet without *Spirulina*. This might be due to some direct effect of β-carotene as well as presence of other carotenoids.

The positive effect of carotenoid supplementation on growth of *T. tambroides* during this feeding regime might be either a metabolic effect with carotenoids acting as biological antioxidants as observed by Torrisen (1984). Interesting fact is that the growth of fish fry fed with 0.20% *Spirulina* diet has declined gradually. This may be beyond of optimum level of *Spirulina* diet for *T. tambroides* fry during their initial stages of life. Watanabe et al. (1990) observed that over-supplementation of dietary *Spirulina* for cultured striped jack caused retardation of growth. This illustrated that utilization of β-carotene depends on the optimum level beyond of which would give the lower efficiency. However, β-carotene was a dominant carotene in this study and it can be postulated that *T. tambroides* fish must have a specific range of β-carotene levels in their different life stages.

References

Choubert G. 1979. Tentative utilization of *Spirulina* algae as a source of carotenoid pigments for Rainbow Trout. Aquaculture 18:135-143.

Jalal K.C.A., M.A. Ambak, C.R. Saad, A. Hasan, and M.A.B. Abol. 1999. Effects of feeding frequency on the growth of Malaysian Mahseer, *Tor tambroides* (Bleeker). Paper presented in World Aquaculture Society Conference held on 26th April-2nd May, 1999, Sydney, Australia.

Liao W.L., S.A. Nur-E-Burhan., O. Shigera, T. Matsui, and K. Yamaguchi. 1993. Pigmentation of cultured Black Tiger Prawn by feeding with *Spirulina* supplementation diet. Nippon Suisan Gakkashi. 59(1):165-169.

Reed J.R., G.L. Samsel, R.R. Daub, and G.C. Lewellyn 1976. Oxidation pond algae as a supplement for commercial catfish feed. In: Proceedings of the annual conference in Southeast Assoc. Game Fish Comm. 27:465-470.

Torrisen O. J. 1984. Pigmentation of Salmonids: Effects of carotenoids in eggs and start feeding diet on survival and growth rate. Aquaculture 43:185-193.

Watanabe T. 1990. Roles of vitamin E in aquaculture. Yukagaku 39:299-306.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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SHRIMP HATCHERIES AND THE ENVIRONMENT

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Introduction

Over the last five years hatcheries have achieved new importance within the shrimp Aquaculture industry. Progress in selective breeding has led to the domestication of stocks, which in some cases already outperform wild shrimp in ponds. As hatcheries supplant the demand for wild postlarvae, it is important to examine other areas of their operation that relate to the environment. Operating a hatchery in an environmentally responsible manner presents challenges that are different from those of pond-based shrimp farms. Because hatcheries require only limited surface area, loss of habitat due to direct utilization of space is not a significant issue. On the other hand, the major areas of potential environmental concern in hatcheries are animal containment, pathogens, and water discharges.

Animal containment

Releasing hatchery-reared juveniles into the ocean is a sound fisheries management tool used to enhance overfished stocks of fish and shellfish. But in certain cases, releasing hatchery shrimp into the environment could cause undesirable consequences. The first, and most obvious case of concern is the release of exotics into the environment. Theoretically, the exotic species could become established and cause ecological problems. The second case is when the genetic makeup of hatchery animals is different from that of the wild populations. Breeding between captive and wild stocks could result in the loss of genetic diversity. The third area of concern would be the introduction of hatchery diseases into the wild. In all these cases it is of critical concern that aquaculturists take every precaution to avoid introducing hatchery animals into wild populations.

In the hatchery, animal containment is of utmost importance. Containment is achieved using mesh-size screens proportional to the size of the larvae. Sand filters and plastic cartridges may also be used at discharge points. Redundancy is desirable and easy to accomplish. Natural disasters, such as hurricanes and

floods, present special challenges to animal containment. For new facilities, locating in areas where the effects of such events are minimal is an important consideration during the site selection process. Of paramount importance is the need for a contingency plan to avoid releasing animals into the environment should a natural disaster occur. Under extreme conditions, the plan must ensure the sanitary destruction of the entire stock.

Pathogens

Disease concerns are best addressed by prevention. It is in the hatcheries' best interest to use stock that is free of significant pathogens. Good management practices, stress avoidance, and bio-security procedures are also part of an effective health management program. For example, effluent disinfection not only protects the health of hatchery animals, it also minimizes the potential for releasing shrimp diseases into the environment. Other examples of good practice are to avoid the mixture of water intake and discharge, and to avoid constructing a hatchery where water intakes are subject to the influence of an existing hatchery or farm.

Discharges

By industrial standards, shrimp hatchery discharges are minimal in volume, with even the largest facilities rarely exceeding 2 000$m^3.d^{-1}$. Hatchery effluents also compare well in concentration to those found in discharge permits for activities other than shrimp farming (Table I). Recirculation systems, common in the broodstock production and maturation components of hatcheries, further contribute towards minimizing discharges. Not only are recirculation systems environmentally sound, they are also economically advantageous due to reduced heating and pumping costs.

Table I. Water quality variables in discharge permits and in shrimp hatchery effluents.

Variable	Maximum values usual in discharge permits for activities other than shrimp farming[(1)]	Values common in shrimp hatchery effluents
C-BOD 5 day	20-60	0.2-6.0
Total Nitrogen	2.0-5.0	0.1-2.0
Total Phosphorus	0.2-0.5	0.1-0.5
Total Suspended Solids	30-100	5-35

[(1)] Boyd and Gautier (2001)

When using chemicals, hatchery operators need to follow local regulations, and adhere to good management practices. Before disposal, fertilizers, antibiotics, and disinfectants must be neutralized, dissipated, or allowed to decompose to non-toxic forms. All dangerous chemicals should be stored according to local regulations and handled with safety in mind. Chemotherapeutants should only be used for treating specific diseases, and then only after a clear diagnosis of the problem has been made. Antibiotic sensitivities and minimum inhibitory concentrations need to be known, or determined before use. Antibiotics should only be used if they are permitted by local regulations. The use of low levels of antibiotics to prevent bacterial diseases is an example of bad practice that should be avoided, as it ultimately defeats its intended purpose. Using antibiotics in such fashion leads to the development of bacterial resistance, and creates a need for increasing dosages, changing of antibiotics, and eventually the complete drying-out of the facility.

Most hatcheries discharge their effluents into the seas, estuaries, or mangrove areas. Percolation ponds and injection wells, where treated discharge is introduced into a saline aquifer, are particularly friendly to the environment. Effluents need to be properly treated before discharging. Depending on the circumstances, treatment can include a combination of particle filtration, disinfection, and temporary retention in ponds, where the discharges oxidize, settle, and/or seep into the aquifer. Water treated prior to discharging can be used to promote mangrove growth.

Conclusion

In upcoming years, hatcheries will play an even more significant role as contributors to world shrimp farming. Already hatcheries are easing the demands for wild postlarvae through production of domesticated stocks. Additionally, sound hatchery management practices lead to preservation of the environment. For example, effective animal containment procedures not only ensure that aquaculturists keep hatchery animals where they belong, but also minimize the possibility of species translocation, loss of genetic diversity, and transmission of shrimp diseases. Proper water treatment and disposal not only preserves the facility's own water supply, but also minimizes concerns over pollution of surrounding waters. Avoiding environmental damage results in long-range benefits for all involved. With good faith, common sense, and use of proper technology, shrimp hatcheries will continue to contribute to world shrimp production, and to help meet world food needs for present and future generations.

References

Boyd C.E. and D. Gautier. 2001. Effluent composition and water quality standards. Global Aquacult. Advocate 5(3):61-66.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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FISH ARTIFICIAL REPRODUCTION FOR INCREASING FISH PRODUCTIVITY IN WATER BODIES IN ARAL SEA BASIN UNDER ECOLOGICAL CRISIS

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Introduction

Before 1960s, in Uzbekistan about 25 000 tons of fish were captured from the Aral Sea. But now the area of the Aral Sea has decreased more than two thirds and has minor fishery importance. The fishery forces were transferred to the other inland lakes with a total water surface of more than 500 000ha, in which modern fish productivity is 3.3-7 $kg \cdot ha^{-1}$, while the potential one is 15-40$kg \cdot ha^{-1}$. Environments are beneficial for fishes, but reproduction is under the negative press of water level changes due to conditions of stocked river flow. Fish artificial reproduction and stocking can increase fish productivity in water bodies. Preferable objects are fishes that occupy first links of food chains. The aim of our work was to induce spawning of common carp *Cyprinus carpio*, silver carp *Hypophthalmichthys molitrix*, grass carp *Ctenopharyngodon idella* in March-April through short time keeping of mature breeders in warm water and hormonal injection instead of usual reproduction season in May-June, and optimize technology of fry stocking into Lake Tuzkan. Silver carp and grass carps cannot reproduce naturally in lakes in Uzbekistan.

Material and methods

Work was carried out in "Balyktchy" fish farm, Uzbekistan. In spring mature fishes 2-5kg in weight of both sexes were selected and stocked in pond before being used in experiments. Each group comprising 4-5 fishes of both sexes were kept in separate tanks (2×1×0.9m). Experimental groups were kept in indoor tanks with heated water inlet. Water temperature was increased by 2°C per day before it reached 18°C for common carp and 20°C for silver carp and grass carp. In such a temperature, fish were kept for 2-3 days. Control groups were kept in outdoor tanks under natural temperature from stock pond.

Water temperature and dissolved oxygen were measured every 6 hours and average daily characteristics were determined. Breeders of both experimental and control groups were simultaneously induced to spawn by the administration of two-time injections 0.5 and in 12-24 hours – 3mg acetone-dried carp pituitary per kg of fish body weight for females and single dose 0.5mg per kg for males. Twelve hours after the last injection fishes were stripped. Ripe eggs and sperm were obtained, fertilized and incubated. Real fecundity was calculated for each female as the number of eggs ovulated. Hatching rate was calculated as the number of eyed embryo from 100 unselected eggs taken from incubation apparatus. Larvae were kept in basins with zooplankton cultivation for feeding.

Results

The first common carp group were taken from the outdoor stock pond with water temperature 14°C and entered into tanks on 20 Mar 2000. Injections were done on Mar 23-24 All males and 3 females from 5 injected gave ripe sex products. Individual real fecundity was 150 – 400 000 (average 301 000) eggs. Beginning of larvae hatching was observed on Mar 27. Hatching rate was 65%.

Second common carp group was taken from water at 14°C and were entered to tanks on Apr 1 at once to warm water temperature 18°C which was increased to 20°C per day. Fish were injected Apr 3-4. Ripe sex products were obtained from all fishes on Apr 5. Real fecundity was 90-230 000 (average 150 600) eggs. Hatching rate was 70%.

Fishes from both control groups had not spawned in response to injections. The first artificial spawning after injections was carried out on Apr 20 and only 5 females from 10 injected gave ripe eggs. Natural spawning in earth ponds in fish farm (without injections) was observed for the first time on Apr 25.

The first silver carp group was taken from stock ponds on Apr 14 when water temperature was 15°C and entered the tanks. After temperature was increased up to 20°C and the fish were kept in such conditions for 2 days they were injected on Apr 18-19. All fishes gave ripe sex products on Apr 20. Individual real fecundity was 250-650 000 eggs. The further eggs and larvae development was normal. Beginning of hatching was observed on Apr 22. Hatching rate was 75%.

The second silver carp group was taken from stock pond when water temperature was 16°C and entered the tanks on Apr 20. Fish were injected on Apr 23-24. Ripe eggs and sperm were obtained Apr 25. Individual real fecundity was 200-610 000 eggs. The beginning of hatching was on Apr 26. Hatching rate was 79%.

Breeders from both control groups did not spawned in response to injections. Fishes from outdoor stock pond spawn for the first time in response of injections only on May 25.

The grass carp group was taken from ponds when water temperature was 15°C and entered the tanks on Apr 15. For 2 days, the temperature was increased up to 19°C, and fish were kept under this temperature for 2 days. Breeders were injected on Apr 19-20. Sex products were stripped on Apr 21. Real productivity varied 25-590 000 eggs. Hatching began on Apr 23. Hatching rate was observed as 70%.

Fish from control group did not respond to injections. The first artificial spawning of grass carp in fish farm was on May 6.

In all tanks during experiments 2.6-5.9g per liter of dissolved oxygen was observed.

On May 2-5 larvae of all species studied were transported in plastic bags to the nursery ponds constructed on the shore of Lake Tuzkan in 350 km from fish farm. Earth ponds with area 0.1-0.2ha each with water coming by gravitation were used. Larvae were extensively reared. One tone of manure was brought per hectare before ponds fill with water. The density of larvae introduction was 2 millions per hectare. Larvae were reared for 20 days. Surviving rate was 50%. When fry reach 1-2g in the end of May they were transferred into a small lake rather constricted from Tuzkan with area about 30 hectares and introduced in a littoral part excluding 10 thousands of fry of each species, which were reared in ponds with a density of 30 000 per hectare up to Autumn. Cut meadow plants grown on the banks and artificial food were used for fingerlings feeding in daily rate 10% of fish body weight. In total 4.5 tons of artificial food was used. In autumn, fingerlings reached 30g in average. Survival ratio was 30%. They were transferred into the lake, too.

In October, during research seinings in the constricted lake, 15 fingerlings of grass carp and 5 of silver carp were caught. They were 300-400g in weight. Due to reproductive biology peculiarities the natural spawning of grass carp and silver carp in Lake Tuzkan is impossible, so they were from our entered fries.

Conclusion

Traditionally, stocking of lakes is carried out by fingerlings that are reared in aquaculture farms. But Arnasay Lake System, into which Lake Tuzkan is included, is 300-400km from the nearest farm “Balyktchy”. It is very expensive to transport fingerlings by special lorries. Normally one lorry takes 20-30 000 of fingerlings. Due to the economic crisis in NIS countries only 1-2 millions of

fingerlings were stocked last years, which is a very small quantity for these big lakes.

Another opportunity is to transport larvae and rear them in small nurseries near the lake. This can provide with a great number of stocked fish. But usually reproductive season in fish farms occurs in May-June, this period conflicts with water level changing regime under conditions of regulated stocked flow in the basin. On the other hand, early reproduction is good for more effective using of plankton in lakes which is reach in April and May.

Our experiments show that it is successful opportunity to induce early spawning of cyprinids by the administration of short time keeping of maturing breeders in warm water and pituitary or hormonal injections. Spawning in March-April and larvae transportation from fish farm to small nurseries near a lake for fry rearing can provide with great amount of fish for stocking.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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SPAWNING OF SOLE IN A RECIRCULATION SYSTEM USING ARTIFICIAL DIETS

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Introduction

Sole (*Solea solea*) spawn easily in captivity and naturally fertilized eggs can be collected regularly during a spawning period. Devauchelle et al. (1987) have published results of 12 years of egg production of sole. Baynes et al. (1993) have reviewed the management of sole broodstock that have been kept in a number of facilities in Europe. In general, sole are kept in flow-through systems using ambient temperatures and photoperiod. Broodstock are fed mainly natural diets like molluscs or polychaetes.

Water recirculation offers considerable advantages in temperature control for out-of-season spawning of sole. In addition, use of artificial diets is less labour-intensive and more hygienic than use of natural feed. This paper reports on the experience during three years with broodstock of sole using a recirculation system and artificial diets.

Materials and methods

Sole were collected in spring of 1997 and 1998 with a beam-trawl. After the initial mortality (67%) was finished, the fish were distributed over 2 round PE tanks with a diameter of ~3m ($7m^2$, $5m^3$). In 1999 the individual fish were weighed and tagged with a transponder. The tanks were connected to a recirculation system with a water volume of $70m^3$ of which the water treatment consisted of a large sand filter ($8m^3$). The total water volume was replaced 5 times per year. The tanks were covered and inside photoperiod was controlled with a 40W lamp. The fish were fed a frozen moist pellet daily which consisted up to mid 1999 of a mixture of cooked mussel meat, a commercial eel pellet and fish meal. After that period, a commercial broodstock mix developed for halibut (Trouw, UK) was used which was mixed with 7% fish oil (red Toby). During the spawning season, the fish received a meal of fresh mussel meat, lugworm (*Arenicola*), or ragworm (*Nereis*) once a week.

Results and discussion

The biomass of the broodstock increased in the period October 1998 till November 1999 with a factor 1.8. At the latter date, when the fish were tagged individually, the average condition factor (Fulton index) was 1.15. In December 2000 individual fish were weighed again. The average condition factor was 1.09 and the average weight had increased from 466g to 546g (*n*=63). Figure 1 shows that the growth of individual fish is variable and roughly half of the population actually looses weight. According to van Beek (1988), the condition factor of female sole in the North Sea at the end of the year is 1.12 on average. These results show that the artificial diet is able to support growth and condition of the broodstock.

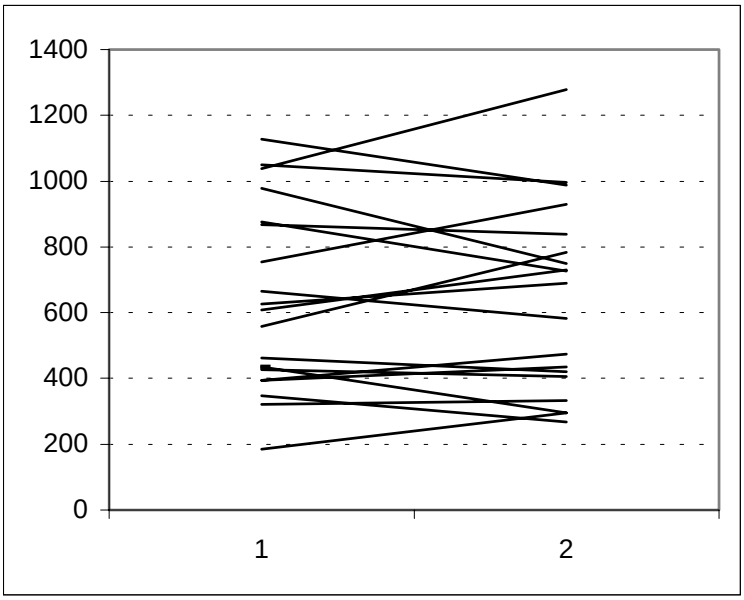

Fig. 1. The growth of individual sole (grams) between November 1999 (1) and December 2000 (2) in one the tanks.

Fish density amounted to 4 fish per m^2 and 1.5 to 3kg per m^2, which is around the upper range mentioned by Baynes et al. (1993). The ratio of males to females was not ascertained precisely. From the mortality during acclimation period this ratio was estimated to be 1:2.

The data concerning the spawning of the sole are presented in Table I.

In the spring of 1997 and 1998 only small batches of eggs could be collected which indicated that fish were only fully acclimated since 1998. The general

pattern of the start of spawning confirms the ideas put forward by Baynes et al. (1993) that a rise in water temperature of 1-2 °C is responsible for the onset of spawning. An exception to this is the spawning in tank A this year. The spawning period lasts in most cases roughly 3 months in which on 3 out of 4 days eggs could be collected. The number of eggs produced per gram of female is above the range indicated in literature.

Table I. An overview of the spawning conditions and egg production of the sole.

	1999		2000		2001	
Tank	A	B	A	B	A	B
Temp. min. (°C)	7	8	8	6	7	7
Start spawning (°C)	10	9	10	7	13	9
DL spawning (h)	15	15	13	10	16	11
Spawning period (d)	85	45	90	85	–	–
No. of batches	62	39	63	69	–	–
Total egg prod. ($\times 10^3$)	1959	563	1711	2984	–	–
Eggs per g female ($\times 10^3$)	228	166	166	209	–	–
% Batches fertilized	30	0	21	1	–	–

However, fertilization is poor in general and tank B only once produced a batch of fertilized eggs. Within fertilized batches of eggs, the percentage fertilization was generally below 25.

Although problems with natural fertilization of sole eggs have been reported frequently in literature, the fertilization rate reported here is exceptionally low. We postulate that the use of a recirculation system is responsible for this phenomenon. Possibly, male behaviour or maturation is inhibited by recirculation of pheromones.

References

Baynes S.M., B.R. Howell, and T.W. Beard. 1993. A review of egg production by captive sole, *Solea solea* (L.). Aquacult. Fish. Manage. 24:171-180.

Devauchelle N., J.C. Alexandre, N. Le Corre, and Y. Letty. 1987. Spawning of sole in captivity. Aquaculture 66:125-147.

van Beek F.A. 1988. On the growth of sole in the North Sea. ICES CM 1988/G:24.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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GROWTH HETEROGENEITY IN PREDATORY FISH LARVAE: PHYSIOLOGICAL AND ENVIRONMENTAL INFLUENCES

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Growth heterogeneity is a common feature during the early development of many teleost fish, both in wild and culture environments. Its impact on the success of larval rearing can be considerable, as it promotes size-dependent dominance hierarchies and cannibalism, even in species that are not typically predatory at a later ontogenetic stage. The factors behind growth heterogeneity among siblings are of genetic, behavioural, and environmental (including trophic) nature, as all of them influence the feeding physiology and metabolism of fish. However their respective importance in governing growth heterogeneity is largely unknown.

This article reviews the role of these factors, with a special comparative emphasis on European sea bass (*Dicentrarchus labrax*) and Eurasian perch (*Perca fluviatilis*) as representatives of marine and freshwater predatory species, respectively. The relative contribution of some environmental (temperature, day length, light intensity), feeding (food composition and availability), and population (hatching time, initial size heterogeneity, stocking density) variables on growth heterogeneity has been investigated, and, for some of them, tentatively correlated to some physiological parameters such as interindividual variations in feed intake, enzymatic activities involved in food digestion, and absorption rates.

In both perch and sea bass, survival and growth were intimately dependent on hatching time, with early hatched larvae generally being better represented after one month of rearing than siblings hatching later. However, in both species, rearing early and late hatched larvae altogether did not significantly influence the heterogeneity of a 30-day-old fish population.

Initial size heterogeneity is generally regarded as a key to feeding hierarchies, growth depression, and subsequent cannibalism. However, it did not significantly influence the survival, growth, individual feed intake, and final size heterogeneity of perch and sea bass larvae, neither when fed *Artemia* nauplii nor

during the weaning period. Similarly, regular size sorting of perch and sea bass larvae did not reduce mortality and final size heterogeneity. Studies of gastric evacuation rate, specific activities of several digestive enzymes (pepsin, trypsin, amylase, leucine-alanine peptidase), and absorption (by feeding perch larvae with *Artemia* nauplii labeled with ^{3}H-phenylalanine) indicated that digestive activities and absorption efficiencies varied between individuals, and could promote growth heterogeneity among perch larvae. This supports the hypothesis that growth heterogeneity among larvae fed in excess does not arise from competition for food, but from discrepancies in their digestion and absorption capacities, which eventually result in a more or less efficient use of ingested food.

The study of stocking density effects in perch larvae provided evidence that low stocking density promoted the differential growth of this species, the faster emergence of incomplete and then complete cannibalism, and caused higher losses due to cannibalism without improving the growth of survivors. On the other hand, stocking density did not affect survival, growth, and weight heterogeneity of sea bass larvae fed *Artemia* nauplii, while it resulted in depressed survival and growth during weaning, without any impact on size heterogeneity.

With regards to environmental variables, day length and light intensity were found to substantially influence survival, cannibalism, and growth of perch larvae, but they had limited effects on size heterogeneity. On the other hand, in sea bass larvae, size heterogeneity as well as mortality due to cannibalism were significantly reduced under continuous day length and food supply, while light intensity had no effect on survival and growth heterogeneity.

By compiling all data within a large database, predictive models of mortality, growth, and size heterogeneity among populations of perch and sea bass larvae have been produced after stepwise multiple-regression analyses. Models were always more accurate for sea bass than for perch in predicting survival and growth heterogeneity, possibly because sea bass has been cultured for decades, whereas the culture of perch is much more recent. Sea bass also exhibits cannibalistic behaviour, but far less intensely than perch, and this may also account for why the growth and mortality models in sea bass were more predictable than their counterparts in perch. However, size heterogeneity was the only variable for which perch was more predictable than sea bass, but this could be accounted for by the contrasted dynamics of cannibalism among these two species.

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POSSIBLE PATHWAYS OF OPPORTUNISTIC MICROFLORA IN THE MARINE FISH LARVAE REARING SYSTEM

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Introduction

The level and ratio of the total aerobic heterotrophs and *Vibrio* group can provide necessary information on possible pathways of pathogenic microflora, as *Vibrio* spp. are considered the most prominent pathogens in marine fish cultivation. Microflora associated with all phases of technological process of the Black Sea turbot (BST, *Scophthalmus maeoticus* Pallas) larvae rearing environment (initial and treated water, microalgae and alive food medium, water from incubators and rearing basins) and organisms (microalgae, alive food and BST eggs and larvae up to the start of weaning) were suspected as possible roots of opportunistic microflora. The objective of this study was to assess the most suspicious roots for infection of BST larvae by opportunistic microflora causing mortality during metamorphosis.

Material and methods

BST (Scophthalmus maeoticus Pallas) larvae rearing . Marine water used for microalgae, alive food and larvae cultivation was previously treated by filtration through course filter followed by cartridge filters (CF 10, 5, 1μm) and UV-sterilization (UV-15 GPD; 30 000mW). Fertilized eggs, obtained from wild broodstock, were gently washed in sterilized marine water and incubated at 15°C at density 1000eggs·l^{-1} in the 100-l flow-through incubators. At the stage of eye pigmentation BST larvae were transferred into the 3m^3 basins with 3-days aged, after UV treatment, water at density of 10 larvae.l^{-1}. Larvae feeding was performed at 17-18°C in "green water": microalgae (10^4cell·ml^{-1}) were introduced into the rearing tanks before the introduction of the BST larvae - *Chlorella marina=Nannochloropsis oculata* (CHLO) (Eustigmatophyceae), and before the start of exogenous feeding - *Isochrysis galbana taitiana* (T-ISO) (Prymnesiophyceae). BST larvae were fed by *Brachionus plicatilis*, followed, from 12dah onwards, by *Artemia* metanauplii (AMN) (pre-fed by T-ISO). Rotifers

supplied for larvae were reared in the separated units on T-ISO and RHO (*Rhodomonas baltica*, Cryptophyceae). Mass cultivation of microalgae was done in 30-l plastic tanks.

Microbiological methods. Monitoring of microflora in the rearing system of the BST larvae was performed using standard microbiological methods for sampling water, larvae and feeding organisms and plating on the standard microbiological mediums applied in mariculture practice (Chowdhury, 1995). Colony forming units (CFU) of the total heterotrophic aerobes (HET) grown on Marine Agar (MA) (DIFCO, USA), and total *Vibrio* group (VIBR) on TCBS agar (Oxoid, England) were registered after 48h of incubation at 25°C.

Qualitative check (growth/absence of colonies on TCBS) was used for plating of: 1) whole fertilized BST eggs in the middle of incubation period; 2) microalgae, stimulated by sterilised peptone water; 3) microalgae, stimulated by sterilised metabolites from rotifer mass culture; 4) sterilized GF/C filters on which 10^7 microalgae cells were concentrated by filtration. Cases (2) and (3) were performed to check possible presence of non-cultivable form of *Vibrio* associated with the surface of microalgae; or (4) within microalgae cell.

Results and discussion

Water filtration through CF provided decrease of total heterotrophs from 10^4.ml^{-1} in the initial seawater to 10^2.ml^{-1} and to 10.ml^{-1} on TCBS. After a week of filtration through untreated CF, in the water after filtration CFU were ten times higher (10^3.ml^{-1}) on MA, and 5 times on TCBS. The organic particles filling CF pores during filtration were supposed to form the rich substrate for development of microflora, revealing the necessity of CF disinfection not less than 2 times a week. UV treatment, yet, reduce CFU both on MA and TCBS to the safe level.

Three variants of microalgae and associated microflora interaction during microalgae mass cultivation were reavealed: slight increase of total heterotrophs growth in the lag-phase after dilution, inhibition of their growth during the exponential phase of microalgae culture (CHLO more than T-ISO), and synchronous growth of microalgae and total heterotrophs in the late exponential phase. In general, in the mass microalgae culture at the end of the exponential phase (5×10^6 for T-ISO, 5×10^7 for CHLO, cells.ml^{-1}) the number of total heterotrophs never surpass 10^4CFU.ml^{-1} on MA. Plated by usual methods, microalgae cultures never revealed CFU on TCBS. After being stimulated, both by peptone water and rotifers metabolites, T-ISO, CHLO, and RHO gave non-identified for *Vibrio* CFU growth on TCBS. Typical variable *Vibrio*-like colonies on TCBS were observed after plating of sterilized GF/C filters with concentrated on them RHO cells (destroyed through filtration being fragile).

Inoculation of 4-day-old rotifer stock culture (5×10^4 CFU.ml^{-1} on MA and 0CFU on TCBS) into T-ISO mass culture in exponential phase ($5x10^6$ cells·ml^{-1} on MA, 0CFU on TCBS), and plating the sample 4 hours after inoculation resulted in appearance of few colonies on TCBS, and within 3 days of mass rotifer cultivation, the number of CFU on MA increased up to 5×10^5 CFU.ml^{-1} with parallel level of 10^3CFU.ml^{-1} on TCBS. In the rinsed rotifers from this culture (used for feeding of the BST larvae) the level less than 10CFU.spec.$^{-1}$ on TCBS, and less then 10^3CFU.spec.$^{-1}$ on MA, was observed, comparable with the bacterial load in untreated *Artemia* nauplii (AN), 18-hours after hatching. After AN were rinsed for 2min in distilled water, CFU on MA decreased 10-fold, and on TCBS, to 0. In 24 hours of feeding on T-ISO mass culture, bacterial load in AMN increased to $5\cdot10^3$CFU.spec.$^{-1}$ on MA, practically equal to that on TCBS (up to 90% consisted of small transparent identical colonies).

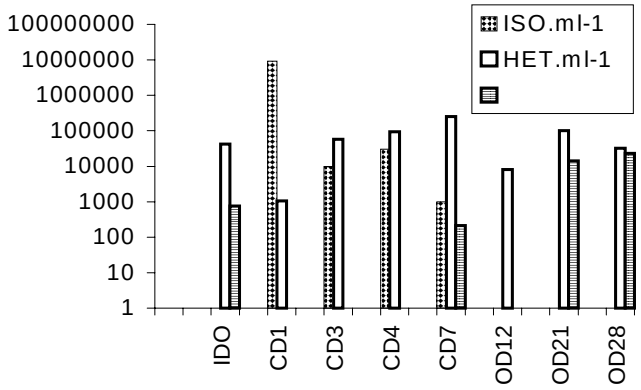

Fig. 1. Number.ml^{-1} of microalgae (ISO), total aerobic heterotrophs (HET), and *Vibrio* (VIBR) in the water during BST rearing: in the incubator before hatching (ID0); before larvae introduction in closed system (3-m^3 basin) (CD1), during rotifers feeding in closed (CD3-7) and opened system (OD12); during the stage of feeding *Artemia* metanauplii in quarantined opened system (0.6-m^3 basin) (OD21-28).

Monitoring of bacterial load in the consecutive volumes of BST larvae rearing phases was provided (Fig. 1). Before introduction of the BST fertilized eggs into incubators, in the water (aged 3 days after sterilization) the level of bacterial load constitute less than 10^2CFU.ml^{-1} on MA and 0 on TCBS. Yet, 20% of developing embryos (3daf) revealed *Vibrio*-suspicious bacterial contamination (growth on TCBS), and at start of BST larvae hatching, the water in the incubators contained $5\cdot10^4$ CFU.l^{-1} on MA and less than 10^3 CFU.ml^{-1} on TCBS (Fig.1, ID0). The "green water" in the 3-m^3 closed system after inoculation of microalgae, before the introduction of the BST larvae contained 10^3CFU.ml^{-1} on MA, 0 – on TCBS (Fig. 1, CD0). After only 1 day of BST larvae feeding rotifers (4dah BST larvae) bacterial press increased to about 10^5CFU.ml^{-1} on MA (Fig.1, CD4), and reached its maximum - over 2.10^5CFU.ml^{-1} on MA and $2\cdot10^2$CFU.ml^{-}

[1] on TCBS - on the 7 day of exploitation of the “green water" 3-m^3 system without water exchange (Fig. 1, CD7). In the opened $3m^3$ with 12dah BST larvae at start of feeding on *Artemia* metanauplii, 3 days of flow-through functioning resulted in disappearance of CFU on TCBS and decrease lower than $10^3 CFU \cdot ml^{-1}$ on MA. In the flow-through 600 l basin for weaning, where the dark-pigmented 21dah BST larvae isolated, *Vibrio*-like microflora increased its number (up to $10^4 CFU.ml^{-1}$ on TCBS) and its input in the number of total heterotrophs from 14% (21dah) (Fig.1,CD21) up to 80% (CD28) (28dah larvae).

Colonization of BST larvae increased continuously during rotifers feeding period from 10^4 $CFU \cdot larvae^{-1}$ up to $10^6 CFU.larvae^{-1}$ on MA, with few $CFU \cdot larvae^{-1}$ on TCBS. In the 19dah black pigmented larvae, fed for 7 days AMN, the growth on TCBS revealed $4 \times 10^5 CFU \cdot larvae^{-1}$ comprising 50% of total heterotrophs. The number of *Vibrio* on TCBS approaching to the number of total heterotrophs on MA usually resulted in the forthcoming mortality (Dehasque et al., 1991). About 90% of CFU grown on TCBS from the environment of moribund 28-dah larvae revealed similarity only with CFU grown on the BST eggs during incubation.

The formation of the initial microflora of the BST larvae at the moment of mouth opening is determined by all components of larvae environment involved in the “green water” rearing system: non-treated cartridge filters without UV-treatment, presence of non-cultivable forms of opportunistic bacteria associated with/or within microalgae cells, maltreated live food. Yet, in presented schedule the most probable infection could be possible pathogen transmitted through the eggs from the wild broodstock. Non-selective artificial decrease of bacterial number due to filtration and UV destroys natural microbial environment of the marine water, and organic load resulting from intensive cultivation medium can stimulate selection and growth of opportunistic bacteria, presented at hatching in negligible number. Presumption of pathogenity of the *Vibrio* sp. (supposed to be *V. haemoliticus* originated from non-sterile eggs) as the main cause of mortality is confirmed by increase up to 80% of *Vibrio* sp. CFU on TCBS (identical to those from 20% of untreated eggs) from the number of total aerobic heterotrophs observed in connection with the moribund 21-28 dah BST larvae.

References

Chowdhury M.J.U. 1995. Probiotic manipulation of the gut microflora in first-feeding larval turbot (*Scophthalmus maximus* L.). MD Thesis. University of Ghent. 72p.

M. Dehasque, L. Verdonck, P. Sorgeloos, J. Swings, P. Leger, K. Kersters, P. Lavens, E. Jaspers, and F. Ollevier. 1991. Determination of bacterial contamination in the live food production systems in marine fish hatcheries in southern Europe. In: Larvi '91. P. Lavens, P. Sorgeloos, E. Jaspers, F. Ollevier (Eds.). Gent, Belgium, EAS Spec. Publ. 15:399-402.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECT OF LOW SALINITY AND TEMPERATURE ON THE LIPID COMPOSITION OF MULLET LARVAE, *MUGIL CEPHALUS*

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Introduction

In Tunisia, millions of mullet larvae are transferred, annually, from coastal areas into inland water and artificial lakes. During the transfer, fish are exposed to some environmental variation, especially salinity, temperature and dissolved oxygen, which may affect the biochemical composition of larvae flesh and organs. The present work concerns the analysis of lipid content and fatty acid composition of *Mugil cephalus* larvae acclimated at different salinities and temperatures.

Materials and methods

Culture. *M. cephalus* larvae, mean total length of 18mm, were collected around the Khelij entrance into the Tunis gulf. After five months of rearing in 75-l aquaria with biologic filtration and natural photoperiod-larvae attained 25.3mm in body length. Then fish were acclimated during four weeks (n=6) at different salinities (35 and 0.5‰) and temperatures (26 and 14°C). Fish were fed daily at the ratio of 10% wet weight.

Lipid extraction and preparation of methyl esters. This was performed according to the Folch method modified by Blight and Dyer (1959). The extracted total lipids were converted to fatty acid methyl esters according to the method of Metcalf et al. (1966).
Gas Chromatography. Methyl esters of fatty acids were separated using a gas chromatograph HP 4890D with a flame ionization detector. Separation was carried out in a capillary column (0.25mm id ~ 30m length). Hydrogen was a carrier gas. The column, detector and injector temperature was 210°C, 250°C, and 220°C, respectively.

Statistical analysis. Data were analyzed for significant differences of means, ANOVA, and inspected by Duncan test at the level of $P<0.05$.

Results and discussion

Effect of salinity on the lipid composition. At 14°C, salinity decrease (from 35-0.5‰) involves a significant decrease of lipid content. At 26°C, the same change in water salinity is followed by a non- significant decrease of total lipid content in *M. cephalus* larvae (Table I). The change of the water salinity implies an osmotic regulation using lipid as an energy source (Brichon et al., 1979). It seems that the amount of energy used for internal and external physiological equilibrium was higher in freshwater compared to seawater (El Cafsi, 2000). This may explain the decrease of total lipid obtained at 14°C. At 26°C, the feeding rate allowed the fish to resist osmotic stress. The fatty acid composition varied also with this change in water salinity; at 14 °C, we note the increase of some fatty acids such as C20: 4, C22: 5, and C22: 6 (Fig l-A).

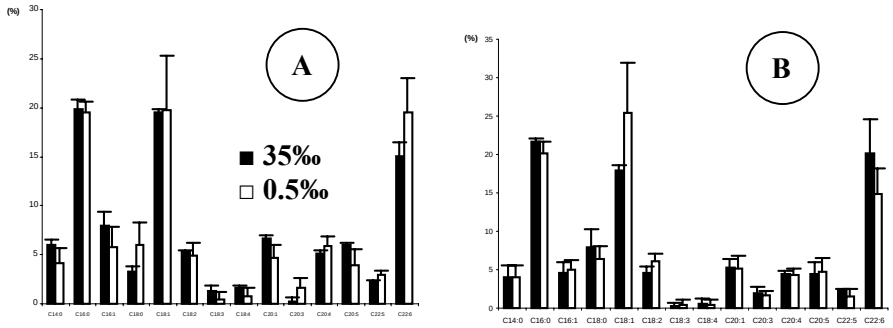

Fig. 1. (A) Variation of fatty acid percentages of *Mugil cephalus*, according to salinity, at 14°C. (B) Variation of fatty acid percentages of *M. cephalus*, according to salinity, at 26°C.

At 26°C, the salinity decrease is followed by an increase of C18: 1, C18: 2, C18: 3 and C20: 5 (Fig 1-B). The increase of those PUFA at 14°C shows that their synthesis from (C18: 2 and C18: 3) is more active in freshwater compared to the seawater. In this context, Sargent (1989) point out that the ω5-and ω6-desaturase activities are inhibited or slackened in seawater. This may therefore block the desaturation and elongation reactions.

However, the high temperatures can also inhibit the synthesis of PUFA in freshwater. The increase of the later at high salinity and high temperature is more related to a feeding increase and not their synthesis increase. In fact, seawater fishes selectively accumulate the DHA present in their diet that they cannot synthesize (Saito et al., 1997).

Table I. Variation of total lipid content (mg.g^{-1} wet weight) according to the water salinity and temperature.

Salinity	Temperature	Total lipid content
35‰	14°C	42.86±8.70
	26°C	13.62±7.40
0.5‰	14°C	19.43±12.49
	26°C	17.10±6.83

Effect of temperature on lipid composition. At high salinity (35‰), total lipid content was significantly higher at 14°C than at 26°C. At low salinity (0.5 ‰), we obtained the same result but the variation was non significant (Table I). The increase of total lipid content when temperature decreased seems to be related to dissolved oxygen, which decreases with high temperature and salinity, in our case 26°C and 35‰, respectively. This may involve physiologic disturbances that fish face by using their lipid reserve. Moreover, many authors have shown that there is a great carbohydrates contribution as fuel via the pentose phosphate shunt pathway and the Krebs cycle (Guederly and Gawlicka, 1992; Kieffer et al., 1998). This also explains the preservation of lipid content at low temperatures. At 0.5‰, low salinity improves dissolved oxygen rate, and fish can shun stress occurred by high temperature. Furthermore, only at low salinity do we note that decrease of temperature involves the increase of PUFA, especially C20:4, C22:5 and C22:6 (Fig 2-B). According to Karahadian et al. (1995), concentrations of EPA and DHA in winter-captured fish (*Morone saxatilis*) were found to be slightly higher compared to their spring counterparts .We can suggest that there is a fatty acid unsaturation increase at cold temperature, especially at low salinity, in *Mugil cephalus* larvae.

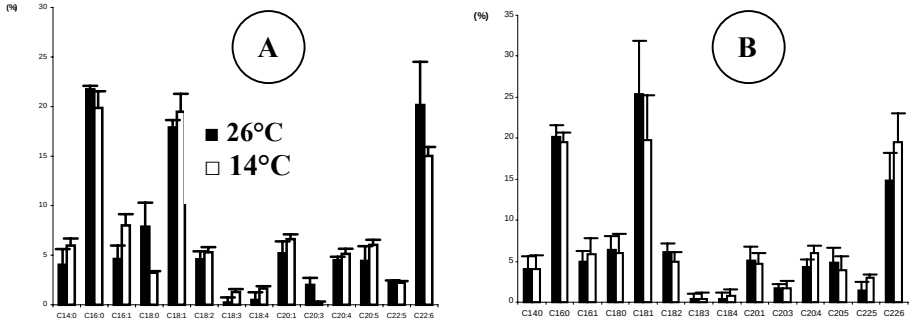

Fig. 2. (A) Variation of fatty acid percentages of *Mugil cephalus*, according to temperature, at 35‰. (B) Variation of fatty acid percentages of *M. cephalus*, according to temperature, at 0.5‰.

This may occur by stimulation of desaturation and elongation enzymes. At high salinity it seems that possible inhibition of this mechanisms by sea salt has an edge on the stimulant effect of low temperature.

Conclusion

The decrease of water salinity and temperature improves PUFA in *M. cephalus* larvae. We can, then, optimize rearing parameters, since we consider an acclimation phase important before the transfer of *M. cephalus* larvae from the marine coastal areas to the continental fresh water lakes. This can therefore improve the production of this fish.

References

Bligh E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Brichon G., G. Zweigelstein, N. Abdul Malak, and R. Meinster. 1979. Effect of seawater on the phosphatidylcholine metabolism in the eels. In: Animals and Environmental Fitness. R. Gilles (Ed.). Pergamon Press, NY. 27p.

El Cafsi M. 2000. Effet de la basse salinité du milieu sur le métabolisme lipidique du Muge. Thèse de Doctorat d'Etat, Université de Tunis, Faculté des Sciences. 177p.

Guderley H., and A. Gawlicka. 1992. Qualitative modification of muscle metabolic organization with thermal acclimation in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 10:123-132.

Karahadian C., K.P. Flower, and D.H. Cox. 1995. Comparison of chemical composition of striped bass *(Morone saxatilis)* from three Chesapeake bay tributaries with those of two aquaculture hybrid striped bass types. Food Chem. 54(4):409-418.

Kieffer J.D., D. Alsop, and C.M. Wood. 1998. A respirometric analysis of fuel use during aerobic swimming at different temperatures in Rainbow Trout *(Oncorhynchus mykiss)*. J. Exp. Biol. 201:3123-3133.

Metcalfe L.D., A.A. Schmitz, and J.R. Pelka. 1966. Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. Anal. Chem. 38:524-535.

Saito H., K. Ishihara, and M. Teruaki. 1997. The fatty acid composition in Tuna (Bonito, *Euthynnus pelamis)* caught at three different localities from tropics to temperate. J. Sci. Food. Agric. 73:53-59.

Sargent J., R.J. Henderson, and D.R. Tocher. 1989. The lipids. p.153-218. In: Fish Nutrition. J.E. Halver, Academic Press, Inc.

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EGG QUALITY CHARACTERISTICS IN FISHES AND MAMMALS – WHAT CAN WE LEARN FROM OTHER SPECIES?

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Abstract

The development of aquaculture for many marine fish species is delayed by high and variable larval mortality rates and a variable juvenile quality. Poor or variable output in the juvenile production can often be linked to problems with egg and larval quality. A strengthened focus on these aspects and how they are related is necessary to obtain a better understanding of biological mechanisms and implications for offspring quality. During the last decade, reliable criteria for egg quality evaluation and effects from egg overripening based on empirical research have been successfully established. For marine fish, assessment of fertilization rates, normal blastomere morphology, and cell symmetry at early stages of cleavage seem to be general indicators of egg quality, and these are promising tools for the prediction of viability potential of developing embryos, larvae, and juveniles in several species.

More recent studies of variations in egg quality throughout spawning periods, egg maturation processes and mechanisms that are possible determinants of egg quality (such as ATP/energy charge, insulin-like growth factors, pH, growth hormones, etc.) and long-term effects of variable egg and larval quality (behaviour, growth, stress tolerance, organ functionality, hormones) all contribute to a better understanding of how biological mechanisms are linked to offspring viability. Development of egg or embryo quality characteristics for diagnostic use seems to have a parallel history for fishes and mammals. In mammals, embryo quality affects the pregnancy rate from *in vitro* fertilization. Established manuals for morphological quality evaluation of early embryonic stages of cattle seem quite similar to those used in fish, as are those criteria used for other mammals where embryo transfer is performed. Also for *in vitro* fertilization in humans the problem of defective oocytes has been studied, and common practice is now to evaluate the early embryo morphology and cleavage state. For all species, current research is performed to reveal causal relations between embryo quality and viability, and the paper will address the potential benefits of comparative work on vertebrates.

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DIGESTIVE ENZYMES IN FISH LARVAE AND JUVENILES – IMPLICATIONS AND APPLICATIONS TO FORMULATED DIETS

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Introduction

During recent years, intensive research has been conducted to find full or partial replacements for the use of live food organisms in larval culture. Although significant improvement was reported in co-feeding live and dry diets, especially at the *Artemia* stage, microdiets (MD) have not at this stage matched the growth and survival demonstrated by larvae fed live feed. It has become increasingly clear that larval nutrition should be examined in the light of ontogenetic development of the larval and juvenile digestive systems.

Microdiet digestion

The small size and rudimentary development of larval marine species and some freshwater species have hampered the success of weaning the fish onto a prepared MD at an early age. Although weaning can be achieved at metamorphosis or 0.5-0.75g in most species, the earlier introduction of MD as the sole replacement for live food has only been met with limited success. The poor performance of MD is related to the inadequate incorporation of nutrients due to poor ingestion, digestion, and/or assimilation (Kolkovski and Dabrowski, 1999).

The relatively low level of enzyme activities and the absence of pepsin-like enzyme activity have been considered some of the reasons for the limited success of MD and the poor growth performance of larvae fed solely on MD (Day et al., 1997; Kolkovski and Dabrowski, 1999).

Co-feeding

Feeding live feed, such as *Artemia* nauplii, a short time before MD are offered has been suggested to increase both digestive enzyme activity and the ability of

larvae to digest the dry diets (Kolkovski et al., 1997a). Kolkovski et al. (1997c) reported a significant increase in MD assimilation when European seabass (*Dicentrarchus labrax*) larvae were co-fed MD and *Artemia* nauplii compared with larvae fed solely on MD.

A possible explanation may be the contribution of digestive enzymes from the live food organisms. Some authors have suggested that 40-80% of the enzymatic activity is donated by the live food organisms (Dabrowski, 1984). However, other studies suggested that the live food contribution to direct digestive enzymes might be negligible (Zambonino-Infante et al., 1996; Cahu and Zambonino-Infante, 1997). It is clear that live food organisms contribute to the digestion and assimilation process in fish larvae. However, their contribution may be in forms other than direct enzyme contribution.

Dietary enzymes

The effect of pancreatic extract supplementation on digestion, assimilation, and growth of gilthead seabream (*Sparus aurata*) was demonstrated by Kolkovski et al. (1993). The authors concluded that supplementation of 0.05% (dw) pancreatin enhanced the MD assimilation by 30% and larval growth by 200%. Other studies assessed the effect of various dietary enzyme supplements in MD fed to different fish species, and overall, the results are not conclusive.

It can be concluded that the effect of dietary digestive enzymes depends on fish age, species, and the type of the dietary enzymes, as well as food habits.

Hydrolysates and Free Amino Acids

An alternative strategy to the supplementation of dietary digestive enzymes in the MD is the use of pre-digested protein sources (hydrolysates; Hardy, 1991). During recent years, studies have been conducted involving a range of variables including: fish species and age, source of protein, and concentrations of hydrolysates and free amino acids (FAA) (Cahu et al., 1999; Kolkovski and Tandler, 2000; Zambonino-Infante et al., 1997). Based on these studies, it would seem that whole protein might be partially replaced by hydrolyzed protein source and/or FAA. It is recommended that the levels of the hydrolysate or FAA in the MD should not exceed 30% of the total protein.

Digestive System Neuropeptides

Another method to activate or enhance digestive enzyme activity has been assessed by numerous authors. It was suggested that dietary neuropeptides supplemented in the MD might increase digestive system activity by increasing

enzyme secretion and peristaltic movements, and hence have a positive effect on MD utilization.

Kolkovski et al. (1997b) found that bombesin (a tetradecapeptide closely related to mammalian gastrin-releasing peptide) activity increased by 300% in gilthead seabream larvae fed *Artemia* nauplii compared to larvae fed dry diets. This would indicate that the nauplii stimulate bombesin activity in some manner, which allows increased peristalsis and assists in the digestion process.

An indirect method to enhance digestive system activity and enzyme secretion was reported by Kolkovski et al. (1999). The authors reported increased total proteolytic activity (trypsin-like) in gilthead seabream and Australian sea bass (*Lates calcarifer*) larvae when chemical stimuli (water from *Artemia* rearing tank, FAA, krill hydrolysate, and other compounds found to be feed attractants) were introduced to the larvae without any feed (dry or live) served. From these results it may be concluded that feed attractants may not only improve the ingestion of diets but also stimulate the fish digestive system.

Conclusions

We now have an appreciation that developing artificial diets for fish larvae is more complicated than just finding the right combination of nutrients. A 'holistic' approach needs to be taken in the development of microdiets for fish larvae. Different aspects of research should be addressed with the incorporation of the ontogenetic development of the larval and juvenile digestive systems, direct or indirect activation of digestive enzymes, and digestibility of MD ingredients, especially proteins, binders, and capsule ingredients. Future research should focus on the chain of events of ingestion, digestion, assimilation, absorption, and the application of this knowledge to diet formulation.

References

Cahu C. and J.L. Zambonino-Infante. 1997. Is the digestive capacity of marine fish larvae sufficient for compound diet feeding? Aquacult. Int. 5:151-161.

Cahu C., J.L. Zambonino-Infante, P. Quazuguel, and M.M. Le Gall. 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. Aquaculture 171:109-119.

Dabrowski K. 1984. The feeding of fish larvae: present "state of the art" and perspectives. Reprod. Nutr. Develop. 24:807-833.

Day O.J., B.R. Howell, and D.A. Jones. 1997. The effect of dietary hydrolysed fish protein concentrate on the survival and growth of juvenile Dover sole *Solea solea* L. during and after weaning. Aquacult. Res. 28:911-921.

Hardy R.W. 1991. Fish hydrolysates: production and use in aquaculture feeds. Proceedings of the Aquaculture Feed Processing and Nutrition Workshop, Thailand. p.109-115.

Kolkovski S., A. Arieli, and A. Tandler. 1997a. Visual and chemical cues stimulate microdiet ingestion in gilthead seabream, *Sparus aurata*, larvae. Aquacult. Int. 5:527-536.

Kolkovski S. and D. Dabrowski. 1999. Diets for fish larvae - present state of art. Proceedings from World Aquaculture Sympmosium, Sydney Australia. 406p.

Kolkovski S., D. Dabrowski, and T. Lam. 1999. The use of krill hydrolysate as feed attractant for fish juvenile. J. World Aquacult. Soc. 31: 81-88

Kolkovski S., W.M. Koven, and A. Tandler. 1997b. The mode of action of *Artemia* in enhancing utilization of microdiet by gilthead seabream *Sparus aurata* larvae. Aquaculture 155:193-205.

Kolkovski S. and A. Tandler. 2000. The use of squid protein hydrolysate as a protein source in microdiets for gilthead seabream *Sparus aurata* larvae. Aquaculture Nutrition. Aquacult. Nutr. 6:11-17.

Kolkovski S., A. Tandler, and M.S. Izquierdo. 1997c. Effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass *Dicentrarchus labrax* larvae. Aquaculture 148:313-322.

Kolkovski S., A. Tandler, G. Kissil, and A. Gertler. 1993. The effect of dietary exogenous digestive enzymes on ingestion assimilation, growth and survival of gilthead seabream *Sparus aurata*, Sparidae, Linnaeus larvae. Fish Physiol. Biochem. 12:203-209.

Zambonino-Infante J.L., C.L., Cahu, A. Peres, P. Quazuguel, and M.M. Le Gall. 1996. Sea bass *Dicentrarchus labrax* larvae fed different *Artemia* rations: growth, pancreas enzymatic response and development of digestive functions. Aquaculture 139:129-138.

Zambonino-Infante J.L., C.L. Cahu, and A. Peres. 1997. Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. J. Nutr. 127:608-614.

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MATURATION AND FECUNDITY OF CRUCIAN CARP, *CARASSIUS AURATUS GIBELIO,* IN UZBEKISTAN

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Introduction

In the 1950s, crucian carp, *Carassius auratus Gibelio,* was introduced into Uzbekistan fish farms from Moscow region, Russia. But after development extensive technology of carp polyculture farmers lost interest for crucian carp and it inhabits in fish farms as trash fish. Crucian carp occasionally entered to rivers and now inhabit in the most freshwater water bodies in the Basin of the Aral Sea. The aim of our study was to determine the reproductive capacity of female crucian carp from wild and fish farm strains in Uzbekistan.

Material and methods

The material was collected in fish farms "Balyktchy" and "Damachy" and in mid-stream of the River Syrdarya in April 1994-1999. Those fish farms are located on the shore of the River Syrdarya in the middle stream. Altogether 50 females at different ages from "Balyktchy", 30 from "Damachy", 40 from the River Syrdarya were analyzed. Body length, weight, and ovary weight was measured for each fish. Age and growth rate was determined by scales. A schemata for classification of fish ovarian development adapted for cyprinid fishes of Nikolsky (1974) was used to determine stage of maturation

The number of ripe eggs was counted in 1g; the result was multiplied by the total weight of ovary in order to determine individual absolute fecundity (AF). Individual relative fecundity (RF) was calculated as number of ripe eggs per g of body weight of the gutted fish. The gonadosomatic index (GSI) was determined as GSI = ovary weight×100×body weight of the gutted fish^{-1}.

Results

In fish farms growth rate was determined as l_1=6.8-8.8cm; l_2=10.2-12.2cm; l_3=13.9-15.1cm and in the River Syrdarya l_1=10cm; l_2=19.9cm; l_3=25.7cm in

average.

Maturation rate depended from age and was similar in ponds and river populations. In the first year, ovaries developed to the stage II when females reached 5-6cm of body length in ponds and 7-8cm in the River Syrdarya. It usually happened in the late Summer-early Autumn. During second year gonads still developed at the stage II. In the autumn, vitellogenic oocytes appeared in gonads that reached stage III. In the next spring, all 2-year-old fish matured and had gonads at stage IV in the April when they reached 8-10cm in length and 11-24g in weight in fish farms and 18-20cm and 100-300g in weight in the River Syrdarya.

GSI of females both in ponds and in river in the first year increased insignificantly and reached 0.01-0.02%. During the second year GSI slowly increased up to 0.1-0.6%. In spring, gonads matured rapidly, and GSI reaches 4-25% just before spawning.

Spawning began in the late April-early May when water temperature reached 16-18°C. Crucian carp used flooded grasses or submerged aquatic macrophytes at a depth of 0.3-1.5 meters as a spawning substrate. Intermittent (2-times) spawning was determined. Second spawning occur in August. The ratio of females to males in the spawning stock was 3-4:1 for river and 6-8:1 for ponds in fish farms.

Crucian carp female absolute fecundity in “Balyktchy” varied 3 000-51 000 (24 000 in average) eggs; in “Damachy”3 000-49 000 (14 000). In each farm the fecundity correlated with body size:

- in Balyktchy: $AF=0.0004\times l^{4.271}$ ($r = 0.96$);
- in Damachy: $AF=0.0049\times l^{3.109}$ ($r = 0.92$).

In the River Syrdarya females were more fecund measured as 9 000-91 000 (46 000), and absolute fecundity also correlated with body size $AF=2.009\times l^{-3.143}$ ($r = 0.53$).

Relative fecundity of females from both fish farms varied from 150 to 500 (280) $egg.kg^{-1}$; there was no significant difference between two pond populations. In the River Syrdarya relative fecundity was 21-590 (190) $eggs.kg^{-1}$. Correlation analyses showed that there was no significant relation between relative fecundity and body size in all populations studied.

Conclusions

Our data showed that Uzbekistan environments are favorable for crucian carp.

In natural water bodies like in the River Syrdarya populations inhabit in completely wild conditions. Crucian carp is commercial fish; arranged about 4% of total country fish capturing from inland water bodies.

In aquaculture fish farms crucian carp is an unwanted fish. Farmers use permits, dewatering, and poisoning the ponds in order to reduce its numbers. But the absolute removal of this fish by such methods in such big ponds (100ha and more) is impossible. Crucian carp inhabits farm channel system and enters fattening ponds every year.

Variation of all biological characteristics studied has been found in populations from different conditions.

In the River Syrdarya the growth rate of crucian carp was significantly much higher then in ponds. Significant difference by t-test ($F<0.01$) in body length between the wild (river) and both two pond populations; but no difference between two fish farm populations studied.

In all populations studied, crucian carp in mass matured at an age of 2 years, but in river fish were larger. River females had significantly higher absolute fecundity than in ponds. At the same time, relative fecundity of females was significantly higher in pond stocks.

References

Nikolsky G.V. 1974. Ecologiya Rib (The Ecology of Fishes). Vischaya Shkola, Moscow. 365p. (In Russian).

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EXPERIMENTAL AND PRACTICAL USE OF MICRODIETS FOR AQUATIC ANIMALS

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Abstract

The review paper focuses on three main subjects. First is the development of carrageenan-microbound amino acid diets and utilization of soybean protein isolates for larval shrimp. The feeding trial was conducted to determine the nutritional value of dietary soybean protein isolates (SPI) and to evaluate the microbound amino acid diets (MAAD) for larval kuruma shrimp. Larvae fed MAAD could successfully metamorphose into the postlarva1 stage. Larvae fed the diets containing different SPI levels (30-60%) with crystalline amino acid supplements also developed and reached the postlarva1 stage. The survival rates were similar among larvae fed the diets containing SPI at 40, 50, and 60%. Although the size of the postlarva1 stage in the MAAD group was smaller than those in SPI fed groups, the number of postlarvae at 10 days post-hatch (dph) was very close. This study demonstrated that the MAAD and SPI diets were acceptable for kuruma shrimp larvae.

The second investigation is into the practical use of microdiets (MD) for larval shrimp. The application of MD for mass seedling production for kuruma shrimp from N_6 to PL_1 stages was evaluated. Four live foods such as *Chaetoceros gracilis*, *Tetraselmis* sp., *Brachionus plicatilis*, and *Artemia* sp. nauplii, and microbound diets (MBD) were used for early stage diets. Five dietary treatments were compared: T1 (commercial MD + *Chaetoceros* + *Tetraselmis* + Brachionus + *Artemia*), T2 (commercial MD + *Chaetoceros* + *Tetraselmis* + *Artemia*), T3 (commercial MD + *Tetraselmis* + *Artemia*), T4 (MF21 MD + *Artemia*), and T5 (MF21 MD). Survival rates were as follows: 76% (T1), 98% (T2), 85% (T3), 79% (T4), and 48% (T5). The growth rates were faster in T1 and T2 than in T3, T4, and T5. Although the complete replacement of live foods with microdiets used in this study is still difficult, this trial indicated that kuruma shrimp larvae can survive and grow reasonably well when fed MD together with *Tetraselmis* and *Artemia*.

Finally, the development of a microbound diet containing lactoferrin for larval red sea bream without *Artemia* was investigated. Although lactoferrin (LF), an

iron-binding glycoprotein, has been reported to have various biological functions in young terrestrial animals, there is limited available information on LF effects on fish larvae. Red sea bream larvae (6dph) were fed MBD together with rotifers until 10dph for 5 days. Those larvae were then fed only MBD containing lactoferrin from 11-30dph. Four MBD with different levels of lactoferrin (0, 300, 700, and 1000 $mg.kg^{-1}$ dry diet) were formulated. Red sea bream larvae fed MD survived and grew similarly to those fed live foods. Larvae fed with LF at 700 and 1000$mg.kg^{-1}$ showed significantly better recovery rates than those fed MBD containing 0 or 300$mg.kg^{-1}$ of LF and live foods. This study suggests the possible sole use of MBD from the early larval stage of red sea bream and that dietary LF can increase the stress tolerance of larval fish.

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THE IMPORTANCE OF ARACHIDONIC ACID, AS A MODULATOR OF STRESS RESISTANCE THROUGH THE HYPOTHALAMUS-PITUITARY-INTERRENAL AXIS, IN DIFFERENT-AGED GILTHEAD SEABREAM LARVAE

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This is a review of a series of studies carried out to investigate the effect of dietary arachidonic acid (20:4n-6, ArA) on survival and growth in developing gilthead seabream larvae and the biological mechanisms involved. Initial studies tested the effect of high dietary docosahexaenoic acid (22:6n-3, DHA) and varying ArA on growth, survival, and resistance to handling stress in 5- to 35-day-old larvae. The results suggest that dietary ArA supplementation did not enhance growth but, when fed to larvae prior to handling stress, markedly improved survival ($P<0.05$) compared to larvae that were fed following handling stress. This implied that ArA, in contrast to other essential fatty acids, whose functionality is tied to the cellular membrane, was involved in biochemical pathways that prepared the larvae to better survive an acute stress event. Moreover, the results suggest the importance of early larval nutrition on later larval and juvenile survival.

A follow-up study investigated the effect of ArA on the acute and chronic stress response in larvae during the stages of pre-metamorphosis (10-20 days old), metamorphosis (20-30 days old), and post-metamorphosis (30-40 days old). In these trials, three levels of dietary ArA were tested during rotifer and *Artemia* feeding. Larvae reared in 400-l V-tanks were first fed rotifers (0.8, 1.3, and 2.7mg ArA.g^{-1} DW) and then *Artemia* nauplii (0.9, 3.8, and 8.4mg ArA.g^{-1} DW). In each of the tested developmental ages, larvae were transferred to 30-l aquaria, defined as a handling stress, and grown for 10 days on the same treatment rotifers and/or *Artemia* nauplii fed to the rearing tanks. In the aquarium system, the three dietary levels of ArA were each tested in replicates of eight. Larvae in

four of the replicates from each treatment were not exposed to any further induced stress other than the initial transfer to the aquaria while larvae in the other four replicates were subjected to a daily cycle of salinity fluctuation (42 to 25 to 42‰). Accumulated mortality was monitored during aquarium rearing while final survival, dry weight, and whole body cortisol were measured 10 days later, at the end of aquaria rearing. In the pre-metamorphosis group, mortality was higher than 80% during aquarium rearing, which masked any dietary ArA effect. However, larvae fed ArA-supplemented *Artemia* in the aquaria during metamorphosis and exposed only to the acute stress of transfer exhibited markedly reduced accumulated mortality ($P<0.05$) and improved final survival ($P<0.05$) at the end of 10 days in the aquaria. On the other hand, dietary ArA fed to larvae subjected to chronic stress showed the reverse trend, demonstrating the highest accumulated mortality ($P<0.05$) and reduced final survival. These survival patterns were also exhibited, but less markedly ($P>0.05$), in the post-metamorphosis group.

A correlation was clearly demonstrated between dietary ArA and cortisol level in larvae at the end of aquarium rearing in both the acute and chronically stressed groups. In fact, cortisol levels in larvae subjected to chronic stress were significantly higher ($P<0.05$) at each ArA dietary level compared to larvae not experiencing daily salinity fluctuation. Further studies carried out on seabream larvae reinforced this observation by showing an elevated stress response in cortisol levels in metamorphosing fish reared on ArA-enriched *Artemia* nauplii after exposure to salinity stress, compared to the non ArA supplemented control. Moreover, larvae fed the ArA-enriched nauplii had slightly elevated basal cortisol levels. The clear correlation between dietary ArA and whole body cortisol suggests a physiological interpretation of the seemingly contradictory effect of this fatty acid under acute or chronic stress. Following stress, cortisol release from the interrenal cells of the developing head kidney is an adaptive response in which cortisol mobilizes energy reserves and helps to maintain ionic balance by stimulating osmoregulatory capacity. We propose that the prostaglandin E_2 (PGE_2), the main metabolite derived from ArA, may be up-regulating cortisol synthesis through the hypothalamus-pituitary-interrenal axis during a stress event expressed as improved survival after acute stress. This may be particularly relevant in marine fish larvae, which are especially vulnerable during metamorphosis and sensitive to osmoregulatory stress. On the other hand, the sustained ArA-mediated release of elevated cortisol during chronic stress can have damaging effects, resulting in increased larval mortality.

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European Aquaculture Society, Special Publication No. 30, Oostende, Belgium, 2001

FEEDING PREDIGESTED PROTEIN TO ATLANTIC HALIBUT LARVAE (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

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Introduction

At first feeding, marine fish larvae seem to have a reduced ability of protein digestion (Munilla-Moran and Stark, 1989), and therefore a supposed need for easily available amino acids. Addition of hydrolyzed protein to formulated diets has been tried with the aim to ease digestion of these types of feed, and encouraging results have been obtained (Zambonino Infante et al. 1997). The hypothesis of the present study was that Atlantic halibut larvae would benefit from inclusion of hydrolyzed protein into a formulated diet. Furthermore, the aim of the experiment was to find an optimal level of hydrolyzed protein in the diet by testing different enzyme treatments and inclusion levels of hydrolyzed protein in the diet.

Materials and methods

Seven isoenergetic and isonitrogenous diets were produced at the Institute of Nutrition, Directorate of Fisheries (Norway). Parts of the dietary protein were separated into three, respectively hydrolyzed with pepsin (P), pepsin + trypsin (PT), or pepsin + trypsin + chymotrypsin (PTC), and then added to the different diets in quantities of 10 or 30%. One diet was kept unhydrolyzed.

The Atlantic halibut larvae were produced following the standard procedure at the Austevoll Aquaculture Station, Institute of Marine Research (Norway). The larvae (start wt. 0.12±0.04g) were weaned on and fed the experimental diets in duplicate raceways from day 40-82 post first-feeding. *Artemia* was used as control.

Dry matter was determined gravimetrically after oven drying at 104°C, and protein (6.25 × N) was determined using a Nitrogen-Analyzer (Perkin Elmer,

USA). The solubility of dietary protein was determined in accordance with Adler-Nissen (1979). Fatty acid composition was analyzed using GLC, and total lipid content in the larvae by using 19:0 as an internal standard in the analysis of fatty acid composition.

$3^1 \times 2^1$ ANOVA (Statistica, StatSoft inc., OK, USA) was used for testing possible effects of the experimental factors on the larvae, and possible effects on the diets were tested by multiple linear regression where also the unhydrolyzed diet was included in the model. Both analyses were followed by the Tukey HSD *post hoc* test. The analyses were based on means±SD of duplicate treatments, and treatments were considered significantly different when $P<0.05$.

Results and discussion

Both the experimental factors (enzyme treatment in the order 0, P, PT, and PTC; inclusion level of hydrolyzed protein) proved to increase the amount of soluble protein in the diets (Fig. 1). However, the levels of soluble protein were hardly comparable with the level in *Artemia* that contained almost three times the values of the formulated diets.

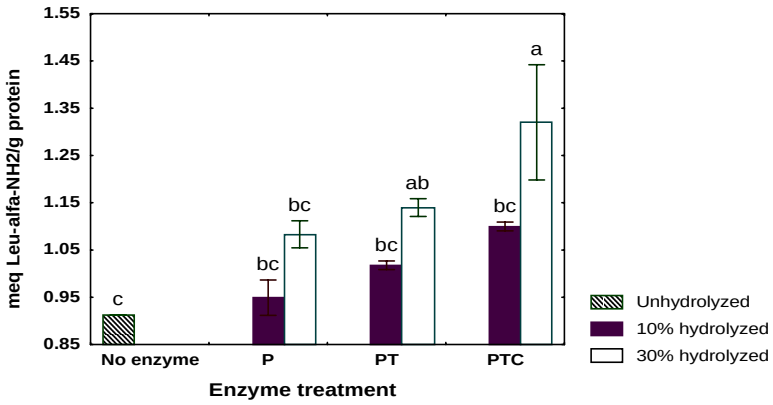

Fig. 1. Content of soluble protein in the experimental diets. *Artemia* = 3.2 meq Leu-α-NH_2 (g.protein^{-1}). Values followed by different letters are significantly different at $P<0.05$. Enzyme treatments: P= pepsin, T= trypsin and C= chymotrypsin.

Survival rates (Fig.2) were significantly decreased both by increasing amount of hydrolyzed protein in the diet and by the enzyme treatment in order P, PT, and PTC. *Artemia* (83±0%) and the diet 10P (10% of dietary protein hydrolyzed with pepsin; 67±4%) seemed most advantageous, although the unhydrolyzed diet and the diet 10PT did not significantly reduce survival compared to the 10P diet.

Including as much as 30% of the hydrolyzed protein into the diet or additional hydrolysis with chymotrypsin impaired the result.

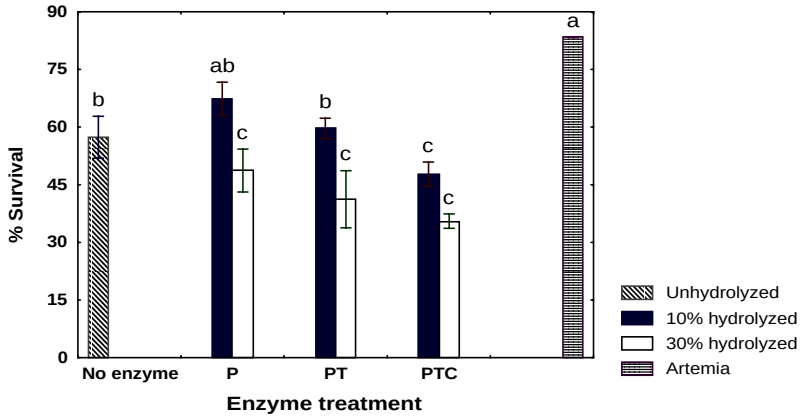

Fig. 2. Survival rates to larvae given the experimental diets. Values followed by different letters are significantly different at $P<0.05$. Enzyme treatments: P= pepsin, T= trypsin and C= chymotrypsin.

The growth pattern (SGR; Fig. 3) appeared similar to the survival rates, but no significant differences were found. Still, the enzymatic treatment with only pepsin tended to improve growth ($P<0.06$) compared to additional hydrolysis.

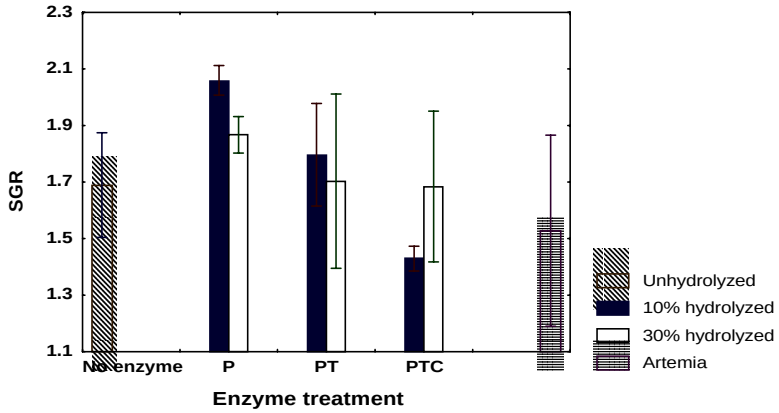

Fig. 3. Specific growth rates (SGR) to larvae given the experimental diets. SGR = [(ln final wt - ln initial wt)/experimental time in days]*100. Enzyme treatments: P= pepsin, T= trypsin and C= chymotrypsin.

Dry matter (162±10mg.g^{-1}) and crude protein (740±58mg.g^{-1} dry wt) did not differ among the larvae fed the different diets. The halibut larvae fed the

formulated diets were lower in total lipid content than those fed *Artemia* (54±8 and 86±2mg.(g dry wt)$^{-1}$, respectively), but higher in EPA (10.7±0.4% and 6.8±0.2% of total fatty acids, respectively) and DHA (21.8±1.5% and 14.5±0.3% of fatty acids, respectively).

Acknowledgements

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References

Adler-Nissen J. 1979. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesuphonic acid. J. Agricult. Food Chem. 27:1256-1262.

Munilla-Moran R. and J.R. Stark. 1989. Protein digestion in early turbot larvae, *Schopthalmus maximus* (L.). Aquaculture 81:315-327.

Zambonino Infante J.L., C.L. Cahu, and A. Peres. 1997. Partial substitution of di- and tripeptides for native protein in sea bass diet improves *Dicentrarchus labrax* larval development. J. Nutr. 127:608-614.

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CHALLENGES IN REARING FISH LARVAE AND OTHER MARINE SUSPENSION-FEEDERS ON MICROPARTICULATE DIETS

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Abstract

Development of satisfactory microparticulate, artificial diets for rearing marine fish larvae and other suspension feeders will make an important contribution to commercial aquaculture. The development of these microparticulate diets is challenging because of difficulties related to the required small size (5-300 microns) of the particles and the poorly developed digestive systems of the larval stages of many marine species. Various types of microcapsules and microbound particles have been used to deliver protein, carbohydrates, and lipids to fish larvae. Unfortunately, leakage of low molecular weight, water-soluble nutrients (e.g., water-soluble vitamins and amino acids) from these microparticle types is typically very rapid due to high surface area to volume ratios and short diffusion paths. Recently, lipid-walled capsules and lipid spray-beads have been developed for the delivery of water-soluble nutrients to fish larvae and other suspension-feeders. These lipid particle types can be incorporated in larger food particles, resulting in a complex particle type that will reduce loss of dietary nutrients through leakage.

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EVALUATION OF DIFFERENT LIVE FOOD ORGANISMS ON GROWTH AND SURVIVAL OF RIVER CATFISH, *MYSTUS NEMURUS* (C&V) LARVAE

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Introduction

Mystus nemurus is one of the most commercially important freshwater fish in Malaysia (Khan et al., 1990). Even though artificial breeding or reproduction of *M. nemurus* is done in private hatcheries around Peninsular Malaysia, inadequate seed supply coupled with relatively high fingerling prices limits its production. Presently, the supply of fingerlings cannot satisfy the demand for fish farming due to some constraints on the larval rearing (Khan et al., 1990), so larval rearing of *M. nemurus* has yet to be improved in terms of nutrition requirement and suitable size of food for the larvae.

At present, the conventional method of fish larviculture using live food such as *Artemia* nauplii (Rottman et al., 1991; Verreth et al., 1992) is being practiced by most Malaysian catfish hatchery operators. Using expensive live food like *Artemia* has made the mass production of catfish fry/fingerlings less profitable. Alternative measures are necessary in order to help minimize importation and use of *Artemia*. Indigenous species of live food organisms, which are great potential as feed and can easily be cultured and mass-produced at low cost, may be used as substitutes. Studies on those live foods are lacking, hence this study was conducted to determine the effect of different live foods on growth and survival of *Mystus nemurus* larvae.

Materials and methods

Three-day-old *M. nemurus* larvae (mean total length 8.4mm and body weight 5.94mg) obtained from a single batch of artificially spawned breeders were stocked in 15 aquaria containing 10 l of water at a stocking rate of 10 l^{-1}. The larvae were fed with *Brachionus* sp., *Chironomus* spp., *P.redivivus, Moina* sp.,

and *Artemia* sp. *ad libitum* three times a day for 16 days. All treatments were triplicated. *Brachionus* sp., *Moina* sp., and *Chironomus* spp. were cultured to assure a continuous supply of feed. *Brachionus* sp. were cultured using greenwater and baker's yeast (Lim and Wong, 1997), while staggered culture of *Moina* sp. and *Chironomus* spp. were done in 300-l fiberglass tanks using chicken manure as a nutrient source. *P. redivivus* was cultured using solid medium composed of oatmeal and saline solution. All aquaria were cleaned daily and water was changed up to 50% of the total volume. Survival rate and growth of fish in terms of total length and weight were determined per aquarium at 4-day intervals. Specific growth rate (SGR) was computed based on the formula of Richer (1979). Data were analyzed using one-way (ANOVA) and Duncan's Multiple Range test was used to compare means.

Results

Survival rate was highest in larvae fed with *P. redivivus*, followed by *Brachionus* sp., *Moina* sp., and *Artemia* sp. fed larvae. Larvae fed with *Chironomus* spp. had significantly lower ($P<0.05$) survival rate compared to other treatments. Final mean weight (256.87mg) was significantly greater ($P<0.05$) for fish fed with *Chironomus* spp. (Table I). Fish given the *Artemia* sp. exhibited the second highest body weight. Significantly lower ($P<0.05$) growth obtained in fish given fed with *P. redivivus* and *Brachionus* sp. compared to the other treatments. Final mean total length was highest in *Chironomus* spp. fed larvae followed by *Artemia* sp., *Moina* sp. and *P. redivivus*, and low in *Brachionus* sp. fed larvae. Specific growth rate (Table I) was significantly higher ($P<0.05$) for fish reared on *Chironomus* spp., followed by those given with *Artemia* sp., *Moina* sp, *P. redivivus*, and *Brachionus* sp. fed larvae had low specific growth rate.

Table I. Survival rate, final mean body weight, final mean total length and specific growth rate of *M. nemurus* larvae fed with different live food reared for period of 16 days.

Treatment	Survival rate (%)	Final mean body weight (mg)	Final total length (mm)	SGR (% day 1^{-1})
Brachionus sp.	94.67±1.33[a]	99.2±3.17 [d]	24.17±0.18[d]	17.57±0.35[d]
Moina sp.	92.67±0.67[a]	120.53±6.8[c]	25.50±0.23[c]	18.79±0.63[c]
Chironomus spp.	82.0±4.0[b]	256.87±4.0[a]	31.83±22[a]	23.54±21[a]
Artemia sp.	93.33±1.33[a]	209.67±1.33[b]	28.83±1.8[b]	22.27±0.17[b]
P. redivivus	96.67±0.67[a]	101.50±2.78[d]	24.50±058[d]	17.73±0.29[d]

Values are means ± SEM. Means with in column and lettered in the same superscript are not significantly different ($P>0.05$). Initial mean total length 8.4±0.1517mm and mean body weight 5.94mg.

Discussion

The feeding of *Chironomus* spp. to *M. nemurus* larvae significantly improved growth rates, but with significantly low survival rates compared to *P. redivivus*., *Brachionus* sp., *Moina* sp., and *Artemia* sp. The differences in growth rates were undoubtedly related to its larger size and nutritional value of the feed. *P. redivivus* was successfully used for rearing *Crangon crangon*, *Cyprinus carpio*, and silver carp. The present experiment showed that feeding *P. redivivus* increased survival rate but exhibited low growth rate. *P. redivivus* are small for the late stage larvae and spend more energy searching for food. Furthermore, *P. redivivus* has a hard cuticle, and this probably affects the feeding activity of the larvae, and apparently its digestibility is low.

Brachionus sp., on the other hand, has been regarded as one of the best food species, particularly for carp (Lubzens et al., 1984; 1989) and dwarf gourami (Lim and Wong, 1997). In this particular study, however, catfish larvae fed with *Brachionus* sp. exhibited lower growth, although the survival rate was high. *Brachionus* sp. are very small and late stage larvae spend more energy searching for food, resulting in lower growth than *Chironomus* spp. and the control (*Artemia*).

Moina sp. was also found to be a suitable food for catfish larvae. Larval growth was similar to those fed *Brachionus* sp. *Moina* sp. has been reported by several authors to give significant effects on growth and survival of milkfish (Villegas, 1990, and *Clarias macrocephalus* (Evangelista, 1996). Moreover, Fermin (1991) has suggested that *Moina* sp. could be a partial or complete substitutes for *Artemia* sp. In this study, despite high survival, *Moina* sp. produced lower growth. It is speculated that more energy was lost during the later larval stage (8 days onward) as live food become too small for the larvae.

Survival rates of *M. nemurus* larvae were generally high and comparable to those obtained by other workers on the utilization of live food for catfishes (Evangelista , 1996; Fermin and Bolivar, 1991) catfish.

Conclusions

From this study, it can be concluded that smaller live food like *P. redivivus*., *Brachionus* sp., and *Moina* sp. should be suitable as an *Artemia* replacement for the early larval stage on (3 to 11 days old) of *M. nemurus*, and larger *Chironomus* spp. for the later stages. However, *Artemia* sp. seems to be most suitable food for all larval stages during a 16-day feeding period.

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References

Evangelista A.D. 1996. Comparison of selected live food organisms and artificial diets as food for larval rearing of catfish (*Clarias macrocephalus* Gunther). M.Sc. thesis, University of the Philippines, Institute of Aquaculture, College of Fisheries, Miagao, Iloilo City, Philippines. 112p.

Fermin A.C. 1991. Freshwater cladoceran *Moina macrocopa* (Strauss) as an alternative live food for rearing sea bass *Lates calcarifer* (Bloch) fry. J. Appl. Ichthyol. 7:8-14.

Fermin A.C. and M.E. Bolivar. 1991. Larval rearing of the Philippine Freshwater catfish, *Clarias macrocephalus* (Guther), fed live zooplankton and artificial diet. A Preliminary Study. Isr. J. Aquacult. 43(3)87-94.

Khan M.S., K.J. Ang, M.A. Ambak, and C.R. Saad. 1990. Reproductive biology of tropical catfish *Mystus nemurus* (C&V) in Chendoroh reservoir, Malaysia. Aquacult. Fish. Manage. 21:173-179.

Lim L.C. and C.C. Wong. 1997. Use of the rotifer, *Brachionus calyciflorus* (Pallas), in freshwater ornamental fish larviculture. Hydrobiologia 358:269-273.

Lubzens E.S., A. Rothbard, G. Blumental, B. Koldny, B. Perry, Y. Olund, Y. Wax, and H. Farbstein. 1987. Possible use of *Brachionus plicatilis* (O.F. Muller). As food for freshwater cyprinid larvae. Aquaculture 60:143-155.

Lubzens E.S., A. Tandler, and G. Minkoff. 1989. Rotifer as food in Aquaculture. Hydrobiologia 186/187:387-400.

Ricker, W. E. 1979. Growth rate and models. In: W.S. Hoat, D.J. Randall and J.R. Brett, (Editors). Fish Physiology, volume VIII. Bio-energetics and growth.Academic Press, New York, USA, p.677-743.

Rottman R.W., J.V. Sheriman, and E.P. Lincoln. 1991. Comparison of three live food and two dry diets for intensive culture of grass carp and bighead carp larvae. Aquaculture 196:269-280.

Verreth J., E.L.S. Torrese, E.J. Spacier, and A.V.D. Swiszen. 1992. The development of a factional digestive system in the African catfish *Clarias gariepinus* (Burchell). J. World Aquacult. Soc. 23(4):286-298.

Villegas C.T. 1990. The effects on growth and survival of feeding water fleas *Moina macrocopa* (Strauss) and *Rotifer* (*Brachionus plicatilis*) to milkfish, *Chanos chanos* (Forskal) fry. Isr. J. Aquacult. 42(1):10-17.

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RECENT ADVANCES IN THE DEVELOPMENT OF MICRODIETS FOR RED DRUM *SCIAENOPS OCELLATUS*

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Introduction

A multidisciplinary approach was employed to evaluate the utilization of microdiets by first-feeding larvae, with the ultimate goal of eliminating the need for zooplankton in the rearing of red drum. Previously, the digestive capacity of the larvae during ontogeny was assessed using histological, biochemical, and molecular techniques (Lazo, 1999). When microdiets were fed as the sole food source, lower growth and survival were observed compared to treatments with live prey, but no dietary-induced differences in digestive enzymes activities were found (Lazo et al., 2000a). In follow-up experiments, red drum were successfully raised on microdiets and algae from first-feeding without zooplankton, and it was determined that the presence of algae improved the performance of microdiets by increasing enzyme activity (Lazo et al., 2000b). To further advance the development of adequate microdiets, the objectives of this study were (1) to develop *in vitro* assays to measure protein digestibility and identify adequate protein sources, (2) to develop and evaluate new microdiets comprised of intact and hydrolyzed protein sources and (3) to develop a technique to measure ingestion rates based on fluorescent markers, to determine whether the presence of algae increases ingestion of microdiets in fish larvae.

Materials and methods

In vitro digestibility assays. Ingredients tested included commonly used protein sources in diets of marine fish larvae (see Table I and Fig. 1). The *in vitro* technique was adapted from Lan and Pan (1993) in which the extent of protein hydrolysis is proportional to the increase in absorbance at 280nm. Enzymes from larval digestive organs were extracted according to Lazo et al. (2000b). Protein digestibility was normalized to casein and expressed as relative protein digestibility ($RPD = \Delta Abs_{ingredient} \div \Delta Abs_{casein} * 100$).

Development of microdiets. Two separate feeding experiments were performed following the rearing protocol described by Holt (1993). For the first feeding trial, two types of microdiets were prepared using the same formulation (Diet A, Table I): a microbound diet (MCB) using zein as binder and a microcoated diet (MCD) using lecithin-cholesterol as the coating factor. The second experiment was performed to evaluate the inclusion of fish protein hydrolysate (FPH) in microbound diets for red drum larvae. Four isonitrogenous (54% protein) and isoenergetic (21kJ.g^{-1}) diets were formulated to contain increasing levels of FPH (15, 20, 25, and 30%). The compositions of the four diets are presented in Table I. A commercial diet (Fry Feed Kyowa, Japan) was used as a control. Three replicate tanks per diet were used. Growth and survival were determined as described in Lazo et al. (2000). The challenge salinity test described by Dhert et al. (1992) was used to evaluate larvae physiological condition at the end of the experiment.

Table I. Composition of test diets (g per 100g dry wt.).

Ingredient	Diet A	Diet15	Diet20	Diet25	Diet30
Casein	15.0	9.0	9.0	9.0	9.0
Menhaden Fish Meal	10.0	40.0	35.0	30.0	25.0
Hake Protein Hydrolysate	15.0	15.0	20.0	25.0	30.0
Yeast		5.0	5.0	5.0	5.0
Red drum muscle	20.0				
Krill	10.0				
Fish oil	14.3	8.0	8.0	8.0	8.0
Aqualip95	4.5	3.0	3.0	3.0	3.0
Aquagrow-DHA+AA		8.5	8.5	8.5	8.5
Vitamin and mineral premix	9.5	9.5	9.5	9.5	9.5
Attractants	1.7	2.0	2.0	2.0	2.0

Ingestion rates. A new technique to measure ingestion rates of microdiets by marine fish larvae was developed based on fluorescent labeling of protein sources in the diet. A commercial diet (Fry Feed Kyowa) was labeled with thioflavin-S adapted from the method of Twinning (1984). Regression analysis was used to derive the equation describing the relationship between fluorescence (pH 8.0, excitation 460nm, and emission 520nm) and quantity of diet. Several tests were performed to evaluate the potential effects of leaching of the dye, saltwater exposure time, and the passage of the diet through the larval digestive system on fluorescence. These factors did not significantly affect fluorescence. The thioflavin-S-marked diet was then used to evaluate the effect of adding algae to the culture tanks of red drum larvae on ingestion. Ingestion rates were quantified in the presence or absence of *Isochrysis galbana* using the feeding protocol described

in Lazo et al. (2000b). Larvae were sampled on days 3, 4, 6, and 8 days after hatching (DAH) and ingestion was evaluated hourly over a 4-h period. All feeding trials described above were evaluated using one-way analysis of variance.

Results and discussion

There were significant differences (P<0.05) in RPD between protein sources evaluated (Fig. 1). Albumin, a commonly used ingredient in diets for red drum larvae, had the lowest RPD. Surprisingly, soybean meal presented a relatively high RPD, suggesting the potential usefulness of soybean meal as a protein source in microdiets for red drum larvae. However, since *in vitro* digestibility assays provide only partial information about the quality of the protein used and do not take into account the amino acid profile of an ingredient and the nutritional requirements of the larvae, it is important to realize the limitations of this approach in selecting adequate protein sources.

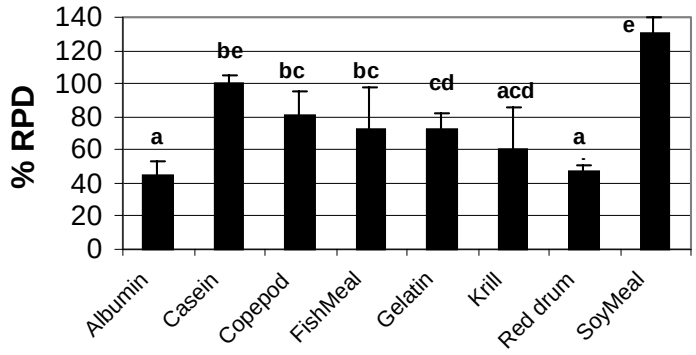

Fig. 1. Relative protein digestibility among protein sources. Means (n=3) with the same superscript are not significantly different from each other. Error bars represent standard deviation from the mean.

Growth, survival and stress response of larvae fed the MCB, MCD, and control diets are presented in Table II. Although the best performance was observed in larvae fed the control diet, no significant differences were found in growth or survival among dietary treatments. However, larvae fed MCD displayed lower growth and survival and a significantly higher stress index compared to the MBD and the control diet.

Significant differences in growth were observed between the experimental diets with different levels of FPH and the control, except for the diet containing 15% FHP (Table II). No significant differences in survival or stress index were observed among dietary treatments. Although not significant (P=0.06), there was

a trend toward reduced growth with increasing dietary content of FPH.

Table II. Performance of red drum larvae fed the experimental diets. Means (±SE) with the same superscript are not significantly different from each other.

Test diet	Final SL	% Survival	Stress Index
Control	8.72 (0.74)	35.80 (16.00)	12.17 (8.16)[a]
Microcoated Diet A	6.79 (0.40)	32.40 (2.20)	55.67 (7.66)[b]
Microbound Diet A	7.69 (0.05)	40.00 (1.41)	19.00 (14.0)[a]
Control	5.84 (0.39)[a]	10.40 (3.09)	22.11 (01.64)
Diet15	4.88 (0.21)[ab]	18.45 (9.32)	27.56 (13.84)
Diet20	4.61 (0.12)[b]	10.52 (4.19)	38.67 (12.23)
Diet25	4.39 (0.06)[b]	16.23 (6.44)	27.89 (10.88)
Diet30	4.53 (0.10)[b]	10.90 (4.32)	39.89 (16.69)

Due to high variability, there was no consistent trend in the quantity of diet ingested by larvae reared in the presence or absence of algae among the 4 hourly measurements completed at various stages of development. However, on any given DAH examined (except d 6), the apparent total amount of diet ingested (summed across the 4-hour period) was significantly higher in the presence of algae particularly on d 3 (94, 19, -30, and 9% higher for 3, 4, 6, and 8 DAH, respectively). This suggests that the presence of algae may have a substantial effect on ingestion early in development. The higher ingestion rates observed here, coupled with higher digestive enzyme activities (Lazo et al., 2000b), could explain the better growth and survival obtained in the presence of algae.

References

Dhert P., P. Lavens, and P. Sorgeloos. 1992. A simple test for quality evaluation of culture fry marine fish. Med. Fac. Landbouww. Univ. Gent. 57:2135-2412.

Holt G.J 1993. Feeding larval red drum on microparticulate diets in closed .recirculating water system. J. World Aquacult. Soc. 42:225-240.

Lan C.C. and B.S Pan. 1993. In-vitro digestibility simulating the proteolysis of feed protein in the midgut gland of grass shrimp (*Penaeus monodon*). Aquaculture 109:59-70.

Lazo J.P. 1999. Development of the digestive system in Red Drum (*Sciaenops ocellatus*) larvae. Ph.D. Dissertation. The University of Texas at Austin. USA.

Lazo J.P., G. Joan Holt and C.R. Arnold. 2000a. Ontogeny of pancreatic enzymes in larval red drum (*Sciaenops ocellatus*). Aquacult. Nutr. 6:183-192.

Lazo J.P., M. Dinnis, C. Faulk, G.J. Holt and C.R. Arnold. 2000b. Co-feeding microparticulate diets with algae: Toward eliminating the need for zooplankton at first feeding in red drum. Aquaculture 188:339-351.

Twinning S.S. 1984. Fluoresccein isothiocyanate-labeled casein assay for proteolytic enzymes. Anal. Biochem. 143:30.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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REVIEW ON ADVANCED BIOTECHNOLOGY IN FINFISH HATCHERIES

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About 20 million metric tonnes of finfish were produced worldwide in 1998, according to Food and Agricultural Organization statistics. We estimated that at least 80 billion juveniles were required to meet this production level. The increasing demand for aquaculture products will require more juveniles to be produced. Numerous studies have been conducted to achieve this objective, in both quantity and quality. A group of experts met in Honolulu, Hawaii in December 1999 to review the status of fry production and discuss the current technical advances in live and inert feed production. This presentation is mainly based on the information presented at that meeting.

In Asia, many finfish species are produced from the hatcheries, more than 90 species in Taiwan and at least 88 species in Japan. In North America and Europe, hatchery operations have concentrated on fewer species. Although hatchery technology follows the same protocols worldwide, modifications have been made to meet specific needs. Modifications of feeding regimes and culture systems are made to improve fish survival and lower production costs. Selecting the appropriate feed, maintaining strict hygienic conditions versus manipulating the natural conditions, and choosing a suitable water exchange system (open, closed, or recirculating) are good examples. Criteria for determining the quality of larvae and juveniles, preferably similar to their natural characteristics in the wild, as well as hatchery techniques to produce such fingerlings, need to be developed for improving the quality of hatchery-produced larvae and juveniles, while at the same time, lowering production costs.

Rotifers and brine shrimp nauplii are the main live food items for the early life stage of the major finfish species, and inert feed is provided at a later stage. Superintensive rotifer culture (up to 190 000 individuals.ml^{-1}) using algal paste was studied to improve the cost-effectiveness of hatchery production. The population growth of rotifers has been shown to increase if the water previously used for their culture is used to inoculate the next generation. In addition to its potential use as an efficient delivery system of antibiotics, the great variation in the nutritional content of *Artemia* from different sources, the need for further

studies on the nutritional requirements of larvae, and the decreasing supply due to increased demand are important factors in calculating the cost-effectiveness of this form of live feed for hatcheries. While studies on live feed production should be continued, reduced demand for live feed was recommended.

In studies on digestive enzymes in fish larvae and formulations of inert feed, formulated feed has showed a potential to replace live feed earlier in the life cycle than is common in current practice. By further developing microdiet formulations, in terms of their physical characteristics, ingestibility, and digestibility, much can be learned about the basic nutritional requirements for fish larvae, provided the diets are of a consistent quality and their source and content are well-documented. Formulated feeds that can be co-fed with live feed, or used as a delivery system for substances such as hormones and vaccines, would be a great asset to hatcheries. A deeper understanding of the roles of microbial processes in culture systems is expected to lead to major breakthroughs in hatchery production in the near future. Although the use of "matured" water (water that has not been disinfected) has been practiced traditionally in Asian aquaculture for decades, it has recently attracted interest as the basis for sound microbial management of aquaculture systems in other regions as well. Management of the microbial community can positively affect live feed production, larviculture, and inert feed application. A better understanding of changes in the microbial community in response to feed could result in the dual benefits of lowering feed costs and preventing the spread of diseases. Further research in this area was recommended.

There was general agreement among the experts that information exchange among researchers and the aquaculture industry, and within these sectors themselves, is essential so that research efforts are more effective and that research results are applied to production. Further details of the recommendations made at the workshop will be presented.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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THE EFFECT OF STARVATION AND FEEDING REGIMES ON SURVIVAL AND GROWTH OF INSTAR 1 PHYLLOSOMA LARVAE OF THE WESTERN ROCK LOBSTER, *PANULIRUS CYGNUS*

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Introduction

There is current worldwide interest in the culture of spiny lobsters. Only a few studies have examined the effects of starvation and feeding on spiny lobster phyllosoma (e.g., Mikami and Takashima, 1993; Mikami et al., 1995; Abrunhosa and Kittaka, 1997). If food deprivation exceeds a maximum period, starved larvae are unable to recover and finish development when re-fed. This point is referred to as the point-of-no-return (PNR). If larvae cannot be fed within a certain time after hatching, continued culture may not be possible due to high levels of mortality. The minimum initial feeding period that is sufficient to ensure development is defined as the point-of-reserve-saturation (PRS) (Dawirs, 1987; Abrunhosa and Kittaka, 1997). This study reports on the estimation of these values which could help improve feeding regimes for spiny lobster larvae.

Materials and methods

Instar 1 larvae were submitted to initial periods of starvation or feeding. Starvation or feeding was stopped at specific times during the intermolt period to determine the periods allowing 50% (PNR_{50} and PRS_{50}) of larvae to molt. Each feeding or starvation period had 10 larvae held individually at 25°C for measurements of growth (intermolt period and postmolt size) and survival (number of larvae molting to the next instar). Larvae that did molt to instar 2 were fed and reared to instar 3 to determine if there were any delayed effects of the initial starvation or feeding periods.

The larvae were kept individually to enable close monitoring of development and feeding. Each day the larvae were checked for molts and deaths and supplied with fresh food. Phyllosoma were fed daily with *Artemia* that had been cultured to ~1.5mm and enriched with Algamac 2000.

Growth data were normally distributed with homogeneous variances. One-way ANOVA was used to test for significance, and a Tukey unequal N HSD test was used for multiple comparisons ("Statistica"). Survival data (%) was analyzed using a Chi-square contingency table and a Tukey-type multiple comparison procedure using angular transformations.

Results and discussion

Point-of-no-return. Survival was high for larvae initially starved for only 0-4 days, however past this point survival decreased rapidly. The PNR_{50} for instar 1 *P. cygnus* phyllosoma was estimated to be 4.66 days (Fig. 1).

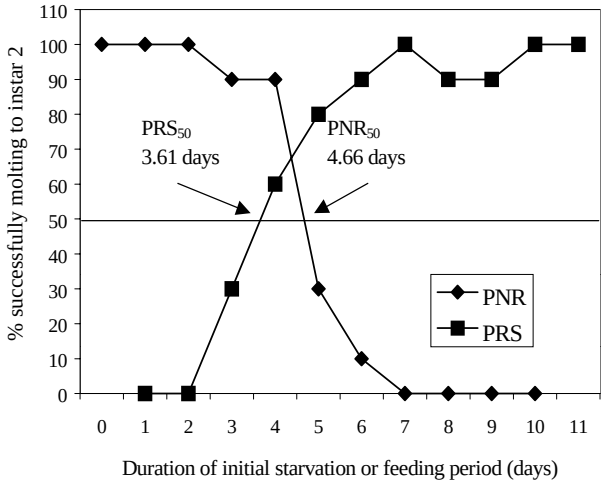

Fig. 1. Percentage (n=10) of instar 1 *Panulirus cygnus* phyllosoma molting to instar 2 subjected to different starvation or feeding periods to determine the point-of-no-return (PNR) or the point-of-reserve-saturation (PRS), respectively.

The PNR_{50} for instar 1 *Jasus edwardsii*, *Jasus verreauxi*, and *Panulirus japonicus* larvae was estimated to be 4.2, 6.5, and 3.43 days, respectively (Mikami et al., 1995; Abrunhosa and Kittaka, 1997). It was speculated that the greater tolerance to starvation of *J. verreauxi* larvae might indicate instar 1 larvae retain more reserves after hatching (Abrunhosa and Kittaka, 1997).

Time to first molt of instar 1 *P. cygnus* larvae was correlated with the duration of the initial starvation period. As the length of the starvation period increased, so did the length of the intermolt period. This correlation was also found in instar 1

J. edwardsii, *J. verreauxi*, and *P. japonicus* phyllosoma (Mikami and Takashima, 1993; Mikami et al., 1995; Abrunhosa and Kittaka, 1997).

Dawirs (1987) found zoea 1 duration of *Carcinus maenas* was delayed by times of starvation plus an additional increasing period, evidently needed to compensate and recover from the increasing starvation periods. Time from first feeding (TFF) to molting for instar 1 *P. cygnus* was relatively stable for larvae starved for up to 3 days. Larvae starved longer exhibited intermolt periods from TFF that gradually increased. Abrunhosa and Kittaka (1997) believed the gradual extension also seen in instar 1 *J. edwardsii* and *J. verreauxi* phyllosoma was due to a lowering of the digestive function or feeding activity of the larvae.

Instar 1 *P. cygnus* larvae initially starved longer than 2 days tended to be smaller at the molt to instar 2. Mikami and Takashima (1993) found *P. japonicus* larvae starved for 3 days were slightly smaller on average than groups fed earlier.

There were no significant effects of the initial starvation periods on the survival or growth of larvae that molted to instar 2 and continued development to instar 3. This may indicate that the larvae were able to accumulate lost reserves and recover before molting.

Point-of-reserve-saturation. Survival was high for instar 1 *P. cygnus* larvae when fed for at least 5 days before starvation. Survival decreased for larvae fed for only 3-4 days, with no larvae molting when fed for only 1 or 2 days. The PRS_{50} for instar 1 *P. cygnus* larvae was estimated to be 3.61 days (Fig. 1).

The PRS_{50} for instar 1 *J. edwardsii*, *J. verreauxi* and *P. japonicus* was estimated as 4.6, 6.7, and 2 days, respectively (Mikami et al., 1995; Abrunhosa and Kittaka, 1997). The differences in the PRS_{50} values, and as well as in PNR_{50} values, are likely to be due to differences in the metabolism between species, and also the rearing conditions (temperature, food quality and amount).

The intermolt period was constant in instar 1 *P. cygnus* between groups fed longer than 4 days, however larvae only fed for 3 days before starvation showed a delay in molting and lower survival than longer fed larvae. Instar 1 *J. edwardsii* and *J. verreauxi* also had constant intermolt periods, however only when larvae were fed for more than 7 and 6 days, respectively, with a delay in molting and increased mortality at molting when feeding stopped before these periods. Abrunhosa and Kittaka (1997) suggested this was due to a deficiency of nutrients needed to molt to the next instar.

Postmolt size of *P. cygnus* larvae was significantly affected by the different initial feeding periods. Larvae showed a gradual increase in size of instar 2 with a longer feeding period up to 5-6 days. The results suggest that the shorter

feeding times only allowed larvae to absorb and accumulate enough reserves to molt, but longer feeding periods allowed larvae to accumulate more reserves that were used for growth. Longer initial feeding periods also resulted in larger instar 2 *P. japonicus* larvae (Mikami et al., 1995).

As with PNR, there were no large differences in the survival or growth of larvae from the different initial feeding regimes that molted to instar 2 and continued development to instar 3.

Conclusions

- Increasing initial starvation periods had a significant effect on decreasing survival of instar 1 larvae and also in increasing the length of the intermolt period, suggesting any delay in feeding should be avoided.
- Instar 1 larvae were able to accumulate sufficient reserves for molting and growth during reduced periods of feeding. As phyllosoma are sensitive to water quality, a reduction in the amount of food added at the end of the instar periods may help maintain water quality.
- Only limited effects of the initial feeding or starvation periods were found on instar 2 larvae. Larvae may need to be cultured for longer periods to rule out any delayed effects.

Acknowledgments

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References

Abrunhosa F.A. and J. Kittaka, 1997. Effect of starvation on the first larvae of *Homarus americanus* (Decapoda, Nephropidae) and phyllosomas of *Jasus verreauxi* and *J. edwardsii* (Decapoda, Palinuridae). Bull. Mar. Sci. 61:73-80.

Dawirs R.R. 1987. Influence of limited starvation periods on growth and elemental composition (C, N, H) of *Carcinus maenas* (Decapoda: Portunidae) larvae reared in the laboratory. Mar. Biol. 93:543-549.

Mikami S. and F. Takashima, 1993. Effects of starvation upon survival, growth and molting interval of the larvae of the spiny lobster *Panulirus japonicus* (Decapoda, Palinuridea). Crustaceana 64:137-142.

Mikami S., J.G. Greenwood, and N.C. Gillespie, 1995. The effect of starvation and feeding regimes on survival, intermoult period and growth of cultured *Panulirus japonicus* and *Thenus* sp. phyllosomas (Decapoda, Palinuridae and Scyllaridae). Crustaceana 68:160-169.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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RECENT DEVELOPMENTS IN THE APPLICATION OF LIVE FOODS IN THE ORNAMENTAL FISH INDUSTRY

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The industrial development of freshwater ornamental fish culture has been hampered by the lack of suitable live foods for feeding the fish at the various production stages. Currently, inert food items such as egg yolk suspension, milk powder, or powdered feeds and artificial fertilization of water to induce plankton bloom are used in larval feeding, and *Moina* and *Tubifex* that are cultured in water enriched with organic manure are fed to bigger fish or broodstock. These traditional practices not only limit the fish stocking density, but also adversely affect fish quality. Many freshwater ornamental fish farmers have shifted to the cleaner *Artemia* nauplii for feeding their young fish, but the size of the nauplii are not appropriate for larvae with small mouths and fish after the fry stage. This paper reports the recent developments in the applications of the freshwater rotifer (*Brachionus calyciflorus*), *Artemia* nauplii, decapsulated *Artemia* cysts, and on-grown *Artemia* in freshwater ornamental fish culture.

Results of larval feeding experiments demonstrate that rotifers are an ideal starter food for dwarf gourami (*Colisa lalia*), a typical freshwater ornamental fish species with larvae that are too small to ingest *Artemia* nauplii or *Moina* at first feeding. Compared with the conventional yolk food, the use of rotifers as a starter food significantly improves the growth and survival of the gourami larvae (Day 2-12), and the beneficial effects are extended to the subsequent *Artemia*-feeding phase (Day 13-32). At metamorphosis, the overall survival rate of the larvae fed on rotifers in indoor tanks (65.1-74.5%) is about 4 times that obtained in extensive open-pond culture (17.5%). The freshwater rotifers are also useful in raising discus larvae in the absence of their parents. In discus, larvae are dependent on the body slime of their parents as a nutrient source during the first two weeks of exogenous feeding. Use of rotifers, followed by feeding with *Artemia* nauplii, facilitates artificial larviculture of the brown discus (*Symphysodon aequifasciata axelrodi*), and their larval growth and survival rates

are comparable to those that rely on parental feeding. The artificial feeding would eliminate the risk of larvae being eaten by the parent fish. It also shortens the brooding interval of the spawners, thereby improving the fry production yield. This feeding protocol is less tedious and more practical than the existing strategies of smuggling the batch of larvae to foster parents or feeding the larvae with egg food.

Work on decapsulated *Artemia* cysts indicates that the cysts could be used as a substitute for *Artemia* nauplii or *Moina* in freshwater ornamental fish culture. The fry of all the five common ornamental fish species tested (guppy, *Poecilia reticulata*; molly, *P. sphenops*; platy, *Xiphophorus maculatus*; swordtail, *X. helleri*; and neon tetra *Hyphessobrycon herbertaxelrodi*) could readily feed on the decapsulated cysts, and their performances in terms of stress resistance, growth, and survival are comparable to or better than those fed on *Artemia* nauplii or *Moina*. Apart from being a hygienic off-the-shelf feed, the direct use of the cysts signifies a new area of application for cysts with poor hatching characteristics in the ornamental fish industry, with concomitant savings in the feed cost.

The key problem in the use of on-grown *Artemia* for feeding ornamental fish is the lack of supply. A culture system has been developed specifically for the use in freshwater ornamental fish farms. The system, using diluted artificial seawater for culture, has a mean production rate of 3 $kg.m^{-3}$ of water in a 12-day cycle and a production capacity of 8 metric tons of on-grown *Artemia* a year. With the system, farmers could produce any specific size of on-grown *Artemia* of up to 5mm to suit the age and size of their fish, by varying the time of harvesting. This characteristic, coupled with the possibility to enhance the nutritional quality of the *Artemia* through bioencapsulation, makes on-grown *Artemia* an ideal organism to replace the unhygienic *Moina* or *Tubifex* in freshwater ornamental fish culture.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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FEEDING OF DISCUS JUVENILES WITH ON-GROWN *ARTEMIA*

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Introduction

Moina and *Tubifex* worms are the most common live food organisms used in the freshwater ornamental fish industry. As these organisms are cultured in ponds enriched with organic manure, they are usually contaminated with pathogenic bacteria and parasites. More and more freshwater ornamental fish farmers have therefore shifted to *Artemia* nauplii and frozen bloodworms (larvae of midge, *Chironomus* sp.) for feeding their fish. *Artemia* nauplii (max. 0.55mm) is only half the size of *Moina* (max. 1.2mm) and bloodworms, being not in live form are less attractive for the fish. It is therefore necessary to look for bigger live food organisms to fill in the size gap. The bigger and older on-grown *Artemia* would be a good alternative live food for use in the hatchery. This paper reports the results of a discus feeding experiment to evaluate the food value of the on-grown *Artemia*, in comparison with *Moina* and frozen bloodworms.

Materials and methods

A 2-week feeding experiment was conducted in triplicate in 45-l aquarium tanks. Six-week-old discus fingerlings (average weight 0.58g) were obtained from a local Discus farm and stocked at a density of 20 fish per tank. The water temperature was maintained at 25-28°C. The fish were fed to satiation, twice a day, with on-grown *Artemia* (4-5mm total length) harvested from an *Artemia* culture reported by Lim et al. (2001). For purpose of comparison, similar experimental tanks were set up for two other groups of discus fed on *Moina* and frozen bloodworms, respectively. The wet weights and survival rates of all the three groups of fish were measured at the end of the experiment. The mean values of the three replicates were tested for statistical significance of differences among the three groups of fish using ANOVA.

Results and discussion

The food value of a live food organism for a particular fish species is primarily determined by its size and form. While a small food organism is desirable for fish larvae in term of ingestibility, the use of a larger organism is more beneficial as long as the size of the food organism does not interfere with the ingestion mechanism of the predator (Merchie, 1996). Fish would take a long time to attain satiation if fed with smaller live food organism, and this would result in poor growth due to inefficient feeding and waste of energy. The advantages of using larger live food organisms for feeding to achieve good performance in fish fingerlings was demonstrated in this study. The feeding response of discus to the on-grown *Artemia* was better than to the *Moina* and frozen bloodworms. Fish fed on *Artemia* attained satiation within 15min and by then most of the fish had displayed a bulging belly. Fish fed on *Moina* and frozen bloodworms did not display bulging belly, although the *Moina* group was seen feeding actively on *Moina*. In terms of wet weight, fish fed on on-grown *Artemia* grew significantly faster than those fed on *Moina*, which in turn grew significantly faster than the group fed on bloodworms (Table I). The mean survival rate of fish fed on on-grown *Artemia* was 12% higher than that fed on *Moina*, but it had no significant difference from the two groups fed on *Moina* and bloodworms respectively.

Table I. Mean and standard deviations of body weight, total length and survival rate of discus after feeding on the respective feeds for 2 weeks.

	Fish feeds		
	On-grown *Artemia*	*Moina*	Frozen bloodworms
Wet weight (g)	0.85 ± 0.01^{a}	0.81 ± 0.01^{b}	0.75 ± 0.01^{c}
Total length (mm)	3.48 ± 0.03^{a}	3.44 ± 0.04^{ab}	3.37 ± 0.04^{b}
Survival rate (%)	90.0 ± 13.2^{a}	78.3 ± 10.4^{a}	91.7 ± 2.89^{a}

Means with different superscript are significantly different at $P<0.05$

The more effective food uptake, arising from bigger and more suitable size of on-grown *Artemia* and its better palatability, could have contributed to the better growth in the *Artemia*-fed group. Besides, on-grown *Artemia* was reported to have superior nutritional digestibility and a thin exoskeleton rich in essential amino acids (Leger et al., 1986). The latter is consistent with the fact that both the total amino acids and essential amino acids in the on-grown *Artemia* were higher than in the *Moina* (Lim et al., 2001). Previous results also show that bioencapsulation technique is very effective in remedying the HUFA deficiency

in on-grown *Artemia*. These characteristics would make on-grown *Artemia* an ideal nursery diet for freshwater ornamental fish.

References

Léger P., D.A. Bengtson, K.L. Simpson, and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Ann. Rev. 24:521-623.

Lim L.C., A. Soh, P. Dhert, and P. Sorgeloos. 2001. Production and application of on-grown *Artemia* in a freshwater ornamental fish farm. Aquacult. Econ. Manage. 5(3/4):211-228.

Merchie G. 1996. Use of nauplii and meta-nauplii. p.137-163. In: Manual on the Production and Use of Live Food for Aquaculture. P. Lavens and P. Sorgeloos (Eds.). FAO Fisheries Technical Paper 361, FAO, Rome.

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C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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ONGROWING AND BIOCHEMICAL COMPOSITION OF BLACKSPOT SEA BREAM (*PAGELLUS BOGARAVEO*) JUVENILES FED WITH DIFFERENT DRY FOOD

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Introduction

Blackspot sea bream, *Pagellus bogaraveo*, ranks as one of the potentially farmable species at commercial level. Over the past few years, considerable progress has been made in terms of reproduction, pre-fattening and ongrowing in tanks and cages (Fernández-Pato et al., 1990; Linares et al., 2001; Olmedo et al., 1997, 2000; Peleteiro et al., 1994, 1997). One of the problems with rearing, particularly at the ongrowing stage, is the amount of fat that builds up in individuals around the inner organs (perivisceral fat) and on the muscle and liver (Linares et al.,2000). This problem is directly linked to feed, so that the solution involves obtaining a suitable diet able to cover the nutritional requirements of Blackspot sea bream.

This work is a comparative study on growth and the biochemical composition of Blackspot sea bream juveniles, fed with four different types of dry feed as regards protein and fat content.

Materials and methods

This experiment conducted over a six month period, was carried out on the premises of the Luso-Hispana de Acuicultura Company in Valdoviño (A Coruña) with a batch of 1200 juveniles, with an initial average weight of 25.06±0.889g, from the Oceanographic Centre in Vigo. Fish were introduced into 8 circular tanks measuring $4m^3$, in groups of 150, with a density of $1kg.m^{-3}$. Each two groups were fed with a different feed. Feeds, supplied by Trouw, had the following composition in terms of percentage protein/fat content: D1

(54/16); D2 (50/24); D3 (42/14) and D4 (43/22). Likewise, the quality of the protein in the feeds was the same and better in feeds D1 and D2, in comparison with feeds D3 and D4, which had a lower quality protein content. Sampling of weight (g) and size (mm) was conducted on a monthly basis, and 3 individuals were extracted from each batch for dissection. Perivisceral fat weight was noted for percentage monitoring of fat in terms of body weight (PF = perivisceral fat weight.body weight^{-1} × 100) and the liver, to calculate the hepatosomatic index (HIS = liver weight.body weight^{-1} × 100). Samples were taken of muscle and liver to subsequent biochemical analysis. Analysis was also made of proteins (Bradford, 1976), total lipids by extraction with chloroform:methanol (2:1) (Blight and Dyer, 1959, modified by Fernández Reiriz et al., 1989) and gravimetric determination. Fatty acids prior to transesterification and metilation (Lepage and Roy, 1986) were analyzed by Gas Chromatography.

Comparison of the results from the groups fed with different feeds was conducted by variance analysis (P<0.05).

Results and Discussion

Figure 1 shows juvenile growth throughout the experience. The juveniles fed with feed D1, at the end of the experience, presented an average weight of 72g, which is significantly higher than that obtained in the juveniles fed with feeds D2, D3, and D4: 41.6, 40.6, and 46.1g, respectively. These results also indicate that the protein quality of the diet exerts an important influence on the growth of juvenile Blackspot sea bream when accompanied by adequate fat content. In the case of the batch fed with D2, despite this having the same protein quality as feed D1, the fat content is excessive, leading to poor growth results.

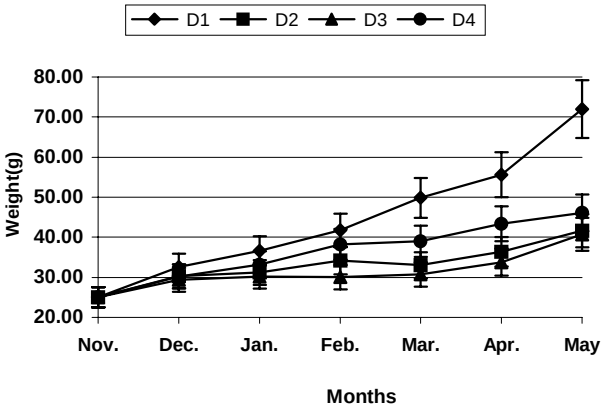

Fig. 1. Growth of juvenile Blackspot sea bream fed with different feeds.

As regards content in perivisceral fat, there appears to be no relationship between the fat content of the diet and the percentage of perivisceral fat found in the juveniles, which ranges from 2.2 to 4.5% of body weight. Nor are there significant differences between the hepatosomatic indices of the juveniles fed with the different diets. The HSIs range from 0.9 to 2%.

In terms of the biochemical composition of juveniles (Table I), the protein content of the liver in the batch fed with diet D1 generally shows slightly higher values than those obtained with the other diets, reaching 30.5% dry weight by the end of the experiments. In the muscle, protein ranged from 64-80%, no differences being noted between the juveniles fed with the different feeds.

Table I. Protein, lipid, and PUFA composition of in Blackspot sea bream juvenile liver.

Feed		Nov	Dec	Jan	Feb	Mar	Apr	May
D1	Proteins (%DW)	24.46	37.26	33.1	24.76	31.48	35.13	30.48
	Lipids (%DW)	55.98	33.65	45.44	37.96	40.97	41.26	43.06
	PUFA (ng.μg^{-1} DW)	23.92	53.45	92.55	63.8	61.45	55.1	61
D2	Proteins	24.46	32.46	32.55	28.32	25.36	29.28	24.15
	Lipids	55.98	37.37	38.08	45.39	50.69	51.49	53.62
	PUFA	23.92	74.05	78.3	100	111.6	88.65	98.3
D3	Proteins	24.46	34.6	32.77	32.53	27.01	27.98	27.22
	Lípids	55.98	42.55	31.49	46.52	55.41	52.62	46.74
	PUFA	23.92	53.8	61.1	71.85	96.3	102.9	77.5
D4	Proteins	24.46	30.51	33.62	23.58	28.17	24.57	30.84
	Lípids	55.98	53.33	44.99	59.66	51.69	57.13	54.57
	PUFA	23.92	68.95	122.2	108.1	91.15	117.2	80.95

The percentage of lipids in liver decreased at the end of the experience in terms of the initial value in the juveniles fed with feeds D1 and D3, from 56 to 43 and 47%, respectively. Conversely, in juveniles fed with more fatty feeds (D2 and D4), although there was an initial reduction in lipids as fish size increased, an increase was subsequently observed. Muscle in juveniles fed with D2 and D4 showed slightly higher lipid values than those fed with D1 and D3. As regards fatty acids, there is no clear relationship between the higher growth obtained with the D1 diet and the content in PUFAs in the liver. In all cases, an increase was noted relative to the initial value (24 ng.μg^{-1} dry weight), and throughout the experience this ranged between 53.5 and 122ng. μg^{-1} dry weight.

Conclusions

- Protein quality in the diet exerts a clear influence on the growth of Blackspot sea bream juveniles.
- A high fat content in the diet delays growth of Blackspot sea bream juveniles.

- There appears to be no relationship between perivisceral fat content and the weight of liver in juveniles with the fat content in the diet administered.
- Protein composition in the juveniles liver is slightly higher in those fed with a diet with a better balance in protein/fat content than in those fed with the other diets.

References

Blight E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Bradford M.M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Fernández-Pato C.A., C. Martínez-Tapia, O. Chereguini, I. García-Banda. 1990. Spawn,hatching,and larvae rearing of black-spot sea bream (*Pagellus bogaraveo* B.). First experiences in the Oceanographic Center of Santander (Spain) in order to its culture. Coun. Meet. ICES 1990/F:30

Fernández-Reiriz M.J., A. Perez-Camacho, M.J. Ferreiro, J. Blanco, M. Planas, M.J. Campos, and U. Labarta. 1989. Biomass production and variation in the biochemical profile (totalprotein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. Aquaculture 83:17-37.

Linares,F.,M.Olmedo,J.B.Peleteiro&A.Ortega.2000.Composición lipídica del besugo(Pagellus bogaraveo) procedente del medio natural y de cultivo intensivo. Resultados preliminares. V Congreso de Ciencias del Mar. Marcuba 2000

Linares F., M. Olmedo, A. Ortega, and J.B. Peleteiro. 2001. Experiencias de engorde de besugo, (*Pagellus bogaraveo* B.) en jaulas y tanques de cultivo. Monografías del Instituto Canario de Ciencias Marinas 4: 206-210.

Olmedo M., F. Linares, N. Ruiz, B. Álvarez-Blázquez, J.B. Peleteiro, A. Ortega, and J.L. Rodríguez. 1997. Preengorde de alevines de besugo (*Pagellus bogaraveo*, B.) nacidos en cautividad. Actas del VI Congreso Nacional de Acuicultura. Cartagena, Spain. pp.: 879-885.

Olmedo M., J.B. Peleteiro, F. Linares, B. Álvarez-Blázquez, C. Gómez, and A. Ortega. 2000. Experience with ongrowing juvenile blackspot seabream (*Pagellus bogaraveo* B.) born in captivity, in tanks at different temperatures. In: Recent advances in Mediterranean aquaculture finfish diversification. Vol. 47, Cah. Options Mediterr. pp.: 153-157.

Peleteiro J.B., M. Olmedo, R.M. Cal, and C. Gómez. 1994. Growth under laboratory conditions of wild juvenile black-spot sea bream (*Pagellus bogaraveo* B.). Aquacult. Eur. 18(4):6-10.

Peleteiro J.B., M. Olmedo, C. Gómez, and B. Álvarez-Blázquez. 1997. Study of reproduction in captivity of blackspot sea bream (*Pagellus bogaraveo* B.). Embryonic development and consumption of vitelline sac. Coun. Meet. ICES 1997/HH19.

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EMBRYOS AND EARLY LARVAE OF *CEPHALOPHOLIS BOENAK* (BLOCH, 1790) (SERRANIDAE)

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Introduction

Little is known of early life of the genus *Cephalopholis*. Two larvae of unidentified *Cephalopholis* species were described at 3.2mm and 5.8mm long (Leis and Rennis, 1983; Leis, 1986). Johnson and Keener (1984) examined three species, *C. cruentatus*, *C. fulvus*, and *C. panamensis* within 5.2–26.5mm standard length. Neither eggs nor early larvae have been described in the genus *Cephalopholis*.

The Chocolate hind, *C. boenak* was not considered of much commercial importance in the past, but it has become one of the more important species in Hong Kong's fish market in recent years, since large groupers have declined. The aim of this study is to provide embryonic and early larval morphology of *C. boenak* from fertilization to day 5 after hatching.

Materials and methods

This study was carried out at the Swire Institute of Marine Science (SWIMS), HK Island. Six adults *C. boenak* were kept in an indoor tank and spawned naturally. Floating eggs were collected and reared as Hussain and Higuchi (1980). The artificial diet (80–200μm) (INVE, Belgium) was fed from early day 3. Morphology and measurements of embryos and larvae were observed and taken under light microscope. The volumes of the yolk and the oil globule were calculated using the volume formulae appropriate for the shapes (Johns and Howell, 1980). Notochord length (NL) of larvae is from the tip of upper jaw to the tip of notochord.

Results

C. boenak spawned around 22:00-01:00 from August to September, 2000 for 35 consecutive days and did not relate to moon phase. The eggs are transparent and pelagic throughout the embryonic developmental period and presented in Fig. 1.

The mean diameter of egg is 724μm, and the volumes of the yolk and the oil globule were $1669\times10^{-4}mm^3$ and $19\times10^{-4}mm^3$, respectively.

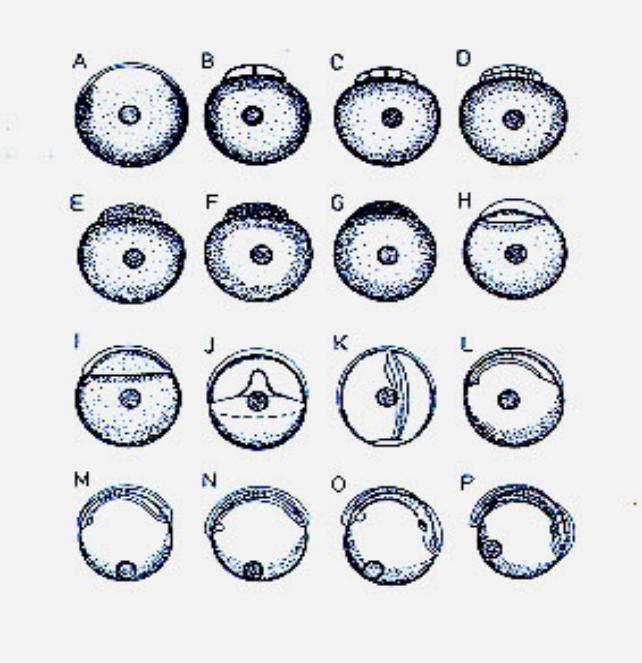

Fig. 1. Embryonic development of *C. boenak*. (A) Fertilised egg, 20min; (B) 2-celled stage, 30min; (C) 8-celled stage, 1 hour and 10min; (D) 32-celled stage, 1h and 50min; (E) 64-celled, 2 hours and 20min; (F) high blastula stage, 3 hours; (G) flat blastula stage, 4 hours; (H) early epiboly stage, 5 hours; (I) 30% epiboly stage, 6 hours; (J) 55% epiboly stage, 7 hours and 30min; (K) 95% epiboly stage, 9 hours; (L) 2 somite stage, 10 hours and 50min; (M) 5 somite stage, 11 hours and 50min; (N) 10 somite stage, 13 hours and 40min; (O) 17 somite stage, 15 hours and 40min; (P) 20 somite stage, 16 hours and 40min.

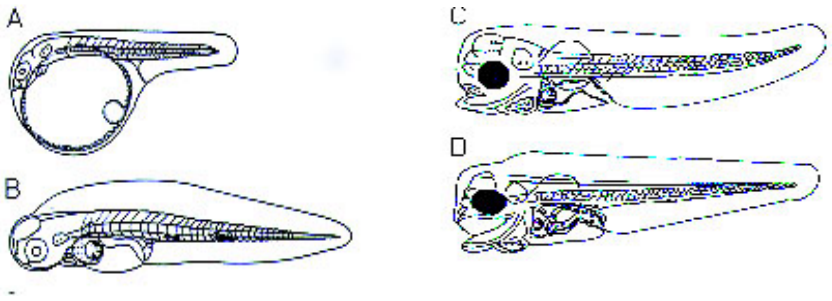

Fig. 2. Early larvae of *C. boenak*. (A) Newly hatched larva, 1.36mm NL; (B) Day 2 larva, 2.04mm NL; (C) Day 3 larva, 1.98mm NL; (D) Day 4 larva, 2.00mm NL.

From fertilization to hatching takes 18-20 hours at 24-27°C. Age of the larva is given in days after hatching. The study ended on day 5 because of heavy mortality from day 3 onwards (Fig. 2). The somite formula of newly hatched larvae is 10+14=24. Two brown dendritic pigments develop on the ventral side of the trunk at the late of day 1. One brown dendritic pigment area appears on the oil globule at day 2. At day 3, the mouth opens (51-56 hours after hatching), the pigments on the ventral side of the trunk disappear and one line of small internal melanophores develops along the ventral portion of the notochord behind the alimentary canal. At Day 4, the yolk is consumed completely. The gut and the stomach contain particles of artificial diet. At Day 5, the oil globule absorbed completely (Fig. 3).

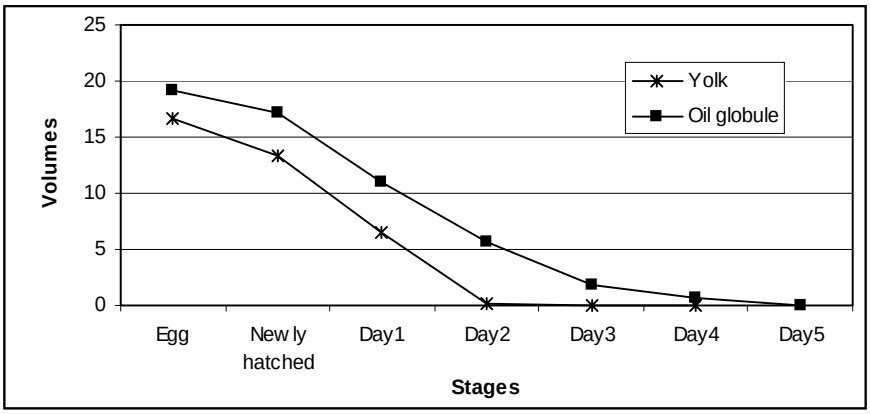

Fig. 3. Consumption of the yolk and the oil globule. The yolk volume is $10^{-2}mm^3$. The oil globule volume is $10^{-4}mm^3$.

Discussion

This is the first description of embryonic and early larval morphological development in *C. boenak*. Small egg size (less than 1000μm) and short hatching time (less than 35 hours) are common in the subfamily Epinephelinae (Ukawa et al., 1966; Hussain and Higuchi, 1980; Powell and Tucker, 1992; Colin et al., 1996). Heavy mortality occurred from Days 3 onward. At Day 3, although the mouth has opened, the upper and the lower jaws are immovable and the stomachs are empty in most of examined larvae. Larvae do not growth during the transition from endogenous nutrition to exogenous feeding (Day 3 to Day 5). This phenomenon was reported in other groupers.

Pigment patterns of early larvae of *C. boenak* differ from those of similar stages of *Epinephelus* larvae. One line of small internal melanophores appears along the ventral portion of the notochord behind the alimentary canal in *C. boenak*. The melanophores on the dorsal part of the gut do not appear until day 5 in *C. boenak*, which appear on day 3 in the genus *Epinephelus*. No small, faint, dendritic melanophores are scattered on the snout in *C. boenak*, whereas they appear on day 1 in the genus *Epinephelus* (Ukawa et al., 1966; Chen et al., 1977; Hussain and Higuchi, 1980; Powell and Tucker, 1992; Colin et al., 1996).

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References

Colin P.L., C.C. Koenig, and W.A. Laroche. 1996. Development from egg to juvenile of the red grouper (*Epinephelus morio*) (Pisces: Serranidae) in the laboratory. p.399-414. In: Biology, fisheries and culture of tropical groupers and snappers. F. Arreguin-Sanchez, J.L. Munro, M.C. Balgos, and D. Pauly (Eds.) ICLARM Conf. Prod., 48, 449p.

Hussain N.A. and M. Higuchi. 1980. Larval rearing and development of the brown spotted grouper, *Epinephelus tauvina* (Forskal). Aquaculture 19:339-350.

Johns D.M. and W.H. Howell. 1980. Yolk utilization in summer flounder (*Paralichthys dentatus*) embryos and larvae reared at two temperatures. Mar. Ecol. Prog. Ser. 2:1-8.

Johnson G.D. and P. Keener. 1984. Aid to identification of American grouper larvae. Bull. Mar. Sci. 34(1):106-134.

Leis J.M. and D.S. Rennis. 1983. The larvae of Indo-Pacific coral reef fishes. New South Wales University Press, Sydney and University of Hawaii Press, Honolulu, 269p.

Leis J.M. 1986. Larval development in four species of Indo-Pacific coral trout *Plectropomus* (Pisces: Serranidae: Epinephelinae) with an analysis of the relationships of the genus. Bull. Mar. Sci. 38(3):525-552.

Powell A.B. and J.W. Tucker, Jr. 1992. Egg and larval development of laboratory-reared Nassau grouper, *Epinephelus striatus* (Pisces, Serranidae). Bull. Mar. Sci. 50(1):171-185.

Ukawa M., M. Higuchi, and S. Mito. 1966. Spawning habits and early life history of a serranid fish, *Epinephelus akaara* (Temminck & Schlegel). Jap. J. Ichthyol. 13(4/6):156-161. (In Japanese with English abstract.)

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ONTOGENY OF α-AMYLASE GENE IN SEABASS (*LATES CALCARIFER*) LARVAE AND THE EFFECTS OF T_3 AND CORTISOL ON GENE EXPRESSION

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Introduction

Several studies have shown that marine fish larvae produce significant amounts of α-amylase enzyme around the time of first feeding; the α-amylase enzyme activity was measured in crude extracts using a biochemical method which is based on the ability of amylase to hydrolyze starch. However, these approaches might not provide specific results, since the measured amylase activity may also reflect changes in other carbohydrate metabolizing enzymes as well as amylase activity present in the ingested prey. Therefore, it would be more reliable to correlate the enzyme activity and its gene expression. The aim of the present study was to characterize the α-amylase gene in seabass, quantify the ontogenic changes in the gene expression during early development, and study the effects of exogenous hormone treatment (T_3 and cortisol) on the gene expression using the real-time RT-PCR method.

Materials and methods

The mRNA levels of α-amylase in developing seabass (*Lates calcarifer*) larvae were studied from hatching until 27 days post-hatch (dph). The changes in the mRNA levels were quantified using a partial α-amylase cDNA sequence and real-time RT-PCR technique. The full length of amylase cDNA sequence was generated using a 5' and 3' RACE kit. The larvae were treated by immersion in T_3 (5nM) or cortisol (100nM) and subsequently, the levels of α-amylase mRNA in the larval samples were analyzed by real-time RT-PCR method.

Results and discussion

The complete cDNA for seabass amylase is 1620bp and the deduced polypeptide is 522 amino acids. The amino acid sequence of seabass amylase shows very high sequence identity to pancreatic amylase and pancreatic amylase precursor followed by salivary amylase of other species. The mRNA transcripts of amylase were first detected in newly hatched larvae and the copy numbers increased from 2 days post hatching (dph), reaching the highest level on 5dph. There was a gradual decrease thereafter. The results indicate that the ontogeny of α-amylase is regulated at the transcriptional level and the high levels of mRNA during early days of larval development suggest a natural predisposition of these larvae to use carbohydrate as a nutritional substrate. Treatment of larvae with T_3 (5nM) and cortisol (100nM) significantly increased the α-amylase mRNA levels, suggesting that endocrine factors may regulate α-amylase at the transcriptional level.

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PRELIMINARY RESULTS OF LARVAL AND FRY ASP (*ASPIUS ASPIUS* L.) REARING IN THE ILLUMINATED CAGES

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Introduction

Aspius aspius L. is a cyprinid piscivorous fish. During last half of the 20th century, asp catches in Poland have steadily decreased, and in several parts of the country, fish are becoming endangered. Recent studies (Babiak et al., 1998; Kujawa et al., 1997; 1998), focused on artificial reproduction and asp rearing enabled to mass fish production for restoration programs and to introduce the asp into aquaculture as new promising object. The purpose of this study was to investigate the growth, mortality and feeding conditions during asp rearing in the illuminated cages.

Materials and methods

The rearing experiment was carried out in Lake Maroz (Northern Poland). Artificial reproduction and incubation of asp eggs were conducted at Experimental Hatchery in Department of Lake & River Fisheries at Olsztyn. Two weeks after hatching asp prefed larvae were counted and stocked into two illuminated cages (illumination 24V, 60W; size 0.5×0.6×1.1m; 0.6-mm mesh) in Lake Maroz. Initial density was 2500 larvae per cage, i.e., 10 larvae.dm^{-3}. Initial size of stocked larvae is presented in Table I. After 2 weeks they were transferred to larger cages (2.0×1.5×2.0m; 1.0-mm mesh), and after following three weeks, into cages of the same volume but with 1.0×2.0-mm mesh size. Initially asps were fed only zooplankton attracted by illumination of cages. From the day 36, fish were fed with supplemental granulated feeds Biomar and Dana Feed. Duration of whole rearing was 95 days. During the experiment, water temperature raised from 15-22°C. A sample of 20 fish was removed at night from the cages every seven days. Zooplankton samples were taken at this same time. Preserved fish were individually measured for total length (TL±0.1mm) and the whole body was wet weighed (±1mg). The alimentary tracks of all sampled fish were dissected and their contents were examined to determine species composition and number of prey items in the diet. The Strauss selectivity

index was used to determine feeding selectivity of asp in relation to main feeding groups.

Table I. Effects of rearing of *Aspius aspius* L. in illuminated cages. The values are the average (± one standard deviation of two replicates).

	Beginning of rearing	End of rearing
Date	May 2	Aug 1
Mean body length (mm)	8.1 (0.0)	63.5 (0.7)
Mean body weight (mm)	3.1 (0.1)	2891.5 (34.6)
Mean number of specimens	2500 (0.0)	1437.7 (99.7)
Survival (%)		57.5 (4.0)

Results and discussion

Similarly as in previous rearing experiment (Kujawa et al., 1998) the zooplankton in Lake Maroz was dominated by cladocerans and copepods (Fig. 1A). Initial high abundance of rotifers decreased after 15 days of rearing. Asps preferred rotifers (positive value of selectivity – Fig. 1B) only during initial period of rearing, when their length was not higher then 11mm. At the same period both Cladocera and Copepoda were intensively selected from the zooplankton. Fish from length range between 11 and 34mm selected also both crustacean groups, dominated in zooplankton structure. Asps larger than 34mm in length more often selected copepods than crustaceans. In fish reaching 40-55mm in length the selectivity index values increased to maximum level over 80. The situation observed confirms earlier observations on asp feeding activity in illuminated cages (Kujawa et al. 1998). From the day 50, asp fry also ate the additional food, represented by flying insects and cyprinid fish larvae. Reared fish accepted also very good artificial feeds given into cages. The survival of asps during whole experiment was relatively high (over 50%) (Table I). The growth rate in body weight of fish was very fast, especially during second half of rearing (Fig. 2). Relatively slow growth rate during initial weeks was caused by lower water temperature (about 15°C). Artificial breeding of asp has been yet developed in Czech Republic (Vostradovsky and Vasa, 1981), and in Iran (Hoseini, 1995). In Poland, the first successful attempts in induced spawning of these fish, originated from pond culture were carried out in 1992. Our studies indicate the possibility to artificial propagation of *Aspius aspius*, and rearing of prefed larvae in lake conditions with the use illuminated cages.

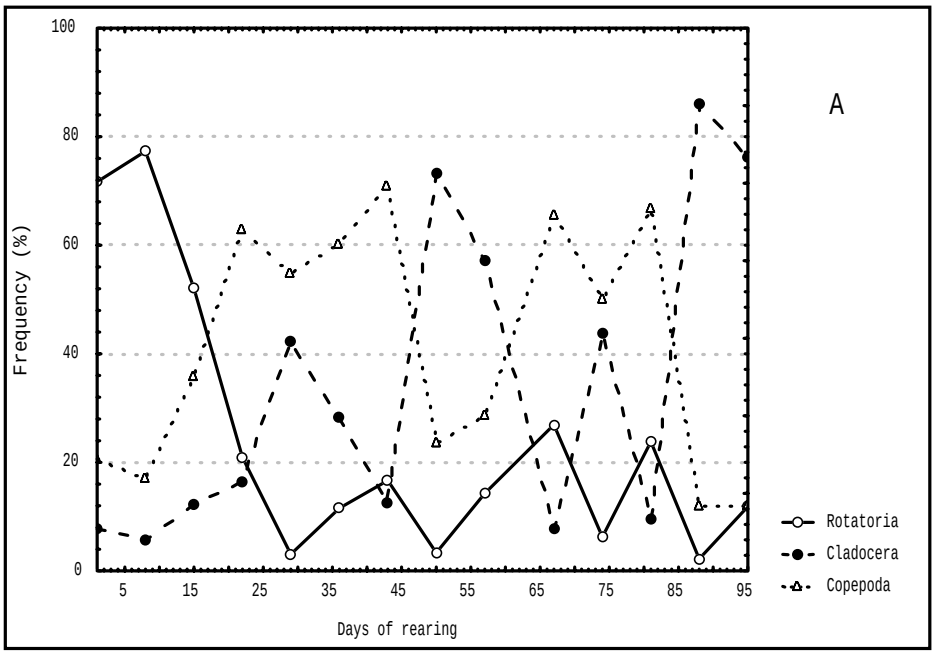

Fig. 1. Frequency of zooplankton in lake (A) and Strauss selectivity index of asp (B) during rearing in the illuminated cages.

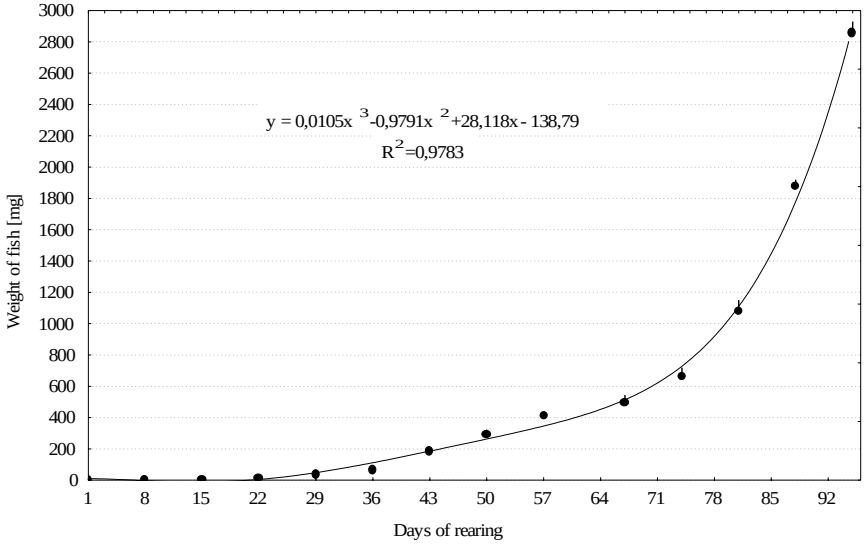

Fig. 2. Growth rate (weight) of asp (*Aspius aspius*) during rearing in illuminated cages.

Conclusions

Preliminary results show that rearing of asps in illuminated cages give the possibility to produce in mass scale good stocking material (larvae or fry) for restoration programs, angling, and aquaculture. Further studies should include determination of optimal densities, behavioral, and feeding aspects of fish growth, and control of possible natural parasitic invasions.

References

Babiak I., J. Glogowski, R. Kujawa, D. Kucharczyk, and A. Mamcarz. 1998. Cryopreservation of sperm from asp *Aspius aspius.* Prog. Fish-Cult. 60:146-148.

Hoseini S.E. 1995. Artificial propagation of *Aspius aspius*. Iran. Fish. Sci. J. 4:4.

Kujawa R., A. Mamcarz, and D. Kucharczyk. 1997. Effect of temperature on embryonic development of asp *Aspius aspius*. Pol. Arch. Hydrobiol. 44:139-143.

Kujawa R., A. Mamcarz, A. Skrzypczak, and D. Kucharczyk. 1998. Feeding habits of asp *Aspius aspius* larvae (Cyprinidae) in Lake Maroz, Poland. Ital. J. Zool. 65:235-236.

Vostradovsky J. and J. Vasa. 1981. Asp (*Aspius aspius* L.) – new object of artificial breeding. Bull. VURH Vodnany 3:10-13 (In Czechoslovakian).

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CURRENT CONDITION OF WHITEFISH (COREGONIDAE) ARTIFICIAL REPRODUCTION IN RUSSIA

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Abstract

The main tendencies of whitefish artificial reproduction in Russia for the last 20 years are observed. At the beginning of 1980 in Russia, there were 24 specialized whitefish farms with production potential of about 8 billions eggs per year. The average annual production volume was 2.8 billions larvae, and the major species produced was the peled (*Coregonus peled*) (54%). In connection with economic reforms in the 1990s, quantity of larvae was reduced down to 2.4 billions per year. Quality changes also occurred with whitefish artificial reproduction. At the expense of Baikal fish-breeding farms, the successful proportion of omul (*Coregonus autumnalis migratorius*) of the total whitefish larval production has increased up to 81%. The production volume of all remaining species was considerably reduced. Nowadays, there are potential technical and economic possibilities to increase whitefish larval production.

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LARVICULTURE OF MARINE SPECIES IN SOUTHEAST ASIA: CURRENT RESEARCH AND INDUSTRY STATUS

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Introduction

The increased requirement for foodfish, the lucrative market for expensive seafood, and the need to conserve marine resources have motivated the rapid pace of larviculture research in Southeast Asia, where various research and academic institutions are carrying out research on commercially important marine species including ten fish, six crustacean, and seven mollusc species. Since fry availability is a major constraint in the development of culture systems, a major research thrust of the SEAFDEC Aquaculture Department is the development of commercially viable technologies for breeding and seed production of important marine species.

Larviculture of marine fish

The intensive larviculture techniques developed for well-studied species such as milkfish and sea bass are currently being applied and modified to suit the requirements of technically demanding species such as groupers, snappers, and rabbit fish. Marine fish larvae are reared in "green water" at densities of 20-50 larvae.l^{-1} and fed suitable sized rotifers at first feeding. Older larvae are fed with *Artemia* nauplii and increasing sizes of *Artemia* until larvae metamorphose to the juvenile phase, when they are fed on minced trash fish (Duray et al., 1996; 1997). Enrichment of rotifers and *Artemia* with supplemental diets containing high levels of HUFA is now a routine hatchery procedure, following studies that demonstrated increased fry survival or enhanced resistance to stress of fry fed HUFA-enriched live food (Gapasin et al., 1998; Gapasin and Duray, 2000). To improve larval survival in hatcheries and reduce production cost, particularly for species that requires extended rearing such as groupers and snappers, current thrusts include screening of candidate zooplankton as alternative live food to replace or supplement the nutritionally deficient rotifers and *Artemia*. The copepods *Acartia tsuensis*, *Pseudodiaptomus* sp., and *Oithona* sp. have been shown to be nutritionally superior to rotifers and *Artemia* and are the preferred live food of fish larvae (Toledo et al., 1997; 1999). Although experimental

production of these copepods has been achieved, problems in mass production of these zooplankton still have to be resolved. Artificial diets to replace *Artemia* have been developed for milkfish and sea bass, while diets for grouper and snapper are currently being tested. A diet supplemented with exogenous enzymes has also been developed for first feeding milkfish larvae.

Survival rates of fry such as groupers and snappers that require extended hatchery rearing are very low due in part to the long duration of metamorphosis and cannibalistic behavior of fry. Metamorphosis in grouper larvae, which occurs after about 45-60 days, can be accelerated through the application of thyroid hormones either by immersion or bioencapsulation in *Artemia*. Four-week-old larvae acquire the features of young juveniles within three days of hormone application (de Jesus et al., 1998). This method has been verified in large-scale production of grouper fry.

Seed production of mud crab

The mud crab is considered a delicacy in Southeast Asia and fish farmers in the region have traditionally practiced the extensive culture of various species. Lack of seeds has hindered the growth of the mud crab industry, hence mud crab breeding and seed production research is being actively undertaken in the region to promote the future expansion of the industry. Wild or pond-reared female *Scylla serrata* fed a formulated diet together with trash fish readily spawn in captivity (Millamena and Quinitio, 1999). Hatchery rearing protocols modified from methods developed for shrimp have been successfully applied to mud crab larviculture (Quinitio et al., 1999). The low and often inconsistent larval survival to the megalopa stage caused by luminous bacterial infection is still a problem, although survival rates have improved with the application of probiotics. Rearing of megalopae to juvenile crabs in tanks pose a problem due to their cannibalistic behavior. A method for further rearing of megalopae to crablets in net cages provided with shelters and set in brackish water ponds, however, has improved survival and enhanced growth rates of crablets (Rodriguez et al., in press).

Other species

A new program thrust of the Aquaculture Department is the restocking of overexploited and endangered species such as the sea horse (*Hippocampus* sp.), windowpane oyster (*Placuna placenta*), and the tropical abalone (*Haliotis asinina*). Although research on these species is at an early stage, there have been encouraging developments in the breeding and larviculture of these species. Several generations of two species of sea horses have been produced in the laboratory and feeding and management schemes have been developed for juveniles and broodstock. Problems on behavioral requirements for mating among hatchery-produced sea horses, however, need to be addressed.

Although the tropical abalone produces large quantities of fertilized eggs, larval settlement is very low and work is currently focused on a search for inducer substances and development of rearing techniques to enhance settlement of competent larvae. Formulated diets developed for broodstock have been shown to improve fecundity and viability of larvae. Further work to improve breeding and seed production will be needed to support future stock enhancement and sea ranching programs for these species.

References

de Jesus E.G.T., J.D. Toledo, and M.S. Simpas. 1998. Thyroid hormones promote early metamorphosis in grouper (*Epinephelus coiodes*) larvae. Gen. Comp. Endocrinol. 112:10-16.

Duray M.N., L.G.Alpasan, and C.B. Estudillo. 1996. Improved hatchery rearing of mangrove red snapper, in large tanks with small rotifer (*Brachionus plicatilis*) and *Artemia*. Isr. J. Aquacult-Bamidgeh. 48:123-132.

Duray M.N., C.B. Estudillo, and L.G. Alpasan. 1997. Larval rearing of the grouper *Epinephelus suillus* under laboratory conditions. Aquaculture 150:63-76.

Gapasin R.S.J., R. Bombeo, P. Lavens, P. Sorgeloos, and H.J. Nelis. 1998. Enrichment of live food with essential fatty acids and vitamin C: effects on milkfish (*Chanos chanos*) larval performance. Aquaculture 162:269-286.

Gapasin R.S.J. and M.N. Duray. 2000. Effect of DHA-enriched live food on growth, survival and incidence of opercular deformities in milkfish *Chanos chanos*. Aquaculture 193:49-63.

Millamena O.M. and E.T. Quinitio. 1999. Reproductive performance of pond-sourced *Scylla serrata* fed various broodstock diets. p.114-117. In: Mud Crab Aquaculture and Biology. C.P. Keenan and Blackshaw (Eds.). Proceedings of an International Scientific Forum, Darwin, Australia; 21-24 April 1997, ACIAR Proceedings No. 78, Canberra.

Quinitio E.T., F. Parado-Estepa, and V. Alava. 1999. Development of hatchery techniques for the mud crab *Scylla serrata* (Forskal): Comparison of feeding Scheme. p.125-130. In: Mud Crab Aquaculture and Biology. C.P. Keenan and Blackshaw (Eds.). Proceedings of an International Scientific Forum, Darwin, Australia; 21-24 April 1997, ACIAR Proceedings No. 78, Canberra.

Rodriguez E., E.T. Quinitio, F.D. Parado-Estepa, and O. Millamena. 2001. Culture of *Scylla serrata* megalopae in brackishwater ponds. Asian Fish. Sci. (in press)

Toledo J.D., S.N. Golez, M. Doi, and A. Ohno. 1997. Food selection of early grouper, *Epinephelus coiodes*, larvae reared by the semi-intensive method. Suisanzoshoku. 45:327-337.

Toledo J.D., S.N. Golez, M. Doi, and A. Ohno. 1999. Use of copepod nauplii during early feeding stage of grouper *Epinephelus coiodes*. Fish. Sci. 65:390-397.

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ECOLOGY OF *VIBRIO* AND *AEROMONAS* DURING THE LARVAL DEVELOPMENT OF THE SPOTTED SAND BASS *PARALABRAX MACULATOFASCIATUS*

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Introduction

Intensive rearing of the spotted sand bass is now an developed process in México (Alvarez-González, 1999), however, the first 15 days after hatch are still the most critical period of rearing, and high mortalities occur (Martinez-Díaz et al., 2001). Until now there not exist evidence of the causes of this mortalities, however, it is probable that bacterial pathogens could be implicated during the first feeding of larvae, since improvements in the sanitary controls of the production have been increased the survival rates of larvae. In this study, the changes of the microflora during the intensive rearing of the spotted sand bass and the ability of selected bacteria to produce mortalities were evaluated.

Materials and methods

Samples of water, feeds (rotifers, copepods, *Artemia*), and larvae were collected during the first 25 days of rearing of spotted sand at CICIMAR IPN México. The samples were analyzed using the standard microbiological procedures and the gastrointestinal colonization of larvae was evaluated using the procedure described by Muroga et al. (1987). Each sample was macerated in a tissue homogenizer under sterile conditions, serial tenfold dilutions of the homogenate were prepared in saline solution (2.5% NaCl), and they were plated by triplicate on Marine Agar (MA), Thiosulfate-citrate-bilis salt-sucrose agar (TCBS; Difco) and MaConkey media. After incubation for 24-48 h at 30°C, representatives of each different colony grown were presumptively identified using standard biochemical tests and by the GN2 plates of BIOLOG®.

Five different bacterial strains isolated from the larval microbiota were used in the infection experiments, *Aeromonas media* C226, *Vibrio* sp. C228, *A. ichtiosmia*

C302, *V. carchariae* C303, and *V. alginolyticus* C390. Also, two controls were included *V. alginolyticus* (C7) isolated during a *Vibrio* outbreak (Martínez-Díaz, 1995) and *V. harveyi* ATCC14126. Juvenile spotted sand bass (48.7g average weight) reared in laboratory, were held in 450-l tanks at 24°C. The water in the tanks was continuously aerated and re-circulated via biofilter. Daily, 2000% of the total water volume was replaced by fresh aerated seawater (SW). Once daily, fish were fed *ad libidum* with formula at 45% protein, according to Alvarez-González (2001). Groups of fifteen fish were intraperitoneally injected for each strain with 0.1ml of bacterial suspension at 10^6 viable bacterial CFU.ml^{-1} in phosphate-buffered saline solution (PBS), 0.85% NaCl, pH 7.5 with 24-h cultures in MA. The concentration in each suspension was photometrically adjusted and standard plate counts were made to determine the inoculated dose. Controls were fifteen fish injected with 0.1ml of sterile PBS. The signs were recorded for 20 days. Moribund fish were bacteriologically analyzed in order to complete the Koch postulates. Also, the effect of the bacteria in larvae was analyzed under monoxenic conditions by a modification of the method of Munro et al. (1995). Bacterium free cultures of rotifer were obtained by removal of eggs from adult rotifers and hatched in sterile seawater at 2ml.l^{-1} of trimethoprim-sulfamethoxasole (Bactrim; Roche) and rinsed in sterile SW during 24 hours. Bacteriological sterile check was made according to Munro et al., (1995); also rotifers were treated with acridine orange (Difco) and viewed under fluorescence to confirm the absence of bacteria. To eliminate the cultivable bacteria from the larvae, fertilized eggs were suspended in a 2% hydrogen peroxide in seawater for 3min. Disinfected eggs were rinsed in sterile SW and allowed to hatch in 250-ml translucent flasks (at 80 eggs per flask in 100ml of sterile seawater). The absence of cultivable bacteria was confirmed as described for rotifers. Overnight cultures of bacteria in MA were resuspended in sterile SW at approximately 1.0×10^9CFU.ml^{-1} (optical density at 640nm, 1.0). Gnotobiotic rotifers were added to the larvae from day 3 post-hatch to give 5 rotifers.ml^{-1}. Bacteria were added at larvae flasks to give a final cell density of 1.0×10^6CFU.ml^{-1} on the first day of feeding. The density of rotifers was maintained at 2.0-3.0 rotifers.ml^{-1} thereafter by the daily addition of axenic rotifers. The mortality was recorded at 48 hours after bacterial inoculation. Five replicates were used by treatment and the experiment was repeated once.

Results and discussion

During the trials the number of bacteria in the water of the rearing units increase progressively, reaching a maximum of 1.5×10^5CFU.ml^{-1} at 15 days after hatch. The presumptive Vibrionaceae is a numerically important component of the cultivable bacterial population (Fig. 1). Further their number was closely related to the total heterotrophic population counted in MA. Members of Vibrionaceae that were isolated from larvae and feeds were identified as *V. alginolyticus, V. campbelli, V. carchariae, V. mediterranei, V. metschnikovii, V. parahaemolyticus, V. proteolyticus, A. allosacharophila, A. hydrophila, A.*

media and *A. veronii*. The numbers of heterotrophic bacteria in the microalgae were higher than found in the rearing units (10^6UFC.ml^{-1}); however, *Vibrio* did not occurs as normal microflora. In rotifers the number of bacteria shown changes between days, and most bacteria from the rotifer microbiota were identified as *Vibrio* which maintain their proportion respect to the total bacterial population. In copepods and in nauplii *Artemia* the microbiota was found composed mainly of non-*Vibrio* bacteria. In larvae it was found a marked variability in the number of bacteria. During the first 3 days the number of bacteria begins to increase and it is apparent that *Vibrio* it is the dominant. This pattern was maintained during the first 8 days after hatch; then the vibrios was displaced by other non-*Vibrio* heterotrophic bacteria. *Aeromonas* occur at inferior levels than *Vibrio*.

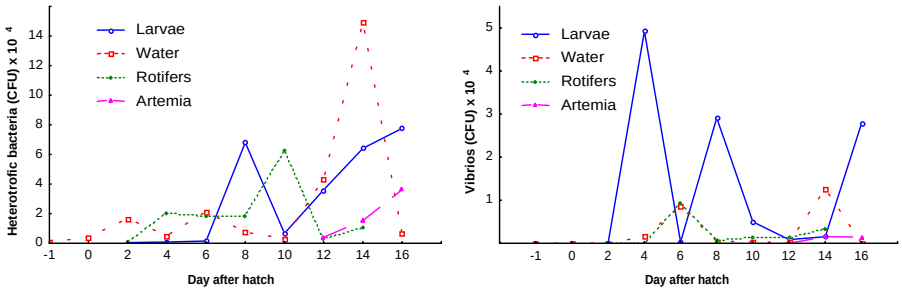

Fig. 1. Abundance of bacteria in different sources during the rearing of the spotted sand bass. A total heterotrophic bacteria and B presumptive *Vibrios*.

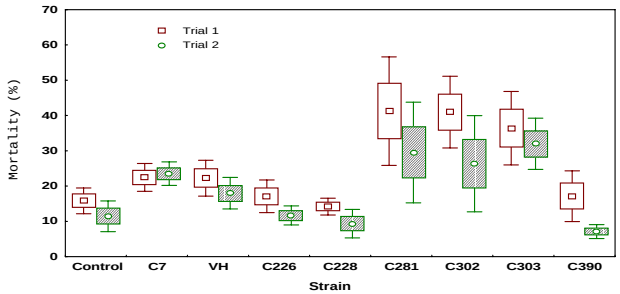

Fig. 2. Mortalities during challenge of spotted sand bass larvae with selected members of their microflora (see text for details).

In the trial with juvenile fish, mortalities were only recorded in the C303 treatment, reaching 100% within 24 hours after injection. Death fish shown anal hemorrhages and extrusion, abdominal inflammation, hemorrhages in the kidney and the intestine was seen full of a transparent fluid. In the larvae challenge no significant mortalities occurred in the C226, C228, and C390 treatments (P>0.05). Variable results were obtained in the C281 and C302 treatments (Fig. 2): during the first trial both strains produces a significant higher mortality than

the controls (P<0.01), however, during the second trial, the average mortality was higher than controls, but no significant differences were found (P=0.06). Only the strain C303 produces significant mortalities of larvae during both trials.

Conclusions

Our results support that different species of *Vibrio* and *Aeromonas* are directly transferred from the live feeds to the larval microflora, where selectively colonize the surfaces and the gut of the larvae. During the first 25 days in the life of the cabrilla were identified two stages in the colonization of the gut, in the first, most bacteria are transient in the digestive tract, and a second stage where some bacteria such as *V. alginolyticus* dominate consistently the microflora and could be considered a stage of transition to juvenile. Apparently the presence of *V. charchariae* should be considered a risk for the rearing of the cabrilla due to their demonstrated pathogenicity for both the larval and juvenile stages.

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References

Alvarez-González C.A., R. Civera-Cerecedo, J.L. Ortíz-Galindo, S. Dumas, M. Moreno-Legorreta, and T. Grayev-Del Alamo. 2001. Effect of dietary protein level on growth and body composition of juvenile spotted sand bass, fed practical diets. Aquaculture 194:151-159.

Alvarez-González C.A 1999. Optimización del cultivo de la cabrilla arenera *Paralabrax maculatofasciatus*. Master´s thesis. CICIMAR IPN. Mexico.

Martínez-Díaz S.F. 1995. Estudio de una enfermedad hemorragico ulcerativa en un lote de reproductores de cabrilla arenera *P. maculatofasciatus*. Master´s. thesis. CICIMAR-IPN. La Paz, México. 108p.

Martínez-Díaz S.F., R. Martínez-Pecero, M.O. Rosales, R. Alvarado-Castillo, H. Pérez-España, and J. Tucker Jr. 2001. Spawning, Early Development and Completion of the Life Cycle of Spotted Sand Bass in the Laboratory. J. World Aquacult. Soc. 32:122-129.

Munro P.D., A. Barbour, and T. Birkbeck. 1995. Comparison of the growth and survival of larval turbot in the presence of *Vibrio anguillarum*, *Vibrio alginolyticus* or a marine Aeromonas. Appl. Envir. Microbiol. 61:4425-4428.

Muroga K., M. Higashi, and H. Keitoku. 1987. The isolation of intestinal microflora of farmed red seabream (*Pagrus major*) and black sea bream (*Acanthopagrus schlegeli*) at larval and juvenile stages. Aquaculture 65:79-88.

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ELIMINATION OF ASSOCIATED MICROBIAL COMMUNITY AND BIOENCAPSULATION OF BACTERIA IN THE ROTIFER *BRACHIONUS PLICATILIS*

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Introduction

In the mass culture of rotifers, a complex bacterial ecosystem exists, and the rotifers are considered a route of bacterial infection to the larvae (Verdonck et al., 1994). The occurrence of bacterial contamination in the live foods, particularly of Vibrionaceae, has been associated with mass mortalities of larvae, however, little is known about the bacteria-larvae relationship during the initial rearing stages. Since rotifers can serve as biocapsules to study the effect of specific bacteria in larvae by oral inoculation, it was our aim to obtain a method to produce bacteria-free cultures of rotifers and evaluate the bioencapsulation of potentially pathogenic bacteria in the rotifer *Brachionus plicatilis*.

Materials and methods

Samples of *B. plicatilis* were obtained from the UPIMA-experimental hatchery at CICIMAR-IPN, México. The cultures were maintained under amictic reproduction as described Rueda-Jasso (1996). The rotifer-associated bacteria were evaluated during the maintenance conditions (19-l carboys) and during the mass production (300-l). Weekly, samples of rotifers were collected in a 35-μm mesh and washed for 10min in running autoclaved seawater (ASW). 100 rotifers were homogenized in 5ml of ASW using a tissue homogenizer under sterile conditions. Tenfold serial dilutions of the homogenate were inoculated in triplicate on plates of Marine Agar and Thiosulfate-citrate-bilis salt-sucrose agar (TCBS; Difco) and incubated at 30°C during 24h. Using the plates with ca. 300 colony forming units (CFU), the numerically most abundant morphotypes were picked off and purified in plates of triptic-casein-soy agar TSA (Difco). The isolates were identified at the genus level using the keys of Muroga et al. (1987).

Two disinfectants and two antibiotic mixtures were tested in order to obtain bacteria-free rotifers. The evaluation was done using adult rotifers and removing amictic eggs. The rotifers or eggs were washed with ASW and dispensed in individual 35-µm sterile meshes at a rate of 3000 individuals.mesh^{-1}. In the mesh, the rotifers or eggs were treated with PVP-Iodine (ISP Technologies) at 0, 0.1, 1, 2, 3, 4, 5, 8, 10, and 15mg·ml^{-1}, hydrogen peroxide (Perhydrol Sigma) at 0.5, 3, 5, and 7%, PEC (Penicillin 100mg + Streptomycin 50mg + Chloramphenicol 10mg in 10ml of ASW) at 0,20,40,60,180,200, 300, and 500µl per tube of 10ml of ASW, or TmSx (trimethoprim-sulfamethoxasole simple Bactrim®-Roche) at 0, 20, 40, 60, 80, 100, 200, and 500µl per tube of 10ml of ASW. Each concentration of disinfectant or antibiotic was assayed in triplicate. Controls were rotifers without disinfectant or antibiotic. Motility and survival were evaluated under 40× magnification. The bacteria after each treatment were evaluated using Brain-Heart Infusion Media (BHI Difco) at 1, 3, 5, 10, 30, and 60min for the disinfectant treatments and 24 and 48h for the antibiotic treatments. At each time, a sample of rotifers was obtained and washed twice for 30 min in ASW. 100 rotifers were homogenized as previously described. Six tubes with 5ml of BHI were inoculated with 1ml of homogenate. 1cm of sterile petrolatum was added to three of the inoculated tubes in order to get anaerobic conditions. The tubes were incubated during 48h at 30°C and the bacterial growth was recorded as the increase in turbidity at 640nm in an spectrophotometer Merck (SQ118).

Gnotobiotic rotifers (from 200µl of TmSx·24h as previously described) were added in triplicate to 250-ml flasks with bacterial suspension (ASW and a target bacteria) at a density of 5 rotifers.ml^{-1} and 10^6 bacterial cells.·ml^{-1}. Controls were bacteria only and rotifer only. Samples of rotifers from each flask were used to evaluate the number of bioencapsulated bacteria at 0, 1.5, 3, 6, 12, and 24 hours of treatment. 20-ml samples from each replicate were collected under sterile conditions and the associated bacteria evaluated as previously described. In this experiment *Vibrio carchariae ATCC*35084, *V. campbellii* ATCC25920, *V. parahaemolyticus* ATCC17802, *V. proteolyticus* ATCC15338, *V. harveyi* ATCC14126 and the local isolates *Aeromonas media* (C281), *V. charchariae* (C280), *V. proteolyticus* (C282), *A. ichtiosmia* (C302), *V. charchariae* (C303) were used as target bacterium.

Results and discussion

Rotifers from the strain stock contained 1.4×10^2CFU.rotifer^{-1} (*n*=15). *Vibrio* and *Pseudomonas* were 4% and 32% respectively, of the culturable bacterial population, and other unidentified bacteria were characterized into seven different morphotypes based on colony morphology; all were Gram(-), catalase(+), and occurred in abundances of 17.3, 21, 18, 3, 23, 3, and 13%,

respectively. Rotifers from the mass production contained 2.3×10^{3}CFU.rotifer^{-1} (*n*=13). The population was found to be composed of 19.95%*Pseudomonas*, 23.20% *Vibrio* spp., and 56.83%other unidentified bacteria. No significant correlations were found between the either the number of *Pseudomonas* and *Vibrio* (R^2=0.005, *n*=13) or *Pseudomonas* and unidentified bacteria (R^2=0.533, *n*=13), however, a significant inverse correlation was seen between the number of *Vibrio* and other bacteria (R^2=0.904, *n*=13).

The effective dose of PVP-I to eliminate the associated bacteria was found to be 5mg.ml^{-1}at 10min of exposure, and at 30min of exposure, all tested concentrations were effective to eliminate the associated bacteria. However, the rotifers and amictic eggs did not survive at these conditions. Similar results were found using hydrogen peroxide, where the concentration to eliminate the bacteria was 3% at 5min of exposure. The antibiotic treatment affected the rotifer survival less. With TmSx, the minimal dosages at which the culturable microbiota of the eggs were completely eliminated were 100µl and 500µl for 24h and 48h, respectively. The apparent motility of the newly hatched rotifers was affected at concentrations higher than 200µl of TmSx. The survival of the rotifers was not affected at 24h of exposure at the different concentrations of TmSx tested, however, dead rotifers were found after 48h of exposure at 100µl of TmSx and survivors were not found after 48h at 500µl TmSx (Fig. 1). In PEC treatments, the survival of rotifers was not affected during the assay, however, the bacteria were not eliminated at either of the tested concentrations (Fig. 1).

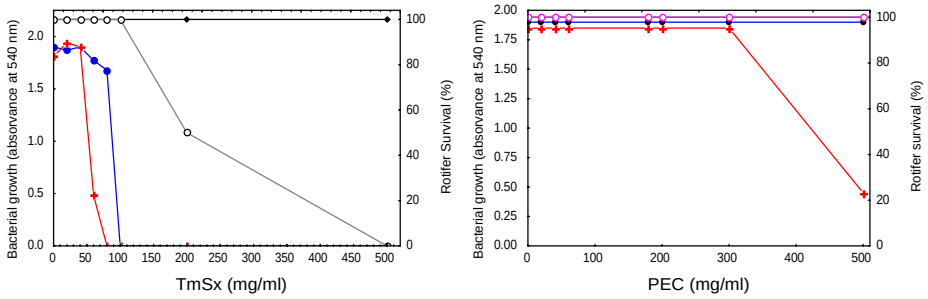

Fig 1. Effect of different antibiotic treatment concentrations on associated bacteria and survival of eggs of *B. plicatilis*. • = bacterial growth in uncovered tubes, + = bacterial growth in covered tubes, ♦= rotifer survival at 24 h and o = rotifer survival at 48 h.

Bacteria were successfully incorporated into gnotobiotic rotifers. A similar pattern of bioencapsulation was found for the different target bacteria; typically, the number of bacteria per rotifer increased during the first 1.5-3h, then dropped to levels near 2000 CFU.rotifer^{-1}, maintaining from 6-24h (Fig 2). No significant differences in the number of bioencapsulated cells per rotifer was found when different strains were used (ANOVA, *P*>0.1). Also, mortality or changes in

motility of the rotifers were not observed during the experiments. At 3h of exposure, the bioencapsulated cells of *V. harveyi* ATCC 14126 reached values 2.5-fold higher than other strains, however, the number decreased rapidly at 6h. The number of biencapsulated cells of *V. carchariae* C303 showed an increase at 3h and the number maintain without changes at 6h, reaching the minimal level at 12h and increasing again at 24h.

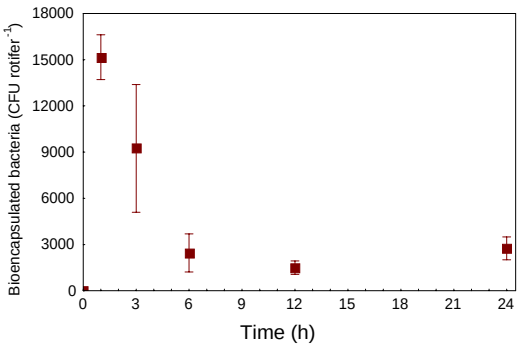

Fig. 2. General pattern of bioencapsulation of different strains of *Vibrio* and *Aeromonas* in the rotifer *B. plicatilis*. (see text for details)

Conclusions

Hatching amictic eggs in a TmSx solution is an alternative to obtain cultures of bacteria-free rotifers. Also, selected bacteria can be incorporated in bacteria-free rotifers. This alternative should be used as a tool to study the effect of selected bacteria on larval health.

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References

Muroga K., M. Higashi, and H. Keetoku. 1987. The isolation of intestinal microflora of farmed red seabream (*Pagrus major*) and black seabream (*Acanthopagrus schelegeli*) at larval and juvenile stages. Aquaculture 65:79-88.

Rueda-Jasso R. 1996. Nutritional effect of three microalgae and one cyanobacteria on the culture of the rotifer *Brachionus plicatilis* Müller:1786. Ciencias Marinas 22:313-328.

Verdonck L., J. Swings, K. Kersters, M. Dehasque, P. Sorgeloos, and P. Leger. 1994. Variability of the microbial environment of rotifer *Brachionus plicatilis* and *Artemia* production systems. J. World Aquacult. Soc. 25:55-59.

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TWO MAIN PROBLEMS IN *ARTEMIA* UTILIZATION: BIOMETRY AND FATTY ACID PROFILE

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Introduction

As it is well known, the main critical factors for the successful use of *Artemia* sp. as a live food are the fatty acid profile and biometry. In spite of this importance, few studies have been done looking at both subjects simultaneously. The present study evaluates the effect of different enrichment strategies on the growth, larval stage dispersion and fatty acid profile of *Artemia franciscana*, by varying the temperature, enrichment period and enrichment products.

Materials and methods

Artemia franciscana cysts from the Great Salt Lake (Aqua-Elite, No: 84110) were hatched under standard conditions (Sorgeloos et al., 1986). Three enrichment products were used: AlgaMac 2000®, Topal®, and micronized freeze-dried S*pirulina* sp.. Three different enrichment periods (6, 12, and 18h) and temperatures (10, 20, and 30ºC) were tested. The enrichment was conducted during 24h, at a maximum density of 200 nauplii.ml^{-1} (0.1g of product in 1 l of seawater).

Total lipid extraction was carried out according to Blight and Dyer (1959) and saponification and esterification of the lipid extracts was done using the method of Metcalfe and Schmitz (1961). The fatty acid methyl esters (FAME) were injected into a capillary column (30m fused silica, 0.32 I.D.) installed in a Varian Star 3400CX gas-liquid chromatograph. GLC data acquisition and handling was done through a Varian integrator 4290. Peak quantification was carried out with a Star Chromatography workstation installed in an IBM PS/1. Peak identification was carried out using as reference well-characterized cod liver oil chromatograms. Duplicate samples were analyzed. Larvae were fixed in Lugol´s solution and the total length (TL), as well as the larval stages were determined according to Amat

(1985), using a stereo microscope (Olympus®, model SZ6045TR) with a calibrated micrometer eyepiece.

Results and discussion

Fatty acid profile. The amount of each fatty acid assimilated by *Artemia* is directly related to its abundance in the enrichment medium (Navarro et al., 1999). In the case of Algamac 2000® this not seems to happen, albeit the highest FAME content as well the highest DHA/EPA/AA and n-3/n-6 ratios were found in this product, the enriched metanauplii do not reflect quantitatively the fatty acid profile of the enrichment diet (Table I). This may be explained by an increase in the HUFA catabolism, which overcomes its anabolism. The short enrichment period, the low enrichment dose and the fact that *Artemia* catabolizes DHA and retroconverts it into EPA during enrichment (Navarro et al., 1999), may explain such a low ratio. The minimum enrichment dose (Dhont et al., 1991) seems to have been achieved with 0.1g.l^{-1} for *Spirulina* sp. and Topal®, but not for Algamac 2000®.

Table I. Composition of the most important (quantitatively) unsaturated fatty acids (μg.mg^{-1} dw ± SE) of the enrichment products and (in parentheses) enriched metanauplii, both at 30°C.

Fatty acid	*Spirulina* sp	Topal®	AlgaMac 2000®
18:2n-6	2.7±0.0	0.665± 0.2	0.16± 0.0
	(5.252±0.1)	(5.665±0.2)	(7.30±0.05)
18:3n-3	0.0	0.01± 0.0	0.12± 0.0
	(33.887±0.8)	(36.196±1.2)	(34.24±0.43)
AA	0.0	0.015± 0.0	1.54±0.2
	(0.566±0.0)	(0.63±0.0)	(2.00±0.08)
EPA	0.11± 0.0	0.08± 0.0	1.72± 0.1
	(2.113±0.1)	(2.286±0.1)	(6.65±0.19)
DHA	0.0	0.02± 0.0	65.6± 6.8
	(0)	(0.014±0.0)	(3.74±0.30)
HUFA	0.385± 0.0	0.34± 0.1	94.02± 9.8
	(6.273±0.2)	(6.92±0.2)	(16.11±0.05)
DHA/EPA	0.0	0.233± 0.1	38.1± 1.3
	(0)	(0.006±0.0)	(0.56±0.06)
n-3/n-6	0.141± 0.0	0.627± 0.1	2.8± 0.0
	(7.643±0.1)	(7.371±0.2)	(4.70±0.01)
FAME	22.735± 0.2	31.22± 10.8	262.2±26.4
	(112.8±2.0)	(120.22±2.4)	(163.42±0.93)

Biometry. The MANOVA revealed that only temperature and enrichment period have a significant influence on larval growth (Fig. 1). The lower growth rate obtained at 10°C can be explained by the decrease of the metabolic activity and by the slow frequency of the antennal movements responsible for larval feeding. Growth rates were not statistically different (P>0.05) at 20 and 30ºC.

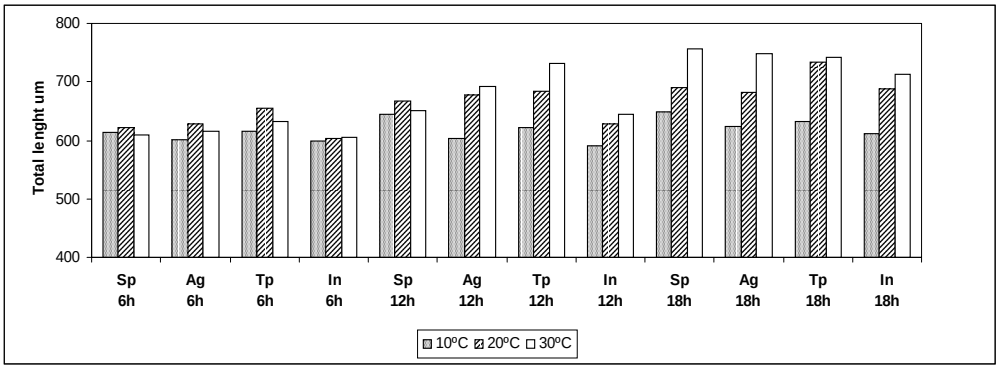

Fig.1. TL of enriched and unenriched (In) metanauplii during 6, 12, and 18h at 10, 20, and 30ºC.

Whenever the *Artemia* size does not cause ingestion problems for the predator, one might expect that the use of large nauplii, with a higher organic weight and energetic content, would be beneficial (Sorgeloos et al., 1986). If the naupliar size is a limiting factor, better results can be achieved by using small nauplii, obtained at lower enrichment temperatures, such as 10°C. The experimental enrichment diets did not affect larval growth (P=0.06), probably due to the short enrichment period.

Larval stage dispersion. During the 18-h enrichment period, only the first three instars were identified, although the instar III was not detected at 10°C. The instar III was detected after 12h of enrichment at 20 and 30°C. The molting process in crustaceans is temperature dependent, being accelerated by increasing temperatures. For the three experimental temperatures, the χ^2 test revealed no differences (P>0.05) between the larval stage dispersion of larvae enriched in different products. This may be a consequence of the short enrichment period and/or the low concentration of the enrichment products.

Conclusions

The present study reveals the possibility of lowering the *Artemia* size, when necessary, by decreasing the temperature of enrichment. Additionally, as expected,

Artemia did not directly reflect the fatty acid profile of the enrichment products, as a result of the DHA catabolism.

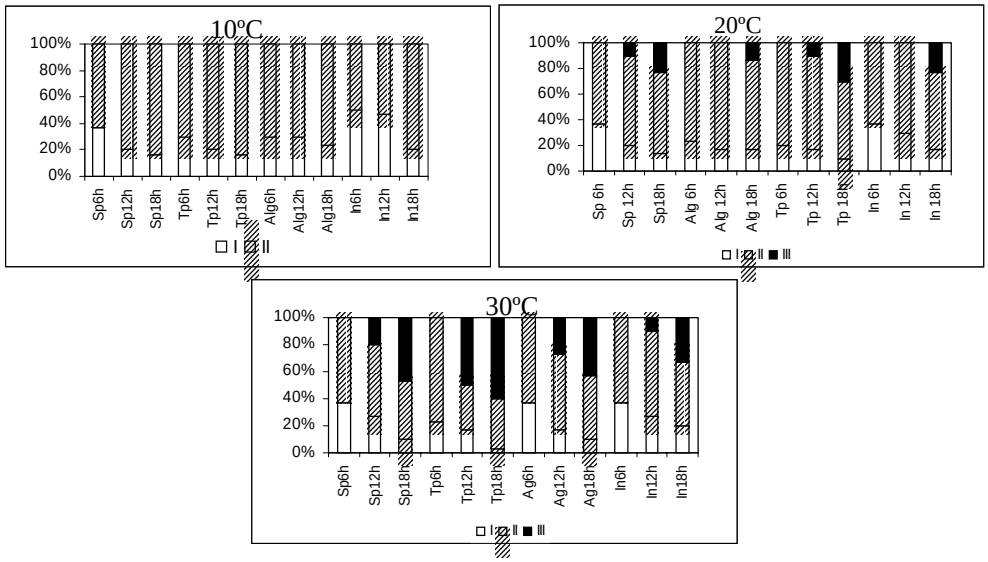

Fig.2. Larval stage proportion after 6, 12, and 18h of enrichment, at 10, 20, and 30ºC.

References

Amat F. 1985. Biología de *Artemia*. Inf. Técn. Inst. Inv. Pesq**.,** 126/127:1-60.

Bligh E.G. and W.J. Dyer. 1959. A rapid method of total lipid extration and purification. Can. J. Biochem. Physiol. 37(8):911-917.

Dhont J., P. Lavens, and P. Sorgeloos. 1991. Development of a lipid-enrichment technique for *Artemia* juveniles produced in an intensive system for use in marine larviculture. p.51-55. In*:* Larvi'91 – Fish and Crustacean Larviculture Symposium. Lavens, P.; Sorgeloos, P.; Jaspers, E. and Ollevier, F. (Eds.). European Aquaculture Society, Special Publication, Gent, Belgium. 15.

Metcalfe L.D. and A.A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatography analysis. Anal. Chem. 33:363-364.

Navarro J.C., R.J. Henderson,. L.A. McEvoy, M.V. Bell, and F. Amat, 1999. Lipid conversions during enrichment of *Artemia*. Aquaculture 174:155-166.

Sorgeloos P., P. Lavens, P. Léger, W. Tackaert, and D. Versichele. 1986. Manual for the culture and use of the brine shrimp *Artemia* in aquaculture. State University of Ghent, Belgium. 319p.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECTS OF TEMPERATURE ON THE CONDITION OF *LOLIGO VULGARIS* AND *LOLIGO FORBESI* (MOLLUSCA: CEPHALOPODA) LATE EMBRYOS AND PARALARVAE

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Introduction

Cephalopods have become promising subjects for commercial culture due to their very high growth rates, high fecundity, and increasing commercial value. However, the high mortality rates of laboratory-reared early life stages are an obstacle to the commercial production of cephalopod species.

The influence of temperature on the embryonic development and growth of cephalopods is relatively known, with increasing temperatures accelerating embryonic development and exponential growth rates at early life stages (Boletzky, 1987).

This paper reports the effects of temperature on the relative embryonic growth and the morphological and biochemical condition of late embryos and planktonic paralarvae of two cephalopod species.

Materials and methods

Spawned *L. vulgaris* egg clusters were collected in the southern coast of Portugal and those of *L. forbesi* in the northwestern coast of Scotland. Eggs from samples A and C were at early embryonic development upon collection, and acclimated to experimental temperature levels at 0.05°C.h^{-1} (Table I). Eggs from samples B and D were at late embryonic stages, therefore embryonic development temperature was that of the seawater in the area of collection.

Natural aerated, filtered, and UV-treated seawater was used in closed systems with biological filters. Salinity was 36-37‰ and the photoperiod was 11L:13D. Paralarvae were fed *ad libitum* live zooplankton twice daily. The duration of embryonic development was calculated in day-degrees. Chorionated egg volume

and body volume (BVol) were calculated using the formula for ovoid objects. Body weight (BW) was measured after blotting excess water, protein was measured by the method of Lowry et al. (1951), and nucleic acids were measured by the fluorimetric method of Clemmesen (1993). The morphological ratio body volume:body weight (BVol:BW) and two biochemical ratios RNA:DNA and RNA:P were calculated and used to assess the relative effects of temperature on the condition of late embryos (stages XVIII-XIX), newly-hatched paralarvae (stage XX), and at three and six days after hatching (3DAH, 6DAH).

Survival at each stage was calculated as the percentage of live and viable embryos in individual egg strings (n=5), and as the percentage of surviving paralarvae in replicate tanks. Student's t-tests were used to compare means at the significance level 0.05. Ratios were arctan transformed before analysis.

Results and discussion

In freshly spawned egg clusters, *L. vulgaris* egg strings contained more eggs (95-128 eggs.string^{-1}) of significantly smaller size (2.76±0.29mm maximum diameter) than those of *L. forbesi* (63-110 eggs.string^{-1}; 3.60±0.21mm maximum diameter).

For most cephalopod species, the duration of embryonic development is directly proportional to egg size and inversely proportional to temperature (Boletzky, 1987). In this study, the embryonic phase was shortened at higher experimental temperature levels, and at similar temperature levels the difference between the two species was between 100 and 200 day-degrees (Table I). The egg volume increases throughout development from 7-60mm^3 in *L. vulgaris* and from 18-120mm^3 in *L. forbesi*.

At the hatching stage XX, *L. forbesi* paralarvae from lower temperature levels were significantly larger than *L. vulgaris* (Table I). For *L. vulgaris* no differences in size were found for temperature levels within the natural range, though paralarvae from higher temperatures carried more internal yolk reserves. Active paralarvae have very high swimming costs (Martins, 1997) and carrying more weight, as internal yolk reserves, will increase such costs. The preying ability is achieved by trial and error during the first days after hatching. Optimum hatching condition would thus be that of larger mantle volume and enough yolk reserves to ensure survival while searching for the first prey. By the third day after hatching (3DAH), most yolk reserves were depleted and the individual dry weight reduced by 10% (Table I).

The morphological ratio BVol:BW shows that embryonic growth and condition at hatching is significantly affected by the temperature of the seawater, especially in species with larger egg size such as *L. forbesi* (Fig. 1A). Values near or above 1 (larger mantle volume and related higher jet propulsion

efficiency) were found at the lower temperature levels of this species' natural temperature range. Higher survival rates occurred at these lower temperature levels (Table I).

Table I. Time from fertilization (T_{dev}) in day-degrees, survival (Sv), body volume (BVol) and weight (BW), and biochemical data at late embryonic stages XVIII-XIX, hatching stage XX and post-hatching age (DAH). Code identifies the sample (A-D), the stage of development and the temperature of embryonic development (8-20ºC). Nucleic acid concentrations are given on a body weight basis.

Code	T_{dev} (Dº)	Sv (%)	BVol (mm^3)	BW (mg)	Protein ($mg.mg^{-1}$)	DNA ($\mu g.mg^{-1}$)	RNA ($\mu g.mg^{-1}$)	N
				Loligo vulgaris				
XVIII-XIX								
A.8	560	20	2.42	5.09	0.016	1.34	2.28	27
A.13	416	98	3.49	4.29	0.046	3.41	3.47	15
A.15	405	97	3.40	4.07	0.035	3.69	3.18	16
A.19	380	95	3.80	4.46	0.029	5.02	4.51	19
B.20	400	98	3.83	4.31	0.035	4.98	5.25	10
XX								
A.13	494	98	4.48	4.95	0.035	3.04	4.31	39
A.15	495	98	4.57	5.17	0.031	3.68	3.29	12
A.19	418	97	4.29	4.94	0.036	4.86	4.24	17
B.20	440	95	4.35	4.96	0.047	5.64	5.91	10
3DAH								
A.13	533	30	4.51	4.84	0.025	3.10	3.53	12
A.15	540	14	4.58	5.21	0.023	3.08	2.57	10
A.19	475	10	4.49	4.87	0.027	3.46	4.01	10
B.20	500	2	4.41	4.06	0.024	4.73	3.98	7
6DAH								
A.13	572	10	5.00	4.79	0.023	3.87	3.07	2
				Loligo forbesi				
XVIII-XIX								
C.8	800	98	14.28	9.20	0.089	8.15	8.79	18
D.13	637	95	5.48	6.07	0.078	9.27	13.09	10
D.15	570	92	4.87	5.03	0.103	6.64	10.26	7
D.19	456	80	2.77	4.95	0.085	4.46	4.98	5
XX								
C.8	920	98	15.97	10.37	0.060	9.53	10.75	10
D.13	728	95	6.77	6.40	0.048	5.44	7.32	10
D.15	690	90	4.90	5.53	0.102	5.81	9.85	5
D.19	532	80	2.89	5.44	0.091	3.73	4.68	5
3DAH								
C.8	944	9	15.31	8.72	0.058	7.98	7.67	10
D.13	767	4	6.53	7.90	0.042	5.02	6.88	5

The biochemical ratio RNA:DNA is lower for paralarvae with BVol:BW approximately 1 (Fig. 1B), suggesting that growth at these stages is by hypertrophy rather than by hyperplasia. The RNA:P ratio was higher for paralarvae with BVol:BW above 1, indicating that these have higher protein synthesis potential. This higher protein synthesis potential is probably related to

digestive function development during late embryonic development and in the transition from yolk to live prey. The most significant effect of temperature on the biochemical condition at hatching was found in the species with larger egg size, *L. forbesi*.

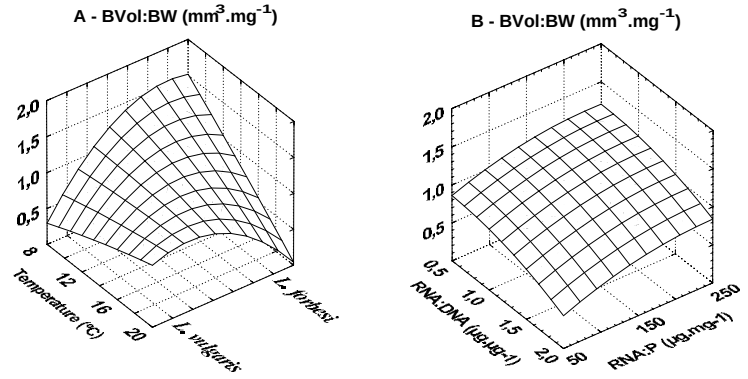

Fig. 1. (A) Effect of temperature during embryonic development on the morphological condition of *Loligo* sp. late embryos and newly hatched paralarvae. (B) Relationship between morphological ratio (Bvol:BW) and biochemical ratios (RNA:DNA and RNA:P) for *Loligo* sp. pre- and post-hatching stages. Quadratic functions fitted to all data pooled (STATISTICA 5.0).

In analogy to fish larvae the first days after hatching represent a critical period for *Loligo* spp. early stages. For higher paralarval survival, the embryos must develop at the lower temperature levels within their natural range.

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References

Boletzky S.v. 1987. Embryonic phase. p.5-31. In: Cephalopod Life Cycles. Vol. 2. Comparative Reviews. Boyle P.R. (Ed). Academic Press, London. 441p.

Clemmesen C. 1993. Improvements in the fluorimetric determination of the RNA and DNA content of individual marine fish larvae. Mar. Ecol. Prog. Ser. 100:177-183.

Lowry O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.

Martins M.C. 1997. Biology of pre- and post-hatching stages of *Loligo vulgaris* Lamarck 1798 and *Loligo forbesi* Steenstrup 1856 (Mollusca, Cephalopoda). PhD Thesis, University of Aberdeen, UK. 179p.

Naef A. 1928. Die Cephalopoden. Fauna e Flora del Golfo di Napoli. Monograph 35B(2). 375p.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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LIPID CLASSES OF *PAGRUS PAGRUS* EGGS AND STARVED LARVAE FROM ADULTS FED ON THREE DIETS

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Introduction

In fish culture, the highest mortality rates always occurs during embryo and larval development, and they are widely influenced by the rearing conditions and broodstock quality. When females are capable of spawning several times, the broodstock diet has a greater effect on egg composition than species with one spawn that requires a longer gonad maturation time. The success of *Sparus aurata* culture has led the way for the culture of similar species like *Pagrus pagrus*.

Many authors stress the importance of lipids during embryo and larval development, due to their role as an energy source and in cell construction. They also suggest the use of a condition index based on triacylglycerol (TG) and polar lipid (PL) function (Lochmann et al., 1995). It is well known that the important lipids remain as long as possible during a starvation period (Rainuzzo, 1993), and this knowledge aids in choosing the starter food or the right enrichment. It is therefore necessary to follow the evolution of lipids from the egg to starvation death.

Materials and methods

Adults of *P. pagrus* were caught in summer of 1998 and kept in floating open-ocean cages until November 1998, when they were separated between six 8 000-l tanks and fed on three different diets: (A) natural food composed of minced fish and mussels; (B) a commercial diet for gonad maturation (Ewos #9); and (C) a mixture of A and B. The spawns were spontaneous and started in January, continuing until the end of June 1999, but only spawns of February are included in this work. The newly spawned eggs were transferred to 100-l tanks with a continuous water flow and natural photoperiod. The temperature varied from 19-21°C and no food was added.

Fertilization samples were taken every six hours until hatch, which took place at 48 hours. For larvae, the samples were taken every twelve hours until 72 hours (three-day-old larvae) and every 24 hours after that until 144 hours (seven days), belonging to the last starvation larvae, although in the present paper, only newly spawned, hatched, and last starved data are offered.

Eggs and larvae were lyophilized before biochemical analysis. The total lipids were obtained in triplicate according to Folch et al. (1957), and neutral (NL) and polar (PL) lipids were separated by adsorption chromatography, passing the samples through a sepak of silica with chloroform and chloroform:methanol 49:1 for NL and methanol alone for PL.

The lipid classes were obtained by TLC using an Iatroscan with a flame ionization detector. NL were developed in hexano:diethylether:formic acid 85:15:0.04 v:v solution, and in chloroform:methanol:distilled water 75:35:3.5 for PL

In this paper, just the major classes in each case are given, and the data are given in percentage of lipid class in relation to 100% neutral or polar lipids (Table I).

Table I. Lipid classes percentage at spawn (sp) hatch (ht), and 72- and 144-hour larvae. TL percentage is related to dry weight and the rest are related to TL.

	Diet A				Diet B				Diet C			
	sp	ht	72h	144h	sp	ht	72h	144 h	sp	ht	72h	144h
SE	18.5	22.96	10.53	5.59	17.81	18.8	7.6	3.19	22.38	22.73	12.44	2.36
TG	25.34	29.31	14.15	0.86	28.45	25.3	15.52	0.23	25.24	19.19	3.75	0.18
Cho	2.75	5.1	13.2	18.25	6.13	5.63	13.27	16.53	3.58	6.55	14.29	18.78
Other NL	TR	0.62	1.08	3.55	0.34	4.85	3.61	7.19	1.96	7.87	12.82	3.45
NL	46.59	57.99	38.96	28.25	52.05	54.58	40.01	27.14	53.9	56.34	43.3	24.77
PC	40.79	34.66	43.89	42.57	38.74	36.53	34.34	39.34	37.55	36.42	40.35	38.86
PE	5.68	2.35	10.82	20.03	3.86	3.76	7.52	16.38	4.16	3.55	8.61	22.97
Other PL	6.94	5.01	6.33	9.16	5.26	5.13	18.14	17.14	4.39	3.69	7.74	13.4
PC/PE	7.18	14.75	4.06	2.13	10.04	9.72	4.57	2.4	9.03	10.26	4.69	1.69
PL	53.41	42.01	61.04	71.75	47.95	45.42	59.99	72.86	46.1	43.66	56.7	75.23
Tl	19.67	24.25	20.04	13.94	19.97	23.55	18.6	10.92	20.05	22.7	19.66	11.36

Results and discussion

Embryo. There was a decrease in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) percentage before hatch, but an increase in NL, probably because the embryo is preparing to utilize more ‘fast’ energy (free fatty acids, triacylglycerols, steryl esters) for swimming. TG decreased in treatments B and C (mixed and commercial diets), but its catabolism released the FFA and

monoglycerids (MG) as an energy source. At hatch, the natural food treatment showed more TG content and SE synthesis.

Newly hatched larvae to start feeding. During this stage, larvae endure exponential growth, increasing the PL to synthesize cell membranes. For diets with natural food (A and C), PC and PE increased until the onset of starvation, but a bit earlier for diet B. Cholesterol became an important class along this period duplicating its percentage in all diets. Brockerhoff (1974) and Rainuzzo et al. (1992) suggest cholesterol and PL or PC form a complex to build the biomembranes.

Starvation. Without a food supply, the more energetic lipids were withered and CHO was the only important NL class. At seven days old, larvae lost almost all the 'fast' energetic classes. Even the FFA, DG, and MG resulted from catabolism of others, and PC could be an alternative metabolic energy source during this condition. The PE is not easily explained simply by PC catabolism in these cases, and probably other classes could release the n-3 HUFA that PE synthesis requires.

References

Brockerhoff H. 1974. Model of interaction of polar lipids, cholesterol and proteins in biological membranes. Lipids 9:645-650.

Lochmann S.E., G.L. Maillet, K.T. Frank, and C.T. Taggart. 1995. Lipid class composition as a measure of nutritional condition in individual larvae Atlantic cod. Can. J. Fish. Aquat. Sci. 52:1294-1306.

Rainuzzo J.R. 1993. Fatty acid and lipid composition of fish egg and larvae. p.43-49. In: Fish Farming Technology.- Proceedings of the First International Conference on Fish Farming Technology, Trondheim, Norway. Reinertsen, H, Dahle, L.A., Joergensen, L., Tvinnereim, K (Eds.). Research Counc. Of Norway, Oslo Norway Rotterdam Netherlands Balkema.

Rainuzzo J.R., K.I. Reitan, and L. Jorgensen. 1992. Comparative study on the fatty acid and lipid composition of four marine fish larvae. Comp. Biochem. Physiol. 103B:21-26.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECT OF SEDIMENT ON THE GROWTH AND SURVIVAL OF *PENAEUS STYLIRROSTRIS* POSTLARVAE

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Introduction

In a culture system, the sediments are important in the nutrient dynamics that affect the development and survival of the organisms (Ray and Chien, 1992). In coastal environments the metal input from anthropogenic activities and erosion is important and could significantly increase the levels of concentration of some metals in sites in which larval growth could be affected. The bioavailability fraction of the metals in the sediment is changed depending on factors, such as pH, oxygen, salinity and temperature together with remaining food with other components of the water (Riley, 1989). Therefore, depending on the nature and concentration of the element in the sediment, the effects of metals on the biota could be beneficial or detrimental. In the present work, the sediment from four areas in Baja California Sur, México, which are currently used for shrimp culture or will be used in the future, were evaluated for their effect on postlarvae growth.

Materials and methods

Cylindrical tanks of 1-m diameter and 150-l capacity were used. In each tank layers of sediments of about 10cm of width were placed. Texture, organic matter, and total and bioavailable phosphorous content were measured (Escoppinichi et al., 1991; Van Loon, 1985). Total and bioavailable fraction of copper, iron, zinc, manganese, and cadmium in sediments were quantified (Van Loon, 1985) using an atomic absorption spectrophotometer BUCK Sci, 200 A, To validate the analytical procedure, samples of marine sediments from the National Research Council of Canada (BCSS-1) were analyzed. Four replicates were used for each sediment with 20 postlarvae per tank. Levels of oxygen, temperature, and pH were monitored daily, and levels of nitrites, nitrates, and ammonium weekly (Parsons, et al, 1982). Data were analyzed by Pearson's correlations and by one-way ANOVA, using the software STATISTICA.

Results and discussion

The texture of the sediments is shown in Table I. During the experiment, the temperature varied by 3°C while the oxygen and pH were constant. The levels of ammonium, nitrates and nitrites had important variations between the treatments, but did not attain levels that affect growth. The sediments with higher levels of organic matter had the higher concentrations of these nitrogen compounds. Total and bioavailable levels of Zn, Cu, Mn, Fe, Cd, and P are shown in Table II. It can be observed that high levels of one element do not necessary mean it has a high bioavailability. However, for the majority the elements analyzed in this study, a high correlation between total concentration and the bioavailable fraction was observed. For example, iron ($r=0.98$; $P<0.01$), cadmium ($r=0.96$; $P<0.05$), manganese ($r=0.94$, $P<0.05$), and copper ($r=0.88$; $P=0.05$) presented significant correlations, while zinc did not ($r=0.26$; $P=0.7$). After one month, significant differences in growth and mortality were obtained between the treatments. Fig. 1 shows significant differences ($F=5.20$; $P<0.01$) in the survival between the four treatments. Sediments 1 and 2 have twice the survival rate than sediments 3 and 4. Higher survival rates were associated with higher levels of total manganese in the sediments ($r=0.92$; $P<0.05$). Also Ritvo et al. (1999) found in soils of shrimp farms a direct relation between manganese levels in soils and shrimp yield. Higher rates of growth were correlated to total and bioavailable phosphorus content ($r=0.94$; $P<0.05$; $r=0.95$; $P<0.05$, respectively). In contrast, Ritvo et al. (1999) found an inverse relation between phosphorus in soil and shrimp yield. Due that the extraction techniques used to estimate the bioavailable fraction of each element are different, it is thus difficult to compare the levels of phosphorus between the soils studied in both studies. Although the sediments with high levels of phosphorus also had a high content of cadmium, this element together others could be first storage in the hepatopancreas and then detoxified from the organism (Vogt and Quinitio, 1994).

Table I. Texture and organic matter (OM) of test sediments.

Sediment	Sand (%)	Clay (%)	Silt (%)	OM ($mg.kg^{-1}$)	Texture class
Zone 1	2.50	22.50	75.00	2.82	Silt loam
Zone 2	5.00	8.75	86.25	1.79	Silt loam
Zone 3	25.00	10.00	65.00	2.00	Silt loam
Zone 4	55.00	40.00	5.00	1.76	Clay-sandy loam

In the present study although no significant correlation was obtained between iron and survival rates, the sediment with higher content of iron had the higher mortality. In this sediment the iron content was almost 6 times higher than in the other sediments. Iron could be precipitated in the gills when ferrous salts are dissolved in the water causing an occlusion of the respiratory lamellae that result in respiratory insufficiency and noted lethargy (Jansen and Groman, 1993). A

negative correlation was obtained between an increase of total copper content of sediments and the final weight of the animals (r=-0.83; $P<0.05$). This is in agreement to Ritvo et al. (1999) whom found in soils of shrimp farm an inverse correlation between copper levels and shrimp yield. In conclusion, the treatments with higher levels of manganese had the higher survival rate while the sediments with higher copper and lower phosphorus levels had the lower growth.

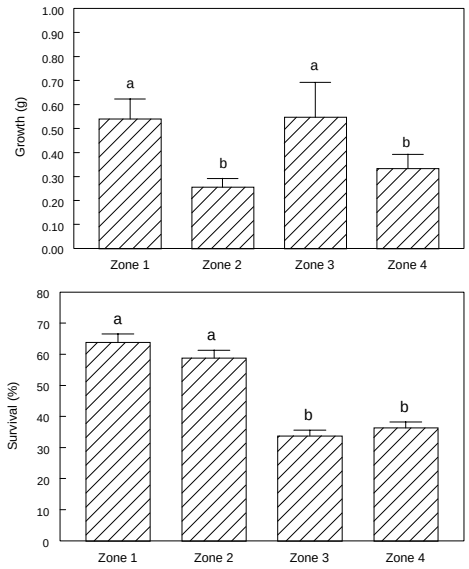

Fig. 1. Effect of sediment on the growth (top) and survival (bottom) of larval *Penaeus stylirrostris*.

Table II. Levels of total and bioavailable elements ($\mu g.g^{-1}$) in test sediments.

		Zone 1	Zone 2	Zone 3	Zone 4
Zn	Total	107	119	55	92
	Bioavailable	5.08 (4.74%)	4.9 (4.11%)	4.46 (8.11%)	2.91 (3.16%)
Cu	Total	21.7	27.8	14.01	31.77
	Bioavailable	0.46 (2.12%)	0.48 (1.73%)	0.24 (1.71%)	0.48 (1.51%)
Mn	Total	368	413	121	235
	Bioavailable	21 (5.71%)	40 (9.68%)	0.99 (0.82%)	11.05 (4.70%)
Fe	Total	1040	931	988	6002
	Bioavailable	6.27 (0.60%)	1.64 (0.18%)	8.35 (0.84%)	35 (0.58%)
Cd	Total	17.19	1.97	1.96	5.73
	Bioavailable	6.90 (40%)	0.41 (20.81)	N.D.	N.D.
P	Total	7362	1052	5259	2546
	Bioavailable	4.32 (0.06%)	2.16 (0.2%)	4.47 (0.08)	2.29 (0.09%)

For bioavailable fractions, percent of total fraction is indicated in parentheses.

References

Escoppinichi R., D.J. Hernández, and B.R. León. 1991. Manual operativo del laboratorio de suelo y agua. UABCS, La Paz. 247p.

Jansen M. and D. Groman. 1993. The effect of high concentrations of iron on impounded American lobsters: A case study. J. Aquat. Anim. Health 5:155-156.

Parsons T.Y, T. Maita, and C.M. Lalli. 1982. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, USA. 173p.

Ray W.M. and Y.H. Chien. 1992. Effects of stocking density and aged on tiger prawn, *Penaeus monodon*, nursey system. Aquaculture 104:231-248.

Riley J.P. 1989. Los elementos más abundantes y menores en el agua de mar. p.61-104. In: Introducción a la química marina. Riley J.P., and R. Chester (Eds.). AGT Editor, S.A. México. 459p.

Ritvo G., F.M Speed, W.H. Nelly, J.B. Dixon, A.L. Lawrence, and A.M. Samocha. 1999. Regression analysis of soil chemical composition for two shrimp farms in Texas. J. World Aquacult. Soc. 30:26-35.

Van Loon J.C. 1985. Selected Methods of Trace Metal Analysis: Biological and Environmental Samples. Wiley-Interscience Publications, New York. 357p.

Vogt G. and E.T. Quinitio. 1994. Accumulation and excretion of metal granules in the prawn, *Penaeus monodon*, exposed to water-borne copper, lead, iron and calcium. Aquat. Toxicol. 28:223-241.

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PRELIMINARY RESULTS ON REARING EXPERIMENTS WITH MACKEREL LARVAE

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Introduction

The Atlantic mackerel (*Scomber scombrus* Linneo, 1758) is a very important commercial species in Europe. An important component in the population dynamics of fishes is recruitment; the number of fishes surviving at an exploitable size. Fisheries managers have historically attempted to predict recruitment from spawning stock size. Mortality during the first month of planktonic life is extremely high, and small percentage changes in survival rate generate extremely high variations in recruitment. This variability in the survival of eggs and larvae masks the effect of the stock size. Survival rate of eggs and larvae depends on environmental factors such as temperature and food.

The study has been designed to check the effect of environmental factors on larvae growth and survival. The objectives in the first stage of that study were (1) to adapt a larvae culture system to our specific studies with mackerel, and (2) to establish a relationship between larval growth and different environmental conditions. Larvae aging will be determined by reading of otolith rings that will be used to validate that technique. In the present work we present preliminary results on mackerel larvae growth and survival from different incubation experiments carried out in 2001.

Material and methods

Adult mackerels were captured using handlines with several lead hooks modified at their end to avoid causing damage to the fish. Mackerels in state of maximum gonadal development were transferred with extreme care to a tank of the quarantine of the Aquarium, where the fertilization was carried out. Also, fertilization was made in the same boat, transferring the already fertilized eggs to

the Aquarium. The obtaining of eggs and sperm was carried out making a smooth abdominal massage in adult fishes. Mature eggs were selected and carefully deposited on a Petri dish where sperm was added for artificial fertilization. In addition, the length and sex frequencies of adult mackerels were registered.

After fertilization, eggs were selected and moved to a 60-l incubator tank in which they remained until hatching of the larvae. Factors like temperature, salinity, dissolved oxygen and pH were controlled daily providing real sea conditions. After hatching, larvae were transferred one by one to larvae rearing tanks of 200 liters were control and handling was carried out. Once the larvae were transferred, 5 larvae of each experimental tank were daily sacrificed and measurements of length and weight were taken. For conservation of the samples, larvae were preserved in 80% buffered ethanol. Dry weight was determined often keeping each specimen 24 hours in heating at 40°C and larvae were weighted with a 0.00001-g precision balance. Both otoliths (sagitta and lapillus) were located using a microscope. Once the otoliths were prepared, ring increments were measured using a TNPC module, specially developed for the analysis of chalky pieces from image analyzer.

Results

Adult Catching. In order to catch adult mackerel in advanced gonadal development, ten consecutive attempts were made. A total of 243 adult mackerels were collected (105 females and 80 males). Fig. 1 shows the length-frequency distribution of caught adult mackerels. Size range was from 27-39cm and one modal size was observed at 35cm with 37 individuals. Mackerel females were larger than males, females ranged from 27-39cm and males from 27-36cm (Fig. 2).

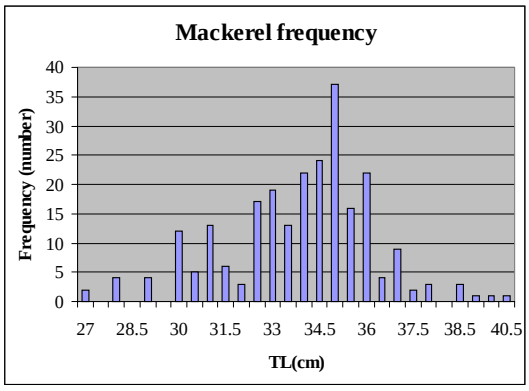

Fig. 1. Length-frequency distribution of adult mackerels sampled.

The relationship between length and weight was described by the power relationship [Weight = a × Length^{b}]. The equation of the fitted model for females was [Weight = 0.0083 × Length $^{3.1124}$], R^2 = 0.8496; and for males was [Weight = 0.007 × Length $^{3.1455}$], R^2 = 0.877.

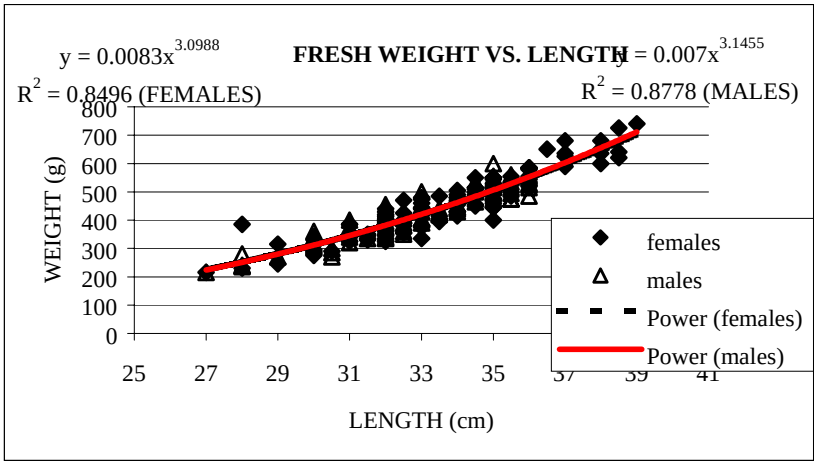

Fig. 2. Relationship between length and fresh weight for adult mackerel collected during the fertilization experiments.

Fertilization experiments. Mean temperature, dissolved oxygen content and salinity of seawater for each fertilization experiments are presented in Table I. Temperature varies 1.3°C between experiments while oxygen content and salinity values were quite stable.

Table I. Physical parameters recorded during the incubation experiments.

Experiment	Temperature (ºC)	Oxygen (mg.l^{-1})	SST (‰)	No. eggs	% hatching	No. larvae hatching
FERTIL. I	15.23	8.87	34.19	15 600	22.6	3525
FERTIL. II	15.44	9.274	33.98	4001	7.2	288
FERTIL. III	16.42	8.636	33.52	11 720	13	1523
FERTIL. IV	16.56	8.22	34.2	10 900	2.9	316

In the 4 experiments of fertilization carried out with 4 different groups of adult mackerels, the hatching time ranged from 99.2 hours at 15.4°C to 82 hours at 16.6°C. Percentage of hatching was very variable, ranging from 22% in the first fertilization experiment to 2.9% in the last one.

Larval growth. Fig. 3 shows the mean larval growth measured as standard length versus time (hours). The total data from the different incubation experiments are presented altogether. Although the physical conditions were quite stable among

incubation experiments, larval survival varied highly between them. Data of length vs. time were fitted to an exponential model as follows: $SL = 3.4938 \times e^{0.0016 \times time}$, $R^2 = 0.6704$. This exponential model shows a growth rate of 3.8% per day.

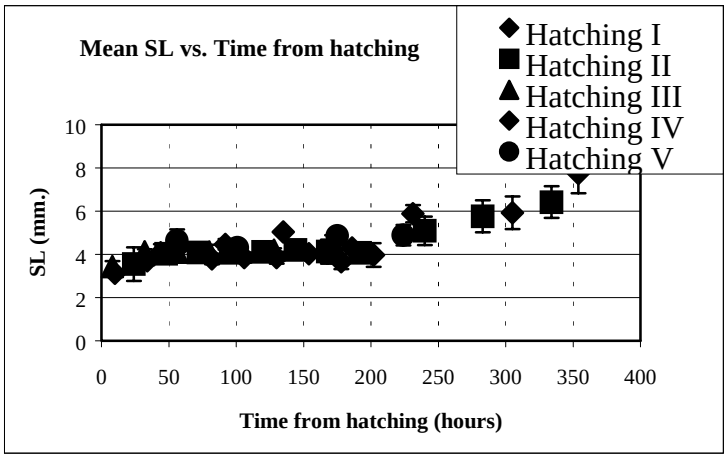

Fig 3. Empirical curve of growth of *Scomber scombrus* (L.) larvae during the incubation experiments. Each point represents SL mean and bars the standard deviation.

Conclusions

- Incubation period until hatching seems to be dependent on temperature. Hence, that length of time varied from 99h at 15.2°C to 82h at 16.4°C.
- Mackerel larvae mortality varied from 14% per day in the third incubation experiment to 6.8% per day in the fourth experiment.
- Growth rate for mackerel larvae versus time follows an exponential model with a growth rate of 3.8% per day. Growth rate of 6.1 and 6.3% per day has been observed in mackerel larvae in natural conditions. However, in these studies the mackerel larvae length ranges were larger than for this work and that could be the reason for the observed differences.

References

Anderson J.T. 1988. A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. J. Northw. Atl. Fish. Sci. 8:55-66.

Heath M.R. 1992. Field investigations of the early life stages of marine fish. Adv. Mar. Biol. 28: 95-115

Kendall A.W. and D. Gordon. 1981. Growth rate of Atlantic mackerel (*Scomber scombrus*) larvae in the middle Atlantic Bight. Rapp. P.-v. Reun. CIEM 178:337-341.

Ricker W.E. 1979. Growth rates and models. Fish. Physiol. 3:677-743.

Ware D.M. and T.C. Lambert. 1985. Early life history of Atlantic mackerel (*Scomber scombrus*) in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 42:577-592.

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CONTROL OF BACTERIAL FLORA OF LARVAE, WATER AND ROTIFER (*BRACHIONUS PLICATILIS*) IN LARVAL REARING OF WHITE SEA BREAM (*DIPLODUS SARGUS*, LINNAEUS, 1758)

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Introduction

A great number of bacteria, belonging to more than 20 genera, are responsible for fish and other aquatic animal illness, most of them present in the aquatic environment as opportunistic pathogens (Menezes, 1995). Despite the promising artificial reproduction and larval rearing results obtained with white seabream (*Diplodus sargus*), using the same technology developed for sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Pousão-Ferreira et al., 1997), sudden and massive larval mortality in the first 15-20 days after hatching (DAH) remains a great problem in hatcheries. The aim of this work was the identification of the bacterial flora in the rearing water of white seabream larvae fed on different diets, considering its close relationship with the larval mortality. Since the main contributor to high bacterial levels in larval rearing is the live food (*Brachionus plicatilis* and *Artemia* sp.*)*, a pre-wash treatment was also analyzed as a way to decrease the bacterial load (Rombaut et al, 1999). Most of the identified bacteria belong to the genus *Vibrio* spp.

Materials and methods

Two experiments were carried out with *Diplodus sargus* larvae, from the day they hatched to 20 DAH, in white 200-l cylindrical-conical tanks. Each experiment had two different treatments, corresponding to two different diets (Tables I and II): live food (rotifer, *Brachionus plicatilis*, and *Artemia* sp.) and a co-feeding regime (live and inert food). Each treatment had three replicates. The identification of the bacterial flora in the larval production water was made at the inlet of the circuit and at one tank, randomly chosen from each treatment. Inlet water of the circuit was UV-treated before entry into the tanks. The samples were collected in triplicate with 200-ml sterile bottles (Table III). The live food (*Brachionus plicatilis*) bacterial flora control was performed through samples taken from the production tanks, followed by its inoculation in TCBS and

Marine Agar culture media. Three treatments were performed – in the first (Treatment 1), there was direct inoculation, in the second (Treatment 2), the sample was previously washed in UV-sterilized water, and the third (Treatment 3) was submitted to an extra wash in sterilized water by bleach and neutralized by sodium thiosulfate. (Table III)

Table I. Feeding regime of the first experiment.

Treatment	Day								
	3	8	10	11	12	13	14	17	20
a	R	R	R	R+A	R+A	A	A	A	A
b	R	R	R	50%A IF	35%A IF	20%A IF	20%A IF	20%A IF	20%A IF

R: rotifers; A: *Artemia*; IF: Inert Food

Table II. Feeding regime of the second experiment.

Treat-ment	Day									
	3	7	8	9	10	11	12	13	15	20
a	R	R	R	R	R	A	A	A	A	A
b	R	50%R IF	35%R IF	20%R IF	20%R 50%A IF	20%R 35%A IF	20%R 20%A IF	20%R 20%A IF	20%R 20%A IF	20%R 20%A IF

R: rotifers; A: *Artemia*; IF: Inert Food

Table III. Methodologies of collecting samples from different experiences.

		Sampling days (DHA)	Hour	Culture Medium	Quantities plated (μl)
Larval production water					
Experiment 1	Inlet	8, 10, and 14	0930	MAgar	10
				TCBS	40
	Treatment a	8, 10, and 14	0930	MAgar	10
				TCBS	40
	Treatment b	8, 10, and 14	0930	MAgar	10
				TCBS	40
Experiment 2	Inlet	8, 10, and 13	0930	MAgar	10
				TCBS	40
	Treatment a	8, 10, and 13	0930	MAgar	10
				TCBS	40
	Treatment b	8, 10, and 13	0930	MAgar	10
				TCBS	40
Brachionus plicatilis water					
	Treatment 1			MAgar	10
				TCBS	40
	Treatment 2			MAgar	10
				TCBS	40
	Treatment 3			MAgar	10
				TCBS	40

Results and discussion

The results of the bacteria present in the larval production water, in the two experiments are represented in Figs. 1 and 2. In MAgar, the co-feeding treatment was the one with the highest counting in both experiments. In TCBS, the growth of *Vibrio* spp. was similar in the two experiments. The contribution of the inlet water was not relevant.

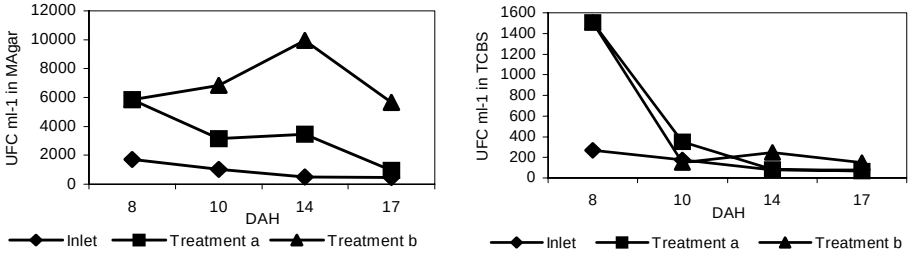

Fig. 1. Number of CFU.ml^{-1} medium on MAgar and TCBS after 24h of incubation at 22ºC in Experiment 1.

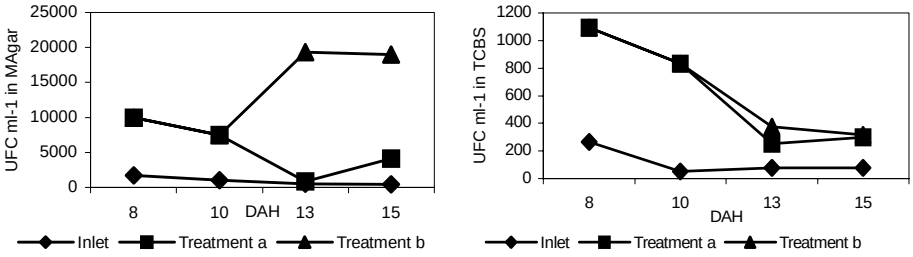

Fig. 2. Number of CFU.ml^{-1} medium on MAgar and TCBS, after 24h of incubation at 22ºC, in Experiment 2.

Using ANOVA from the STATISTICA /W 5.0® Module Switcher (Windows 98), we could see that, over time, the differences between treatments in the two experiments became more significant, becoming significantly different at the end (P=0.000001) of the experimental period.

As for the rotifer culture, the best results were obtained with treatment 3 (9 000-20 000 UFC.ml^{-1} on MAgar and 2 000-6 000 UFC.ml^{-1} on TCBS). Treatment 1 was responsible for a significant contamination – between 71 000-95 000 UFC. ml^{-1} on MAgar and 12 000-40 000 UFC.ml^{-1} on TCBS. The statistical analysis results are represented in Table IV, where it can be seen that there are significant

differences between treatments 1 vs. 2 and treatments 1 vs. 3, but not between treatments 2 vs. 3.

Table IV. Results of the statistical analysis with STATISTICA /W 5.0® Module Switcher, of Windows 98, of bacterial growth in live food, *Brachionus plicatilis.*

ANOVA	Treatment 1	Treatment 2	Treatment 3
Treatment 1	–	0.0397*	0.0078*
Treatment 2	0.0397*	–	0.4398
Treatment 3	0.0078*	0.4398	–

* $P<0.05$ i.e., significant differences between treatments

Conclusions

Being the first exogenous feed administrated to the larvae, the maintenance of rotifer quality is of great importance. The results obtained in these experiments indicate that a significant decrease of the bacterial load can be achieved through an effective and accurate rotifer wash.

It was expected that the tanks fed with live food would present greater contamination. However, this was not confirmed by the present experiments. Co-feeding revealed to be the worst regime, as it caused the highest microbial contamination. These results can be explained by the fact that the inert food, in spite of being administrated in several rations, sums up to a total that exceeds the feeding capacities of the larvae. This excessive inert food sinks and accumulates in the bottom of the tanks, being a substrate for bacterial contamination and contributing to the deterioration of the water quality.

References

Menezes J. 1995. Methods for disease control in fish culture. Bol. IPIMAR 1: 95-100

Pousão-Ferreira P., E. Dores, and J. Amaral. 1997. White Sea Bream (*Diplodus sargus*) juvenile production: perspectives for commercial aquaculture. Short communications and abstracts of contributions presented at the International Conference “Martinique 97 – Island Aquaculture and Tropical Aquaculture” Les Trois Ilets, Martinique, French West Indies, May 4-9, 1997, pp. 241-242.

Rombaut G., Ph. Dhert, J. Vandenberghe, L. Verschuere, P Sorgeloos, and W. Verstraete. 1999. Selection of bacteria enhancing the growth rate of axenically hatched rotifers (*Brachionus plicatilis*). Aquaculture 176:195-207.

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ACCLIMATIZATION OF THE ROTIFER *BRACHIONUS PLICATILIS* FOR REARING OF WINTER FLOUNDER LARVAE

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Introduction

Winter flounder (*Pleuronectes americanus*) is a common inshore finfish that was identified as a candidate for cold-water marine aquaculture a few years ago (Litvak, 1994). Today, larval rearing has been accomplished but not optimized. The winter flounder larvae are fed with two live preys: first a rotifer (*Brachionus plicatilis*) and then a brine shrimp (*Artemia salina*). The 24°C optimal growth temperature of these two species is not compatible with the larvae's 10°C rearing temperature. When live prey are given to larvae, they suffer a thermal shock that is thought to reduce their vigor and nutritional value. Inert food was attempted as a total replacement of live prey but larvae did not digest it until their gut loop development was completed (Ben Khemis et al., 2000). Partial use of inert food allows the avoidance of brine shrimp (Ben Khemis et al., unpublished data). The aim of this study was to improve the quality of the live prey *B. plicatilis* until inert food could be used reliably.

Materials and methods

Chlorella sp. culture. A *Chlorella* sp. strain from the St. Lawrence estuary (Canada) was isolated and run semi-continuously at 18°C with f/2 medium (Guillard and Ryhter, 1962). The culture density was kept at about 15×10^6 cells.ml^{-1}.

B. plicatilis rearing and acclimatization. *B. plicatilis* rearing was done in 18.9-l carboys supplied with filtered water (10μm) maintained at 24°C. *B. plicatilis* were fed each morning with *Chlorella* sp. and supplemented with the formulated diet "Culture Selco" (Inve Aquaculture). At the end of the day, a predetermined quantity of *B. plicatilis* was taken off and acclimatized at 10°C overnight.

Larval rearing. Sexually mature winter flounder from the St. Lawrence estuary (Canada) were caught in May 2000 and kept in 200-l tanks. The eggs of three females were fertilized with the sperm of three males and placed in three different incubation tanks. After 10 days of incubation, eggs hatched and larvae were split between four 60-l cylindro-conical tanks, supplied with filtered water maintained at 10±1°C and 27±1PSU. Gentle aeration was provided in the center of each tank to create a smooth upwelling current, and a natural photoperiod (12L:12N) was applied. At mouth opening, two diets were tested in duplicate: *B. plicatilis* reared at 24°C and *B. plicatilis* from the same rearing but acclimatized overnight at 10ºC. A solution of *Chorella* sp. was added to each tank to keep larvae in green water and to prevent larval wall syndrome (Naas et al., 1992; Hernández-Cruz et al., 1994; Reitan et al., 1997). Prey was given in excess density (3-5 prey.ml^{-1}) according to Laurence (1977). The present study was conducted from hatching (day 0) to complete larval gut loop development (day 26). At this last stage, larvae measured from 5.5-6.3mm and their enzymatic activity was developed enough to allow digestion of inert food (Ben Khemis et al., 2000).

Sampling and analysis. Sampling was performed at hatching, mouth opening, and then every seven days (days 0, 5, 12, 19, and 26). Sampling took place early in the morning to minimize contamination by gut contents. For each sampling, 20-60 larvae were rinsed three times in millipore-filtered seawater before being pooled into 1.5-ml Eppendorf tubes and frozen at -80°C. All samples were run in duplicate. RNA, DNA, and total and soluble protein contents were determined from the samples and results were expressed as μg.larvae^{-1}. Trypsic activity was determined and reported per μg of soluble protein to obtain the specific trypsin activity. Lipid composition (triacylglycerol, sterol, and phospholidid) and fatty acids were also analyzed at day 0, 12, and 26. At each sampling, ten larvae were measured *in vivo* with a micrometer under a binocular microscope. Statistical analysis was carried out using ANOVA and regressions. A significance level of 5% was used throughout.

Results and discussion

Complete yolk sac absorption and mouth opening occurred on day 5. First feeding was observed on the same day.

Growth. A linear relationship is observed between the logarithm of larval growth and age. Slopes are parallel (ANCOVA, $P>0.05$) which demonstrates the same growth performance in each diet.

Ribonucleic acid contents. Ribonucleic acid statistical analyses do not indicate differences in efficiency of one diet compared to another. RNA and DNA have nearly the same three-stage profiles: decrease between day 0 and day 5, slight increase between day 5 and day 12, and high increase between day 12 and day 26. RNA/DNA ratio is not significantly different between the two diets tested and at day 26, RNA is 5 times higher than DNA.

Total and soluble protein contents. No significant difference in total or soluble protein is observed between the two diets. Total protein profile shows nearly the same pattern as RNA profile. This is not surprising since RNA is implied mostly in protein synthesis. Soluble proteins increase from hatching to day 26. Soluble proteins/total proteins ratio also increases during the entire experiment.

Trypsic activity. No significant influence of a diet compared to another is noticed. Trypsic activity increases from hatching to day 26, slightly between day 0 and day 12, and then faster between day 12 and day 26. Trypsin specific activity shows a declined profile. This could be explained by the increased synthesis of other enzymes and other soluble proteins during larval development. To date, this is the first known trypsic measurement on winter flounder larvae.

Lipid and fatty acid composition. Both diets yielded equal larval content of triacylglycerols, sterols, and phospholidids. The ratio of triacylglycerols/sterols is about 1 at day 0, 0.13 at day 12, and 0.37 at day 26. EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) contents are similar between the two diets.

Conclusion

Biochemical composition (ribonucleic acids, protein, and lipids), trypsic activity, and growth of winter flounder larvae were similar with the two diets tested. Acclimatization does not improve the quality of *Brachionus plicatilis* and therefore does not optimize food conditions before the use of an inert diet. Acclimatization of *Brachionus plicatilis* is not a future issue for the rearing of winter flounder larvae.

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References

Ben Khemis I., J. de la Noüe, and C. Audet. 2000. Feeding larvae of winter flounder *Pseudopleuronectes americanus* (Walbaum) with live prey or microencapsulated diet: linear growth and protein, RNA and DNA content. Aquacult. Res. 31:377-386.

Hernández-Cruz C.M., M. Salhi, H. Fernández-Palacios, and M.S. Izquierdo. 1994. Improvements in the culture of *Sparus aurata* L. larvae in relation to the use of antibiotics, phytoplankton and rearing system. Aquaculture 124:269-274.

Gulliard R.R.L. and J.H. Ryther. 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hudtedt, and *Detonula confervacea* (Cleve) Gran.. Can. J. Microbiol. 8:229-239.

Laurence G.C. 1977. A bioenergetic model for the analysis of feeding and survival potential of winter flounder, *Pseudopleuronectes americanus*, larvae during the period from hatching to metamorphosis. Fish. Bull. 75:529-546.

Litvak M. 1994. Winter flounder. Bull. Aquacult. Assoc. Canada 94:4-8.

Naas K.E., T. Naess, and T. Harboe. 1992. Enhanced first feeding of halibut larvae (*Hippoglossus hippoglossus*) in green water. Aquaculture 105:143-156.

Reitan K.I., J.R. Rainuzzo, G. Øie, and Y. Olsen. 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155:207-221.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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INFLUENCE OF THE SPAWNING TIME DURING THE REPRODUCTION PERIOD ON THE LARVAL QUALITY OF EURASIAN PERCH, *PERCA FLUVIATILIS*

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Introduction

In Eurasian perch, a broodstock can spawn over 6-7 weeks during the natural reproduction period, between March-April in the east of France (Sulistyo et al., 1998). During this period, the eggs and larvae quality highly fluctuated (Kestemont et al., 1999). In fact, numerous factors, such as feeding, storage conditions, stress, strain, etc., of the broodstock can affect the larvae quality (Bromage, 1995). In Eurasian perch, it has been demonstrated that the levels of some fatty acids (20:5n-5, 22:6n-3) must be sufficient in the broodstock food to produce healthy larvae (Abi-Ayad et al., 1997). Kestemont et al. (1999) observed a progressive increase of the cathepsin L activity in 7-day-old eggs, linked to a progressive decrease of the hatching/fertilization ratio, during the Eurasian perch spawning period. Such results also suggested a progressive decrease in larval quality during the spawning period. To improve the hatchery management, a study has been conducted to determine the effect of the spawning time on the larval quality.

Materials and methods

A Eurasian perch broodstock was harvested in a pond (Moselle, France) and stored in a floating cage during the winter (with roach as live preys) and early spring. During the 3-week spawning period in April 2000 and from the first harvested spawnings (April 10), 3 ribbons of eggs were harvested every 3 days, then transferred to the laboratory and incubated in an experimental hatchery (17°C). Firstly, 24 hours after spawning, fertilization rates were estimated on samples (3 samples per ribbon, 30 eggs per sample). Then, the resistance of newly hatched larvae (5.5-6.3mm) was determined through challenge tests: osmotic (in solution with 2% seawater) and starvation stress tests (Abi-Ayad, 1998). The tests were performed in triplicates at 17°C in 1-l recipients. For the osmotic test, the experiment was conducted on 30 larvae.l^{-1}, and survival rates

were determined after 90 minutes. For the starvation test, the experiment was realized on 100 larvae.l^{-1} while recording the duration inducing 50% of mortality (LC_{50}).

Data were compared by three-way hierarchical model of analysis of variance with date effect hierarchies by bloc effect and ribbon effect hierarchies with bloc and date effects (General Linear Models procedure, SAS 1989; Univariate procedure, SAS 1990). The adjusted Ls_{means} (adjusted means) were compared to the adjusted Scheffé test.

Results and discussion

Results showed high fertilization rates between 70 and 95% during all the spawning period (Fig. 1). No effect of the spawning time was observed during this spawning period.

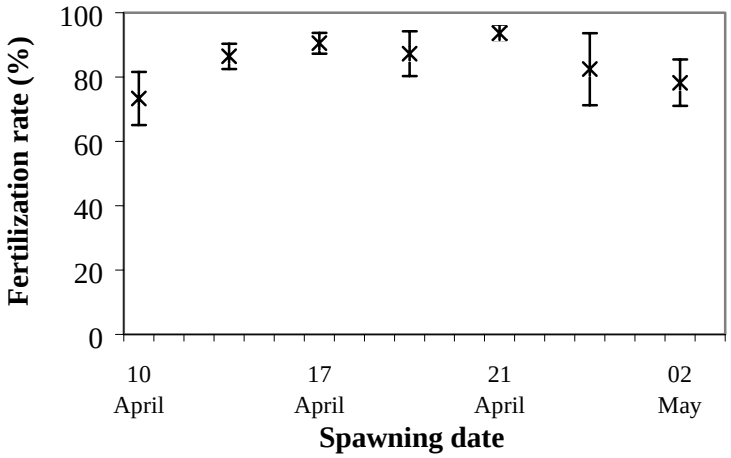

Fig. 1. Variation of the fertilization rate during an annual spawning period of a *Perca fluviatilis* broodstock (mean±SD, *n*=3).

The larvae resistance (LC_{50}) to starvation tests (Fig.2) fluctuated during the spawning period from 64.7 (April 19) to 189.6 hours (April 26) (Fig. 2). The resistance to the osmotic test varied from 4.5 (April 19) to 49.7 % (April 14) (Fig. 3). Our results were in accordance with those obtained by Abi-Ayad (1998) on wild larvae. He recorded values fluctuating between 44 and 240 hours in the case of the starvation test and between 6.8 and 57.7% for the osmotic test. During the spawning period, the larvae resistance to osmotic and starvation stress tests varied in a considerable way. In fact, our results indicated higher larvae quality at the beginning of the spawning period and a sudden decrease in larval quality at the middle of the spawning period ($P<0.05$).

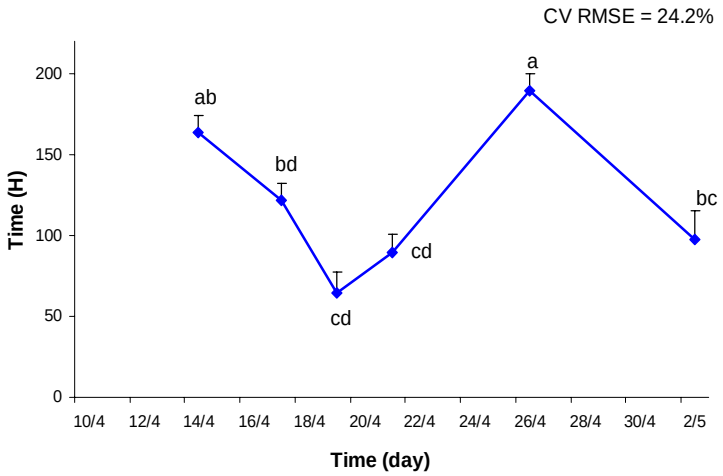

Fig. 2. Variation of newly hatched larvae resistance (LC_{50}) to a starvation test, expressed in hours, during the annual spawning period (mean ± SD, *n*=3).

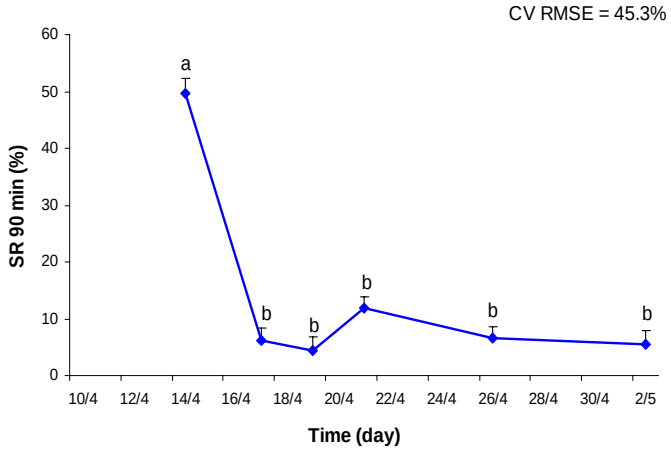

Fig. 3. Variation of the newly hatched larvae resistance to an osmotic test, expressed as the survival rate SR (%) after 90 minutes, during an annual spawning period (mean ± SD, *n*=3).

After April 19, when considering the larvae resistance to a starvation stress, the larval quality again seems of higher quality, just before the ultimate decline at the end of the spawning period. Such variations suggest the existence of two successive spawning periods corresponding to two different groups in the

broodstock. However, this hypothesis is not confirmed by the data relative to the osmotic stress test.

References

Abi-Ayad S.M. 1998. Etude expérimentale de la biologie de la reproduction de la Perche Fluviatile (*Perca fluviatilis*). Effet de la composition en acides gras de la série (n-3) dans l'alimentation des géniteurs sur la qualité des œufs et des larves. Thèse Université de Liège, Belgium, 147p.

Abi-Ayad S.M., C. Mélard, and P. Kestemont. 1997. Effects of n-3 fatty acids in Eurasian perch broodstock diet on egg fatty acid composition and larvae stress resistance. Aquacult. Int. 5:161-168.

Bromage N. 1995. Broodstock management and seed quality – General considerations. p.1-24. In: Broodstock management and egg and larval quality. N.R. Bromage and R.J. Roberts (Eds.), Blackwell Science Ltd., Oxford.

Kestemont P., J. Cooremans, S.M. Abi-Ayad, and C. Mélard. 1999. Cathepsin L in eggs and larvae of Perch *Perca fluviatilis*: variations with developmental stage and spawning period. Fish Physiol. Biochem. 21:59-64.

SAS Institute Inc. 1989. SAS/STAT User's Guide. Version 6, Fourth Edition, Volume 2, Cary, NC:SAS Institute Inc., 846p.

SAS Institute Inc. 1990. SAS Procedures Guide. Version 6, Third Edition, Cary, NC:SAS Institute Inc., 705p.

Sulistyo I., J. Rinchard, P. Fontaine, J.N. Gardeur, B. Capdeville, and P. Kestemont. 1998. Reproductive cycle and plasma levels of sex steroids in female Eurasian perch *Perca fluviatilis*. Aquat. Living Resour. 11:101-110.

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INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON THE BROODSTOCK GONADOGENESIS, SPAWNING AND LARVAE QUALITY OF EURASIAN PERCH, *PERCA FLUVIATILIS*

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The Eurasian perch, *Perca fluviatilis*, has been identified as a new species intended for diversification of freshwater aquaculture (Kestemont and Dabrowski, 1996). In Northern and Western Europe, aquaculture development is mainly linked to intensive rearing in recirculating systems. Today, production mainly relies on mature breeders captured from natural habitats. In order to meet market requirements, reproduction cycles must be controlled so as to delay spawning and yield fingerlings throughout the year.

Among environmental factors, temperature and photoperiod appear to be the most important elements in the control of the reproductive cycle and spawning in temperate species (Scott, 1979; Bromage, 1993). Compared to other percids such as yellow perch, *P. flavescens* (Dabrowski et al., 1996), and walleye, S*tizostedion vitreum* (Malison et al, 1998), little attention has been paid to the reproduction of Eurasian perch, and there are very few data dealing with their environmental control. Furthermore, for the last 5 years, research in the Animal Science Laboratory has focused on (1) the characterization of the annual male and female reproductive cycle in natural habitat, and (2) the subsequent environmental control of the reproduction.

This paper is meant to present the main results relative to the environmental and hormonal control in their reproduction.

Variations of the gonadosomatic index (GSI) and seasonal changes of plasma sex steroids were studied for both sexes in their natural habitat (Sulistyo et al., 1998; 2000). In females, after the sexual resting period from May to August, oogenesis began in September, when the water temperature and daylength decreased. The GSI and oocyte diameter (OD) increased progressively until spawning (GSI=25%, OD=850µm) in April. Spawning occurred when

temperature and daylength reach 12-14°C and 13-14 hours, respectively. Plasma levels of testosterone (T), estradiol-17β (E_2), and 17,20β-P (DHP) were low during the sexual resting period. E_2 levels increased significantly at the onset of oogenesis in September, then raised abruptly in November (3-4ng.ml^{-1}). In December, when temperature is still decreasing and daylength is the shortest the T levels increased rapidly to 15-20ng.ml^{-1}. The T and E_2 levels remained very high during vitellogenesis until spawning. During the periovulatory period (daylength and temperature increasing), a peak of E_2 (4ng.ml^{-1}) was observed, while T levels decreased. DHP concentrations remained low (0.2-0.6ng.ml^{-1}) during vitellogenesis from September to February, then, for the first time, some significant peaks were assayed (4ng.ml^{-1}), which strongly suggested the role of DHP in oocyte final maturation in Eurasian perch. As for males, the GSI increased during summer and reached its maximum in September. Males spermiated from January to the spawning season. From April to November, plasma testosterone (T) and 11-ketotestosterone (11-KT) levels were low (<0.5ng.ml^{-1}). Then, plasma levels of both steroids increased (12.3 and 4.9ng.ml^{-1} in T and 11-KT, respectively) in early winter and decreased towards the spawning season in April.

According to the hypothesis that temperature changes could induce gonadal development and control the main stages of the Eurasian perch reproductive cycle, several out-of-season experiments were conducted. Photoperiod was fixed at 12L:12D (200 lux at the water surface) and different thermal regimes were tested. It appears that a cooling period from 22 to 6°C of 6 weeks, a long chilling period at 6°C of 5 months and a short warming phase to 14°C (one month) allows better gonadal development (Migaud et al., 2001). Moreover, spontaneous out-of-season eggs and larvae were obtained, but only a few females developed their gonads (30%) and some atresia was observed. As far as larvae quality is concerned, weight (0.4-0.5mg), length (4.7mm), osmotic and starvation stress test resistances were lower than in natural spawnings. Plasma T and DHP levels, in developed females and spermiating males, were similar to those observed in the wild. Higher values were observed in late winter (end of cold chilling period) just before spawning (approximately 14 and 9ng.ml^{-1} for females and males, and 2-3 and 4ng.ml^{-1}, respectively for both steroids) and early spring just after spawning, whereas plasma T levels were lower in immature females (from 2-6ng.ml^{-1}). The same pattern as in wild conditions was observed for E_2 in developed females with higher levels in late winter (3-4ng.ml^{-1}). These experiments provide some important contributions concerning the environmental control of Eurasian perch reproduction, especially for the chilling and warming period durations. These studies have also shown that delayed spawnings in Eurasian perch can be obtained by thermo-periodic manipulation, but the results are not convincing enough, due to the low female development rate, high heterogeneity, low spawning rate, as well as the egg quality (low fertilization rate, high embryo mortality, etc.).

In order to improve the artificial program developed, meant for inducing delayed spawnings, the role of photoperiod was tested. A 10-month experiment has been carried out to investigate the effects of different photoperiod regimes on gonadal growth, spawning and larvae quality, while temperature changes remained natural. The natural and pseudo-natural treatments have given the same results in terms of gonadal development and number of spawnings, although a little delay was observed in the case of the second treatment. Egg quality seems to be affected by photoperiod regimes and artificial lighting, especially during fertilization and hatching processes. As far as the last treatment is concerned, fixed photoperiod has shown the same phenomenon as in out-of-season conditions with very heterogeneous results: spermiating males, developed females, and immature fishes were observed. Few females spontaneously spawned and among others, atresia has been observed. Therefore, photoperiod is as crucial a factor as temperature in the reproductive cycle control of Eurasian perch.

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References

Kestemont P. and K. Dabrowski. 1996. Recent advances in the aquaculture of percid fish. J. Appl. Ichthyol. 12 (3-4):137-200.

Scott D.B.C. 1979. Environmental timing and the control of reproduction in teleost fish. Symp. Zool. Soc. Lond 44:105-132.

Bromage N. 1993. Environmental control of reproduction in salmonids. Rec. Adv. Aquacult. IV:55-65.

Dabrowski K., A. Ciereszko, L. Ramseyer, D. Culver, and P. Kestemont. 1996. Reproductive physiology of yellow perch (*Perca flavescens*): environmental and endocrinological cues. J. Appl. Ichthyol. 12:139-148.

Malison J.A., L.S. Procarione, T.B. Kayes, J.F. Hansen, and J.A. Held. 1998. Induction of out-of-season spawning in walleye (*Stizostedion vitreum*). Aquaculture 163:151-161.

Migaud H., P. Fontaine, I. Sulistyo, P. Kestemont, and J.N. Gardeur. 2001. Induction of out-of-season in Eurasian perch *Perca fluviatilis*: effects of rates of cooling and cooling durations on female gametogenesis and spawning. Aquaculture (in press).

Sulistyo I., J. Rinchard, P. Fontaine, J.N. Gardeur, B. Capdeville, and P. Kestemont. 1998. Reproductive cycle and plasma levels of sex steroids in female Eurasian perch *Perca fluviatilis*. Aquat. Living Ressour. 11(2):101-110.

Sulistyo I., P. Fontaine, J. Rinchard, J.N. Gardeur, H. Migaud, B. Capdeville, and P. Kestemont. 2000. Reproductive cycle and plasma levels steroids in male Eurasian perch *Perca fluviatilis*. Aquat. Living Resour. 13(2):99-106.

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INFLUENCE OF DAYLENGTH AND FEEDING RATE ON SURVIVAL, CANNIBALISM AND GROWTH RATES OF EURASIAN PERCH *PERCA FLUVIATILIS* LARVAE

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Introduction

During its early stages, the Eurasian perch is pelagic and diurnal (Thorpe, 1977; Colette et al., 1977). The environmental factors (daylength, light intensity, tank wall colour) were very important in successful Eurasian perch larviculture (Tamazouzt et al., 2000). Jourdan (1999) showed that, with a similar feeding regimen, an increase in the daylength (DL) improved the survival and growth rates of larvae. As the survival of newly hatched larvae highly depends on the first feeding and, subsequently, on the food availability (Blaxter, 1965), a complementary study has been conducted to determine the influence of daylength increase in relation to the different feeding rates (FR).

Materials and methods

A 21-day experiment was conducted using newly hatched larvae obtained from an egg ribbon harvested in the Leman Lake, on 19 May 2000. One day after the mass hatching time, the larvae (initial body weight: 1.1mg) were transferred to 12 cylindro-conical tanks (30 l, 625 larvae.tank^{-1}) functioning in a recirculated system. The larvae were subjected to four treatments in triplicates: two daylengths (8 or 24h, 400 lux) and two different feeding rates (40 or 80 % of the dry biomass). These treatments were applied in reference to the study by Jourdan (1999) and Fiogbé (1996). Water temperature was maintained between 18 and 22°C, and the water quality was checked every two days. Fish were automatically fed with *Artemia* nauplii (one meal per lighting hour), according to the protocol of Jourdan (1999). Mortalities were checked daily. Growth was checked on days 7, 14, and 21 (30 larvae per tank). At the end of the experiment, fish were counted individually weighed and measured.

Data was compared by two-way variance analysis followed by the Newman-Keuls test: significant differences were found at a 0.05 level.

Results and discussion

The final mean weight (FMW) was significantly higher for the 24 h-DL / 80 % FR treatment than for the 24 h DL / 40% FR treatment ($P < 0.05$, Fig.1). Compared to the other treatments (8 h DL / 40% FR and 8 h DL / 80% FR), these treatments induced significantly higher final mean weights ($P<0.05$). The interaction between daylength and feeding rate was also significant as far as growth and cannibalism rates were concerned.

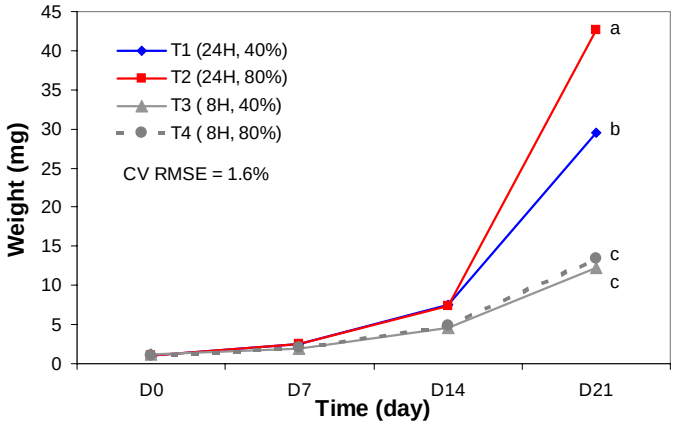

Fig. 1. Comparison of growth of *Perca fluviatilis* larvae (mean weight, n=3) in relation to daylength and feeding rate.

The cannibalism rate (CR) was significantly lower under the 8 h-DL / 80% FR treatment (Table I). The survival rate (SR) was significantly higher for the 24 h-DL / 80 %-FR treatment.

Table I. Effect of the feeding rate and the daylength on the final mean body weight, cannibalism and survival rates (mean ± SD, n=3) of *Perca fluviatilis* larvae ($P<0.05$).

Treatment	24h DL 80% FR	24h DL 40% FR	8h DL 80% FR	8h DL 40% FR	CV RMSE
FMW (mg)	42.7 ± 0.4^{a}	29.5 ± 0.5^{b}	12.4 ± 0.4^{c}	12.2 ± 0.1^{c}	1.6%
CR (%)	19.5 ± 6.2^{b}	31.5 ± 4.0^{ab}	1.6 ± 2.6^{c}	39.4 ± 10.3^{a}	28.1%
SR (%)	18.3 ± 1.6^{a}	5.2 ± 2.1^{c}	8.9 ± 2.1^{b}	2.3 ± 0.5^{d}	16.4%

Even if these survival and growth rates are lower than those obtained by Jourdan (1999), our results show that an increase in the food availability improved the growth and survival of Eurasian perch larvae. The increase in the daylength

(with a higher number of meals per day) appears to be a more effective way for improving the growth and survival of Eurasian perch larvae. However, for reducing cannibalism, an increase of the feeding rate is more effective than a lengthening of the feeding period. In fact, the CR seems to be related to prey density.

References

Blaxter J.H.S. 1965. The feeding of herring larvae and their ecology in relation to feeding. Atlas California Cooperative Oceanic Fisheries Investigations 10:79-88.

Colette B., M.A. Ali, K.E.F. Hokanson, M. Nagiec, S.A. Smirnov, J.E. Thorpe, A.H. Wethearley, and J. Willemsen. 1977. Biology of the Percids. J. Fish. Res. Bd. Canada 34:1890-1899.

Fiogbé E.D. 1996. Contribution à l'étude des besoins nutritionnels chez les larves et juvéniles de la perche fluviatile (Perca fluviatilis L.). Thèse F.U.N.D.P., Namur, Belgium, 334p.

Jourdan S. 1999. Influence de facteurs abiotiques, la photopériode et l'intensité lumineuse, sur la survie et la croissance de larves, post-larves et juvéniles de perche commune *Perca fluviatilis* L.. Thèse I.N.P.L., Nancy, France. 147 p.

Tamazouzt L., B. Chatain, and P. Fontaine. 2000. Tank wall colour and light level affect growth and survival of Eurasian perch larvae (*Perca fluviatilis* L.). Aquaculture 182:85-90.

Thorpe J. 1977. Daily ration of adult perch, *Perca fluviatilis* L., during summer in Loch Leven, Scotland. J. Fish Biol. 11:55-68.

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EFFECT OF FEEDING ASCORBIC ACID ENRICHED ZOOPLANKTON ON SURVIVAL, GROWTH AND TISSUE BIOCHEMICAL COMPOSITION OF *LABEO ROHITA* LARVAE

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Introduction

High mortality accompanied by stunted growth and skeletal deformities are common problems encountered during controlled nursery rearing of rohu (*Labeo rohita*, Ham.) (Jhingran, 1986; Jena et al., 1998). Sub optimal nutrition is one attributed reason, which also can affect viability in later stages of growth. Larval growth depends to a great extent upon rearing practices ensuring good water quality, distribution of adequate nutritional factors based on live food and also feeding management to suit the rapid transit rates and high growth rates of larvae (Charlon and Bergot, 1984; Kaushik, 1985). Optimization of the feeding strategy to function in accordance with the feeding behaviour and nutritional requirement of extremely fragile and sensitive larvae might improve the larval rearing condition (Sorgeloos and Leger, 1992). Vitamin C is an essential dietary nutrient during larval stages of the carp rohu. The near complete dependence on live food (mainly the zooplankton) by carp larvae constitutes a bottleneck in successful larval rearing (Mookherji and Rao, 1991). The nutritional profile of zooplankton from CIFA farm ponds indicated only trace amount of ascorbic acid compared to the fairly high ascorbic acid requirement of carp larvae. In this context an attempt has been made to study the effect of dietary supplementation of vitamin C enriched live food on growth, survival and biochemical changes in rohu larvae.

Material and methods

Mixed zooplankton (*Moina, Daphnia, Cyclops* and *Diaptomus*) culture was maintained in four 1000-l outdoor tanks of the larval culture unit using manure doses adopted by Jena et al. (1998). Cultured plankton were then suspended at density of 150-200ml^{-1} in 10 l well aerated-water. Enrichment was carried out for 12 hours using vitamin C fortified (10%, 20% and 30% ascorbyl palmitate inclusion) formulated diet (yeast, cod liver oil, sunflower oil, soy lecithin, ascorbyl palmitate, tween 80, and water) at 0.1gl^{-1}in two equal doses at 0 and 6 hours. The plankton given different diets were sampled regularly for analysis of vitamin C (Jagota et al., 1982) and moisture content. Induced bred rohu larvae (3 days old, 2.20mg av. wt.) were reared in twelve 10-l well aerated glass jars

containing de-chlorinated water for a period of 15 days with 10 000.m^{-2} stocking density and four feeding regimes. T_1 group fed with normal zooplankton without enrichment, and the T_2, T_3, T_4 groups were fed with zooplankton enriched with 10%, 20%, and 30% ascorbyl palmitate included diet respectively. During the rearing period water temperature and dissolved oxygen were 29.9±1°C and 7.6mg.l^{-1}, respectively. Every day approximately two third of the water was exchanged with clean freshwater and new batch of non-enriched and enriched plankton were administered to the larvae *ad libitum*. The larval survival and growth increment in all treatments were assessed by recording mortalities, counting remaining fish in jars and by measuring initial and final length and weight respectively. Proximate composition (%DM) of initial and final fish samples were analyzed following standard methods. Muscle DNA and RNA contents were estimated (Burton, 1956; Ceriotti 1955). Survival and growth rates were analyzed using SAS Software to determine significance of differences among mean treatment values.

Results

Survival of rohu larvae under different diet treatments indicated a variation range from 62-90% (Table I) without significant difference (P>0.05) between treatment groups. Live weight gain (%), length increment (%), and specific growth rate (SGR) were highest in T_3 group followed by T_4, T_2, and T_1, with significant difference (P<0.05) between the groups.

Table I. Growth performance and survival (%) of Rohu (average initial length and weight 4.98mm, 2.20mg) larvae fed different experimental diets for 15 days. (Different superscripts in a row differ significantly P<0.05).

Attributes	Diets			
	T_1	T_2	T_3	T_4
Final length (mm)	10.33±0.68^d	15.10±0.10^c	29.83±0.67^a	20.0±0.1^b
Length increment (%)	106.66±13.61^d	196.07±1.96^c	506.36± 13.53^a	308.16±2.04^b
Final weight (mg)	19.45±0.79^d	64.64±1.48^c	208.67±5.91^a	162.89±8.33^b
Live weight gain (%)	783.93±35.71^d	2838.33±67.36^c	9384.39±269.02^a	7048.32±841.23^b
SGR (%.day^{-1})	14.52±0.28^d	22.53±0.14^c	30.35±0.19^a	28.69±0.35^b
Survival (%)	62.33±0.58^b	64.0±.00^b	90.0±4.93^a	88.33±0.58^a

Table II. Carcass chemical composition and muscle nucleic acid content of experimental fish (Different superscripts in a row differ significantly P<0.05).

Composition	T_1	T_2	T_3	T_4
Dry matter	13.72± 0.03^c	16.78 ± 0.03^b	17.11± 0.02^a	17.08 ± 0.01^a
Protein (N x 6.25)	63.41± 0.34^c	63.76 ± 0.12^c	70.11± 0.02^a	69.06 ± 0.15^b
Lipid (ether extract)	15.82 ± 0.05^c	16.44 ± 0.09^b	16.86 ± 0.07^a	16.79 ± 0.70^a
DNA (mg.g^{-1})	0.18 ± 0.01^a	0.17 ± 0.01^b	0.16 ± 0.00^c	0.15 ± 0.00^c
RNA(mg.g^{-1})	0.99 ± 0.01^d	1.01 ± 0.01^c	1.23 ± 0.02^a	1.11 ± 0.01^b
DNA:RNA	5.67 ± 0.15^d	5.95±0.01^c	7.55 ± 0.05^a	7.26 ± 0.07^b

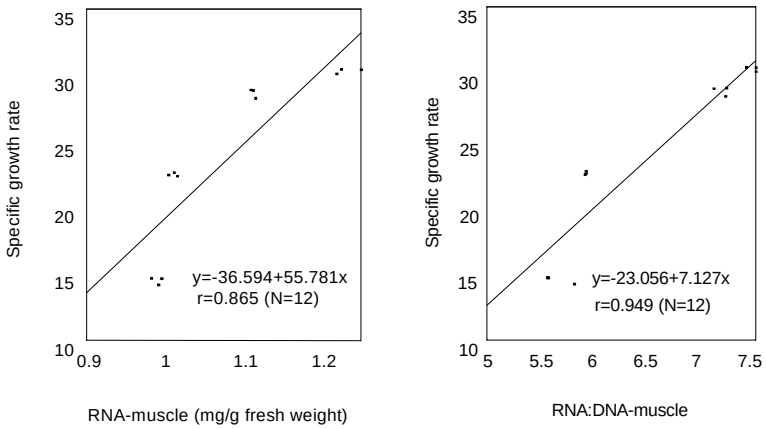

Fig.1. Linear correlations between SGR and both muscle RNA content muscle RNA:DNA.

Carcass composition (Table II) showed highest crude protein (70.64%) and lipid (16.86%) content in T_3 group, with a decreasing trend of by $T_4 > T_2 > T_1$. RNA:DNA ratio also followed the similar trend as carcass composition. Significant positive correlation (r = 0.865 and 0.949) were found in muscle RNA content and muscle RNA:DNA ratio, respectively, with SGR of larva in different treatments (Fig. 1). No significant difference (P>0.05) was found in tissue ascorbic acid level in between enriched plankton fed groups (Table III).

Table III. Vitamin C content (µg ascorbic acid.g^{-1} dry weight) of enriched zooplankton and rohu larva fed experimental diets.

Enrichment level	Vitamin C content in mixed zooplankton	Vitamin C content of rohu larva before feeding	Vitamin C content of rohu larva after feeding
0%	10	20-25	<2
10%	750 –815	20-25	99
20%	1495-1575	20-25	108
30%	2010-2510	20-25	102

Discussion

Better survival and growth, which resulted in raising quality stock of fry in T_3 and T_4 groups than others were due to extra amounts of vitamin C in the diet (enriched zooplankton), which helped the larvae withstand high stocking density (10 000.m^{-2}). With this high stocking density, Jena et al. (1998) found 34.6-45.7% survival in rohu larva for a 15-day rearing period. The present investigation showed comparatively higher values (90 and 88%) in T_3 and T_4 groups. Carcass chemical composition and muscle nucleic acid analyses showed the superiority of T_3 and T_4 groups than others. Strong correlation between

specific growth rate with RNA:DNA ratio and RNA content in rohu larvae of different groups represent the different protein synthesis capacities (Bastrop et al.,1992) due to feeding of different level of vitamin C enriched zooplankton. No significant (P>0.05) difference was found in tissue ascorbic acid level, in different treatment groups, suggesting a saturation of the body ascorbic acid pool at 10% (750-815µg ascorbic acid.g^{-1} dry weight) enriched zooplankton feeding which agrees with Merchie et al., (1997). This might be the optimum dietary ascorbic acid requirement of rohu larvae at this high stocking density. Since larval survival is the most important aspect during nursery rearing, this kind of enriched plankton feeding method will have great relevance in nursery rearing for raising quality rohu fry with minimum cost and time. Moreover, a distinct improvement in survival and growth suggested that ascorbic acid, essential for the larvae is sufficiently available in enriched live zooplankton to ensure better larval survival and healthy fry production, the main goal of hatcheries anywhere in the world.

References

Bastrop R., K. Jurss, and R. Wacke. 1992. Biochemical parameters as a measure of food availability and growth in immature rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. and Physiol. 102A:151-161.

Burton K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-323

Ceriotti G. 1955. Determination of nucleic acids in animal tissues. J. Biol. Chem. 214:59-70.

Charlon N. and P. Bergot. 1984. Rearing system for feeding fish larva on dry diets. Trial with carp (*Cyprinus carpio* L.) larvae. Aquaculture 41:1-9.

Jagota S.K. and Dani H.M. 1982. A new colorimetric technique for the estimation of vitamin C using folin phenol reagent. Clin. Biochem. 127: 178-182.

Jena J.K., P.K. Aravindakshan, and W.J. Sing. 1998. Nursery rearing of Indian major carp fry under different stocking densities. Indian J. Fish. 45 (2):163-168.

Jhingran V. 1986. Aquaculture of Indian major carps. In: Aquaculture of cyprinids Billard, R, and J. Marcel (eds.), INRA, Paris.

Kaushik S.J. 1985. Some aspects of larval nutritional des cyprinides physiology in carp. p.215-226. In: Aquaculture. Billard, R., Marcel, J. (eds.). INRA Publ.

Merchie G., P. Lavens, and P. Sorgeloos. 1997.Optimization of dietary vitamin C in fish and crustacean larvae: A review. Aquaculture 55:165-181.

Mookherji N. and T.R. Rao. 1991. Survival and growth of Rohu (*Labeo rohita*) and Singhi (*Heteropneustes fossilis*) larvae fed on dry and live foods. p.148-150. In: Larvi 1991 Fish and Crustacean larviculture symposium Vol. 15, Lavens, P., P. Sorgeloos, E. Jaspers, and F. Ollevier. (eds.), European Aquaculture Society Special Publication. Belgium.

Sorgeloos P. and P.L. Leger. 1992. Improved larviculture outputs of marine fish, shrimp and prawn. J. World Aquacult. Soc. 23:251-264.

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ENRICHMENT OF *ARTEMIA* NAUPLII IN PUFA PHOSPHOLIPIDS AND WATER-SOLUBLE NUTRIENTS USING LIPOSOMES

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Introduction

Artemia nauplii lack long-chain polyunsaturated fatty acids (PUFA), which are essential for the proper nutrition of marine larvae. Consequently, this widely used prey needs to be enriched. This supplementation is achieved treating the nauplii with lipid emulsions, microparticles, or microalgae with high PUFA content. Enrichment materials are passively filtered by *Artemia* nauplii and their digestive tract is slowly loaded with any particle of suitable size present in the medium. Thus, nauplii become vehicles of the enrichment products.

The use of liposomes as enrichment products provides different advantages and possibilities (Hontoria et al., 1994). They are discrete particles with an appropriate size for the naupliar filtering abilities. It is possible to encapsulate into these lipid vesicles – water-soluble substances in the aqueous phase between the lipid bilayers – as well as hydrophobic molecules into the hydrocarbon chain moiety of the phospholipids. In addition, the polar lipids that make up part of liposomes can be also easily introduced under this form in the trophic chains utilized in larviculture. The main problem encountered when highly unsaturated phospholipids are used is the loss of stability in the walls of the vesicles, causing the aqueous phase to leak, and any substance dissolved in it to be lost in the external medium. This effect increases with the unsaturation level.

Previous studies have shown that it is possible to encapsulate considerable amounts of PUFA into liposomes (McEvoy et al., 1996). However, other previous experiments conclude that the leakage found in highly unsaturated liposomes is more evident during the enrichment process, since it is particularly aggressive (high temperature and aeration). Consequently, the retention of water-soluble products inside the liposome is lost. These studies have also ruled out the unsaturated chain autoxidation as the instability source (Albaladejo et al., 1998).

We present the results of leakage tests (during enrichment) on different types of liposomes, varying in composition, lamellarity, and type. New data on PUFA bioencapsulation in *Artemia* nauplii using liposomes are also presented.

Material and methods

Three different phospholipid sources have been used in the preparation of liposomes: dipalmitoyl phosphatidylcholine (DPPC), krill phospholipid extract (KPC), and soy bean lecithin (SBL). In some cases, certain amounts of cholesterol (CHO) have been included as membrane stabilizer. Multilamellar vesicles (MLV) have been prepared by the method proposed by Bangham et al. (1965), but using seawater as the aqueous phase. Two different compositions have been assayed: KPC:CHO (2:1 w/w) and DPPC:KPC:CHO (1:1:1 w/w). Two methods have been used to form large unilamellar liposomes (LUV), following the methods from New (1990): by extrusion through polycarbonate membranes (two preparations with the same composition as MLV) and by detergent solubilization (two more samples made of pure SBL and pure KPC). These liposomes have been subjected to stability tests. For the additional enrichment trials, three different mixtures of SBL and KPC (1:1, 3:7, and pure KPC) have been utilized with the aforementioned method of detergent solubilization. Commercial Super Selco emulsion has been assayed as control.

The ability to maintain water-soluble molecules encapsulated inside the liposomes (liposome stability) has been estimated by means of the retention of carboxyfluorescein with fluorescence methods as described in Hontoria et al. (1994). Triplicate 100-ml tubes under enrichment conditions (28°C, strong aeration and 300 *Artemia* nauplii per ml) have been periodically sampled during 24h and the increase in fluorescence, proportional to the leakage from the liposomes, measured.

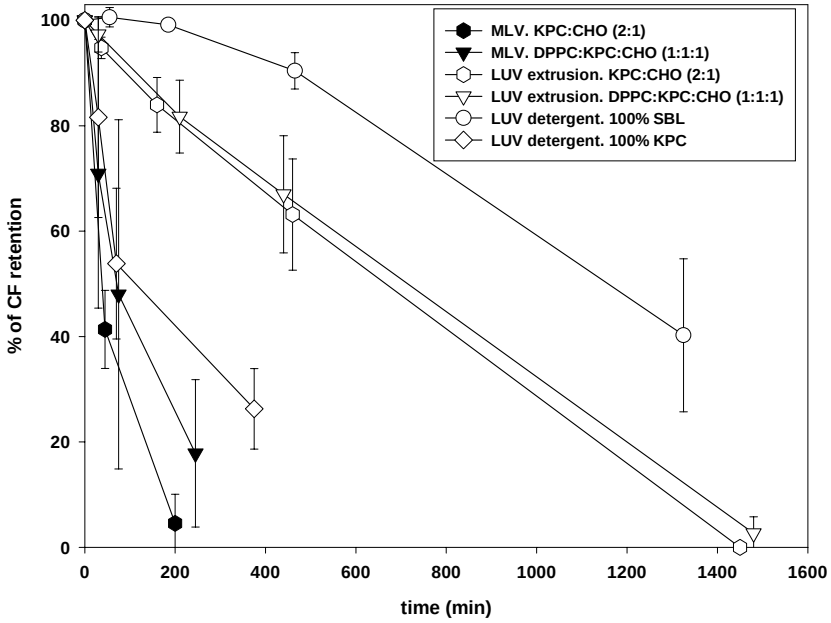
The *Artemia* nauplii enrichment has been achieved after 24h treatment in 100-ml tubes in the same temperature, aeration, and density conditions above mentioned. Final lipid concentration was 0.6mg.ml^{-1}.

Lipid extraction and fatty acid analyses have been carried out following the methods described in Navarro et al. (1992).

Results and discussion

Liposomes rich in PUFA show an important loss of the water-soluble vehiculated materials under enrichment conditions (Fig. 1). In a few hours, the water-soluble fluorescent marker is completely lost. These results show an important difference compared to the evidence found previously with liposomes

stored at room temperature and stable conditions (Albaladejo et al., 1998). Under these conditions, the PUFA rich liposomes retained the fluorescent markers for several days. This is an important issue to take into account, since the possibility to bioencapsulate water-soluble molecules has been one of the main advantages proposed for the use of liposomes as enrichment product for *Artemia* nauplii. The reduction of PUFA rich phospholipids do not decreases the leakage, unless PUFA phospholipids are totally absent. The preparation method does not interfere either. However, some unilamellar vesicles with cholesterol in its composition seem to have a slightly better retention. At least, they can maintain a retention around 60% for 10h. This is probably due to the proximity



between the different bilayers in MLV liposomes, situation that can be avoided with LUV.

Fig. 1. Percentage of carboxyfluorescein (CF) retention of different liposome preparations in enrichment conditions. See text for compositions and liposome type.

Liposomes with high PUFA content are able to modify Artemia nauplii lipid profile. As shown in Table I, eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids contents in nauplii enriched with pure KPC liposomes are similar to the amounts found in nauplii enriched with the control emulsion.

Liposomes seem an adequate vehicle to deliver different products to marine larvae through bioencapsulation in live preys. However, more efforts are necessary to devise better formulations that allow a single particle type useful both for water-soluble and lipophilic molecules.

Table I. Selected fatty acids from total lipids (weight %) of *Artemia* nauplii enriched with three liposome products and the emulsion Super Selco.

Fatty acid	SBL:KPL (1:1)	SBL:KPC (3:7)	100% KPC	Super Selco
16:0	10.9	12.9	11.9	8.1
16:1	3.3	11.7	10.5	10.5
18:0	4.1	4.7	4.2	3.7
18:2n-6	19.4	10.2	5.5	5.8
18:3n-3	16.1	3.1	2.4	2.3
20:5n-3	6.0	10.2	16.3	14.1
22:6n-3	1.8	0.8	5.4	5.8

References

Albaladejo M.A., F. Amat, F. Hontoria, and J.C. Navarro. 1998. Stability of liposomes as enrichment diets for live food. VIII International Symposium on Nutrition and Feeding of Fish. Las Palmas de Gran Canaria (Spain).

Bangham A.D., M.M. Standish, and J.C. Watkins. 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13:238-252.

Hontoria F., J.H. Crowe, L.M. Crowe, and F. Amat. 1994. Potential use of liposomes in larviculture as a delivery system through *Artemia* nauplii. Aquaculture 127:255-264.

McEvoy L.A., J.C. Navarro, F. Hontoria, F. Amat, and J.R. Sargent. 1996. Two novel *Artemia* enrichment diets containing polar lipid. Aquaculture 144:339-352.

New R.R.C. 1990. Liposomes. A practical approach. IRL Press, Oxford. 301p.

Navarro J.C., M.V. Bell, F. Amat, and J.R. Sargent. 1992. The fatty acid composition of phospholipids from brine shrimp, *Artemia* sp., eyes. Comp. Biochem. Physiol. 103B:89-91.

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LARVAL NUTRITION OF *PALAEMON SERRATUS* (PENNANT, 1777): GROWTH AND FATTY ACID PROFILE

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Introduction

Palaemon serratus Pennant, 1977 (Palaemonidae), is a caridean prawn common in the Mediterranean and in the Atlantic. It is an important fishery resource in southern Europe, due to its high commercial value and it seems to be a suitable species for extensive culture in salt marshes (Rodriguez, 1981). However it is necessary to optimize larval rearing, which is still considered one of the main constraints for commercial exploitation. Although some work has been published on *P. serratus* larval rearing in the last decades (Campillo, 1979, Rodriguez, 1981; Luis and Narciso 1987; 1990; Narciso, 1996), little as been done to understand the fatty acids requirements of this species.

The experiment reported in this paper evaluates the influence of six diets, using *Artemia franciscana* nauplii and enriched metanauplii, on the growth and fatty acid profile of *P. serratus* larvae.

Materials and methods

The larvae were obtained from wild ovigerous females, maintained in the dark in a 240-l hatching tank at 21±1ºC and 30±1‰, with a removable larvae concentrator covered with a 390-µm nylon mesh (Luis and Narciso, 1987). Larvae were placed in 6 l conical flasks, at a density of 50 zoea.l^{-1}, a temperature of 25±1ºC, a salinity of 30±1‰. The photoperiod was 8 hours light: 16 hours dark. Seawater was renewed every 2 days.

Six live diets were tested (Table I). All treatments were tested in duplicate. The *Artemia franciscana* cysts (GSL, Utah) were decapsulated and hatched under standard conditions (Sorgeloos et al., 1986). Enrichments were carried in 6-l conical tanks during more 24h. The food density was maintained at 5 *Artemia* nauplii/ml in the larval rearing flasks.

Growth was expressed in terms of total length (TL), from rostrum to tailfin, under a stereo microscope with a calibrated micrometer. Total lipid extraction was carried out according to Blight and Dyer (1959) and saponification and esterification of the lipid extracts was done using the method of Metcalfe and Schmitz (1961). The fatty acid methyl esters (FAME) were injected into a capillary column (30m fused silica, 0.32 I.D.) installed in a Varian Star 3400CX gas-liquid chromatograph. GLC data acquisition and handling was done through a Varian integrator 4290. Peak quantification was carried out with a Star Chromatography workstation installed in an IBM PS/1. Peak identification was carried out using as reference well-characterized cod liver oil chromatograms. Duplicate samples were analyzed.

Table I. Experimental feeding regimes.

Larval stages	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Zoea I	*Artemia* nauplii	*Artemia* nauplii	*Artemia* nauplii	*Artemia* nauplii	*Artemia* nauplii	*Artemia* nauplii
Zoea II onwards	*Artemia* nauplii	*Artemia* metanuplii in Algamac2000®	*Artemia* metanuplii in *Spirulina* sp	*Artemia* metanuplii in SuperArtemia®	*Artemia* metanuplii in Frippak®	*Artemia* metanauplii in SuperRotifer®

Results and discussion

Significant differences (P<0.05) were found in growth from zoea IV onwards in all treatments (Fig. 1).

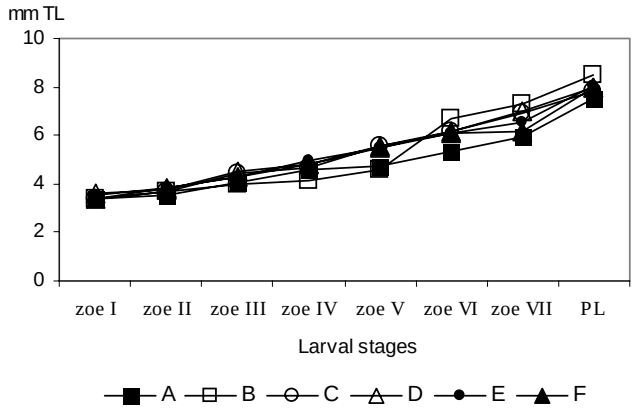

Fig. 1. Larval growth (TL) of *Palaemon serratus* fed on 6 different diets.

From the total length data, Hiatt diagrams (Forster, 1970) were composed to understand how growth type was affected by the diets (Fig. 2) Through the

regression equations it can be concluded that all diets were responsible for a progressive geometric growth. These results seem to point out that all diets are suitable to feed *Palaemon serratus*. Nevertheless, the best growth results were achieved with diet B (*Artemia* metanauplii in Algamac 2000).

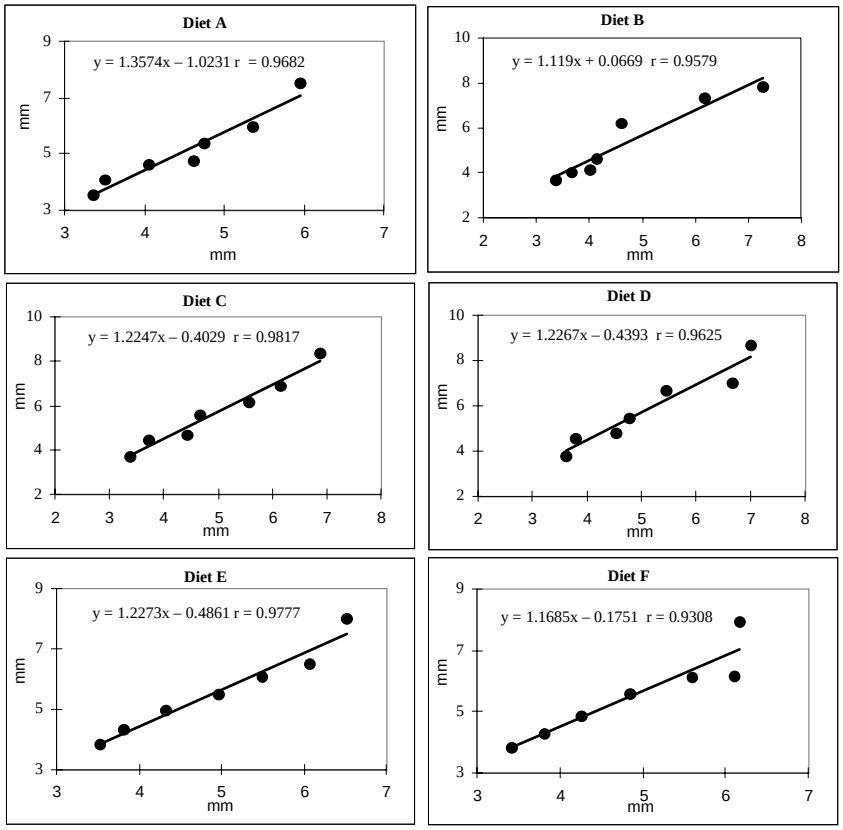

Fig. 2. Hiatt diagrams for *Palaemon serratus* larval stages fed on the 6 diets.

The fatty acid profile of post-larvae presents small DHA amounts in all treatments (Fig. 3). This result can be due to the fact that all diets are composed of *Artemia* nauplii and metanauplii which hasn't DHA in their composition and when supplied by diet it is rapidly catabolized (Lavens et al., 1995).

The higher values were shown for diet B (metanauplii enrich with Algamac 2000 (1.78μg.mg^{-1} of DHA and 0.3 DHA/EPA ratio), explained by the fact of this product being extremely rich in PUFA (40%) which 70% are DHA.

Conclusions

The results obtained in this study with larval rearing of the prawn *Palaemon serratus* clearly showed the importance of nutrition on larvae growth and the importance of *Artemia* enrichment, mainly with Algamac 2000. With this diet, it was achieved the highest mean total length. It can also be assumed that the addition of lipids, mainly high unsaturated fatty acids to the diet, through *Artemia* enrichment it is a very recommended procedure.

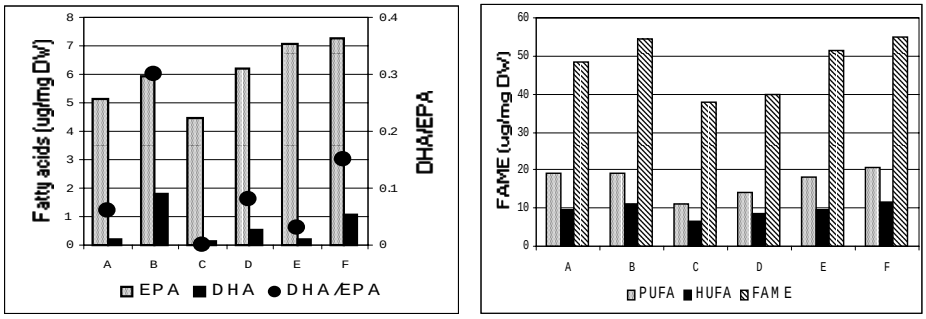

Fig. 3. Fatty acid profile of post-larvae fed on the six live diets.

References

Blight E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Campillo A. 1979. Contribution à l'étude de la crevette rose *Palaemon serratus* Thèse Doctorat en Science, Nat., Univ. Sci. Tech. Languedoc. 247p.

Forster, J.R., 1970. Further studies on the culture of the prawn *Palaemon serratus*, with emphasis on the post-larval stages. Fish. Invest. Lond. Ser. 2, 26(6), 40 pp.

Lavens P., P. Sorgeloos, P. Dhert, and B. Devresse. 1995. Larval foods in: Broodstock management and egg and larval quality. p.373-379. In *Broodstock management and egg and larval quality* Bromage N.L. and Roberts R.J. (Eds) Blackwell.

Luis O.J. and L. Narciso. 1987. Growth, survival and fatty acidsof *Palaemon serratus* larvae fed four livediets reared in large scale volume. Cuad. Marisq. Publ. Téc. 12:295-300.

Luis O.J. and L. Narciso. 1990. Efeito da dimensão das presas no crescimento e sobrevivência de larvas de *Palaemon serratus* cultivadas em grande volume. Actas III Congresso Nac. Acuicult.:325-330

Metcalfe L.D. and A.A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatography analysis. Anal. Chem. 33:363-364.

Narciso L. 1993. Growth and survival of *Palaemon serratus* larvae fed eight live feeds. Actas IV Congresso Nac. Acuicult.:263-268.

Rodriguez A. 1981. Growth and sexual maturation of *Penaeus kerathurus* and *Palaemon serratus* in salt ponds. Aquaculture 19:325-337.

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THE EFFECT OF DIFFERENT LIVE DIETS IN THE FIRST ZOEAL STAGES OF THE NORWAY LOBSTER *NEPHROPS NORVEGICUS* (L.) (CRUSTACEA: DECAPODA).

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Introduction

The Norway lobster (*Nephrops norvegicus* (L.)) is a species of high commercial interest in the NE/E Atlantic Ocean, from Greenland to Morocco and in the Mediterranean Sea. Despite the early interest (in the 70's) on the larviculture of this species, few studies have been concerned with zootechnical and nutritional requirements. One of the main problems concerning crustacean's larviculture is the larval nutrition. Nevertheless, most of the previous experiments have not been conducted in order to study the nutritional requirements of early larval stages. The vital importance of some fatty acids in larval development has been revealed and recent studies (Pousão-Ferreira et al., 1999), have shown that the docosahexaenoic acid (DHA; C22:6n-3) / eicosapentaenoic (EPA; C20:5n-3) ratio of the eggs and early larval stages of marine organisms are similar. Therefore, the fatty acid profile of the eggs can easily be used to evaluate the quality of different diets used in larval rearing. The study of the fatty acid profile of the first zoeal stages can be an excellent way to evaluate larval quality and therefore predict the suitability of the diet to the demanding larval stages.

Being *Nephrops norvegicus* a deep cold water species, special attention must be paid to its highly unsaturated fatty acid (HUFA) profile and DHA/EPA ratio.

The main objective of the present work was to study the effect of different live diets (unenriched *Artemia* nauplii and metanauplii enriched with *Spirulina* sp. and Algamac 2000®) on larval survival, growth and fatty acid profile.

Materials and methods

Ovigerous females, caught at 400-600m depth, were brought to the laboratory after being landed by commercial boats. Eggs in an advanced development (stage D_f, according to the scale proposed by Figueiredo et al. (1983)) were

sampled for biochemical analysis. The females were placed in the dark until hatching, at 35‰ salinity and 12ºC and with moderate aeration. The larvae were placed in 2-l beakers, with moderated aeration, at a density of 15 larvae.l^{-1}. Salinity was maintained at 35‰, the photoperiod was 8h light:16h dark and a constant temperature of 12ºC was used.

Artemia franciscana (Kellogg, 1906) cysts from Prime Artemia Inc® strain (lot 552530) were hatched under standard conditions (Sorgeloos et al., 1986). Two different enrichment products were used: freeze dried microparticulate *Spirulina* sp. and Algamac 2000® (Aquafauna – Biomarine Inc). The enrichment was conducted during 16h in 1 l beakers, with strong aeration and a maximum density of 50 nauplii.ml^{-1}; 0.1g of product was used in 1 l of seawater. Food was daily supplied at a density of 3000 prey.l^{-1}.

Surviving larvae of the second zoeal stage were sampled and carapace length was measured, under a stereo microscope (Olympus®, model SZ6045TR) with a calibrated micrometer eyepiece.

The samples were processed according to procedures described Blight and Dyer (1959) and Metcalfe and Schimtz (1961). The fatty acid methyl esters (FAME) were injected into a capillary column (30m fused silica, 0.32I.D.) installed in a Varian Star 3400CX gas-liquid chromatograph (Varian Analytical Instruments, CA). Helium was used as carrier gas at a flow rate of 1ml.min^{-1}, the oven temperature was 180ºC for 7min and then 200ºC (with a temperature gradient of 4ºC.min^{-1}) over a period of 71min. Both the injector and the FID detector were set at 250ºC. Peak quantification was done with a star chromatography workstation installed in an IBM PS/1.

Variance analysis (ANOVA) was used and differences between treatments means were determined using the Tukey-Kramer's test. All results were considered statistically significant at the 0.05 probability level.

Results and discussion

The larvae fed on different live diets showed similar survival rates (P>0.05) - unenriched nauplii (52%), metanauplii enriched in *Spirulina* sp. and Algamac 2000® (42%). Nevertheless, these results are far from the survival rates presented by Anger and Püschel (1986) (92% to the second larval stage).

Larvae on zoeal stage II fed on unenriched *Artemia* nauplii presented a significantly higher carapace length (2.53mm) than the larvae fed on metanauplii enriched in *Spirulina* sp. (2.4mm) and Algamac 2000® (2.35mm) (P=0.0005 and P=0.0082, respectively). This difference could be explained by the relative

protein/lipid uptake from the diet, since higher lipid content can lead to a lower prey ingestion, in order to meet the energetic requirements.

The biochemical analysis revealed, as expected, some similarities between the fatty acid profile of advanced eggs (D_f) and newly hatched larvae, especially in the DHA/EPA and n-3/n-6 ratios (Table I). Therefore, the fatty acid profile of stage D_f eggs can be used to evaluate the nutritional quality of the diets used in *N. norvegicus* larval rearing, as well as the nutritional quality of the reared larvae.

Table I. Fatty acid profile ($\mu g.mg^{-1}$ ± standard deviation) of D_f eggs, newly hatched larvae and larvae reared on different diets.

	Stage Df eggs	Newly hatched larvae	Larvae fed on unenriched nauplii (12ºC)	Larvae fed on metanauplii enriched in Algam.2000 (12ºC)	Larvae fed on metanauplii enriched in *Spirulina* (12ºC)
18:2n-6	2.19±0.10	1.00±0.14	5.03±0.68	4.27±0.16	3.95±1.68
18:3n-3	0.79±0.05	0.37±0.04	11.13±7.24	6.12±0.17	7.04±1.94
20:4n-6	2.66±0.11	3.04±0.31	1.74±0.75	2.07±0.21	1.43±0.13
20:5n-3	13.94±1.33	13.18±0.52	7.09±1.87	7.20±0.63	5.19±0.55
22:6n-3	16.38±1.07	11.40±0.83	2.48±1.06	4.12±0.04	1.94±0.07
Saturated	29.10±1.18	20.40±1.70	19.54±9.23	15.84±1.53	11.94±2.47
Monounsat.	54.64±2.40	32.23±2.31	21.87±11.87	16.47±0.95	13.41±3.20
Diunsat.	3.39±0.17	1.67±0.18	5.68±0.45	4.79±0.20	4.39±1.70
PUFA	41.89±2.88	32.66±2.22	35.26±13.11	29.25±1.46	24.44±5.06
HUFA	38.46±2.81	30.84±2.04	17.01±5.30	17.30±1.05	12.01±1.02
Branched	1.13±0.01	0.97±0.01	3.23±2.60	1.49±0.23	1.25±0.32
Σn-3	35.09±2.55	27.38±1.66	26.94±12.33	21.27±1.00	17.88±3.34
Σn-6	6.80±0.33	5.29±0.56	7.85±0.51	7.58±0.42	6.25±1.69
n-3/n-6	5.16±0.12	5.19±0.23	3.39±1.35	2.81±0.03	2.90±0.25
DHA/EPA	1.18±0.04	0.86±0.03	0.34±0.06	0.57±0.06	0.38±0.03
FAME	130.14±6.73	87.92±6.42	85.56±36.36	67.83±4.37	55.41±12.75

Although the larvae fed on metanauplii enriched in Algamac 2000® showed the highest HUFA content and DHA/EPA ratio, all treatments presented a lower fatty acid content than stage D_f eggs (Table I). These results may be explained by the hard task of providing C20 and C22 HUFA's to the larvae. Several authors have reported that it is quite difficult to achieve a high DHA/EPA ratio when enriching *Artemia* nauplii. This difficulty is mainly due to the rapid

catabolism of DHA during the enrichment process and the starvation following enrichment (Narciso et al., 1999).

Conclusions

Since none of the tested diets completely fulfilled the estimated larvae's nutritional requirements, other diets should be used in future studies (e.g., *Artemia* metanauplii enriched in *Dunaliella terttiolecta* used by Anger and Püschel (1986)). These studies allow comparisons between the fatty acids profile of reared larvae with the eggs, in order to evaluate the nutritional suitability of different diets for *N. norvegicus* larval rearing. Nevertheless, different prey species (i.e., small adult *Artemia* and other zooplankton) and different enrichment products should also be analyzed, in order to solve some of the unanswered questions presented by this study.

References

Anger K. and C. Püschel. 1986. Growth and exuviation of Norway lobster (*Nephrops norvegicus*) larvae reared in the laboratory. Ophelia 25(3):57-167.

Blight E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Figueiredo M.J., O. Margo, and M.G. Franco. 1983. The fecundity of *Nephrops norvegicus* (L.) off the Portuguese West coast. Boletim do Instituto Nacional de Investigação das Pescas 9:5-16.

Metcalfe L.D. and A.A. Schimtz. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Analytical Chemistry 33:363-364.

Narciso L., P. Pousão-Ferreira, A. Passos, and O. Luis. 1999. HUFA content and DHA/EPA improvements of *Artemia* sp. with commercial oils during different enrichments periods. Aquacult. Res. 30:21-24.

Pousão-Ferreira P., S. Morais, E. Dores, and L. Narciso. 1999. Eggs of gilthead seabream *Sparus aurata* L. as a potential enrichment product of *brachionus* sp. in the larval rearing of gilthead seabream *Sparus aurata* L. Aquacult. Res. 30:751-758.

Sorgeloos P., Ph. Lavens, P. Léger, W. Tackaert, and D. Versichele. 1986. Production of *Artemia* nauplii and decapsulated cysts for use as food source in the hatchery production of fish and crustaceans. p.69-117. In: FAO Manual for the Culture of Brine Shrimp *Artemia* in Aquaculture. University of Ghent, Ghent, Belgium. 319p.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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A COMPARISON OF RETINOL, RETINAL, AND RETINYL ESTER CONCENTRATIONS IN LARVAE OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.) FED *ARTEMIA* OR ZOOPLANKTON

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Introduction

Vitamin A is one of several important factors participating in transcription of genes and is known to be teratogenic in both excess and deficiency. It is essential to cell differentiation and proliferation regardless of species. Metamorphosis in Atlantic halibut represents major changes (e.g., eye migration, pigmentation). To achieve a successful metamorphosis, there is probably a specific requirement for hormones and micronutrients that participates in cell division and gene regulation such as vitamin A. Zooplankton is the natural feed for the larvae, but *Artemia* is regularly used as feed in the production of Atlantic halibut larvae. *Artemia* and zooplankton contains different forms of carotenoids (Rønnestad et al., 1998) which fish can metabolize into vitamin A (Guillou et al., 1989).In this study we wanted to investigate whether the two different live organisms resulted in different vitamin A concentrations and compositions in the larvae.

Materials and methods

The Atlantic halibut larvae feeding experiment was carried out at the Institute of Marine Research, Austevoll Aquaculture Research Station, Storebø, Norway. At 260 degreedays (6°C) the larvae were stocked in four 1500-l upwelling tanks at approximately 5000 larvae.tank^{-1}. The larvae in two of the tanks were fed enriched *Artemia* (RH-type, INVE Aquaculture NV, Belgium enriched for 20h with one dose of 0,3 g.l^{-1} DC-DHA-Selco (INVE, Ghent, Belgium), + vitamins) and the larvae in the two other tanks were fed zooplankton collected from a fertilized seawater pond (Svatatjønn) at Storebø, Norway.

Samples for vitamin A analyses of the larvae were collected on days 0, 15, 29, 45, and 60 after the first feeding. Two parallel samples of larvae were taken from each

tank. Samples of zooplankton were taken only at days 0, 45, and 60, while samples of *Artemia* were taken at days 0, 29, 45, and 60. Two different HPLC methods were used to analyze total vitamin A (adapted and modified from Nöll 1996) and retinol, retinal, and retinyl esters (adapted and modified from Bankson, 1986).

Results and discussion

Malpigmentation, free dorsal fin, and eye migration were registered at the end of the feeding experiment (Table I). Larvae fed *Artemia* had a higher incidence of unsuccessful metamorphosis.

Table I. Pigmentation, metamorphosis success and larvae with free dorsal fin in Atlantic halibut larvae fed zooplankton or *Artemia* for 60 days (% of population n = 60).

	Artemia	Zooplankton
Malpigmentation	93	32
Minor or no eye migration	90	12
Larvae with free dorsal fin	82	33

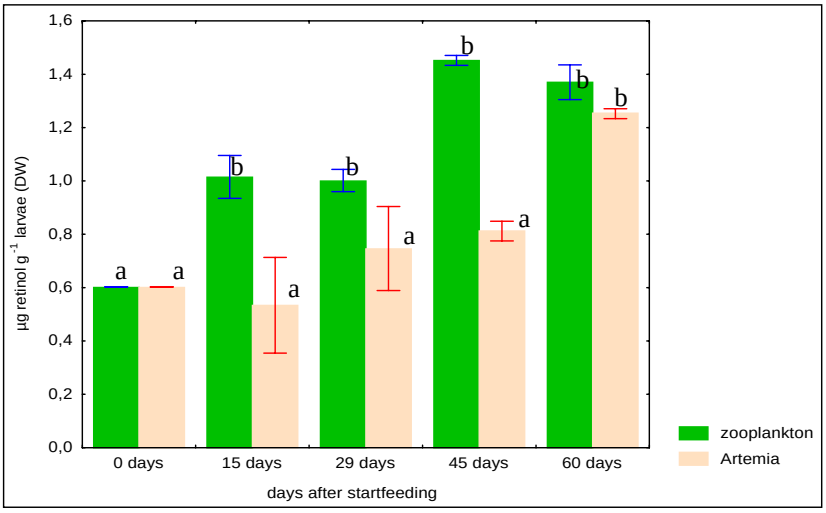

Fig. 1. Concentration of total vitamin A in Atlantic halibut larvae fed *Artemia* or zooplankton (n=2). Different letters indicate significant differences (Tukey HSD test, P< 0.05)

Although *Artemia* contain more vitamin A than zooplankton (Table II), larvae fed zooplankton had a higher level of vitamin A than larvae fed *Artemia* (Fig. 1). This difference might be explained by the low digestibility of *Artemia* or by the different carotenoids that might be metabolized to an unequal extent.

Table II. The concentration of vitamin A in the feed (*n*=2,exept for *, where *n*=3).

Type of feed	Total vitamin A concentration µg retinol/g feed (DW)			
	start	29 days	45 days	60 days
Artemia	1.31±0.04*	2.0±0.2	2.3±0.9*	4.2±0.2*
Zooplankton	0.23±0.02	Not analyzed	0.27±0.03	2.7±0.5

Regardless of the reason, the result in this experiment was as expected: a lower level of vitamin A in larvae fed *Artemia*. Exogenous retinoic acid has been shown to alter the pigmentation in Japanese flounder (*Paralichthys olivaceus*) only when the treatment was given to the larvae at late premetamorphosis and early postmetamorphosis (Satoshi and Yamano, 1999). Estevez and Kanasawa (1995) enriched *Artemia* with vitamin A and found the best pigmentation rates in the group of *Scophtalmus maximus* L. fed an overdose of vitamin A. Although a certain level of vitamin A probably is one of the requirements for successful pigmentation, this study has revealed that *Artemia*-fed larvae contain a considerable amount of vitamin A. To our knowledge, the requirement for vitamin A has not been established for Atlantic halibut larvae. Consequently, the difference seen in this study cannot lead to the conclusion that *Artemia* result in vitamin A deficiency. On the contrary, one needs to know more about the function, the metabolism and the requirements of vitamin A. The analyses of free retinol, retinal, and retinyl esters are not completed but will be presented and discussed at the conference.

References

Bankson D.D., R.M. Russell, and J.A. Sadowski. 1986. Determination of retinyl esters and retinol in serum or plasma by normal phase liquid chromatography: method and applications. Clin. Chem. 32:35-40.

Estevez A. and A. Kanasawa. 1995. Effect of (n-3) PUFA and vitamin A *Artemia* enrichment on pigmentation of turbot, *Scophtalmus maximus* (L). Aquacult. Nutr. 1:159-168.

Guillou A., G. Choubert, T. Storebakken, J. Delanoue, and S. Kaushik. 1989. Bioconverion pathway of astaxanthin into retinol-2 in mature Rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 94:481-485

Nöll G.N. 1996. High-performance liquid chromatographic analysis of retinal and retinol isomers. J. Chromatogr. 721:247-259

Rønnestad I., G-I. Hemre, R.N. Finn, and Ø Lie. 1998. Alternate sources and dynamics of vitamin A and its incorporation into the eyes during early endotrophic and exotrophic larval stages of Atlantic halibut (*Hippoglossus hippoglossus* L.). Comp. Biochem. Physiol. 119A:787-793.

Satoshi M. and K. Yamano. 1999. Retinoic acid stimulates development of adult-type chromatophores in the flounder. J. Exp. Zool. 284:317-324.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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GENE EXPRESSION AND BIOCHEMICAL ACTIVITY OF VARIOUS DIGESTIVE ENZYMES DURING LARVAL DEVELOPMENT IN ATLANTIC COD (*GADUS MORHUA*) AND HADDOCK (*MELANOGRAMMUS AEGLEFINUS*)

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Introduction

Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) are being developed for aquaculture in Atlantic Canada. As with other marine species, larval cod and haddock production depends upon live foods as the primary diet. Since bacteria associated with live feeds can have a negative impact on larval health, and the production of live feed is labour-intensive and expensive, there is a great deal of interest in replacing live feed with less costly dry diets. Unfortunately many of these diets do not result in good performance.

Biochemical studies on digestive activities of marine fish larvae are relatively common and techniques for enzyme activity studies are widely available (Gawlicka et al., 2000). In addition, a number of genes coding for key digestive enzymes in winter flounder have been isolated (Douglas et al., 1998; 1999a; 1999b; 2000) and shown to work well as probes for *in situ* hybridization studies (Gawlicka et al., 2001). In our lab, we are using molecular, *in situ* hybridization, and biochemical techniques to determine the timing and localization of digestive enzyme gene expression during the ontogeny of a variety of marine fish species under different feeding regimes. The goal of this research is to provide relevant information to aid in formulating new larval feeds designed to replace live feeds, thereby enabling the industry to increase the efficiency of juvenile production. In this paper, we present preliminary results for both gene expression and biochemical studies of cod and haddock larvae collected from 8-45 days post-hatch (DPH).

Materials and methods

Atlantic cod larvae were obtained from the Aquaculture Research and Development Facility (ARDF) at the Ocean Science Centre, Memorial University of Newfoundland. Haddock larvae were obtained from the National Research Council Aquaculture facility, Sandy Cove, Nova Scotia. Larvae were reared at 10-12°C following a standard protocol, and fed enriched rotifers and *Artemia*. Samples were collected in the morning prior to feeding. Biochemical studies were conducted on extracts obtained from pools of 100-300 whole larvae. Total protease, trypsin, pepsin, and alkaline phosphatase activity was determined according to Parent (1998).

Larvae (30-40) were fixed for *in-situ* hybridization studies in Ambion *in situ* fixative (10% formalin in Tris, pH 7.0) following the manufacturer's suggested procedure. Serial sections (7 μm) were cut, placed on glass slides treated with 3-aminopropyl triethoxysilane, and baked overnight in a 60°C oven. Pepsinogen (IIa and IIb) and trypsin RNA probes were produced by *in vitro* transcription as described (Gawlicka et al., 2001) using a digoxigenin UTP labeling kit (Roche). Probes were based upon clones isolated from a winter flounder cDNA library (Douglas and Gallant, 1998; Douglas et al., 1999b) and the *in situ* hybridization was performed as described by Murray (2000).

Results and discussion

In situ hybridization results for pepsinogen IIa and IIb and pancreatic trypsinogen showed that RNA probes based upon winter flounder cDNAs hybridize to homologues in unrelated species such as cod and haddock and can therefore be used to study gene expression in these species (Fig. 1).

Using biochemical techniques, total protease activity of whole body homogenates of haddock larvae generally declined from 8 DPH (Fig. 2). This decline may be due to increasing levels of protease inhibitors in larger larvae or a decline in the portion of body mass contributed by the digestive tract in older haddock. Trypsin activity was evident in haddock at 8 DPH and there was a marked increase in trypsin activity at 25 DPH (Fig. 2) that remained high through 45 DPH. These observations compare well with the results of the *in situ* hybridization study in which trypsinogen gene activity could be identified in the youngest (10 DPH) samples examined for haddock. Trypsin activity in older (45 DPH) haddock and cod was present in pancreatic tissue distributed between the pyloric caeca (Fig. 1C,D).

Variable levels of pepsin activity could be demonstrated biochemically in haddock from 8 to 25 DPH. Pepsin activity increased markedly in older haddock larvae (Fig 2). A similar pattern of activity was seen using *in-situ* hybridization. Pepsin expression was restricted to the glandular region of the cardiac stomach in 45-day-old haddock and cod (Fig. 1A,B), identical to that described for winter flounder juvenile stomach using the same probes (Gawlicka et al., 2001). Signal

was detectable at 35 days but was minimal at 25 days for both species, in agreement with previous descriptions of histologically identifiable gastric glands in these species (Hamlin et al., 2000).

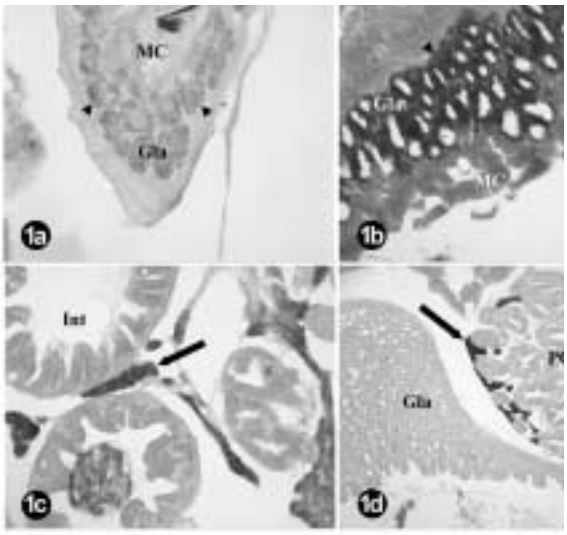

Fig. 1. Tissue distribution of pepsinogen (1a,b) and trypsinogen (1c,d) gene expression in 45 DPH Atlantic cod (1a,c) and haddock (1b,d). Gla, gastric glands, MC, mucous cells, Int, intestine.

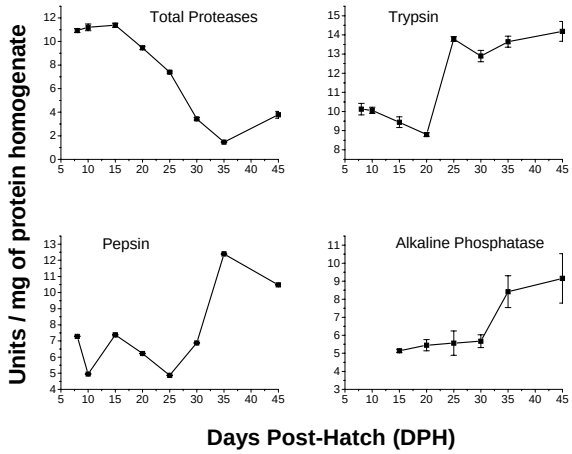

Fig. 2. Biochemical analysis of total protease, trypsin, pepsin, and alkaline phosphatase activity in whole body homogenates of larval haddock.

This trend suggests that the stomach becomes functional in the cod and haddock at 25-35 DPH. Pepsin-like activity determined using biochemical analysis in younger haddock could be due to the activity of lysosomal source enzymes like cathepsins. Alkaline phosphatase activity in haddock increased from the earliest

sample examined (15 DPH) through 45 DPH (Fig. 2).

Conclusions

We have demonstrated that probes generated from winter flounder cDNA can be used to localize pepsinogen and trypsinogen gene expression in cod and haddock. In general, there was good agreement between the results of the biochemical studies and those of the *in situ* hybridizations. Biochemical methods can be biased due to enzyme activity from the live prey items within the gut. *In situ* hybridization studies can identify and localize gene expression that may or may not correspond to the expression of active protein. Together, these two techniques provide a good picture of the development of the digestive process in larval fish.

References

Douglas S.E. and J.W. Gallant. 1998. Isolation of cDNAs for trypsinogen from the winter flounder, *Pleuronectes americanus*. J. Mar. Biotechnol. 6:214-219.

Douglas S.E., A. Gawlicka, S. Mandla, and J.W. Gallant. 1999a. Ontogeny of the stomach in winter flounder: characterization and expression of the pepsinogen and proton pump genes and determination of pepsin activity. J. Fish. Biol. 55:897-915.

Douglas S.E., C.E. Bullerwell, and J.W. Gallant. 1999b. Molecular investigation of aminopeptidase N expression in the winter flounder, *Pleuronectes americanus*. J. Appl. Ichthyol. 15:80-86.

Douglas S.E., S. Mandla, and J.W. Gallant. 2000. Molecular analysis of the amylase gene and its expression during development in the winter flounder, *Pleuronectes americanus*. Aquaculture 190:247-260.

Gawlicka A., B. Parent, M.H. Horn, N. Ross, I. Opstad, and O.J. Torrissen. 2000. Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): indication of readiness for first feeding. Aquaculture. 184:303-314.

Gawlicka A., C.T. Leggiadro, J.W. Gallant, and S.E. Douglas. 2001. Cellular expression of the pepsinogen and gastric proton pump genes in the stomach of winter flounder as determined by *in situ* hybridization. J. Fish Biol. 58:529-536.

Hamlin H.J., I. Hunt von Herbing, and L.J. Kling. 2000. Histological and morphological evaluations of the digestive tract and associated organs of haddock throughout post-hatching ontogeny. J. Fish Biol. 57:716-732.

Murray H.M. 2000. The ontogeny of Type I antifreeze protein expression in winter flounder (*Pleuronectes americanus*). Ph.D. Thesis. Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Parent B. 1998. Determination of activity of digestive enzymes in Atlantic halibut and winter flounder using spectrophotometry and zymography. Report. National Research Council's Institute for Marine Biosciences, Canada.

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SPAWNING AND LARVAL REARING OF THE MARINE PURPLE SNAIL *PLICOPURPURA PANSA* (GOULD, 1853)

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Introduction

The carnivorous marine purple snail *Plicopurpura pansa* (Gould, 1853) inhabits exposed rocky shores along the Pacific coast of the Americas from the northwest coast of Mexico to northern Peru. In 1988, the Mexican government declared *P. pansa* as a protected species because of its over-exploitation for "Tyrian Purple".

Despite the economic importance of the purple snail, little is known about the spawning and the conditions necessary for rearing larvae and juveniles. This information is a prerequisite for the development of techniques for restocking and recovery of natural stocks.

Materials and Methods

Adult snails (300: shell length 27.61±4.79mm, wet weight: 3.41±1.74g) were collected between October and December 1999 from the Pacific coast, at Playa Cerrito, Baja California Sur, Mexico (23°19´54"N and 110°10´38"W), from intertidal rocks in the low intertidal area with high impact waves. The snails were transferred to the laboratory (CICIMAR, La Paz) and randomly distributed into six inverted glass bottles with cut-off bottoms filled with 10 l of seawater (salinity: 30-34‰, daily water exchange; temperature range: 21-23°C). Snails were fed daily with unlimited amounts of squid, mussels, or beef heart.

In February 2000, the first egg capsules were found attached to the walls of the glass bottles. The peak of reproduction was between May and June. A second spawning season occurred between September and October. Mature two months old egg capsules were opened to liberate the fully developed veliger larvae. The larvae were transferred into culture vessels (0.8 l and 8.0 l) made out of PVC with bottoms of nylon filter. Mesh aperture of the nylon filters was varied during development from 180µm during the first weeks of culture to 300µm and later 500µm, floating in 50-l containers filled with 5-µm filtered and UV-treated

seawater. Salinity was adjusted with brine to 35‰ and the temperature was maintained at 22-23°C. A continuous flow of oxygenated water was obtained via airlift pumps installed in the containers. The water was changed every 3-4 days. The larvae were fed daily with a final concentration of 10 000 cells.ml^{-1} of the microalgae *Isochrysis galbana* (Taihiti strain) and *Thalasiosira weissflogii* (formerly *T. fluviatilis*) (1:1). The growth and survival rate was estimated weekly.

Results and Discussion

The egg capsules of the purple snail are laid in a compact mass of multiple capsules (up to 30 capsules per deposition) firmly attached to the glass of the culture vessels. More capsules are added daily to the periphery of previously deposited capsules so that eventually, egg clusters with hundreds of capsules of different ages spawned by different females are formed. During a culture period of five months, 15 females deposited more than 2000 capsules on the wall of one glass bottle.

The capsules are the size and shape of lentils (*n*= 45, length: 4.36±0.73mm, width: 3.34±0.78mm, height: 1.66±0.32mm). The oval escape aperture (length 0.6mm, width 0.45mm) is placed centrally on top of the egg capsules. Freshly spawned egg capsules are gelatinous and harden after a few days. Immediately, capsule walls were white and moderately translucent with the eggs inside clearly visible. With aging, the colour of the capsules changed to coffee brown. Capsules containing non-viable embryos turned purple. A single capsule, depending on size, contains an average of 439 ± 160 eggs (*n*=19) with as diameter of 188±13µm (*n*=50). The intracapsular development to a free-swimming veliconcha stage at 22-23°C occurs in 6-8 weeks and hatching occurred asynchronously among capsules. At the time of hatching torsion was already completed so that the larvae were able to retract its head and foot into the shell. At the time of hatching the shell measured 0.35×0.25mm, appeared translucent allowing observation of the beating heart and intestine.

The shell size nearly doubled from 282 to 477µm during the first four weeks, and during the following four weeks to 902µm. After this rapid initial increase the size of the shell did not increase significantly during the following 7 weeks. The shell size increased to only a little bit more than 1mm (1004µm).

At the time of hatching the velum of the veliconcha was bilobed (area 37µm^2) and increased nearly ten times to a “butterfly-shaped” velum during the following three weeks (347µm²). After this period the growth rate followed the size increase of the shell and reached a surface area of 937µm^2 after six weeks, and 976µm^2 after 20 weeks.

As a means to control and improve the culture conditions we determined the survival rate of the larvae. A steady, but slight decrease if the number of animals was observed. After 11 weeks of the experiments, we obtained a survival rate of more than 50%.

In our experiments we were not successful to induce naturally metamorphosis through natural substrates, strong wave actions, and films from adult snails attached to the culture vessels. However, the addition of 15mM KCl to the seawater found to be effective with four months old pediveligers. The larvae responded with velar convulsions and erratic swimming for a few minutes, and withdrew partially into the shell. A few hours later the larvae exhibited substratum-searching behaviour, and after 12 hours started to crawl on the bottom of the glass jar. However, the animals did not shed their velum. It stayed functional and after 24 hours was fully expanded again. A second 15mM KCl treatment resulted after 24 hours a gradual loss of the velum, however, after three days the animals were found dead, may be due to the KCl treatment, or due to starvation after the loss of the velum.

With *P. pansa* the long time needed for intracapsular development and for larval development until metamorphosis should not be surprising. Benthic marine organisms with a large number of small eggs undergo a long period of intracapsular development. In tropical areas hatch rocky shore muricids as veligers. Since small-sized veliconchas need a long period until they become physiologically competent to metamorphose, they remain planktotrophic for a long period.

The many months needed for the larval development of *P. pansa* and the danger of contamination with predators, like copepods, makes the larval rearing under laboratory conditions difficult. Nearly sterile culture conditions, frequent water changes, and removal of dead microalgae, are needed to secure an appropriate culture environment. By triggering the metamorphosis it seems possible to reduce the time needed from hatching until settlement, and by this to reduce the period needed for larval rearing.

These preliminary results for the rearing of the larvae of *P. pansa* showed us the problems that will need to be solved during the next experiments. In experiments with the Chilean muricid *Concholepas concholepas*, it took many years and much effort until the first successful metamorphosis and settlement of larvae of this species could be reported. Similar hurdles have to be overcome in rearing the larvae of the purple snail, however the first basic steps are made.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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THE NEED FOR MORE DIVERSITY IN *ARTEMIA* CYST RESOURCES: VARYING CHARACTERISTICS A HANDICAP OR AN OPPORTUNITY FOR THE OPTIMAL USE OF *ARTEMIA* IN FISH AND SHELLFISH LARVICULTURE

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The decreasing cyst production and harvest restrictions on the Great Salt Lake in the late 1990s has emphasized not only the critical role that *Artemia* continues to play in larval aquaculture, but also the risks of relying on a single source for its supply. In recent years, numerous new resources are successfully being explored.

The broader spectrum of available *Artemia* has produced a large variety in cyst and naupliar characteristics. Differences in cyst decapsulation behavior, hatching characteristics, separation of unhatched cysts, nauplius size and weight, HUFA and vitamin C contents as well as their enrichment kinetics, etc. may all complicate the proper evaluation of cysts (nauplii) quality and hold a potential risk for upsetting the routine procedures of many hatcheries if not properly dealt with.

This paper shows that the nauplius yield of cysts of different origin and/or different harvest seasons cannot be compared by using one of the commonly applied hatching criteria. To objectively evaluate a cyst batch, one should be able to quantify the cyst and naupliar characteristics. First of all there is the number of cysts per gram and either the hatching percentage or the hatching efficiency combining the former two criteria. Equally indispensable are the individual dry weight of the instar I nauplii and their hatching rate (e.g., expressed as the ratio instar I/instar II upon 18h and 24h of cyst incubation). The variation of HUFA and vitamin C contents is documented not only between strains but also within the same strain between different harvest seasons. It is also clear that substantial differences in enrichment kinetics may influence the efficiency of the enrichment process

For practical purposes, it is also demonstrated that certain cysts have excellent buoyancy, to such an extent that even decapsulation for cyst shell removal can be omitted. Others, however, may require severe adaptations of the decapsulation procedure in order to obtain the desired result or not to affect embryo viability.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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THE LIPID NUTRITION OF THE SENEGAL SOLE (*SOLEA SENEGALENSIS* KAUP 1858) LARVAE

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Introduction

The intensification in the production of European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) in the South of Portugal and Spain has caused a decline in the market value of these species. This has lead to the search for potential new species for aquaculture. *Solea senegalensis* Kaup 1858 has been found to have good prospects as an alternative species, given its high price and demand in European markets and high growth rates. Natural tank spawning of broodstock is now routine and larval rearing has been achieved with good survival rates but there are still problems concerning the larval and juvenile nutritional requirements (Dinis et al., 1999).

In the present work, three feeding trials using different enrichment products – Super HUFA®, Arasco® oil emulsion, sunflower oil emulsion, and an algal mixture – were conducted, with the objective of studying the effect of the prey nutritional profile on the larval fatty acid composition. The fatty acid profile of the *S. senegalensis* eggs was also analyzed, in order to gather some clues about the nutritional requirements of the first larval stages.

Materials and methods

Three independent feeding experiments were carried out using naturally spawned *Solea senegalensis* eggs from captive broodstock kept at the IPIMAR/CRIPSul hatchery. During the 24h incubation and larval rearing period, the eggs and larvae were stocked in 200 l cylindrical-conical tanks, in a flow-through rearing system (0.8-1 $l.min.^{-1}$), with gentle bottom aeration and a temperature of 20±1ºC, a salinity of 36±1‰ and an oxygen level of $8mg.l^{-1}$. Larvae were reared under a photoperiod of 14:10. Initial larval density was 25 $larvae.l^{-1}$.

Each treatment was conducted in triplicate and was initiated at the onset of

exogenous feeding (2 days post-hatch). Larvae were fed on *Brachionus plicatilis* from 2-5 days after hatching (DAH), on *Artemia* AF® type (Artemia Systems Inc.) from 3-10 DAH, followed by *Artemia* EG® type (Artemia Systems Inc.) enriched in different products, from 8 DAH until the end of the experimental period. *Artemia* density was kept at approximately 1 nauplii.ml^{-1} in the larval culture tanks. *Brachionus plicatilis* were cultured on baker's yeast and enriched in Protein Selco® (Artemia Systems Inc.) before being fed to the larvae. *Artemia* cysts were decapsulated and incubated under standard conditions (Sorgeloos et al., 1986) and the enrichment was conducted during 24h, at a density of 200 nauplii.ml^{-1}, a temperature of 27±1ºC and a salinity of 36±1‰.

In each trial two treatments (enrichment products) were tested:

Trial 1 - Non-enriched *Artemia vs.* Super HUFA® (Salt Creek Inc.);

Trial 2 - Sunflower oil emulsion *vs.* microalgae (50% *Tetraselmis chui* and 50% *Isochrysis galbana* aff. "Tahitian");

Trial 3 - Arasco® oil (Martek Biosciences) *vs.* microalgae (50% *Tetraselmis chui* and 50% *Isochrysis galbana* aff. "Tahitian").

Biochemical analysis were performed to determine the fatty acid composition of the *Solea senegalensis* eggs, of the enriched *Artemia* and of larvae 20 and 26 DAH in trials 1 and 2 and 26 and 36 DAH in trial 3. Total lipid extraction was carried out according to Blight and Dyer (1959) and saponification and esterification of the lipid extracts was done using the method of Metcalfe and Schmitz (1961). The FAME analyses were conducted as described in Pousão-Ferreira et al. (1999). Duplicate samples were analyzed.

Results and discussion

In Table I the fatty acid profile of *Solea senegalensis* eggs and of the preys used in the different trials can be seen. The fatty acid profile of the Senegal sole eggs indicates a requirement for high dietary DHA/EPA ratios, although the absolute content of both DHA and EPA is not very elevated. The (n-3)/(n-6) ratio is relatively low, in comparison with typical marine fish eggs.

The enrichment products tested in the present work are probably not the most suitable for the larval rearing of Senegal sole. All products were below the required DHA/EPA ratio; the highest value was obtained in *Artemia* enriched with Super HUFA®, which is far from the values obtained in the eggs. As for the (n-3)/(n-6) ratio, the results were also less than desirable – Super HUFA® was the only product that induced a (n-3)/(n-6) ratio similar to that of the eggs.

A decrease in the FAME level during larval development can be observed in all trials (Table II). This is explainable by the characteristic fast metabolism and

high nutritional demands of fish larvae, which use lipids as a source of metabolic energy and essential fatty acids.

Table I. Fatty acid composition (μg.mg^{-1} of dry weight) of *S. senegalensis* eggs and of enriched and non-enriched (N.E.) *Artemia* used in the feeding trials.

	FAME	HUFA	ARA	DHA	EPA	DHA/ EPA	(n-3)/ (n-6)
Solea senegalensis eggs	70.2	14.2	0.7	8.4	2.0	4.2	5.4
N.E. *Artemia*	57.0	3.0	0.6	0.2	1.4	0.12	3.0
Artemia in microalgae	121.7	7.9	1.3	0.4	3.7	0.10	3.8
Artemia in sunflower oil	142.5	8.7	1.6	0.1	5.1	0.02	2.5
Artemia in Super HUFA	93.5	18.2	1.3	4.9	8.1	0.61	5.3
Artemia in Arasco oil	130.2	16.5	9.8	0.1	4.7	0.02	1.9

FAME: fatty acid methyl esters; HUFA: highly unsaturated fatty acids; ARA: 20:4(n-6), arachidonic acid; DHA: 22:6(n-3), docosahexaenoic acid; EPA: 20:5(n-3), eicosapentaenoic acid.

The larval fatty acid profile generally reflected the composition of their diet; when larvae were fed a diet with a relatively low fatty acid content, such as preys enriched with a mixture of microalgae or non-enriched *Artemia*, their fatty acid composition was also lower. On the other hand, when using Super HUFA®, Arasco® oil emulsion or sunflower oil emulsion, the larvae present a higher level of fatty acids in their composition. The exception seems to be DHA; the larvae always present this essential fatty acid in considerable amounts, independently of the dietary treatment.

Table II. Fatty acid composition (μg.mg^{-1} of dry weight) of *S. senegalensis* larvae at different ages, submitted to different dietary treatments.

	Treatment	FAME	HUFA	ARA	DHA	EPA	DHA/ EPA	(n-3)/ (n-6)
Trial 1	N.E. *Artemia* 20 DAH	109.5	24.2	4.7	5.2	5.9	0.9	2.3
	N.E. *Artemia* 26 DAH	112.9	27.6	5.1	6.1	6.0	1.0	2.4
	Super HUFA 20 DAH	172.1	41.7	5.3	13.7	10.1	1.4	2.7
	Super HUFA 26 DAH	106.7	27.6	3.7	9.6	7.0	1.4	3.2
Trial 2	Sunflower oil 20 DAH	170.0	24.3	4.5	3.7	6.0	0.6	1.8
	Sunflower oil 26 DAH	121.6	17.9	3.3	3.3	3.7	1.0	1.6
	Microalgae 20 DAH	172.5	27.3	5.4	4.2	6.4	0.7	2.5
	Microalgae 26 DAH	99.6	19.4	3.7	4.0	3.9	1.0	2.2
Trial 3	Arasco oil 26 DAH	116.5	25.6	12.3	3.0	3.1	1.0	1.2
	Arasco oil 36 DAH	74.0	16.4	5.8	2.6	2.5	1.1	1.7
	Microalgae 26 DAH	95.0	19.5	4.0	3.5	3.7	1.0	2.2
	Microalgae 36 DAH	59.3	13.2	2.7	2.6	2.4	1.1	2.0

When using products which induce high DHA levels in the enriched preys (e.g., Super HUFA®) and, consequently, in the reared larvae, this fatty acid seems to be metabolized, although in limited amounts, during larval development. However, when the products were responsible for a low DHA content in the enriched *Artemia*, the larvae seemed to accumulate (in the N.E. *Artemia* treatment) or conserve this essential fatty acid during larval development.

As for EPA, it was consumed in most treatments during larval development, except when larvae were fed non-enriched *Artemia* with a very low EPA content. In this case, the EPA level was probably below the minimum requirement and, therefore, it has been conserved by the larvae during its development. As a result of the DHA and EPA conservation/consumption patterns, a slight increase in the DHA/EPA ratio could be observed in all treatments.

Conclusions

The egg composition is most probably related with the benthic diet of the broodstock and reveals the need to include high levels of DHA in the larval diet. This is probably the biggest challenge in the nutrition of the first larval stages, given the selective DHA catabolism by *Artemia* and the difficulty in manipulating the DHA content independently of EPA. Further studies should be conducted to find more suitable enrichment products for the rearing of the first larval stages.

Solea senegalensis larvae have the ability of accumulating and conserving DHA and EPA when it is present in very low amounts in the diet but metabolize these fatty acids, particularly EPA, when abundant in the diet.

References

Blight E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Dinis M.T., L. Ribeiro, F. Soares, and C. Sarasquete. 1999. A review on the cultivation potential of *Solea senegalensis* in Spain and in Portugal. Aquaculture 176:27-38.

Metcalfe L.D. and A.A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatography analysis. Anal. Chem. 33:363-364.

Pousão-Ferreira P., S. Morais, E. Dores, and L. Narciso. 1999. Eggs of gilthead seabream *Sparus aurata* L. as a potential enrichment product of *Brachionus* sp. in the larval rearing of gilthead seabream *Sparus aurata* L. Aquaculture Research 30:751-758.

Sorgeloos P., Ph. Lavens, P. Léger, W. Tackaert, and D. Versichele. 1986. Production of *Artemia* nauplii and decapsulated cysts for use as food source in the hatchery production of fish and crustaceans. p.69-117. In: FAO Manual for the Culture of Brine Shrimp *Artemia* in Aquaculture. University of Ghent, Ghent, Belgium. 319p.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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"PHYTOBLOOM": MICROALGAE SOLUTION FOR AQUACULTURE

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Abstract

Phytobloom is an innovative microalgal concentrate developed by Necton, in the range of 10% dry weight. The product is based on *Nannochloropsis oculata* cultured in advanced proprietary photobioreactors, concentrated, and preserved at 0-4°C with addition of a preservative. The microalgal concentrate can be kept for 5 months without significant changes in the biochemical composition. The preservation system is food-grade, appropriate for rotifer nutrition, and was the result of a 4-year research project and 25 different tested systems. The product was evaluated and is currently used by several hatcheries for a wide range of microalgal applications in aquaculture, such as an inoculate for start-up, rotifer growth, and for green water techniques.

Introduction

Microalgae are used in aquaculture as live feeds for all growth stages of molluscs, for larval stages of crustaceans and some fish species, and for zooplankton used in aquaculture food chains. The microalgal-related production costs can range from 20-50% of a bivalve hatchery's operating costs (Coutteau and Sorgeloos, 1992), becoming a significant production cost especially in smaller hatcheries. Consequently, there has been much effort directed at examining alternatives to the production of microalgae in hatcheries. In our company, a significant effort has been done to develop a live microalgal concentrate product that could be a substitute for regular microalgal production.

The aim of this study is to present 'Phytobloom' applications resulting from a new technique for microalgae preservation, evaluating its suitability as a starter in algal cultures as food for zooplankton and in green water techniques.

Materials and methods

Microalgal culture. The microalga *Nannochloropsis oculata* was cultured in outdoor 'flat-panel flow-through photobioreactors' (500-2000 l). A daily exchange rate of 20% was carried out once the culture reached the stationary phase, and daily harvested cells were concentrated using a Westfalia separator, yielding a microalgal paste of 25-35% dry weight.

Preservation system. The harvested microalgal paste was diluted to obtain a 10% dry weight (DW) concentrate and a complex food-grade preservation system was added.

Analysis. The nutritional quality of the product was determined by biochemical analysis of the major components. Protein content was measured using the Lowry method (Lowry et al., 1951), carbohydrates by the phenolsulfuric acid method (Kochert, 1978), and lipids by the charring method (Marsh and Weinstein, 1966).

Applications. After determination of a suitable preservation method, Phytobloom was tested as an inoculate for start-up and in applications to rotifer nutrition and green water techniques.

Inoculate for start-up. The microalgal concentrate was diluted in autoclaved seawater media (1, 2, and 4ml.l^{-1}) and cultured for 5 days in 1-l bottles, in triplicate. Initial and final optical density (OD=560nm) was determined. The experiment was carried out every 2 weeks.

Rotifer nutrition. About 50 rotifers.ml^{-1} (*Brachionus plicatilis*, strain S) were cultured in 1 l seawater and 3 Phytobloom concentrations were assayed (1, 2, and 3ml.l^{-1}). The experiment was carried out in triplicate and rotifer concentration was measured daily.

Green water techniques. Phytobloom was used for green water techniques on sea bream (*Sparus aurata*) cultures (3000 l). Several Phytobloom concentrations were used and larval survival rate was determined at the end of the experiment.

Results and discussion

Once the preservation system was defined, and the concentrate lifetime determined (at least 5 months), the evolution of the nutritional quality was determined and the several applications were tested.

To evaluate the changes in the nutritional quality of the microalgal concentrate, the biochemical composition was determined monthly. The content in the major components was still without significant changes for 4 months and only in the fifth month was a significant decrease observed (Fig. 1).

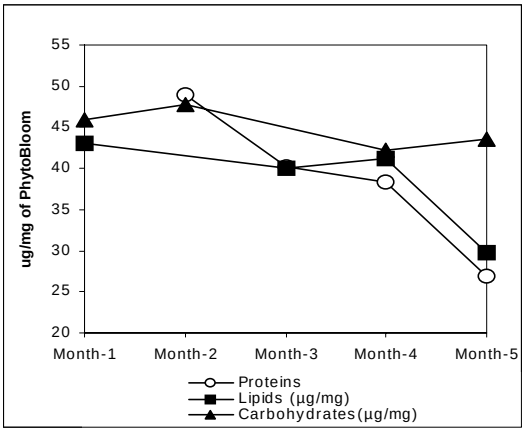

Fig. 1. Evolution of the PhytoBloom biochemical composition.

The experiments using the concentrated microalgae as an inoculate demonstrated that the microalgae could keep their viability for at least 3½ months (Table I), and could be used at hatcheries for starting new cultures. Some hatcheries prefer to use the Phytobloom as 'inoculate' for their microalgal mass culture systems, to reactivate the cells and ensure the cell viability.

Table I. Some applications of the PhytoBloom.

Applications	$ml.l^{-1}$ of PhytoBloom	Product shelf life
Inoculate	1-4 $ml.l^{-1}$	3.5 months
Rotifers culture	2-3 $ml.l^{-1}$	4-5 months
Green water	0.08-0.1 $ml.l^{-1}$	5 months

Concentrated and preserved *Nannochloropsis* was used for rotifer culture and the results show that the preserved microalgae are suitable for biomass production (Table I). Rotifer production with Phytobloom depends on the microalgal concentration in the culture medium, increasing the rotifer reproductive rate with the Phytobloom concentration. Using the Phytobloom at a concentration of $3ml.l^{-1}$ (60×10^6 $cell.ml^{-1}$), it is possible to obtain high-density rotifer cultures ($920.ml^{-1}$) in 5 days. The use of preserved *Nannochloropsis* in rotifer production could reduce or eliminate the rotifer enrichment, since *Nannochloropsis* contains substantial amounts of EPA.

The suitability of Phytobloom for green water techniques was also demonstrated. An increase of 3-5% in sea bream larval survival was achieved when 80–100µl.l^{-1} of Phytobloom was used in the culture tanks. For green water techniques, the preserved microalgae can be used for at least 5 months after production.

The use of preserved *Nannochloropsis* (Phytobloom) offers a solution to several fish culture problems:

1. The use of Phytobloom as an inoculate reduces microalgal production costs, since the cultures can be started directly in high volumes (1 l of Phytobloom can inoculate a 250-l plastic bag). This allows the hatcheries to save substantial amounts of work in the microalgal scale-up process.
2. Preserved *Nannochloropsis* can successfully replace live microalgae in supporting rotifer biomass production – fast bloom and high-density rotifer cultures can be obtained.
3. The use of Phytobloom for green water techniques substantially reduces the space and labour required for microalgae culture, and also allows the extension of the green water period time, thus increasing larval survival rate.
4. Phytobloom can be used as a back-up system during winter (when outdoor microalgal productivities are low), for microalgae crashes in summertime, for weekends and holidays, or simply when more microalgae are needed.

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References

Bligh E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Coutteau P. and P. Sorgeloos. 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: An international survey. J. Shellfish Res. 11:467-476.

Dubois M., K.A. Gilles, L.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Lowry O.H., H.J. Rosebroug, A.L. Farr, and R.J. Randal. 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193:265-275.

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FACTORS CONDITIONING THE NUTRITIONAL VALUE OF *ARTEMIA* CYSTS: EFFECT OF DIET

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Introduction

Lavens et al. (1989) found that it was possible to manipulate the fatty acid profile of *Artemia* cysts and nauplii produced in intensive controlled systems using inert diets, and Navarro and Amat (1992) obtained similar results using algal cultures. The main conclusion of these works is that although there is a clear phenotypic influence of food in the fatty acid composition of the offspring, a genotypic effect cannot be ruled out.

On the other hand, there is no available information comparing the fatty acid composition of the food, of the broodstock, or of the cysts in the wild. Under this scope it is important to note that *Artemia* can feed not only on the phytoplankton of the waters but also on halophilic bacteria that thrive in hypersaline environments.

Here we report information on: a) the effect of culturing *Artemia* from different geographical locations with the same algal diet on the fatty acid composition of the cysts; b) the fatty acid composition of food matter (<60μm), *Artemia* biomass, and cysts collected from the wild in one coastal and one inland Spanish biotope; and c) the fatty acid composition of a bacterial diet (*Halobacterium salinarum*) cultured with different media.

Materials and methods

Variation of the fatty acid composition of the cysts. Cysts from different locations – China, Iran, Italy, Spain, Africa, and six Latin American countries – were hatched under standard conditions and the nauplii kept in culture with a mixture of *Dunaliella* sp. and *Tetraselmis* sp. at 60g.l^{-1} of salinity. The cysts

produced when the population reached adulthood were harvested, dehydrated, and stored until fatty acid analysis was performed.

Fatty acids were analyzed using the protocols described in Navarro et al. (1992) and the results statistically processed using discriminant analysis according to the procedures described in Navarro et al. (1995). For the sake of clarity, only the data of seven Latin American populations will be reported here, given that the results were similar for the rest of the samples.

Fatty acid composition of biomasses, cysts and particles of less than 60μm. Two Spanish biotopes were sampled: Laguna de La Mata, a coastal lagoon next to the Mediterranean coast in Alicante; and Laguna Salada de Petrola, an inshore athalassic lagoon from Albacete (details in Navarro et al., 1992).

Biomass and cysts harvested from the biotopes were processed according to standard protocols (Navarro et al., 1992). Water samples were filtered through a 60-μm plankton mesh and filtered again through glass microfibre filters, from which lipid extraction and fatty acid analyses were performed.

Fatty acid composition of *Halobacterium salinarum*. *H. salinarum* was isolated from La Mata lagoon and cultured in the laboratory with four culture media: Nutrileaf, Guillard, *Artemia* extract, and Yeast extract. The cultures were concentrated with the aid of an open flux centrifuge. The lipids of the pellets obtained were extracted and the fatty acids analyzed as described in Navarro et al. (1992).

Results and discussion

Irrespective of the geographical origin of the cysts and their original fatty acid composition, the culture with algae rich in certain fatty acids – particularly 16:0 and 18:3n-3 – produces offspring rich in these fatty acids. This can be graphically and statistically seen in Fig. 1 as the grouping produced by the discriminant analysis of the centroids with respect to those corresponding to the original fatty acid composition (Navarro et al., 1995).

The comparison of the fatty acid profiles of biomasses, cysts, and food reveals a lack of correlation among all of them, i.e., predominant fatty acids of food are not necessarily present in the broodstock profile nor “transmitted” to the offspring (Table I). This happens in both biotopes and suggests: a) an independent metabolic lipid pathway from the brood stock towards the offspring, b) the possibility of variable composition of the food, not necessarily reflected in a single analysis, and/or c) a certain degree of selectivity in the feeding strategies of the brood stock, i.e., grazing in the upper layer of the bottom where halophilic bacteria concentrate.

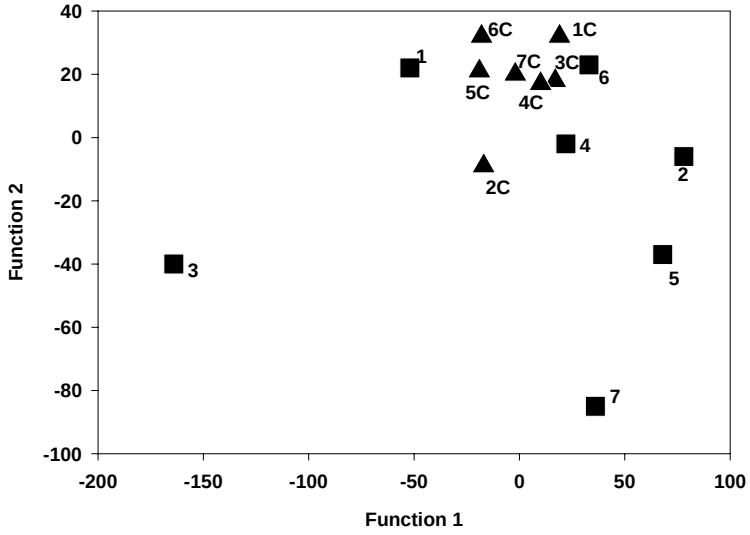

Fig 1. Graphical representation of the centroids obtained for the first two discriminant functions of fatty acid data from original cysts (squares, 1: Argentina, 2: Brazil, 3: Peru, 4: Venezuela, 5 and 6: Cuba, 7: Mexico) and cysts obtained after culture in the laboratory (triangles and "C").

Table I. Selected fatty acids (% of total fatty acids) of food (particulate matter <60μm), *Artemia* biomass, and cysts of two Spanish biotopes. No data means not detected.

Fatty acid	Biotopes					
	La Mata			Petrola		
	Food	Biomass	Cysts	Food	Biomass	Cysts
14:0	3.6	1.2	1.4	8.0	2.11	2.0
16:0	24.6	11.0	12.2	18.1	20.2	12.8
16:1n-7	13.7	6.3	12.9	17.2	3.6	20.7
18:0	5.6	8.2	3.9	2.1	23.4	3.7
18:1n-9	8.0	17.3	21.0	4.6	24.1	17.7
18:1n-7	11.1	17.1	8.8	3.3	4.2	10.2
18:2n-6	5.4	4.4	6.4	1.9	2.7	3.4
18:3n-3	0.8	6.5	3.8	8.8	0.8	3.2
18:4n-3		3.6	1.7		1.6	1.0
20:1n-9	0.5	0.3	0.4	0.2	0.3	0.3
20:4n-6	0.3	1.5	1.6	1.2	0.4	1.8
20:5n-3	2.0	15.5	8.0	7.1	2.7	10.5
22:5n-3	0.9	0.2		0.7		0.1
22:6n-3		1.0	0.3	2.0	0.4	0.1

Table II. Selected fatty acids (% of total fatty acids) of *Halobacterium salinarum* cultured with different media: Nutrileaf, Guillard, *Artemia* extract, and Yeast extract. No data means not detected.

Fatty acid	Media			
	Nutrileaf	*Artemia*	Guillard	Yeast
14:0	0.5	1.6	2.2	2.0
16:0	17.6	34.8	46.0	17.3
16:1n-7	8.0	2.4	1.5	8.4
18:0	1.6	6.8	7.8	8.0
18:1n-9	3.4	8.6	4.8	9.9
18:1n-7	11.7	3.5	1.8	11.1
18:2n-6	1.6	7.9	2.7	
18:3n-3	16.0	10.9	4.4	6.0
18:4n-3				
20:1n-9				
20:4n-6				
20:5n-3	0.1			
22:5n-3				
22:6n-3				

The last point may be further substantiated by the fact that *Artemia* can be cultured on a laboratory scale with monocultures of halophilic bacteria (Olivares et al., unpublished). These types of bacteria (e.g., *H. salinarum*) show a variable fatty acid profile depending on the culture conditions (Table II). Studies are underway in our laboratories to characterize the fatty acid composition of the sediments of hypersaline ecosystems and to broaden all the points here described.

References

Lavens P., Ph. Léger, and P. Sorgeloos. 1989. Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems. p.731-739. In: Aquaculture - a Biotechnology in Progress. De Paw, N., Jaspers, E., Ackefors, H., Wilkins, N. (Eds.). European Aquaculture Society, Bredene.

Navarro J.C. and F. Amat. 1992. Effect of algal diets on the fatty acid composition of brine shrimp, *Artemia* sp. cysts. Aquaculture 10: 223-227.

Navarro J.C., F. Amat, and J.R. Sargent. 1992. Fatty acid composition of coastal and inland *Artemia* sp. populations from Spain. Aquaculture 102: 219-230.

Navarro J.C., L.A. McEvoy, F. Amat, and J.R. Sargent. 1995. Effects of diet on fatty acid composition of body zones in larvae of the sea bass *Dicentrarchus labrax*: a chemometric study. Mar. Biol. 124: 177-183.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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THE FATTY ACIDS OF EARLY STAGES OF *OCTOPUS VULGARIS*

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Introduction

Data from fatty acid compositions of hatchlings of different cephalopods species and from some preliminary feeding trials reveal that these molluscs have high requirements for long-chain polyunsaturated fatty acids (PUFA; Navarro and Villanueva, 2000). To further clarify these requirements, in particular in *Octopus vulgaris*, we have undertaken the study of the fatty acid composition of: a) the ovary and eggs, b) the fatty acid composition of wild, recently settled juveniles, and c) the fatty acid composition of planktonic paralarvae reared with different diets.

On one hand, together with the available composition of the hatchlings, the fatty acid composition of the ovary, eggs, and wild juveniles of different sizes will help to establish a theoretical framework of the ideal fatty acid profile, i.e., the "natural" fatty acid profile, during the early life of the species.

On the other hand, the time course variation of the fatty acid composition of the paralarvae fed enriched *Artemia* and co-fed enriched *Artemia* and experimental inert diets will help to understand how the artificial larval rearing of the species deviates from the above mentioned framework.

Materials and methods

Egg masses were obtained from a brood stock maintained as described in Navarro and Villanueva (2000). The egg masses, ovary and samples of paralarvae and food were analyzed for lipid and fatty acid composition as described in Navarro and Villanueva (2000).

Wild juveniles were captured from the wild by scuba diving. Their dry weights ranged from 0.3-14.2g. Ten of these specimens were freeze dried upon arrival in

the laboratory, their lipids individually extracted, and the fatty acids analyzed as stated above.

Four rearing experiments were carried out. Experiments 1 and 2 were carried out during 20 days and experiments 3 and 4 during 30 days. All experiments were conducted at a mean of 20°C in a semi-closed filtered seawater system. Paralarvae were reared in 25-l PVC tanks at a density of 32 l^{-1} under constant illumination.

In experiment 1, larvae were fed *Artemia* nauplii ($10.ml^{-1}$) enriched with Super Selco. In the rest of the experiments, co-feeding techniques were tested. Enriched *Artemia* nauplii and a microdiet food was supplied together from Day 0 to the paralarvae. The microdiet was offered in the form of millicapsules, made by a process of gelification and coacervation, by Lipotec S.A. (l'Hospitalet de LLobregat, Barcelona). Three types of millicapsules were used differing on the basis of moisture, formulation and colorant addition. The microdiet composition was: squid (*Todarodes sagittatus*) powder (Rieber and Son), DC Super Selco (Artemia Systems), freeze-dried crab (*Carcinus maenas*) meal, krill extract (Avanti lipids), cholesterol (Sigma), vitamin complex (Kurios) and mineral complex (Warner Lambert). Ingredients were ground and mixed with an electric blender. The colorant red carmine was added to the millicapsules used in experiment 4, to test a possible increase of millicapsule acceptance by visual stimulus. Millicapsules of experiments 2 and 3 were beige in colour. All millicapsules were ovoid in form, 1295 ± 414µm (minimum) and 1960 ± 610µm (maximum) diameters. Millicapsules were supplied each hour, $24h.d^{-1}$, using an automatic delivery system. From Day 0-20, a total of $7.5g.d^{-1}$ of fresh millicapsules was supplied to each rearing tank, and from Day 21-30, $15g.d^{-1}$. The total amount of food ingested by the paralarvae was not quantified.

The survival rate (SR) was calculated as $SR = 100\ S\cdot(I-B)^{-1}$, where S was the number of surviving individuals on day x, I was the initial number of individuals in the culture, and B was the total number of individuals killed for sampling purposes to day x.

Some selected fatty acids important from the nutritional point of view, found in the total lipids of the millicapsules were: 17-20% of 22:6n-3, 12-17% of 20:5n-3, ~1% of 20:4n-6, ~8% of 18:1 monoenes, 18-23% of 16:0, and ~2% of 16:1 monoenes. Enriched nauplii showed some differences in their fatty acid profile as compared to the millicapsules: 5-6% of 22:6n-3, 12-17% of 20:5n-3, 1.6-1.8% of 20:4n-6, 25-27% of 18:1 monoenes, ~9% of 16:0, and 11-13% of 16:1 monoenes.

Paralarvae were sampled every 5 days from day 10 to the end of experiment for growth determination, and an aliquot was separated for lipid analysis.

Results and discussion

In general, the fatty acid profile of the ovaries, eggs, juveniles and hatchlings (Navarro and Villanueva, 2000) of *O. vulgaris* is similar in terms of the major fatty acids (Table I), except for the higher 18:2n-6 in the hatchlings.

Table I. Selected fatty acids (% of total) of the ovary, eggs and juveniles of *Octopus vulgaris*.

Fatty acids	Ovary	Eggs	Juveniles
16:0	22.6	27.1	18.5
16:1	2.5	0.3	1.0
18:0	4.9	6.3	9.3
18:1	4.4	5.7	4.3
18:2n-6	0.2	0.3	0.7
20:4n-6	5.2	7.8	5.0
20:5n-3	13.6	8.7	14.1
22:6n-3	23.0	19.9	20.4

Table II. Time course of variation of selected fatty acids (% of total) of *Octopus vulgaris* paralarvae reared with different diets in 4 experiments.

Expt.	Days	Fatty acid							
		16:0	16:1	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:6n-3
1	10	17.9	9.8	9.8	22.5	2.0	2.5	8.1	8.7
	15	17.3	9.7	10.1	22.9	2.0	2.6	7.7	7.2
	20	16.5	8.5	10.7	22.6	2.5	3.2	9.9	6.7
2	10	16.6	9.1	9.1	21.5	2.1	2.8	8.3	8.7
	15	16.1	10.4	9.5	23.8	1.8	2.6	7.2	7.3
	20	16.7	9.3	10.1	21.8	2.0	2.8	8.1	6.8
3	10	14.7	9.1	8.7	21.1	3.2	3.4	11.6	8.1
	15	13.7	10.8	8.5	22.4	3.2	3.2	13.0	7.1
	20	12.5	9.1	8.0	21.7	3.6	3.2	16.1	6.7
	25	13.3	8.9	8.6	21.1	3.2	3.5	14.8	7.1
	30	13.8	7.5	8.9	18.7	2.9	3.8	15.6	8.0
4	10	15.2	8.6	8.7	21.0	2.8	2.9	10.9	8.2
	15	13.2	10.5	8.2	23.7	3.3	3.0	12.3	6.6
	20	13.9	10.9	8.6	24.2	4.0	3.4	9.3	5.1
	25	14.8	11.7	9.6	25.2	3.5	3.1	7.5	4.8
	30	15.7	9.1	10.6	23.2	3.0	3.8	8.6	6.3

The fatty acid profiles of the juveniles were consistent, showing coefficients of variation of less than 25% for the major fatty acids. The presence of high levels of 22:6n-3 in the juveniles and a general fatty acid profile similar to the early stages, indicates that the fatty acid composition of the cultured paralarvae obtained in the present work (Table II), and in Navarro and Villanueva (2000),

are a deviation from the natural conditions, induced by the composition of the artificial food. This deviation from the ideal fatty acid composition of the food can explain the low survivals obtained at the end of the feeding trials: 3.3±1, 3.5±3, 0.8±2.3, and 2.6±3.2 for experiments 1 to 4, respectively. Navarro and Villanueva (2000) also reported low survivals after 30 days of culture.

The effect of the food changing the fatty acid composition of the paralarvae is rapid (10 days) and remains constant along the experimental period irrespective of the food combinations. Except for subtle differences, the trend in the fatty acid composition of the paralarvae is similar in all four experiments (Table II).

The artificial feeding conditions induce a decrease in 16:0, a very marked increase in 18C monoenes, increase in 18:0, decrease in 20:4n-6 and clear decrease in 22:6n-3, the levels of 20:5n3 remaining similar to the wild profile.

The fatty acid composition of the millicapsules parallels more that of the wild profile than the composition of the enriched nauplii. The presence in the fatty acid composition of the paralarvae of certain fatty acids abundant in *Artemia* (18C monoenes), together with the decrease in 22:6n-3, abundant in the millicapsules, may be an indication that the inert food is not efficiently ingested or digested, or even that the paralarvae selectively prey on the moving enriched *Artemia* in spite of the colorants added to the millicapsules.

More research is needed to fine-tune the lipid requirements of cephalopods or at least to effectively supplement essential fatty acids to their live prey diets.

References

Navarro J.C. and R. Villanueva. 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. Aquaculture 183:161-177.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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CONTROL OF NODAVIRUS INFECTION IN FARMED ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*)

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Abstract

One of the greatest obstacles to successful Atlantic halibut culture in Norway at present is the recurring mass mortality of halibut larvae and juveniles due to VER (viral encephalopathy and retinopathy) caused by nodavirus infection. Three main approaches to overcome the problems in the hatcheries are discussed: (1) minimizing the challenge by the pathogen from outside, (2) destruction of the viruses when entering the hatchery, or (3) immunizing fish against the disease.

Nodavirus is among the smallest viruses known. It consists of an icosahedral capsid 25-35nm in diameter containing two single-stranded positive-sense RNA molecules: RNA1 (3100nt) and RNA2 (1400nt), where the former encodes the RNA-dependent RNA polymerase and the latter encodes the capsid protein.

The entire genome of the nodavirus affecting Atlantic halibut in Norway has recently been cloned and sequenced by our group. This has enabled us to establish a specific and highly sensitive RT-PCR method for the detection of nodavirus in tissue samples. A semi-quantitative method based on ultra-centrifugation followed by RT-PCR has also been developed to detect nodavirus in seawater. RNA2 has also been subcloned into a plasmid vector for the expression of recombinant capsid protein in *E. coli* bacteria. This recombinant protein has been further purified and utilized in an ELISA test. Hence, the detection of fish serum antibodies to Atlantic halibut nodavirus is made possible. The purified recombinant capsid protein was also used to immunize rabbits in order to obtain specific polyclonal antibodies against Atlantic halibut nodavirus, which is applied for the detection of the virus by immunohistochemistry and immunoblot techniques.

To be able to prevent entry of the pathogen into the rearing facilities, one has to understand the paths of virus transmission. With this in mind, we followed several groups of reared Atlantic halibut throughout a commercial production cycle, starting with hatching of the eggs, through the larval yolk-sac stage, start feeding with live food, metamorphosis, and weaning to inert food, until the fry have reached a body weight of approximately 5g, when the animals seem to be fairly refractory to a challenge by nodaviruses. Samples of fish and seawater from the holding facilities were collected at regular intervals. The samples were fixed in formalin and/or frozen at -20°C. The events and samples preceding overt disease related to nodavirus were studied retrospectively by the methods mentioned above, in order to trace the onset of the virus challenge and replication. Our studies revealed a very high concentration of nodaviruses in the overspill of seawater coming from rearing units containing infected fish. There were also strong indications of vertical transmission of a low quantity of nodaviruses from the brood stock to the offspring. However, as the virus replicates in a few individuals, who again will shed large numbers of infective virons into the surrounding seawater, horizontal transmission to fish naive to the pathogen will occur at a large scale.

We also followed the production cycle at a different commercial Atlantic halibut hatchery where eggs were treated by ozonated seawater before transfer to silos for hatching. The inlet seawater was also treated by ozone and subsequently disinfected with sodium thiosulfate. Signs of nodavirus-related disease were not observed clinically, nor indicated by any laboratory analyses specified above. However, one could not conclude that the indicated absence of nodaviruses is solely due to the ozonation.

We are currently beginning a preliminary screening program for broodstock halibut by ELISA and RT-PCR methods, in order to detect and eliminate latent carriers of nodavirus, and hence reduce the risk of vertical transmission.

As nodavirus may affect halibut larvae at a very early stage prior to immunocompetence, it would prove difficult to base prophylaxis upon vaccination of the young. However, vaccination of virus-free broodstock may contribute to reducing the likelihood of vertical transmission of nodavirus-related disease. Our group has developed a DNA vaccine based on the gene encoding the capsid protein subcloned into a plasmid vector behind the strong eukaryote CMV promoter. We have also made a recombinant vaccine based on the capsid protein expressed in *E. coli* bacteria. We are presently testing out these vaccines in a challenge experiment.

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EFFECT OF LIPID EMULSIONS ON LARVAE AND EGG PRODUCTION OF THE CHILEAN SCALLOP, *ARGOPECTEN PURPURATUS* (LAMARCK, 1819)

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Introduction

The specific nutritional requirements of bivalves remain poorly defined, mainly due to the lack of suitable experimental diets that allow testing of specific components. Molluscs seem to require n-6 and n-3 polyunsaturated fatty acids (HUFA), especially 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA), as they are considered to play a critical role in the determination of the nutritional value of bivalve diets (Caers et al., 1998). It is still under discussion however whether they are used as a source of metabolic energy or as a source of essential nutrients and structural components. Comparison with a diet rich in saturated fatty acids therefore leads to a better understanding of the specific role of n-3 HUFA's for bivalves.

As part of a wider study dealing with the Chilean scallop *Argopecten purpuratus* (Caers et al., 1999), this paper describes results of various experiments with larvae and broodstock animals. The use of standard ICES emulsions (both saturated and polyunsaturated (Caers, 1998; 1999)), allowed us to change the lipid composition of the diet without affecting the other dietary components.

Materials and methods

The adult scallops that were used in the experiments were brought from Coquimbo (Region IV, North Chile) to the field laboratory in Calbuco (Region X, 1500km to the south). The experimental tanks were filled with filtered (0.5μm) and UV-treated seawater. Water was changed three times a week and gentle aeration was provided.

Larvae experiments. Broodstock animals were conditioned in the laboratory

during several weeks. The D-larvae were collected on a 63-µm sieve, counted and distributed evenly amongst the experimental tanks at a density of 8-10 larvae.ml^{-1}. Each treatment consisted of 3 randomly distributed replicates.

The standard diet consisted of a mixture of *Isochrysis galbana (T-Iso)* and *Chaetoceros neogracile* (1:1, based on cell number) harvested in the exponential phase. The daily food ration (16 cells.µl^{-1}) was given once a day as well as 2mg.l^{-1} of antibiotics. Temperature was kept at 18±1°C.

Broodstock conditioning experiments. Recently spawned scallops were kept in conical tanks of 50 l. 4mg.l^{-1} of antibiotics was administrated with every water change. The standard diet of 3×10^9 algal cells.animal^{-1}.day^{-1} consisted of a mixture of *Isochrysis galbana* (T-Iso) and *Chaetoceros neogracile* (3:1 based on cell number). The daily food ration was given by means of a drip-system. Temperature was kept at 17±1°C. After the experimental period, the scallops were induced to spawn by thermal shock and the use of algal bloom.

Data analysis. Percentage of lipid supplementation or substitution was calculated on basis of the dry weight (DW) of algae fed to the scallops. The daily growth rate of the larvae before reaching metamorphosis was calculated as the linear increase of the ventral-dorsal shell length (Delaunay et al., 1992), using multiple regression. 1-way ANOVA and the post-hoc Tukey-test were applied to determine significant differences between treatments after log-transformation of the data. Percentages underwent an arcsine-transformation. In case data were not homogenous, the non-parametric Kruskal-Wallis ANOVA was used and sets of 2 groups were compared with the Mann-Whitney U-test.

Results and discussion

Supplementation experiments with larvae. Table I summarizes the effect of lipid supplementation on the parameters evaluated. Supplementation of the Standard diet with a 20% mixture of DHA and EPA emulsions had no positive effect on larvae growth or survival. No significant differences were observed in DW, ash content or percentage of pediveliger larvae with eyespot at the end of the experiment. Supplementation of 50% lipids had an adverse effect on larvae growth, reflected in a lower DW and fewer larvae with eyespot. Hence, the addition of lipid emulsions do not produce an additional effect, probably because the algae diet of *Isochrysis galbana* and *Chaetoceros neogracile* fulfills with the dietary needs of larvae before reaching the metamorphosis.

Substitution experiments with larvae. There was no significant difference in growth rate and general larvae performance when 20% (Standard-20%) and even 40% (Standard-40%) of the algae were replaced by the emulsion mixture of

DHA and EPA (Table II). A slower growth rate was observed however when 60% (Standard-60%) of the algae were replaced, leading to smaller larvae and fewer pediveliger larvae with eyespot after 2 weeks.

Table I. Effect of lipid supplementation on various parameters of *Argopecten purpuratus* larvae.

Diet	Shell height (μm)	DW (ng)	%eyespot	Growth rate (μm/day)	Survival (%)
Experiment 1 (14 days)					
Standard	153.8[a] (±44.7)	445[ab] (±127)	51.6 (±2.1)	8.55 (±0.27)	80.9 (±4.4)
Standard+20%	153.1[a] (±41.7)	454[a] (±35)	46.0 (±16.5)	8.32 (±0.72)	70.3 (±7.1)
Standard+50%	140.4[b] (±37.2)	302[b] (±55)	29.0 (±12.8)	6.73 (±2.0)	75.9 (±12.9)
Experiment 2 (17 days)					
Standard	156.8[a] (±38.4)	455[a] (±19)	11.7 (±3.8)	5.27 (±1.06)	90.7 (±1.5)
Standard+20%	164.0[a] (±38.2)	459[a] (±15)	15.0 (±5.0)	5.84 (±1.11)	84.0 (±2.0)
Standard+50%	141.3[b] (±37.2)	359[b] (±14)	12.3 (±6.0)	4.50 (±1.26)	77.7 (±10.2)

Values in one column followed by a different letter are significantly different (Tukey HSD-Test, $P<0.05$)

Table II. Results of algal substitution with lipid emulsions for *Argopecten purpuratus* larvae.

Diet	Shell height (μm)	DW (ng)	%eyespot	Growth rate (μm/day)	Survival (%)
Experiment 1 (14 days)					
Standard	175.8 (±22.5)	403 (±83)[a]	55.0	6.97 (±0.48)	93.2 (±1.2)
Standard-20%	174.5 (±28.3)	309 (±67)[a]	56.0	7.7 (±1.01)	93.9 (±1.8)
Standard-40%	177.0 (±32.7)	206 (±68)[b]	57.0	6.65 (±0.61)	93.0 (±1.8)
Experiment 2 (14 days)					
Standard	179.0[a] (±33.9)	734 (±65.1)	62.2[a] (±4.7)	7.75 (±0.42)	77.2 (±6.8)
Standard-40%	181.1[a] (±32.2)	707 (±41.0)	63.0[a] (±8.9)	7.87 (±0.43)	74.5 (±6.6)
Standard-60%	172.8[b] (±36.1)	663.0 (±9.9)	51.2[b] (±2.6)	7.28 (±0.11)	67.4 (±8.5)

Values in one column followed by a different letter are significantly different

Experiments with broodstock conditioning. Table III summarizes the effect of lipid emulsions on spawning quality and quantity. Both experiments showed that lipid treatment did not affect maturation time, egg size, egg ash content or dry weight. Supplementation of the algae diet with 50% lipids, be it DHA solely or 25% DHA + 25% EPA, tended to lead to a higher egg production, although the difference was not statistically significant. A significantly higher egg production however was observed when 50% of a saturated lipid emulsion (predominantly C12) was supplemented to the algae diet. This would be a strong indication that

the scallops use the lipids as an energy source for the production of gametes, more than as structural components, but further research is required.

When 50% of the algal diet was replaced with DHA lipid emulsion, only 30% of the scallops spawned. Egg production per animals was significantly lower than for those fed on the Standard diet supplemented with 50% DHA, but not significantly different from the scallops which received pure algae.

Table III. Spawning results of *Argopecten purpuratus* conditioned with different lipid supplementation.

Treatment	Egg size (μm)	Egg DW (ng)	Eggs per animal ($\times 10^6$)	% scallops spawning eggs
Experiment 1 (34 days)				
Standard	55.8 (±3.8)	19.6 (±2.7)	5.36^{ab} (±2.53)	100
Standard+50%DHA	54.7 (±2.5)	20.2 (±2.7)	8.62^{a} (±3.44)	40
Standard-50%DHA	55.2 (±3.2)	24.8 (±9.2)	2.18^{b} (±1.85)	30
Experiment 2 (82 days)				
Standard	54.8 (±4.7)	24.2 (±5.1)	2.81^{a} (±1.0)	75
Standard+25%DHA +25%EPA	52.0 (±3.7)	27.4 (±8.0)	4.86^{a} (±3.0)	85.7
Standard+50%satur. lipid	52.6 (4.2)	23.7 (4.9)	7.83^{b} (±2.7)	92.8

Values in one column followed by a different letter are significantly different (Tukey HSD-Test, $P<0.05$)

Acknowledgements

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References

Caers M., P. Coutteau, P. Lombeida, and P. Sorgeloos. 1998. The effect of lipid supplementation on the growth and fatty acid composition of *Tapes philippinarum* (L.) spat. Aquaculture 162:287-299.

Caers M., P. Coutteau, K. Cure, V. Morales, G. Gajardo, and P. Sorgeloos. 1999. The Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). II. Manipulation of the fatty acid composition and lipid content of the eggs via lipid supplementation of the broodstock diet. Comp. Biochem. Physiol. 123B:97-103.

Delaunay F., Y. Marty, J. Moal, and J.-F. Samain. 1992. Growth and lipid class composition of *Pecten maximus* (L.) larvae grown under hatchery conditions. J. Exp. Mar. Biol. Ecol. 163:209-219.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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INFLUENCE OF THE CONTENT AND RATIO OF ESSENTIAL HUFA'S IN THE LIVE FOOD ON LARVICULTURE SUCCESS OF THE MUD CRAB (*SCYLLA PARAMAMOSAIN*) IN THE MEKONG DELTA (VIETNAM)

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Introduction

Previous experiments have proven that live prey (rotifers and *Artemia*) enriched with HUFA's (highly unsaturated fatty acids) increased the survival and performance of crab larvae. Especially, these enriched live prey could prevent the mass mortality often observed in later larval stages (from Zoea 4 onwards). In order to define that relationship and to improve survival and molting or metamorphosis success, further studies on the effect of HUFA content and the ratios of specific fatty acids (mainly DHA: docosahexaenoic acid, 22:6n-3; EPA: eicosapentaenoic acid, 20:5n-3; and ARA: arachidonic acid, 20:4n-6) in the live feed were warranted.

Materials and methods

The experiments were carried out in the indoor demo hatchery of AFSI in 30-l and 100-l fibreglass containers in recirculation mode with the stocking density was 50 and 100 zoea 1 (Z1) per litre in experiment 1 and 2, respectively.

In the first experiment, rotifers were the only live food for all stages of crab larvae. In the second experiment, Z1 and Z2 (D0-D6) were fed with rotifers and with instar II *Artemia* nauplii (Vinh Chau strain, Vietnam) from the Zoea 3 stage onwards. Rotifers were cultured in an outdoor integrated recirculation rotifer culture system consisting of two 10-m³ tanks stocked with *Tilapia* (fed by locally made pellets) and *Chlorella* (growing on the waste products from Tilapia and uneaten feed), connected to a 4-m³ rotifer-culture fibreglass tank.

Rotifers and *Artemia* were enriched using standard reference emulsions (ICES –

Working Group on the Mass Rearing of Juvenile Fish, 1994). Five types of ICES emulsions were used: 30/0.6 (containing 30% total HUFA with a DHA/EPA ratio of 0.6), 30/4, 50/0.6, ARA (containing 30% HUFA with a DHA/EPA/ARA ratio of 1/1/1) and COCO (a coconut emulsion, free of HUFA, mainly consisting of saturated fatty acids) serving as control. Two additional treatments were used for the purpose of comparison: STAN - Standard treatment using DIS (Dry Immune Selco, an experimental enrichment product of INVE Technologies NV, Belgium) and micro-algae (*Chlorella* for rotifers and *Chaetoceros* for *Artemia*) to enrich live feed on alternating days and CONT – control (feeding freshly harvested rotifers and then *Chaetoceros*-enriched *Artemia*).

The 7 treatments were initially run in 6 replicates. On D6 of rearing (D0 is the day of hatching), half of the replicates were sacrificed randomly for FAME analysis. Samples of live feed and crab larvae were analyzed by a direct transmethylation method according to Lepage and Roy (1984).

Treatments were further evaluated based on survival and larval stage index (LSI, average larval stage of 10 larvae from each tank).

LSD test in STATISTICA 5.5 was used for post-hoc comparisons of means of survival rates and LSI. In the graphs, data having the same superscript letter are not significantly different at P level < 0.05.

Results and discussion

To date, two replicate experiments are completed. They were canceled on D12-D15 at Z4-Z5 stage due to low overall survival. Further general improvements will have to overcome this in order to investigate the effect on the later larval stages. Samples of crab larvae and live feed before and after enrichment are currently being analyzed.

Experiment 1 (female 450). Fig. 1 clearly shows that on all sampling days, treatment “COCO” has the lowest LSI, while the other treatments had higher values. It can be concluded that HUFA is important for growth and molting of crab larvae even at early stages. Treatment “CONT”, feeding freshly harvested rotifers (cultured on *Chorella*) and *Artemia* enriched with *Chaetoceros* also resulted in high LSI’s. These micro-algae apparently play an important role in the improvement of the live feed quality for crab larvae. On the later sampling days (D9, D12 and D15), treatments “30/4” and “STAN” gradually revealed the positive effect of the high DHA/EPA ratio in the enrichment products. Previous analysis of FAME (fatty acid methyl ester) demonstrated that the ratio DHA/EPA of rotifers enriched with DIS could reach a value of over 2, while it is around 1-1.5 for eggs and Z1 of the mud crab. The results also show that an enrichment product with high HUFA content but low DHA/EPA ratio performed

worse (comparing treatment “50/0.6” with “30/0.6” and “30/4”).

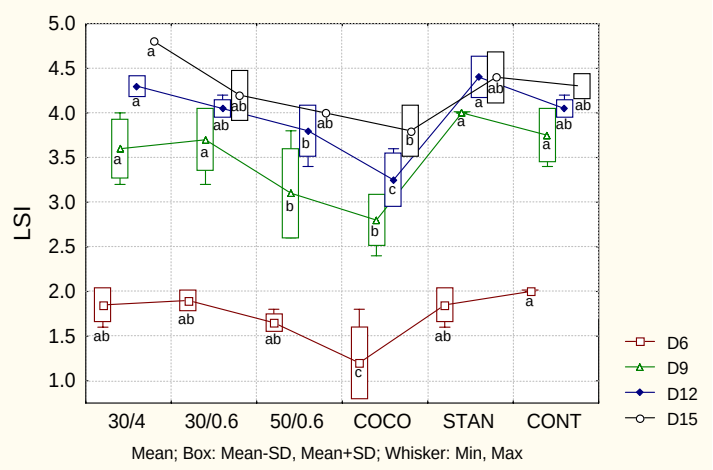

Fig. 1. LSI's of larvae in experiment 1.

The survival rates of all treatments were not significantly different on any of the sampling days (for all treatments, ranging 9.07-18.23% on D13, the last sampling day). This proves that even within a wide range of total HUFA levels and ratios of specific fatty acids, the crab zoea can survive, but the molting process is retarded. This phenomenon is common in larval rearing of crustaceans (f. ex. *Macrobrachium rosenbergii*)

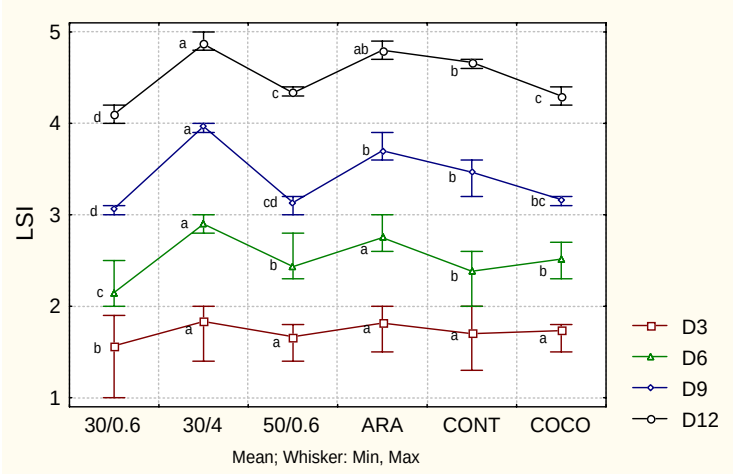

Fig. 2. LSI’s of the larvae of experiment 2.

Experiment 2 (female 09P). LSI’s on D12 revealed that treatment “30/4” and “ARA” resulted in the highest values. A similar trend existed on other sampling

days (Fig. 3). The results of this experiment confirmed the first one that an enrichment product with proper HUFA contents and high ratio of DHA/EPA (ICES emulsion 30/4) results in the best performance of the crab larvae. Treatment "ARA" also brought a good molting. In the future, more attention should therefore be given to other essential HUFA's, to make more balanced diets for the larvae.

The average survival rates on the last sampling day (D12) for all treatments ranged 1.17-10.74%. On D3, the survival rates of all treatments were still similar. On D6 it was lowest for "50/0.6". Treatments "ARA" and "COCO" exhibited a slightly better survival. On later sampling days (D9 and D12), all treatments were rather similar. 30/0.6 and COCO had however the highest survival. As these were the treatments with the lowest LSI, this could point out an interaction with growth.

Conclusions

This is the start of a series of experiments into more fundamental research on the nutritional aspect of mud crab larviculture. Low and variable survival rates of the larvae (due to the variability in hydraulic regime between individual rearing containers, delayed mortality of the slow-molting larvae, infections, etc.) make it difficult to explain effects based on this parameter. The LSI is a more reliable criterion to evaluate various treatments, because it seems less affected by the above-mentioned factors. It is preliminarily concluded that:

- Proper HUFA content (30%) and high ratio of DHA/EPA (4) results in quick molting during zoeal stages of the crab larvae.
- More balanced diets (DHA/EPA/ARA ratio) and micro-algae (Chlorella and Chaetoceros) enriched live feed could improve the growth rate of crab zoea.
- Even with imbalanced diets, crab zoea can survive for a long time even though the molting process is retarded.

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VARIABILITY IN EGG COMPOSITION IN CAPTIVE BROODSTOCK OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*) FROM ICELAND AND NORWAY

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Introduction

Malnutrition has been hypothesized to cause some of the variability observed in egg quality from captive halibut broodstock. The ideal diet composition is not yet known, but several important dietary constituents and their interactions have been identified (Watanabe et al., 1984; Bruce et al., 1993; Mangor-Jensen et al., 1994; Fernàndez-Palacios et al., 1995; Navas et al., 1995). The present study was undertaken to determine the present variability in egg composition in farmed halibut from two different locations as a basis for developing improved broodstock diets. This paper presents a portion of the data illustrating the range of the levels of various constituents and the change in egg composition at both locations over time.

Materials and methods

Unfertilized eggs were collected during the period of 1997-2000 from Atlantic halibut females of different age and nutrition status after feeding in Austevoll Aquaculture Research Station, Norway, or a fish farm in Iceland. They were analyzed for the contents of free amino acids, fatty acids, minerals and vitamins. Eggs from the same groups were also fertilized and hatched by normal routines. Feed samples from the same locations and from half a year before spawning were also sampled and analyzed for the same chemical contents as in the eggs.

Results and discussion

The nutritional contents of the eggs showed a wide range in several parameters, according to origin, between locations, among females from within the same location, and between egg batches from the same female (Table I).

Also, the feed composition showed considerable variation for several important

components due to changes in strategy from feeding whole herring with added vitamins to formulated feeds in both locations. This change in feed composition was partly reflected in the egg composition, illustrated by the eggs' changing vitamin C levels (Fig. 1). Vitamin C has several important functions during maturation, such as its role in steroid hormone synthesis, thereby influencing vitellogenesis and its antioxidative functions. Vitamin C has also been shown recently to influence lysozyme activity in some stages of embryos and larvae, reflecting activation of the non-specific immune system (Cecchini et al., 2000). Since several nutritional components are changing simultaneously, it is not easy to predict the effect on egg quality and larval survival.

Table I. Minimum and maximum values of some nutrients in eggs from captive Atlantic halibut in the period 1997-2000. Values are in wet weight, except the trace elements (Cu, Fe, Zn, Se, P, and I) given in dry weight.

	%Dry matter	Thiamin $\mu.g^{-1}$	Folate $\mu.g^{-1}$	Vit. C $\mu.g^{-1}$	Vit. E $\mu.g^{-1}$	Vit. A $\mu.g^{-1}$	ARA $mg.g^{-1}$
Min.	4.4	<0.1	0.02	5	2.5	0.01	0.09
Max	13.9	3.0	0.13	42	23.1	0.03	0.21
	EPA $mg.g^{-1}$	DHA $mg.g^{-1}$	n-3:n-6 $mg.g^{-1}$	Asp $\mu.g^{-1}$	Glu $\mu.g^{-1}$	Asn $\mu.g^{-1}$	Gln $\mu.g^{-1}$
Min.	0.59	1.52	11.7	0.05	0.47	0.22	0.19
Max	1.40	3.03	17.8	0.38	1.15	1.43	1.14
	Cu $\mu.g^{-1}$	Fe $\mu.g^{-1}$	Zn $\mu.g^{-1}$	Se $\mu.g^{-1}$	P $mg.g^{-1}$	I $\mu.g^{-1}$	
Min.	1.3	<8.0	50.9	0.87	8.5	0.36	
Max	10.1	18.8	76.4	2.97	13.6	1.38	

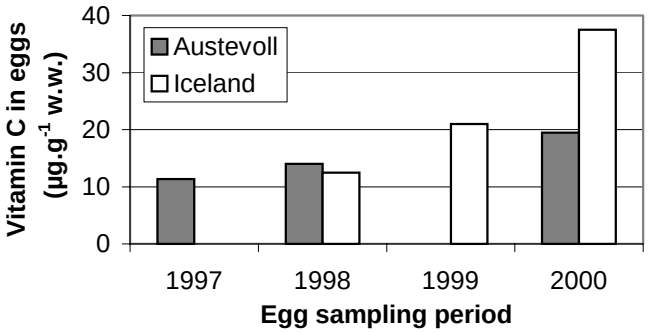

Fig. 1. Average vitamin C concentration in several (n = 3-15) egg groups from captive Atlantic halibut in Iceland and Norway in the period 1997-2000.

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Aquaculture Research Station, Norway and the staff at the fish farm in Iceland for egg sampling from the broodstock. In addition we thank Vibecke Asphaug, Torill Berg, Annbjørg Bøkevoll, Jorun Haugsnes and the rest of the technical staff at Institute of Nutrition, Directorate of Fisheries, Norway for carrying out all the chemical analyses. Funding was provided by the Norwegian Research Council and the Nordic Ministry Council.

Conclusions

- The largest variation was observed for levels of thiamin, vitamins C and E, and copper
- A significant range was also observed in the other constituents
- The egg groups from both locations showed a general increase in the contents of vitamins in the period from 1997 to 2000, partly reflecting changes in the feed composition
- Further studies on these data will estimate the correlations between egg composition and egg quality.

References

Bruce M.P., R.J. Shields, M.V. Bell, and N.R. Bromage. 1993. Lipid class and fatty acid composition of eggs of Atlantic halibut, *Hippoglossus hippoglossus* (L.), in relation to egg quality in captive broodstock. Aquacult. Fish. Manage. 24:417-422.

Cecchini S., G. Terova, G. Caricato, and M. Saroglia. 2000. Lysozyme activity in embryos and larvae of sea bass (*Dicentrarchus labrax* L.), spawned by broodstocks fed with vitamin C enriched diets. Bull. Eur. Assoc. Fish Pathol. 20(3):120-124.

Fernàndez-Palacios H., M. Izquierdo, L. Robaina, A. Valencia, M. Salhi, and J. Vergara. 1995. Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead seabream (*Sparus aurata*). Aquaculture 132:325-337.

Mangor-Jensen A., J.C. Holm, G. Rosenlund, Ø. Lie, and K. Sandnes. 1994. Effects of dietary vitamin C on maturation and egg quality of cod *Gadus morhua* L. J. World Aquacult. Soc. 25(1):30-40.

Navas J.M., M. Thrusj, J. Ramos, M. Bruce, M. Carillo, S. Zanuy, and N. Bromage. 1995. Effect of the lipid composition of the diet on hormonal levels and spawning performance of sea bass (*Dicentrarchus labrax*). p.108-110. In: Proceedings of the 5th International Symposium on the Reproductive Physiology of Fish. Goetz, F. and P. Thomas (Eds.). The University of Texas at Austin, USA.

Watanabe T., S. Ohashi, A. Itoh, C. Kitajima, and S. Fujita. 1984. Effects of nutritional composition of diets on chemical components of red sea bream broodstock and eggs produced. Bull. Jap. Soc. Sci. Fish. 50:503-515.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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HATCHERY SEED PRODUCTION OF THE ORIENTAL HARD CLAM *MERETRIX MERETRIX* (L.1758) IN THAILAND

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Introduction

The oriental hard clam *Meretrix meretrix* (L. 1758) (Family Veneridae) is one of the commercial bivalves being exploited in Thailand, the Philippines, and Indonesia (Carpenter and Neim, 1998). In Thailand, it has been used not only as a food source, but its beautiful glossy shells with variable colours and patterns are also valuable for the shell craft industry. The harvest, even though collected by hand during low tide, may exceed a ton per day in some areas both in the Gulf of Thailand and the Andaman coasts. Overexploitation has caused rapid decline of hard clam populations in every area of its natural distribution. The Thailand Department of Fisheries initiated experimental hatchery breeding of the oriental hard clam *M. meretrix* in 1985 (Sahavacharin et al., 1987). It was met with success first in laboratory scale, but no continuing activity after that until 1999. Study on hatchery breeding of the clams was carried out to develop the techniques for hatchery seed production to supply stock enhancement and provide a basis for mariculture development.

Materials and methods

Seawater and food preparation. Coastal seawater (32-34ppt salinity) from a subsurface intake was pumped into a concrete sedimentation reservoir, then filtered and stored in 200-m^3 concrete tanks without chemical treatment. Fine filtration and sterilization were subsequently done according to the requirement for each step of hatchery procedure (Fig. 1).

Unicellular algae – *Isochrysis galbana*, *Chaetoceros calcitrans*, and *Tetraselmis* sp. – were produced by batch culture. The culture to be used for feeding the larvae to settlement was performed in 20-l glass carboys under sterile conditions. Mass culture in 500 to 1000-l fiberglass tanks was used for feeding the older juveniles and broodstock. Mass culture production with low cell density was

harvested by continuous centrifuge to remove culture media as well as to increase cell densities before feeding to the clams.

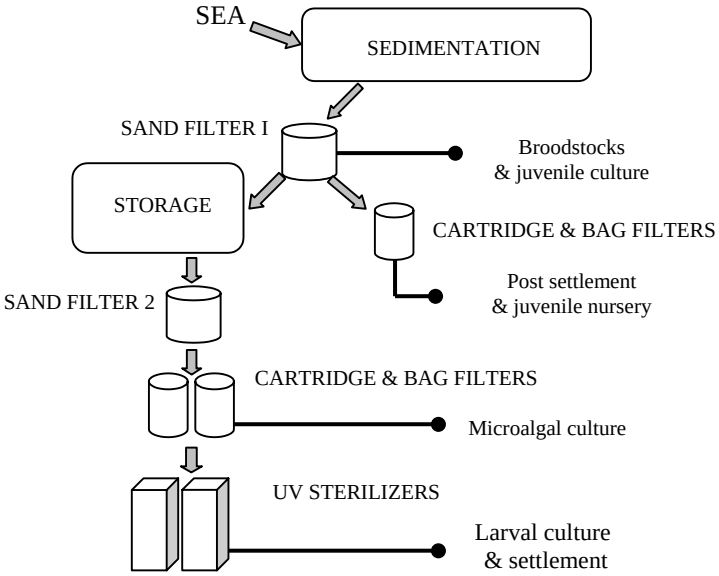

Fig. 1. Diagram showing seawater system in clam hatchery

Broodstock, breeding, and larval rearing. Adult clams were collected from coastal areas in Prachuap Khiri Khan Province. Either spawning was induced, or the clams were maintained for conditioning. In the latter case, the clams were held in 200 to 500-l shallow fiberglass trays with a sand substrate, seawater flow, and mixed microalgae for feeding. They were taken for breeding by random sampling without inspection of gonadal maturity prior to spawning induction. Two methods of induction were tried, either by serotonin (5-Hydroxy tryptamine creatinine sulfate complex, SIGMA Chemicals) injection (Gibbons and Castagna, 1984; Nugranad et al., 2000), or by short-period desiccation (Nugranad and Promjinda, 1997). Fertilized eggs were incubated at a density of 2-5 eggs.ml^{-1} in 500 to 1000-l cylindrical fiberglass tanks filled with UV-sterilized seawater with gentle aeration. Larvae were cultured using intensive bivalve larval rearing techniques with an initial density of 1-3 larvae.ml^{-1}. The larvae were fed twice a day with *Isochrysis* and *Chaetoceros* at a density of 1-3×10^4 cells.ml^{-1} at a time. When the larvae developed into a pediveliger stage, identified by the development of a foot and crawling behaviour, they were graded using nylon screen sieves and transferred

into 300 to 500-l settling tanks. No particular substrate was provided. *Tetraselmis* was supplemented for feeding.

Post-settlement and juvenile cultures. Newly settled juveniles were held in 30-cm diameter PVC trays with nylon-screen bottoms placed in 200-l shallow rectangular fiberglass tanks, or in larger 2-layered 300-l cylindrical tanks with nylon-mesh bottoms at the inner layer. Nursery units with airlifted water circulation were used as a closed down-flowing system. An open flow-through seawater system was used after 2 weeks following complete settlement. Food addition was provided 2-3 times daily, and water flow was stopped for an hour during feeding. Fully developed juveniles were reared in the down-flow nursery system to a size of 2-3mm, or cultured in the shallow flow-through tanks with sand substrate to 5-10mm, which is likely to be an appropriate size for natural release.

Results and discussion

Table I. Summary of hatchery seed production of the oriental hard clam *M. meretrix* in 1999-2000.

Batch No.	D-shaped larvae	Pediveligers	Survival[a)] (%)	2-5mm juveniles	Survival[b)] (%)
24-05-99- 1	810 000	256 800	31.7	5 000	0.6
26-05-99- 2	660 000	564 000	85.5	80 000	12.1
21-06-99- 3	910 000	474 000	52.1	139 000	15.3
23-06-99- 4	9 136 000	4 420 000	48.4	250 000	2.7
16-07-99- 5	3 342 000	2 454 000	73.4	22 000	0.7
10-08-99- 6	4 900 000	1 530 000	31.2	1 300 000	26.5
11-08-99- 7	310 000	210 000	67.7	165 000	53.2
05-04-00- 1	2 210 000	340 000	15.4	63 240	2.9
11-04-00- 2	2 100 000	428 000	20.4	< 5 000	-
21-04-00- 3	4 320 000	2 802 000	64.9	310 200	7.2
05-05-00- 4	650 000	430 000	66.2	59 400	9.1
21-05-00- 5	140 000	60 000	42.9	5 000	3.6
22-06-00- 6	310 000	150 000	48.4	17 200	5.6
29-06-00- 7	2 398 000	1 466 000	61.1	< 5 000	-
Average	2 299 714	1 113 200	50.7	201 336*	11.6*

Notes: [a)] from D-shaped to pediveligers; [b)] from D-shaped to 2-5mm juveniles
* excluding results from batches no. 11-04-00- 2 and 29-06-00- 7

No success was achieved from induced spawning by desiccation while serotonin injection was very effective. Incidence of injected clams spawning in each trial

ranged from 15% (6 of 39 in May 2000) to a maximum of 95% (43 of 45 in June 1999). A total of 14 culture batches was achieved in 1999-2000, yielding a few thousand to over a million millimeter-sized juveniles. Survival to the pediveliger stage was rather high compared to other bivalves cultured in the same hatchery. Production of seeds from each batch is shown in Table I.

Although the hatchery techniques were applicable for this clam species, shown by good survival and production in some batches, further studies on several aspects are still needed. Serotonin injection, although effective, might be not suitable for a large number of broodstock, and is also costly. The classic thermal stimulation method has been tried formerly but with no success.

Post-settlement nursery techniques are another important topic to consider. Upwelling nurseries, which have proved to be very effective for rearing the spat or juveniles of many other bivalves, was not applicable for *M. meretrix*. Such an obstacle was due to drifting behaviour of the juveniles by secretion of mucous into the water, which still remained in clams over 5mm in shell length. The down-flow nursery system was used to overcome this problem.

Results obtained from these two-year studies revealed that the clam *M. meretrix* is a species with a potential to develop mass seed production from the hatchery. Further experiments should be investigated to improve survival during and after settlement to gain higher production.

References

Carpenter K.E. and V.H. Neim (eds.). 1998. FAO species identification guide for fishery purposes: The living marine resources of the Western Central Pacific. Volume 1. Seaweeds, corals, bivalves and gastropods. Rome, FAO. p.1-686.

Gibbons M.C. and M. Castagna. 1984. Serotonin as an inducer of spawning in six bivalve species. Aquaculture 40:189-191.

Nugranad J., S. Noodang, W. Ratanachurdchai, K. Promjinda, S. Phanna, and S. Chantara. 2000. Breeding of the oriental hard clam *Meretrix meretrix* (L., 1758). Phuket Mar. Biol. Ctr. Spec. Publ. 21(1):203-210.

Nugranad J. and K. Promjinda. 1997. An experiment on hatchery seed production of the scallop *Chlamys senatoria* Gmelin. Phuket Mar. Biol. Ctr. Spec. Publ. 17(1):241-249.

Sahavacharin S., Silapajarn, K., Limsurat, S. and S. Klangnarong. 1987. Induced spawning of hard clam. Technical paper No. 47/2530. Prachuap Khiri Khan Brackishwater Fisheries Station. 9p.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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DIGESTIVE CAPABILITY WITH DIET TYPE IN ATLANTIC COD LARVAE

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Introduction

A major bottleneck in the mass production of cod (*Gadus morhua*) is the high mortality associated with the larval period, where starvation is a major factor. This is generally assumed to be due to problems related to feeding, digestion, nutrition, bacterial loading, or a combination of these factors. Foraging models predict success for fish based on contact with appropriate food at critical points in the life cycle. One examination of this is the "match/mismatch" hypothesis, which suggests that the degree of overlap between larval fish and their prey affects larval growth, survival, and recruitment (Cushing, 1990). For example, cod have a relatively fixed spawning period, while peak production of their prey varies yearly, which can result in a 'match' or 'mismatch' between the two. Potentially important, yet neglected, components of prey selection are the nutritional quality of the food, and the ontogenetic shifts in food preference displayed by the larvae. Recent experiments have shown that the importance of food to the growth and survival of cod varies through ontogeny, with older larvae being more vulnerable to mismatches with food than young larvae (Gotceitas et al., 1996). However, comprehensive information on young fish is rare because of the difficulty with culturing and experimenting on the small delicate larvae. We propose to investigate the survival, growth, foraging behaviour, functional development of feeding, and onset of digestive enzyme activity in cod larvae in response to exposure to different enrichment formulations and prey types.

Materials and methods

Cod broodstock are housed at the Ocean Science Centre, Memorial University of Newfoundland. Eggs will be collected from broodstock collectors, disinfected and incubated in circular tanks with water flow and aeration following established protocols. Dead eggs will be removed daily. When 50% of the eggs have hatched, the larvae will be transferred to experimental tanks, and this will

be taken as Day 0 of the experiment.
The fifteen experimental tanks to be used are 30-l rectangular glass aquaria (38cm deep) with three sides covered in black opaque plastic. The front will not be covered to facilitate the behavioural observations. Initially, the tanks will be filled with filtered UV-treated seawater. Flow rate to these tanks will be increased as larvae develop. Light intensity will be maintained at 2000 lux during the first three weeks of feeding, and lowered after this. A continuous photoperiod will also be used, based on published protocols. Temperature data will be collected over this period.

The larvae will be separated into five groups (with replicates) based on feeding regime from day of hatching: 1) high lipid content rotifers, 2) low lipid content rotifers, 3) high lipid content rotifers and high lipid *Artemia* (used after day 30), 4) low lipid content rotifers and low lipid *Artemia* (used after day 30), and 5) no food. Prey densities will be maintained as 4000 prey.l^{-1}. Cultured prey are naturally poor in highly unsaturated fatty acids. Details for formula of prey enrichment will be determined later. Fatty acid analysis of dietary enrichments and prey types will be done for all diets and prey types used.

Ten larvae will be sampled on day 0 and thereafter, five larvae from each tank (15 per treatment) will be arbitrarily chosen for morphometric measurements and dry weights at five-day intervals over the duration of the experiment. Using a dissecting microscope, the following will be recorded: standard length, head depth, eye diameter, and myotome height (all in mm). The presence or absence of food in the gut, in proportion to volume, will also be recorded. After measurements, the larvae will be rinsed in freshwater and placed on preweighed aluminum foil and dried in an oven for 24-48h at 65°C. To calculate the larval dry weight, larvae and foils will be weighed to the nearest 0.0001mg using an electro-microbalance.

Behavioural observations will be recorded from day 1 to day 45 post-hatch and will terminate when the majority of the larvae are past metamorphosis. Metamorphosis will be determined by the external disappearance of the continuous fin fold and the subsequent formation of discrete fins. Observations will be collected twice a week, and all the observations are to be made by an observer seated in front of each tank between 1000 and 1200h. During each observation period, a larva will be observed for 1 minute. The occurrence (beginning and end of an event) of any five Modal Action Patterns (MAP; swim, motionless, orient, bite, success, miss, pass) or two activities (swim or motionless) performed by the larva will be recorded. At the end of the experiment, the number of surviving larvae will be recorded.

The enzymatic activity of larval whole body homogenates will be studied using a variety of biochemical techniques. Samples will be analyzed for trypsin,

pepsin, a-amylase, total protease, esterase, and alkaline phosphatase activity using techniques outlined in Parent (1998). Additional samples will also be collected for studies of gene expression (using RT-PCR or DNA chips) as well as *in-situ* hybridization studies to localize digestive enzyme gene expression.

Results and discussion

We suggest that older larvae are more vulnerable to mismatches with food than are young larvae. This may be due to the fact that young larvae have poorly developed sensory and digestive capabilities and a small mouth gape. Thus, we hypothesize that the nutritional quality of food becomes more important to the growth of fish larvae in later ontogenetic stages. There is evidence in the literature that the growth of fish larvae is positively influenced by increased prey quality (van der Meeren and Naess, 1993), and that larvae have evolved behavioural and physiological mechanisms to deal with this variability in prey quality (Brown et al., 1997).

It has been observed that if fish larvae do not successfully initiate and maintain feeding behaviour by a critical point in time ("point of no return") after yolk sac absorption, then swimming, foraging, and survival will be reduced (Puvanendran and Brown, 1999). The flexibility of foraging behaviour in larval cod has been demonstrated in response to prey selectivity and hunger (Munk, 1995). We will examine this further with the benefit of digestive enzyme analyses to see what physiological differences exist between fish fed varying diets. We are particularly interested in examining the enzyme profiles of the unfed and fed groups to see if there is an underlying correlation between digestive ontogeny and expressed foraging behaviour.

To get a more comprehensive picture of functional feeding in the cod larvae, behaviour, growth, performance, and enzyme assay results will be examined together. Behavioural data aids in the interpretation of quantitative findings and will further put the results into an appropriate aquaculture context and application. In addition, the possible influence of prey quality on these factors will be examined. This study is part of a larger project undertaken by AquaNet, which will be the first comprehensive examination of larval feeding to be performed anywhere in the world.

References

Brown J.A., D. Wiseman, and P. Kean. 1997. The use of behavioural observations in the larviculture of cold-water marine fish. Aquaculture 155: 297-306.

Cushing D.H. 1990. Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Adv. Mar. Biol.

26:249-293.
Gotceitas V., V. Puvenandran, L. Leader, and J.A. Brown. 1996. An experimental investigation of the ‘match/mismatch’ hypothesis using larval Atlantic cod. Mar. Ecol. Progr. Ser. 130:29-37.
Munk P. 1995. Foraging behaviour of larval cod (*Gadus morhua*) influenced by prey density and hunger. Mar. Biol. 122:205-212.
Puvanendran V., and J.A. Brown. 1999. Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. Aquaculture 175:77-92.
van der Meeren T. and T. Naess. 1993. How does cod (*Gadus morhua*) cope with variability in feeding conditions during early larval stages? Mar. Biol. 116:637-647.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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LIPID AND CARBOHYDRATE DIGESTION IN SCALLOP (*PECTEN MAXIMUS*) JUVENILES

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Introduction

Scallop (*Pecten maximus*) juveniles are regularly grown in intensive or semi-intensive, land-based systems until they can tolerate cultivation in the open sea. The growth of scallop juveniles is dependent on the fed microalgal species and its nutritional value. The content of lipid, protein, and carbohydrate varies between microalgal species and the cultivation conditions (Reitan et.al., 1994). In order to choose the optimal algae for feed, it is important to gain knowledge about the digestive capacity of the juvenile scallops. The aim of this study was to characterize the activity of lipases and β-1,3 glucanase in scallop juveniles, which were fed microalgae with different chemical compositions. β-1,3-glucan is the main carbohydrate in these algae (Myklestad, 1974).

Material and methods

In the first experiment, the activities of neutral lipase and phospholipase A_2 were determined in juvenile scallops (2-mm shell height) after being fed *Isochrysis galbana, Chaetoceros mülleri*, and *Tetraselmis* sp., or starved. The algae were cultivated at low growth rates (2-10% of maximum specific growth rate; Reitan et al., 1994). The second experiment was performed to study the dietary effects of algae cultivated with different growth limitations (5% and 60% of maximum specific growth rate) on the digestion in scallop juveniles (4-mm shell height). The experimental diets consisted of (A) 100% *C. mülleri* (60% of μ_{max}), (B) 70% *C. mülleri* and 30% *I. galbana* (both 60% of μ_{max}), (C) 100% *C. mülleri* (5% of μ_{max}), and (D) 70% *C. mülleri* and 30% *I. galbana* (both 5% of μ_{max}). Activities found in 4-mm scallops were compared to activities found in older scallops (40-50mm).

The feeding experiments were conducted in 50-l tanks using filtered seawater (1µm, 16-17°C, 35ppt salinity) that was exchanged constantly with a daily exchange rate of 100%. Homogenous water circulation was ensured using

circulation pumps in the tanks. The shell height was measured at the start and end of the experiment, and the daily growth rate was calculated based on shell height measurements.

The activities of digestive enzymes were analyzed *in vitro*, both in scallops and the fed algae. Activities of neutral lipase and phospholipase A_2 were analyzed using a modified method of Roberts (1985) and Izquierdo and Henderson (1998). β-1,3-glucanase activity was measured as described by Myklestad et al. (1982). The total lipid content and the total carbohydrate in algae were analyzed as described by Reitan et al. (1994) and Dubois et al. (1956).

Results and discussion

No significant difference was found in neutral lipase activity between the different scallop groups, while small differences in phospholipase A_2 activity were observed (Table I). The activities of neutral lipase and phospholipase A_2 in the algal cells were low compared to that of the scallops.

The contents of both total carbohydrates and total lipids in the algae were higher at lower growth rates than at higher growth rates (Table II). *I. galbana* had a higher total lipid content than *C. mülleri*. The juvenile scallops showed higher growth when they were fed algae produced at lower growth rates (groups C and D) than at higher growth rates (A and B). A positive correlation was found between the content of carbohydrates in the diet and the growth of scallops (Fig. 1). No such correlation was found between total lipid content of the diet and growth of the scallops.

Table I. Activities of neutral lipase and phospholipase A_2 in scallop juveniles fed the algae *I. galbana*, *C. mülleri* and *Tetraselmis* sp., and in scallops starved for 3 days.

Diets	Neutral lipase activity (nmol 4-MU $ind^{-1}min^{-1}$)	Phospholipase A_2 activity (nmolC_6-NBD $ind^{-1}min^{-1}$)
Starving condition	0.90 ± 0.23	0.036 ± 0.011
I. galbana	1.07 ± 0.28	0.022 ± 0.006
C. mülleri	1.04 ± 0.27	0.047 ± 0.013
Tetraselmis sp	0.87 ± 0.23	0.045 ± 0.012

The activity of β-1,3-glucanase was low in all groups of 4-mm scallops compared to larger scallops (40-50mm, Fig. 2). On the other hand, higher specific activity of neutral lipase was observed in the smaller ones than in the larger scallops. These differences may suggest that the lipid digestion capacity is not limiting for the scallop juveniles. The low β-1,3-glucanase activity in the juveniles together with the positive effect of increased content of carbohydrates may suggest that carbohydrate digestion can limit the growth of the scallop juveniles.

Table II. Content of total carbohydrates and lipids in the diets and daily growth rate of scallops (%). DW, Dry weight.

Content (% of DW)	Low growth rate (5% μ_{max})		High growth rate (60% μ_{max})	
	C. mülleri	*C. mülleri+ I. galbana*	*C. mülleri*	*C. mülleri+ I. galbana*
	C	D	A	B
Total carbohydrate	14.9 ± 0.7	14.0 ± 0.7	10.6 ± 0.3	10.0 ± 0.4
Total lipid	15.5 ± 0.3	18.5 ± 0.4	12.0 ± 0.9	15.9 ± 1.3
Daily growth increase (%)	7.4	5.6	4.6	3.7

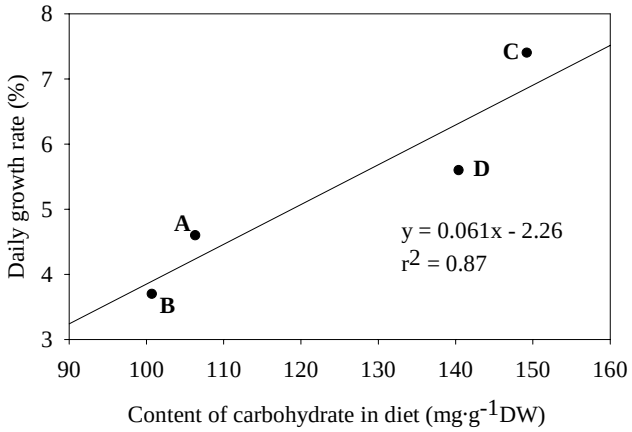

Fig. 1. Correlation between the content of total carbohydrates in the diets and the daily growth rate of the scallops.

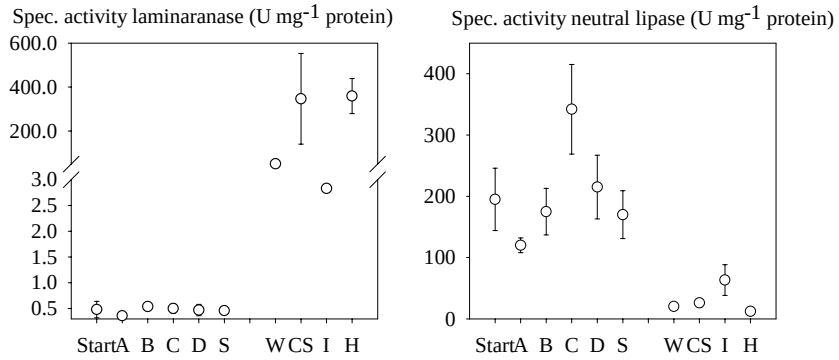

Fig. 2. Specific activity of β-1,3-glucanase and neutral lipase in the different groups of scallop. Start, A-D: fed with algae and S: starved 4 mm scallops, together with W: whole body, CS: crystalline style, I: intestine and H: stomach/digestive glands of 40-50 mm scallops.

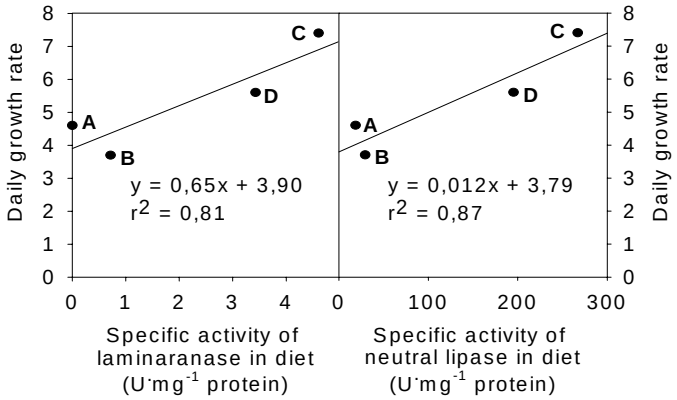

Fig. 3. Correlation between daily growth rate of 2-mm scallops and activity of 1,3-glucanase and neutral lipase.

The daily growth rate of scallop juveniles showed a positive correlation with the specific activities of both β-1,3-glucanase and neutral lipase in the algae (Fig. 3).

This may not only be important for the choice of the right algal species as feed, but also for the development of formulated diets for scallop juveniles.

Acknowledgements

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References

Dubois M., K.A. Gilles, J.K. Hamiltin, P.A. Rebers, and F. Smith. 1956. Method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Izquierdo M.S. and J.R. Henderson. 1998. The determination of neutral lipase and phospholipase activities in gut content of turbot (*Scphthalmus maximus*) by fluorescence-based assays. Fish Physiol. Biochem. 19:153-162.

Myklestad S. 1974. Production of carbohydrates by marine planktonic diatoms. I. Comparison of nine different species in culture. J. Exp. Mar. Biol. Ecol. 15:261-274

Myklestad S., R. Djurhuus, and A. Mohus. 1982. Demonstration of *exo*-(β-1,3)-D-glucanase activity in some planktonic diatoms. J. Exp. Mar. Biol. Ecol. 56:205-211.

Reitan K.I., J.R. Rainuzzo, and Y. Olsen. 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. J. Phycol., 30, 972-979.

Roberts I.M. 1985. Hydrolysis of 4-Methylumbelliferyl Butyrate: A convenient and sensitive fluorescent assay for lipase activity. Lipids 20:243-247.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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FEEDING STRATEGIES FOR EARLY WEANING OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

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The use of live food, rotifers, *Artemia*, or harvested wild zooplankton is currently considered obligatory for successful culture of halibut larvae. Cultivation and enrichment methods of live organisms such as *Brachionus* and *Artemia* are well documented and commercially employed by the industry. However, their use is costly, and their nutritional value is less than adequate for halibut, as the rate of deformities is high (incomplete metamorphosis and skin pigmentation) and growth rates are suboptimal. The development of formulated feeds for halibut larvae from day one or to reduce the period with live feed are essential for the development of the halibut farming industry.

A feeding experiment was conducted on halibut larvae with five different feeding regimes: (1) dry diet from day one, (2) seven days with *Brachionus plicatilis* and weaning to dry diet, (3) fourteen days with *Brachionus plicatilis* and weaning to dry diet, (4) *Brachionus plicatilis* seventeen days, and then *Artemia* from day seventeen, (5) six days with *Artemia* and then copepods.

The total survival rate in the group fed live feed varied from 57-60%. In the group weaned to dry diet on day fourteen, the survival rate was 98% during the weaning period. The survival rate during weaning period, when the weaning started on day seven, was 49%. In the group fed dry diet from day 1, there was total mortality on day nine.

The specific growth rate (SGR) was highest the first week in the group fed rotifers (9.6%). In the *Artemia* group, the SGR was 5.3%. The growth in the early-weaned group was half that in the groups fed live feed. At day 55-post-first-feeding, post-metamorphic characteristics such as eye migration and pigmentation were measured. The feeding regime with rotifers, *Artemia*, and dry diet increased the amount of normal looking halibut juveniles from 7-70% compared to feeding with only *Artemia* enriched with DHA-Selco.

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C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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GROWTH AND SURVIVAL OF COD LARVAE AND JUVENILES – EFFECTS OF PARENTAL ORIGIN

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Abstract

This poster summarizes some of the preliminary results from the EU FP5-project "Demonstration of maternal effects of Atlantic cod: combining the use of unique mesocosm and novel molecular techniques" (http://macom.imr.no). Growth and survival are important viability measures, both for aquaculture and for recruitment to wild stocks. Several factors determine these characteristics, and in this project we look particularly into the importance of the maternal factors (effects related to phenotypic characteristics of the mother fish). Cod is a batch spawner that can produce about 15 batches during the two-month spawning season, and it will usually spawn for several years. In nature, egg size is generally smaller and egg quality poorer from recruit (first-year) spawning females compared to repeat spawning females.

We have followed the offspring from 26 families of cod where the female was a recruit spawner in 13 families, and a repeat spawner in 13 families. The offspring from all 26 families were kept in the same rearing units throughout their life cycles – the first few months in a semi-natural environment (a mesocosm) and further on in indoor tanks. Maternal identity of larvae and early juveniles was established with DNA fingerprinting methods using microsatellites. Important viability measures include growth rates, condition (RNA/DNA ratio), and survival. Egg quality during the spawning season was also monitored, and parental fecundity will be compared with offspring fecundity when these reach maturity in spring 2002.

Preliminary results from the first of two experimental spawning seasons (spring 2000) indicate few significant differences in offspring viability between recruit- and repeat spawners. The size of the mother fishes in the two groups was, however, similar. On the other hand, family variation was large and indicates the importance of breeding in the development of cod aquaculture. It also emphasize the importance individual variation has for early life survival likelihood in the field. The results also demonstrate very well the suitability of modern DNA technology as a tool in marine aquaculture breeding programs.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
European Aquaculture Society, Special Publication No. 30, Oostende, Belgium, 2001

FEED INGESTION PREFERENCES IN JUVENILE COD ESTIMATED BY LANTHANIDE MARKERS

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Abstract

Presently, there are indications that low ingestion rate of formulated feed may be a limiting factor in the growth of juvenile fish, including cod. This has particularly been observed during the weaning period, when the fish must change from a presumable palatable live prey to a usually dry and lifeless diet. The physical property of the diet seems to be an underestimated area of research, compared to the comprehensive work that has been done on diet nutrition. A series of experiments employing lanthanide markers as a tool in estimating diet preferences have been performed on juvenile cod. This poster summarizes some of the results where effects of dietary moisture content on ingestion rates have been examined. A fishmeal-based diet was produced with five levels of water, giving diets with 35, 42, 54, 65, or 95% dry weight. Each diet was marked with a small amount of lanthanide oxide. A short-term feeding experiment was performed on 0.8-g cod. The fish were hand-fed on an equal-weight basis mixture of the five diets. Ingestion rates were then estimated by killing half of the fish after one day and the rest after four days of feeding. The whole fish with gut contents were homogenized and lanthanide content was measured by ICPMS. The amount ingested of each of the five diets could then be calculated from these figures. Even though there is quite a high variation in diet selection between individuals, the results clearly indicate a preference for moist feed. Ingestion rates increased more than just the compensation for reduced energy in the moist feeds, which could be expected.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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LIPID COMPOSITION DURING EMBRYOGENESIS AND EARLY LARVAL DEVELOPMENT IN SHRIMP (*PENAEUS VANNAMEI*)

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Introduction

In a previous work, it was shown that eggs which produced zoea larvae with a higher survival had higher levels of triacylglycerol and lysophosphatidylcholine (LPC) (Palacios et al., 2000). The objective of the present study was to analyze the changes in fatty acid composition of neutral and polar lipid fractions and for each phospholipid class that occur from recently spawned eggs to zoea.

Methods

Ablated females were matured as in previously described conditions (Palacios et al., 2000). The spawn from four mature females (46.2±3.8g) were individually reared to zoea stage (fertilization = 68.1±18.7%). Tanks were sampled periodically after spawning; at 1h (eggs, 254±3µm), 7h (embryos, 269±1µm), 13h (NI, 297±4µm), 33 h (NIII, 367±4µm), 57h (NIV, 448±5µm), and 79h (ZI, 493±6µm). Samples were pooled for each stage and extracted with chloroform:methanol (2:1 v/v). Phospholipid classes were separated on a HPLC with an UV as described by Soudant et al. (1995). Fatty acids (FA) were transesterified by BF3:methanol, and analyzed on a Chrompak 9001 GC equipped with a flame ionization detector.

Results and discussion

The proportion of FA in the neutral (NL) and polar lipid fraction (PL) in each stage are presented in Table I. Saturated FA increased though development. The sum of (n-3) decreased slightly, but the sum of (n-6) remained relatively constant, thus the ratio (n-3):(n-6) decreased. The proportion of PUFA present in the PL increased during development at the expenses of monounsaturated fatty acids (MUFA). The sum of both (n-3) and (n-6) increased, and thus the ratio (n-3):(n-6) remained constant.

When expressed on a μmol.g^{-1} basis, total FA of the NL decreased by 67% from eggs to ZI. The sum of saturated FA decreased from 82.1-31.8μmol.g^{-1} (61%), the MUFA from 60.4-19.0μmol.g^{-1} (68%), and the PUFA from 59.7-15.7μmolg^{-1} (74%, Fig. 1A). Thus, the PUFA of the NL are used at a higher rate than saturated FA during shrimp larval development.

Table I. Distribution (molar %) of FA for each stage (h after spawn).

Fatty acid	Neutral lipid fraction						Polar lipid fraction					
	1	7	13	33	57	79	1	7	13	33	57	79
14:0	3.4	3.5	3.1	3.2	2.7	2.7	1.1	1.4	1.1	1.0	0.8	1.3
16:0	31.7	31.5	32.4	32.7	33.5	37.8	24.0	22.8	23.8	23.1	25.0	24.9
18:0	4.1	3.8	4.2	4.2	5.0	6.6	9.5	9.9	9.8	9.4	8.6	8.3
16:1n-7	8.4	9.2	8.5	8.6	7.3	5.6	6.7	5.7	6.2	5.4	4.6	2.7
18:1n-9	15.6	15.2	16.3	15.7	16.2	16.1	15.6	16.2	15.8	14.3	12.2	10.6
18:1n-7	4.6	4.4	4.8	4.8	5.0	5.4	4.8	5.1	5.1	4.8	4.1	4.1
20:1	2.2	2.0	2.2	1.8	2.2	1.9	3.6	2.8	2.9	2.7	1.7	2.0
18:2n-6	2.9	3.0	3.4	3.5	3.4	3.1	2.7	3.1	2.9	3.4	3.3	2.8
20:2n-6	0.7	0.7	0.8	0.8	0.9	1.0	1.1	1.3	1.3	1.4	1.4	1.6
20:4n-6	1.1	1.1	1.1	1.2	1.2	1.2	2.3	2.4	2.3	2.7	3.1	3.6
20:5n-3	7.5	7.5	6.8	7.4	6.6	5.7	11.8	12.4	12.1	14.4	17.4	19.4
22:5n-3	1.1	1.1	1.0	0.9	0.8	0.6	1.1	1.1	1.0	1.0	0.7	0.4
22:6n-3	14.7	15.0	13.6	13.7	13.4	10.5	12.9	14.0	13.8	15.0	15.4	16.4
Σ Saturates	39.3	38.8	39.7	40.1	41.3	47.2	34.6	34.2	34.8	33.5	34.5	34.4
Σ MUFA	30.8	30.8	31.9	30.8	30.7	29.0	30.6	29.8	29.9	27.2	22.5	19.4
Σ PUFA	29.9	30.4	28.4	29.1	28.0	23.9	34.8	36.1	35.3	39.3	43.0	46.2
Σ (n-6)	4.2	4.2	4.7	4.9	4.8	4.4	5.1	5.7	5.4	6.2	6.8	7.0
Σ (n-3)	24.1	24.6	22.2	22.8	21.5	17.7	26.8	28.4	27.7	31.0	34.2	37.3
(n-3):(n-6)	5.8	5.8	4.7	4.7	4.5	4.0	5.2	5.0	5.1	5.0	5.1	5.4

In the PL, total FA were reduced by 24% during development to the ZI stage. However, this decrease was observed only for MUFA (51%) and saturated FA (24%, Fig. 1B), in accordance to the reported use of some phospholipids, especially phosphatidylcholine (PC), for energy supply during crustacean development (Coutteau, et al., 1997). However, PUFA levels remained constant, indicating retention throughout larval development, in accordance to their role in membrane phospholipids (Sargent, 1995). Transfer of PUFA from NL to PL has been suggested for penaeid shrimp larvae (Cahu et al., 1988), and could be the case of the present work, as shown by the increase in 22:6n-3 from nauplii stage I to III, and 20:5n-3 from eggs to zoea.

PC was the major phospholipid (from 40-58%), followed by the LPC (13-23%), phosphatidylinositol (PI, 8-18%), lysophosphatidylethanolamine (LPE, 6-10%), phosphatidylethanolamine (PE, 4-9 %), and phosphatidylserine (PS, 2-4%).

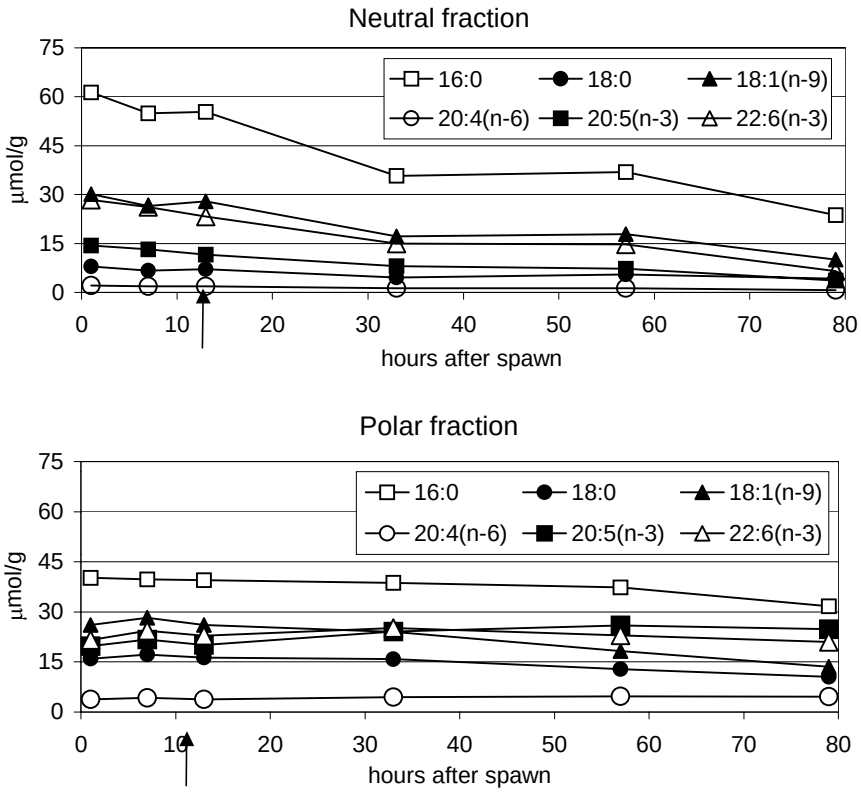

Fig. 1. Concentration (µmol.g^{-1}) of the main FA (The arrow represents hatching).

In the PC, the major FA were 16:0>18:1n-9>22:6n-3, and the proportion of 16:0 increased during development while the 18:0 decreased. The proportion of MUFA decreased throughout the development. The proportion of PUFA increased from embryo to NIII, but decreased to ZI. In the LPC, the major FA were 16:0>20:5n-3>22:6n-3. The proportion of PUFA was highest in ZI, at the expense of a decrease in the proportion of MUFA. In the PE and the LPE, the major FA were 20:5n-3>22:6n-3>16:0. In PE, the lowest proportion of total PUFA was found in the ZI stage, with a higher proportion of MUFA and saturated FA. In the PI, the highest proportion of 20:4n-6 was found in the egg and the lowest after hatching. The proportion of 18:0 and the ratio (n-3):(n-6) increased during development, while the other FA tended to remain stable in this fraction. In the PS, the major FA were 18:0>16:0>20:5n-3>22:6n-3. The sum of saturated FA increased, essentially at the expense of MUFA.

Table II. FA (molar %) in phospholipid classes during development (h after spawn).

		Σ Saturates	Σ MUFA	Σ PUFA	Σ (n-6)	Σ (n-3)	(n-3):(n-6)
PC	7	36.3	33.8	29.9	5.0	23.3	4.7
	33	37.0	30.0	33.0	5.6	25.7	4.6
	57	35.3	26.5	38.1	6.3	30.3	4.8
	79	41.7	24.3	34.1	5.9	26.6	4.5
PE	7	35.4	20.1	44.6	5.9	37.6	6.4
	33	33.9	15.6	50.6	5.9	43-9	7.5
	57	28.1	17.7	54.2	6.2	47.5	7.6
	79	44.5	22.0	33.5	5.7	27.5	4.8
LPC	7	34.9	22.8	42.2	5.7	35.8	6.3
	33	34.4	25.5	40.1	5.6	33.1	5.9
	57	39.5	22.8	37.7	6.1	30.9	5.1
	79	37.1	12.9	50.0	6.3	42.8	6.8
LPE	7	23.8	17.8	58.3	5.6	52.4	9.4
	33	25.4	10.7	63.9	6.2	57.4	9.3
	57	26.5	20.4	53.0	6.6	45.9	7.0
	79	25.2	13.2	61.6	7.8	52.5	6.7
PS	7	38.5	26.0	35.6	4.8	30.2	6.3
	33	46.9	16.8	36.3	4.7	30.8	6.6
	57	45.0	28.1	26.9	3.2	22.5	6.9
	79	53.1	11.5	35.4	5.6	28.8	5.1
PI	7	20.2	29.8	49.9	17.1	29.2	1.7
	33	27.2	28.5	44.3	11.3	25.2	2.2
	57	27.4	23.9	48.6	13.4	31.4	2.3
	79	36.7	21.0	42.4	10.4	30.1	2.9

References

Cahu C., A. Severe, and P. Quazuguel. 1988. Variation de la composition en lipides de *Penaeus indicus* au cours de son development larvaire. Comité de la Mariculture, C.M. 1988/F:22.

Coutteau P., I. Geurden, M.R. Camara, P. Bergot, and P. Sorgeloos. 1997. Review on the dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture 155:149-164.

Palacios E.**,** I.S. Racotta, H. Heras, Y. Marty and J.F. Samain. 2000. Relation between lipid composition of eggs and larval survival in shrimp (*Litopenaeus vannamei*, Boone, 1931). V International Symposium on Aquaculture Nutrition, November 19-22, 2000 Mérida, Yucatan, México. p.88.

Sargent J.R. 1995. Origins and functions of egg lipids: Nutritional implications. p.353-372. In: Broodstock Management and Egg and Larval Quality. N.R. Bromage and R.J. Robert (Eds.) Blackwell Science, Cambridge.

Soudant P., Y. Marty, J. Moal, and J.F. Samain. 1995. Separation of major polar lipid in *Pecten maximus* by high performance liquid chromatography and subsequent determination of their fatty acids using gas chromatography. J. Chromatogr. 673:15-26.

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TOLERANCE RESPONSE OF *SPARUS AURATA* AND *SOLEA SENEGALENSIS* LARVAE TO DIFFERENT pH VALUES

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Introduction

Most fish larvae are very sensitive to any kind of environmental changes (Westernhagen, 1988). Changes in water characteristics can increase the rate of mortality during the first developmental stages. Water quality is also of special interest in aquaculture. Waste in aquaculture is generated primarily by food and excretion processes (Cho et al., 1994). The pH has been considered an environmental factor that strongly affects the survival during the first developmental stages. Seawater has a high buffering capacity, and the pH is maintained in a narrow range, however the rearing technologies and procedures (recirculation systems, partial or intermittent water renovation, microalgae addition, animal density, use of artificial diets, etc.) can induce changes in water pH. Westernhagen (1988) described the effects of low pH values in different developmental stages of larval fish, but there is scarce information about the effect of high pH values. Therefore, furthering knowledge of the tolerance limits of fish larvae to different abiotic factors is a necessary step to improve new rearing systems. The aim of this paper is to define the 24-h mortality responses in early *Sparus aurata* and *Solea senegalensis* larvae when exposed to different pH values.

Materials and methods

Sparus aurata and *Solea senegalensis* eggs were obtained by natural spawning from captive broodstock. Different egg batches were used. Eggs were incubated at 19.5ºC, and the newly hatched larvae were transferred to 300-l tanks with permanent illumination and 33g.l^{-1} salinity. Constant aeration was provided. Initial larval density ranged from 50-70 larvae.l^{-1}. The rotifers *Brachionus rotundiformis* and *B. plicatilis* were used as live food in *Sparus aurata*, and *B. plicatilis* and *Artemia* nauplii in *Solea senegalensis*.

Sterilized seawater with 33g.l^{-1} salinity and pH 8.2 was used for the tests. Analysis of the seawater showed low levels of ammonia-N and nitrite-N (0.32ppm and 0.20ppm, respectively). Modified seawater with different pH values for testing was obtained using tris and phosphate-citrate buffer.
Tolerance experiments were conducted with 12-day-old *Sparus aurata* larvae and 7-day-old *Solea senegalensis* larvae with mean dry weights of 67±15 and 103±9µg, respectively. These ages were chosen in an attempt to avoid the mortality associated with first feeding and to compare larvae with a similar developmental stage. Thirty larvae were collected from the rearing tanks, transferred gently to the experimental beakers (1 l) with a specific modified pH, and kept under constant light for 24h with a constant temperature of 19.5±0.5ºC. No food was supplied during the experiment. The pH was measured at the beginning and at the end of the experimental period, and the average pH value obtained was used for later calculation. After 24h of exposure, the dead larvae were counted in each beaker. Control mortality was taken into account to calculate the corrected mortality (Abel and Axiak, 1991). Three replicates were conducted with each initial pH value. The lethal pH values affecting 50% of the population in 24h (LpH_{50}) was determined from the linear regressions between the corrected mortality probits and logarithmic concentration, as well as the 95% confidence limits (Abel and Axiak, 1991).

Results and discussion

Larval response was analyzed in a 24-h test because in preliminary experiments we verified that mortality increased rapidly when larvae were maintained in the experimental beakers for more than 1 day. Larval mortality vs. pH showed a double sigmoid response curve in *Sparus aurata* and *Solea senegalensis* (Fig. 1a and Fig. 2a). Both species showed different responses when exposed to low and

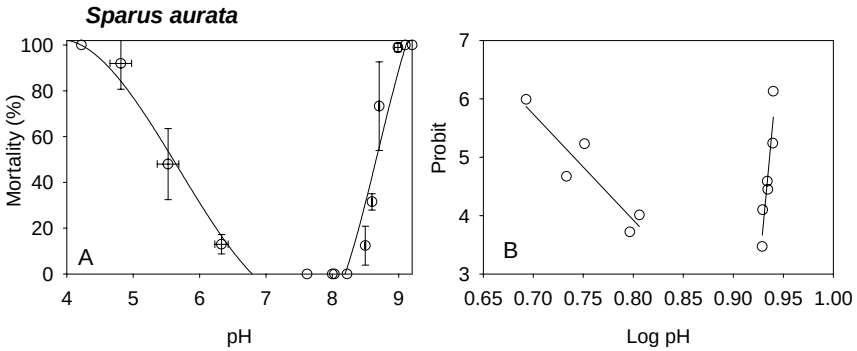

Fig. 1. (A) Corrected mortality after 24-h test under different pH values and (B) regression lines obtained with log-transformation of pH values vs. probit transformation of corrected mortality in 12-day-old *Sparus aurata* larvae after 24-h test.

high pH values. Higher mortalities take place in a narrower interval at high pH values and with a quicker response than at low pH values (Table I). Total mortality occurred when pH was lower than 4.5 in both species. When larvae were exposed to high pH values *Sparus aurata* showed total mortality over pH 8.9, while *Solea senegalensis* did so over pH 9.5. The pH range tolerated by *Sparus aurata* and *Solea senegalensis* larvae was relatively wide and similar to the results showed by Brownell (1980) for other marine fish larvae at first-feeding, although the tolerance range reported by this author is a little wider (4.74-5.06 for low pH_{50} and 9.04-9.24 for high pH_{50}). They resists similar low pH values (5.55 and 5.76 Low pH_{50}, respectively) (Fig. 1b and Fig. 2b) (Table I). However, the high pH_{50} was lower in *Sparus aurata* larvae than in *Solea senegalensis* (8.66 and 8.94 high pH_{50}, respectively) (Table I).

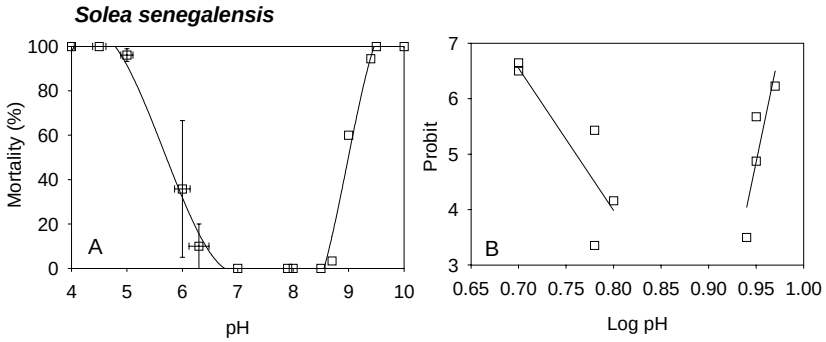

Fig. 2. (A) Corrected mortality after 24-h test under different pH values and (B) regression lines obtained with log-transformation of pH values vs. probit transformation of mortality in 7-days-old *Solea senegalensis* larvae after 24-h test.

Table I. Tolerance regression models of 12-day-old *Sparus aurata* larvae and 7-day-old *Solea senegalensis* larvae exposed to different pH. The 24-h pH_{50} values and confidence limits (95% CL) are included.

	pH	regression	24-h pH_{50} (95% CL)
Sparus aurata	Low	y = 15.28-13.81x r^2 = 0.73; *n* = 6	5.55 (5.87 - 5.24)
	High	y = -107.52+120x r^2 = 0.87; *n* = 7	8.66 (8.60 - 8.72)
Solea senegalensis	Low	y = 24.50-25.65x r^2 = 0.73; *n* = 5	5.76 (5.86 – 5.65)
	High	y = -72.85+81.81x r^2 = 0.76; *n* = 4	8.94 (8.90 – 8.98)

Larvae of *Solea senegalensis* exhibited a higher tolerance to other toxins – such ammonia and nitrite – than *Sparus aurata* larvae (Parra and Yúfera, 1999), but in

the present study both species showed similar tolerance response to pH changes. It has been described that low pH values induce changes in calcium metabolism and alter the osmotic activity, while the high pH values interfere with certain enzymes activities and their optimum pH, and also in the osmoregulation (Albers, 1970). As larvae have to invest more energy trying to modify those alterations, growth, development, and survival are affected. Values for high LpH_{50} observed in the present study are very close to those that are usually present in larval rearing systems. Therefore, small fluctuations in the water pH can be the cause of development problems and mortalities.

References

Abel P.D. and V. Axiak. 1991. Ecotoxicology and the marine environment. Ed. Ellis Horwood De., London 264p.

Albers C. 1970. Acid-Basic Balance. p.173-208. In: Fish Physiology. Vol. IV. Hoar and Randdall. Academic Press.

Brownell C.L. 1980. Water quality requirements for first-feeding in marine fish larvae. II pH, oxygen and carbon dioxide. J. Exp. Mar. Biol. Ecol. 44:285-298.

Kamler E. 1992. Early life history of fish. An energetic approach. Chapman and Hall. Fish Fish. Ser. 4. 267p

Parra G. and M. Yúfera. 1999. Tolerance response to ammonia and nitrite exposure in larvae of two marine fish species (gilthead seabream *Sparus aurata* L. and Senegal sole *Solea senegalensis* Kaup). Aquacult. Res. 30:857-863.

Westernhagen H. 1988. Sublethal effects of pollutants on fish eggs and larvae. In: Fish Physiology. Vol.XI. Part A. Hoar and Randall. Academic Press.

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COMPARATIVE STUDY ON THE LARVAL BEHAVIOUR OF CUTTLEFISH *SEPIALLA INERMIS* AND *SEPIA PHARAONIS*

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Abstract

The hatchlings of *Sepia pharaonis* are benthic and prefer hideouts, adhering to the substratum with the ventral surface of their mantle. Contrarily, *Sepialla inermis* are planktonic, very active, and swim hovering at an angle of 50-60º. The arms of *Sepia pharaonis* are longer than *Sepialla inermis*, and the first two arms are always pointed. *Sepia pharaonis* prefers prey twice the size of its body. *Sepialla inermis* hatchlings and paralarvae exhibit food sharing behaviour while the other cuttlefish is highly competitive. The behaviour, pattern of feeding, and growth of the paralarvae of both the species are compared and discussed.

Introduction

The spineless cuttlefish (*Sepialla inermis*) and pharaoh cuttlefish (*Sepia pharaonis*) of the family Sepiidae are two commonly occurring species in the coastal waters of Tuticorin (southeast coast of India). These are commercially important and play an significant role in the export market. Though they are abundant, much attention has not been paid to their larval development and culture methods. Few attempts have been made to study the development of Big fin squid (Nabhitabhata, 1996) and spineless cuttlefish (Nabhitabhata, 1997, Sivalingam et al., 1993 and Sivalingam 1999). As the hatchlings resemble a miniature adult, the term "paralarva" was introduced for the planktonic young of cephalopods that meet certain ecological – and in some cases, morphological – criteria (Sweeney et al., 1992). Because cephalopod culture has gained momentum in many Asian countries, the studies on the larval behaviour will be very useful to hatchery rearing and pond growout. The present study was undertaken to study and compare the larval behaviour of these two species belonging to the same family.

Materials and methods

The egg masses of cuttlefish *Sepialla inermis* used for the study were spawned in the laboratory, while eggs of *Sepia pharaonis* were collected from crab nets

set around Tuticorin. Eggs were maintained separately in 50-l capacity fiberglass tanks with clean seawater and sufficient aeration. Light was avoided using nets to prevent any algal growth. Water temperature, salinity, and pH were maintained constant during the incubation period. After hatching, the hatchlings were initially fed mysids and later with postlarvae, small size adult shrimps, and fish fry. Growth rate based on the total length was studied for 51 days. PVC pipe pieces were provided in the tanks as shelters for the hatchlings. Behavior relating to feeding, swimming, defense, cannibalism, and others were studied and compared for both species.

Results and discussion

The spineless cuttlefish deposits its eggs in clusters. Each egg capsule is black in colour, opaque, round, and possesses a tip and a long stalk. The incubation period was 18 days. The pharaoh cuttlefish egg capsules are single, white in colour, and have striations, possessing a tip and short stalk. The incubation period of these eggs was 21 days.

Sepialla inermis hatchlings are planktonic and were found congregating to the sides of the tank at the surface. Swimming was found to be a 'hovering' fashion with the head pointed downward. This was explained as a 'hold behaviour' to a peculiar angle of 60-80º to the floor (Nabhitabhata, 1997). The same behaviour has also been observed in the big fin squid *Sepioteuthis lessoniana* (Nabhitabhata, 1996). They never prefer hideouts and swim in groups, whereas the hatchlings of *Sepia pharaonis* are benthic and prefer hideouts. The size of these hatchlings is bigger than those of *Sepialla inermis*.

In *Sepialla inermis*, the chromatopores are normal, dark brown and white or transparent. The function of the ink sac was normal as in adults. In the posterior end of the mantle, a characteristic granular pore is present and it secretes a coffee-brown coloured fluid when under stress. Norman and Reid (2000) reported that the function of the granular pore was unknown. Nabhitabhata and Polkhan (1983) observed the release of brown fluid from the anal pore after abrupt change to low salinities. In the case of *Sepia pharaonis*,, the chromatopores were observed as a band from the posterior to anterior end when they swam and landed in a new place.

Both cuttlefish hatchlings started to feed voraciously from the second day onwards, using their tentacles. The arms and tentacles of *Sepia pharaonis* are larger and the first two arms are in a pointed fashion. In *Sepialla inermis*, an interesting behaviour of food sharing was observed, where 2-4 paralarva of different sizes shared one prey. This is not observed in *Sepia pharaonis* and the paralarvae displayed feed competition. The initial mantle length of *Sepialla inermis*. was 6.9mm, while in *Sepia pharaonis* it was 10.5mm. The growth rate

was much faster in the pharaoh cuttlefish and it reached a mantle length of 64.75mm after only 50 days. The percentage growth rate was higher in *Sepia pharaonis* than *Sepialla inermis* (Table I).

Table I. Percentage increase of mantle length of *Sepialla inermis* and *Sepia pharaonis* larvae cultured for 50 days.

Days	*Sepialla inermis*		*Sepia pharaonis*	
	Mantle length (mm)	% increase	Mantle length (mm)	% increase
Initial	6.9	–	10.5	–
10	8.1	1.60	16.0	4.15
20	11.7	2.58	30.0	4.90
30	20.6	3.00	42.75	4.48
40	29.9	3.27	53.30	3.19
50	30.6	2.47	64.75	2.82

In *Sepialla inermis*, heavy cannibalism was observed. The weaker and smaller ones were eaten by the stronger and larger paralarvae. This phenomenon occurred when there was a feed demand. Herny and Camou, (1989) observed crustaceans (75%), fishes (15%), and cephalopods (5%) in the stomach contents of young *Sepia officinalis*. Cannibalism was not observed in *Sepia pharaonis*, and they also never accepted dead fish even after 30 days, reported earlier by Sivalingam 1993. But the paralarvae of *Sepialla inermis* accepted dead fish when there was lack of live feed such as mysids and fish fry.

It is necessary to do further research and development on cephalopod culture in order to assess growout feasibility. Among the two cuttlefish species studied, *Sepia pharaonis* was found to be a suitable species for mass culture in growout ponds due to the absence of cannibalism and a fast growth rate.

References

Henry J. and B.Camou. 1989. Diet of very young *Sepia officinalis* L. in Bay of Seine. In: The cuttlefish – First International Symposium on the cuttlefish Sepia- Caen, June 1-3, 1989. Caen, France. 195 p.

Nabhitabhata J and P. Polkan. 1983. Biological studies on economic Cephalopods II. Survival and growth of cuttlefish, *Sepialla inermis* in various salinity conditions. Technical paper 4. Rayong Brackishwater Fisheries Station, Brackishwater Fisheries Division, Department of fisheries. 48 p.

Nabhitabhata J. 1996. Life cycle of cultured bigfin Squid *Sepioteuthis lessonisna*, Lesson. Phuket Mar. Biolog. Ctr. Spec. Publ. 16:83-95.

Nabhitabhata J.1997. Life cycle of three generations of spineless cuttlefish *Sepialla inermis* (Ferussac and D'Orbingy 1848), Phuket Mar. Biolog. Ctr. Spec. Publ. 17(1):289-298.

Norman M.J. and A .Reid. 2000. A guide to Squid, Cuttlefish and Octopuses of Australasia, CSIRO Publication. Australia. 96p.

Sivalingam D, K Ramadoss, A. D Gandhi and M.Enose. 1993. Hatchery rearing of the Squid, *Sepioteuthis lessoniana* and the cuttlefish *Sepia pharaohnis.* Mar. Fish. Inf. Serv. 22:12-14.

Sivalingam D.2000. Seed production of spineless cuttlefish *Sepiella inermis* - A breakthrough in cephalopod culture. Seafood Export J. 30(2):3-4.

Sweeney M.J., C.F.E Roper, K.M. Mangold, M.R.Clarke and S.V. Boletzky. 1992. Larval and juvenile cephalopods - A manual for their identification. Smithson. Contrib. Zool. 513.

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BIOCHEMICAL INDICATOR OF QUALITY OF SCALLOP LARVAE *PLACOPECTEN MAGELLANICUS*

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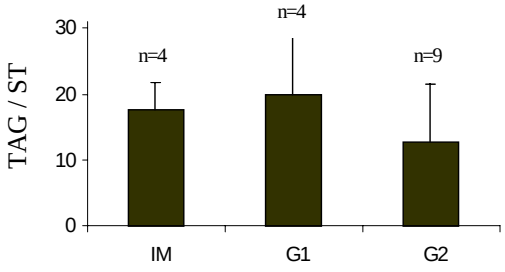
Introduction

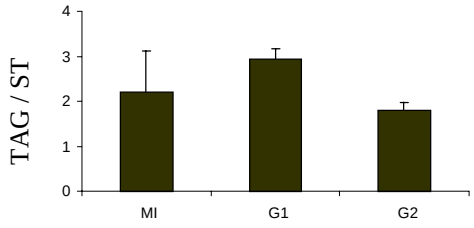
In order to sustain the increasing demand of juveniles of the scallop *Placopecten magellanicus*, hatcheries need to produce reliable quantities of eggs and larvae. However, egg and larval quality, defined as a physiological characteristic, is highly variable and may have profound effects on growth, survival, and success of metamorphosis. Total lipid is a good indicator of quality, since it has been correlated with growth and viability of bivalve larvae (Gallager *et al.* 1986). Indeed, during embryogenesis, larvae depend on endogenous reserve, and lipid account for 47.6% of energetic needs (Whyte *et al.* 1991). When larvae are able to feed from exogenous sources, excess energy is stored mainly as triacylglycerol, the major storage lipid in animal cells (Lehninger et al., 1993). During metamorphosis, neutral lipids, particularly triacylglycerol (TAG; Holland, 1978; Gallager and Mann, 1986), are the primary energy reserve, although proteins are used later. Thus, lipids and proteins account for approximately 95% of the total energy requirement (Holland and Spencer 1973). Based on these results, attention has been paid to quality assessment using as physiological indicator ratio of lipid classes (Fraser, 1989; Delaunay et al., 1992; Miron et al., 2000). TAG content can be correlated with larval quality, but it cannot be used directly since it is dependent on larval size. Use of TAG-sterol (ST), TAG-DNA, or TAG-organic matter ratios have been suggested to take into account the size dependency of TAG content, since there are positive correlations between ST, DNA, or organic matter content with larval weight.

The purpose of our preliminary study is twofold: 1) to produce a biochemical indicator of gonad, egg, and larval quality based on lipid class, and 2) to verify if this indicator can predict growth, mortality, or success of metamorphosis.

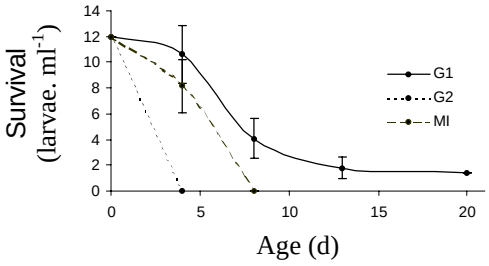
Materials and methods

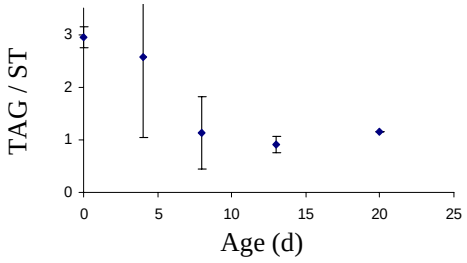
Experimental design. *P. magellanicus* larvae were obtained from parents originating from two stocks, Magdalen islands (MI) and Gaspé peninsula (G) (Quebec, Canada). Gonad maturity level was assessed before spawning according to visual observations. Broodstock from MI was induced to spawn in mid-July, but those of Gaspé were induced at the end of July (G1) and the beginning of September (G2). Triplicate samples of gonads of each spawned individual were taken and stored at -80°C until lipid class analysis. We used different populations and spawning periods in order to optimize the potential differences of gonad quality. Larvae were reared at the experimental hatchery of Grande Rivière (Québec) in 15-l batch culture with light bubbling at 12°C. Densities vary from 1-12 larvae.ml^{-1} depending on the developing stage. Larvae were fed with a mixture of the diatom *Chaetoceros mulleri* and the flagellate *Isochrisis* sp. (T-Iso) at approximately 15 000 cells.ml^{-1}. Triplicate samples of eggs and larvae were taken at day 4, 8, 13, 20, and 28 after fertilization, and





stored at -80°C until lipid class analysis. At the same time, we collected approximately 300 organisms in 10%formaldehyde for growth measurements





and larval density assessment.

Lipid analysis. The solvent system for lipid separation was CH_2Cl_2-MeOH-H_2O (2:1:1; v/v/v). The homogenates were centrifuged at 2000rpm for 2min and the lipid fraction was removed after each wash and transferred to a clean tube. The solvent was evaporated under a nitrogen flow and lipids suspended in 10µl CH_2Cl_2. Total extracts were spotted onto S-III Chromarods using a Hamilton syringe. Four different solvent systems were used to obtain four chromatograms per rod according to the methods described by Parrish (1987) and Innis (1981). These combined methods allowed us to separate aliphatic hydrocarbon, ketone, triacylglycerol, free fatty acid, free sterol, diglyceride, acetone mobile polar lipid, and different phospholipid. Chromarods were scanned by the flame

ionization detection system (FID) of the analyzer Iatroscan Mark-V (Iatron Laboratories Inc., Tokyo, Japan).

Results and discussion

We present results based on triacylglycerol (TAG)-sterol (ST) ratios to assess quality. Based on this ratio, variation of gonad quality (Fig. 1) are reflected in eggs (Fig. 2) despite a decrease by a magnitude of ten from gonad to egg. However, differences of gonad and egg quality as measured by TAG-ST ratio were not significant ($P<0.109$ and $P<0.246$, respectively). Our three batches of larvae had survival times of 4, 20, and 8d for MI, G1, and G2, respectively. These seemed to be related to egg quality. Indeed, a focus on the quality of G1 larvae shows that survival decreased in the same way as the TAG-ST ratio. Not only is this tendency typical of larvae in poor physiological condition (Fraser 1989), it is also consistent with the fact that no batch has reached metamorphosis and growth was negligible or had stopped. It is impossible to determine the relative importance of egg quality or environmental conditions in explaining this result. This study shows that the TAG-ST ratio offers some promise to hatchery practices in order to predict larval survival. The potential use of this indicator will be verified in a major study in the summer of 2001 involving larvae of more variable quality until metamorphosis.

References

Delaunay F., Y. Marty, J. Moal, and J.-F. Samain. 1992. Growth and lipid class composition of *Pecten maximus* (L.) larvae grown under hatchery conditions. J. Exp. Mar. Biol. Ecol. 163:209-219.

Fraser A.J. 1989. Triacylglycerol content as a condition index for fish, bivalve and crustacean larvae. Can. J. Fish. Aquat. Sci. 46:1868-1873.

Gallager S.M., R. Mann, and G.C. Sasaki. 1986. Lipid as an Index of growth and viability in three species of bivalve larvae. Aquaculture 56:81-103.

Holland D.L. and B.E. Spencer 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* L. during larval development, metamorphosis and early spat growth. J. Mar. Biol. Assoc. UK 53:287-298.

Innis S.M. and M.T. Clandinin. 1981. Separation of phospholipids on chromarods. J. Chromatogr. 205:490-492.

Lehninger A.L., D.L. Nelson, and M.M. Cox. 1993. Lipids. Principles of Biochemistry. I. Worth publisher. New York.: 1013.

Miron G., L. Walters, R. Tremblay, and E. Bourget. 2000. Physiological condition and barnacle larval behavior: a preliminary look at the relationship between TAG/DNA ratio and larval substratum exploration in *Balanus amphitrite*. Mar. Ecol. Progr. Ser. 198:303-310.

Parrish C.C. 1987. Separation of Aquatic Lipid Classes by Chromarod Thin-Layer Chromatography with measurment by Iatroscan Flame Ionization Detection. Can. J. Fish. Aquat. Sci. 44:722-731.

Whyte J.N.C., N. Bourne, and N.G. Ginther. 1991. Depletion of nutrient reserves during embryogenesis in the scallop *Patinopecten yessoensis* (Jay). J. Exp. Mar. Biol. Ecol. 149:67-80.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EXTENSIVE REARING OF SEABASS LARVAE USING *ARTEMIA*

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Introduction

Although intensive production of seabass fry is successfully performed in the Southeast Asian region, results are not always predictable. Massive mortality occurring around d15-19 often causes big losses in commercial hatcheries in Australia, Indonesia, and Thailand. Viral infections and unexpected mortality, such as over inflation of the swimbladder, bone deformation, accumulation of excessive fat deposit in the liver, high sensitivity of larvae etc., are often observed (Danayadol, 1984; Bagarinao and Kungvankij, 1986). Unstable nutritional quality is one of the disadvantages of using live food. Animals fed on diets consisting of more than one species of live food grow faster and more vigorously than those fed on single species diets. The synergistic interaction of the mixed diet component seem most likely related to the availability and balance of fatty acids, amino acids, or micronutrients such as vitamins and minerals. Maneewong et al. (1987) reported a survival rate of 40% during the intensive hatchery and nursery phase, using *Brachionus* and *Artemia* as live food. Nutritional enhancement of the highly unsaturated fatty acids (HUFA) in live food resulted in significant improvement of survival and physiological condition of the larvae, consequent to the lower disease susceptibility and higher yield (Dhert et al, 1990). Increasing food concentration can partially help reduce grading and stress during this period. This study investigated the possibility of rearing seabass larvae in earthen ponds using *Artemia* as a single live food. We expected that the natural condition of the earthen ponds could overcome the handicap of using the single species diet. Cost and return analysis was done in order to show the net income and profit of the production system.

Materials and methods

Seabass (*Lates calcarifer*) larvae were cultured in the 800-m^2 earthen ponds at 3 different stocking densities (37.5, 75, and 112.5 larvae.m^{-2}, respectively). Each

pond was installed with 4 lines of airpipes placed 10cm above the pond bottom. The ponds were dried and limed with 187.5kg.ha^{-1} for 3 days, thereafter seawater was filled to 120cm depth. Each pond was disinfected with 30ppm calcium hypochlorite and aerated 7d before adding the fish.

Fifteen-day-old seabass larvae were fed with *Artemia* nauplii 3 times a day (09.00, 16.00, and 24.00h) *ad libitum* during the first week, followed by adult *Artemia* during the second week. Water level was maintained at 120cm and water parameters such as salinity, alkalinity, and pH were checked daily. Total length and mortality were measured and cost and return analysis were calculated at the end of two-week experiment.

Results

Growth and survival. Larval seabass growth was categorized into 3 groups according to their total lengths (TL; small fish 1.50-2.5cm, medium fish 2.5-3.5cm, and large fish 3.5-5cm). The percentage of big fish increased according to decrease of the stocking density, where the highest ratio of big fish (14.87%) was at the lowest stocking density (37.5 larvae.m^{-2}) (Table 1). The results revealed that seabass larvae stocked at 37.5 larvae.m^{-2} had the highest survival rate (56.04%), followed by high and medium stocking densities, which had survival rates of 53.70 and 44.55%, respectively.

Table I. Percentage of seabass larvae cultures in three different stocking densities.

Total length	37.5 larvae.m^{-2}	75 larvae.m^{-2}	112.5 larvae.m^{-2}
Small (1.5-2.5 cm)	16.18	30.89	35.22
Medium (2.5-3.5 cm)	68.32	57.24	54.24
Large (3.5-5 cm)	14.87	11.87	10.54

Cost and return. Cost and return of seabass nursing in earthen ponds was analyzed after the experiment. Calculations were done using only operational costs, since the experiment took place in a government research station. The results show that the variable cost per crop of seabass larvae cultured in the earthen ponds were 13 500, 22 200, and 41 900 Baht.crop^{-1}, while the highest cost in all stocking densities was the feed cost (*Artemia* cyst and adult), which comprised 53.33, 51.35, and 63.48% at stocking densities 37.5, 75, and 112.5 larvae.m^{-2}, respectively (Table II).

The analysis showed that the highest production cost per larva was 0.867Baht at the highest stocking density (112.5 larvae.m^{-2}), while the greatest benefit per larva was 0.687Baht at the lowest stocking density (37.5 larvae.m^{-2}).

Table II. Cost and return per crop of the seabass production in earthen ponds (43Baht = 1USD).

Cost and return	37.5 larvae.m^{-2}		75 larvae.m^{-2}		112.5 larvae.m^{-2}	
	Baht	%	Baht	%	Baht	%
Seabass seed	4 500	33.33	9 000	40.54	13 500	32.22
Artemia cyst	3 150	23.33	6 300	28.38	14 100	33.65
Adult *Artemia*	4 050	30.00	5 100	22.97	12 500	29.83
Miscellaneous	1 800	13.33	1 800	8.11	1 800	4.30
Total cost	13 500		22 200		41 900	
Total income	25 054.25		37 548.75		66 532.5	
Total benefit	11 554.25		15 348.75		24 632.5	

Discussion

Seabass larvae from this experiment showed good growth performance at all stocking densities. The total length of the larvae varied from 1.5-5cm. The ratio of big fish was increased according to decreasing stocking density. The highest ratio of the big fish and survival rate was seen in the lowest stocking density, consequent to the highest benefit per larvae. This study reported good growth performance and high survival rate of the seabass at 15 days old (5mm TL), of which the pigmentation and ontogenesis have been documented. The results showed good survival rate (56.04%) compared with previous experiments: 4.66% (Jungyampin et al., 1985), 18.8% (Tansuwan and Boonkhaewkhuon, 1999), and 40% (Maneewong, 1987).

Russell and Garrett (1983) suggest that juvenile seabass in the natural nursery condition appear to have high tolerance to temperature and salinity fluctuations. The good growth potential and survival of postlarval barramundi is relative with the abundance and availability of their food and microscopic organisms of increasing size. Cannibalism in seabass is one important loss in seabass production, caused by their piscivorous habit that develops at 1.25cm TL. Adoption of an appropriate feeding regime and increasing food concentration can limit the degree of cannibalism. The lack of cannibalism during extensive pond rearing is in contrast to intensive tank rearing, where it is necessary to constantly grade the population after metamorphosis to prevent cannibalism (Maneewong, 1987).

Many studies have shown the importance of n-3 fatty acids in the diet of marine fish larvae. The essential fatty acids of marine fish are generally considered to be the C20 and C22 unsaturated fatty acids. Kendall et al. (1984) and Rimmer and Reed (1989) suggest that 22:6n-3 deficiency does not adversely affect survival

of seabass during the rotifer feeding phase, but such a deficiency would cause extensive mortalities during the brine shrimp feeding phase, when larval organogenesis is well advanced and the metabolic functions of larvae are presumably more competent.

This experiment revealed the good possibility of extensive seabass fry production in earthen ponds, shown from the growth performance and survival rate. Although the price of *Artemia* is presently unpredictable, the profit of the system is still assured.

References

Bagarinao T. and P. Kungwankij. 1986. An incidence of swimbladder stress syndrome in hatchery-reared seabass (*Lates calcarifer*) larvae. Aquaculture. 51:181-188.

Danayadol Y. 1984. Study on the diseases of seabass, *Lates calcarifer*, in south Thailand, 1981-1983. P.140-144. In: Report of Thailand and Japan Joint Coastal Aquaculture Research Project, Songkhla, Thailand, Apr 1981-Mar. 1984. Japan International Cooperation Agency, Tokyo.

Dhert P., M. N. Duray, P. Lavens, and P. Sorgeloos.1990. Optimized feeding strategies in the larviculture of the Asian seabass *Lates calcarifer*. Proc. The Second Asian Fisheries Forum.1989. (In press)

Jungyampin S., B. Sirikul., C. Chanchuklin, S. Taechanarawong, W. Wattanakul, and S. Kochasing. 1985. Study on nursing seabass larvae (*Lates calcarifer* Bloch) in the earthen pond. Technical paper No. 16/1985. National Institute of Coastal Aquaculture. Department of Fisheries. Thailand. 12p.

Kendall A.W.Jr., E.H. Ahlstrom, and H.G. Moser. 1984. Early life history and stages of fishes and their characters. In: Ontogeny and Systematics of Fishes, special publication No. 1, Amer. Soc. Ichthyol. Herpetol.

Maneewong S. 1987. Research on nursery stages of seabass (*Lates calcarifer*) in Thailand. In: J.W. Copeland and D.L. Grey (Editors), Management of Wild and Culture Seabass/Barramundi (*Lates calcarifer*). Proceeding of the International Workshop held at Darwin, N.T., Australia, 24-30 September 1986. ACIAR Proceeding No. 20:138-141.

Rimmer M.A. and A. Reed. 1989. Effect of nutritional enhancement of live food organisms on growth and survival of Barramundi/Seabass *Lates calcarifer* (Bloch) larvae. Advances in Tropical Aquaculture. Tahiti, Feb. 20- March 4, 1989. AQUACOP. IFREMER. Actes de Collogue 9:611-623.

Russel D.J. and R.N. Garrett. 1983. Use by juvenile barramundi, *Lates calcarifer* (Bloch), and other fishes of temporary supralittoral habitats in a tropical estuary in Northern Australia. Aus. J. Mar. Freshwat. Res. 34:805-11.

Tunsuwan S. and B. Boonkhaewkhuon. 2000. Study on nursing of seabass (*Lates calcarifer* Bloch) fry in the earthen pond. Technical paper No. 23/2000. Patani Coastal Aquaculture Station. Department of Fisheries, Thailand. 16p.

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INCREASED LEVELS OF DHA (22:6n-3) AND CHOLESTEROL OXIDATION AND DECREASED LEVEL OF ASTAXANTHIN IN ATLANTIC SALMON, *SALMO SALAR* L., EGGS EXHIBITING REPRODUCTION DISTURBANCES IN THE BALTIC SEA

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Introduction

Fish ovaries and eggs are rich in polyunsaturated fatty acids (PUFA) and cholesterol. Aside from being important in biological membranes, PUFA and cholesterol function as precursors for eicosanoid and hormone syntheses, respectively. Cholesterol (cholest-5-en-3β-ol) is the main sterol in fish and is readily susceptible to oxidation. Cholesterol biosynthesis is controlled in the liver and intestine by means of a feedback mechanism in fish, as in mammals. Fish also possess the ability to convert cholesterol to bile acids and steroid hormones in a similar way as in mammals.

Temperature, light, storage, air, pro-oxidizing agents, and deficiency of antioxidants enhance oxidation of cholesterol in both food products and living tissues. Several cholesterol oxidation products (COPs) have a variety of biological effects both *in vitro* and *in vivo* and have been linked to human diseases and cholesterol metabolism (Addis et al., 1996). Considering the similarity between fish and mammalian lipid metabolism, it is feasible to assume the toxic effects of COPs, found in man, are to be true also for fish.

Levels of certain PUFA, particularly 20:5n-3 (EPA) and 22:6n-3 (DHA), are high in fish and are susceptible to oxidation, producing free radicals and peroxy radicals that can accelerate cholesterol oxidation. The endogenous natural antioxidants like tocopherols, vitamin C, selenium, polyphenols, and carotenoids, have significant roles in preventing production of COPs *in vivo* (Smith, 1996).

M74 (swim-up fry mortality syndrome) in Baltic salmon was initially suggested to be a result of oxidative stress, as an effect of a depleted antioxidant system

(Börjesson et al., 1996). Besides M74, other reported reproduction disturbances are believed to relate directly or indirectly to the high load of xenobiotics in the Baltic Sea (Norrgren et al., 1998). The aim of this work was to see if levels of COPs in eggs from females exhibiting the M74 syndrome were higher in eggs from females not exhibiting the M74 syndrome.

Materials and methods

Salmon eggs were obtained from hatcheries at three Swedish rivers (6 females per river). About 1g of each egg sample was homogenized in a Potter-Elvehjem homogenizer, and the lipids were extracted. Fatty acid (FA) composition, cholesterol content, carotenoids, and cholesterol oxidation products (COPs) were analyzed. GC (FA, cholesterol, and COPs) and HPLC (carotenoids) were applied for the analytical procedures.

Results

Two of the stocks were of Baltic Sea origin, where reproduction disturbances demonstrated as mortality in swim-up larvae have been observed. In eggs from batches yielding affected larvae, we found elevated levels of COPs ($P<0.0289$) and docosahexaenoic fatty acid (22:6n-3, DHA) ($P<0.0056$) and low levels of astaxanthin ($P<0.0078$) compared to eggs yielding non-affected larvae and eggs from the Atlantic salmon stock, that do not exhibit swim up mortality. In addition, higher level of DHA and total PUFA n-3 was found in eggs of the two Baltic stocks (28.15% and 26.25%, $P<0.0056$; 44.4% and 40.8%, $P<0.0292$, respectively) compared to the Atlantic stock. No differences were found in the content of cholesterol between any stock or year class.

Discussion

Accumulation of oxidation products has been linked to the M74 syndrome in the affected fry. As our sampling was performed directly post-fertilization, we conclude that the increased content of both oxidation products as COPs and DHA found in M74 affected eggs are caused by an increased maternal incorporation and formation, respectively (Cowey et al., 1985). The low level of astaxanthin and total carotenoids may also be linked to an increase of oxidative processes. There are several possible explanations to the underlying mechanisms of the increased incorporation of DHA. First, a higher level could be due to an elevated desaturation rate, as a response to an evolutionary adaptation towards a low river temperature as argued in Pickova et al. (1998). This as DHA enhances the fluidity of biological membranes at low temperatures. Second, the higher desaturation could be a result of a metabolic switch caused by an increased xenobiotic exposure, towards higher amount of unsaturation, most likely as an effect of an

increased activity of the Δ-5 and Δ-6 desaturases. Polychlorinated biphenyls (PCBs) are of special interest in this respect as it has been shown in the liver of widely different animal species to cause changes in lipid metabolism, resulting in an elevated level of DHA (Borlakoglu et al., 1990). Whether xenobiotics have the same metabolic effect in fish is presently not known. The increased formation of COPs is then an effect of the higher content for oxidation-susceptible substrates, in this case DHA. In addition, the carotenoid and astaxanthin levels were decreased in this situation, which may add to the vulnerability to the oxidative processes. This unbalance between the substrates for oxidation and antioxidative capacity will deteriorate the situation.

Results from the present study of an increased occurrence of COPs in M74-affected eggs tally with earlier findings that this reproduction disturbance is mainly related to decreased antioxidative capacity. The alternative mechanisms as suggested above are to be explored.

References

Addis P.B., P.W. Park, F. Guardiola, and R. Codony. 1996. Analysis and health effects of cholesterol oxides. p.199-240. In: Food lipids and health. McDonald R.E. and Min D.B. (Eds); Marcel Dekker Inc. New York.

Borlakoglu J.T., Edwards-Webb, J.D. and Dils, R.R. 1990. Polychlorinated biphenyls increase fatty acid desaturation in the proliferating endoplasmatic reticulum of pigeon and rat livers. Eur. J. Biochem. 188:327-332.

Börjesson H., L. Förlin, and L. Norrgren. 1996. Investigation of antioxidants and prooxidants in salmon affected by the M74 syndrome. Swedish Env. Prot. Agency, Rep. No. 4534:95-96.

Cowey C.B., J.G. Bell, D. Knox, A. Fraser, and A. Youngson. 1985. Lipids and antioxidant systems in developing eggs of salmon *(Salmo salar).* Lipids 20:567-572.

Norrgren L., P. Amcoff, H. Börjesson, and P-O. Larsson. 1998. Reproductive disturbances in Baltic fish: A review. In: Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. McDonald, G., Fitzimons, J.D. and Honeyfield, D.C. (Eds.) Am. Fish. Soc. Symp. 21:8-17.

Pickova J., A. Kiessling, A. Pettersson, and P.C. Dutta. 1998. Comparison of fatty acid composition and astaxanthin content in healthy and by M74 affected salmon eggs from three Swedish river stocks. Comp. Biochem. Physiol. 120B:265-271.

Smith L.L. 1996. Review of progress in sterol oxidations: 1987-1995. Lipids 31:453-487.

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CONTROL OF METAMORPHOSIS IN FLATFISH

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The processes leading to the final phenotype and the settled flatfish have their origins in the early life stages, and can be modified by the nutritional and environmental factors to which the developing larvae are exposed. In an experiment designed to elucidate the effects of an inadequate diet and induce poor quality, natural copepods or enriched *Artemia* were given to 2 and 2 groups of halibut larvae for 68 days. The quantitative effects were not significantly different for standard length, myotome height, or dry weight until nearly 60 days after commencement of feeding (postmetamorphosis), whereas at prometamorphosis, significant qualitative differences were seen in pigmentation, eye migration, and skeletogenesis. There were no significant differences in fatty acid profile, tyrosine, or phenylalanine, but there were significant differences in the iodine content of the feed (700× more in zooplankton than enriched *Artemia*) and the larvae, as well as in selenium and the thyroid hormone profiles. This suggests the importance of dietary micronutrients integral to the synthesis of hormones prior to metamorphosis, when gills are immature.

Two experiments looked at the addition of exogenous thyroid hormone (T_4) between 15-40mm SL. The first showed a peak of T_3 in larvae between 24-26mm SL given exogenous T_4, indicating peak deiodinase activity occurred in this size range. Further, exogenous T_4 did not increase endogenous T_3 before 20-22mm SL. In another experiment, exogenous T_4 was added over a 2-week-long period at pre-, pro-, or climax metamorphosis. Eye migration was accelerated at prometamorphosis, when the larvae were between 16-18mm standard length, indicating neural change to be one of the first metamorphic events subject to heterochrony. Thus, eye migration cannot be used as an index of metamorphic stage or success. The experiments suggest that T_4 is more important during initial metamorphosis than T_3.

Two experiments were also conducted to investigate photoperiodic stimulation of metamorphosis by reducing the photophase from constant to 12L:12D at

various ages. When a diel photoperiod was introduced at 22 days post-first feeding (DPF) (prometamorphosis), eye migration was initially accelerated and growth slowed. Hemoglobin showed heterochrony, appearing first in the group receiving constant photoperiod and not correlating with size. There were no long-term disadvantages of reduced photoperiod on growth rate of metamorphosing halibut, as both groups attained the same size. In the second experiment, when photoperiod was reduced at 12, 21, 30, and 42DPF, eye migration was accelerated when photoperiod was reduced at 30DPF. Later manipulations had little effect. The results indicate the existence of a window of opportunity of about 18-20mm SL (prometamorphosis), during which the final phenotype is being determined.

The general order of metamorphic events in halibut is (1) neural change, (2) growth and skeletal change, (3) change in circulatory elements, and (4) final establishment of the pigmentation pattern. Preceding these changes would be the environmental and endocrine cues, some of which are necessary within a "window of opportunity" or a critical size suggested to be 18mm SL (Solbakken et al., submitted). Photoperiods of 12L:12D or 24L:0D may induce a heterochrony of some metamorphic events such as eye migration and the appearance of hemoglobin in halibut, and these changes are more related to age than size. The action may be through the photoperiod-melatonin-thyroid axis and the outcome of manipulations may be stage-dependent. There is no long-term reduction in growth due to shortened photoperiod in metamorphosing halibut larvae. Further juvenile quality may be limited by diet quality, particularly micronutrients such as iodine and selenium at pre- and prometamorphosis.

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EVALUATION OF FRESH AND PRESERVED *PAVLOVA LUTHERI* CONCENTRATES AS LARVAL FEED FOR THE PACIFIC OYSTER *CRASSOSTREA GIGAS*

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Introduction

Microalgae cultivation represents a bottleneck for bivalve production in hatcheries because it requires heavy human and economic investments (Myers and Boisvert, 1990). In order to solve this problem, substitutes of live fresh microalgae are sought and many experimental studies have been carried out in the past to find the best alternatives (see reviews by Coutteau and Sorgeloos, 1992; Robert and Trintignac, 1997). Among several techniques of preservation, refrigeration of concentrated microalgae appears to be one of the most interesting, especially for feeding highly demanding bivalve species. In this study, the food quality for *Crassostrea gigas* larvae of both fresh and preserved *Pavlova lutheri* was investigated. Monospecific and bispecific diets in combination with fresh *Chaetoceros calcitrans* forma *pumilum* were used. Two cultivation techniques (batch and semi-continuous) were compared to find out possible nutritional differences.

Materials and methods

Microalgae were produced using standard batch culture methods in 2 to 10-l glass carboys, or in a semi-continuous mode using 4-l flat alveolar photobioreactors (Tredici and Materassi, 1992). Microalgae were grown at 20°C under continuous illumination (50-150μmol photons.m^{-2}.s^{-1}) in sterilized Conway media, aerated with a 2% CO_2-air mixture. The salinity was 34ppt for *P. lutheri* and 25ppt for *C. calcitrans* forma *pumilum*. The algal cultures directly obtained from the photobioreactor at a concentration of 2.5 to 4g.l^{-1} dw, were stored in the dark at 1°C or 4°C, in 2-l polycarbonate carboys, aerated by air-

bubbling. When used as feed, *P. lutheri* suspensions were 9-27 days old. Two trials were carried out on *C. gigas* larvae, each lasting 12 days. At each time, 10 000 2-day-old larvae were placed into 2-l hard glass beakers, each containing 1.8 l of 1-µm filtered seawater at 34ppt salinity, and kept at 24°C. In order to limit bacterial contamination, chloramphenicol was added at a concentration of 4mg.l^{-1}. In the first trial, eight diets, each run in triplicate, were compared: unfed (control); fresh *P. lutheri* produced in photobioreactor (Pfp); fresh *P. lutheri* produced in batch (Pfb); *P. lutheri* produced in photobioreactor and stored at 4°C (Pcp4); *Chaetoceros calcitrans* forma *pumilum* (Cp), and the bispecific combinations Pfp+Cp, Pfb+Cp and Pcp4+Cp. In the second trial, two additional conditions were considered: *P. lutheri* stored at 1°C and used in the monospecific (Pfp1) or bispecific diets (Pfp1+Cp). Based on microalgae cellular volume (1 *P. lutheri* cell ≡ 2 *C. calcitrans* forma *pumilum* cells), the standard daily food ration gave an initial cell concentration of 10^5 cells.ml^{-1} (*P. lutheri* equivalent). Bispecific food ratios were 50/50 for the first trial and 80/20 for the second trial, 20% corresponding to the supplementation of fresh *C. calcitrans* forma *pumilum*. Food was added three times a week, at each water change. Larvae were sampled at the start of trials (day 2 after fecundation) and on day 14, for determination of mortality (by counting the translucent larval shells under the microscope) and growth (by measuring the shell length (SL) by means of image processing software (Image SXM®)).

Data were analyzed by ANOVA (Statview® software), and significant differences between diets were detected by Scheffé test ($P<0.05$).

Results

In trial 1 (Fig. 1), no significant differences in growth were noticed between unfed larvae (84.7µm) and those receiving fresh *P. lutheri* produced in batch (93.6µm) or preserved *P. lutheri* produced in the photobioreactor (90.4µm).

Growth was slightly improved when larvae were fed fresh *P. lutheri* produced in the photobioreactor (106.7µm). *C. calcitrans* forma *pumilum* gave the best performance both in monospecific (156.7µm) and in bispecific diets containing *P. lutheri* produced in photobioreactor, both fresh (156.7µm) and preserved (149.2µm). At the end of trial 1 (day 14), survival was high (70-90%), with the exception of the unfed larvae (41%) and those fed preserved *P. lutheri* (19%).

The poor food value of *P. lutheri* for *C. gigas* larvae was confirmed through the second experiment (Fig. 2). Indeed, no significant differences in growth were noticed between unfed larvae (86.6µm) and those fed a monospecific diet of *P. lutheri* produced in photobioreactors, either as fresh culture (89.1µm) or as preserved suspensions (90.5-96.0µm). Growth was slightly improved when

larvae were fed fresh *P. lutheri* produced in batch (105.6µm). The good food value of *C. calcitrans* forma *pumilum*, both as a bispecific and as a single diet, was also confirmed. Substitution of 80% of that ration with fresh *P. lutheri* did not improve larval development, whereas the use of concentrates enhanced growth (155.4µm-161.5µm). The two preserved diets did not show any significant differences among them. Perhaps advantages of storage at the lower temperature (1°C) would have been appreciated in biomass stored for a time longer than the 9-27 days considered.

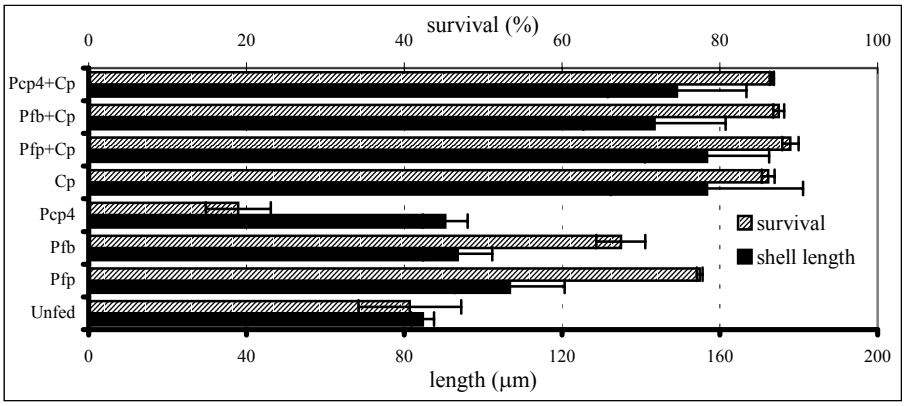

Fig. 1. Trial 1: Length (means ± IC 95%) and survival (average ± sd; *n* = 3) of *C. gigas* larvae fed different diets. Cp= Fresh *Chaetoceros calcitrans* forma *pumilum;* P= *Pavlova lutheri* (fb: fresh produced in batch; fp: fresh produced in photobioreactor; cp4: concentrated and preserved 4°C). Initial shell length: 82.5µm.

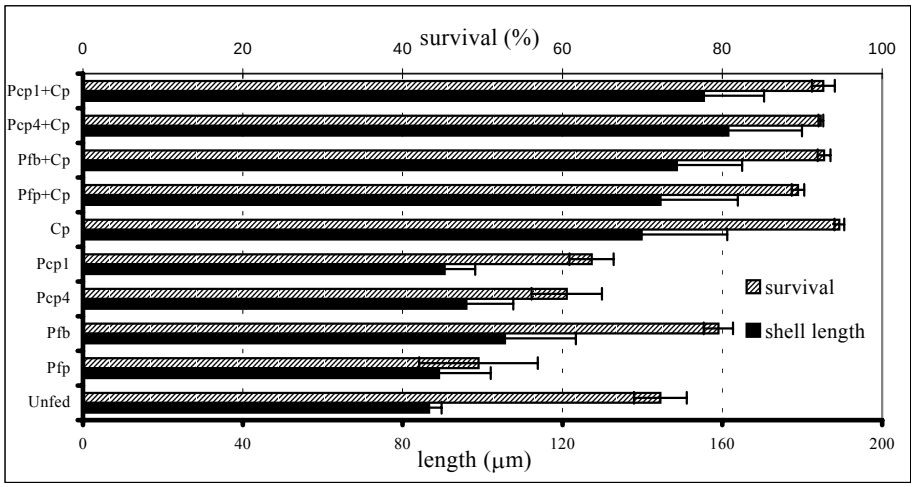

Fig. 2. Trial 2: Length (means ± IC 95%) and survival (average ± sd; *n* = 3) of C. *gigas* larvae fed different diets. Cp= Fresh *Chaetoceros calcitrans* forma *pumilum. P*=

Pavlova lutheri (fb: fresh produced in batch; fp: fresh produced in photobioreactor; cp4: concentrated and preserved 4°C; cp1: concentrated and preserved 1°C). Initial shell length: 85.0μm.

At the end of trial 2, good survival rates resulted in larvae fed the diatom in monospecific or in bispecific diets (>89%), whereas the lower survival was recorded in larvae fed fresh *P. lutheri* produced in photobioreactors (49%).

Conclusions

The results of this work clearly show that a monospecific diet of *P. lutheri*, either as fresh or as preserved suspensions, has poor food value for young *C. gigas* larvae. In contrast, *C. calcitrans* forma *pumilum*, used as a single diet, has a good nutritional value for the Japanese oyster as already reported by Robert *et al*. (1989). In any case, a bispecific diet based on concentrates of *P. lutheri*, preserved at low positive temperature for 9-27 days, allowed a substitution of 80% of this valuable diatom without compromising neither larval growth nor survival.

References

Coutteau P. and P. Sorgeloos. 1992. The use of algal substitutes and the requirement for live algae in hatchery and nursery rearing of bivalve molluscs: an international survey. J. Shellfish Res. 11:467-476.

Myers J.A. and R.N. Boisvert. 1990. The economics of producing algae and bivalve seeds in hatcheries. Aquaculture 86:163-179.

Robert R., T. Noel and R. Galois. 1989. The food value of five unicellular diatoms to the larvae of *Crassostrea gigas* Thumberg. In: Proc. Aquaculture Europe ’89. Bordeaux, France, 2-4 October, 1992. EAS Spec. Publ. 10:215.

Robert R. and P. Trintignac. 1997. Substitutes for live microalgae in mariculture: a review. Aquat. Living Resour. 10:315-327.

Tredici M.R. and R. Materassi. 1992. From open ponds to vertical alveolar panels: the Italian experience in the development of reactor for the mass cultivation of phototrophic microorganisms. J. Appl. Phycol. 4:221-231.

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LIPID REQUIREMENTS OF THE WHITE SEABREAM (*DIPLODUS SARGUS* LINNAEUS, 1758) LARVAE

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Introduction

The white seabream (*Diplodus sargus* Linnaeus, 1758) is a common species in the Mediterranean area and one of the most appreciated and highly valued species in Southern European markets. Good artificial reproduction and larval rearing results have been obtained with this species, using the same technology as for seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*) (Gómez and Ros, 1993; Pousão-Ferreira et al., 1997). However, as in most marine species, the lipid nutrition of the larval stages is probably one of the main bottlenecks to the development of a successful commercial activity. Having this in mind, the present study was conducted with the objective of studying the lipid requirements of white seabream larvae. The fatty acid profile of *D. sargus* eggs and newly hatched larvae is analyzed and compared with other marine species. Four feeding experiments were also conducted, in order to test the effect of different feeding regimes – non-enriched *Artemia*, enriched *Artemia*, an inert diet, and a co-feeding regime – on the larval fatty acid composition.

Materials and methods

Four independent feeding experiments were carried out during 20 days, using *Diplodus sargus* eggs obtained by natural spawning from captive broodstock kept at the IPIMAR/CRIPSul (Portugal) hatchery. Eggs were placed in 200-l incubation tanks, at a density of 2g.l^{-1}, a temperature of 19±1ºC, a salinity of 36±1‰, and an oxygen level of 8mg.l^{-1}, until hatching (±36h). The larvae were reared in 200-l cylindrical-conical fiberglass tanks, in a semi-closed system, with mechanical, biological, and UV filtration. The tanks were gently aerated from the bottom and the "green water" method was used. Oxygen, salinity, and temperature were measured daily and maintained at 8mg.l^{-1}, 30±1‰ and 20±1ºC, respectively. The photoperiod was 14h L:10h D, and larval density ranged between 5-35 larvae.l^{-1}.

Table I shows the experimental feeding regime followed in each treatment. All treatments were tested in triplicate. Rotifers (*Brachionus plicatilis*) were cultured on baker's yeast and enriched in Protein Selco® (Artemia Systems Inc.) before being fed to the larvae, twice a day, at a density of 5 prey.ml^{-1}.tank. *Artemia* E.G. (Artemia Systems Inc.) was enriched with DHA Super Selco (Artemia Systems Inc.) and was kept at a density of approximately 0.1 nauplii.ml^{-1} in the larval culture tanks. The experimental microparticulate diet (200-400µm) was prepared at the Department of Zoology and Anthropology, University of Porto (Portugal) and 0.1-1g.day^{-1} was added to each tank. In the co-feeding regime, larvae were fed on the inert diet as well as rotifers and enriched *Artemia* (at 20% of the live diet treatments).

Table I. Experimental feeding regime followed in each treatment.

Treatment	2 DAH	10 DAH	15 DAH
Non-enriched *Artemia*	Rotifers	Rotifers + *Artemia* AF	Rotifers + *Artemia* AF
Enriched *Artemia*	Rotifers	Rotifers + *Artemia* AF	Rotifers + *Artemia* EG
Inert diet	Rotifers	Inert diet	Inert diet
Co-feeding	Rotifers	Rotifers + *Artemia* AF + Inert diet	Rotifers + *Artemia* EG + Inert diet

At 20 days after hatching (DAH), samples of the larvae submitted to the different dietary treatments were collected for fatty acid (FA) analysis. The fatty acid composition of the *Diplodus sargus* eggs and newly hatched larvae was also determined and compared with other marine species' eggs and larvae. Total lipid extraction was carried out according to Blight and Dyer (1959) and saponification and esterification of the lipid extracts was done using the method of Metcalfe and Schmitz (1961). The fatty acid methyl esters (FAME) were injected into a capillary column (30µm fused silica, 0.32 I.D.) installed in a Varian Star 3400CX gas-liquid chromatograph. GLC data acquisition and handling was done through a Varian integrator 4290. Peak quantification was carried out with a Star Chromatography workstation installed in an IBM PS/1. Peak identification was carried out using as reference well-characterized cod liver oil chromatograms. Duplicate samples were analyzed.

Results and discussion

In Table II, the fatty acid profile of *Diplodus sargus* eggs and newly hatched larvae (NHL) in comparison with other marine species can be seen. The fatty acid profile of the *D. sargus* eggs indicates a requirement for high dietary DHA/EPA ratios, with high absolute amounts of DHA and EPA. *D. sargus* are similar to *Solea senegalensis* in the fact that they have a higher DHA/EPA ratio than typically observed in marine fish eggs (around 2-3). However, *D. sargus* are more similar to *Sparus aurata* regarding the absolute amounts of these two essential fatty acids

(FA's). In terms of the (n-3)/(n-6) ratio (another important parameter for the diet formulation), *D. sargus* eggs have quite a high (n-3)/(n-6) ratio, comparable to *S. aurata* eggs. As for the NHL, *D. sargus* presents the same FA profile as the eggs, although the absolute amounts of the analyzed FA's are double those found in the eggs.

Table II. Fatty acid composition (μg.mg^{-1} of dry weight) of some marine species eggs and newly hatched larvae (NHL).

	PUFA	DHA	EPA	DHA/ EPA	(n-3)	(n-6)	(n-3)/ (n-6)
Diplodus sargus eggs	47.6	30.2	8.9	3.4	43.7	3.9	11.2
Sparus aurata eggs	44.8	22.7	10.8	2.1	43.0	3.5	12.3
Solea senegalensis eggs	14.1	8.4	2.0	4.2	13.0	2.4	5.4
Diplodus sargus NHL	88.8	58.4	16.6	3.5	82.6	6.3	13.1
Sparus aurata NHL	45.5	21.7	8.3	2.6	43.8	3.1	14.1

PUFA - polyunsaturated fatty acids; DHA - 22:6(n-3), docosahexaenoic acid; EPA - 20:5(n-3), eicosapentaenoic acid

Table III shows the fatty acid composition of *D. sargus* larvae 20 DAH, after being reared on different dietary regimes. The results reveal that when white seabream larvae are fed enriched *Artemia*, there is a higher incorporation of (n-3) PUFA, particularly of DHA and EPA. Nevertheless, the DHA/EPA ratio remains quite low and equivalent (even slightly lower) to that of the larvae with a diet of non-enriched *Artemia*. When using an inert microparticulate diet in complete replacement of live food from 10 DAH onwards, there is no substantial difference in the larval FA profile, comparative to larvae fed on non-enriched live preys. This indicates that the inert diet (originally formulated for *S. aurata*) may not completely satisfy *D. sargus* FA requirements. However, when the inert diet was complemented with live prey (co-feeding regime), far better results were achieved. With the co-feeding regime, the highest absolute levels of PUFA, namely of DHA and EPA, as well as the highest DHA/EPA and (n-3)/(n-6) ratios, were achieved.

Table III. Fatty acid composition (μg.mg^{-1} of dry weight) of *D. sargus* larvae 20 DAH, submitted to different dietary treatments.

Treatment	PUFA	DHA	EPA	DHA/ EPA	(n-3)	(n-6)	(n-3)/ (n-6)
Non-enriched *Artemia*	31.9	2.7	6.9	0.4	17.6	14.3	1.2
Enriched *Artemia*	43.7	3.9	11.3	0.3	27.3	15.5	1.8
Inert diet	28.6	2.8	6.6	0.4	18.1	9.8	1.9
Co-feeding	45.0	10.0	13.1	0.8	34.0	10.1	3.4

Despite these preliminary results suggesting that a co-feeding regime may induce a better larval fatty acid profile than a diet based solely on enriched *Artemia*, attention should be drawn to the fact that the experiments were conducted with different batches of eggs and, therefore, the initial egg

biochemical composition may have influenced the results. However, it is possible that a more stable and balanced formulation may be accomplished with an inert diet than with *Artemia* enrichment procedures, given the *Artemia* DHA catabolism and the consequent difficulty in maintaining high DHA/EPA ratios.

The exclusive use of an inert diet at such an early stage of development as the one used in this study has been attempted with other species, and the results have been far from satisfactory. Much better results are usually accomplished by co-feeding live and formulated diets, as found here with white seabream. It is believed that live prey enhance the acceptability and facilitate the digestion and assimilation of inert feeds (Rosenlund et al., 1997).

Conclusions

As a consequence of the amount of information available on the *S. aurata* FA requirements and the existence of well established methodologies for seabream culture, there might be a tendency to apply the same techniques and diets to white seabream larval culture. However, the differences in the FA profile of *D. sargus* and *S. aurata* eggs should be taken into account when formulating an inert diet or designing live prey enrichment strategies for white seabream larval rearing. Therefore, special consideration should be given to the inclusion level of essential FA's, particularly DHA, and to the DHA/EPA ratio in the diet.

In the present study, the tested inert diet does not seem appropriate for the complete live food replacement in the larval rearing of white seabream. However, much better results were achieved, in terms of the larval FA profile, with a co-feeding regime. There is therefore scope for improvement and co-feeding formulated diets, and live preys may be a good way of producing a nutritionally well balanced diet for *D. sargus*.

References

Blight E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Gómez A.G. and A.O. Ros. 1993. Revisión general del cultivo de nuevas espécies piscícolas en el área mediterránea y experiencias realizadas en el centro oceanogáfico de Murcia. Inf. Téc. Inst. Esp. Oceanogr. 141:32p.

Metcalfe L.D. and A.A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatography analysis. Anal. Chem. 33:363-364.

Pousão-Ferreira P., E. Dores, and J. Amaral. 1997. White sea bream (*Diplodus sargus*) juvenile production: Perspectives for commercial aquaculture. Island Aquaculture and Tropical Aquaculture, EAS-WAS-CAA-ADA-ADAM. Martinique, May 4-9,1997, Short Communications and abstracts: 241-242.

Rosenlund G., J. Stoss, and C. Talbot. 1997. Co-feeding marine fish larvae with inert and live diets. Aquaculture 155:183-191.

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SHRIMP LARVAL QUALITY AS A FUNCTION OF BROODSTOCK CONDITION

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This review focuses on the different criteria currently used to assess the larval quality of penaeid shrimp and the factors that affect it. The term 'larval quality' is widely used, generally refers to the physiological condition of the larvae, and is related to survival and growth rates during several larval developmental stages. Criteria fit into five general categories, depending on the approach used;

1. *Biochemical* — includes analysis of total or specific lipid content, vitellin levels, RNA/DNA ratio, etc.
2. *Morphological* — measurements of size, weight, deformities, etc.
3. *Behavioral* — positive phototropism, negative geotaxis, swimming activity.
4. *Production* or *yields* — fecundity, fertilization and hatching rates, survival through several stages.
5. *Survival to adverse environmental conditions* (stress tests) — low salinity, low temperature, formalin, high ammonia concentrations, low dissolved O_2, etc.

Condition or quality of eggs, embryos, and nauplii (spawn quality) are almost exclusively dependent on broodstock condition and environmental conditions in spawning and hatching tanks. Culture conditions, especially nutrition, are considered the main determinants of the quality of later larval and postlarval stages, although some existing evidence indicates the condition of the broodstock could affect those stages.

Several variables at the broodstock management level are known (or suspected) to affect larval quality. These can be divided into variables that can be controlled by producers or researchers, and uncertain factors that are not easily controlled. For the controlled variables, the following categories could be considered:

a) *Broodstock nutrition* — represents the most investigated variable, with some studies evaluating more than just spawn quality.
b) *Environmental conditions* — temperature, salinity, dissolved oxygen, photoperiod, and general water quality (e.g., nitrogenous compounds, heavy

metals, etc.). In general, standard values of each of these variables have been recommended for shrimp maturation.

c) *Spawner size* — probably the most widely used criteria for broodstock selection, although only few studies have thoroughly analyzed its influence on the resulting larval quality. This variable is generally linked to age, which can be known only for pond-reared populations.
d) *Origin* — wild vs. pond-reared populations, from different capture sites for wild populations, or different grow-out conditions for pond-reared populations. Presently, the use of pond-reared populations is increasing because of the associated advantages over wild populations (ecological and sanitary safety, feasibility of genetic improvement programs, continuous availability). Comparisons between wild and pond-reared stocks for reproductive performance and larval quality are still under study.
e) *Endocrine manipulations* — although currently the most used method is unilateral eyestalk ablation, its consequences on larval quality are still controversial. Administration of several compounds related to endocrine function (methylfarnesoate, serotonin, endocrine gland extracts, steroid hormones, retinoids, eyestalk peptides, antibodies for inhibiting hormones, etc.) has been tested with satisfactory results under experimental conditions. However, few of them evaluated the resulting larval quality.

For uncertain or difficult-to-control variables, we can consider the following ones:

a) *Age* or *season of the year* — for wild populations, age cannot currently be assessed. Natural reproductive cycles are clearly seasonally dependent. Although this is not an unknown variable, it still represents a source of uncertainty of reproductive performance and resulting larval quality, even if eyestalk ablation is used and optimal environmental conditions for maturation are established.
b) *Genetic variability* — although it is generally not now considered, there is evidence of individual (genetic) differences in reproductive performance and associated larval quality. These genetic differences are mainly related to physiological traits such as endocrine function, metabolic pathways involved in gametogenesis, and digestive or assimilation capacity.
c) *Reproductive exhaustion of spawners* — refers to the decline in reproductive capacity under intensive maturation conditions, and could be occurring in both sexes as a function of time spent in these conditions or to successive rematurations. Controversies about such a decline exist in the literature. The occurrence of such a decline depends on management of several controlled conditions (e.g., broodstock diet, use of eyestalk ablation, origin, and environmental factors) and on genetic variability.

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SUPPLEMENTATION OF GROUPER (*EPINEPHELUS COIOIDES*) WEANING DIET WITH DIFFERENT SOURCES OF PHOSPHOLIPIDS

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Introduction

In a rapidly growing fish, phospholipid requirements are high due to rapid membrane development. At an early stage, larvae cannot synthesize large amounts of phospholipids for membrane development, which should therefore be supplemented into the diet to attain maximum growth (Kanazawa et al., 1983a; 1983b). This study determines the effect of supplementation of different sources of phospholipids on the growth and survival of 30 days post hatched (dph) grouper, *Epinephelus coioides.*

Materials and methods

Post-hatched *E. coioides* (25 days old) from the hatchery at SEAFDEC/AQD, Philippines were stocked in oval fiberglass tanks, fed newly hatched *Artemia*, and gradually weaned to a phospholipid-free diet for a week.

Three phospholipids (PL) sources namely marine fish roe (MFRPL), *Acetes* (SHPL), and soybean (SBPL) were supplemented into a basal diet containing 50% crude protein (CP) and 10% lipid (Table I). A no PL diet was used as a negative control.

Thirty-five grouper larvae (ABW=0.163g) were randomly stocked in 250-l conical tanks, filled with 200 l filtered seawater, with gentle aeration, and PVC shelters. Test diets were given at a daily rate of 15% total biomass four times a day for 54 days. Tanks were cleaned daily before the first feeding and 1/3 of the total rearing water was changed. Temperature ranged from 28-32°C and salinity 30-32ppt. Sampling was done every 2 weeks to assess the weight and adjust the feed ration.

Total lipid of the brain, eye, and muscle were determined according to Folch et al. (1957) and expressed as % wet weight. Fatty acid profile was determined using a GC (Shimadzu 9A) with a hydrogen FID attached to a Chromatopack 7A

integrator. Fatty acids were identified using known standards (PUFA-1 and PUFA-2, Supelco USA, and standard cod liver oil) and expressed as percent of total identified fatty acids.

Table 1. Composition of the basal diet.

Ingredients	Composition g per 100 g diet
Basal mixture[1]	73.0
Bread flour	6.2
Carrageenan	5.0
Vitamin mix	3.0
Mineral mix	2.0
α-tocopherol	0.01
Astaxanthin	0.25
Vitamin C	0.40
Lipid	7.0
Phospholipids[2]	3.0
BHT	0.05
Filler	0.09
Nutrient composition (%)	
Crude protein	50
Lipid	10

[1] Basal mixture: white fishmeal 36g; shrimp meal 17g; squid meal 10g; soybean meal 10g

[2] Phospholipid sources: MFRPL, SHPL, and SBPL

Percent weight gain, survival, and FCR were subjected to one-way analyses of variance (ANOVA) and Duncan multiple ranged test (DMRT) to determine differences between means.

Results

A significant (P<0.05) difference was observed in weight gain over post-larval grouper fed no PL diet. Fish fed MFRPL (marine fish roe) and SHPL (*Acetes*) had better weight gain compared to SBPL (soybean PL) and no-PL (Table II).

Table II. Weight gain, increase in length, survival, SGR, and FCR of grouper post-larvae* fed diets supplemented with different sources of phospholipids.

Diets	Weight gain (%)	Increase in length (cm)	Survival (%)	SGR	FCR
No-PL	1231± 1.6^{a}	2.8± 1.30	25±5.5^{ab}	0.217	3.3^{a}
SBPL	1597± 3.1^{ba}	3.2± 0.40	29±1.4^{b}	0.285	3.1^{a}
SHPL	1724± 0.7^{b}	3.6± 0.04	15±1.4^{a}	0.308	2.9^{ab}
MFRPL	1858± 0.6^{b}	3.6± 0.20	31±2.0^{b}	0.331	2.0^{b}

* ABW= 0.163g

Post-larval grouper fed MFRPL had significantly (P<0.05) higher survival compared to other treatments. FCR with the MFRPL diet was the best among treatments (Table II).

Table III. Total lipid (% wet weight) and fatty acid composition retained in the brain, eye, and muscle tissues of post-larval grouper weaned on diets supplemented with different phospholipid sources*.

Fatty acids	Brain				Eye				Muscle			
	A	B	C	D	A	B	C	D	A	B	C	D
16:00	22.38	26.55	18.81	17.91	16.34	–	2.42	5.57	18.87	16.13	17.07	17.86
18:1n-7	_	15.17	11.48	15.70	12.11	14.86	17.38	9.66	1.47	1.95	–	–
18:2n-6	5.31	6.10	4.45	4.88	7.07	6.43	8.58	8.34	6.83	5.26	6.48	7.41
18:3n-3	1.64	1.95	3.50	4.90	–	6.96	1.64	1.98	1.98	4.07	1.77	4.33
20:4n-6	–	2.41	1.99	1.47	–	–	2.89	3.38	–	1.10	–	1.08
20:5n-3	4.56	4.70	5.23	4.16	–	–	1.75	3.06	–	–	–	–
22:6n-3	–	14.37	16.71	16.74	–	5.36	14.21	34.20	–	–	0.13	0.27
Total lipid	2.09	2.35	3.56	4.35	1.15	1.64	1.32	1.65	2.16	2.65	2.25	2.66

*A = No PL ; B = SBPL ; C = SHPL ; D = MFRPL

High amount of total lipid (wet weight) was extracted from the brain and lowest was from the eye tissue (Table III). The brain from MFRPL fed fish had high amount of total lipids (4.35%), followed by SHPL (3.56%). The eye of fish fed no-PL diet had the lowest amount of total lipids. Incorporation of DHA and EPA was high in the brain and eye of grouper weaned on diets MFRPL and SHPL compared to No-PL and SBPL fed grouper post-larvae (Table III).

Discussion

Supplementation of PLs of marine origin significantly supported growth of grouper post-larvae and effectively weaned them from live food onto dry diet by day 30. This effect was also observed in early studies conducted on fish and crustacean larvae (Koven et al., 1993; Guerden et al., 1995; Pascual et al., 1986). PL of marine origin triggered grouper post-larvae to accept dry diet at an early age. This stimulation effect of PL on the diet resulted in an increased consumption and assimilation of the weaning diet, resulting in high weight gain of grouper fed MFRPL and SHPL. Lack of feeding stimulants in the weaning diet may account for the poor acceptability of the dry diet for larval fish (Baskerville-Bridges and Kling, 2000). The improved survival and FCR when the weaning diet was supplemented with PL in this experiment, was in agreement with those observed in rainbow trout and Atlantic salmon fry (Poston 1990 a &b).

Incorporation of the total lipid and fatty acids in selected tissues of post-larvae was influenced by the PL source. The DHA and EPA preferentially retained in

the brain and eye tissue after weaning may indicate its importance in neural development and photoreceptor function (Neuringer et al., 1988; Bazan, 1990). This is the essential role that these fatty acid plays during developmental stage particularly predatory species like grouper, in which prey capture is also essential at and beyond first feeding.

The occurrence of “shock syndrome” was decreased after four weeks of weaning on PL-supplemented diets. Grouper post-larvae fed diets supplemented with PL became highly resistant to sampling-related stress. Dietary PL was found to influence stress sensitivity in post-larval *P. vannamei* (Coutteau et al., 1996).

Our study showed that PL of marine origin has a positive effect on larval growth, survival, and FCR. Furthermore, it enhances the incorporation of DHA and EPA into the brain and eye tissues of grouper post-larvae.

References

Baskerville-Bridges B and L.J. Kling. 2000. Early weaning of Atlantic cod (*Gadus morhua*) larvae onto a microparticulated diet. Aquaculture 189:109-117.

Coutteau P., M.R. Camara, and P. Sorgeloos. 1996. The effect of different levels and sources of dietary phosphatidylcholine on the growth, survival, stress resistance, and fatty acid composition of postlarval *Penaeus monodon*. Aquaculture 147:261-273.

Folch J., M. Lees, and G.S. Stanley. 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-507.

Geurden I., P. Coutteau, and P. Sorgeloos. 1997a. Effect of dietary phospholipids supplementation on the growth and fatty acid composition of Eruropean seabass (Dicentrarchus labrax L.) and turbot (*Scophthalmus maximus*) juveniles from weaning onwards. Fish Physiol. Biochem. 16:259-272.

Kanazawa A., Sh. Teshima, T. Kobayoshi, M. Take, T. Iwashita, and R. Ushara. 1983a. Necessity of dietary phospholipids on growth and survival of the larval Ayu. Mem. Fac. Fish, Kagoshima Univ. 32:109-114.

Koven M.W., S. Kokovski, A. Tandler, G.W. Kissil, and D. Sklan. 1993. The effect of dietary lecithin and lipase, as a function of age, on n-9 fatty acid incorporation in the tissue lipids of Sparus aurata larvae. Fish Physiol. Biochem. 10(5):357-364.

Metcalfe D., A.A. Schmitz, and J.R. Pelka. 1966. The rapid preparation of fatty esters for gas chromatographic analysis. Anal. Chem. 38:514.

Piedad-Pascual F. 1986. Effect of supplemental lecithin and lipid sources on the growth and survival of *Penaeus monodon* juveniles. p.615-618. In: The first Asian Fisheries Forum. J.L. MacLean, L.B. Dizon, and L.V. Hosillos (Eds.) Asian Fisheries Soc., Manila, Philippines.

Poston H.A. 1990a. Performance of rainbow trout fed supplemental soybean lecithin and choline. Prog. Fish Cult. 52:218-225.

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MORPHOLOGY OF THE POSTLARVA OF *FENNEROPENAEUS INDICUS* (H. MILNE EDWARDS)

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Introduction

In aquaculture, the development of postlarvae is commonly referred to age in days from metamorphosis to PL1. However, this identification is limited and unspecified since growth trends may change with culture environment making it difficult to assess the quality of PLs. The taxonomy of *Fenneropenaeus indicus* has been studied for larval development (Mohamed et al., 1968; Muthu et al., 1978). Both also described the first postlarva sub-stage (PL1) of *F. indicus* in comparison to other penaeid species. To date no study has described the complete morphological development of *F. indicus* from postlarva PL1 to juvenile stage, making it difficult to study natural resources and to manage the nursery culture. The present study attempts further identification and characterisation of *F. indicus* postlarval development. Postlarvae reared in captivity from known parentage are examined for morphological development from sub-stage PL1 to juvenile. The growth and appearance of morphological characters is related to each sub-stage of development and size (total length) of PLs.

Materials and methods

Postlarvae (PL) of *F. indicus* used in this study were obtained from induced spawning of wild brood collected from the coast of Saudi Arabia. After metamorphosis to PL1, postlarvae were transferred to new 60-l tanks provided with filtered seawater at 33‰ salinity, 28±1°C temperature and 7.9-8.2pH. The total length, body width, length of carapace, length of abdomen (total) and of 1st to 5th and 6th abdominal somites, the length of rostrum and telson were measured to the nearest 0.01mm. The length of the rostrum was measured from the tip to the post-rostral ridge. The rostrum teeth (dorsal and ventral), telson spines, segments of antennular flagella, and branchiae were counted and examined for each PL stage. The ratio between morphometric characters was

assessed for postlarval development. Morphological characterisation for each PL sub-stage covered the following body parts: carapace, rostrum, antennules, abdomen, pereiopods, pleopods, telson, uropods, and branchiae.

Results and discussion

The relationships between total length (Table I) and body width, total length and carapace length, carapace length and telson length, and between carapace length and the length of the 6th abdomen somite were fitted to the following linear functions, respectively:

Width = 0.103 × total length - 0.146mm; R=0.995; N=220; SE of slope=0.003
Carapace = 0.191 × total length - 0.0008mm; R=0.990; N=220, SE of slope=0.002
Telson = 0.563 × carapace + 0.205mm; R=0.995; N=220; SE of slope=0.01
6th abdomen somite = 0.609 × carapace + 0.771mm; R=0.989; N=220; SE of slope=0.02

The growth of the carpus of the 3rd pereiopod relative to length of carapace of *F. indicus* postlarvae increased linearly and fitted to the following function:

Length of carpus = 0.908 × carapace length - 0.861mm; R=0.986; N=22; SE of slope=0.049

The number of rostral teeth increases from sub-stage PL1 but the ventral teeth only appear from the 9th postlarva onwards. Muthu et al. (1978) observed reared *F. indicus* PL1 with 1-2 dorsal rostrum teeth but in these studies a second dorsal rostral tooth was only observed from sub-stages PL2 and PL3. The complete rostral teeth composition is observed at PL22 with 8 dorsal teeth and a maximum of 7 ventral teeth.

Table I. Total length of *F. indicus* post-larval sub-stages. Mean and size range in mm estimated from 10 PLs.

PL1	PL2	PL3	PL4	PL5	PL6
4.84 (4.57-5.15)	5.50 (5.48-5.81)	6.51 (6.14-6.81)	7.39 (7.06-7.55)	7.96 (7.64-8.27)	8.63 (8.38-8.88)
PL7	PL8	PL9	PL10	PL11	PL12
9.27 (9.05-9.46)	9.70 (9.46-10.03)	10.43 (10.21-10.62)	10.97 (10.71-11.29)	11.48 (11.20-11.79)	12.12 (11.79-12.53)
PL13	PL14	PL15	PL16	PL17	PL18
13.34 (12.86-13.69)	14.29 (13.86-14.79)	15.46 (14.80-15.95)	17.10 (16.58-17.67)	18.64 (17.85-19.03)	20.32 (19.27-21.25)
PL19	PL20	PL21	PL22		
22.81 (21.86-23.66)	24.56 (23.27-25.58)	27.16 (25.20-28.66)	31.63 (29.97-34.04)		

The exopod of pleopods develops very fast and increases in number of setae during early PL development, which indicates the increasing function of pleopods for swimming after metamorphosis. The endopod was only observed at PL10, initially with a few and very small setae. For *P. japonicus* (Hudinaga, 1942) and *P. kerathurus* (Hiramatsu, 1984) the endopod of pleopods occurs earlier at sub-stages PL8 and PL7, respectively.

The complete set of branchiae was observed at moult stage PL14, but full development of respiratory gills is only achieved from sub-stage PL16, 20-27 days after metamorphosis. *F. indicus* like other penaeids is a euryhaline species and postlarvae have some ability to adapt to gradual and relatively small changes in salinity (Kumlu, 1995). Large postlarvae usually perform better than small PLs (Mair, 1980; Charmantier et al., 1988; Briggs, 1992). Small poorly developed PLs do not have respiratory gills fully developed and are therefore limited response to drastic changes in salinity, resulting in high mortality. Ontogenetic development of gills and change in osmoregulation efficiency may explain the frequent variability of results in salinity stress tests and better resistance of larger PLs.

These studies were completed with specimens of known parentage reared in captivity, therefore differences may be found in comparison with wild postlarvae. Differences in morphology between wild and reared postlarvae were observed for rostral length and occurrence of rostral teeth for *P. vannamei* (Kitani, 1993b), and *P. californiensis* (Kitani, 1993a).

The morphogenesis process after metamorphosis is continuous and postlarval development of *F. indicus* comprises 22 morphological sub-stages and last for 4-5 weeks. Most of morphological development of *F. indicus* postlarva is achieved after 2 weeks from metamorphosis to PL1, but some body structures such as respiratory gills seem only to reach full structural development from sub-stage PL16, 20-27 days after metamorphosis. Some of the most important morphological characters for PL development of *F. indicus* are the rostrum teeth, telson spines, ratio of the 6th abdominal segment to the 1st-5th abdominal segments and to carapace length, and the branchial composition.

Conclusions

The morphogenesis for postlarval development of *F. indicus* last for 4-5 weeks after metamorphosis, and the postlarva develops through 22 morphological sub-stages before reaching the juvenile stage. For PL development size is highly related to moult sequence, and growth in total length, carapace length and abdomen length, can be used to identify the morphological stage of development in penaeid postlarvae. The growth and modification of other body structures

such as rostrum teeth, telson spines, and branchiae are also additional and useful morphological indicators for identification of *F. indicus* PL moulting stage. Age of postlarvae in days differs from moult sequence hence postlarvae of same age may represent different morphological stages and perform differently during nursery culture.

References

Briggs M.R.P. 1992. A stress test for determining vigour of postlarval *Penaeus monodon* Fabricius. Aqua. Fish. Manag. 23:511-515.

Charmantier G., M. Charmantier-Daures, N. Bouaricha, P. Thuet, D.E. Aiken, and J.-P. Trilles. 1988. Ontogeny of osmoregulation and salinity tolerance in two decapod crustaceans: *Homarus americanus* and *Penaeus japonicus*. Biol. Bull. 175:102-110.

Hiramatsu K. 1984. Development of the postlarva of *Penaeus kerathurus* (Forskål, 1775) in comparison with *Penaeus japonicus* Bate, 1888 (Decapoda Natantia). Crustaceana 46(1):55-68.

Hudinaga M. 1942. Reproduction, development and rearing of *Penaeus japonicus* Bate. Japanese J. Zool. 10(2):305-393.

Kitani H. 1993a. Morphology of postlarvae of the yellowleg shrimp *Penaeus californiensis*. Nippon Suisan Gakkaishi 59(2):217-221.

Kitani H. 1993b. Morphology of postlarvae of the yellowleg shrimp *Penaeus vannamei*. Nippon Suisan Gakkaishi 59(2):223-227.

Kumlu M. 1995. Physiology of decapod crustacean larvae with special reference to diet. Ph.D. Thesis. School of Ocean Sciences, University of Wales, Bangor, UK. 256p.

Mair J.M. 1980. Salinity and water-type preferences of four species of postlarval shrimp (*Penaeus*) from West Mexico. J. Exp. Mar. Biol. Ecol. 45:69-82.

Mohamed K.H., P.V. Rao, and M.J. George. 1968. Postlarvae of penaeid prawns of Southwest coast of India with a key to their identification. FAO Fish. Rep., 57(2):487-503.

Muthu M.S., N.N. Pillai, and K.V. George. 1978. Larval development of Indian penaeid prawns. III Larval development – *Penaeus indicus* H. Milne Edwards. Bull. Central Mar. Fish. Res. Inst. India 28:12-21.

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EFFECT OF FEEDING COTTONSEED MEAL-CONTAINING DIETS TO BROODSTOCK RAINBOW TROUT AND IMPACT ON GROWTH OF THEIR PROGENIES

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Introduction

During recent decades, fish nutritionists have evaluated alternate sources of animal and plant protein in fish diets as partial or total replacement for fishmeal. Among plant proteins, cottonseed meal (CSM), a by-product of the cottonseed processing industry, seems to be an economically valuable substitute for fish meal. CSM is widely available and ranked second among plant proteins produced in the United Stated (Robinson and Li, 1995). Nutritionally, CSM contains high levels of protein and is very palatable to fish (Robinson and Li, 1995). However, the gossypol, a yellow polyphenolic pigment found in the whole cotton plant, and the lysine deficiency in CSM limit its use in diet formulation for monogastric animals.

In short-term (4 and 8 months) feeding experiments, Dabrowski et al. (2000; 2001) and Blom et al. (2001) revealed that broodstock rainbow trout (*Oncorhynchus mykiss*) growth and survival of both genders were not affected by complete replacement of fishmeal by CSM. Moreover, CSM did not deteriorate reproductive indicators (i.e., plasma sex steroid hormones, sperm concentration and motility, and sperm fertilizing ability). In the present study, we evaluated the quality of the offspring from broodstock fed CSM over a two-year period in terms of survival at the eyed-stage, growth performance, and sex ratio.

Materials and methods

Male and female rainbow trout were fed over a two-year period with five experimental diets in which fishmeal protein was replaced with CSM protein (0, 25, 50, 75 and 100%). At spawning, sperm and ova were collected from 10 males and 4-11 females per dietary treatment. Two experiments were conducted

to evaluate fertilizing ability in regard to paternal impact. Subsamples of approximately 100 ova from 3 females fed a commercial diet (London State Fish Hatchery) were fertilized with fresh (1×10^6spermatozoa.ova^{-1}, experiment 1) or cryopreserved (3×10^6spermatozoa.ova^{-1}, experiment 2) sperm from each male. Sperm was cryopreserved as described by Ciereszko and Dabrowski (1996). To evaluate the maternal impact, a third experiment was carried out in which approximately 100 ova from each female were fertilized with sperm from fish fed a 0% CSM diet. Percentage survival was determined at the eyed-embryo stage.

In each experiment, progenies from multiple parents per dietary treatment were combined and distributed in 3-4 replicate tanks (40-l volume, experiments 1 and 3) or aquaria (20-l volume, experiment 2). Fish were then fed daily a commercial diet (Biodiet, BioOregon, OR) over a 4- or 6-month period at a rate of 4% body weight. Fish were weighed every two weeks and feeding rate adjusted accordingly. At the end of experiments 2 and 3, rainbow trout were fixed in Bouin's solution to evaluate how the diet fed to broodstock could affect sex ratios of their progenies. Histological analyses were performed following routine procedures. Gossypol concentration in spermatozoa (experiment 1) and in embryo at eyed stage (experiment 3) was determined by HPLC (Kim and Calhoun, 1995; Dabrowski et al., 2000; 2001).

Results

Table I. Gossypol concentration in spermatozoa of broodstock fish fed cottonseed meal diet, survival at eyed embryo stage, and final body weight in progenies analyzed for paternal effect (fresh sperm, experiment 1). Same superscript letters are not significantly different (P>0.05).

	Dietary treatment				
	CSM0%	CSM25%	CSM50%	CSM75%	CSM100%
Gossypol (μg.g^{-1})					
Total	nd	0.77±0.32^{a}	1.61±0.85^{b}	4.37±3.77^{bc}	5.04±1.46^{c}
(+)-isomer	nd	0.56±0.21^{a}	1.10±0.61^{b}	3.12±2.67^{bc}	3.58±1.44^{c}
(–)-isomer	nd	0.21±0.13^{a}	0.51±0.24^{b}	1.25±1.14^{bc}	1.46±0.33^{c}
Survival (%)	77.0±5.5^{a}	75.6±6.8^{a}	73.7±12.0^{a}	45.6±16.9^{b}	38.1±14.5^{b}
Final body weight (g)	8.1±0.5^{b}	9.7±0.5^{a}	9.6±0.5^{a}	9.6±1.0^{a}	8.4±0.7^{b}

The spermatozoan gossypol concentration significantly (P<0.05) increased with increasing levels of CSM in diets fed to male rainbow trout (Table I). Spermatozoa accumulated predominantly (+)-gossypol (68-73%). Fertilizing ability of sperm, expressed as survival of embryos at the eyed stage, was significantly affected by the increasing levels of dietary CSM (75 and 100%). The paternal origin (fresh sperm, experiment 1) had a highly significant effect on growth performance of progenies, and progenies from males fed CSM25, 50, and 75% grew significantly

(P<0.05) faster than progenies from males fed CSM0 and 100% (Table I).

Table II. Survival at eyed embryo stage, final body weight and sex ratio in progenies analyzed for paternal impact (cryopreserved sperm, experiment 2). Control referred to fresh sperm from CSM0%. Same superscript letters are not significantly different (P>0.05). *indicates that sex ratio differed significantly (P<0.05) from 1:1.

	Dietary treatment			
	Control	CSM0%	CSM50%	CSM100%
Survival (%)	35.7 ± 3.0[a]	11.8 ± 8.8[b]	10.4 ± 9.2[b]	11.1 ± 9.0[b]
Final body weight (g)	3.3 ± 0.1[a]	3.3 ± 0.5[a]	3.6 ± 0.3[a]	3.3 ± 0.3[a]
Sex ratio (F:M)				
Number	7:8	1:13	6:11	2:12
Percentage	47:53	7:93*	35:65	14:86*

Survival at eyed stage of embryos produced using cryopreserved sperm was low regardless of the dietary levels of CSM. Moreover, it significantly (P<0.05) differed from the survival of eyed-stage embryos produced using fresh sperm (Table II). Growth performance of progenies produced using cryopreserved sperm was not affected regardless of the CSM levels fed to male rainbow trout. The sex ratio of the progenies from males fed CSM0 and 100% significantly differed from the expected sex ratio 1:1 (Table II), and a majority of males was observed in all experimental groups besides control.

Table III. Concentration of gossypol in embryo and survival at eyed embryo stage, final body weight, and sex ratio of progenies analyzed for maternal effect (experiment 3). Same superscript letters are not significantly different (P>0.05). *indicates that sex ratio differed significantly (P<0.05) from 1:1.

	Dietary treatment				
	CSM0%	CSM25%	CSM50%	CSM75%	CSM100%
Gossypol ($\mu g.g^{-1}$)					
Total	nd	0.57±0.11[a]	0.88±0.51[a]	5.34±0.98[b]	10.4±2.46[c]
(+)-isomer	nd	0.33±0.08[a]	0.46±0.25[a]	3.87±0.76[b]	7.60±1.74[c]
(-)-isomer	nd	0.24±0.04[a]	0.42±0.25[a]	1.47±0.36[b]	2.80±0.78[c]
Survival (%)	56.7±15.6[a]	20.5±24.9[a]	11.4±18.6[a]	48.8±25.9[a]	39.6±31.1[a]
Final body weight (g)	7.9±0.9[a]	7.6±1.0[a]	5.5±0.9[b]	7.2±0.6[a]	7.7±0.4[a]
Sex ratio (F:M)					
Number	3:10	0:18	1:15	3:9	2:13
Percentage	23:77	0:100*	6:94*	25:75	13:87*

nd: not detected

Gossypol was transferred from the female to the embryos regardless of the dietary levels of CSM fed to female. The highest levels were found in progenies of fish

fed CSM100% (Table III). Moreover, the (+)-isomer accounted to 52-73%. No significant differences were observed in the survival of embryos of different maternal origin (Table III). Progenies from females fed a diet containing CSM50% grew significantly ($P<0.05$) slower than the other groups (Table III). The sex ratio of the progenies from females fed CSM25, 50, and 100% significantly ($P>0.05$) differed from the expected 1:1 sex ratio (Table III). Moreover, a majority of males was observed in all groups, including control.

Discussion

The response to increasing dietary CSM levels as fishmeal substitution over a 24-month period of reproductive performance was gender specific. In contrast to the fertilizing ability of the ova, which was not significantly affected regardless of the CSM levels, sperm fertilizing ability was significantly decreased when CSM exceeded 50% protein replacement. Thus, those results indicated that long-term feeding with CSM in comparison to short-term feeding experiments (Dabrowski et al., 2000; 2001) altered male reproduction. Growth of progenies exhibited different patterns in the case of the paternal or maternal origin. However, we do not presently have any answer to explain this phenomenon. Regardless of maternal or paternal origin, males dominated among the progenies. Thus, we postulate that other substances such as flavenoids, present in the CSM, might affect the sex ratio in favor of males. Further studies are needed to evaluate the effects of the phytochemicals on the sex differentiation of rainbow trout.

References

Blom J.H., K.-J. Lee, J. Rinchard, K. Dabrowski, and J. Ottobre. 2001. Reproductive efficiency and maternal-offspring transfer of gossypol in rainbow trout (*Oncorhynchus mykiss*) fed diets containing cottonseed meal. J. Anim. Sci. 79:1533-1539.

Ciereszko A. and K. Dabrowski. 1996. Effect of a sucrose-DMSO extender supplemented with pentoxifylline or blood plasma on fertilizing ability of cryopreserved rainbow trout spermatozoa. Progr. Fish Cult. 58:143-145.

Dabrowski K., J. Rinchard, K.-J. Lee, J.H. Blom, A. Ciereszko, and J. Ottobre. 2000. Effects of diets containing gossypol on reproductive capacity of rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod. 62:227-234.

Dabrowski K., K.-J. Lee, J. Rinchard, A. Ciereszko, J.H. Blom, and J. Ottobre. 2001. Gossypol isomers bind specifically to blood plasma proteins and spermatozoa of rainbow trout fed diets containing cottonseed meal. Biochem. Biophys. Acta 1525:37-42.

Kim H.L. and M.C. Calhoun. 1995. Determination of gossypol in plasma and tissues of animals. In: Proceedings of 86th AOCS Annual Meeting; 1995; San Antonio, TX. Abstract B 487.

Robinson E.H. and M.H. Li. 1995. Use of cottonseed meal in aquaculture feeds. p.157-165. In: Nutrition and utilization technology in aquaculture C. Lim and D.J. Sessa (Eds), AOCS press, Champaign Illinois.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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WILL THE INTESTINAL MICROFLORA OF FISH PLEASE STAND UP: ELECTRON MICROSCOPY OF FISH GASTROINTESTINAL TRACT

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Abstract

The role, and even the existence, of stable indigenous microbiota in fish were not accepted until the seventies. The last decade has increased our understanding of adhesion and translocation of bacteria in fish gut, and electron microscopy has contributed significantly to this knowledge. This review summarizes the information available on gut-associated bacteria and on the translocation of bacteria in fish gastrointestinal tract.

In several studies on various fresh- and saltwater fishes, bacteria in the intestinal lumen and epithelium-associated bacteria have been demonstrated by using transmission electron microscopy (TEM) and/or scanning electron microscopy (SEM). Some of these studies have demonstrated translocation of bacterial cells by endocytosis in the gastrointestinal tract of larvae and adult fish as well as uptake of intact bacterial antigens. Endocytosis of bacteria in the digestive tract is highly relevant, as the gastrointestinal tract is a potential port of entry for pathogens.

Introduction

It is now accepted that fish possess specific intestinal microbiota consisting of aerobic, facultative anaerobic, and obligate anaerobic bacteria, and that the bacterial complement and composition may change with age, nutritional status, and environmental conditions. The intestinal microbiota have been classified as autochthonous or indigenous (when they are able to colonize the host's gut

epithelial surface) or as allochthonous, or transient. In this context, light and electron microscopic examinations of gut samples are important tools for investigating the microbial ecology of the gastrointestinal (GI) tract ecosystem and determining the presence of autochthonous or allochthonous microbiota. Several studies on various fresh- and saltwater fishes using TEM and/or SEM have demonstrated bacteria in the intestinal lumen and associated with the intestinal epithelium. Furthermore, some studies have revealed translocation of bacterial cells by endocytosis in the GI tract of both fish larvae and adult fish. Uptake of intact bacterial antigens has also been detected.

This review provides an overview of electron microscopical studies on gut-associated bacteria together with a critical evaluation of the results obtained so far. This is highly relevant, as the digestive tract is a potential port of entry for pathogens. Finally, directions for further research are proposed.

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EFFECTS OF *ARTEMIA* FEEDING REGIMES ON GROWTH AND SURVIVAL OF PHYLLOSOMA LARVAE OF SOUTHERN ROCK LOBSTER (*JASUS EDWARDSII*)

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Introduction

It is impractical to feed natural diets of zooplankton to phyllosoma larvae of rock lobster on a large aquaculture scale (Tong et al., 1997). Therefore, *Artemia* and shellfish have been used to feed larvae through to the puerulus stage (Kittaka, 1999). These foods are easily produced and dispensed for larval phyllosoma culture. *Artemia* may also be manipulated by growing to a larger size and enrichment to alter their biochemical composition.

Although the optimum number and size of *Artemia* fed to individually held larvae has been determined (Tong et al., 1997; 2000), this has not been investigated in mass culture. This study examined the feeding of different densities and sizes of *Artemia*, either alone or in combination with mussel flesh, and either alive (swimming) or incorporated in alginate pellets, on growth and survival of phyllosoma from hatch until Stage VIII.

Materials and methods

Newly-hatched phyllosoma were collected from broodstock holding tanks, disinfected with 25ppm formaldehyde, and counted into culture vessels (n = 200-1000). Larvae were cultured as described by Ritar (2001). This entailed flow-through seawater filtered to 1μm, heated to 18°C and UV-disinfected, entering circular 35-l plastic vessels via jets equally-spaced at the base and exiting through a screen positioned on the wall of the vessel. Phyllosoma were fed either *Artemia* alone (alive or in an alginate base) or in combination with chopped mussel (0.5-1.0mm pieces, disinfected with 100ppm sodium hypochlorite for 30min). *Artemia* were grown up to 2.5mm (9 days old) before enrichment in algae or commercial oil emulsion. Uneaten food and debris were removed daily from the culture vessels. Larvae were counted and sampled regularly to determine survival and body length. Stages of larval development were determined according to Lesser (1978).

Results and discussion

For larvae cultured from hatch to Stage VI, the body length (7.71±0.06mm) and survival (33.5±8.4%) did not differ significantly when 1.5-mm *Artemia* were fed at densities of 1.5, 3, or 6.ml^{-1}. However, for phyllosoma fed from Stage III to VI with 1.5-mm *Artemia* at 0.3, 1, or 3.ml^{-1}, larval size increased with increasing *Artemia* density (Table I), although survival was not affected.

Table I. Larval length and survival of *J. edwardsii* phyllosoma fed different densities of 1.5-mm *Artemia* from Stages III to VI.

Density	Length (mm) at Stage VI	Survival (%) to Stage VI
0.3ml^{-1}	7.18±0.06 ^{a}	32.9±1.7
1ml^{-1}	7.53±0.07 ^{b}	43.2±3.6
3ml^{-1}	7.77±0.07 ^{c}	40.6±4.8

Values with different superscripts within parameter differ significantly (P<0.05).

Phyllosoma growth and survival were significantly affected by the feeding of different sized *Artemia* (Table II). Larvae fed from hatch to Stage III on 0.8-mm *Artemia* were smaller and had lower survival than those fed larger *Artemia*. The size of *Artemia* fed to larvae did not affect the growth and survival between Stages III to V or between Stages VI to VIII. For all larval stages, a control treatment of 1.5-mm *Artemia* with a mussel supplement was not advantageous, and tended to have lower growth and survival than the best treatments of *Artemia* alone. This was unexpected and contrary to the findings by Kittaka (1999).

Table II. Effect of feeding treatment on larval length (mm) and survival (%) of phyllosoma of *J. edwardsii* from hatch to Stage VIII.

Size and density of *Artemia*	Length (survival) at St III	Length (survival) at St III-V	Length (survival) at St VI-VIII
0.8mm@9.ml^{-1}	3.79±0.03 ^{a} (20.4±10.2 ^{a})	–	–
0.8mm@18.ml^{-1}	3.81±0.03 ^{a} (21.9±6.5 ^{a})	–	–
1.5mm@3.ml^{-1}	4.05±0.02 ^{b} (63.0±3.7 ^{bc})	6.18±0.04 (38.5±11.1)	9.05±0.25 (18.9±1.7 ^{ab})
2.5mm@1.ml^{-1}	4.05±0.05 ^{b} (66.0±10.3 ^{c})	6.25±0.05 (52.7±9.6)	8.88±0.21 (20.8±1.4 ^{b})
2.5mm@2.ml^{-1}	4.05±0.02 ^{b} (66.2±11.6 ^{c})	6.24±0.07 (46.7±16.4)	–
1.5mm@3.ml^{-1} +mussel*	3.98±0.02 ^{ab} (34.3±12.3 ^{ac})	6.15±0.04 (44.8±9.5)	8.60±0.52 (11.4±2.9 ^{a})

* mussel supplemented at 400 pieces.culture vessel^{-1}

Values with different superscripts within parameter differ significantly (P<0.05).

From Stage VI to VIII, some larvae were fed alginate pellets incorporating live *Artemia*. The survival (32.8±1.4%) and size (9.37±0.15mm) of these larvae were significantly greater (P<0.05) than for larvae fed 1.5-mm or 2.5-mm *Artemia*.

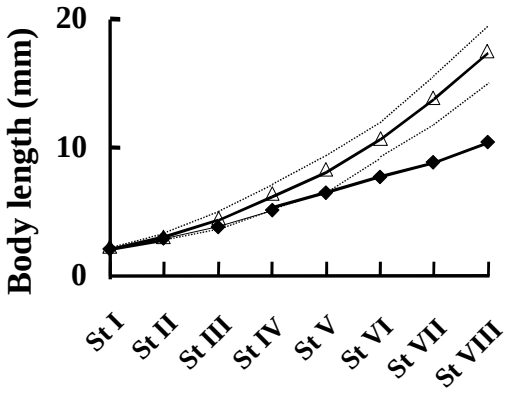

Fig. 1. Body lengths of phyllosoma in culture (diamonds, this study) or captured from the wild (triangles; dashed lines show high and low extremes; from Lesser, 1978).

The lengths of cultured phyllosoma were less than those captured from the wild from Stage II onwards (Fig. 1). By Stage VIII, wild-caught larvae were at least 50% longer than hatchery-reared animals.

Conclusion

There appeared to be no improvement in growth and survival when phyllosoma were fed 1.5-mm *Artemia* at densities between 1.5 to 6.ml^{-1}; but at 1.ml^{-1} or less, larvae could not gather sufficient prey to satisfy their nutritional requirements. Similarly, the lower growth and survival of phyllosoma fed small (0.8-mm) *Artemia* did not appear to be due to diet composition, but an inability of larvae to capture sufficient prey. The improved growth and survival of advanced larvae presented with *Artemia* in alginate pellets may be due to the inert nature of the food or to an immunostimulatory effect of the alginate binder. For all treatments, the size of cultured phyllosoma remains markedly smaller than for animals captured from the wild, and this is probably due to differences in nutritional regimes.

Acknowledgments

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References

Kittaka J. 1999. Importance of three major factors for successful phyllosoma culture. p.37-41. In: Proceedings of the 1999 FRDC Rock Lobster Propagation Workshop, Fisheries Research and Development Corporation. Hart, P. R. and R. van Barneveld. (Eds.), Hobart, Australia. 29-31 January 1999.

Lesser J.H.R. 1978. Phyllosoma larvae of *Jasus edwardsii* (Hutton) (Crustacea: Decapoda: Palinuridae) and their distribution off the east coast of the North Island, New Zealand. NZ J. Mar. Freshwat. Res. 12:357-370.

Ritar A.J. 2001. The experimental culture of phyllosoma larvae of southern rock lobster (*Jasus edwardsii*) in a flow-through system. Aquacult. Eng. 24:149-156.

Tong L.J., G.A. Moss, M.M. Paewai, and T.D. Pickering. 1997. Effect of brine-shrimp numbers on growth and survival of early-stage phyllosoma larvae of rock lobster *Jasus edwardsii*. Mar. Freshwat. Res. 48:935-940.

Tong L.J., G.A. Moss, and M.M. Paewai, 2000. Effect of brine shrimp size on the consumption rate, growth, and survival of early stage phyllosoma larvae of the rock lobster *Jasus edwardsii*. NZ J. Mar. Freshwat. Res. 34:469-473.

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THE FOOD VALUE OF MICROALGAL CONCENTRATES FOR LARVAE AND POSTLARVAE OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

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Introduction

In mollusc hatcheries and nurseries, live microalgae are traditionally used as feed for bivalves (Brown et al., 1997). Their production by conventional photoautotrophic means is high, and represents nearly 20-50% of mollusc hatcheries' operating costs (Coutteau and Sorgeloos, 1992). In an attempt to improve cost-efficiency and to simplify hatchery-nursery procedures, alternatives to live microalgae have been developed over the past few years (Robert and Trintignac, 1997). In general, these products are unsuitable as complete feeds, except microalgal concentrates, which represent the most promising off-the-shelf alternatives (Heasman et al., 2000). To date, concentrates have mainly been prepared by centrifugation. A disadvantage is that this process can damage cells, leading to a rapid deterioration of nutritional value. In this study, we assessed a novel method for preparing microalgal concentrates based on chemical flocculation (Knuckey, 1998). We applied the process to seven microalgae and tested five of these as an 80% component diet for Pacific oyster (*Crassostrea gigas*) larvae and juveniles.

Materials and methods

Actively growing starter cultures (6 l) were inoculated into perspex columns containing 300 l of 1µm-filtered seawater (34-35ppt) enriched with Conway Media. Cultures were grown under continuous illumination (160µmol photon.m^{-2}.s^{-1}) at 21°C and aerated with a 2% CO_2/air mixture to maintain pH between 7.6 and 8.1. Additional 300-l cultures were grown to late logarithmic phase for preparation of concentrates by a flocculation technique. To achieve this, the pH of cultures was first progressively increased to 10.2-10.4 by adding 1M NaOH. An anionic polyacrylamide solution (300ml of 0.05% MagnaFloc LT-25; Ciba

Specialty Chemicals) was then added to the cultures. After 15min, approximatively 80-95% of the cells had collected at the base of the cylinder and were drained off and stored into carboys. The pH of the floc was immediately adjusted to between 8.5 and 8.9 by the addition of 1M HCl. For most microalgal species, this dark floc underwent further rapid sedimentation, and after 30min the bulk of the supernatant was removed by siphoning. This gave a final volume of 2-4 l; i.e., about a 100-fold concentration. The pH was then further adjusted down to 7-7.5. The concentrates were then stored in carboys at 4°C in the dark for periods of up to 30 days. Prior to use, the required amount of concentrate was removed and dispensed in seawater into a 50-ml plastic centrifuge tube, gently shaken for 2min to disaggregate the cell suspensions.

Larvae at the density of $5.ml^{-1}$ were reared in 20- to 35-l cylindro-conical tanks. The 1µm-filtered seawater was maintained at 24°C at ambient salinity (34-35ppt) and renewed each second day. Food was added daily to give an initial cell concentration of 100 $cells.\mu l^{-1}$ (T.Iso equivalent), comprising 80% of test diet (live algae or concentrate) to 20% alternative live algae component. Diets were fed on the basis of cellular volumes: 1 *Skel*. cell = 2 T.Iso or *C.cal*. cells = 4 *C.pum*. or *C.ten*, while the average age of the concentrates ranged from 9.5 to 20.5 days. Subsamples of oysters were collected on days 9 and 16 for assessment of length (100 individuals) and mortality (200 individuals) under a profile projector (Nikon V12) equipped with an electronic micrometer. Four experiments were completed with larvae assessing live or concentrated microalgae, as a major component of a binary diet. Each experiment assessed either 4 or 5 dietary treatments, with duplicate larval tanks assigned to each treatment.

C. gigas post-larvae (~2mm) were divided into 12 groups of 16.5ml volume (~10.4g wet weight) and placed into rectangular PVC chambers with a 400-µm nylon-meshed base. Each chamber was suspended 10mm from the base of a tub filled with 26 l seawater. Seawater (18°C, 34-35ppt) was constantly dripped into the top of each chamber at a flow rate of $650ml.min^{-1}$. Duplicate chambers were assigned to each of six dietary treatments. Food was added continuously to give a final concentration (after dilution with the seawater) of 50 $cells.\mu l^{-1}$ (T.Iso equivalent), comprising 80% of test diet (live algae or concentrate) to 20% alternative live algal component (10 $cells.\mu l^{-1}$ of T.Iso). Subsamples of oysters were collected on days 16 and 28 for assessment of length (100 individuals).

Results

Five Bacillariophyceae (*Chaetoceros muelleri, C. calcitrans, C. calcitrans* forma *pumilum, C.* sp. ("*tenuissimus*-like"), *Skeletonema costatum*) and one Haptophyceae (*Pavlova lutheri*) were successfully harvested with 58-81% efficiency while *Isochrysis affinis galbana* exhibited low efficiency (31%). *C.*

calcitrans and *C. calcitrans* forma *pumilum* readily dispersed to single cells by gently mixing in storage for up to 3 weeks and appeared accordingly to have the best storage characteristics. *C. muelleri*, *C.* sp. ("*tenuissimus*-like"), and *S. costatum* concentrates had this property for 1-2 weeks; thereafter there was a progressive increase in the degree of cell clumping. *I. aff. galbana* and *P. lutheri* deteriorated rapidly.

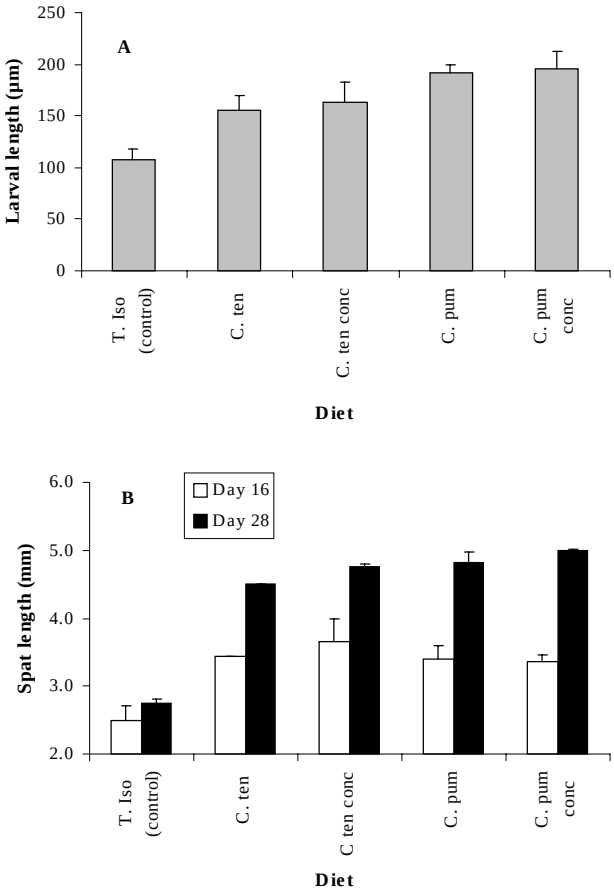

Fig. 1. Effects of mixed diets (20% fresh T. Iso + 80% fresh or concentrated diatoms)) on the growth (mean ± SE) of *Crassostrea gigas* larvae on day 14 (A) and of spat (B) on days 16 and 28. T. Iso = *Isochrysis affinis galbana* ; C. ten = *Chaetoceros sp " tenuissimus*-like " ; C. pum = *Chaetoceros calcitrans* forma *pumilum* ; conc = concentrate.

The performance of concentrates on larvae varied between species and experiments. On three occasions, concentrates performed worse than the equivalent live microalgae (*S. costatum*, *I. aff. galbana* and *C. calcitrans* forma *pumilum* from exp.1). On other occasions, concentrates gave an equivalent performance (*C.* sp "*tenuissimus-like*", *C. calcitrans* forma *pumilum* from exp. 3

(Fig. 1A) and *C. calcitrans* from exp. 4) or better (*C. calcitrans* from exp. 1) than live microalgae. The 28-d feeding experiment with oyster spat showed no difference between *C. calcitrans* forma *pumilum* or *C.* sp "*tenuissimus*-like", whether live or concentrated (Fig. 1B).

Discussion and conclusions

This study demonstrated that microalgae concentrates prepared by flocculation were used successfully as major dietary components for both larval and juvenile Pacific oysters. Such encouraging results have been also reported by Heasman et al. (2000) who found that 7 to 8 week-old centrifuged concentrates of *P. lutheri* in combination with *C. calcitrans or S. costatum* gave 85 to 90% of the growth of an optimal live microalgae reference diet. Moreover, Knuckey (1998) showed that 1 to 4 week-old flocculated concentrates of *Thalassiosira pseudonana* gave better *C. gigas* spat growth compared to other centrifuged preparation, but 70% of that of the live microalgae.

Of the different species tested, concentrates of *C. calcitrans* forma *pumilum* appeared the most promising. Their cells were readily resuspended upon dilution in seawater, and they had equivalent nutritional value for oysters in two of the three experiments in which they were assessed. In conclusion flocculation is therefore presented as a cost-effective and less damaging alternative to centrifugation for preparing microalgal concentrates of high nutritional value, especially suited for on-site production by hatcheries.

References

Brown M.R., S.W. Jeffrey, J.K. Volkman, and G.A. Dunstan. 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151:315-331.

Coutteau P. and P. Sorgeloos. 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. J. Shellfish Res. 11(2):467-476.

Heasman M., J. Diemar, W. O'Connor, T. Sushames, and L. Foulkes. 2000. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs – a summary. Aquacult. Res. 31:637-659.

Knuckey R. 1998. Australian microalgae and microalgal concentrates for use as aquaculture feeds. Ph.D. thesis, University of Tasmania. 254p.

Robert R. and P. Trintignac. 1997. Substitutes for live microalgae in mariculture: a review. Aquat. Living Res. 10:315-327.

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REPRODUCTION OF SAND SMELT (*ATHERINA BOYERI*, RISSO), AN AUTOCHTHONOUS EURYHALINE SPECIES OF THE ADRIATIC SEA, UNDER LABORATORY CONDITIONS

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Introduction

The sand smelt (*Atherina boyeri*, Risso) is small, sailing fish belonging to *Atheriniformes*. This species occurs from the Mediterranean Sea to the coast of Holland and is common along the Italian coasts, especially estuarine and brackish lagoons of the North Adriatic Sea. With regard to size, studies and samples carried out confirm adults rarely overtake 14-cm length; Mistri and Colombo (1988) studied the morphometric aspects in individuals coming from different waters. There is economic interest in *Atherina boyeri* because it is consumed both as fresh and processed; unfortunately, this autochthonous species is in jeopardy in the wild, especially in the brackish and lagoon waters of the North Adriatic Sea.

The aim of this study was to optimize a reliable breeding technique for *A. boyeri* under laboratory conditions in order to provide availability of good-quality larvae and juveniles for use in ecotoxicological tests carried out in brackish and marine waters, according to the recent Italian law no.152 of 1999. This law introduces the regulation to carry out toxicity tests (acute), forecasting the use of different bioindicators to evaluate the quality of waters and discharges.

Materials and methods

Five hundred sand smelts were captured at "lavoriero" of Val Pisani (Rovigo) throughout October-November. After an initial mortality during fishery

operations, fish were transferred to the laboratory, utilizing a transport technique by cooling water to 7±1°C. On arrival, they were arranged into 4 1-m^{-3} black fibreglass tanks and briefly quarantined (10d) in order to observe eventual pathologic signs and mortality consequent to capture stress. After this period, fish were weighed, measured, and stocked at 1 fish.l^{-1} in 6 120-l (100×40×30cm) aquaria, in partially closed circuit to guarantee a total water exchange every 3h. Light was provided by fluorescent lamps at a natural photoperiod. Temperature and salinity were maintained at 20±2°C and 21±1ppt, respectively.

In January, during 4 weeks, photoperiod was gradually increased to 16hL:8hD while the other environmental parameters were not modified. When females were ready, eggs were gently stripped into a glass culture dish containing seawater; milt from 3 males was stripped into the culture dish and mixed with the eggs by tilting the dish from side to side. In order to let the embryos adhere, one end of a nylon screen (50-μm mesh) was dipped into the dish and the embryos were then transferred into 20-l rectangular Plexiglas incubators at 20±1°C. The hatching rate was checked by counting live larvae for each incubator chamber. At 48h post-hatch, larvae were transferred between two 200-l aquaria, with a partially closed recirculation system with mechanical and biological filtration and UV sterilization. For the first 3 days, the larvae were reared in 16hL:8hD conditions. Water temperature and salinity were maintained at 20±1°C and 20ppt, respectively. During the trial, the quality of eggs and larvae was evaluated until 14 days after the hatching using a binocular microscope (4×) connected to an Olympus digital camera.

Results and discussion

After the quarantine phase, the artificial feed was readily accepted by most of the fish within few weeks. The females were 4±1 years old and were a larger size (9.8±1cm) than males (6.5±1.5cm), which were 3±1 years old. The development of only the right gonad in both the sex – characteristic of this species – was also observed. After 4 weeks conditioning, about 70% of the broodstock matured without hormonal stimulation; males were considered sexually mature when they released sperm on stripping. Before fertilization (Fig. 1), the eggs were spherical, with an outspread attachment of filaments arising from the surface by means of which they adhere to substrate. Their mean diameter was 1.45±0.3mm and they contained about 30 oil globules which, after fertilization, remained as a distinct aggregation opposite the vegetative pole. Initial embryo development was rapid and the blastodisc differentiated into a cylindrical structure along the yolk by day 2. Developing eyes and auditory sac (Fig. 2) appeared after 48h from hatching, coinciding with the development of 5-6 melanophores on the yolk sac. At day 5, eye pigmentation had begun and melanophores were observed in the supra-orbital and occipital region. At day 8,

the pectoral fins began movement and the embryo measured 1.5 times the circumference of the egg.

Fig. 1. Unfertilized egg of *A. boyeri*.

The hatching rate amounted at 80% and occurred after 10d from spawning when all the yolk sac had been consumed. The mean length was 6.51±0.4mm. The gut was short and spherical and the anus was positioned one quarter of the way back along the body. These observations were very similar to those reported for *Atherina presbyter* (Bamber et al., 1985).

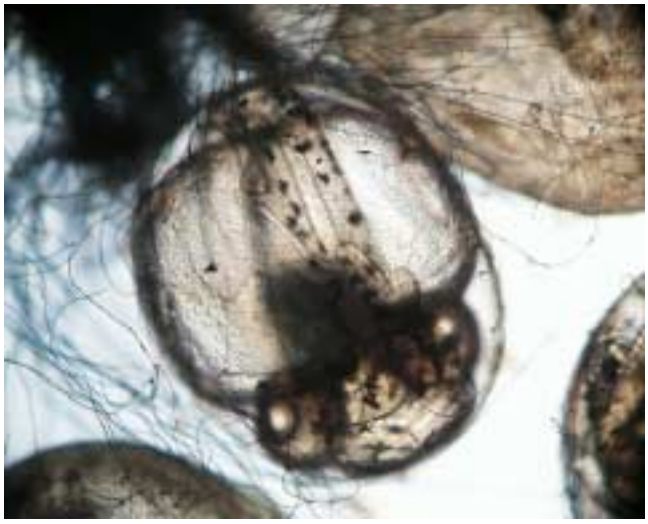

Fig. 2. Eye development at day 2.

This experiment demonstrated that it is possible to optimize a reliable capture and transport technique of *Atherina boyeri*, a species extremely sensitive to manipulation. In the present study, the environmental factors, especially photoperiod, appear to exert an important influence upon the timing of breeding in this species, whereas temperature seemed to have a lesser effect as observed in salmonids and euryhaline species. It is also possible to get reproduction in captivity without any hormonal treatment. The laboratory-induced maturation protocol provided availability of spawn, reducing the demand from the wild during the natural spawning season. These results are very important because simplify culture practices for embryos and larvae, and they permit the availability of larval stages necessary to carry on the optimization of acute toxicity tests in brackish and marine waters.

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References

Bamber R.N., P.A. Henderson, and A.W.H. Turnpenny. 1985. The early life history of the sand smelt (*Atherina presbyter*). J. Mar. Biol. Assoc. UK 65:607-706.

Mistri M. and G. Colombo. 1988. Morphometric variability in sandsmelt, *Atherina boyeri* Risso 1810, populations from different Italian sites. Boll. Zool. 3:129-132.

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ONTOGENY OF DIGESTIVE FUNCTION OF MARINE FISH LARVAE

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The digestive tract of larval fish plays a central role in nutrient acquisition and is the basis for body growth following the depletion of yolk reserves. However, this organ is still rudimentary at the onset of first feeding in marine fish larvae, where many species lack a stomach and pyloric caecae, limiting the total digestive capacity. A fully functional digestive tract is acquired during metamorphosis, when the stomach develops and the protein digestion capacity consequently increases. In this paper we will present recent data within two topics: (1) protein and amino acid digestion, absorption, and assimilation in marine fish larvae, and (2) hormonal regulation of digestive function, including peristaltic activity and enzyme release.

Due to their rapid growth rate, fish larvae require a full complement of all amino acids necessary for efficient protein synthesis. Amino acids also serve as an important energy source (Rønnestad et al., 1999). Consequently, there is a critical demand for considerable levels of amino acids in the diet. Using an *in vivo* set-up for controlled tube-feeding of fish larvae together with metabolic tracer studies (Rønnestad et al., 2001), we have shown that fish larvae absorb free amino acids (FAA) up to 10 times faster than protein from the digestive tract (Rojas-García and Rønnestad, 2000), reinforcing previous results in the Senegal sole (Rønnestad et al., 2000a). A recent study (Barr et al., unpublished results) suggests that protein digestion efficiency declines rapidly with increasing amounts of dietary protein in the intestine. Taken together, these findings suggest that the availability of amino acids from intestinal digestion of complex proteins may be insufficient to satisfy the metabolic demands of the rapidly developing fish larvae.

Very little is known about the control of intestinal function, peristalsis, and gut transit time in fish larvae. Cholecystokinin (CCK) is a key hormone in the regulation of digestion, gallbladder contraction, pancreatic enzyme secretion, and intestinal peristalsis. Our recent data, based on immunohistochemistry, suggest that this key intestinal regulatory peptide hormone is not present in the gut until two weeks after first feeding in Atlantic halibut, a species that spawns pelagic eggs (Kamisaka et al., 2001), but is present at first feeding in herring, a species which spawns benthic eggs (Kamisaka, 2001). These latter findings were supported by Koven et al. (unpublished results) who reported a CCK response in early-feeding herring larvae tube-fed soluble protein and/or selected FAA. However, the question remains how digestion is regulated during this vital developmental window, particularly in halibut, and whether there is a phylogenetic component involved.

We have recently found a marked retrograde peristaltic activity in the pyloric region of Atlantic halibut juveniles and proposed that it could provide a mechanism to fill the pyloric caecae and/or mix the chyme with digestive secretions (Rønnestad et al., 2000b). The retrograde peristalsis may be especially important for increasing digestion efficiency in the short larval intestine. CCK immunoreactive cells were found in the anterior midgut, particularly in the region adjacent to the pyloric caecae, but not posterior to the first curvature (Kamisaka et al., 2001). It is interesting to note that the retrograde peristaltic waves originate in the first intestinal curvature, thus the distribution pattern of CCK-immunoreactive cells may have functional relevance to digestive secretions and the control of the digestive processes.

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References

Kamisaka Y. 2001. Ontogenetic development of cholecystokinin (CCK)-producing cells: A comparative study of fish larvae with different types of gut development. MSc thesis. Kyoto University, Kyoto, Japan.

Kamisaka Y., T. Kurokawa, T. Suzuki, M. Tagawa, M. Tanaka G. Totland, and I. Rønnestad. 2001. Ontogeny of cholecystokinin producing cells in Atlantic halibut (*Hippoglossus hippoglossus*) larvae. Gen. Comp. Endocrinol. (in press).

Rojas-García C.R. and I. Rønnestad. 2000. Assimilation of dietary free amino acids, peptides and protein in early stages of Atlantic halibut (*Hippoglossus hippoglossus*) studied by in vivo tube-feeding. Comp. Biochem. Physiol. 126A:S128.

Rønnestad I., L.E.C. Conceição, C. Aragão, M.T. Dinis. 2000a. Free amino acids are absorbed faster and assimilated more efficiently than protein in postlarval Senegal sole (*Solea senegalensis*). J. Nutr. 130:2809-2812.

Rønnestad I., C R. Rojas-Garcia, and J. Skadal. 2000b. Retrograde peristalsis, a possible mechanism for filling the pyloric cecae? J. Fish Biol. 56:216-218.

Rønnestad I., C R. Rojas-García, S. Tonheim, and L.E.C. Conceição. 2001. In vivo studies of digestion and nutrient assimilation in marine fish larvae. Aquaculture (in press).

Rønnestad I. A. Thorsen, and R.N. Finn. 1999. Fish larval nutrition: Recent advances in amino acid metabolism. Aquaculture 177:201-216.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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THE USE OF *ARTEMIA FRANCISCANA* (KELLOG 1906) IN THE RESEARCH OF DISEASES IN AQUATIC ORGANISMS

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Introduction

Live nauplii of *Artemia* spp. have been used as vectors for delivering compounds of diverse nutritional and/or therapeutic value to larval stages of aquatic animals, a process known as bioencapsulation. Recently, *Artemia* has been used as a model crustacean since its biofiltering features make it easy to consistently reproduce bacterial infections.

The present project looks at different aspects where *Artemia* can be used either as vehicle of antibiotics or bacteria or as a model crustacean for disease studies.

Materials and methods

Standardization of bioencapsulation of bacteria (Gomez-Gil et al., 1998). The objective was to investigate whether bioencapsulation of bacteria was a feasible technique to be used in the future to deliver probiotics, or to use in bacterial challenges. Two different bacteria were inoculated in *Artemia*: a potential pathogen (*Vibrio parahaemolyticus)* and a potential probiotic (*V. algynolyticus)*.

Oral challenge (Roque et al. 2000). The objective was to design a reproducible experimental infection technique for postlarvae that was reproducible. Since the oral route is thought to be the main route of infection in young animals, the bacteria were fed with the feed.

Challenges of *Artemia*. The objective was to use *Artemia* nauplii as a test organism to characterize the bacteria assessed in relation to their virulence. Seventeen luminous bacterial strains were tested. *Artemia* nauplii were hatched in sterile conditions, and six glass test tubes with 20ml of sterile seawater were inoculated with 20 nauplii (control). The remaining nauplii were divided into Petri dishes with 18ml of sterile seawater each. Two ml of each experimental strain was added to the corresponding Petri dish to obtain a bacterial density of 10^5CFU.ml^{-1}. After 1h, the nauplii were washed and 20 were placed in a test

tube with six replicates per bacterium. All experimental tubes were incubated for 24h, and afterwards, the number of live nauplii was counted. Each experiment was carried out with 4 strains and a control.

Treatment with Enrofloxacin. The objective was to evaluate the therapeutic effects of enrofloxacin during an experimental infection with *V. harveyi* in *Artemia franciscana.* Treatments used were: (1) *Artemia* nauplii challenged with *V. harveyi*, (2) *Artemia* nauplii treated with enrofloxacin, (3) *Artemia* nauplii treated with enrofloxacin and 4h later challenged with *V. harveyi*, and (4) *Artemia* nauplii challenged with *V. harveyi* and 24h later treated with enrofloxacin. Survival rates were estimated after 48h. Treatments had 4 replicates, and the experiment was performed twice.

Artemia as a carrier of antibiotics (Gomez-Gil et al., 2001). The objective was to standardize the bioencapsulation of enrofloxacin and oxytetracycline (OTC) in *Artemia* nauplii to further be delivered to larvae of aquatic organisms. Number of hours in enrichment solution, amount of antibiotic to be offered to the *Artemia* nauplii, and time the antibiotic remained in the *Artemia* nauplii once it was transferred into clean seawater were determined.

Potential of bioencapsulation of oxytetracycline as a therapeutant. The objective was to further standardize the bioencapsulation of OTC, evaluating whether the levels of OTC incorporated were higher or lower in the presence of Rich®. The presence of Rich® was also evaluated for the loss of antibiotic in clean seawater. Finally, *Artemia* bioencapsulated with OTC with or without Rich® was offered to shrimp postlarvae and the levels incorporated in the shrimp compared. Another treatment was added to this experiment – a medicated bath with 20 $\mu g.ml^{-1}$ of OTC – with the objective of comparing the incorporation of antibiotic into the shrimp body through two delivery routes. Samples from all replicates of all treatments were taken daily to be analyzed through high performance liquid chromatography (HPLC). All experiments had 4 replicates per treatment and were carried out twice.

Results

Standardization of bioencapsulation of bacteria. Significant differences between the bacterial suspension inoculated were observed; *V. parahemolyticus* gave almost 10 fold higher than *V. algynolyticus* (1.5×10^8 and 3.17×10^7, respectively). In both experiments with *V. parahaemolyticus*, when the nauplii were maintained in the bacterial broth, the quantity of bacteria bioencapsulated increased after 30min and it kept a sustained level until around 8h, when it started decreasing. For *V. algynolyticus*, the isolate was rapidly bioencapsulated, reaching a peak at 45min. The concentration of bacteria per nauplius decreased slowly until it reached a minimum at 24h.

Oral challenge. After seven days, the mortality levels were significantly different (P<0.05) between the two treatments, averaging 9.4 and 15.3% for the experimental treatment and 1.6 and 0% for the control treatment, for trials 1 and 2, respectively.

Challenges of *Artemia*. From the challenge assays with *Artemia* nauplii, 8 out of 17 isolates induced significant (P<0.05) mortality, which ranged from 45-80%, when inoculated at 10^5-10^6CFU.ml^{-1}. The maximum bacteria bioencapsulated varied between 10^1-10^4CFU.ml^{-1} in all assays.

Treatment with Enrofloxacin. Survival rates after 48h were as follows: 29% for *Artemia* nauplii challenged with *V. harveyi*, 99% for *Artemia* nauplii bioencapsulated with Enrofloxacin, 88% for *Artemia* nauplii bioencapsulated with enrofloxacin and 4 h later challenged with *V. harveyi*, and 85% for *Artemia* nauplii challenged with *Vibrio harveyi* and 24h later bioencapsulated with enrofloxacin.

Artemia as a carrier of antibiotics. The optimal concentration of antibiotic to be offered to the nauplii was found to be 40% for enrofloxacin; at this time, 1.01 and 1.07ng per nauplius of the antibiotic was incorporated in both experiments. Significant differences were observed among all the treatments (P<0.01). For OTC, 80% was the optimum, with 9.32ng per nauplius in both experiments.

Potential of bioencapsulation of oxytetracycline as a therapeutant:. In all experiments and by both techniques, no antibiotic was detected in the control and Rich® treatments. The results of experiment 1 showed a higher incorporation of OTC in the treatment where Rich® was used (57.81μg.ml^{-1} of *Artemia* extract by HPLC), whereas for the treatment where OTC was offered with no Rich®, the incorporation was 43.14μg.ml^{-1}.

In experiment 2, the loss of OTC in clean seawater was proportional. The amount of OTC in the nauplii where Rich® was used was 41.33μg.ml^{-1} by HPLC after 240min in clean seawater, whereas for the treatment with no Rich®, the amount of antibiotic in the *Artemia* after 240min in clean seawater was 15.32μg.ml^{-1}.

In the third experiment, greater incorporation of OTC in the shrimp was found in the bath treatments. Values did not vary much from day to day within the treatments. Rich® improved the amount of OTC incorporated by the shrimp. By day 7, antibiotic determinations were as follows: OTC – 5.77±0.6μg.ml^{-1}; Rich®+OTC – 18.98±2.8μg.ml^{-1}, and bath – 26.3±8.1μg.ml^{-1} of shrimp extract.

Discussion

The initial part of this work looks at *Artemia* as a model organism to study

vibriosis. The traditional way to study disease involves reproducing the disease under experimental conditions. So far, this has been difficult to achieve, at least reproducibly. Having a reproducible challenge method would make it easy to identify virulent strains of *Vibrio* as opposed to non-virulent, and it would be possible to investigate treatments. Results show it is possible to set up challenge techniques using *Artemia* nauplii and once this technique is standardized, it is possible to test chemicals as therapeutants.

The second part of the work looked at *Artemia* as carrier of antibiotics to shrimp. Much work has been done in this field, but no established protocol is available referring to the amount of antibiotic offered to the *Artemia*, or the length of time the antibiotic should be available to the *Artemia*. Available literature refers mainly to Trimethoprim+sulfamethoxazole and its potential use for sea bass diseases (e.g., Touraki et al., 1999). These antibiotics are not used in Mexico or in shrimp culture. One issue not addressed in the literature consulted was once *Artemia* was removed from an environment with antibiotic and placed in seawater without it, how long the antibiotic remained in its system. It is believed that the bioencapsulation of antibiotics in *Artemia* would bring environmental and economic advantages, however this has not been shown in the scientific literature. For the case of oxytetracyline, results indicate that it is in theory possible to achieve 1.5 times the MIC (Minimum inhibitory concentration) of this antibiotic for Vibrios isolated from shrimp when *Artemia* is used as a carrier. However, the results from HPLC analysis on the shrimp body showed that only 0.15 of the MIC was in fact detected in the shrimp tissues. OTC is water-soluble, and perhaps its bioencapsulation for treatment purposes is not feasible. Generally, it is recommended to offer at least 4 times the MIC in order to achieve therapeutic doses in the organisms to be treated. For Enrofloxacin, 18 times the MIC can be theoretically incorporated into the shrimp, but unfortunately no work to confirm this has been carried out.

References

Gomez-Gil B., M.A. Herrera-Vega, F.A. Abreu-Grobois, and A. Roque. 1998. Bioencapsulation of two different Vibrio species in nauplii of the brine shrimp (Artemia franciscana). Appl. Environ. Microbiol. 64:2318-2322.

Goméz-Gil B., J. Cabanillas-Ramos, S. Paez-Brambila, and A. Roque. 2001. Standarization of the bioencapsulation of enrofloxacin and oxytetracycline in *Artemia franciscana* Kellog 1906. Aquaculture 196:1-12.

Roque A., A. Mazari, and B. Gomez-Gil. 2000 Infección oral experimental de *Penaeus vannamei* Boone, 1931 a través de bioencapsulación de *Vibrio parahaemolyticus* en *Artemia franciscana*. Rev. Cienc. Mar. 26:65-77

Touraki M., I. Niopas, and C. Kastritsis. 1999. Bioaccumulation of trimethoprim, sulfamethoxazole and N-acetyl-sulfamethoxazole in *Artemia* nauplii and residual kinetics in seabass larvae after repeated oral dosing of medicated nauplii. Aquaculture 175:15-30.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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SEX CONTROL IN EURASIAN PERCH, *PERCA FLUVIATILIS*

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Introduction

In fish, phenotypic sex can be modified by hormonal treatment. This technique of steroid-induced sex reversal is widely used for the production of monosex populations in aquaculture. The success of hormonal sex reversal treatments depends of three major factors: hormonal doses, treatment duration, and moment of application (Malison et al., 1986).

Eurasian perch, *Perca fluviatilis*, display a sexual growth dimorphism in which females grow faster and reach a larger ultimate size than males (Craig, 2000). The development of a process to produce all-female Eurasian perch populations would increase the commercial aquaculture production potential of this species (Kestemont and Mélard, 2000). Since the mechanism of sex determinism in Eurasian perch is supposedly female homogametic (XX) – male heterogametic (XY), monosex female populations should be obtained by a hormonal sex reversal treatment producing males with a XX genotype, which, when crossed with XX female, would thus give 100% female progenies.

In this study, hormonal doses, initiation period, and treatment duration were investigated and optimized for the hormonal sex reversal treatment and production of monosex female populations of Eurasian perch by use of hormonally sex-reversed male breeders.

Materials and methods

In early May 1999, 11 groups of 2000 fish were used to test the efficiency of sex reversal treatments using variable hormonal doses (40, 60, and 80mg.kg^{-1} diet) of 17α-methyltestosterone (17MT). Fish weight at the time of application was 43, 71, 190, and 205mg, and the treatments lasted 30-80 days. 17MT was dissolved in 95% ethanol and incorporated into formulated feed for larval fish 24

hours before use. During the experiment, fish were reared at 22-23°C, and food was delivered in excess during the daylight period. After treatment, control and experimental groups were fed a non-treated diet. After 194 rearing days, 100 fish were randomly sampled in each treatment, dissected, and their gonads were removed for gross morphological examination. The 17MT-treated populations were reared at 22-23°C over six months, then exposed to a simulated natural thermal regime during winter (6-10°C) to enable sexual maturation.

In April 2000, males from the 17MT-treated populations were killed prior artificial fertilization, and genotypic sex was determined based on gonad morphology. The gonads were cut into small fragments and milt was extracted with a syringe. Females were anaesthetized in quinaldine sulfate, and egg ribbons were collected by abdominal stripping. Eggs were fertilized using the dry fertilization technique, incubated on clays over 6 days at 16-17°C, and then transferred to a recirculated larval rearing facility at 20°C. Perch larvae were progressively trained to accept formulated feed, and then transferred into 0.3-m^3 tanks at 22-23°C for ongrowing. After 6 months, fish were killed and sex ratios determined.

Sex ratios in different treated group were compared using chi-square (χ^2) tests, at a $P<0.05$ level of significance.

Results and discussion

Fig. 1. Gonad types identified in the treated groups: (a) male with 2 testes, (b) XX male with an abnormal single twisted testis, (c) a single gonad with male and female tissues (ovotestis), and (d) female with a single ovary.

Production of hormonally sex-reversed male breeders. Five types of gonads were identified in the treated groups: female with a single ovary, male with 2 testes,

XX male with an abnormal single twisted testis (Fig. 1), or a single gonad with male and female tissues (ovotestis). Fish were considered sterile when the gonads were undeveloped and the sex could not be identified. Abnormal XX male gonad morphology was also reported by Malison et al. (1986) in yellow perch.

Sex ratio of the control group did not differ significantly from 50:50. Complete sex reversal (100% male progenies) with 17MT was obtained exclusively when the hormonal treatment was applied in fish initially ranging from 40-71mg, regardless of doses used (40 or 60mg kg^{-1}) and treatment duration (30 or 80 days). Treatments applied in fish initially ranging from 190-205mg gave variable proportions of males (45-62%), females (0-29%), XX males (16-40%), fish with ovotestis (0-18%), and sterile individuals (0-27%). As for yellow perch, the initial body weight of fish appears the most important factor for the success of hormonal sex reversal (Malison et al., 1986).

Table I. Sex ratios of progenies originating from XY male or XX male parents.

No.	Male gonad morphology	Male genotype	Number of fish sexed	Mean weight (g)	% females	% males	χ^2
1	Double	XY male	100	17.34	47.0	53.0	0.18
2	Double	XY male	100	18.70	47.0	53.0	0.18
3	Double	XY male	100	18.21	65.0	35.0	4.60*
4	Double	XY male	100 + 369**	15.90	39.3	60.7	10.99*
5	Double	XY male	100	16.90	50.0	50.0	0.01
6	Double	XY male	100	21.50	48.0	52.0	0.14
7	Double	XY male	76	8.90	57.9	42.1	0.95
8	Double	XY male	60	12.90	41.7	58.3	0.84
9	Double	XY male	49	15.00	32.7	67.3	3.01
10	Single	XX male	100	14.50	**97.0**	**3.0**	**56.70***
11	Single	XX male	100	15.72	**100**	**0.0**	**66.66***
12	Single	XX male	65	22.85	**98.5**	**1.5**	**39.78***
13	Single	XX male	56	10.30	**100**	**0.0**	**37.33***
14	Single	XX male	46	18.04	**97.9**	**2.1**	**27.28***

*P<0.05. **100 fish were first sexed in order to determine the population sex ratio, which was deviated towards males. In order to confirm this result, we sexed all the remaining population.

Production of all-female populations. Table I summarizes the different crosses performed in April 2000 and the sex ratios of resulting progenies according to the genotype of the male. Batches 1-3 were obtained from natural in-tank spawning. Sex ratios of the progenies from the batches 1 and 2 did not differ significantly from a balanced sex ratio (47% females:53% males), while the progenies originating from batch 3 displayed a significantly higher (P<0.05) proportion of females (65% female:35% male). Groups 4-9 were obtained from

artificial fertilization with semen from XY males containing double testes. Sex ratios in groups 5-9 did not differ significantly from a balanced sex ratio, while the sex ratio in group 4 was 40% females:60% males ($\chi^2 = 11.21$; $P<0.05$). In all progenies originating from males with a single twisted testis (groups 10-14), the proportion of female fish ranged from 95-100%, supporting the idea that these males were indeed genotype XX.

Conclusions

Under our culture conditions, oral administration of 17MT at 40 $mg.kg^{-1}$ food, at 70mg body weight (612 degree-days at 17°C) for 30 days is effective to induce masculinization in Eurasian perch (100% male). This technique of sex reversal induction allows the production of males with a XX genotype which, when crossed with normal females, give 100% female progenies. However, the unbalanced sex ratio of the progenies obtained in group 3-4 – originated from an XY male – and those obtained in group 10, 12, and 14 (97-98.5% females) – originating from a XX male – suggest the action of other sex determination factors (polygenic sex determinism or autosomal genes) or the effect of environmental factors (e.g., temperature; see review by Baroiller et al., 1999) on the sex determinism mechanism.

References

Baroiller J.-F., Y. Guigen, and A. Fostier. 1999. Endocrine and environmental aspects of sex differentiation in fish. Cell. Mol. Life Sci. 55:910-931.

Craig J.F. 2000. Percid Fishes – Systematics, Ecology and Exploitation. Fish and Aquatic Resources Series 3. Blackwell Science, Oxford, England. 352 p.

Kestemont P. and C. Mélard. 2000. Chapter 11 – Aquaculture. p.191-224. In: Percid Fishes – Systematics, Ecology and Exploitation. J.F. Craig (Ed.), Blackwell Science, Oxford, England. 352p.

Malison J.A., T.B. Kayes, C.D. Best, and C.H. Amundson. 1986. Sexual differentiation and use of hormones to control sex in yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 43:26-35.

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THE UTILIZATION OF PLANT-BASED PROTEIN FOR THE GROWTH AND SURVIVAL OF *MACROBRACHIUM ROSENBERGII* (DE MANN)

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Abstract

A study was conducted to determine the effect of partial or complete substitution of fishmeal (FM) by soybean meal (SBM) on the growth and survival of *Macrobrachium rosenbergii*. The postlarvae (PL 28) with an average individual length and weight of 16.20±0.20mm and 0.032±0.002g, respectively, were stocked in aquaria filled with 5.0 l of water at the rate of 30 PL for a period of eight (8) weeks. Six types of diets with different percentages of FM:SBM ratios were fed to the postlarvae of *M. rosenbergii*. The ratios – 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0 – were treated as diet 1, diet 2, diet 3 and diet 4, diet 5, and diet 6, respectively. The results showed that there were significant differences ($P<0.05$) in terms of body weight and orbital length between diets 2, 3, 4, and 5 and diets 1 and 6. Diet 3 showed the highest mean weight gain (0.71g) followed in decreasing order by diets 4, 2, 5, 1, and 6. A significant difference in terms of survival rate was also observed in this study. Thus, the study reveals that soy meal could be instrumental for enhancing the growth of *M. rosenbergii*.

Introduction

The freshwater prawn, *M. rosenbergii*, has been studied and developed over the past 20 years throughout the world, and has received considerable attention for its attractive characteristics as an aquaculture species. This species is also widely cultured (55 443 tons.year^{-1}; FAO, 1998), although much less than marine shrimp (around 900 000 tons.year^{-1}; FAO, 1998). Knowledge of the nutritional requirements of *M. rosenbergii* is necessary for the development of cost-effective diets, which can be formulated with some flexibility in the choice of ingredients. Research has provided some information concerning their nutritional requirements (Tidwell et al, 1993; Tidwell et al, 1994; Cavalli et al,

1999). The purpose of our study was to evaluate the effects of partial and total dietary replacement of fishmeal with soybean meal.

Materials and methods

M. rosenbergii with an average orbital length of 16.2±0.20mm and weight of 0.03±0.002g were studied in 60×30×30-cm concrete tanks. The experiment was conducted with six triplicated treatments. A complete randomized design was employed in all treatments. Initial body weight and orbital length of the prawns were recorded prior to the start of the experiment. All the prawns were weighed individually at 2-week intervals. Specific growth rate and survival rate were estimated following the method given by Chiu (1989). Food conversion ratio (FCR), protein efficiency ratio (PER), and proximate composition were estimated following the method described by AOAC (1985). Experimental diets were formulated to contain 40% crude protein. The compositions of the six experimental diets are presented in Table 1. Prawns were fed a percentage of body weight based on a feeding schedule suggested by D'Abramo et al. (1989). All data on growth, survival, FCR, PER, and carcass composition were analyzed using one-way ANOVA, and the differences between the means were tested using Duncan's New Multiple Range Test (Duncan, 1955).

Table 1. Ingredient and chemical composition of experimental diets.

Ingredient	Diet					
	1	2	3	4	5	6
Fish meal (55%)	0	17.02	32.65	47.06	60.38	72.73
Soybean meal (45%)	88.89	68.09	48.98	31.37	15.09	0
Plam oil	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin mix	1	1	1	1	1	1
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5
Mirenal	1	1	1	1	1	1
CMC	3	3	3	3	3	3
Celufil	4.11	7.89	11.89	14.57	17.53	20.27
Protein	40.12	40.25	40.03	40.26	40.43	40.48
Lipid	1.38	2.93	4.74	4.45	5.39	6.35
Ash	23.44	23.14	18.41	17.95	24.75	24.77
Moisture	11.52	16.06	13.53	16.78	14.35	13.44
Fiber	0.62	0.79	0.83	0.81	0.76	0.68

Results and discussion

Growth, survival, FCR, and protein efficiency ratio of *M. rosenbergii* fed with varying percentages of soybean meal after 56 days of feeding are presented in Table 2. During the period of study, there were significant differences ($P<0.05$) in terms of body weight between diets 2, 3, 4, and 5 and diets 1 and 6 (Table 2). Diet 3 showed the highest mean final body weight (0.74g) followed by diets 4, 2,

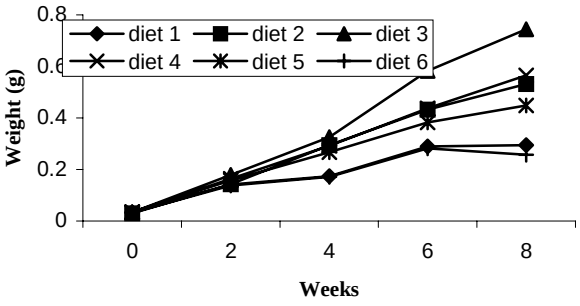

Fig. 1. Growth performance of *M. rosenbergii* fed on experimental diets with varying percentages of soybean meal for 8 weeks.

5, 1, and 6, with values of 0.56g, 0.54g, 0.44g, 0.29g, and 0.25g, respectively (Fig. 1). The specific growth rate in diet 3 was higher and showed significant difference (P<0.05) compared to the other diets (Table 2). The survival rate in diet 3 was satisfactory, as it was closed to diet 6. Similar studies have also been conducted by Tidwell et al. (1993), where they observed higher survival rates during the replacement of fishmeal with soybean meal. Tai (1991) found that *M. rosenbergii* postlarvae attained higher growth and survival rates in cage culture than in ponds and tanks. No significant differences (P>0.05) were observed in FCR and PER for all the treatments (Table 2). It was shown that diets without the addition of soybean meal and fishmeal did not produce high growth performances as compared to diet with the combination of both ingredients. Forster and Beard (1973) also found that there was increase in the weight of *Palemon serratus* with complete substitution of fishmeal with soybean meal. Weight increase dropped when the level of soybean in formulated feed was increased, or reduced by less than 40%. Lawren et al. (1986) found that it was possible to substitute fish and shrimp meal with a soybean meal (20-50%), without any effect on the growth and survival of *Penaeus schmitti*, *P. setiferus*, and *P. vannemei*. Thus, our findings agree with other studies that soybean meal has a profound effect on the growth and survival of prawn, and it may also reduce production costs.

Table 2. Growth, survival, food conversion ratio, and protein efficiency ratio of *M. rosenbergii* fed on experimental diets.

Diet	Initial Wt. (g)	Final Wt. (g)	Survival (%)	SGR (%.day^{-1})	FCR	PER
1	0.034	0.294±0.012	50.56^{b}	0.464±0.160^{b}	3.01±0.32^{a}	0.971±0.053^{a}
2	0.030	0.532±0.013	48.89^{b}	0.896±0.413^{a}	2.46±1.31^{a}	1.020±0.256^{a}
3	0.033	0.744±0.022	61.11^{a}	1.270±0.403^{a}	2.16±2.12^{a}	1.140±0.161^{a}
4	0.035	0.565±0.003	46.67^{b}	0.946±0.104^{a}	2.39±0.67^{a}	1.000±0.025^{a}
5	0.034	0.448±0.042	50.67^{b}	0.739±0.179^{a}	2.59±1.16^{a}	1.187±0.273^{a}
6	0.032	0.257±0.027	70.00^{a}	0.400±0.228^{b}	3.07±0.06^{a}	0.997±0.092^{a}

Note: Values in each column having the different superscripts are significant different (P<0.05).

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References

Association of Official Analytical Chemists (AOAC). 1985. Official Methods of Analysis. AOAC, Washington, DC. 1263 p.

Cavalli R.O., P. Lavens, and P. Sorgeloos. 1999. Performance of *Macrobrachium rosenbergii* broodstock fed diets with different Fatty acid compositon. Aquaculture 179:387-442.

Chiu Y.N. 1989. Considerations for Feeding Experiments to Quantity Dietary Requirements of essential Nutrients in Fish. In Finfish Nutrition in Asian. (ed. S.S De Silva, Special Publ. No. 4 Asian Fisheries Soc. Manila. p.46-57.

Duncan D.B. 1955. Multiple Range & Multiple F-Test. Biometrics. 11:1-42.

D'Abramo L.R., J.M. Heinen, H.R. Robinette, and J.S. Collins. 1989. Production of the freshwater prawn *Macrobrachium rosenbergii* stocked as juveniles at different densities in temperate zone ponds. J. World Aquacult. Soc. 20:81-89.

FAO. 1998. Food Agriculture Organisation. Aquaculture production statistical: 1987-1996. FAO Fisheries Circular No. 815, Revision 10, 197 p.

Forster J.R.M. and T.W. Beard.. 1973. Growth experimcnts with the prawn *Palemon serratus*, pennant fed fish and compounded foods. Fish Invest. Min. Agric. Fish. Foods. London. Ser. II. 27(7):1-16.

Lawrence A.L., F.L. Casiille, L.N. Sturmer, and D. Akiyatna. 1986. Nutritional responses of marine shrimp to different levels of soybean meal in feeds. USA – ROC and ROC – USA. Economic Council Tcnth Anllivcrsary Joint Business Conference Taipei. Taiwan. December. 1986. 21p.

Tai A.B.M. 1991. Aspek pengeluaran udang galah *Macrobrachium rosenbergii* (de Man) diperingkat semaian. M.Sc. Thesis. Universiti Pertanian Malaysia. 63p.

Tidwell J.H., C.D. Webster, L. Goodgame-Tiu, and L.R. D'Abramo. 1994. Population characteristics of *Macrobrachium rosenbergii* fed diets containing different protein sources under coolwater conditions in earthen ponds. Aquculture 126:271-281.

Tidwell J.H., C.D. Webster, D.H. Yancey, and L.R. D'Abramo. 1993. Partial and total replacement of fish meal with soybean meal and distillers' by-products in diets for culture of the freshwater prawn (*Macrobrachium rosenbergii*). Aquaculture 118:119-130.

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CASA: A QUANTITATIVE AND RAPID METHOD FOR THE ASSESSMENT OF SPERM QUALITY IN FISH

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Abstract

In the past decade, attention of the fish farming industry has been directed much more towards the quality of eggs and larvae than to that of sperm, even though rearing conditions may affect the sperm quality of male broodstock. Motility is most commonly used to evaluate the quality of sperm, since they must be motile to achieve fertilization. However, the assessment of sperm quality in fish has for long relied on subjective estimates of motility characteristics, the value of which were and are still questionable in predicting fertility. Computer Assisted Sperm Analysis (CASA) systems were initially developed to examine sperm quality in mammals and birds and have only recently been applied to fish sperm. CASA can play an important role in aquaculture as it can rapidly and quantitatively examine the effects of water chemistry and food quality, temperature manipulation, photoperiod, or holding conditions on sperm quality and hence, fertilizing ability of farmed fish. CASA has, for example, been used to select the most appropriate extenders and cryoprotectants for freezing fish sperm and to assess the viability of spermatozoa after storage in liquid nitrogen. The use of computer-calculated motility as a measure of sperm quality in fish is reviewed.

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MAGIC SOLUTIONS TO BACTERIAL PROBLEMS IN EARLY LIFE STAGES: DO THEY EXIST?

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Many different approaches are applied to counteract bacterial problems in intensive larval marine production systems. During recent decades, attention has been paid mainly to methods aiming to reduce the level of bacteria either into or within the larval system. Due to the severity of the problem, 'new' methods become very popular once they are introduced and are often considered as 'the solution' to the problem. As many of the applied methods are counterproductive in controlling bacterial levels, more focus is now paid to the necessity of taking basic ecological principles into consideration when microbial management strategies are developed. At present there is a growing interest for methods that seek to manipulate the bacterial community using biofiltration technology or by direct addition of beneficial bacteria.

The microbial environment in the non-feeding period is, in particular, critical for the establishment of a beneficial primary flora and for further realization of the larval growth potential. During hatching, there is an upward shift in available organic carbon when as much as one third of the egg carbon may be released as dissolved organic matter. Bacteria proliferate due to the increased substrate levels, and a very low bacterial diversity is characteristic at this critical point when a primary microflora is established on larval mucosal surfaces. Bacteria and microalgae are ingested in the yolk-sac period and this uptake at an early point in development could have an important effect on the establishment of a primary protective flora as well as on immunological and nutritional functions. Microalgae have an indirect effect on the larval microflora through their interactions with the bacterial community of the water, either by the antibacterial metabolites or by the bacteria that are supplied to the system along with microalgal addition. The bacterial flora of

the water and the microalgal cultures are thus important sources for the larval primary microflora.

The potential of different approaches to manipulate the bacterial community will be discussed in an ecological context, taking into consideration the three factors that are present in the non-feeding period: egg/larvae, water, and microalgae. Focus will be paid to the dynamics of the bacterial community of the water and how it is affected by different factors.

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NUTRITION AND REPRODUCTION OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS*: MAIN RESULTS OF THE EUROPEAN PROJECT GIGANUGA (*GIGAS* NUTRITION AND GAMETOGENESIS)

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This project aims at the improvement of broodstock management in oyster hatcheries through a better understanding of the role of nutrition among other non nutritional factors on oyster reproductive process and quality of early life stages. The first step was to document problems in current hatchery practice by comparison of nutritional aspects of reproduction under natural and artificial conditioning.

Cellular aspects during the reproductive cycle of *C. gigas* in nature (Marennes–Oleron) and in hatchery

This study showed that a preliminary phase of gonial mitosis for 5 months (from November to March) was followed by meioses, gamete maturation, and spawning in early August. First mature gametes (in both sexes) are present in the gonad around 4 months before the spawning period. The storage tissue, constituted by only one cell type (i.e., vesicular cells) is developing since fall until February, then decreased during last spring and summer where storage tissue development is never possible. In hatcheries, gamete production at spring is easy and cellular events are similar to nature, but not in autumn (after the natural spawning time) where egg quality is not good or reproduction remains difficult. Availability of mature broodstock all along the year in controlled

environmental conditions implicates (i) to be able to induce storage tissue reorganization and development, and (ii) to induce gonial mitosis.

What is the biochemistry of a normal reproductive cycle today?

The first step of a new sexual cycle after the spawning in summer was the fall somatic storage of neutral lipids and steryl esters in vesicular cells from mantle in November. Then, a deep decrease in vitamins B_1 and B_2 in the same organ was observed from November to February, simultaneously to the increase in glycogen storage capacity and gonial mitoses. During vitellogenesis, neutral and polar lipids increased simultaneously in gonads with a specific accumulation of 22:6(n-3) and 20:5(n-3) in the polar lipids, under natural or hatchery diets. Steryl esters (42% of sterols), were the sterol storage form in gonads, with cholesterol being preferentially accumulated. Vitamins B_1, B_2 and E accumulated also in the same organ at that period. All these molecules were mainly stored in oocytes. FA and sterol composition of the diet was reflected respectively in neutral lipids, and in free sterols of gonads. This result allowed the estimation of the FA and sterol composition of the broodstock diet in the field and a comparison of natural and hatchery diets. This comparison showed that the proportions of 22:6(n-3) and 20:5(n-3) from neutral and polar lipids of oysters conditioned artificially were significantly lower than those in nature. Cholesterol and vitamins were similar to the field but variable in eggs in the two conditions.

Methods for supplementation of these molecules

Different types of microparticles to deliver lipids and hydrosoluble vitamins to broodstock and larvae of *Crassostrea gigas* were tested. Lipid emulsions, (Coutteau et al., 96) were tested on spat (Soudant et al., 2000) to define the optimal distribution conditions for ingestion and absorption without perturbation of algal filtration rate. Absorption was time and dose-dependent and decreased with an increasing supply of emulsion. A low absorption efficiency of about 10% was observed. However emulsion must be added in excess to obtain effective incorporation to an emulsion concentration of $1.2 mg.l^{-1}$ to prevent algal filtration perturbation. Supply of hydrosoluble compounds as vitamin B_1 and B_2 was also tested using liposomes (Cansell et al., 2000) and spray beads (Langdon et al., 2000). Leakage of thiamine was very rapid from liposomes. Retention of riboflavin in spraybeads was 25% after 24 hours, which allowed using them and B_2 for feeding experiments. Demonstration of digestion and absorption of riboflavin from spraybeads was performed by measuring the increase in hemolymph and mantle tissue of broodstock after 6-hour treatment

Effect of artificial supplementations during conditioning

In a first experiment performed in spring, broodstock was fed a standard hatchery diet (mixed algal diet) and was supplemented with emulsion in attempt to correct identified deficiencies. At 2.5, 5, and 10% emulsion/algal DW using ICES 30/0.6/E5 emulsion rich in EPA and n-3 PUFAs, and 5% cholesterol, no significant effect was observed on PUFA and sterol composition of gonad and eggs, nor an effect on the reproduction and the larval development. This result corroborated the low absorption efficiency of emulsions observed in the same time. However, the same levels of emulsion increased significantly the rate of the gametogenesis process in the fall, when oysters had little storage before conditioning. This suggests the importance of prestored lipid quality on the reproductive process. A second set of experiments was dedicated to test the possibility to supplement a low cost algal diet (i.e., *Skeletonema costatum*) characterized by a DHA deficiency. Another emulsion (ICES 50/5) rich in DHA was tested at the levels of 20 and 40%. The DHA deficiency induced by the *S. costatum* monospecific diet was compensated by this protocol. Supplementation in an open circulating system led to promising results as D-larvae yield increased, and abnormalities decreased as in a multispecies diet, compared to the *S. costatum* diet. However mortalities were observed in an enclosed system showing limits of the use of emulsions in these conditions

Reproduction at fall: a combination of physical and nutritional factors

A conditioning experiment one month after the summer spawning with oysters maintained at 19°C, fed a high phytoplanktonic diet and in natural decreasing photoperiod, showed that no storage, nor gonial mitoses and gametogenesis, was evidenced in these conditions, even after 4 months conditioning. This observation would suggest an impossibility to reinitiate a storage or vitellogenesis process, when the stock of germinal cells has been completely mobilized for a major summer spawning. We have demonstrated that it was possible to reinitiate experimentally gonial mitoses of *C. gigas* after the main spawning, by a decrease in temperature at 9°C for 5 weeks and a decreasing natural photoperiod as described by Dupuy et al. (1977) on *C. virginica*. When conditioned thereafter, oysters began to restore their reserves first, but had a very slow vitellogenesis process. Those supplemented by emulsions even at low concentrations and containing PUFAs led to an acceleration of vitellogenesis dose-dependent. These results demonstrated that essential nutriments play a role in vitellogenesis rate.

For practical aspects, supplementation of essential nutriments to oysters is a promising tool to standardize quality of conditioning algal diets and reduce the phytoplankton costs. Different other artificial particles are understudy to improve retention and absorption efficiency of lipo- and hydrosoluble molecules

today. A simple manipulation of oyster reproductive cycle through temperature and photoperiod seems possible to reactivate gonial mitoses, but conditions for restoring in the same time storage tissue should be studied before obtaining an acceptable conditioning at fall.

References

Cansell M., E. Bailhache, J.F. Samain, J. Moal, J.P. Gouyou, and B. Entresangles. 2000. Potential water soluble vitamin delivery using liposomes based on marine lipids. Proceedings of the Symposium: Marine Lipids, 19-20nov 1998, Brest, France. p.212-220.

Coutteau P., J.D. Castell, R.G. Ackman, and P. Sorgeloos. 1996. The use of lipid emulsions as carriers for essential fatty acids in bivalves : a test case with juvenile *Placopecten magellanicus*. J. Shellfish Res. 15:259-264.

Dupuy J.L., N.T. Windsor, and C.E. Sutton, 1977. Manual for design and operation of an oyster seed hatchery for the American oyster *Crassostrea virginica*. Special report N° 142 in Applied Marine Science and Ocean Engineering, Virginia Institute of Marine Science, Gloucester Point, Virginia. p.13-23.

Langdon C.J, C. Seguineau, B. Ponce, J. Moal, and J.F. Samain. 2000. Delivery of riboflavin to larval and adult pacific oyster, *Crassostrea gigas* Thunberg, by lipid spray beads. J. Shellfish Res.19:129-132.

Soudant P., M. Van Sanles, C. Quere, J.R. Le Coz, Y. Marty, J. Moal, J.F. Samain, and P. Sorgeloos. 2000. The use of lipid emulsions for sterol supplementation of spat of the pacific oyster, *Crassostrea gigas*. Aquaculture. 184:315-326.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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A SEMI-INTENSIVE LARVAL REARING SYSTEM FOR TROPICAL MARINE FISH

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Introduction

We have developed a semi-intensive larval rearing system for the production of two tropical marine fish species: barramundi (*Lates calcarifer*) and golden snapper (*Lutjanus johnii*). The system maximizes production of juvenile fish whilst minimizing inputs, particularly labour and live feeds (algae, rotifers, and *Artemia*).

The semi-intensive system is based on the extensive method of marine fish culture, where earthen ponds are filled and fertilized to encourage algal and zooplankton blooms before the addition of first feeding larvae. In our case, we used outdoor 40 000-l fiberglass tanks filled with sea water and cultures of algae and zooplankton, which were added at a low density several days prior to the addition of first-feeding larvae, and allowed to bloom naturally in the tanks. The resultant blooms were usually self-sustaining for the majority of the rotifer feeding period, however, backup tanks of rotifers and algae provided extra security and reliability. The larval rearing system was normally operated by one person for three to six hours per day.

Results from the system so far have been excellent, with regular production from two 40 000-l tanks of more than 125 000 weaned barramundi per tank (greater than 70% survival), and 18 000 weaned juveniles per tank of the difficult-to-rear golden snapper (25-30% survival).

Methods and materials

Tank Setup. Two rectangular fiberglass tanks (8m×4m×1.5m deep) with an operational volume of 40 000-l were used for the larval rearing process. The tanks were situated outside, subjected to natural daylength and light conditions, and two-thirds covered by knitted shadecloth (80% light reduction) to assist temperature and algal bloom control. Adjacent to the larval rearing tanks were

six 7000-l round fiberglass tanks and 2 raceway-style 20 000-l fiberglass tanks, used for production of supplementary algae and zooplankton and as storage for chlorine-disinfected seawater.

Barramundi production method . Four days prior to the addition of first feeding larvae, the two 40 000-l tanks were filled with chlorine-disinfected, sand-filtered sea water at a salinity of 30‰ and an ambient temperature of 28-32ºC. The following day, 1000 to 2000 l of a dense culture (> 5×10^6 cells.ml^{-1}) of *Nannochloropsis oculata* were added to the tanks to give a final cell density of 5×10^5 cells.ml^{-1}. Twenty four hours later, 1×10^7 rotifers were added (0.3 rotifers.ml^{-1}). On the day the rotifers were added, floating artificial aquatic weeds (Aquamats™, Meridian Applied Technology Systems, USA) were added to the larval rearing tanks. The algae and rotifers were left to bloom for 2-3 days, and then 180 000 first feeding barramundi larvae were added (4.5 larvae.l^{-1})

Water quality (salinity, temperature, oxygen, ammonia, and nitrite) and live food densities were monitored daily. If algal density fell below 5×10^5 cells.ml^{-1} and/ or rotifer density was below 1.ml^{-1}, extra live feed was added as necessary from one of the 20 000-l or 7 000-l feeder tanks that were operated concurrently as reserve feed supplies. These reserve tanks were simply filled, inoculated, fertilized, and left to bloom until required, without any further daily maintenance.

The larval rearing tanks had no water exchange until D9 or D10, or until total ammonia levels rose above 0.5 mg.l^{-1}. After this time, at least 10% of the water in the tanks was changed daily by the addition of new, chlorine-disinfected sea water. From D17 the tanks were put on continuous water exchange with sand-filtered sea water.

From D12, newly hatched instar I *Artemia* were fed at 0.3 nauplii.ml^{-1} in one meal. Between D12 and D24, the amount of *Artemia* fed increased by meal frequency (up to 3 by the end of the larval rearing), density (up to a maximum of 3 nauplii.ml^{-1}), and nutritional value (*Artemia* boosted with *Isochrysis* Tahitian sp. (T.iso) for more than 24 hours replaced freshly hatched *Artemia* from D17).

The larval diet Proton, (INVE Aquaculture) was added as a liquid suspension from D6. Proton was initially given as one meal per day, increasing in amount, meal frequency, and particle size over the next 15 days. From D17, a commercial barramundi starter diet (Pivot 400-600µm) was fed via automatic belt feeders. By D24-28, the fish were fully weaned and ready for harvest and transfer to the nursery stage. The method is summarized in Figure 1.

Golden snapper production method. The rearing method for golden snapper was essentially the same as for barramundi, with a couple of key differences. The major difference between the methods is that rotifers were replaced as the first feed item by nauplii of the copepod *Acartia* sp., which have proven to be essential for the early survival of golden snapper larvae (Schipp et al., 1999a). Adult copepods from our hatchery-based stock cultures (Schipp et al., 1999b) were added two days prior to the larvae, together with the microalgae *N. oculata* and T.iso, giving a final density of 50-60 adult copepods.l^{-1} and a combined algae density of 5×10^5 cells.ml^{-1}. On D2, 80 000 first feeding *L. johnii* larvae (2 larvae. l^{-1}) were added to the cultures. By the time the larvae were in the rearing tanks, the copepods had bred and produced sufficient nauplii for the larvae to eat (at least 0.3 nauplii.ml^{-1}). After feeding on *Acartia* sp. nauplii for 3-4 days, the larvae were able to consume and digest the rotifers. Once the larvae commenced feeding on rotifers, the rearing regime for snapper was similar to barramundi, with the exception of Aquamats™ and Proton diet, which have yet to be tried with the snapper.

The other main difference between barramundi and golden snapper is that the latter has a more extended larval rearing period (35-40 days, as opposed to 24-28 days; Fig. 1).

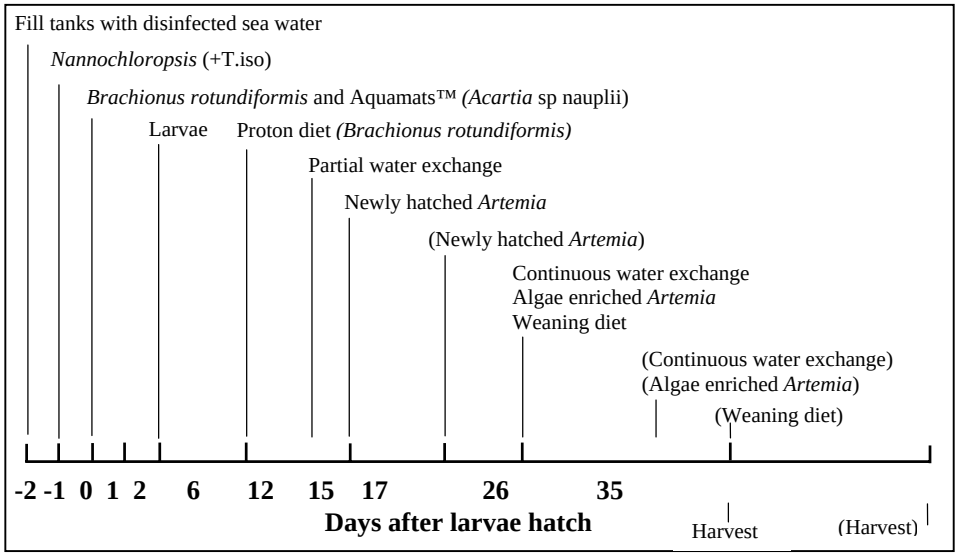

Fig. 1. Summary of the semi-intensive larval rearing regime used for production of barramundi, *Lates calcarifer* and golden snapper, *Lutjanus johnii*. Parentheses are used to distinguish those events that are specific to snapper production.

Results and discussion

The main features of the larval rearing system are its simplicity and the high yielding results. The barramundi method consistently produced survival rates above 70% to harvest (the best result has been 86%). Survival for the snapper method has varied from 25-35% for this difficult species.

The fact that the system is normally operated by one person for 3-6 hours per day represents a large efficiency increase over the traditional intensive method, where separate staff are usually required to operate larval rearing and live feed cultures. We were also able to do away with the time consuming and laborious live feed boosting protocols without affecting the quality and/or number of fish produced.

The addition of Aquamats™ to the rearing tanks and feeding of the Proton diet from Day 5 are two recent improvements to the barramundi method. The Aquamats™ have been observed to assist the larvae by providing a substrate for growth of supplementary feed (e.g., *Lotica* sp.) during the early exogenous feeding stage, as well as acting as 'water-conditioners' or in-tank biofilters, and as protection for the fish once they reach the juvenile stage, when the fish become highly cannibalistic.

Addition of the Proton diet has assisted the ease of the weaning process for barramundi, with the larvae interested in the diet from the first day it is offered. There has been a significant drop in weaning mortalities, reduced from 15% prior to using Proton down to 2.5%

In summary, the semi-intensive larval rearing system represents a cost-effective method for the production of high-quality juveniles of two tropical marine fish species.

References

Schipp G.R., J.M.P. Bosmans, and C.J. Pitney. 1999a. A semi-intensive production method for the culture of golden snapper, *Lutjanus johnii* (Bloch). Book of Abstracts, World Aquaculture ’99. World Aquaculture Society. p.681.

Schipp G.R., J.M.P. Bosmans, and A.J. Marshall. 1999b. A method for the hatchery culture of tropical Calanoid copepods, *Acartia* spp. Aquaculture 174:81-88.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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CULTURE OF THE PACIFIC MULLET (*MUGIL SOIUY* BASILEWSKY) LARVAE IN WATER BODIES OF VARIOUS SALINITIES

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The Pacific mullet is a bi-environment fish spawning in brackish estuarine water with optimal salinity of 20-23ppt (psu) (Semenenko et al., 1996) and migrating to rivers for overwintering in freshwater. Embryonic and larval development occurs in estuarine waters of relatively high salinity, while 0+ juveniles are already able to migrate in fall to the freshwater. Thus, salinity tolerance of the species has to change during ontogenetic development. A short description of these changes in tolerance observed along development from freshly hatched embryo to migrating juvenile was the aim of the present contribution.

A set of experiments (using 50 individuals per one salt concentration) showed that 100% of freshly hatched Pacific mullet embryos, when transferred directly to freshwater, died after 70h exposure. However, in another experiment, a change to freshwater on the third day of development (when embryos start to feed exogenously) for 120h caused their survival rate reach as much as 33% under unchanged conditions, optimal temperature (in our case it was 23°C), and stepwise acclimation to the diluted medium.

In case of transfer to water with the low salinity of 4psu, only 20% survived, in 8psu – 60%, in 16psu – 80% , in 18psu – 81%, and in the range of 23-33psu – 95-100 % of newly hatched, free embryos.

In salinities of 4-8psu, the hatched embryos sank and lay immobile at the bottom of experimental tanks. In 16psu, only 5-10% of them were able to swim up to the water surface.

When incubated in unsuitable, too-low salinities, some embryos showed morphological deviations, e.g., they did not have a dorsal membrane. They were retarded in growth and metamorphosis, the swimming vesicle did not developed, and when the fat droplet in the yolk sack nearly disappeared, they turned their

abdomens up and could not feed exogenously at the water surface, even at the presence of abundant zooplankton. As a rule, they died in 12-15 days, while some larvae survived until the 20-25th day.

At near-to-optimum salinity of 18psu, all the embryos swam up to the water surface with the fat droplet up, similar to when kept during egg incubation. The embryos were developing normally, except 5-10% with some deviations. That was, however, routinely met in aquaculture of this species.

In suitable temperature and salinity, on the 2nd day after hatching, the embryos were leaving the water surface membrane and migrating down into the water column. On the 3rd day, the majority of them had well-developed guts appearing and gut peristaltic movements distinctly increased. The hatched embryos were 2.2-2.7mm in length. During the following 70-74 hours, they start to feed on zooplankton and utilize the remnants of their vitelline store.

After that period, they transformed into larvae, 0.9 – 0.95mm high and 3.2-3.6mm long. The ingested exogenous food was well visible through the transparent body walls. The swimming vesicle was already formed, but the fat droplet and the hydrosinus were still to come. The 390-550-μm fat droplets help larvae to stay and swim in the surface water layer.

Small larvae, 2.8-3.0mm long and 0.73mm high, had small fat droplets of only 200-360 μm in size in the period of transition to exogenous feeding and, in cases of suboptimal salinity and high trophy in the natural habitat, were unable to develop the swimming vesicle, and fell to the bottom where they died from oxygen depletion or simply from hunger.

Salinities of 23-33psu were undoubtedly optimal for embryos and larvae of the first generation obtained from the spawners from the Sea of Japan. Such salinities allowed larvae to easily stay at the surface water layer with high abundance of small zooplankton. The big larvae are sometimes capable to feed on copepodid stages as early as on the 2nd or 3rd day after hatching.

Larvae grew very fast; on the 6th day they reached 4.1-4.6mm, and on the 9th, 5.2mm. In 11-day-old and 6-mm-long larvae, the swimming vesicle was $0.12mm^3$ in volume and the fat droplet disappeared. The larvae of that age are capable to feed on zooplankton up to 1mm in size. Simultaneously, they become more tolerant to decreased salinity (15-12psu) met at entrance to the sea.

Juveniles 20-22mm long are able to migrate to the Sea of Azov, which has salinity as low as 12psu. Two months later, they go for overwintering in a river. Genetic and anatomical background of changes in salinity tolerance observed in Pacific mullet during its ontogenetic development, as well as individual

differences in tolerance of diluted medium, is a subject of further studies. A hypothesis of genetically encoded tolerance thresholds (Styczynska-Jurewicz, personal communication) and linked to genetic polymorphism in the studied fish stock will be tested.

Conclusions

- Biotechnology of Pacific mullet larvae aquaculture in the period of early ontogenesis is closely connected with suitable salinity of the water
- For that reason the breeding installations should be constructed in the marine areas where the salinity is close to optimum, i.e., 20-23psu
- However the breeding and rearing of larvae is possible also in artificial water bodies of lower salinity when suitable amounts of salts will be added to the water
- Larvae 10- to 12-days-old may be transferred to the water of 8psu after stepwise acclimation
- In case of a strong deficit of suitable brackish or marine water the rearing of larvae is possible, but the initial reduction of the stock might be as high as 60-70%
- Grown juveniles tolerate the freshwater to which they migrate at fall for overwintering

Reference

Semenenko L.I., E.M. Fitingov, and E.I. Mirosnichenko. 1996. Ecological aspects of culture of the estuarine Pacific mullet „pilengas" (*Mugil so-iuy*, Basilewsky 1855) introduced to the Sea of Azov, Ukraine. In: Proc. 2nd International Estuary Symposium, Gdansk. E. Styczynska-Jurewicz (Ed.). Issues of the Marine Biology Centre „Crangon" 1:91-100.

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ABUNDANT FISH OFFSPRING – AN EXAMPLE OF PACIFIC MULLET ACCLIMATIZATION AND AQUACULTURE

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Aquacultural acclimatization of the Pacific mullet was initiated in the fall of 1978, when 2000 juveniles caught in the Sea of Japan were transported by air to the city of Berdiyansk (Ukraine), and then released to the Molochnoiy Liman of the Sea of Azov. This brackish water area of 20 000ha and salinity gradient of 18-23ppt (an optimal salinity range for spawning of the Pacific mullet) has been selected as a suitable habitat for mariculture of that species (Semenenko et al., 1977; Semenenko et al., 1996). Another transfer of juveniles was done in 1979. In 1983, 7000 juveniles were released to the Liman, previously kept in cages located in special ponds joining with the waters of the Liman. When released to the Liman, they averaged 6.3cm in length and 3.6g in weight (Table I). In addition, 50 pairs of sexually mature spawners, 37cm in length and on average 600g in weight, were released in the same period.

Table I. Size distribution within Pacific mullet 0+ juvenile population introduced in 1978 to the cages hold in Molochnoiy Liman, the Sea of Azov.

	Length (cm)						Mean
	4-5	5-6	6-7	7-8	8-9	9-10	6.3
Number of specimens	13	120	152	41	10	4	
Mean ind. weight (g)	1.2	2.8	3.5	5.5	10	12	3.6
% of total number	3.7	34.3	46.3	11.7	2.8	1.2	

In the cages, another 2500 young fishes of the maternal stock were grown, from which 2277 grown juveniles, plus some spawners injected with sexual hormones, were also released into the Liman. From the spawners kept in cages (or freely in the ponds filled with Liman water) and stimulated in ample time with gonadotropic hormones, a progeny of 3000 juveniles (15cm in length and 17g in weight) were released to the Liman. In 1988, there were also 5 million eggs (with embryos obtained in semi-industrial aquaculture), 6 million 3- to 4-day-old larvae, and 133 000 juveniles released into Molochnoiy Liman (Semenenko, 1991).

In 1991-1992, the maternal stock of the second generation (2500 individuals) nearly ready to spawn, was been also released to the Liman.

Over the whole period (1991-98) 0.3-1.7 million 25- to 30-day-old juveniles from aquaculture were released. At present, that artificially induced population breeds spontaneously in that liman (Semenenko et al., 1996). Evidence for occurrence of natural spawning was recorded starting with 1989, when, for the first time, a mass migration of juveniles from the liman into the Sea of Azov and Black Sea for overwintering was observed. The abundance of that migrating juvenile generation was estimated at 300-500 million.

In 1992, after the well-fed and hormone-stimulated spawners were released into the liman, an extremely abundant generation appeared in the breeding area. Besides the aforementioned spawners, the earlier released spawners also participated, having adapted to their new home, in the Molochnoiy Liman, rather than returning to their native habitat in the Sea of Japan (Semenenko et al., 1996). Cortisol content in blood plasma of spawning migrants is kept on average at the level of 145nmol.l^{-1}. This increased level, by about one order of magnitude, indicates that catabolic metabolism is a dominating physiological state of fishes during reproductive migration and spawning (Styczynska-Jurewicz et al., 1999).

A reduced abundance of zooplankton noted in June allowed estimation of the number of older mullet larvae to be 9 million, which were already capable of feeding on zooplankters (500-1000μm) in size in Molochnoiy Liman. High survival of larvae in the liman may be explained not only by suitable environmental conditions, certainly being close to optimal, but also by good condition of the spawners, which were able to produce high-quality larvae. The close coincidence of the peak of spawning with the peak of zooplankton development in the liman was also an important factor. The basic natural food of larvae consisted of naupliar and copepodid stages of several copepods, particularly *Acartia clausi*, a predominating component of zooplankton in the Sea of Azov, as well as in the liman.

Table II. Abundance (number.m^{-3}) and biomass (mg.m^{-3}) of zooplankton in Molochnoiy Liman from April-July 1992.

Organisms	April	June	July	%
Cladocerans and copepods	1578, 77.9	11 984, 464.4	7003, 92.7	25.2, 13.6
Temporarily occurring other zooplankters	58 553, 3918.6	2 452, 64.1	3, 0.001	74.8, 86.4
Total	60 131, 3996.6	14, 446±528.5	7006, 92.7	100, 100

A high biomass of zooplankton is recorded every year in June and July in Molochnoiy Liman, which is when the mullet larvae are actively preying on it. This illustrates the high availability of adequate food (Table II). As well, gut

fullness of the larvae feeding ranged from 50-220%.
When the hatched mullet embryos begin feeding exogenously, efficient growth of the larvae can be accomplished by providing ample zooplankton of suitable size. The food organisms should be 50-100μm in size. For that reason, cultivated rotifers (e.g., *Brachionus plicatilis*) which have the body length of 100-150μm are not suitable as food for small larvae, as the larvae die of starvation. In such a case when food particles are too large, the whole juvenile generation is classified as "scarce". Infusoria, copepod nauplii, eggs, and mollusc, worm, and crustacean larvae, etc. can be ingested by mullet larvae only if they are smaller than 100μm. Burlachenko (1987) and Dushkina (1998) have determined the suitable amino acid composition of food for mullet juveniles (Table III).

Table III. Amino acid contents in full-quality foods for larvae and juveniles of mullets at the 50% requirement level (according to Dushkina, 1998).

Amino acid	% dry weight (variation range)
Leucine	3.1 - 3.9
Arginine	2.8 - 3.5
Lysine	2.4 - 3.0
Valine	2.4 - 3.0
Isoleucine	2.3 - 2.9
Threonine	2.2 - 2.6
Phenylalanine	2.3 - 2.9
Methionine	1.4 - 1.7
Histidine	1.2 - 1.6

Presently, artificial feeds specific for early juveniles of the Pacific mullet are commonly used. Such feeds, with 30% protein (animal or plant origin) and 10-15% unsaturated fatty acids, can compensate for a lack of sufficient protein in the natural food. This makes the aquaculture of the Pacific mullet economically more efficient for farmers.

One study has shown good growth potential in 0+ mullet juveniles fed easily available local feeds. Twenty to thirty-day-old larvae (20-25mm long and 30-60mg weight) were fed 7 times a day with a paste-like feed made composed of small, fresh fish (acerinas and gobiids), waste of mill production, and waste of plant oil processing (Semenenko, unpublished data). The juveniles fed this mixture over a 60-day period grew up to 20cm in length and 70g in mass. While the natural potential for growth in 0+ juveniles might not be realized in all individuals, these juveniles easily exceeded 100g and 19-20cm when released in the fall.

Summary and conclusions

1. Successful aquacultural acclimatization of the Pacific mullet has been performed in the Sea of Azov/Black Sea with culture of larvae obtained

either from parents relocated as juveniles from the Sea of Japan, or spawners from the same generation kept in cages in the new habitat.

2. Gametogenesis in females may be promoted by stimulation with injected gonadotropic hormones, eliminating the homing instinct in relocated spawners.
3. A high abundance of larvae obtained from good-quality spawners in optimal extensive farming conditions may be obtained only when there is an availability of suitable food organisms for the larvae.
4. In intensive farming, live food organisms can be fed to larvae until 15-20 days after hatching, after which artificial feeds with 30% protein are recommended (Burnachenko, 1987; Semenenko, 1989). There exists a possibility for preparation of industrial feeds from local sources.
5. In recent years, the catch of Pacific mullet, acclimatized and naturalized in the Azov-Black Sea, reached more than 7000t.yr^{-1} (Volivik et al., 1998). Fortunately, this has provided ample funding for scientific research and experimental introduction. Based on these results, it may be possible to introduce this species in other countries with less cost and over a shorter time.

References

Burlachenko I.W. 1987. Opyt primeneniya iskustviennykh kormov dlia ranneiy molodi kefaleiy. Voprosy fizyiologii i biokhimii pitaniya ryb. VNIIPRKH, Moskva, p.160-168.

Dushkina L.A. 1998 (ed.) Biologicheskiye osnovy marikultury. VNIRO, Moskva, 320p.

Semenenko L.I. 1989. Sposób poucheniya lichinok kefalevykh ryb (pilengasa). Avtorskoiye svidetelstvo ob izobrstenii nr 1739540. Moskva, USSR.

Semenenko L.I. 1991. Akklimatizatsiya i rybokhozaystvennoiye osvoiyeniye pilengasa.VNIIERKH, Vypusk 2, Seria: Akvakultura, Moskva, 80p.

Semenenko L.I., E.C. Proskurina, and I.Ya. Dubrovin. 1977. Perspektivy razvitiya akvakultury i povysheniye bioproduktivnosti Mirovogo Okeana. Tezisy dokladov sympoziyuma, Moskva – Batumi, p.322-327.

Semenenko L.I., E.M. Fitingov, and E.I. Myroshnichenko. 1996. Ecological aspects of culture of the estuarine Pacific mullet «pilengas» (*Mugil soiuy* Basilewsky, 1855) introduced to the Sea of Azov (Ukraine). In: Estuarine ecosystems and species. E. Styczynska-Jurewicz (Ed). Iss. Mar. Biol. Ctr. PAS in Gdynia, «Crangon» 1:91-100.

Styczynska-Jurewicz E., L.I. Semenenko, W. Kulinski, E.I. Myroshnichenko, and E. Kusiak. 1999. Nekoryie novyiye dannye po fiziyologii proizvoditieli tikhookeanskoiy kefali pilengasa (*Mugil soiuy* Basilewsky) posle akklimatizatsii vida v Azovskom basseinie.Vtoroiy miezdunarodnyiy sympoziyum «Resursosberegayushchiye tekhnologii v akvakulture». Materialy dokladov. Adler, Russia, p.101-102.

Volovik C., B. Kotenew, and E. Mikodina, 1998. Pilengas – novyiy obiekt promysla. Rybnoiye Khozaiystvo, (Moskva) 5/6:45-46.

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EFFECTS OF DIET TRANSITION REGIMEN ON SURVIVAL AND GROWTH CHARACTERISTICS OF INTENSIVELY REARED ATLANTIC COD (*GADUS MORHUA*) LARVAE

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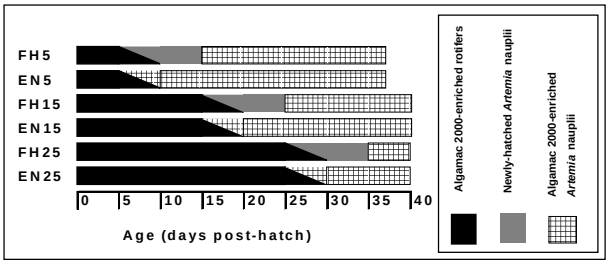
Introduction

Extensive methods for the large-scale cultivation of juvenile Atlantic cod have recently been superseded by intensive rearing techniques. While the intensive approach enables greater control over culture parameters, problems have been encountered with mortality among newly metamorphosed cod, associated with swimbladder over-inflation. The current study was conducted to determine whether there is a dietary basis for this phenomenon.

Materials and methods

Naturally fertilized Atlantic cod embryos were stocked into 24 100-l black cylindrical polyethylene rearing tanks (height 70cm, diameter 50cm) at a density of 75.l^{-1}. Water temperature was maintained at 8.8±0.2°C and salinity at 34.2±0.1ppt. Continuous overhead lighting was provided via PAR 38 tungsten lamps. Each tank received continuous aeration via a single airstone, and 50% of water volume was exchanged per day, using a 5-µm-filtered, UV-sterilized water supply. Microalgae (*Nannochloris* sp.) was added daily to each tank to maintain a density of circa 1 million cells.ml^{-1}. Hatching was completed 5d after stocking (experiment day 0), at which time 6 experimental regimens were assigned at random (4 replicates per treatment), as illustrated in Fig. 1.
Enriched rotifers were first presented to cod larvae on d1 post-hatch (ph). Larvae were subsequently transferred from rotifers (*Brachionus plicatilis*) to *Artemia* (Great Salt Lake cysts) on either d5, d15, or d25ph. On each occasion, larvae were either transferred directly to 16h-enriched *Artemia* or via freshly hatched nauplii. Gut contents were examined for a sample of larvae 24h after each diet transfer. Both rotifers and *Artemia* were enriched using Algamac 2000™ (Aquafauna Biomarine Inc) and were fed to larvae twice daily.



FH = fresh-hatched *Artemia*.
EN = enriched *Artemia*.
Numbers represent age-at-transition

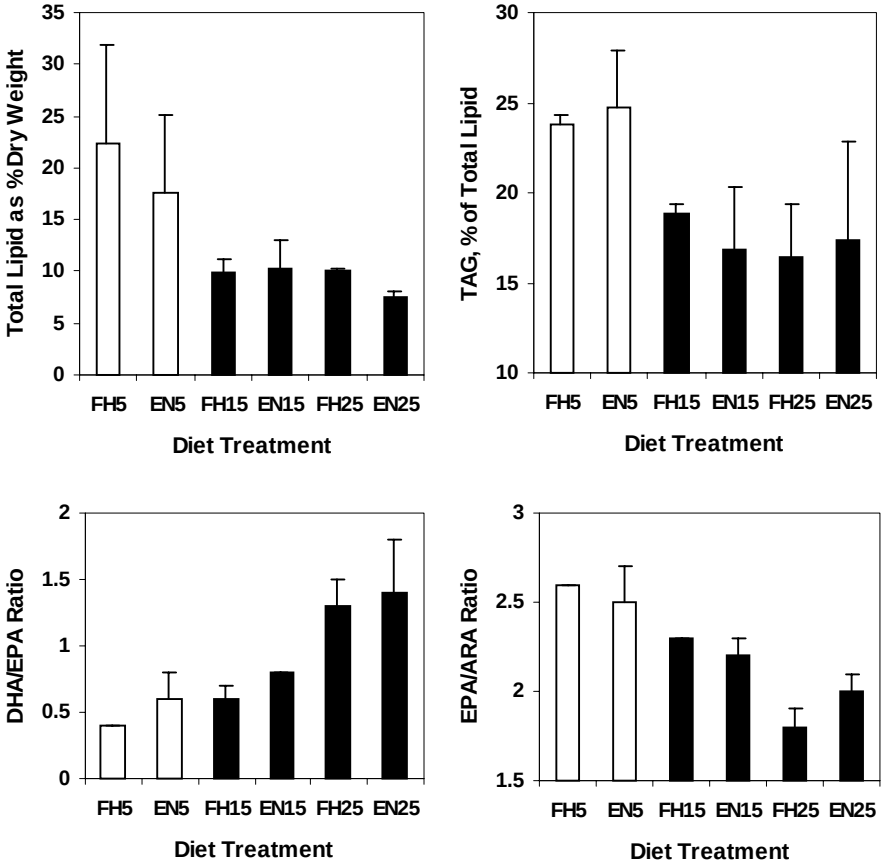
Fig. 1. Experimental diet transition regimen applied to Atlantic cod larvae.

Samples of rotifer-fed larvae were collected for size measurement prior to each diet transition. Treatments FH-5 and EN-5 were terminated on d36ph in response to high mortality rates, while the remaining treatments were retained until d40ph. Surviving fish were enumerated and samples collected for length and weight measurement and lipid biochemistry. Total lipid content, lipid class composition, and fatty acid composition of total lipid were analyzed using methods described by Shields et al. (1999). Surviving cod from the d15 and d25 transition groups were pooled into 4 nursery tanks and survival rate quantified 24h post-transfer. Results for the FH5 and EN5 treatments (end point d36ph) were compared using Student's t-test, while two-way ANOVA was applied to data from the d15 and d25 groups (end point d40ph). Data were tested for normality, with arc-sin transformation of percent data. A significance level of 0.05 was used throughout.

Results and discussion

Cod larvae receiving *Artemia* nauplii from d5ph did not ingest any nauplii within 24hr of diet transition, and only 27% of sampled larvae contained nauplii after 48hr (d7ph). In contrast, circa 90% of sampled cod larvae in the d15 and d25 transfer groups had ingested nauplii 24hr after introduction.

Larvae transferred to *Artemia* on d5ph suffered a high incidence of swimbladder over-inflation, beginning circa d20ph. Subsequent mortality in both the FH5 and EN5 groups resulted in lower cumulative survival rates than all other groups (Table 1). The surviving cod were also smaller in size and exhibited lower specific growth rates than cod transferred to *Artemia* on d15 or d25ph. Larvae receiving *Artemia* from d15ph had a significantly lower mean survival rate than those transferred on d25. Furthermore, fish from the d15 groups suffered higher mortality following transfer to nursery tanks on d40ph (Table 1). The type of *Artemia* offered during diet transition (fresh-hatched versus enriched) did not significantly affect survival rate, size, or specific growth rate at any of the 3 transfer ages.



Surviving cod from the d5 transfer groups had a greater total lipid content and contained a higher proportion of triacylglycerol (TAG) than cod from all other groups (Fig. 2a,b). For cod in the d15 and d25 transfer groups, there were no significant effects of age-at-transition, or *Artemia* type on total lipid content or percentage TAG. However, ratios of the essential fatty acids (EFAs) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) differed according to age-at-transition (Fig. 2c,d). DHA/EPA ratios increased with age-at-transition, while EPA/ARA ratios decreased, reflecting the relative quantities of these EFAs in *Schizochytrium*-enriched rotifers and *Artemia* (Barclay and Zeller, 1996). The type of *Artemia* offered during diet transition did not significantly affect cod lipid composition at any of the 3 transfer ages.

Table I. Survival rate, size and weight-specific growth rate of Atlantic cod larvae in relation to diet transition regimen. Values are means of 4 tank replicates.

	FH5†	EN5†	FH15‡	EN15‡	FH25‡	EN25‡
Standard length at transition (mm)	5.5	5.5	8.1	8.1	9.8	9.8
% Survival d5-d36/d40	0.6 ^{A}	0.9 ^{A}	6.6 ^{a}	8.0 ^{a}	18.7^{b}	17.5 ^{b}
Standard length (mm), d36/40ph	11.1 ^{A}	12.0 ^{A}	14.3 ^{a}	13.4 ^{a}	13.5 ^{a}	14.2 ^{a}
Dry weight (mg), d36/40ph	1.4 ^{A}	1.4 ^{A}	4.0 ^{a}	3.4 ^{a}	3.6 ^{a}	4.0 ^{a}
SGR, d5-d36/40ph	3.7 ^{A}	4.5 ^{A}	7.0 ^{a}	6.3 ^{a}	6.6 ^{a}	6.9 ^{a}
% Survival, 24h after nursery transfer	N/A	N/A	83.9	75.9	95.9	97.4

† end point d36ph. ‡ end point d40ph. SGR (%.day^{-1}) = [(ln final dry weight – ln initial dry wt)/days]×100. Values within a row having the same superscript are n.s.d. at P<0.05. Upper case superscripts refer to FH5 and EN5 treatments only.

We conclude that survival and growth rates of Atlantic cod are indeed influenced by diet transition regimen and that mortality associated with over-inflated swimbladders can be avoided by withholding *Artemia* until the larvae's digestive capabilities are sufficiently developed (Kjørsvik et al., 1991). These results concur with Baskerville-Bridges and Kling's (2000) observations that the growth rate of cod larvae offered microparticulate diet was improved by prolonged co-feeding with rotifers (to d22ph). In the current study, no advantage was gained by offering freshly hatched nauplii during the transition from rotifers to *Artemia*, and it is recommended that cod larvae are fed enriched nauplii directly.

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References

Barclay W. and S. Zeller 1996. Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia* nauplii by feeding spray-dried *Schizochytrium* sp. J. World Aquacult. Soc. 27:314-322.

Baskerville-Bridges B. and L.J. Kling, 2000. Early weaning of Atlantic cod (*Gadus morhua*) larvae onto a microparticulate diet. Aquaculture 189:109-117.

Kjørsvik E., T. van der Meeren, H. Kryvi, J. Arnfinnson, and P.G. Kvenseth 1991. Early development of the digestive tract of cod larvae, *Gadus morhua* L., during start-feeding and starvation. J. Fish Biol. 38:1-15.

Shields R.J., J.G. Bell, F.S. Luizi, B. Gara, N.R. Bromage, and J.R. Sargent. 1999. Natural copepods are superior to enriched *Artemia* nauplii as feed for halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. J. Nutr.129:1186-1194.

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LARVAL REARING OF CHUB, *LEUCISCUS CEPHALUS* (L.), USING DECAPSULATED *ARTEMIA* CYSTS AS DIRECT FOOD

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Abstract

The use of decapsulated *Artemia* cysts as food for chub larviculture was investigated. After three days feeding with the rotifer *Brachionus calyciflorus*, the larvae were fed on different diets: (a) dried decapsulated *Artemia* cysts, (b) *Artemia* nauplii, (c) rotifers for 7 more days and then *Daphnia* collected from a pond, and (d) an artificial diet. After a 24-day rearing period, the highest survival rate was obtained with the larvae receiving decapsulated *Artemia* cysts. Feeding of the larvae with an artificial diet resulted in a significantly lower survival rate compared to the other groups. At the end of the experiment, the larvae fed on *Artemia* nauplii yielded significantly higher mean length compared to the other groups. Feeding an artificial diet resulted in significantly lower average weight and mean length gain compared to the other groups.

Introduction

Chub, *Leuciscus cephalus*, (L), is a cyprinid species that lives in European running waters. The chub is listed as an endangered species by the International Union for the Conservation of Nature and Natural Resources (IUSN, 1994). Little is known about the larviculture of chub (Calta, 2000; Kujawa et al., 2000).

The brine shrimp *Artemia* is widely used as a live food organism for many larval fish cultured in intensive systems. However, increased demand for good quality *Artemia* cysts and recent fluctuations in world harvests have increased prices sharply. As a result, attention is concentrating again on new alternative diets to *Artemia* nauplii.

Low quality *Artemia* cysts might represent a potential alternative to *Artemia* nauplii. The outer layer of the *Artemia* cyst is non-digestible by predator organisms, but this can be quickly removed with hypochlorite treatment, a

procedure called decapsulation. Decapsulated *Artemia* cysts have been successfully fed to fish larvae (Vanhaecke et al., 1990; Pector et al., 1994).

The aim of the present study was to investigate the suitability of decapsulated *Artemia* cysts for chub larvae during their early feeding stage.

Materials and methods

Chub eggs were obtained from broodfish held at the Fish Culture Centre (Linkebeek). Fertilized eggs were incubated on an artificial substrate using aerated and dechlorinated tap water at 18°C. Larvae hatched after 4 days of incubation. Five days after hatching, the larvae were fed *ad libidum* with the rotifer *B. calyciflorus* for three days. Afterwards, the larvae were exposed to different feeding conditions: (a) decapsulated *Artemia* cysts, (b) freshly hatched *Artemia* nauplii ($4.ml^{-1}$), (c) rotifers for 7 more days and then *Daphnia* collected from a pond, or (d) a granulated trout starter diet. The larvae were stocked (10 $larvae.l^{-1}$) at random in aquaria using a flow-through system. Average wet body weight were 8.52±0.28mm and 3.53mg, respectively. There were three replicates per treatment. For length measurements, 10 larvae were collected randomly from each replicate. Survival of the larvae was recorded by counting the fish in the aquaria on day 7, day 14, and at the end of the experiment.

Cysts were decapsulated following Bruggeman et al. (1980).

The granulated diet was prepared from a trout starter food. Larvae were fed with the artificial diet *ad libidum*.

Data were analyzed with a computerized statistical program (S-Plus, 2000). Analysis of variance was performed to determine any significant differences among the treatments. Significant differences among treatments were determined by Tukey's multiple range test ($P<0.05$).

Results

At day 7 and day 14, the mean size of larvae fed freshly hatched *Artemia* nauplii and decapsulated cysts were not significantly different ($P>0.05$). However, a significantly better growth in *Artemia* nauplii-fed larvae was observed at the end of the experiment. Both diets produced significantly faster growth than the artificial diet. After 24 days of culture, the larvae fed on artificial diet had the lowest mean length (Table I).

At day 7, the mean weights of the larvae (Table II) fed on freshly hatched *Artemia* nauplii and decapsulated cysts were not significantly different ($P>0.05$). However, a significantly better growth in *Artemia* nauplii-fed larvae was

observed at day 14 and at the end of the experiment. The larvae fed on artificial diet had the lowest wet weight.

Table I. Length (mm) of chub larvae measured on day 7, day 14, and day 24 of the experimental course (Mean±S.D.).

Treatment group	Day 7	Day 14	Day 24
(a) (decapsulated cysts)	10.62±0.43	13.82±0.82	17.82±0.81
(b) (*Artemia* nauplii)	10.79±0.44	14.21±0.90	19.70±1.03
(c) (Rotifer + *Daphnia*)	9.70±0.69	12.64±1.23	16.46±1.13
(d) (Artificial diet)	9.20±0.37	10.30±0.50	11.71±1.06

Table II. Wet weight (mg) of chub larvae measured on day 7, day 14 and day 24 of the experimental course (Mean±S.D.).

Treatment group	Day 7	Day 14	Day 24
(a) (decapsulated.cysts)	7.20±0.07	18.44±0.27	46.42±1.26
(b) (*Artemia* nauplii)	7.26±0.37	20.73±0.23	57.52±4.11
(c) (Rotifer + *Daphnia*)	4.61±0.33	12.45±1.37	30.56±4.15
(d) (Artificial diet)	4.26±0.25	7.11±0.60	10.92±1.40

No significant difference was observed in larval survival at day 7 among the treatments fed *Artemia* nauplii, decapsulated cysts, and rotifers (Fig. 1). The survival rate of the larvae fed on the artificial diet was significantly lower compared to the latter groups (Fig. 1).

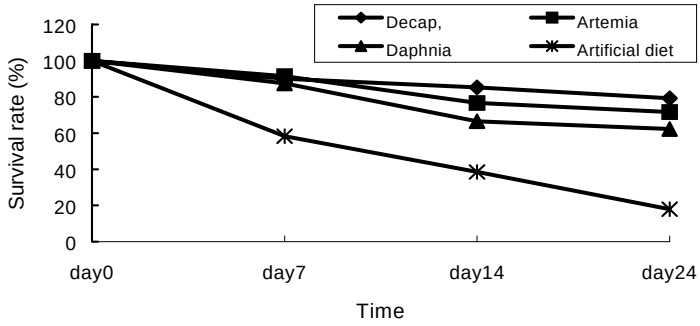

Fig 1. The survival rate of larvae counted on day 7, day 14, and at the end of experiment (Mean±S.D.).

Discussion

Dried decapsulated cysts exhibited a positive effect on growth and survival of chub larvae. After two weeks, the larval mean size was similar using *Artemia*

nauplii and decapsulated cysts. At the end of the experiment, however, mean size and wet weight of the larvae was better when fed on *Artemia* nauplii in comparison to decapsulated cysts. During long-term feeding (beyond 14 days post-hatching), decapsulated cysts did not result in as much growth in chub larvae as did live nauplii. The difference in growth after 14 days of culture may be attributed to the limited size of the decapsulated cysts. The handicap of a small food item may limit the growth of the larvae. A similar argument was also reported by Vanhaecke et al. (1991) in feeding of carp larvae on undersized decapsulated cysts in a later stage of larval growth.

Slow growth and high mortality of larvae fed on artificial diets may be related to the absence of a stomach and low digestive capacity at the beginning of their development. Kujawa et al. (2000) also found low survival and growth rates of chub larvae fed on artificial diets. They suggested that the transfer of chub larvae from live food to artificial diet should start after 12 days of exogenous feeding.

No significant difference was seen between the survival of larvae fed on *Artemia* nauplii and those on decapsulated cysts, though the latter was slightly higher

In conclusion, the results of the present work demonstrate that dried decapsulated *Artemia* cysts appear to be a suitable food for the early developmental stage of chub larvae.

References

Bruggeman E., P. Sorgeloos, and P.Vanhaecke. 1980. Improvements in the decapsulation techniques of *Artemia* cysts. pp.261-269. In: The brine shrimp *Artemia*. Ecology, culturing, uses in aquaculture Vol.3. G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press: Wetteren, Belgium 428p**.**

Calta M. 2000. Morphological development and growth of chub, *Leuciscus cephalus* (L.) larvae. J. Appl. Ichthyol. 16:83-85.

Kujawa R., D. Kucharczyk, and A. Mamcarz. 2000. The rearing methods of chub (*Leuciscus cephalus* L.) on artificial diets. p.356. In: Responsible aquaculture in the new millennium. Abstracts of contributions present at the international conference. R. Flos, and L. Creswell (Eds). Aqua 2000. European Aquaculture Society, Special Publication 28, Oostende. Belgium.

Pector R., W. Tackaert, P. Abelin, F. Ollevier, and P. Sorgeloos. 1994. A comparative study on the use of different preparations of decapsulated *Artemia* cysts as food for rearing catfish (*Clarias gariepinus*) larvae. J. World Aquacult. Soc. 25(3):366-370.

Vanhaecke P., L. De Vries, W. Tackaert, and P. Sorgeloos, 1990. The use of decapsulated cysts of the brine shrimp *Artemia* as direct food for carp C*yprinus carpio* (L.) larvae. J. World Aquacult. Soc. 21(4):257-262.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECTS OF TEMPERATURE ON GROWTH, SURVIVAL, AND DAILY FOOD CONSUMPTION OF CHILEAN FLOUNDER *PARALICHTHYS ADSPERSUS* LARVAE

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Abstract

The effects of three different temperature (16, 18, and 20ºC) on growth, survival, and daily rotifer consumption of Chilean flounder (*Paralichthys adspersus*) larvae were studied at 10-25 days post-hatching. Growth and survival were significantly related to temperature. Maximum growth rates were observed at 20ºC, while survival tended to decrease with increasing temperature. The number of rotifers consumed daily was also related to temperature. The total amount of food eaten daily by larvae increased from 460 (16ºC) to 620 (20ºC), while temperatures between 16-18ºC had no significant effects on daily food consumption. The results suggest that optimal water temperatures for growth and food consumption are 18-20ºC. However, advances in the ability to obtain better larval fish survival to this temperature are necessary

Introduction

Chilean flounder are native, high–value flatfish present off the coasts of Perú and Chile that are commonly exploited by artisanal fishermen. In Chile, demand for Chilean flounder exceeds supply due to harvest restrictions on wild population. As a result, there is an increased interest in them as an aquaculture species. Temperature is the most important environmental factor in fish culture (Stickney 1979). Temperature can affect development, survival, feed consumption, and fish growth (Smith, 1989**;** Bry et al., 1991; Mihelakakis and Yoshimatsu, 1998; Tidwell et al., 1999). The optimal conditions for Chilean flounder larviculture as related to water temperature are unknown. The objective of this study was to analyze the effects of temperature on growth, survival, and food consumption of Chilean flounder larvae.

Materials and methods

Chilean flounder larvae were obtained from eggs of wild-caught broodstock held in captivity in the fish culture laboratory of the Universidad Catolica del Norte, Coquimbo. Approximately 700 10-day-old larvae (5.5mm mean TL) were distributed among nine 5-l plastic tanks at a density of $15.l^{-1}$. Three temperatures – 16, 18, and 20ºC – were tested in duplicate. Seawater (34‰) used was filtered through a 1-µm-pore filter and UV-treated. Tank volumes were exchanged at a rate of $90ml.min^{-1}$ from three 250-l tanks maintained at each of the experimental temperatures. The water temperature in each tank was checked daily and carefully regulated to minimize day-to-day fluctuations. Larvae were fed *Brachionus plicatilis* from day 10-25 (10 $prey.ml^{-1}$.day), which were fed *Isochrysis galbana* and *Saccharomyces cerevisiae* and enriched for 12h with DHA Selco (INVE Aquaculture, Belgium). Rotifer concentration, temperature, and pH were recorded daily and the organic matter and dead larvae siphoned off. The experiment lasted 15 days, after which a final count was taken and 100% of the larvae were sampled. Total length was recorded, as well as the stage of development according to the classification of Zúñiga and Acuña (1992).

The average daily rotifers consumption at the same temperature was estimated from changes in rotifer density through hourly observation for 15 hours of 3 additional 5-l plastic tanks containing 15-day-old larvae (65mm mean TL) using the same above protocol. Two additional tanks without larvae were used to adjust natural fluctuation in rotifer density. Daily food consumption rates defined by Kitajima and Hayashida (1984) were estimated as (consumption × rotifer weight. larvae $weight^{-1}$) × 100.

Results and discussion

Growth and survival were significantly related to temperature (Figs. 1 and 2). Mean total length and growth rate were significantly highest ($P<0.05$) at 20ºC (8.7mm and 3.1%) than at 18ºC (7.2mm and 1.8%) and 16ºC (6.3mm and 0.9%), while survival tended to decrease with increasing temperature, respectively. At 16ºC and 18ºC, survival (20.5% and 13.3% respectively) was significantly higher ($P<0.05$) than at 20ºC (9.3%). The number of rotifers consumed daily was also related to temperature. The daily total amount of food eaten by larvae increased from 460 (16ºC) to 620 (20ºC), while temperatures between 16-18ºC had no significant effects on daily food consumption. Similar results were reported for the larvae of *Paralichthys olivaceus* (Yasunaga, 1971), *P. dentatus* (Johns et al., 1981), and *Pagrus major* (Kitajima et al., 1976). A positive effect of temperature on food consumption by automatic increase in basal metabolism and activity could allow an increase in larval growth rate. However, this was not true for small larvae at high temperature, where food consumption was more affected by food competition (hierarchies), therefore smaller larvae could not eat sufficiently,

resulting in higher mortality. Also when culture temperatures are too high, susceptibility to pathogens is increased. The results suggest that optimal water temperatures for growth and food consumption are 18-20ºC. However, advances in the ability to obtain better survival at this temperature are necessary.

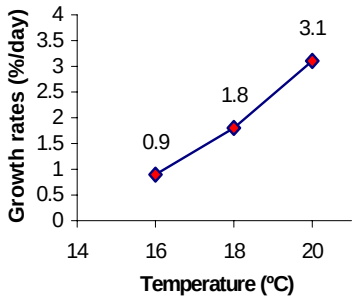

Fig. 1. Growth rate (%/day) of larval Chilean flounder *Paralichthys adspersus* cultured for 15 days at three different temperatures.

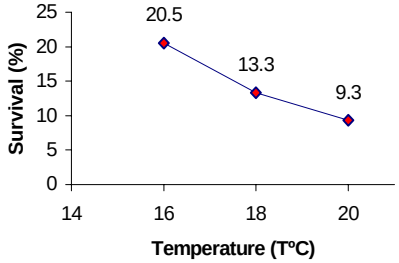

Fig. 2. Survival (%) of larval Chilean flounder *Paralichthys adspersus* cultured for 15 days at three different temperatures. Mean values are for the three replicate groups.

References

Johns D.M., W.H. Howell, and G. Klein-Macphee. 1981. Yolk utilization and growth to yolk sac absorption in summer flounder (*Paralichthys dentatus*) larvae at constant and cyclic temperatures. Mar. Biol. 63:301-308

Kitajima C., K. Fukusho, H. Iwamoto, and H. Yamamoto. 1976. Amount of rotifer, *Brachionus plicatilis*, consumed by red sea bream larvae, *Pagrus major*. Bull. Nagasaki Pref. Inst. Fish. 2:105-112

Kitajima C. and G. Hayashida. 1984. Number of rotifer, *Brachionus plicatilis*, and *Artemia salina* nauplius consumed daily by a larva and juvenile of puffer, *Takifugu rubripes*. Bull. Nagasaki Pref. Inst. Fish. 10:41-48

Mihelakakis A. and T. Yoshimatsu. 1998. Effects of salinity and temperature on incubation period, hatching rate and morphogenesis of the red sea bream. Aquacult. Int. 6:171-177.

Smith L.S. 1989. Digestive functions in teleost-fishes. Pages 331-421 in J.E.Halver, editor. Fish Nutrition. Academic Press, New York, New York, USA.

Stickney R.R. 1979. Principles of warmwater aquaculture. John Wiley & Sons, New York, New York, USA.

Tidwell J., S.D. Coyle, J. Evans, Ch. Weibel, J. Mckinney, K. Dodson, and H. Jones. 1999. Effect of culture temperature on growth, survival, and biochemical composition of yellow perch *Perca flavescens*. J. World Aquacult. Soc. 30(3):324-330

Yasunaga Y. 1971. Studies on the feeding habit and growth of the plaice *Paralichthys olivaceus,* in the larval stage. Bull. Tokai Reg. Fish. Res. Lab. 68:31-44

Zúñiga H. and E. Acuña. 1992. Larval development of two sympatric flounder *Paralichthys adspersus* (Steindachner, 1867) and *Paralichthys microps* (Gunther, 1881) from the Bay of Coquimbo, Chile. Fish Bull. 90:607-620.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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FEEDING EARLY LARVAL STAGES OF FIRE SHRIMP *LYSMATA DEBELIUS* (*CARIDEA, HIPPOLYTIDAE*)

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Introduction

High larval mortality and the long larval cycle are constraints to *Lysmata debelius* commercial rearing. After hatching, the zoea seems to have reserves for the first 24h, thus food is normally not provided during day 1. However, in the wild, where a wide range of preys is available, it is possible that young larvae show some level of feeding activity immediately after release. Newly hatched captive larvae showed peristaltic hindgut movements, indicating swallowing of water and a capacity to ingest food immediately after hatching. Without food, they lost coloration, reduced swimming activity, and some died before completing one day. This indicates an early start of feeding activity, even before the reserves are exhausted. Thus, it was hypothesized that supplying microalgae during the first 24h and complementing *Artemia* nauplii with microalgae during the following days would prevent their decline, speed their growth, and improve their survival, although they are insufficient on their own to support *Lysmata* larval development. It was also hypothesized that microalgae on the first day may boost energy resources and improve the gut condition, resulting in better digestive capacity later in ontogenic development. The present work evaluates differing larval stocking densities within the first 5 days after hatching, early feeding during 24h after hatching, and the effect of feeding different microalgal combinations (*Tetraselmis chuii, Skeletonema costata,* and *Rhinomonas reticulata*) at days 3 and 4 after hatching.

Materials and methods

L. debelius broodstock, caught in Sri Lanka, were kept in 20-l tanks (S 35.3-35.6‰; T 26-28ºC; pH 8.0-8.2), and fed fresh and frozen diets. The larvae were collected 15-45min after hatching and those showing rapid response to light were selected for feeding experiments. In the stocking density experiment, newly hatched larvae were kept for 24h in two aerated 8-l tubs and fed with *Artemia* nauplii 18-20h after hatching at 5 nauplii.ml^{-1}.day. Larvae in 1 of the tubs were

also fed with *T. chuii* at 50 cells.µl^{-1}.day. On the day 2 after hatching, the larvae were stocked in 2-l flasks at 25, 50, 75, and 100 larvae.l^{-1} and reared for 5d under the two different feeding regimes. Every other day, 50% of the water and the remaining food were replaced by new water and fresh nauplii or microalgae, accordingly. The survival and larval development was estimated on days 3 and 5 from 3 replicates of each stocking density. The ingestion rate of Z1 fed *T. chuii* and nauplii was assessed for the first 24h, and the effect of feeding during day 1 was compared to non-fed larvae for final TL and survival. The ingestion rate was assessed from individual Z1 stocked in 50ml beakers (salinity 33‰; temperature 28±1°C; pH 8.2) and fed *T. chuii* at 50 cells.µl^{-1}.day and newly hatched *Artemia* nauplii at 3 nauplii.ml^{-1}.day. The daily ingestion rate (cells or nauplii.larva^{-1}.day) was estimated after 24h. To calculate the energy intake, the energy content of *T. chuii* was assumed to be 3.4×10^{-6} J.cell^{-1} (Kurmaly et al., 1989) and that of *Artemia* 4.2×10^{-2} J.nauplii^{-1} (Ribeiro, 1998). The growth (TL) and survival of larvae fed on micro algae (ALG) or *Artemia* nauplii (ART) and that of non-fed larvae (SEM) were estimated from 150ml beakers stocked with 15 Z1 fed the same food concentrations used for assessment of ingestion rates. The effect of different foods on larval development and survival was evaluated at the molt to stage Z2, 48h after hatching. Newly hatched larvae Z1 were stocked in 2L flasks at 50 larvae.L^{-1} and reared for 48h. After 24h, prior to addition of fresh algae and *Artemia* nauplii, 25% of the water was renewed and the remaining *Artemia* removed. The following feeding groups were tested: SEM – larvae reared without food; SEM1 – no food given during the first 24h; 2nd day fed with 3 nauplii.ml^{-1}.day; ALG – *T. chuii* at 50 cells.µl^{-1}.day; ART – *Artemia* at 3 nauplii.ml^{-1}.day; ART+ALG – *Artemia* at 3 nauplii.ml^{-1}.day and *T. chuii* at 25 cells.µl^{-1}.day; and ART+DIS – *Artemia* at 3 nauplii.ml^{-1}.day 48h enriched (DIS)

Table I. Total length and survival (mean±SE) of *Lysmata debelius* larvae Z2 (48h after hatching) reared in 2-l round flasks with different dietary treatments. Different superscripts = significant differences (P=0.05; N=number of replicates at TL 10 measurements each.

Diet	N	Total length (mm)	Z1 (%)	Z2 (%)	Survival (%)
SEM	3	2.70 ± 0.01^{a}	91	9	77 ± 4^{a}
SEM-1	3	2.81±0.01	92	8	79±2
ALG	3	2.82 ± 0.01^{b}	21	79	87 ± 4^{ab}
ART	3	2.87 ± 0.01^{c}	9	91	94 ± 1^{b}
ART+ALG	3	2.94 ± 0.01^{d}	7	93	94 ± 0^{b}
ART+DIS	3	2.84 ± 0.01^{bc}	14	86	93 ± 2^{b}

The effect of different microalgal food combinations on early larval survival and molting to stage Z2 was evaluated 72 and 96h after hatching. Newly hatched Z1 larvae were stocked in 2-l flasks at 50 larvae.l^{-1} and reared for 96h. Larvae were fed 50 cells.µl^{-1}.day of each microalgae and 5 nauplii.ml^{-1}.day. Water was renewed (50%) and fresh algae and enriched nauplii (DIS) supplied after 24h. On days 3

and 4, survival was assessed. Survival on day 4 was calculated with reference to initial stock and to the number of live larvae on day 3. The following feeding groups were used: Not: larvae reared without food; Naup/DIS enriched (DIS) *Artemia* nauplii; Ske+Naup/DIS *S. costata* and enriched (DIS) *Artemia* nauplii; Rhi+Naup/DIS *R. reticulata* and enriched (DIS) *Artemia* nauplii; SkeRhi+ Naup/DIS *S. costata, R. reticulata* and enriched (DIS) *Artemia* nauplii; Tet+Naup/DIS *T. chuii* and enriched (DIS) *Artemia* nauplii; Tet+Ske+Naup/DIS *T. chuii, S. costata* and enriched (DIS) *Artemia* nauplii; Tet+Rhi+Naup/DIS *T. chuii, R. reticulata* and enriched (DIS) *Artemia* nauplii; and Tet+Ske+Rhi+Naup /DIS *T. chuii, S. costata, R. reticulata* and enriched (DIS) *Artemia* nauplii.

Results

The results for survival of Z1 larvae stocked at different densities show that the best results for larvae fed with *Artemia* nauplii were achieved at 50 larvae.l^{-1}. Larvae fed with algae and *Artemia* had the highest survival rates (100% and 98% on day 3 and day 5, respectively) at densities equal or below 75 larvae.l^{-1}. The survival rate of larvae fed exclusively on *Artemia* was significantly (P<0.05) negatively affected when stocking density increased above 50 larvae.l^{-1}, but larvae supplemented with algae produced the best results up to 75 larvae.l^{-1}. The negative effect of density on larval survival becomes more critical on day 5 regardless of feeding. The ingestion rates for newly hatched (24h) Z1 fed on *T. chuii* (50 cells.μl^{-1}) is 27 500±6614 cells.$larva^{-1}$.h, equivalent to 2.244±0.54 J.$larva^{-1}$.day, and those fed on *Artemia* nauplii (3 nauplii.ml^{-1}) is 26.7±0.8 nauplii.$larva^{-1}$.day, equivalent to 1.320±0.038 J.$larva^{-1}$.day. Energy intake of larvae fed on microalgae was 70% higher than that calculated amongst larvae fed on *Artemia*. While larvae fed on microalgae showed food in the gut 20min after being fed, the larvae feeding on *Artemia* remained with an empty gut for over 2h, indicating low efficiency at capturing and ingesting nauplii.

Discussion and conclusions

Growth and survival of *L. debelius* larvae is not affected when stocking density does not exceed 50 larvae.l^{-1}. During early stages, the addition of algae sustains slightly higher densities, although not exceeding 75 larvae.l^{-1}. Results indicate the advantage in feeding from the first day, despite the fact that the larvae hatch with apparent high reserves of energy. During day 1, the energy intake of larvae feeding on *T. chuii* was higher than that observed among larvae feeding on newly hatched nauplii, and this may explain the larger size (TL) of larvae feeding on algae. Larvae fed on algae ingested food immediately after being fed and showed full guts, whereas larvae preying on *Artemia* commenced feeding over 1h later and rarely presented a full gut. Although an algal diet does not support fast growth and development to Z2, results indicate that supplementing *Artemia* nauplii with algae

significantly increases growth and supports a higher young larval survival, and that it is only during the second day that *Lysmata* larvae improve predation on zooplankton to the level where they can succeed as exclusive carnivores. Growth and development of larvae feeding on enriched *Artemia* nauplii was slightly lower than that supported by newly hatched nauplii, though not significantly different. Results show that algae are necessary for the initial stages, and that maximization of predator/zooplankton encounters through an appropriate feeding rate is insufficient to meet the larval feeding requirements. Cannibalism occurred during the molt to Z2 in starved and algae-only-fed groups and not observed in the group fed algae+nauplii. *Lysmata* larval growth and survival is density and diet dependent. Feeding with nauplii sustains high survival rates with stocking densities at or below 50 larval.l^{-1}. Feeding early stages with algae reduces initial mortality and supports higher stocking densities (up to 75 larvae.l^{-1}). Larvae fed from day 1 on microalgae show both higher growth and survival. Supplementing nauplii with algae further enhances larval survival up to Z2.

Table II. Survival rate (mean±SE) and stage distribution of *Lysmata debelius* larvae fed on enriched *Artemia* nauplii and supplemented with different microalgae. (1)3 and (1)4 – Survival on day 3 and 4 with reference to initial stock. (3)4 – Survival on day 4 with reference to larvae alive on day 3. Different superscripts = significant differences (P =0.05); d3 = day 3; d4 = day 4.

Group	Survival (%±SE)			Stage composition (%)			
Description	(1)3	(1)4	(3)4	$Z1_{d3}$	$Z2_{d3}$	$Z1_{d4}$	$Z2_{d4}$
Without Food	59±5^{a}	9±5^{c}	15±8^{f}	100	0	97	3
Naup/DIS	28±3^{a}	16±7^{c}	59±26^{f}	100	0	2	98
Ske+Naup/DIS	33±6^{a}	25±4^{d}	77±4^{g}	0	100	0	100
Rhi+Naup/DIS	32±5^{a}	28±6^{d}	87±11^{g}	1	99	0	100
Ske+Rhi+Naup/DIS	31±1^{a}	29±3^{d}	93±7^{g}	0	100	0	100
Tet+Naup/DIS	55±9^{b}	53±6^{e}	96±6^{g}	0	100	0	100
Tet+Ske+Naup/DIS	62±6^{b}	60±6^{e}	97±1^{g}	0	100	0	100
Tet+Rhi+Naup/DIS	58±3^{b}	50±2^{e}	87±8^{g}	0	100	0	100
Tet+Ske+Rhi+Naup/DIS	61±7^{b}	58±8^{e}	95±2^{g}	2	98	0	100

References

Kurmaly K., A.B. Yule, and D.A. Jones. 1989. An energy budget for the larvae of *Penaeus monodon* (Fabricius). Aquaculture 81:13-25.

Ribeiro F.L.T. 1998. The postlarval development, growth and nutrition of the Indian White prawn *Penaeus indicus* (H. Milne Edwards). Ph.D. thesis. Univ. of Wales- Bangor, UK, p.176.

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OPTIMAL STOCKING DENSITY OF *LITOPENAEUS VANNAMEI* EARLY POSTLARVAE UNDER CLOSED RECIRCULATION

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Introduction

Recirculation systems reduce water discharges from aquaculture sites, control levels of toxic substances, such as ammonia and nitrites, and help stabilize temperature, salinity, pH, and dissolved oxygen in culture water. Recently, they have also been used to reduce water intake as a further control measure to prevent disease outbreaks. Most of the technical aspects for the implementation of these systems have already been studied, however, many biological aspects, such as stocking density, have not yet been completely examined. Recent work has determined a range of recirculation rates under completely closed conditions providing encouraging results in terms of growth and survival of early *Litopenaeus vannamei* postlarvae using moderate stocking densities (Martinez et al., 2000). This study presents further information on factors influencing the optimal stocking density of this species under recirculation conditions.

Materials and methods

Initial stocking densities ranging from 0-1420 animals.m^{-2} were tested with 95% continuous water renewal (average flow of 45ml.min^{-1}) in 20-l white polyurethane tanks (41cmL×34cmW×30cmH), for 28 days with constant aeration. Animals were fed high protein pellets 3 times per day. Residuals in the tanks were not siphoned. The recirculation system consisted of one 700-l reservoir connected to a 50-µm cartridge filter, a fluidized sand biofilter and a foam fractioner in closed circuit with a submersible pump. Water was then distributed to the 21 tanks described above with the help of a magnetic pump. The total volume of the system was approximately 1300 l. The experimental design using regression analysis approach is shown in Table I. Initial PL weight was obtained from a sample of 50 animals (2.14±0.78mg). The total body weight of 3 randomly selected animals from each tank was measured every 7 days together with survival estimates of each experimental tank and feeding adjusted accordingly. After 28 days all

survivors were weighed. Temperature, oxygen and water flow were monitored daily. Salinity, pH, ammonia and nitrites were measured every 4-5 days. Suspended solids were collected in 1.4-µm filters at the end of the experiment.

Results

Temperature was maintained at approximately 27.5±0.5°C during the experimental period. Oxygen concentrations and pH averaged 6.0 and 8.1 respectively at the beginning and steadily decreased to 5.5 and 7.9 towards the end of the experiment. Salinity rose from 37 to 38‰ despite occasional addition of fresh water to compensate high evaporation. Individual tank flows varied, but were re-calibrated every day. Suspended solids at the end of the experiment increased proportionately with increasing initial stocking density (ISD). Accumulated debris on the bottom of the highest ISDs tanks towards the end of the experiment was sufficient for shrimp to bury in it.

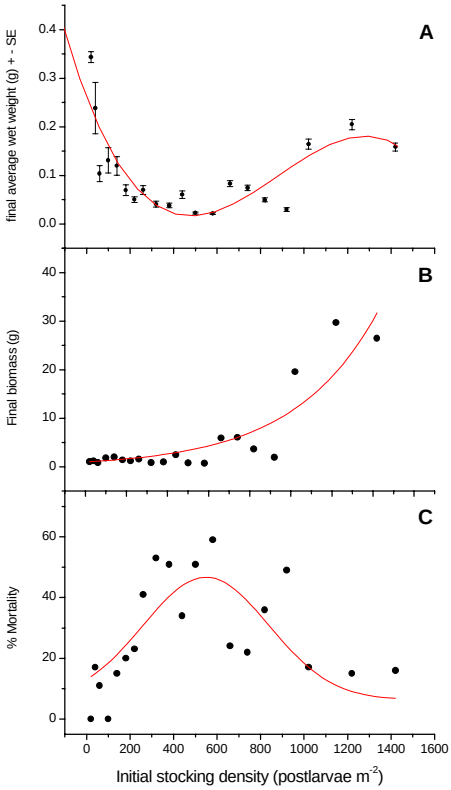

Fig. 1. Effects of initial stocking density of early *Litopenaeus vannamei* postlarvae (PL_{5-10}) on final average wet weight, final biomass, and % mortality after 28 days under closed recirculation conditions and fed high protein pellet. Adjusted lines only represent trends.

Ammonia concentrations in the control tank without animals attained 11mg.l^{-1} on day 10-14, but from the 21st day onwards decreased to as low as 3-4mg.l^{-1}. Nitrites concentration rose steadily to reach a maximum of 8.9mg l^{-1} on day 14, after which it decreased to 2.5mg.l^{-1} at the end of the experiment. Water flow, however, was not enough to maintain ammonia and nitrite concentrations as low as in the control, and consequently concentrations rose linearly with ISD. Maximum ammonia (27mg.l^{-1}) and nitrite concentrations (9mg.l^{-1}) were attained earlier in tanks with the highest ISD than in the control tank. These concentrations declined shortly after, but flattened at levels higher than the control, indicating the establishment of a complementary nitrifying bacterial community in the tanks.

Final weight after 28 days presented an exponential decay in relation to ISD up to 800-1000 animals.m^{-2} (Fig. 1A). However, the three tanks with the highest ISD produced larger animals in average than all other tanks, except the first two.

In Fig. 1B, final biomass per tank is shown to be nearly linear up to ISD of 600 animals m^{-2} mainly due to the heavier animals from the lower ISD treatments. Final biomass in tanks with ISD above 600 animals.m^{-2} increased markedly reaching 20+ grams per tank.

The Gaussian distribution of Fig. 1C indicates that amongst tanks with ISD of 200 to 900 animals m^{-2}, percentage mortality attained values above 20%. Zero mortality occurred only in tanks with the lowest ISDs, whilst in tanks with 1000 animals m^{-2} or more, mortality seemed to stabilize around 17%.

Discussion

The decline of oxygen concentration towards the end of the experiment (despite increased aeration) clearly reflects the intense bacterial activity degrading uneaten food and faeces, as these were not siphoned out from the tanks. This situation was more obvious in tanks with large quantities of suspended solids. Under these conditions, ammonia and nitrite concentrations reached values above reported safe levels, although only momentarily, and after gradual acclimation. This situation certainly affected growth rate, as indirectly seen on the final weight data presented in Fig. 1A. Postlarvae in treatments of 200 animals.m^{-2} and above grew well below the maximum observed final weight (0.25-0.35g). However, animals from the three highest ISD unexpectedly grew larger, indicating a beneficial effect of large feed quantities throughout the experiment. One hypothesis explaining these results is that postlarvae undergo early acclimation to high ammonia and nitrite concentrations, and are thereafter more resistant. A complementary explanation is that these animals grow under the best nutritional conditions based on extra nutrients from abundant microorganisms growing on uneaten food and faeces.

High concentrations of ammonia and nitrite were not solely responsible for the observed mortality values. Many postlarvae died trapped on the dry sides of tanks with the highest density treatments during the first days of the experiment. As animals grew, overcrowding in these tanks induced a general chain reaction of escape responses that eventually caused mortality by jumping out of the tanks. This was a consequence of stress behaviour rather than poor water quality. Despite mortality and reduced growth, highest stocking densities yielded considerably more biomass than standard industry densities for these stages (Fig. 1B). This study was subsequently repeated with *Farfantepenaeus brasiliensis* obtaining similar results to be published elsewhere, together with further analysis of present data.

Conclusion

Reasonable weight gain and relatively low mortalities demonstrate that the highest tested densities ($1200.m^{-2}$) are viable for super-intensive rearing of early *Litopenaeus vannamei* postlarvae using closed recirculation conditions.

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References

Martínez Y.G., N. Simões, D.A. Jones, G. Gaxiola, L.E. Hidalgo, and M. Mascaró. 2000. Continuous recirculation versus batch water exchange in shrimp postlarvae culture: Which method is best and how much percent volume should be exchanged? p.39. In: Advances in Aquatic Nutrition. L.E. Cruz-Suarez, D. Ricque-Marie, M. Tapia-Salazar, M. Olvera-Novoa, and R. Civera-Cerecedo (Eds.). Proceedings of the V International Symposium on Aquatic Nutrition, 19-22 November 2000, Merida, Yucatan, Mexico.

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PRELIMINARY DATA ON BACTERIAL GUT COLONIZATION OF FIRST FEEDING PENAEID LARVAE STAGES

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The initiation of exogenous feeding in penaeid larvae (Z_1) has been identified as a critical phase in terms of survival and growth as the larvae are exposed internally to environmental microbial communities for the first time at the molt from N_{5-6} to Z_1. Measurements of larval gut volume and clearance rates are given together with a review of the potential for the disinfection of eggs and first-stage larvae. Several fluorescent stains including DAPI, DTAF, and Acridine Orange were tested in their effectiveness to mark and follow live bacteria within the larval gut using two bacterial strains isolated from shrimp hatcheries in Mexico. Bacteria numbers and position in the larval digestive tract were estimated after the mouth opened at first feeding and after the loss and regeneration of portions of the gut during molting. Results are compared with examination of the virgin gut prior to the mouth opening. The following 3 hypotheses were tested: H_1: The internal tissues of shrimp nauplii are bacterially sterile; H_2: The bacterial population composition of a Z_1 gut is identical to the bacterial population present in the surrounding culture water; and H_3: bacteria remain in the larvae gut by attachment to the gut inner walls.

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IMMUNOSTIMULATION AS COUNTERACTION TO BACTERIAL PROBLEMS IN FIRST FEEDING OF MARINE LARVAE

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Introduction

Intensive culture of marine fish is, for many species, restricted and unpredictable because of mass mortalities, suboptimal growth, and poor disease resistance of larvae. These problems are often caused by opportunistic bacteria that benefit from the enriched cultivation conditions and proliferate in the water, live food, or gut of larvae. Marine fish larvae may become rapidly colonized by such bacteria, and improved technology for microbial management is needed. Nonspecific immunostimulation is expected to become important in a strategy to obtain better control of the microbial conditions in intensive culture of marine fish larvae (Vadstein, 1997; Skjermo and Vadstein, 1999).

Several compounds can be used for nonspecific immunostimulation. Alginates containing high percentages of mannuronic acid polymers (high-M-alginates) have been documented to be nonspecific immunostimulants, through stimulation of the cytokine production by human monocytes (Otterlei et al., 1991), and by increasing the superoxide production in macrophages from Atlantic salmon (*Salmo salar*) *in vitro* (Rokstad et al., 1996). *In vivo* immunostimulation of halibut (*Hippoglossus hippoglossus* L.) larvae and juvenile turbot (*Scophthalmus maximus* L.) with high-M-alginate have also shown positive effects (Vadstein et al., 1993; Skjermo et al., 1995).

This paper discusses how nonspecific immunostimulation can be used as a counteraction to the microbial problems in the earliest stages of intensive culture of marine larvae. *In vitro* and *in vivo* studies of immunostimulation of turbot macrophages have been performed, as well as *in vivo* experiments with larval

turbot. High-M-alginates, bacterial lipopolysaccharides (LPS), and β-1,3-glucan were used as immunostimulants.

Stimulation of fish macrophages

Because of the size of marine larvae, macrophages for immunological studies have to be isolated from kidneys of adult fish. High-M-alginates were used as immunostimulants, comparative to LPS in *in vitro* and *in vivo* studies with turbot macrophages. Responses of increasing doses on the macrophages *in vitro* indicate that high-M-alginates stimulate turbot macrophages to increase production of superoxide in NBT-assay and enhanced phagocytosis efficiency, whereas only weak effects on production of acid phosphatase are observed. Comparable to human immunology, factors in the serum participate in the nonspecific stimulation of turbot macrophages. No responses were measured after *in vivo* immunostimulation by intravenous injection of high-M-alginates, probably due to suboptimal exposure times or doses.

High-M-alginates are shown to be milder immunostimulants than bacterial LPS, and toxic effects on the fish cells have not been observed in the assays, even with the highest concentrations of the alginates.

Stimulation of fish larvae

Because marine fish larvae hatch without a definite immune system, nonspecific immunostimulation should be performed early, either by introduction of the stimulants directly into the rearing water or by bioencapsulation and delivery via live food organisms. Techniques are adjusted for delivery by rotifers and *Artemia*, depending on size and feed preference of the fish larvae.

In experiments with turbot larvae, rotifers (*Brachionus plicatilis*) have been used as vectors for oral administration of immunostimulants. Introduction of high-M-alginates as one first meal induced up to 38% increase in survival of 12-day-old larvae, and up to 55% increased survival in challenge tests run at the same age. The stimulation also improves growth (up to 24% increase measured) and food uptake. Administration of the high-M-alginate directly to the water is shown to be less effective for stimulation of turbot larvae, but for larvae with a long yolk sac phase, such administration can be suitable.

A water-soluble β-1,3-glucan, added directly to the rearing water, has also been tested in first feeding of turbot. The treatment resulted in enhanced bacterial growth in the rearing water and in the gut of larvae, and decreased growth and survival, and was thereby not regarded as a suitable immunostimulant for the earliest stages of turbot larvae.

Conclusions

Nonspecific immunostimulation can be used to enhance larval resistance in the earliest stages in intensive culture. Positive effects on larvae are obtained.

High-M-alginates stimulate the non-specific immunity of marine fish, do not induce bacterial growth or have toxic effects on fish, and are suitable as immunostimulants for marine fish larvae.

Immunostimulation can be performed in rearing water (yolk-sac stage) or by bioencapsulation and delivery via live food organisms at first feeding.

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References

Otterlei M., K. Østgård, G. Skjåk-Bræk, O. Smidsrød, P. Soon-Shion and T. Espevik. 1991. Induction of cytokine production from human monocytes stimulated with alginate. J. Immunotherapy 10:286-291.

Rokstad A.M., T. Espevik, G. Skjåk-Bræk, and O.Vadstein. 1996. Polymannuronic acid (polyM) and lipopolysaccharide (LPS) induced superoxide anion production in Atlantic salmon (*Salmo salar* L.) macrophages and the dependence of serum."International symposium on Fish Vaccinology", 5-7 June 1996, Oslo.

Skjermo J. and O. Vadstein. 1999. Techniques for microbial control in intensive larval rearing. Aquaculture 177:333-343.

Skjermo J., T. Defoort, M. Dehasque, T. Espevik, Y. Olsen, G. Skjåk-Bræk, P. Sorgeloos, and O. Vadstein. 1995. Immunostimulation of juvenile turbot (*Scophthalmus maximus* L.) using an alginate with high mannuronic acid content administered via the live food organism *Artemia*. Fish Shellfish Immunol. 5:531-534.

Vadstein O. 1997. Application of immunostimulation in marine larviculture: Possibilities and challenges. Aquaculture 155:401-417.

Vadstein O., G. Øie, Y. Olsen, I. Salvesen, J. Skjermo, and G. Skjåk-Bræk. 1993. A strategy to obtain microbial control during larval development of marine fish. p.69-75. In: Proceedings of the First International Conference on Fish Farming Technology. H. Reinertsen, L. A. Dahle, L. Jørgensen, and K. Tvinnereim (Eds.) Rotterdam: A. A. Balkema.

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EFFECTS OF SIZE VARIATION AND PREY DENSITY ON CANNIBALISM IN JUVENILE ATLANTIC COD (*GADUS MORHUA*)

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Introduction

Cannibalism has been reported in an increasing number of fish species, many of which are important for aquaculture. Cannibalism in Atlantic cod (*Gadus morhua*) has been demonstrated to have a significant impact on juvenile production (Folkvord, 1991). Cannibalism is particularly problematic during metamorphosis, when size differences become apparent. Grading is often postponed during metamorphosis because it can cause severe losses due to mortality. Previously, it has been found that both size variation and stocking density influence the rate and extent of cannibalism (Folkvord and Ottera, 1993; Hecht and Pienaar, 1993). We will set up experiments to expand on previous studies examining the effects of size variation and stocking density on the incidence of cannibalism in juvenile Atlantic cod. Cannibalistic behaviour will be examined, focusing on the modes of larval prey capture and ingestion. Protocols for mitigating cannibalism by the adjustment of favorable conditions will be presented.

Materials and methods

Larval cod will be obtained from broodstock at the Ocean Science Centre, St. John's, Newfoundland, Canada. During the pre-experimental period, larvae will be reared in 280-l conical tanks until the largest fish are approximately 30mm standard length. Fish will be hand-graded for transfer to experimental containers according to size, where larger fish will be used as cannibals and smaller fish will be used as conspecific prey.

In the first experiment, we will examine the effects of size variation on cannibalism. Each treatment will pair two fish with different standard lengths in a 3-l plastic

bucket to examine the occurrence of cannibalism at five cannibal:conspecific prey length ratios (1.6:1, 1.4:1, 1.3:1, 1.2:1, and 1:1). These cannibal:conspecific prey length ratios are above and below the theoretical minimum ratio proposed by Ottera and Folkvord (1993). Cannibal standard length will range from 10-30mm and conspecific prey standard length will range from 6-30mm. Standard length, height, and body depth will be measured by videotaping individual cannibals and conspecific prey in a container with 1-cm water depth and a measured grid on the bottom. The experimental buckets will be kept under conditions of continuous light, and temperature will be maintained at 8ºC, with water flowing through from a wet bench. Cannibals will be transferred to buckets 24h prior to the introduction of conspecific prey. The introduction of conspecific prey to the buckets will be videotaped for 5min in order to obtain observational data on cannibalistic behaviour. The buckets will be checked every hour for 24h to determine the time of cannibalism. Uningested dead conspecific prey will be examined under a microscope for signs of attack by the cannibal. Cannibal mouth gape will be measured after the experiment

The second experiment will examine the effects of density on cannibalism in graded juveniles. Three groups will be established using a grid grading apparatus: small cod (width < 3mm), large cod (width > 3mm), and medium cod (4mm > width > 3mm). Each treatment group will be replicated at low density (0.75 fish.l^{-1}), medium density (1.5 fish.l^{-1}) and high density (3 fish.l^{-1}). The fish will be measured by the method stated in the above experiment. Experimental tanks will be 38-l glass aquaria, maintained at 10ºC under a continuous light regime. Fish will be fed to satiation four times a day at approximately two-hour intervals during the period from 09.00 to 16.00h. Dead fish will be removed from the tanks, counted, and measured daily. Dead fish will be classified as having died due to cannibalism if they show visible signs of injury. Cannibalism will be assessed from the difference between the initial number of fish and the survivors plus the removed dead fish. The experiment will be terminated when approximately 50% of the cod have been ingested. All fish will be individually measured at the end of the experiment.

Results and discussion

Size variation will probably have a pronounced effect on cannibalism. Cannibalism is expected to increase as cannibal:conspecific prey length ratio increases, possibly occurring at a lower cannibal:conspecific prey length ratio for cannibals around 20mm. These results would support what other studies have suggested – that allometric changes in mouth gape:body height with length make cod juveniles around 20mm susceptible to cannibalism (Ottera and Folkvord, 1993). It is

important that size variation be kept at a minimum in order to reduce losses during production of cod juveniles. Strict size grading has been shown to reduce cannibalism (Folkvord and Ottera 1993). Less size variation, and therefore less cannibalism, is expected in the graded medium cod since there is an upper limit (4mm) and a lower limit (3mm) on fish width. Cannibalism appears to be positively correlated with density in many species (Hecht and Pienaar 1993). Less cannibalism is expected in the low-density (0.75 fish.l^{-1}) treatment.

References

Folkvord A. 1991. Growth, survival and cannibalism of cod juveniles (*Gadus morhua*): effects of feed type, starvation and fish size. Aquaculture 97:41-59.

Folkvord A. and H. Ottera. 1993. Effects of initial size distribution, day length, and feeding frequency on growth, survival, and cannibalism in juvenile Atlantic cod (*Gadus morhua* L.). Aquaculture 114:243-260.

Hecht T. and A. G. Pienaar. 1993. A review of cannibalism and its implications in fish larviculture. J. World Aquacult. Soc. 24:246-261.

Ottera H. and A. Folkvord. 1993. Allometric growth in juvenile cod (*Gadus morhua* L.) and possible effects on cannibalism. J. Fish Biol.43:643-645.

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DEVELOPMENT OF LARVICULTURE TECHNIQUES FOR THE ENHANCED PRODUCTION OF GROUPER *EPINEPHELUS COIOIDES*

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Introduction

Since 1985, larval grouper rearing in the laboratory has been established in Taiwan (Lin et al., 1986), and commercial seed production was over ten millions fry in 1995. However, the survival rate was less than 1% and production fluctuated due to viral nervous necrosis disease (VNN) and iridovirus-like infection (Chao and Pang, 1997; Chi et al., 1997). An almost standardized rearing procedure of grouper *Epinephelus coioides* has been developed after many improvements, and a constant production of fry without viral symptom was achieved. This report describes the developed techniques for larviculture of grouper performed in Tungkang Marine Laboratory, TFRI, Taiwan.

Materials and methods

Hygienic, nutritional, and zootechnical studies were applied to improve the larviculture of grouper *E. coioides*. Eggs spawned naturally from pond-reared broodstock from the commercial hatchery were brought into the laboratory in the morning. There were three methods used to treat eggs. First, eggs were soaked in 0.3ppm O_3-treated seawater (50g eggs, 10.l^{-1}) for 10min ($O_3$10min, Table I). Second, eggs were washed continuously with sand-filtered seawater (9 l.min^{-1}, 100g, 300.l^{-1}) for 60min (SFW1h). Third, eggs were soaked in 0.3ppm O_3-treated seawater for 1min ($O_3$1mW). Two methods were used in hatching the eggs. In one method, the treated eggs were hatched directly in larval rearing tanks (3 tons volume with 2 tons water) ($O_3$10min, SFW1h). The other involved hatching the eggs in a flowing through tank (150g, 180.l^{-1}), using O_3C water with flow rate of 6 l.min^{-1} initially and then 2 l.min^{-1} after the eggs have just hatched ($O_3$1minW). After all eggs had hatched, larvae were brought into the larval rearing tank. The O_3C water was 0.3ppm O_3-treated seawater with total residue oxidant (TRO) removed by active carbon. Rearing water disinfected with sodium hypochlorite and neutralized by sodium thiosulphate (OCL) was used in 1998-9, while O_3C

water was used in 2000. Rotifers were cultured with *Tetraselmis* and enriched with DHA Protein Selco (1998-9) or *Isochrysis* (2000). Fertilized oyster eggs were applied from d3 (3rd day after hatching) to d5 (1998-9), or replaced by super small rotifers at d3-d9 (2000). Small rotifers were applied from d3 (1998-9) or d6 (2000) to d25. *Isochrysis* was added daily from d3-d25. *Artemia* nauplii fed from d15 and those enriched with commercial product (DHA Selco-1998, DC Selco-1999, DC DHA Selco-2000) or copepod were fed from d18. The commercial diet (Inve NRD) was applied from d7, d19, or d25. From d8 onwards, debris, feces, and dead fish were siphoned off the tank bottom, causing 32%-220% water volume exchange. Tanks were mildly aerated (1.0-2.2 $l.min^{-1}$). Dissolved oxygen, pH, NO_2-N, and NH_4-N were measured daily or twice a week. Viral infection was checked by PCR (Chi et al., 1997).

Table I. Survival and biomass of grouper fry at harvest during 1998-2000.

Year	Egg treatment	Rearing water	Fry[a] density at harvest (l^{-1})	Survival rate (%)	Harvest day	Total length (cm)
1998	$O_3$10min	OCL	2.10	5.60	D36	1.42±0.47
1998	$O_3$10min	OCL	2.65	6.80	D36	1.59±0.42
1998	SFW1h	OCL	1.35	3.91	D36	1.64±0.41
1998	SFW1h	OCL	1.80	5.22	D36	1.53±0.36
1999	$O_3$10min	OCL	1.36	3.39	D30[b]	1.83±0.31
1999	$O_3$10min	OCL	1.98	4.94	D37	1.92±0.37
1999	$O_3$10min	OCL	1.62	4.05	D37	1.85±0.36
2000	$O_3$1minW	O_3C	3.06	9.56	D35	1.34±0.25
2000	$O_3$1minW	O_3C	2.92	12.72	D35	1.28±0.20
2000	$O_3$1minW	O_3C	2.73	13.49	D35	1.37±0.20
2000	$O_3$1minW	O_3C	0[c]	0	D18	

[a]Water volume is 2000 l; [b]Copepod was fed; [c]*Artemia* nauplii fed at D13

Results and discussion

The fry production was constant and almost uniform in all tanks (Table I). Fry density at harvest ranged from 1.35-3.23.l^{-1}, with survival rates from 3.91-13.49%. Better survival was obtained when eggs were hatched in the tank with flowing water than that in stagnant water with aeration. The maximum capacity for fry at harvest in the 2-ton-volume tank was nearly 6000, no matter the density incubated. Higher survival was due to lower larval density at the start. Fry size produced in 1999 was bigger due to the larger broodstock. With the same broodstock, fry fed with copepods had the same size earlier (d30) than fed with *Artemia* (d37). Fry fed with *Artemia* nauplii too early (d13; TL 4.8mm) all died at d18. The super small rotifer enriched with *Isochrysis* and used as the first feed for grouper larvae (2000) had the same good performance as using the oyster trochophore (1998-9). *Isochrysis*, having a high DHA content, supporting satisfactory nutritional levels for larviculture of grouper (Su et al., 1997a) and

being better than *Nannochloropsis*, was confirmed in this three-year study. Commercial diet (Inve NRD) could be fed at d7, but better results were shown when fed after d25 due to difficulties in maintaining feed in suspension early in the feeding period. Water quality parameters were as follows: temperature 25.5-28.8°C, salinity 24-33ppt, pH 7.70-8.46, NO_2-N 0.01-0.48ppm, and NH_4-N 0.01-2.11ppm. The established techniques for grouper larviculture in this study include egg incubation, rearing water treatment, feed regime, and zootechnique. The strict hatchery hygiene control measures conducted in this study were effective in restricting the spread of viral disease as that which has occurred in seabass in the Mediterranean (Dhert et al., 2000). To enhance fry production of grouper, elimination of floating mortality at the first feeding period (d3-d6) and cannibalism at the metamorphosis stage (after d30) are necessary, and fine tuning of some physical factors like aeration, water current, and light are ongoing.

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References

Chao C.B, and V.F. Pang. 1997. An outbreak of an iridovirus-like infection in cultured grouper (*Epinephelus* spp.) in Taiwan. J. Chin. Soc. Vet. Sci. 23:411-422 (in Chinese).

Chi S.C., C.F. Lo, G.H. Kou, P.S. Chang, S.E. Peng, and S.N. Chen. 1997. Mass mortalities associated with viral nervous necrosis (VNN) disease in two species of hatchery-reared grouper, *Epinephelus fuscogutatus* and *Epinephelus akaara* (Temminck & Schlegel). J. Fish Dis. 20:185-193.

Dhert P., M. Rimmer, M. Harel, and P. Sorgeloos. 2000. Progress in live food production and use in commercial seabass hatcheries. EAS Spec. Publ. No.28, p.181.

Lin K.J., C.L. Yen, T.S. Huang, C.Y. Liu, and C.L. Chen. 1986. Experiment of fry nursing of *Epinephelus salmonoides* and its morphological study. Bull. Taiwan Fish. Res. Inst. 40:219-240 (in Chinese).

Su H.M., M.S. Su, and I. C. Liao. 1997. Preliminary results of providing various combinations of live foods to grouper (*Epinephelus coioides*) larvae. Hydrobiologia 358:301-304.

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BIOLOGICAL, TECHNICAL, AND ECONOMICAL FEASIBILITY OF A ROTIFER RECIRCULATION SYSTEM

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Abstract

A feasibility study was performed on the use of a recirculation system for the mass culture of rotifers at an industrial level. Rotifer culture systems were operated at three different stocking densities (3000, 5000, and 7000 individuals.ml^{-1}) in a completely closed recirculation system. At all operating rotifer densities, a reliable production of 2.2 billion rotifers could be obtained on a daily basis during three weeks. Excellent water quality was maintained by the use of protein skimmers, ozone, and a submerged biofilter. The microbial counts remained stable during the whole culture period (10^6CFU.ml^{-1} on marine agar and 10^4CFU.ml^{-1} on TCBS after 15 days and 23 days, respectively). No difference in HUFA and protein content were obtained between rotifers harvested from the recirculation system or from a conventional batch culture system. Compared to a commercial batch culture system, the use of a recirculation system can contribute to a 30% saving on the capital investment and the annual operation cost. By using this system, capital investment cost is reduced considerably by 31%. Savings are also made on labour (65%) and feed costs (10%) during a one-year production.

In general terms, it can be stated that by using a simple recirculation system, a cost-effective technology and a reliable rotifer culture can be obtained.

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NATURAL SPAWNING AND MASS LARVICULTURE OF MOUSE GROUPER, *CROMILEPTES ALTIVELIS*, IN CAPTIVITY IN INDONESIA

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Abstract

This is the first report on the success of natural spawning and larval rearing of mouse grouper, *Cromileptes altivelis*, in captivity. Wild-caught broodstock (5 male and 25 female) were naturally spawned in captivity at 26.5-31.0°C in a flow-through system where seawater was exchanged at a rate of 300-400% daily. Spontaneous spawning occurred successively 4-7 times a month throughout the year. Spawning mostly occurred during the new moon phase, and the fish usually spawned at 22h00 and midnight. The hatched larvae were reared in 10-m^3 rectangular cement tanks with initial larval density of 5-10.l^{-1}. The larvae were fed initially on SS-type rotifer, followed by S-type rotifer, and then weaned from *Artemia* nauplii onto artificial diets. Larviculture trials to 50d produced juveniles of 24.1-24.8mm mean total length, at a survival rate ranging from 11.0-53.9% (25.13% average). Spreading squid oil on the water surface minimized floating death of the larvae at initial stage. Cannibalism was not the main factor of mortality in this species. The success of larval rearing mostly depends on the control of Viral Nervous Necrosis infection. The results indicate that the present technique could be used for mass seed production of mouse grouper.

Introduction

Cromileptes altivelis, also known as mouse grouper, humpback grouper, high-finned grouper, and barramundi cod, is a protogynous marine hermaphrodite, and an important coral reef fish in the Indo-Pacific region. Among live fish trade in Hong Kong, this fish commands the highest commercial value, with a price of US$91.4±12.2.$kg^{-1}$ (Lau and Jones, 1999). Smaller (5-10cm total body length) fish are used for aquarium fish. Due to its high commercial value, the fish is considered a desirable species for aquaculture, especially in Indonesia. The juveniles used for culturing are usually collected from local coastal waters, but the supply of juveniles is not sufficient, and this is becoming a major limiting factor in

the development of *C. altivelis* culture. No success has been reported so far in rearing of the larvae. The present study reports the results of experiments on natural spawning of *C. altivelis* in captivity during 2000-2001, as well as larval rearing systems.

Materials and methods

C. altivelis broodstock were collected from the wild using a trap net in the coral reef areas of Eastern Indonesia. Thirty fish (25 females and 5 males) used for spawning studies were stocked into a circular concrete tank (100-m^3 capacity, 2-m water depth). The females weighed 1.48-2.32kg and males weighed 2.93-3.38kg. The spawning tank was equipped with a drain water pipe, water inlet and outlet (over-flow) pipes, an egg collection tank with a fine net (400μm) that was connected with the outlet water pipe, and an aeration system. The system was flow-through, achieving 300-400% water exchange daily. The broodstock were fed fresh and frozen fish (mainly *Sardinnella* sp.) and squid that was mixed with 1% vitamin mix and fed 4 times a week at 2-3% of their total body weight (BW) at each feeding.

Only floating eggs were used for larval rearing. The rearing tank shape is rectangular, rounded in each corner, made of concrete cement, and has a 10-m^3 capacity. The tanks were roofed and each tank was covered with transparent plastic sheet. Initial stocking densities were 5-10 fertilized eggs.l^{-1}. The larval rearing protocols are summarized in Fig.1

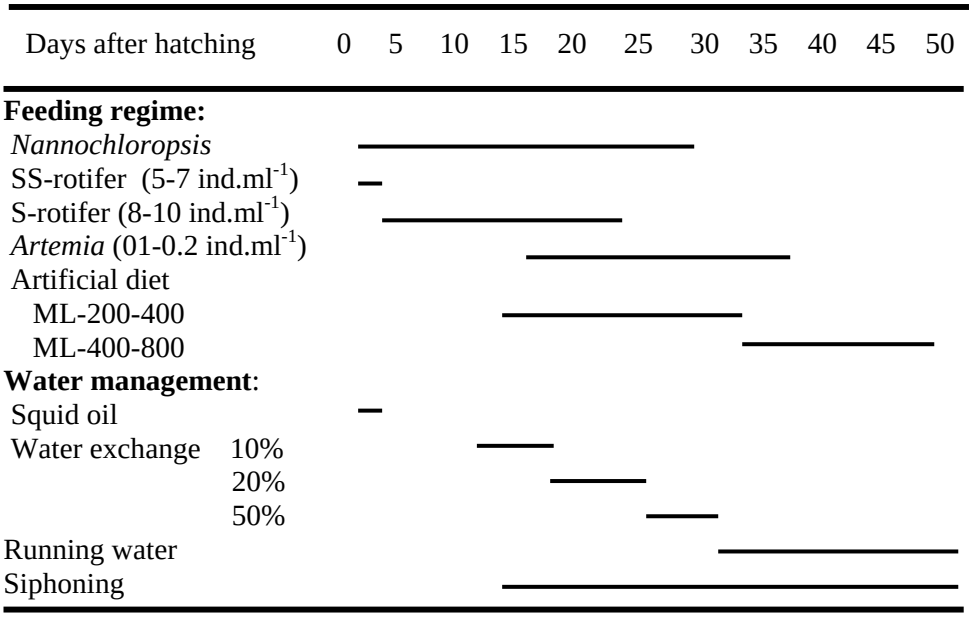

Fig. 1. Tank management procedures for the larviculture of mouse grouper, *Cromileptes altivelis*.

Results and discussion

The broodstock started to spawn naturally 4 months after stocking, and spawning occurred every month usually within 7-10 days before and after the new moon phase. Normally spawning occurred successively 4-5 times a month and took place between 22h00 and midnight. The salinity and temperature of the water in the spawning tank was 34-35ppt and 26.6-31.0°C, respectively. The total number of eggs spawned in 2000 ranged between 4.2-8.0 million, and only floating eggs (fertilized) were used for larval rearing. The diameter of fertilized eggs ranged from 812-889μm, and at a water temperature of 28-29°C, the eggs hatched after 18-20h of incubation. The newly hatched larvae measured 1.52±0.002mm total length. The newly hatched larvae stocked in rearing tanks were checked for Viral Nervous Necrosis (VNN) using Polymerase Chain Reaction technique and only negative VNN lots of larvae were continue to rear.

In a 1999 experiment (data not shown), 40-60% mortality of the larvae occurred during the initial 1-5d after hatching. Larval mortality was mainly caused by floating death, as the newly hatched larvae were easily trapped at the water surface by surface tension (Sugama et al., 2001). As well, the trapped larvae would be stressed and produce mucus which would accelerate the trapping of other larvae. In the 2000 experiment, the squid oil seemed to reduce such mortalities.
Beginning on d10, larvae have an elongated dorsal and pelvic fin spines, which often entangle larvae, especially when they swim to a common place in the tank wall near the water surface, probably in response to the light condition. Here, they severely aggregate and clump together – which may be accelerated by the mucus – and eventually die. Consequently, a high mortality (20-40%) frequently occurred between d10-25. To prevent larval aggregation, four fluorescent tube lamps (40 watt each) were hung above the larval rearing tank with light intensity of 1000 lux. The light intensity was adjusted as even as possible on the water surface. The color of larval rearing water was maintained green by inoculation of *Nannochloropsis* at density of 300×10^3 cell.ml^{-1}. This condition might reduce larval aggregation.

Gradual larval mortality was usually observed after d25, which was suspected due to nutritional deficiency. To prevent this problem, early weaning of larvae onto artificial diets that have sufficient nutritional value was done. In 2000-2001 experiments, artificial diets were introduced at d15 prior to feeding *Artemia*, and this minimized the demand of *Artemia* as food (Fig.1). The remaining *Artemia* in the larval rearing water should not be kept for more than one day. With this feeding management, mortality could be reduced and resulted in absence of lordosis. Based on our observation, cannibalism was not the main factor of mortality in this fish.

The success of larval rearing for this fish mostly depended on the control of VNN. Once VNN broke out during larval rearing, all larvae died within a few days. No special treatment method for VNN is presently available. In our experience, VNN diseases mostly occurred between d15-30. To avoid VNN infection, use only VNN-free broodstock, examined by checking oocytes and sperm by PCR. The VNN check was also carried out for the newly hatched larvae. Only VNN-free larvae were continued to rear. An effort was made to reduce larval stress by decreasing the stocking density, followed by maintaining water colour and feeding the larvae diets containing sufficient nutritional value, such as early weaning onto artificial diets.

Table I summarizes the results of the larviculture trials conducted from January 2000 to February 2001. The survival rates ranged from 11.0-53.9% (25.13% mean) and larvae reached a total length of 24.1-24.7mm after 50d of rearing.

Table I. The results of the rearing trials of larval *Cromileptes altivelis* for 50d using 10-m3 capacity of tanks. SE = standard error.

Trial number	Number of eggs stocked	Stocking density (egg.l^{-1})	Number of surviving juveniles	Survival (%)	Average total length (mm)
1	100 000	10.0	18 250	18.25	24.5
2	100 000	10.0	21 080	21.08	24.3
3	100 000	10.0	30 010	30.01	24.6
4	50 000	5.0	9 500	19.00	24.7
5	50 000	5.0	5 500	11.00	24.6
6	100 000	10.0	25 050	25.05	24.4
7	50 000	5.0	9 000	18.00	24.4
8	100 000	10.0	19 750	19.50	24.7
9	54 000	5.4	29 100	53.90	24.7
10	70 000	7.0	25 020	35.74	24.1
Mean		6.8		25.15	24.5
SE		3.1		12.24	0.2

References

Lau P.F.P. and R.P. Jones, 1999. The Hong Kong trade in live fish for food. TRAFFIC East Asia. WWF for Nature. Hong Kong. 65p.

Sugama K., Trijoko, B. Slamet, S. Ismi, E. Setiadi, and S. Kawahara 2000. Manual for seed production of *Cromileptes altivelis*. JICA ATA 379. Central Research Institute for Fisheries of Indonesia. Dept. of Marine Affairs and Fisheries of Indonesia. 37p.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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FEEDING STRATEGY OF DECAPSULATED *ARTEMIA* CYSTS AS FOOD SOURCE FOR COMMON CARP (*CYPRINUS CARPIO* L.) LARVAE

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Introduction

Decapsulated *Artemia* cysts can be handled as an inert diet, do not leach, and have a chemical composition comparable to freshly hatched nauplii. The aim of this experiment was to study its food value and feeding strategy for common carp (*Cyprinus carpio* L.) larvae.

Materials and methods

Carp larvae were cultured in 25-l PVC tanks within a re-circulation system. Temperature was 23-25°C and initial stock density was 40 larvae.l^{-1}. Feeding was done manually 6 times per day at 2-h intervals. The experiments were designed with different *Artemia* nauplii feeding periods (0, 1, 2, 3d) and different feeding levels. Feeding level was calculated by:

$$R_f = \Delta y \times \frac{DM_f}{DM_a} \times FCR$$

where R_t = food requirement per fish on day t (mg of wet weight)
DM_f = dry matter content of fish
DM_a = dry matter content of diet
FCR = estimated diet conversion rate ($2g.g^{-1}$)

The growth of fish [$\Delta y = y_{(t+1)} - y_t$] was estimated by the linear growth equation [$y_t^{1/3} = y_o^{1/3} + gt$]. Feeding levels were chosen according to growth rates (g), predicted by theoretical g=0.2, 0.3, 0.4, and 0.6 in different treatments. The growth of fish was measured by sampling at three-day intervals.

Results and discussion

The effects of live food feeding period at g=0.3 and g=0.4 are shown in Fig. 1.

One-way ANOVA showed there was no significant difference among 0, 1, 2, and 3 days feeding live food at g=0.3 and g=0.4 (P<0.05). This indicates that decapsulated cysts can be successfully used as a start food for carp larvae, moreover *Artemia* nauplii feeding at the start of exogenous feeding does not significantly benefit carp larvae growth (P<0.05).

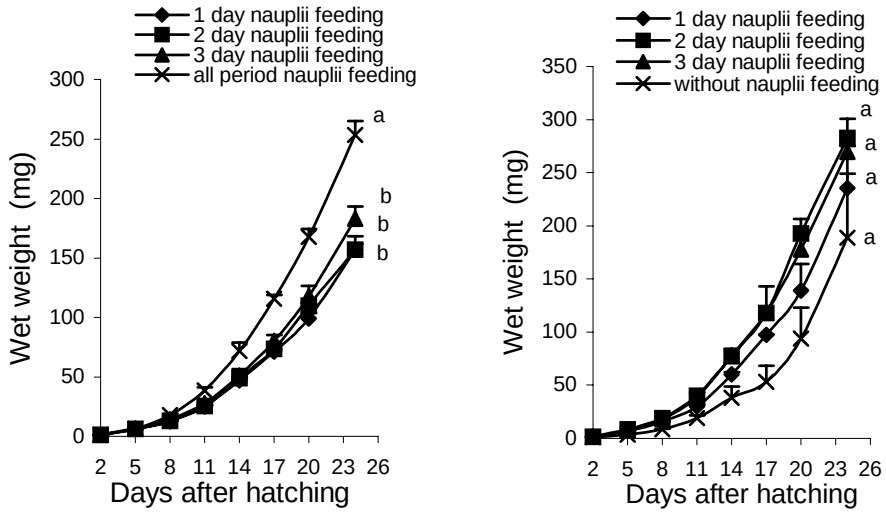

Fig. 1. Growth of *Cyprinus carpio* in wet weight during 24-day rearing at feeding level g=0.3(left) and g=0.4(right). Means at day 24 with different letter are significantly different (P<0.05).

Growth of fish with two days of *Artemia* nauplii feeding at different feeding levels is shown in Fig. 2. One-way ANOVA showed there is significant difference among different feeding levels (g=0.2, 0.3, 0.4, and 0.6). The higher the feeding level, the better the fish growth obtained.

The actual growth rate (g*), food conversion ratio (FCR), and average specific growth rate (SGR) in dry weight of fish is presented in Table I. Fish fed with decapsulated cysts at a higher feeding level had higher SGR. g* was lower than theoretical g . The higher g was, the more difference between g and g* was obtained. This indicates that actual growth rate (g*) followed a smaller amount to the higher expected feeding levels at higher g, which is also expressed in a higher FCR.

Since larval fish dry matter content changes considerably and its specific growth rate decreases continuously during a very short period (in a few days, larval weight increases twenty to fiftyfold), daily feed ration should be adjusted accordingly. The linear equation is based on the assumption that growth of larval fish can be linearized over the entire larval culture period, which actually has been

successfully used by Verreth and Den Bieman (1987) for African catfish (*Clarias gariepinus*). In the present study, the linear regression analysis shows high linear relation (R^2>0.99) between weight gain and rearing period (P<0.05). It concludes that the linear equation [$y_t^{1/3} = y_0^{1/3} + gt$] can be successfully used to express the growth of carp larvae.

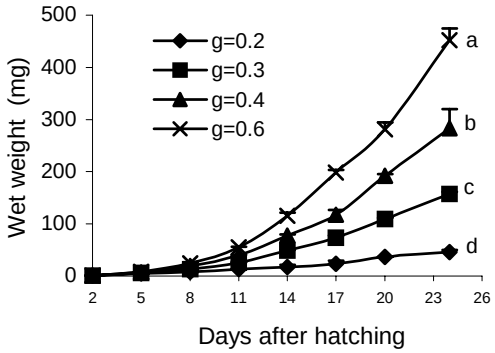

Fig. 2. Growth of *Cyprinus carpio* in wet weight during 24-day rearing (with two-day *Artemia* nauplii feeding). Means with different letters are significantly different (P<0.05).

Table I. Actual growth rate (g*), FCR, SGR in dry weight of *Cyprinus carpio*.

Nauplii feeding day	g	g*[1]	Actual FCR[2]	Average SGR (% dry weight.day^{-1})		
				Day 2-8	Day 2-14	Day 2-24
24	0.3	0.25	2.25±0.10	40.95	33.09	24.46
2	0.2	0.12	4.31±0.51	36.53	29.95	22.20
1	0.3	0.20	3.04±0.22	36.41	29.90	22.18
2	0.3	0.20	3.03±0.11	36.67	30.18	22.25
3	0.3	0.21	2.72±0.15	37.91	30.59	22.93
1	0.4	0.23	3.34±0.59	39.57	31.97	24.14
2	0.4	0.26	3.20±0.40	43.46	34.16	25.03
3	0.4	0.25	3.29±0.44	41.72	34.13	24.71
2	0.6	0.31	4.21±0.25	47.53	37.76	27.23

[1] g* was obtained by linear regression analysis

[2] FCR = dry weight of diet per dry weight gain of fish

According to Vanhaeche et al (1990), the quantity of cysts needed per carp larva, using live nauplii and decapsulated cysts, can be expressed as:

2.25mg of GSL cysts material = 1mg DW of nauplii biomass
1.47mg of GSL cysts material = 1mg DW of decapsulated cysts

It has been known that the similar growth were obtained for the fish fed with *Artemia* nauplii at feeding level g = 0.3 and with decapsulated cysts at feeding level g = 0.4 at day 24. The evaluation of cysts requirement are shown in Fig. 3.

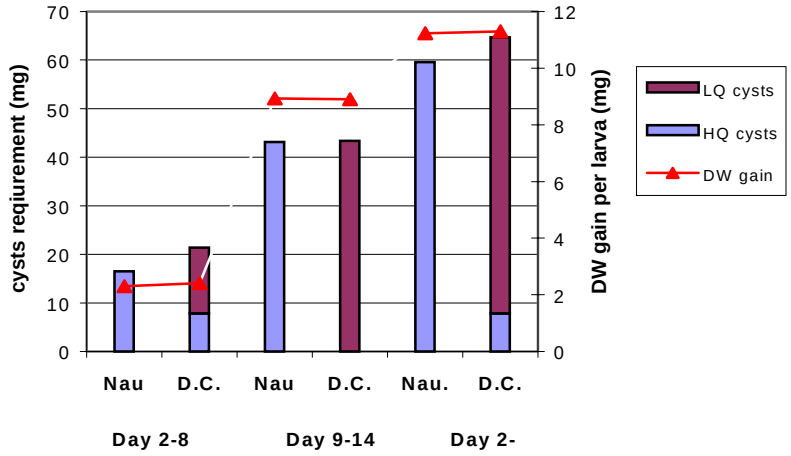

Fig. 3. Cysts requirement (high and low quality) and dry weight gain during *Cyprinus carpio* larvae rearing.

Taking into account the large price difference between high quality cysts (from which nauplii biomass comes) and the low quality cysts (from which decapsulated cysts come), we can conclude that the carp larvae can be more cheaply reared using non- or low- hatching decapsulated cysts. Moreover, the labor and time saved in decapsulation and drying of the cysts is largely rewarded, because daily incubation, separation, harvest, and storage of nauplii during feeding becomes unnecessary. All above advantages further confirm the marketing opportunity of large stock, low quality *Artemia* cysts in USSR, USA, and China.

References

Vanhaecke P., L. De Vrieze, W. Tackaert, and P. Sorgeloos. 1990. The use of decapsulated cysts of the brine shrimp *Artemia* as direct food for carp (*Cyprinus carpio* L.) larvae. J. World Aquacult. Soc. 21(4):257-262.

Verreth J.A.J. and H. Den Bieman. 1987. Quantitative feed requirements of African catfish (*Clarias gariepinus* Burchell) larvae fed with decapsulated cysts of *Artemia* I. The effect of temperature and feeding level. Aquaculture 63:251-267.

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GROWTH PERFORMANCE OF THE WRECKFISH (*POLYPRION AMERICANUS*) IN CAPTIVITY

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Introduction

The wreckfish has a very wide geographic distribution, living on both sides of the Atlantic Ocean and in the Mediterranean, but also in the Indian and the Pacific oceans (Sedberry et al., 1999). Wreckfish are large fish (maximum length 2m, maximum weight recorded 100kg). Juveniles are pelagic until 30-60cm and are often associated with floating objects. Adults are caught at depths ranging from 120-800m (Vinnichenko, 1997). Annual landings are close to 2 000 tons (FAO, 1996; G.R. Sedberry, pers. comm.). On account of its rapid growth (Kentouri et al., 1995), the great quality of its flesh, and its high wholesale price (France 12 Euro.kg^{-1} in 1999), wreckfish is a good candidate for finfish aquaculture. However, rearing performances of wreckfish have not been reported in the literature. The purpose of this work was to test the growth performances of juveniles and adults wreckfish in captivity. Furthermore, reproductive features of adults were followed during an annual cycle.

Material and methods

Young wreckfish (<5kg) were caught during tuna fishing using drift nets (fishing zone: 46°N-49°N). Larger specimens were fished using demersal longlines. Air pressure damage (decompression) was reduced by pricking the swimbladder. Because the survival of these specimens was low, large wreckfish were also obtained from public aquaria.

Twelve young wreckfish (mean initial weight ± SD: 3.8±0.7kg) were reared in a 15-m^3 cylindrical tank and submitted to natural variations of photoperiod (8L:16D/16L:8D) and temperature (8.9-17.7°C). Water exchange was 10% per

hour. Wreckfish were daily fed *ad libitum* on frozen-thawed moist pellets composed of 48% crushed commercial turbot pellets and 48% fresh mackerel. A vitamins complex (1%) and alginate (1%) were also added. Dry weight and protein content of pellets were obtained after desiccation (105°C, 24h) and the use of the Dumas method (analyzer NA 2000, Fison Instrument), respectively. The food intake was recorded daily. From February 2000 to March 2001, fish were weighed and individually measured monthly.

Seventeen larger wreckfish (11.7±3.7kg) were reared in one 40-m^3 outdoor tank. Rearing and sampling conditions were the same as those described in the previous paragraph. In addition, the stock was fed *ad libitum* two times a week on fresh trash fish (sardines and mackerel). From January 1999 to February 2000, oocyte development was monitored by biopsies using an endometric device, with the diameter of the larger 30 oocytes recorded. Then, blood samples were taken and estradiol-17β was assessed by radioimmunoassay.

Results and discussion

In young wreckfish, an annual growth of 42% was recorded (Fig. 1). In the wild, a weight of 1.5kg was recorded 15s month after hatching (Kentouri et al., 1995). Food conversion ratio [total dry weight of distributed food × total fish biomass $gain^{-1}$ = 2.56] was high. On the other hand, daily food intake [100(total dry weight of distributed food per day × mean total fish $biomass^{-1}$) = 0.25], specific growth rate [100(ln final weight – ln initial weight)$days^{-1}$ = 0.09], and protein efficiency ratio [total biomass gain × $protein\ intake^{-1}$ = 0.69] were low. The highest monthly specific growth rate was recorded for the highest water temperatures (from 15.6-17.7°C).

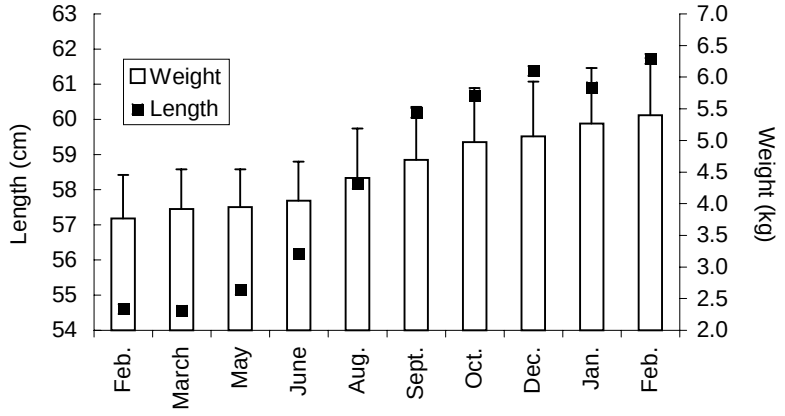

Fig 1. Mean monthly changes in length and weight (±SD) of young wreckfish.

In larger wreckfish, an annual growth of 26% was recorded. Mean annual gains of length and weight were 3±1cm and 2.5±0.8kg, respectively. From August to January, an increase in oocyte diameter was observed in the 3 females successfully biopsied (Fig. 2). From November, dark pellets, possibly vitelline globules, appeared in the cytoplasm of the larger oocytes in two females. An increase in plasma estradiol levels was observed from July to January (Fig. 3). The concomitance of these elements suggests the initiation of vitellogenesis in these females. However, neither eggs nor sperm were collected from the present stock. In the wild, Sedberry et al. (1999) reported that only fish bigger than 85-90cm were mature. Furthermore, mature wreckfish were caught in depths ranging from 450-800m (Vinnichenko, 1997; Sedberry et al. 1999). These observations suggest two hypotheses: (1) specific environmental conditions including hydrostatic pressure are required during the reproductive period of wreckfish, and (2) the present stock has not yet undergone puberty (mean length of these fish: 77.7±5.7cm).

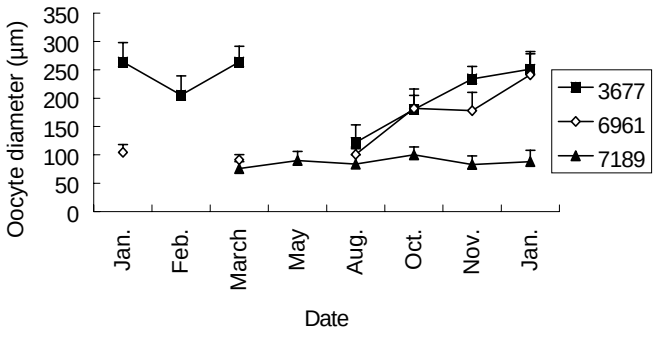

Fig. 2. Mean (±SD) monthly changes in oocyte diameter of large female wreckfish.

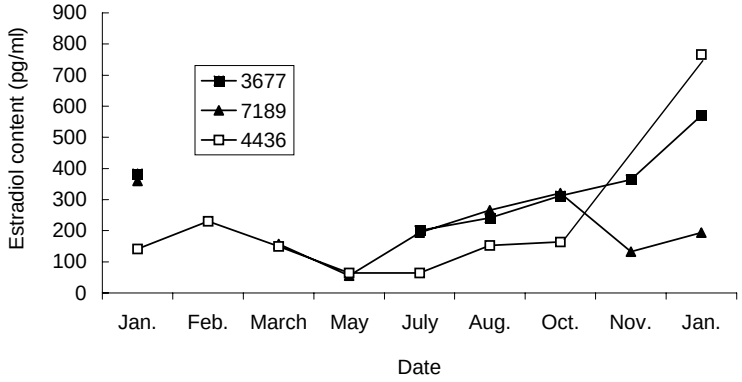

Fig. 3. Mean monthly changes in plasma estradiol-17ß levels of large female wreckfish.

In conclusion, wreckfish juveniles present a high growth rate in captivity. This result supports the selection of wreckfish for rearing. However, the lack of reproduction observed in the captive stock and the high food conversion rate recorded during the growout phase necessitate further research.

Acknowledgements

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References

FAO. 1996. Fishery statistics. Vol.82.

Kentouri M., N. Papandroulakis, and P. Divanach. 1995. Specific diversification in Greek finfish mariculture. Cah. Opt. Méditerr. 14:129-136.

Sedberry G.R., C.A. Andrade, J.L. Carlin, R.W. Chapman, B.E. Luckhurst, C.S. Manooch, G. Menezes, B. Thomsen, and G.F. Ulrich. 1999. Wreckfish, *Polyprion americanus*, in the North Atlantic: fisheries, biology and management of a widely distributed and long-lived fish. Amer. Fish. Soc. Symp. 23:27-50.

Vinnichenko V.I. 1997. Russian investigations and deep water fishery on the corner rising seamount in subaera 6. Sciences Council Studies 30:41-49.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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SOME ASPECTS OF LARVAL IMMUNITY

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It is important to accumulate the knowledge on defense mechanisms of larvae for successful seed production in aquaculture. Here, I try to make a brief overview of larval immunity, particularly of marine fish, which spawn floating eggs.

Defense by immunoglobulin transferred from mother fish to larvae

Larvae of marine teleosts spawning floating eggs are extremely primitive in the function of immunity. They hatch out from eggs within a short time after spawning – e.g., one day or a few days – with a primitive body form. In addition, it takes a long time before they possess functional lymphoid tissues – e.g., several weeks after hatching. This is quite different from salmonid fish, which spend a long period from spawning to hatching and from hatch-out to absorption of yolk. The hatch-out larvae of salmonid, called fries, have already primitive lymphoid organs and antibody producing ability. These suggest the existence of alternative way of defense mechanisms in such marine fish species. Transfer of maternal immunity into larvae was a possible answer to this question, since fish egg contains immunoglobulin (IgM) of maternal origin.

Red sea bream was used for the analyses. The fish spawns small floating eggs, 0.91-1.03mm in diameter, and the larvae hatch out at two days after spawning. Only a limited immune function was found to develop during the larval period for about one month with no lymphocytes in the kidney and the spleen.

IgM was detected in the eggs and hatch out larvae by western blotting analysis. Eggs and hatch out larvae from immunized mother fish with *Vibrio* vaccine had antibody against the *Vibrio.* The titer, however, acutely disappeared within two or three days after hatches, simultaneously with the acute decrease of IgM. The IgM in larvae increased again after 20-30 days after hatch, although the antibody titer never rose again.

These results showed that the transfer of maternal immunity into larva has no

defensive value in the red sea bream and may also in other marine teleost, which spawn floating eggs. They must have other systems to defend themselves from infections.

Defense at the gut surface

Defense mechanisms on the route of infection, such as on the gut and the skin, may have much importance in fish larvae that have less ability of internal immunity. Gut epithelium of larvae is known to take up food protein. The protein might be transported into blood, since protein administered directly into the gut appear as intact and antigenic macromolecule in the blood of adult fish. This suggests that fish larvae can not prevent the invasion of pathogens from the gut.

To clarify the quantitative changes in transport of orally derived protein into blood circulation as macromolecules in developing juvenile eel (*Anguilla japonica*), IgY extracted from hen's egg was administered via oral route to elvers. At the first experiment, conducted before the commencement of artificial feeding, oral delivery of 2.0μg per 0.1g body weights of IgY resulted in rapid increase of plasma IgY to the maximum of 2.03μg.ml^{-1}. However, transport of IgY into blood decreased significantly at the following experiments, which were performed at 12, 25, and 42 days later. In this period, body weight increased approximately eight times larger, and the rapid growth of stomach was observed histologically. The decrease of protein transport into blood seems to be mainly caused by the development of digestibility, not by the disappearance of the absorptive ability of enterocytes. Leptocephalous eel larvae have thus much less ability to prevent invasions of foreign antigenic materials derived from food, for more than a half year.

Defense at the skin surface

Skin surface is another route of infections. Fish skin is covered with mucus, which washes away pathogens attached to the body surface by active secretion. In addition, the skin mucus contains many bioactive substances, which are included in the defense mechanisms. Such substances will be much more important in larvae having extremely underdeveloped internal immunity.

Development of skin lectin was studied in the leptocephalous larvae of the Japanese eel, *A. japonica*; specimens ranging in total length of 11-58mm were captured in the Pacific Ocean. Their lymphoid tissues concerning immune functions showed delayed development excepting the thymus. On the other hand, the skin of them contained extremely active lectin, which agglutinated rabbit red blood cells just like adults. Club cells, known as lectin secreting cells, were also recognized in the epidermis of leptocephali, although the shape was

not elongated club form but oval. The cells were confirmed to contain lectin in the secretory vacuole by an immunofluorescence technique. The lectin in the cells was also recognized in the preleptocephalous larvae of eight days post hatch, which was obtained from an artificially spawned eel, suggesting the importance of the lectin in the early larval development.

Bioactive substances on the skin surface may thus compensate the delayed development of internal immune function. However, knowledge on the defense mechanisms of marine fish larvae is still very limited to utilize it for improved aquaculture.

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ONTOGENY OF PROTEOLYTIC DIGESTIVE ENZYME GENE EXPRESSION IN DEVELOPING SEABASS (*LATES CALCARIFER*) LARVAE

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Introduction

The transition from endogenous feeding to exogenous feeding, often referred as first feeding, is a critical period in most marine fish larvae. The proteolytic digestive capacity of the larvae is limited and most of the larvae are inefficient in breaking down encapsulated diets. The mortality of larvae at this stage is high and is often presumed to be due to insufficient enzyme production. The larvae do not have a functionally differentiated stomach at first feeding and hence there is no acid secretion or peptic digestion; and stomach differentiation and subsequent acid secretion and pepsin digestion occur after metamorphosis. Detailed information on the basic digestive physiology, specifically on the proteolytic digestive enzymes, is necessary in order to develop formulated larval diets suitable for different marine fish larval stages. The present paper reports the results of molecular ontogeny of proteolytic digestive enzymes, trypsin, aminopeptidase N, and pepsin in seabass larvae.

Materials and methods

Fertilized seabass eggs were collected soon after natural spawning in floating net cages and the buoyant eggs were separated and hatched in 15-l buckets. After hatching, the larvae were stocked in 300-l fiberglass tanks filled with filtered and UV-irradiated seawater. Larvae were fed with rotifers ($15.ml^{-1}$) daily from 3 days post hatching (3 dph). From 10dph onward, larvae were fed with *Artemia* nauplii ($5.ml^{-1}$). The larval samples were collected in triplicate on 1, 2, 3, 4, 5, 8, 10, 12, 15, 17, 19, 21, 23, 25, 27, and 30 dph. Total RNA from the samples was extracted using TRI Reagent.

Total RNA from the gut of 64-dph juvenile seabass was extracted and a cDNA library was constructed using Thermoscript RT-PCR system. Degenerate primers for trypsinogen, aminopeptidase N, and pepsinogen were designed and used to clone and sequence trypsinogen, aminopeptidase N, and pepsinogen cDNA fragments from seabass cDNA library. Using these sequences, intron flanking oligonucleotide primers were designed for seabass trypsinogen, aminopeptidase N, and pepsinogen, and were used in the semi-quantitative RT-PCR analysis to measure the relative mRNA levels of these genes during seabass ontogeny.

Results and discussion

The PCR amplification of seabass whole gut cDNA using degenerate primers yielded a 530-bp fragment of trypsinogen, a 539-bp fragment of aminopeptidase N, and a 836-bp fragment of pepsinogen. Their identities were verified by sequencing and subsequent BLASTn.

The semi-quantitative RT-PCR results showed that the expression of all three genes (trypsinogen, aminopeptidase N, and pepsinogen) was low at 1dph. There was a steep increase in the mRNA levels of trypsinogen by 3dph and remained at that level until 10dph; followed by a further increase when the larvae were switched from rotifers to *Artemia* nauplii. The mRNA levels of aminopeptidase N increased by 2dph and remained at higher levels until 15dph, followed by a gradual decrease thereafter. Pepsinogen transcripts were detected even at 1dph at low levels, and remained low until 15dph. The levels increased rapidly from 15dph onwards and reached a maximum by 20dph, and these levels remained high thereafter. The increase in the pepsinogen transcript levels coincided with the decrease in aminopeptidase N and trypsinogen.

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EFFECTS OF TRIIODOTHYRONINE AND CORTISOL ON THE EXPRESSION OF PROTEOLYTIC DIGESTIVE ENZYME GENES IN DEVELOPING SEA BASS (*LATES CALCARIFER*) LARVAE

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Introduction

First feeding and stomach development during metamorphosis are two critical stages in the development of marine fish larvae for their survival and growth. Larvae of most marine fish species at first feeding are inefficient in breaking down encapsulated diets. At this stage, their digestive capacity is limited since the digestive system (gut) is not well developed yet and generally lacks a functional stomach until after metamorphosis. This results in limitations on the types of feed that the larvae are able to digest, and they feed mainly on live food organisms before being weaned on to formulated feeds after metamorphosis. During this period, trypsin and aminopeptidase N (intestinal brush border enzyme) are the main enzymes involved in protein digestion, and peptic digestion comes in later at metamorphosis.

The role of thyroid hormones and cortisol has been extensively studied in amphibian metamorphosis. These hormones have also been shown to accelerate organogenesis and metamorphosis in several species of fishes, with improved larval survival and growth.

In the present study, we have evaluated the effects of triiodothyronine (T_3) and cortisol on proteolytic digestive enzymes at the transcription level, at two different stages of critical development in sea bass larvae: around the time of first feeding and around the time of metamorphosis.

Materials and methods

Experiment 1. Newly hatched sea bass larvae were distributed into fifteen 20-l

conical glass tanks. The larvae were reared in seawater alone or in seawater containing T_3 (5nM and 10nM) or cortisol (100nM and 200nM) with each treatment in triplicate. The treatments were administered on day 1 post hatching (1dph) and the media were not replaced until 3dph. From 4dph, 25% of the water in each tank was replaced daily with fresh filtered seawater until the experiment was terminated on 8dph. The larvae were fed with rotifers at a density of $15.ml^{-1}$ from 3dph onwards. The larval samples were collected on 1,3, 5, and 8dph for RNA extraction.

Experiment 2. Fifteen-day-old sea bass larvae were stocked in 20-l tanks at a density of $15.ml^{-1}$ and reared in the same treatments as in Experiment I. Each treatment was triplicated and the media were changed and replaced with fresh media daily. The larvae were fed with *Artemia* nauplii *ad libitum* daily. The larval samples for RNA were collected on 15, 17, 21, 23, 26, and 28dph.

Total RNA from the larval samples was extracted using TRI Reagent and 1µg was reverse transcribed in a total volume of 10µl. PCR amplification was performed on 0.5µl cDNA using trypsinogen-, aminopeptidase N-, and pepsinogen-specific intron-flanking oligonucleotide primers. PCR products were run on ethidium bromide-stained agarose gel and the band volume measured using a Gel-Doc2000 system and Quality one software (Biorad).

Results and discussion

In first feeding larvae, the levels of aminopeptidase N gene expression were significantly higher in all hormone-treated larvae compared to those in control treatment on 3dph, and on 5dph, all the treatments except 10nM T_3 showed significantly higher levels. On 8dph, only the 200-nM F treatment showed significantly higher levels than in the control. During first feeding, when the stomach is not yet developed, intestinal brush border enzymes are important for protein digestion and the upregulation of aminopeptidase N by hormone treatments during this period may be beneficial for the larvae in terms of better digestion. However, T_3 (5nM and 10nM) and cortisol (200nM) significantly downregulated the expression of trypsinogen. Compared to the control, cortisol did not show any significant effect on pepsinogen transcript levels. However, T_3 significantly downregulated pepsinogen expression levels.

In metamorphosing larvae, the relative amounts of mRNA for pepsinogen were significantly higher in all hormone-treated larvae compared to the control larvae on 17dph, and a similar increase was observed in control larvae on 21dph. On 23dph, pepsinogen transcript levels decreased significantly in all hormone treatments and a similar decrease in control treatment was observed on 26dph. No significant differences were subsequently found between control

and hormone-treated larvae on 26dph and 28dph. These results show that upregulation of pepsinogen gene expression was brought forward by 3 days in all the hormone treatments. The results indicate that T_3 and cortisol may be useful in the acceleration of stomach maturation and the switch over to peptic digestion in the larvae. This may be useful in earlier weaning of larvae to formulated diets. These results may further emphasize the potential for the use of hormones to accelerate gastrointestinal function and increase the feeding efficiency, and thus survival, in marine fish larvae.

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USE OF *ARTEMIA* BIOMASS IN SHRIMP BROODSTOCK FEEDING

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Abstract

Two trials were carried out – (I) different fresh foods (squid, shrimp, and *Artemia* biomass) combined with balanced food (Nippai); and (II) a combination of *Artemia* biomass with squid, shrimp, and Nippai – with the purpose to evaluate the effect of *Artemia* biomass on reproduction of white shrimp (*Litopenaeus schmitti*). The experiments were performed in the Shrimp Hatchery of Santa Cruz del Sur, Camagüey, with wild shrimp. The evaluation of each treatment included the number of coupling.tank^{-1}.day, eggs.spawn^{-1}, nauplii.spawn^{-1}, and hatching percent. In trial I, the best results were obtained with the combination of shrimp + Nippai (2.7 coupling.tank^{-1}.day), though no statistical differences were found (P>0.05) in relation to the number of nauplii.spawn^{-1} for all treatments. In trial II, the highest hatching percentage (42.55%) was obtained in the treatment of squid + *Artemia* biomass, though no statistical differences were found in relation to the other parameters. Based on the final results, *Artemia* biomass could be employed to feed broodstock with positive economic and productive results.

Introduction

The use of adult *Artemia* biomass has been applied in a limited way as food in shrimp cultivation. Such a supplement in broodstock diet of penaeid shrimps can contribute to induce the maturation.

In Cuba, investigations of maturation and reproduction of white shrimp, *Penaeus schmitti*, are directed to the search of foods that can substitute partial or wholly imported products. The objectives of this work are to evaluate the effects of different natural foods, particularly *Artemia*, on the reproduction of white shrimp.

Materials and methods

The experiments were carried out in 3.36-m black circular tanks in the Shrimp

Hatchery of Santa Cruz del Sur (province of Camagüey) with wild-caught shrimp. Unilateral ablation of the ocular peduncle was applied to females. Two experiments were carried out, with three replicates for each treatment. Fresh food was given at a rate of 15% of the total biomass distributed three times a day (02:00 hours; 08:00 hours; 20:00 hours), while dry food Nippai was added at a rate of 1.5% of the biomass distributed in two portions (14h00 and 24h00).

For Trial I, *Artemia* biomass from Caimanera saltwork (Guantánamo province) was used with whole shrimp smaller than 8g from culture ponds. In Trial II, *Artemia* biomass from intensive cultures fed with torula yeast was used.

Table I. Trial I experimental conditions.

Treatment	Nippai + squid	Nippai + shrimp	Nippai + *Artemia*
Weight (g; M/F)	30.3/47.5	30.3/47.5	30.3/47.5
Shrimp/tank	60	60	60
M:F ratio	1:1	1:1	1:1
T (days)	43	43	43
Photoperiod (L/D)	14/10	14/10	14/10

Table II. Trial II experimental conditions.

Treatment	*Artemia* + squid	*Artemia* + shrimp	*Artemia* + Nippai	Control Nippai + squid
Weight (g; M/F)	27.2/41.3	27.2/41.3	27.2/41.3	27.2/41.3
Shrimp/tank	60	60	60	60
M:F ratio	1:1	1:1	1:1	1:1
T (days)	32	32	32	32
Photoperiod (L/D)	14/10	14/10	14/10	14/10

Shrimp were acclimatized for 10 days before experiments began. After selection to detect copulated females, they were placed in 200-l tanks with seawater filtered by a 5-µm cartridge and 10mg.l^{-1} of EDTA. Immediately after spawning, the females were returned to the origin tank. After 12h, eggs were collected and counted, and placed in 100-l cylindro-conical tanks under similar conditions as the broodstock. Nauplii were harvested the following day in stage III-IV. The count of eggs and nauplii was carried out using the volumetric method, taking eight 1-ml samples from each tank.

Daily (08h00 and 20h00), temperature, salinity, dissolved oxygen, and pH were monitored. The following parameters were analyzed: coupling.tank^{-1}.day; eggs.spawn^{-1}, nauplii.spawn^{-1}, and hatching percentage.

After checking the normality of the data, a totally randomized ANOVA and Duncan's multiple range test ($P<0.05$) were applied to evaluate all the parameters except the hatching percentage, for which a chi-squared (χ^2) test was used.

Results and discussion

The average values of environmental parameters in both experiments (Table III) are considered adequate for reproduction in this species.

Table III. Environmental parameters (ranges).

	Trial I	Trial II
Temperature (°C)	23.6 - 27.5	26.2 - 28.6
Salinity (‰)	36 - 38	35 - 38
Oxygen (mg.l^{-1})	5.8 - 7.7	4.3 - 6.2
pH	7.9 - 8.6	7.9 - 8.4

The results of Trial I are shown in Table IV. With respect to the eggs.spawn^{-1} and nauplii.spawn^{-1} statistical differences were not found ($P>0.05$) between treatments. However, in the Nippai + shrimp treatment, the highest coupling.tank^{-1}.day was achieved. Peréz and Ramos (1992) have reported a maximum coupling frequency of 1.75 spawn.female^{-1}.month for *Liptopenaeus schmitti*, while for *P. vannamei*, 2.5 spawn.female^{-1}.month was reported (Fauvel, 1986) mixing the results reached with natural and artificial fecundation. The hatching percentage did not show significant differences among the treatments.

Table IV. Trial I results.

Treatment	Nippai + squid	Nippai + shrimp	Nippai + *Artemia*
Coupling.day^{-1}	1.2^{b}	2.7^{a}	1.3^{b}
Number of couplings	51	145	54
Eggs.spawn^{-1}	210 200^{a}	202 400^{a}	189 800^{a}
Nauplii.spawn^{-1}	110 900^{a}	94 800^{a}	89 900^{a}
Hatching percentage	52.8^{a}	46.8^{a}	47.4^{a}

Equal letters indicate no statistical difference ($P>0.05$)

In Trial II (Table V), the highest average coupling.tank^{-1}.day was achieved with fresh squid + dry food Nippai (2.5), though no significant differences ($P>0.05$) were found among the rest of the treatments. However, with the *Artemia* biomass + squid treatment, the biggest hatching percentage was reached (42.6%), and the average number of nauplii.spawn^{-1} was similar to that of the *Artemia* biomass + shrimp and squid + dry food Nippai treatments. In the

Artemia biomass + dry food Nippai treatment, the worst results for hatching percentage and the nauplii.spawn^{-1} were seen.

Table V. Trial II results.

Treatment	*Artemia* + squid	*Artemia* + shrimp	*Artemia* + Nippai	Control Nippai + squid
Coupling.day^{-1}	2.1^{a}	2.1^{a}	1.9^{a}	2.5^{b}
Number of couplings	108	111	99	129
Eggs.spawn^{-1}	101 200^{b}	118 000^{b}	123 200^{b}	128 800^{b}
Nauplii.spawn^{-1}	43 000^{a}	42 630^{a}	22 370^{b}	44 140^{a}
Hatching percentage	42.6^{a}	38.7^{ac}	18.2^{b}	34.3^{c}

Equal letters means no statistical differences (P>0.05)

In general, the results reached in the second experiment were inferior with respect to both those reported by other authors for white shrimp and those reached in Trial I concerning eggs.spawn^{-1} and hatching percentage, although the number of coupling.tank^{-1}.day can be considered satisfactory.

References

Fauvel C. 1986. Hatcheries Techniques: Control of maturation and reproduction. In: Intern. Workshop on Marine Crustacean Culture, Development and Management. Venezia, Nov. 5-7. 12p.

Pérez L. and L. Ramos.1992. Determinación de la proporción de sexos Penaeus schmitti en tanques de maduración inducida para el incremento de la cópula natural. Rev. Inv. Marinas. Univ. de La Habana. 13(2):167-176.

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COMPARATIVE STUDY OF THE ANTIOXIDANT DEFENCE SYSTEM AND GROWTH PERFORMANCE OF FISH FED VARIABLE LEVELS OF OXIDIZED OIL AND VITAMIN E

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Introduction

Lipid and polyunsaturated fatty acid (PUFA) peroxidation is highly deleterious, resulting in damage to biomembranes and is implicated in several pathological conditions in fish. Physiological antioxidant protection involves both endogenous components such as free radical scavenging enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) (Winston and Di Giulio, 1991), and exogenous dietary micronutrients such as vitamin E. The objective of the present study was to determine oxidative stress and physiological responses in juveniles of marine fish to varying levels of dietary oxidized oil with and without supplemental vitamin E. The species studied were sea bream (*Sparus aurata*), turbot (*Scophthalmus maximus*), and Atlantic halibut (*Hippoglossus hippoglossus*), with different temperature tolerances and growth characteristics.

Materials and methods

Juvenile fish were grown for 7 weeks (at 19, 20, and 14°C for sea bream, turbot, and halibut, respectively) on diets containing 50% protein, 20% oil and 7% dry wt. as n-3 HUFA with variable peroxide values and vitamin E levels (Table I). Liver enzymes were assayed in homogenate supernatants as described in detail by Mourente et al. (2000). The levels of 8-isoprostane and TBARS were determined in homogenates of liver and whole fish as described by Mourente et al. (2000). Protein contents in homogenates and supernatants were determined by the Folin-phenol reagent method (Lowry et al., 1951). Results are presented as means ± SD (n=3). Differences between mean values were analyzed by two-way analysis of variance for effects due to oxidized oil and vitamin E. Differences were reported as statistically significant when $P<0.05$ (Zar, 1984).

Table I. PUFA and vitamin E contents, unsaturation index, and peroxide values of diets.

Diet name	PUFA ($g.kg^{-1}$ diet)	Vit E ($mg.kg^{-1}$ diet)	PUFA/vit E (mol/mol)	Unsaturation index	Peroxide value
HO	86.2±3.8	37.9±3.0	3063±609	343.1±0.1	9.2±0.5
HXO	82.9±2.5	35.3±7.3	3795±926	346.1±1.7	42.0±1.6
HXE	76.5±0.3	260±38	467±64	342.5±2.3	42.0±1.6
HE	78.3±2.7	197±5	628±11	346.3±3.0	4.5±0.4

Results and discussion

Both the sea bream and turbot showed excellent growth, with specific growth rates (SGRs) varying between 2.9 and 3.7, whereas growth was poorer in halibut and an SGR of 3 was only observed with diet HE (Table II). Dietary oxidized oil significantly reduced growth in turbot and especially halibut, but not sea bream. Vitamin E improved growth in sea bream fed oxidized oil but not in turbot or halibut. However, vitamin E addition appeared to improve survival in all three species.

Table II. Effects of dietary oxidized oil and vitamin E on growth and survival of sea bream (80 days old), turbot (75 days old), and halibut (67 days old).

		HO	HXO	HXE	HE	Significance		
						ox. oil	Vit. E	inter.
Sea bream	Initial weight (g)	1.52±0.21	1.52±0.21	1.52±0.21	1.52±0.21			
	Final weight (g)	4.12±0.5	4.20±0.34	4.55±0.04	4.05±0.58	Y	Y	Y
	SGR ($\%.day^{-1}$)	3.30±0.44	3.38±0.27	3.66±0.03	3.24±0.50	Y	Y	Y
	Survival (%)	99.0±0.9	99.2±1.0	99.5±0.5	99.5±0.9	N	Y	N
Turbot	Initial weight (g)	0.95±0.22	0.95±0.22	0.95±0.22	0.95±0.22			
	Final weight (g)	3.02±0.65	2.61±0.65	2.52±0.69	2.74±0.59	Y	Y	N
	SGR ($\%.day^{-1}$)	3.47±0.68	3.04±0.72	2.92±0.78	3.20±0.63	Y	Y	N
	Survival (%)	90.0	75.0	99.0	91.0			
Halibut	Initial weight (g)	0.31±0.11	0.31±0.11	0.31±0.11	0.31±0.11			
	Final weight (g)	0.66±0.24	0.58±0.33	0.51±0.31	0.89±0.41	Y	N	Y
	SGR ($\%.day^{-1}$)	2.2±0.3	1.8±0.2	1.4±0.2	3.0±0.3	Y	Y	Y
	Survival (%)	57.0	29.0	41.0	59.0			

In sea bream, catalase, SOD, and GPX were increased by feeding peroxidized oil and reduced by vitamin E, and in turbot, GST and GR were increased by feeding oxidized oil and catalase, and SOD and GST activities were reduced by vitamin E (Table III). Contrarily, in halibut only GST increased with feeding oxidized oil and only SOD was reduced by feeding vitamin E. Interestingly, GR was generally increased by dietary vitamin E in all three species (Table III).

Table III. Effects of dietary oxidized oil and vitamin E on the activities of liver antioxidant enzymes.

		HO	HXO	HXE	HE	Significance		
						ox. oil	vit E	inter.
Sea bream	Catalase	437±10	667±23	520±25	307±7	Y	Y	N
	SOD	5.7±0.0	6.5±0.2	6.0±0.8	2.8±0.1	Y	Y	Y
	GPX	101.6±5.9	84.7±3.7	89.8±5.6	85.7±1.5	Y	N	Y
	GST	895±80	802±100	944±61	833±15	N	N	Y
	GR	26.3±1.6	59.2±2.7	71.3±7.2	77.8±28.9	N	Y	N
Turbot	Catalase	129±21	202±36	127±35	117±17	N	Y	N
	SOD	4.6±0.3	5.1±0.7	4.0±0.2	4.0±0.3	N	Y	N
	GPX	0.93±0.19	0.81±0.09	0.83±0.08	0.79±0.07	N	N	N
	GST	104±3	117±5	102±8	86±9	Y	Y	N
	GR	8.5±1.5	13.1±0.9	11.5±1.4	10.2±0.6	Y	N	Y
Halibut	Catalase	170.1±11.6	170.5±24.3	158.0±2.8	178.8±4.6	N	N	N
	SOD	3.3±0.2	3.0±0.2	2.4±0.0	3.2±0.2	Y	Y	Y
	GPX	38.7±17.3	n.d.	36.6±0.0	14.9±6.8	N	N	Y
	GST	154.0±5.9	158.0±5.1	190.0±1.4	155.0±6.1	Y	Y	Y
	GR	9.1±3.0	6.2±0.9	9.6±0.4	14.2±0.8	Y	Y	N

Catalase, (μmol.min^{-1}.mg protein^{-1}); GPX (glutathione peroxidase), GR (glutathione reductase) and GST (glutathione-S-transferase) are all nmol.min^{-1}.mg protein^{-1}; SOD, superoxide dismutase (Units.min^{-1}.mg protein^{-1}).

Feeding oxidized oil increased lipid peroxidation products in halibut, but generally not in sea bream or turbot (Table IV). Furthermore, lipid peroxidation products were generally reduced by dietary vitamin E in both sea bream and turbot, but not in halibut (Table IV).

Halibut liver antioxidant defence enzymes did not respond to dietary oxidized oil or vitamin E as occurred in turbot and, especially sea bream. Perhaps this resulted in increased levels of lipid peroxides in halibut compared to turbot and sea bream in fish given dietary oxidized oil. In addition, supplemental vitamin E did not reduce lipid peroxides in halibut as it did in turbot and sea bream. The increased peroxidation stress in halibut may account for their poorer growth and survival in comparison to turbot and especially sea bream. Halibut were reared at a lower temperature, although relatively high for halibut, than either turbot or sea bream but they were also slightly younger/smaller fish and possibly, therefore, more developmentally immature, and either or all of these factors may be important in the lack of response of the liver enzymes in halibut.

Conclusions

The capability of the antioxidant system appeared to be species-dependent, and may be related to developmental stage. Halibut may have a higher requirement

for vitamin E compared to the other 2 species, perhaps related to culture temperature and HUFA requirement.

Table IV. Effects of dietary oxidized oil and vitamin E on lipid peroxidation products in liver and whole fish.

		HO	HXO	HXE	HE	Significance		
						ox. oil	vit E	inter.
Sea bream	TBARS							
	liver	5.4±0.5	5.0±0.5	4.2±0.3	3.3±0.1	N	Y	Y
	Isoprostane							
	liver	110±6	188±3	125±22	177±3	N	N	Y
Turbot	TBARS							
	liver	0.66±0.18	1.20±0.14	0.54±0.18	0.60±0.20	Y	Y	Y
	fish	10.2±0.8	9.6±3.1	7.2±1.0	7.3±1.9	N	Y	N
	Isoprostane							
	liver	79.1±1.0	64.3±5.0	64.0±14.5	77.9±3.2	Y	N	N
	fish	42.4±7.7	46.0±10.6	21.2±1.6	18.1±5.6	N	Y	N
Halibut	TBARS							
	liver	0.18±0.02	0.22±0.01	0.21±0.01	0.19±0.01	Y	N	N
	fish	1.6±0.5	10.7±2.4	3.5±0.6	6.4±0.8	Y	N	Y
	Isoprostane							
	liver	58.3±4.3	57.1±6.7	83.9±5.8	60.8±6.5	Y	Y	Y
	fish	14.7±5.7	52.1±0.7	37.7±2.8	32.8±7.2	Y	N	Y

Thiobarbituric acid-reactive substances (TBARS), mmols.mg^{-1} protein; Isoprostane (pg.mg^{-1} protein).

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References

Lowry O.H., N.J. Roseborough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Mourente G., E. Díaz-Salvago, D.R. Tocher, and J.G. Bell. 2000. Effects of dietary polyunsaturated fatty acid/vitamin E (PUFA/tocopherol) ratio on antioxidant defence mechanisms of juvenile gilthead sea bream (*Sparus aurata* L., Osteichthyes, Sparidae). Fish Physiol. Biochem. 23:337-351.

Winston G.W., and R.T. Di Giulio. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicol. 19:137-161.

Zar J.H. 1984. Biostatistical Analysis, 2nd Ed. Prentice-Hall, Englewood, NJ.

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VITAMIN E CONTENT OF *ISOCHRYSIS* T-ISO UNDER DIFFERENT CULTURE REGIMES

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Introduction

Vitamin E is the collective term for certain close related substances identified as tocopherols and tocotrienols. These vitamers function as lipid-soluble chain-breaking antioxidants and free radical scavengers preventing and blocking the membrane polyunsaturated fatty acid peroxidation (reviewed by Huo, 1998). Vitamin E is synthesized mainly by photosynthesizers with the predominant form α-tocopherol, associated in plants with chlorophyll-containing tissue. The other three tocopherols and the tocotrienols can be found in the non-chlorophyll parts and in the oils. Animals obtain vitamin E from their diet and its incorporation into the tissue function as antisterility factor promotes immune responses and the health status in general (e.g., Valk, 2000). Several studies have classified vitamin E deficiency syndromes in fish, birds, and mammals. Particularly for aquaculture, vitamin E is used in the formulated diets to enhance growth and stress resistance in different fish and penaeid shrimps (e.g., Zheng et al., 1997).

Microalgae synthesize vitamin E at levels seemingly species-specific (e.g., De Roeck-Holtzhauer et al., 1991), also possibly influenced by environmental conditions (e.g., Seguineau et al., 1993). In this study, we report on the effects of various culture regimes on α- and γ-tocopherol of T-ISO.

Materials and methods

The prymnesiophyte *Isochrysis* aff. *galbana*, "Tahitian strain" (T-ISO), was obtained from the Guernsey Farms SA hatchery, UK, and stocked as a monospecific culture in the Artemia Reference Center.

Batch cultures of T-ISO were grown at a high photon flux density of 460μmol photon.m^{-2}.s^{-1}. Different culture regimes were established under either 24:0h L:D

or 12:12h L:D, 25°C or 19°C, and 22 or 32 g.l^{-1} salinity. Sampling took place at the end of the exponential phase and at the end of the post-exponential phase. All cultures were left to acclimate under each regime for as many generations necessary to obtain identical specific growth rates. Details on culture methodology as well as on growth assessment and fatty acid analysis can be found in Tzovenis et al. (1997).

The analysis of α- and γ-tocopherol was carried out after Huo et al. (1997). Statistics were done using Statistica software (StatSoft, 1995).

Results and discussion

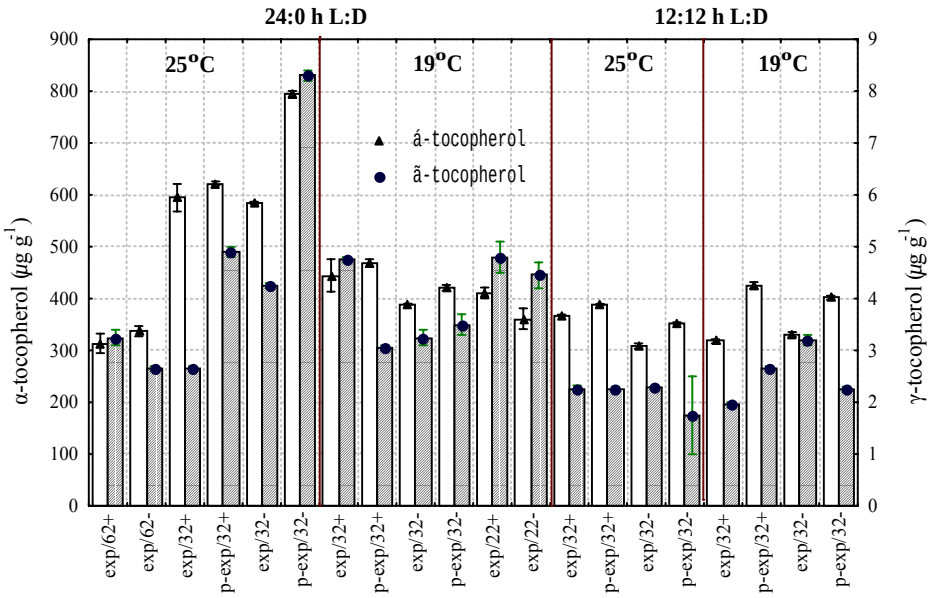

Fig.1 Vitamin E (α- and γ- tocopherol) content of T-ISO under different culture regimes. Codes: Growth phase exp: exponential; p-exp: post-exponential; Salinity 62, 32, 22 g.l^{-1}; CO_2 regime '+', '-' 1% and ambient aeration.

Fig. 1 summarizes results concerning the α- and γ-tocopherol content of T-ISO. ANOVA confirms the hypothesis that the tocopherol levels are influenced by different environmental conditions. Results showed that the α- and γ-tocopherol vitamer contents maximize under continuous light and 25°C. At 19°C, the vitamer contents were lower and at levels comparable with the ones obtained under 12:12h L:D. Under the latter photoperiod, there was no effect of temperature. Except for the significant effect of the temperature:photoperiod interaction on the α-tocopherol content, that vitamer did not seem to be influenced by the other variables tested. In contrast, high salinity (62g.l^{-1})

negatively influenced the γ-tocopherol content, which was found highest at 32g.l^{-1}. CO_2 regimes (ambient aeration or 1% addition) did not seem to have any effect. During the post-exponential phase in cultures of 22g.l^{-1} salinity, the α-tocopherol content increased while there was no difference in 32-g.l^{-1} cultures.

As the experimental design was not complete, interactions between the main factors could be revealed with safety only for particular combinations. Therefore, the effects of each factor are presented in Table I as correlations (P<0.05) to show the main trends. α-tocopherol is positively influenced mainly by the length of the light phase, while γ-tocopherol is positively influenced also by the temperature and, to a lesser extent, by growth phase.

Table I. Correlations of the vitamers with the different factors. Marked correlations are significant at P<0.05; n=60

factors	α-tocopherol	γ-tocopherol
L:D	**0.46**	**0.60**
temperature	**0.46**	0.09
growth phase	**0.35**	0.09
salinity	0.11	0.25
CO_2	-0.03	0.11

In order to explore the relationship between the fatty acids and the different vitamers, the correlation approach was again used (Table II). Results show that both vitamers increase with the accumulation of highly unsaturated fatty acids (ω3 and ω6 HUFA) in T-ISO. The same strong trend was evident with the ω6 fraction, resulting in a negative correlation with the ω3/ω6 ratio. γ-tocopherol seems to also be positively correlated with the ω3 HUFA fraction.

Table II. Correlations of the vitamers with the fatty acid groups. Marked correlations are significant at P<.05; n=60.

response	α-tocopherol	γ-tocopherol
SAFA	0.03	-0.13
MUFA	0.23	0.05
PUFA	-0.22	0.02
HUFA	**0.72**	**0.87**
ω3	-0.17	-0.02
ω6	**0.73**	**0.75**
ω3 HUFA	-0.02	**0.29**
ω3/ω6	**-0.52**	**-0.51**

From these data, evidence is provided that vitamin E components accompany the membrane unsaturated fatty acids in a proportion capable to protect them against peroxidation. Interesting is that the ω6 fraction is strongly correlated with tocopherols in a manner similar to that found in shrimps (Huo, 1998), possibly related with the eicosanoid oxygenase cycles as in animals (De Duve and Hayashi, 1978).

Conclusions

The vitamin E content of T-ISO (α- and γ-tocopherol) is influenced by culture conditions. Continuous light exerts a positive trend on both vitamers.

Vitamin E correlates positively with HUFA and ω6 fatty acids, supporting a hypothesis that its function is to protect and block polyunsaturated fatty acid peroxidation. Regulation of vitamin E synthesis may be related with the fluctuation of HUFA.

Acknowledgements

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References

De Duve C. and O. Hayashi. 1978. Tocopherol, oxygen and biomembranes. Elsevier-North-Holland, Amsterdam. p.363-366.

Huo J.Z. 1998. Bio-analytical study of vitamin E and indicators of antioxidant status in aquacultural research. Ph.D. thesis, Gent University, Belgium, 159p.

Huo J., H.J. Nelis, P. Lavens, P. Sorgeloos, and L. De. 1997. Determination of E vitamers in microalgae using high-performance liquid chromatography with fluorescence detection. J. Chromatogr. A 782:63-68.

StatSoft, Inc. 1995. STATISTICA for Windows [Computer program manual]. Tulsa, OK

Tzovenis I., N. De Pauw, and P. Sorgeloos. 1997. Effect of different light regimes on the docosahexaenoic acid (DHA) content of *Isochrysis* aff. *galbana* (clone T-ISO). Aquacult. Int. 5:489-507.

Valk Esther E.J. 2000. Relationship between vitamin E requirement and polyunsaturated fatty acid intake in man: A review. Int. J. Vit. Nutr. Res. 70(2):31-42.

Zheng F., T. Takeuchi, M. Kobayashi, J. Hirokawa, and T. Watanabe. 1997. A study of vitamin E absorption in rotifer and cod larvae and the effect of DHA content in rotifer for cod larvae. Nippon Suisan Gakkaishi 63:77-84.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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EFFECT OF SUBSTRATE AND DIET IN THE NURSERY PHASE OF MUD CRAB (*SCYLLA PARAMAMOSAIN*) PRODUCTION

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Introduction

The strong cannibalistic behaviour of juvenile crabs may lead to low survival during post-settlement in the wild and in the nursery phase of aquaculture of many species. In natural crab populations at higher densities, conspecific encounter rates and cannibalism are significant causes of mortality (Perkins-Visser et al., 1996). Moksness et al. (1998) found that juvenile shore crabs (*Carcinus* sp.) were extremely efficient predators on small conspecifics. Structured habitats, such as seagrass, blue mussels, and filamentous algae, have been documented to provide significant shelter from predation and cannibalism in crabs in the wild (Moksnes et al., 1998; Perkins-Visser et al., 1996; Heck and Thoman, 1981). Similarly, providing physical substrates or shelters, adequate feeding, and reducing stocking density in nursery systems are all factors which could mitigate the high level of cannibalism that is typical of the post-larval culture of mud crabs. The aim of this study was to compare the effects of stocking densities, provision of sand substrate, 3-dimensional shelters, and feeding regimes on improving the survival of juvenile *Scylla paramamosain* during the nursery phase.

Materials and methods

Experiment 1 examined the effect of stocking density (110, 175, and 230 crabs.m^{-2}) on growth and survival with a sand substrate. Hatchery-reared stage 1 crabs (CW 4.4±1.1mm) were stocked in twelve flat-bottomed 15-l PVC tanks, with four replicates per treatment. All tanks were connected to a common water recirculation system. Crabs were fed *Artemia* until day 3, together with peeled shrimp.

Experiment 2 compared the effects of sand substrate and clay brick shelters on crab growth and survival. The bricks were 8cm long × 7cm wide, with four circular holes (diameter 2.5cm) along the length of the brick. The experiment

was set up in 4-m^2 cement tanks connected to a biofiltered recirculation system. Three replicates per treatment were used. Crabs (CW 3.7±0.1mm) were stocked at 100.m^{-2} of tank bottom. In the sand substrate tanks, a 2-cm layer of sand was spread over the entire tank bottom. In the shelter treatment, 100 bricks were placed in each tank. Crabs were fed *ad libitum* with peeled shrimp.

Experiment 3 compared three dietary treatments (fresh marine fish, peeled shrimp, and a combination of both) using the brick shelter system in 2m × 2m concrete tanks (as in Experiment 2). Each treatment had three replicates, and 400 crabs from the same batch used in Experiment 2 (CW 3.7±0.1mm*)* were stocked in each tank.

At the end of Experiment 1, all surviving crabs were counted and measured. In Experiments 2 and 3, samples of 15 crabs from each tank were weighed and measured. Differences between treatments were compared by ANOVA and Turkey's pairwise comparison of means.

Results and discussion

In Experiment 1, after 15 days, survival of crabs stocked at the two lower densities was higher than for crabs stocked at the highest density (71.3, 61.7, and 57.5 %, respectively), but there was no significant difference in either survival or growth among treatments (Fig. 1).

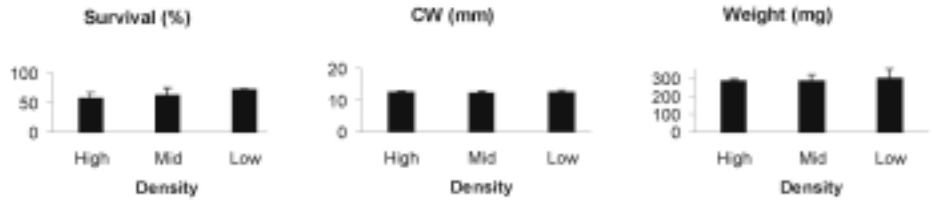

Fig. 1. Survival and growth of crabs after 15 days at three stocking densities – high (230m^{-2}), mid (175m^{-2}) and low (110m^{-2}) – on a sand substrate. No significant differences were seen among treatments.

In Experiment 2, there were highly significant differences in survival (P<0.001) and growth (P<0.001) between brick shelters and sand substrate. Crabs stocked in a brick shelter had higher survival (47.8±2.4% vs. 18.4±2.7%) but lower growth than those in sand (Fig. 2).

In Experiment 3, there were significant differences in survival (P<0.001) among diets, with best results using shrimp and worst using fish. The mixed diet supported intermediate survival (Fig. 2). Crabs fed shrimp exhibited lower

growth in terms of weight gain than those fed either the fish or the mixed diet.

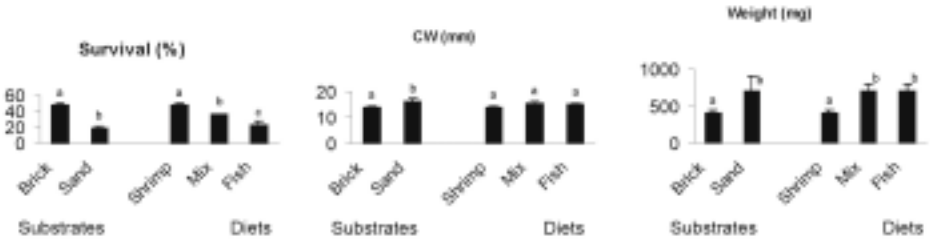

Fig. 2. Survival and growth after 24 days of crabs grown with brick shelters or sand substrate (Experiment 2) and brick substrate and three diets (shrimp, fish, and mixed shrimp and fish) (Experiment 3). Within each experiment, values bearing different superscripts are significantly different (P<0.05).

Substrates and shelters are known to be important in reducing cannibalism in mud crabs in pond grow-out, where seaweeds (such as G*racilariopsis*) and bamboo shelters have been used (Triño et al., 1999). In the present study, the bricks provided a better shelter than sand, probably because crabs were observed to molt on the surface of the sand substrate and were unable to re-bury immediately while still soft. In contrast, the 3-dimensional shelter of the bricks provided refuges for newly molted crabs. Similarly, Barshaw et al. (1994) found that for postlarval lobster, *Homarus americanus*, cobble provided better shelter than peat or sand.

The higher growth observed with the sand substrate probably reflects cannibalistic feeding, selection of larger faster-growing individuals, and the reduction in density for survivors (Perkins-Visser et al., 1996). Inclusion of shrimp in the diet (either alone or mixed with fish) supported higher survival, probably reflecting greater attractiveness and palatability, as the shrimp was more rapidly consumed than the fish. Growth was significantly better in the crabs fed fish, probably as the result of increased cannibalism and reduced densities rather than any direct dietary effect.

In conclusion, the present study indicates that improved mud crab production (in terms of survival and homogeneity in size range) can be promoted by provision of a three-dimensional hard substrate with refuge for molting crabs, together with a palatable and nutritionally adequate diet. Within the tank system used, the influence of higher and lower stocking densities remains to be evaluated.

References

Barshaw E.D. et al.. 1994. Salt marsh peat reefs as protection for postlarvae lobster *Homarus americanus* from fish and crabs predators: comparisons with other substrate. Mar. Ecol. Progr. Ser. 106:203-206

Heck K.L. Jr and T.A. Thoman. 1981. Experiments on predator-prey interactions in vegetated aquatic habitats. J. Exp. Mar. Biol. Ecol. 53: 125-134

Moksnes P-O, L. Pihl, and J. van Montfrans. 1998. Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. Mar. Ecol. Progr. Ser. 166:211-225.

Perkins-Visser E., T.G. Wolcott, and D.L. Wolcott. 1996. Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathbun). J. Exp. Mar. Biol. Ecol. 198:155-173.

Triño A.T., O.M. Millamena, and C. Keenan . 1999. Commercial evaluation of monosex pond culture of the mud crab *Scylla* species at three stocking densities in the Philippines. Aquaculture 174:109-118.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
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BIOCHEMICAL COMPOSITION OF COPEPODS: SEASONAL VARIATION IN LAGOON-REARED ZOOPLANKTON

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Intensive rearing methods in fry production of coldwater species like cod and halibut require live prey of good quality. Mostly rotifers and *Artemia* are used, but in halibut fry, quality seems to be better in terms of pigmentation and eye migration when copepods are used. This suggests that the nutritional composition of prey may play a major role in larval and juvenile quality, and that copepods provide the best nutrition for the pelagic stages of marine fish. In Norway, copepods are cultured in large lagoon systems, over a period of 5-7 months a year. The important question arising then is what variability exists in the biochemical composition of copepods, both through season, between species, between lagoons, and between years. This investigation deals with all these aspects of variability, and the poster presents some of the data for the first year of the project. Biochemical components analyzed were lipids, fatty acids, proteins, amino acids, pigments, vitamins, and hormones (cortisol and thyroid hormones).

Copepods were collected every fifth day (late May to early November 2000) from the "Svartatjern" lagoon (pond) at the Institute of Marine Research, Austevoll Aquaculture Research Station (approximately 40km southwest of Bergen, Norway). The pond was emptied and refilled with seawater from the outside fjord during mid-February and mid-July. To enhance algal and copepod production, the pond was fertilized with Cl-free NPK complex. A UNIK-900 wheel-filter was used to concentrate and fractionate copepods. Only copepods going through 800-µm but retained on 250-µm mesh sizes were used (mostly CIV-CVI copepod stages). The dominating species were *Eurytemora affinis*, *Centropages hamatus*, and *Acartia grani*. In some periods, more than 80% of the collected copepods were dominated by one of these species.

The copepods were kept alive during sampling for the different biochemical analyses. Copepods were filtered with a slight vacuum on a 60-µm plankton net and rinsed with 1-µm filtered seawater adjusted to 10‰ salinity. This procedure ensured that the copepods were undamaged until frozen either in liquid nitrogen (lipid analyses) or in a -80°C freezer (other analyses).

Copepod dry weight (DW) was on average 15.1% of wet weight through the whole sampling period, varying between 12.8 and 17.6%. The total lipid content was between 7.6 and 22.1% of copepod dry weight, averaging 11.5%. The lipids were dominated by polar lipids (62-68%, mostly PE and PC) in spring and summer, with increasing fraction of neutral lipids during autumn, to about 60% (mostly TAG) in late October. Total n-3 PUFA varied very little and was between 53 and 67% of total lipids. The sum of EPA and DHA averaged 56%, and the DHA/EPA ratio was between 0.88 and 3.23, averaging 2.05. Similarly, the EPA/AA (arachidonic acid) ratio was between 5 and 36%, averaging 20%.

The total protein content was quite stable, around 38% of copepod dry weight (minimum and maximum of 33 and 42%, respectively). Free amino acid (FAA) concentration averaged 464nmol.mg^{-1} DW, varying between 352 and 768nmol.mg^{-1} DW. Levels of FAA were highest in August, during a period of elevated salinity. The most abundant FAAs were arginine, glycine, and taurine, which constituted on average 61% of total FAAs (ranging between 46% and 73%). The essential/non-essential FAA ratio varied between 0.21 and 0.37, averaging 0.32. Astaxanthin was identified to be the most abundant pigment in the copepods, ranging between 11 and 816µg.g^{-1} DW, with an average of 358µg.g^{-1} DW. Astaxanthin constituted on average 82% of total pigments (from 67-87%). Astaxanthin was lowest in August and September, with average contents of 17µg.g^{-1} DW during this period. This was only 3.2% of the average level observed for the rest of the samples.

Among the water-soluble vitamins, mean values of 23, 28, and 473µg.g^{-1} DW were observed for B_1 (thiamin), B_2 (riboflavin), and vitamin C (ascorbic acid), respectively. In the lipid-soluble vitamins, mean observed value of vitamin A (retinol) was 0.2µg.g^{-1} DW, while vitamin E (total tocopherol) averaged 111µg.g^{-1} DW. The most abundant vitamin E was α-tocopherol, accounting on average for 96.2% of the total vitamin E. Vitamin D_3 (cholecalciferol) was not detected in any of the samples. No strong seasonal effect was observed for the vitamins. Correcting for an extraction efficiency of 60%, average levels of thyroid hormones found in the copepods were 0.034 and 0.063µg.g^{-1} DW for T_3 and T_4, respectively. Analyses of cortisol are still in progress. The sampling and analyses will be repeated during 2001. This will give possibilities for year-to-year comparisons of the biochemical content of copepods. This study has been supported by the Norwegian Research Council, project no. 138379/120 and the marine fish producer Norsk Kveite AS, (Austevoll Marine Yngel), N-5392 Storebø, Norway.

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SEASONAL SHIFT IN SPAWNING OF COD BROODSTOCKS BY LIGHT MANIPULATION: EGG QUALITY AND LARVAL REARING

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Fish from two year classes of extensively produced cod (1997 and 1998) were transferred in late January 2000 from net pens to indoor tanks for control of light regime and spawning. In addition to natural light, the 1998 group received continuous artificial light (two 400W underwater metal halogen lights) in the net pen 4.5 months before transfer, while the 1997 group only received natural light. At transfer, the fish were checked for gonad development by ultrasound. The 1997 group was close to spawning at transfer, and 25 fish (10 males and 15 females) were stocked in each of two 3-m (7.5-m^3) tanks. The largest fish were stocked in one tank and the smaller fish in the other. The 1998 group showed no maturation and 40 fish were stocked in each of another two 3-m tanks. Gonad development in this fish was checked again with ultrasound in late June, and as most fish were maturing, spawning stocks were established in the two tanks similar to the 1997 group. All tanks were provided with egg collection units sampling from both the drain and at the tank surface. Once eggs were found in the collectors, feeding was stopped to minimize the organic load in the egg collectors. The indoor light regime was similar for all the tanks. The first three weeks after transfer, light was continuous (two 36W light tubes on the ceiling), then shifted to two 36W tubes 60cm above each tank, controlled by a dimmer (30min L-D) and a manual timer. Within one week, light was displaced 6 months according to the natural light regime.

The 1997 group spawned naturally from late February to mid May. Total amounts of eggs collected from the two tanks were 45.6 and 67.2 l, with a fertilization rate of 53.6 and 54.6% for the small and large fish, respectively. Similarly, total fecundity was 907 and 934 $eggs.g^{-1}$ of pre-spawning body weight. The 1998 group spawned from late July to early November. Total amounts of eggs collected were 34.5 and 62.9 l (24.4 and 22.7% fertilization) for the small and large fish, respectively. Fecundity was close to the spring-spawning fish, with 885 and 913 $eggs.g^{-1}$ of pre-spawning body weight.

The low fertilization rate in the autumn-spawning fish (1998 group) may partly

be related to temperature. Temperature for the spawning stock rose to a maximum of 13.7°C in September, resulting in reductions of fertilization rate when temperature exceeded 10°C. Also, a higher rate of mortality, deformities, and abnormal development was observed in the fertilized eggs when temperature exceeded 10°C. Temperature was then reduced below 10°C in one of the spawning tanks, resulting in a significant increase in fertilization and normal development.

Temperature's important role in regulation of fertilization and egg development was also demonstrated for the 1997 group, which started to spawn again in September. Temperature in one of the spawning tanks for this group was reduced to <8°C, resulting with fertilization and development rates comparable to the spring spawning. In the other tank with temperature >10°C, fertilization and egg development were lower and comparable to the poor results of the autumn spawning in the 1998 group. Monitoring of the second spawning in the 1997 group was not completed, as exhaustion of some individuals required feeding to be restarted.

Several egg batches were incubated and hatched. Yolk-sac larvae were transferred to black 1-m (0.5-m^3) tanks for start-feeding. Continuous addition of algae was used for most tanks, but two groups were also reared without algae. Rotifers (*Brachionus plicatilis*) grown on Rotimac were used as initial food and supplied 1-2 times a day. After 3-5 weeks, standard DHA-Selco-enriched *Artemia* was used along with rotifers for one week, and then shifted to *Artemia* only. Addition of algae was usually stopped at this shift in prey. Rearing temperature was between 10-12°C, and continuous light from an 18W light bulb ca. 70cm above the tank was used (1.08$W.m^{-2}$ at surface, or approximately 250 lux). Stocking densities ranged between 8 and 40 larvae.l^{-1}.

Larvae in the rearing tanks were counted and sampled for growth measurements between day 42 and 51 post-hatch. In the spring, 5 tanks were used for one larval group. High larval mortality occurred in this group (3.1 and 7.7%), probably due to a large temperature difference at transfer from the incubator (6°C) to the rearing tanks (10°C). During start-feeding of the autumn groups, larvae were transferred at equal temperature, with an increase of maximum 1°C per day up to the rearing temperature of 12°C. Four groups were start-fed, each in two replicates. Survival in the first group was 9 and 14%. In the next 3 groups survival ranged between 29.3 to 46.6%, with a density of 4-14.6 ind.l^{-1} at sampling. Larval size at sampling was between 12-15mm. Growth rate was low compared to previously reported growth at the given rearing temperature, indicating a considerable potential for optimization related to larval feeding dynamics and procedures. Another intriguing aspect was that the highest output in terms of larval density occurred in groups with the highest initial stocking densities. Even higher output may be expected with stocking densities above 40 larvae.l^{-1}. Finally, the autumn rearing showed that algae might be reduced to a minimum (less than two weeks after hatching) or not used at all in intensive larval rearing of cod. This study was supported by the Norwegian Research Council, project no. 134069/120.

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DEVELOPMENT OF A NUTRITIONAL FLAKE FOR SHRIMP LARVICULTURE

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Introduction

Flakes for shrimp culture are commonly available in the markets of Asia and the Americas, and have received considerable interest from the industry. However, they are typically designed as low-end products, often aimed at water colouring only. Obviously, low-end flakes have a limited *Artemia*-replacing capacity (ARC). This study summarizes the effort to develop "nutritional" flakes – i.e., flakes with a high nutritional value. This was achieved by careful raw material selection and formulation, but also by the use of an innovative flaking technology which allows maximized use of nutritional ingredients and minimized loss of essential nutrients (e.g., vitamins) during processing. Several diet formulations have been tested during preliminary trials, not presented in this paper. One formula was selected and flaked applying this new technique for comparison with the classic flaking technique. In a first small-scale experimental set-up, the diets were tested at 100% of the total feeding regime. In a second experiment, a large-scale set-up was used to evaluate the ARC of these flakes in typical commercial culture conditions of *Penaeus monodon* postlarvae.

Materials and methods

Experiment 1 (research application). *P. monodon* PL1 were stocked in 150-l circular fiberglass tanks at a density of 60ind.l^{-1} and grown for a period of 20 days. Temperature and salinity averaged 30.5°C and 26g.l^{-1}, respectively. Animals were fed 6 times daily (every 4 hours). Five dietary treatments, with three replicates each, were compared. These treatments consisted of a 100% *Artemia* nauplii control (treatment ART), a reference dry diet fed at 25% and 100% of the total feeding regime on dry weight basis (treatments REF25 and

REF100, respectively), and two treatments with experimental flakes fed at a 100% feeding rate: a flake produced according to a classic flaking technology (treatment FL-classic) and the same flake formula produced with an innovative flaking technology (treatment FL-innov). The reference dry diet fed at 25% (REF25) is the standard positive control used by our research team. Survival, individual length, and individual dry weight were determined at the end of the trial. Statistical analysis was done with one-way ANOVA. Tukey's multiple range test was applied to detect significant differences among means ($P<0.05$).

Experiment 2 (commercial-scale application). The design and environmental conditions in experiment 2 were similar to those described for experiment 1, but with the use of a typical commercial culture protocol. *P. monodon* PL1 were stocked in 2.5-MT concrete, flat bottom tanks at a density of 75ind.l^{-1}. "FL-classic" and "FL-innov" flakes were fed at a 40% *Artemia* replacement level (on a dry weight basis) and compared to treatments ART and REF25.

Results and discussion

In experiment 1, survival rates (Table I) were not significantly different ($P>0.05$) among the *Artemia* control (ART) and the flake treatments FL-classic and FL-innov. However, a tendency towards better performance of treatment ART could be appreciated. Both flake treatments obtained survival rates equal to the reference artificial diet treatments REF25 and REF100. Treatment REF100, however, resulted in a significantly lower larval survival rate ($P<0.05$) than treatment ART. Mean dry weights and lengths (Fig. 1) did not reveal significant differences ($P>0.05$) among treatments. Dry weights and lengths of animals receiving flake FL-innov were higher than those of animals receiving flake FL-classic, although not significantly ($P>0.05$).

Table I. Survival of *P. monodon* postlarvae fed different dietary treatments in Experiment 1 (research application).

	% Artificial diet	Survival (%)
ART	0	93.6^{a} (4.7)
REF25	25	75.9^{ab} (5.2)
REF100	100	62.0^{b} (12.0)
FL-classic	100	69.4^{ab} (7.4)
FL-innov	100	68.9^{ab} (2.5)

Values are means (standard deviations). Values within a column with the same superscript are not significantly different ($P>0.05$).

In experiment 2, survival rates (Table II), dry weights, and lengths (Fig. 2) were not significantly different among treatments (P>0.05). Both flakes have an ARC of at least 40%. Again, there was a tendency (P>0.05) towards better growth performance of the animals fed the flake FL-innov as compared to those fed FL-classic (mean dry weights of 2.044mg and 1.733mg, respectively), confirming the importance of using appropriate flaking techniques. Additionally, the dry weights obtained in the flake treatments were higher, although not significantly (P>0.05), than the dry weight obtained in the positive control REF25. Similar findings were found in a small-scale experiment (results not presented) in which *Artemia* replacement levels of 65% did not affect the larval performance. Further research and development into this area is ongoing.

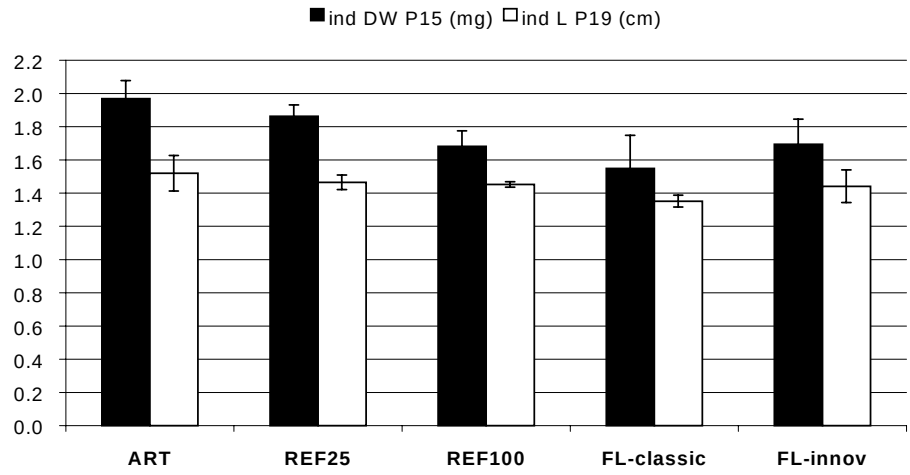

Fig.1. Individual dry weight (DW) and length (L) of *P. monodon* postlarvae fed different dietary treatments in experiment 1 (research application). Standard deviation bars are shown.

Table II. Survival of *P. monodon* postlarvae fed different dietary treatments in Experiment 2 (commercial-scale application).

	% *Artemia* replacement	Survival (%)
ART	0	95.2[a] (11.3)
REF25	25	81.2[a] (17.6)
FL1-classic	40	79.3[a] (5.9)
FL1-innov	40	84.2[a] (10.4)

Values are means (standard deviations). Values within a column with the same superscript are not significantly different (P>0.05).

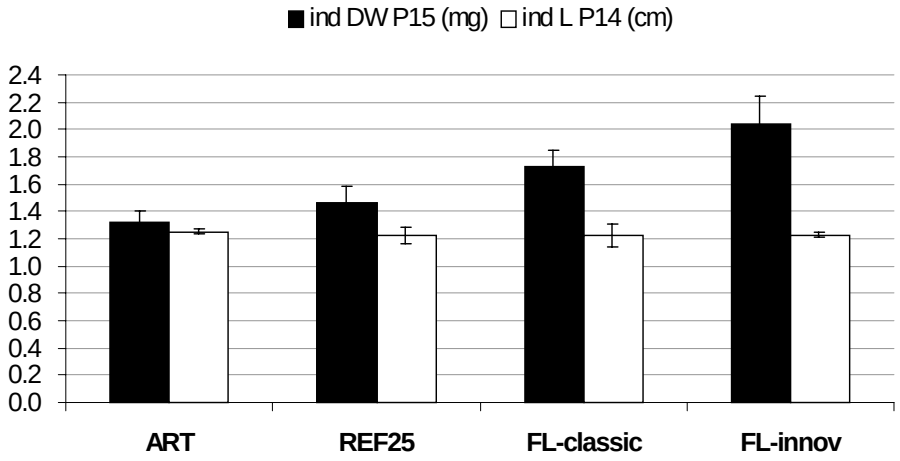

Fig.2. Individual dry weight (DW) and length (L) of *P. monodon* postlarvae fed different dietary treatments in experiment 2 (commercial-scale application). Standard deviation bars are shown.

Conclusion

Contrary to the majority of the shrimp flakes on the market, a flake with a balanced nutritional composition produced with a superior flaking technique can replace live *Artemia* nauplii by at least 40% and help improve the larviculture performance.

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C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
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PHENOTYPIC DIVERSITY AMONGST *VIBRIO* ISOLATES FROM MARINE AQUACULTURE

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Abstract

The total number of 1829 *Vibrio* isolates were collected from different continents. Isolates originated mainly from larvae of molluscs, shrimps, fish, their food (microalgae, *Artemia* sp., rotifers, ration), and culture environment (i.e., tank water, inlet water, sediment). All isolates were phenotypically characterized using the Biolog GN technique. Isolates were found forming 90 clusters of which 33 were identified. Biolog could not distinguish *V. harveyi* from *V. aestuarinus*, *V. alginolyticus* from *V. hollisae*, and *V. ichthyoenteri* from *V. penaeicida*. 57 other groups (481 isolates) did not cluster with any of the included type strains and remained unidentified indicating that the genus *Vibrio* harbors a great diversity of phenotypes and that several taxa from the aquaculture environment are yet to be described.

Introduction

Vibrios are readily isolated in a wide range of marine and estuarine environments including rearing systems (Vandenberghe *et al*., 1999). Many pathogenic *Vibrio* species have become a great threat for fish and shellfish hatcheries, and are responsible for significant economical losses (Austin and Austin, 1999). Although vibrios have been widely reported as opportunistic pathogens (Salvesen et al., 2000), some *Vibrio* species have been found as symbiotic [e.g., *V. halioticoli* and the abalone *Haliotis hannai hannai,* (Sawabe et al., 1995)] or probiotic [e.g., *V. alginolyticus* and larvae of shrimp *Litopenaeus vannamei*, (Vandenberghe et al., 1999)]. Moreover, it was shown that vibrios form the dominant culturable microflora in the gut of fish, molluscs, and shrimps probably playing an important role in digestion and nutrition (Ringo and Birkbeck, 1999; Moss et al., 2000). The proper use of probiotic *Vibrio*

strains and the control of *Vibrio* related epidemics in hatcheries depends on reliable identification systems, which should be built specially for these purposes. Phenotype based techniques for the screening of high numbers of isolates have been intensively used in studies on the diversity of the microflora associated to the aquacultural environment (Moss et al., 2000; Vandenberghe et al., 1998; Vandenberghe et al., 1999), however the shortcomings of this technique for identification of Vibrios have not yet been addressed. Our aims in this study were to analyze the phenotypic diversity among 1829 *Vibrio* isolates from the marine aquacultural environment using Biolog GN.

Material and methods

Strain collection. During the past 16 years, 1829 *Vibrio* isolates from the marine aquacultural environment were collected in all continents. Isolates originated from various species of molluscs (mussel, scallop, oyster), shrimps and fish larvae, sea urchins, microalgae, *Artemia*, rotifers, seaweeds, seawater, ration and from the aquaculture environment (tank water, sediments, incoming water). A full strain list, containing all strain information can be obtained by request to the authors.

Storage of isolates. Suspensions of pure cultures were stored in a deepfreezer at -80°C or in a nitrogen container at -140°C after addition of Marine broth or Trypticase Soy Broth (Becton Dickinson, Cockeysville, USA) supplemented with 2.0% NaCl and 15% glycerol (w/v). All strains were stored at the BCCM™/LMG Culture Collection (Ghent University, Ghent, Belgium).

Isolate characterization. Gram staining was performed using the methodology described by Smibert and Krieg (1981). The further phenotypic characterization was performed using the Biolog® GN technique (Biolog Inc., Hayward, CA., USA) as described by Austin et al. (1995). For identification, the metabolic fingerprints of the isolates were compared to the metabolic fingerprints of 33 *Vibrio* type strains, *Listonella anguillarum*, *Listonella pelagia*, *Photobacterium damselae* sups. *damselae* and *Salinivibrio costicola*. Numerical analysis was performed using the Pearson product moment correlation coefficient and UPGMA (Sneath and Sokal, 1973) clustering method. Clusters were delineated at 80%r and isolates clustering together at this level with type and reference strains were considered to belong to the same species. Clusters that did not harbour type or reference strains were named according to one central isolate in the cluster.

Results and discussion

The 1829 isolates analyzed, clustered in 90 different groups of which 33 could be identified. The other 57 groups (consisting of 481 isolates) did not cluster

with any of the included type and reference strains and thus remained unidentified. These clusters consisted of isolates with completely different phenotypes. A recent study on the diversity of psychrotrophic vibrios in the Japanese coast found that most isolates could not be identified using 16S rDNA RFLP (Urakawa et al., 1999). The present study points out that the genus *Vibrio* harbors a great diversity of phenotypes and that many taxa isolated from the aquacultural environment remain unknown. This is one of the main reasons which could explain the difficulty of identifying isolates through phenotypic based techniques. Besides, it was found that type and reference strains of *V. harveyi* and *V. aestuarinus*, *V. alginolyticus* and *V. hollisae*, and *V. ichthyoenteri* and *V. penaeicida* had very similar Biolog fingerprints and were thus in the same Biolog clusters, indicating that Biolog is of limited value for identification of those species. On the other hand, Biolog seems to be reliable for identification of certain species e.g. *V. cincinnatiensis*, *V. fischeri*, *V. halioticoli*, *V. mediterranei*,*V. parahaemolyticus*, *V. pectenicida*, *L. pelagia*, *Photobacterium damselae* subsp. *damselae* and *S. costicola* which showed distinct and homogeneous Biolog profiles. The application of a polyphasic approach, combining Amplified Fragment Length Polymorphisms (Janssen et al., 1996), 16S rDNA sequencing and DNA-DNA hybridisation is a reliable alternative when dealing with identification of *Vibrio* isolates. Currently, representative isolates from each Biolog clusters are being analyzed using these techniques. New taxonomic proposals in the genus *Vibrio* will be further undertaken based on phenotypic and genomic results.

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References

Austin B. and D.A. Austin. 1999. Bacterial fish pathogens. Disease of farmed and wild fish. Third edition. Springer-Praxis series in aquaculture and fisheries, Chichester.

Austin B., M. Alsina, D.A. Austin, A.R. Blanch, P.A.D. Grimont, J. Jofre, S. Koblavi, J.L. Larsen, K. Pedersen, T. Tiainen, L. Verdonck, and J. Swings. 1995. Identification and typing of *Vibrio anguillarum*: A comparison of methods. Syst. Appl. Microbiol. 18:285-302.

Janssen P., R. Coopman, G. Huys, J. Swings, M. Bleeker, P. Vos, M. Zabeau, and K. Kersters. 1996. Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. Microbiology 142:1881-1891.

Moss S., B.R. LeaMaster, and J.N. Sweeney. 2000. Relative abundance and species composition of Gram-negative, aerobic bacteria associated with the gut of juvenile white shrimp *Litopenaeus vannamei* reared in oligotrophic well water and eutrophic pond water. J. World Aquacult. Soc. 31(2):255-263.

Ringo E. and T.H. Birkbeck. 1999. Intestinal microflora of fish larvae and fry. Aquacult. Res. 30:73-93.

Salvesen I., K.I. Reitan, J. Skjermo, and G. Oie. 2000. Microbial environments in marine larviculture: Impacts of algal growth rates on the bacterial load in six microalgae. Aquacult. Int. 8(4):275-287.

Sawabe T., I. Sugimura, M. Ohtsuka, K. Nakano, K. Tajima, Y. Ezura, and K. Christen. 1998. *Vibrio haliotioli* sp nov, a non-motile alginolitic marine bacterium isolated from the gut of the abalone *Haliotis discus hanai*. Int J. Syst. Bacteriol. 48:573-580.

Smibert R.M. and N.R. Krieg. 1981. General Characterization, pp. 409-443. In: Manual of Methods for General Microbiology, Washington, American Society for Microbiology.

Sneath P.H.A.and R.R. Sokal. 1973. Numerical Taxonomy. In: The Principles and practice of numerical classification, Baltimore, W.H. Freeman.

Urakawa H., K. Kita-Tsukamoto, and K. Ohwada. 1999. 16s rDNA restriction fragment length polymorphisms analysis of psychrotrophic vibrios from japanese coastal water. Can. J. Microbiol. 45:1001-1007.

Vandenberghe J., Y. Li, L. Verdonck, J. Li, P. Sorgeloos, H.S. Xu, and J. Swings. 1998. Vibrios associated with *Penaeus chinensis* (Crustacea: Decapoda) larvae in Chinese shrimp hatcheries. Aquaculture 169:121-132.

Vandenberghe J., L. Verdonck, R. Robles-Arozarena, G. Rivera, A. Bolland, M. Balladares, B. Gomez-Gil, J. Calderon, P. Sorgeloos, and J. Swings. 1999. Vibrios associated with *Litopenaeus vannamei* Larvae, postlarvae, broodstock and hatchery probionts. Appl. Env. Microbiol. 65:2592-2597.

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TOWARDS THE DEVELOPMENT OF A PROTOCOL FOR THE ISOLATION AND SELECTION OF PROBIOTIC BACTERIA FOR MARINE FISH LARVICULTURE

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Introduction

At hatching, the larvae of marine fish generally have a rudimentary digestive tract consisting of a tube with limited digestive capabilities (Timmermans, 1987). At this time, the gut is sterile and the composition of the subsequent intestinal microflora can be attributed to the diet and surrounding water (Munro et al., 1993; Bergh et al., 1994). Although bacteria considered to be part of the normal flora may not harm the fish, other bacteria are considered abnormal and may lead to disease (Salvesen et al., 1999). The known disease conditions in larviculture are closely related to the type of competition between pathogens and the healthy microflora, as well as to the species and development stage of the fish.

Current techniques used in the isolation and selection of probiotics are simple and usually only involve the ability of the organism to produce metabolites which are antagonistic to pathogens. Although this ability is important for selection, other aspects such as growth rate, attachment ability and production of beneficial compounds need to be considered (Ringø and Birkbeck, 1999).

Materials and methods

Bacteria from the stomach and intestine of five adult common clownfish (*Amphiprion percula*) were isolated on a variety of media (Marine Agar, TCBS Agar, MacConkey Agar, *Pseudomonas* isolation agar, MRS agar) under sterile conditions. After 48h incubation, colonies were then streaked onto Marine Agar.

To test for the production of anti-microbial metabolites by the isolates, eight known fish pathogens (Table I) were incubated in marine broth and made into pour plates. Wells were cut into the agar and marine broth cultures of the isolates

were placed into them. The presence of anti-microbial metabolites produced by the isolates inhibited the growth of the pathogen, producing a clear zone of inhibition around the well.

For the growth studies, the 23 bacteria found to produce anti-microbial metabolites were cultured in marine broth, and then inoculated into sterile broth placed in a 96-well microtitre plate reader with diode array and incubation facilities. Each isolate was inoculated in triplicate and the pathogens in duplicate. The absorbance was recorded every thirty minutes for a total of 46 hours at a primary wavelength of 600nm at a constant 26°C.

The growth curves for each bacteria were averaged and the specific growth rate (μ) and doubling time were calculated from the semilog plots of absorbance (Table I).

Results and discussion

Table I. Specific growth rates (μ_n), doubling times and antagonistic potential of seven probionts (AP1-AP7) and eight fish pathogens. Pathogens were not tested against each other in antagonism study.

Bacteria	Inhibition of pathogens (listed below)	μ_1	Doubling time (hh:mm)	μ_2
AP2	8	0.392	1:46	
AP5	1, 2, 3, 5, 6, & 7	0.342	2:02	
AP4	3, 7 & 8	0.204	3:24	
AP6	1, 2, 3, 5, 6, & 7	0.174	3:59	
AP7	8	0.148	4:41	
AP3	3 & 7	0.144	4:49	
AP1	4	0.091	7:39	0:12
1. *Vibrio anguillarium*		0.289	2:24	
2. *Aeromonas salmonicida*		0.287	2:25	
3. *Vibrio damsela*		0.272	2:33	
4. *Vibrio harveyi/alginoloyticus*		0.252	2:45	
5. *Vibrio harveyi*		0.248	2:48	
6. *Aeromonas hydrophila*		0.212	3:16	
7. *Vibrio damsela* (2^{nd} strain)		0.186	3:43	
8. *Carnobacterium piscicola*		0.145	4:47	

Of the 75 isolates tested, 23 displayed zones of inhibition to the pathogens. From the growth study, a number of strains were excluded, as they appeared to have similar antagonistic properties and growth characteristics to other strains. Seven candidate probionts (named AP1 to AP7) were selected (Table I) based on their

different antagonistic properties and growth curves.

Probiont AP1 showed a multiphase growth curve (Table I), suggesting an ability to switch its energy source once the first choice of substrate has been completely utilized. Although it had the slowest doubling time, it showed antagonism to *V. harvey/alginolyticus*, and its ability to utilize various energy sources may increase its capacity to out-compete pathogens.

The probionts AP2 and AP5 both had specific growth rates greater than any of the pathogens, suggesting that they should be able to out-compete any of the tested pathogens either by producing antagonistic metabolites and/or by growing quicker.

This information will be used in subsequent experiments to further refine the selection of suitable probionts depending on their growth characteristics and ability to utilize different energy sources.

Conclusions

These experiments mark the beginning of a protocol for the selection of suitable probiotics in larviculture. The initial step of isolating bacteria that produce anti-microbial metabolites is common practice. However, experiments determining at what stage of growth the bacteria produce the anti-microbial metabolites and whether the bacteria are able to compete for attachment sites (Vanbelle et al., 1990) have been rarely performed. These experiments are necessary as bacteria may only produce the metabolites during the stationary phase (Monaghan et al., 1999), which may not be achieved in the gut due to constant flushing but may occur *in vitro* thereby creating a false impression of their ability to inhibit pathogens. Additionally, the inability to compete for attachment sites on the gut wall suggests that the bacteria may be flushed out at a rate greater than they are able to multiply.

Current experiments are investigating the growth rates of the isolates and pathogens in intestinal mucus and the adhesion of the bacteria to intestinal mucus. Competitive exclusion experiments are being conducted to further narrow down the selection of suitable probionts. Experiments investigating at what stages of the growth cycle anti-microbial metabolites, essential fatty acids and enzymes are being produced will be performed as will species identification of the probionts using 16s mRNA analysis.

Once a “suite” of suitable probiotic bacteria has been identified, further *in vitro* and *in vivo* experiments will be done on clownfish larvae to establish whether the protocol can be used effectively for clownfish and other marine fish species.

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References

Bergh Ø., K.E. Naas, and T. Harboe. 1994. Shift in the intestinal microflora of Atlantic halibut (*Hippoglossus hippoglossus*) larvae during first feeding. Can. J. Fish. Aquat. Sci. 51:1899-1903.

Monaghan R.L., M.M. Gagliardi, and S.L. Streicher. 1999. Culture preservation and inoculation development. p.29-48. In: Manual of industrial microbiology and biotechnology. A.L. Demain and E. Julian (Eds.) American Society for Microbiology, Washington.

Munroe P.D., T.H. Birkhead, and A. Babour. 1993. Influence of the rate of bacterial colonisation of the gut of turbot larvae on larval survival. p.85-92. In: Fish Farming Technology. H. Reinertsen, L.A. Dahle, L. Jørgensen, and K. Tvinnereim (Eds.) A.A. Balkema, Rotterdam.

Ringø E. and T.H. Birkbeck. 1999. Intestinal microflora of fish larvae and fry. Aquacult. Res. 30:73-93.

Salvesen I., J. Skjermo, and O. Vadstein. 1999. Growth of turbot (*Scophthalmus maximus* L.) during first feeding in relation to the proportion of *r*/K-strategists in the bacterial community of the rearing water. Aquaculture 175:337-350.

Timmermans L.P.M. 1987. Early development and differentiation in fish. Sarsia 72:331-339.

Vanbelle M., E. Teller, and M. Focant. 1990. Probiotics in animal nutrition: a review. Arch. Anim. Nutr. 40:543-567.

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HIGH-HEALTH LARVAL REARING OF MARINE SHRIMP *FENNEROPENAEUS CHINENSIS* IN CHINA

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Introduction

High-health larval (HHL) rearing is of increasing importance for a sustainable developing shrimp farming industry, especially considering the challenges the global industry has had to deal with in recent years. The marine shrimp *Fenneropenaeus chinensis* has been the most widely cultured species in Northern China, with a highest annual production of around 200 000 metric tones in the early 1990's. Every effort was made to recover this industry since 1993, when serious disease problems were suffered by the industry. High-health larvae are one of the critical elements considered to be the basis for healthy shrimp farming. This presentation briefly introduces the protocols we employed to produce high-health *F. chinensis* larvae, including using genetically improved and/or selectively bred spawners, treating fertilized eggs with organic disinfectant to inactivate possible pathogens, feeding larvae with tested feed, carefully controlling water quality during larval rearing (especially for desirable microalgal composition and manipulation), etc. Some biotechnological methods such as PCR and DNA probes were developed and used for ensuring the high-health larval rearing of *F. chinensis.*

Materials and methods

Two populations were genetically selected in order to cultivate HHL in *F. chinensis.* The first population was aimed at growth rate selection (GRS). The initial spawners were selected from wild populations in the Yellow Sea. Selective intensity for GRS was controlled to an index of 3-5%. The second population was aimed at disease resistance selection (DRS). The spawners were selected from farming ponds where serious WSSV infection occurred and most shrimp died. The remained survivors were collected and used for DRS purposes.

An elaborately designed protocol based on a series of experiments and practices was adopted for HHL rearing in shrimp hatcheries. Eggs were treated with organic

disinfectants to inactivate possible pathogens after spawning. All feed used during larval rearing – including microalgae, rotifers, *Artemia* and artificially produced microfeeds – were tested regularly, especially for live feed used during mysis and postlarval stages. Seawater was thoroughly treated by filtration and inorganic disinfectants to prevent possible pathogens from invading the hatchery system.

Results and discussion

Selective breeding on growth rate of shrimp *F. chinensis* has been conducted since 1997, and promising results were achieved. After 4 generations of selection, selected populations showed obvious performance benefits over the control population in terms of both average body length and body weight (Table I).

Table I. Average performance of *F. chinensis* after 4 generations of selection.

Pond No.	Materials	Body length range (cm)	Average body length (cm)	Average body weight (g)	Average body length increase (%)	Average body weight increase (%)
I-1	Selected	11.0-16.4	13.97	32.35	10.79	16.45
I-2	Selected	11.8-16.2	13.66	36.67	8.33	32.00
I-6	Selected	11.8-15.2	13.22	32.26	4.84	16.13
I-7	Selected	12.0-15.6	13.47	32.67	6.82	17.60
	Average	11.0-16.4	13.58	33.49	7.70	20.55
II-10	Control	9.4-14.4	12.61	27.78	–	–

Selective breeding on disease resistance of *F. chinensis* dramatically increased the survival rates in both larval rearing and growout stages. In a WSSV challenge test, the population from the third selected generation showed a much higher survival rate than unselected shrimp (Fig. 1).

Disease control was essentially conducted in two categories: pathogen detection and health management. New techniques such as PCR, DNA probes, and T-E stain were used to detect pathogens during the larval rearing period. Well-received WSSV dot blot diagnostic kits were developed for quickly checking the existence of WSSV during larval rearing. Health management refers to the employment of ecological manipulation measures during shrimp larval rearing to maintain desirable levels of DO, pH, salinity, temperature, microalgal composition and density, COD and NH_3-N, and so on, and prevention of bacteria and viral diseases from seriously infecting the larvae.

Results showed that growth rate and disease resistance of shrimp *F. chinensis* are genetically inherited. Although the genetic variation is low, progress could be achieved after successive generations of selection. Modern biotechnology has a wide application in the development of shrimp farming industry, and classic selective breeding also may play an important role in healthy shrimp farming. Both population-selective and family-selective breeding are important for obtaining desirable spawners and rearing high-health shrimp larvae.

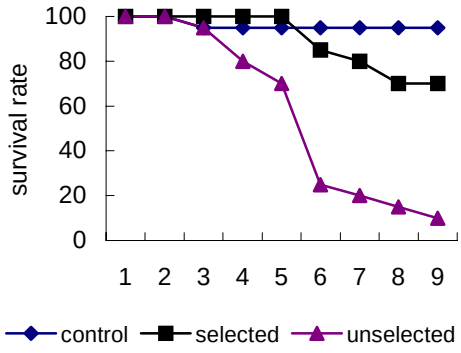

Fig. 1. WSSV challenge test of selected and unselected *F. chinensis*.

A sustainable developing shrimp farming industry depends on healthy shrimp larvae. Results showed that using selectively bred and/or genetically improved spawners were very important for producing healthy larvae. Although the studies were only carried out for four years, and some aspects need to be further improved, these initial results have established a foundation for further investigation. With the increasing human population and market demand, the shrimp mariculture industry undoubtedly has a bright future. What we need to do is to keep the industry developing towards sustainability, stability, and healthiness. New technology – especially biotechnology –together with classic techniques will play an even bigger role than ever in the development of the shrimp farming industry.

References

Crocos P., N. Preston, and S. Lehnert. 1999. Genetic Improvement of Farmed Prawns in Australia. The Advocate 2(6):62-63.

Gjedrem T. 1997. Selective breeding to improve aquaculture production. World Aquacult. 28(1):33-45.

Karunasagar I., 1998. Applications of Biotechnology in Aquaculture. Aquaculture Asia 3(3):5-9.

Khan T.A. and M.P. Bhise. 2000. Genetic Improvement of Farmed Shrimp for Sustainable Aquaculture. INFOFISH International 2000, No.1:41-44.

Wang Qingyin, Cai Shengli and Li Jian. 1997. The shrimp farming industry in China. World Aquacult. 28(1):23-29.
Wyban J. 1999. Selective Breeding for TSV-Resistant Shrimp. The Advocate 2(6):30.

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BROODSTOCK NUTRITION RESEARCH ON MARINE FINFISH IN JAPAN

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Abstract

Broodstock diet formulations are essential for the development of marine fish breeding and propagation. Japanese research has focused on four commercially important species and indicated that the required quantities of essential dietary components may vary according to species. The more recent work carried out on yellowtail and striped jack consolidated the trend already observed in red sea bream. In yellowtail, soft-dry pellets were used and astaxanthin was the determining factor for good egg quality, albeit at a critical supplemental level of around 30mg.kg^{-1}. Paprika powder supplementation further improved the spawning performance of yellowtail in terms of egg production, egg quality and larval survival. Squid meal inclusion also showed potential as an effective ingredient. Work on the development of dry pellets for striped jack broodstock revealed that egg production and quality were affected by separate nutrients. Even though striped jack eggs did not contain carotenoids, dietary astaxanthin increased fecundity whereas egg quality was improved by squid meal. The combination of these two ingredients in dry pellets produced a spawning performance matching that of the widely used raw fish mix. Fatty acids, especially n-3 HUFA in fertilized eggs were dependent on dietary lipids for all the species studied.

Gonad development of different marine fish is dependent on specific needs and the present information can be utilized for the development of wholesome dry pellet diets for different species of marine broodstock.

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ARACHIDONIC ACID REQUIREMENTS FOR LARVAL SUMMER FLOUNDER (*PARALICHTHYS DENTATUS*)

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Introduction

Despite the incorporation of n-3 highly unsaturated fatty acids (HUFA) into the enrichment protocols of live feeds, the larval stage for summer flounder continues to be a period characterized by high mortality and susceptibility to environmental stress. Recently, the long chain n-6 HUFA arachidonic acid (20:4n-6, AA) has been implicated as an essential fatty acid for a variety of developing marine species (Copeman et al., 1999, Estevez et al., 1999, Koven et al., 2001). Given the role of AA as a precursor to eicosanoid biosynthesis and the impacts its metabolites may have on the hypothalamic-pituitary-interrenal (HPI) axis (Abou-Samra et al., 1986, Bernardini et al., 1989), its incorporation into live feed enrichment protocols was examined. The present study intends to evaluate the dietary effect of various AA levels on the survival, growth, and resistance to salinity stress of larval summer flounder.

Materials and methods

Table I. Experimental emulsions and levels of fatty acids (%) used in the enrichment of rotifers and *Artemia*. Emulsions containing AA have fixed DHA/EPA ratios of $3.52.1^{-1}$. All emulsions contain 5% Soya PC, 2% Tween 80, 2% alginic acid, 1% vitamin C, 1% vitamin E, and 1% silicone.

Diet	AA0	AA3	AA6	AA9	AA12
EPA	0	6.6	6.6	6.6	6.6
DHA	0	23.2	23.2	23.2	23.2
AA	0	3	6	9	12
Olive Oil	88	22.5	15	7.5	0
DHA/AA	0	7.73	3.87	2.58	1.93

Experimental emulsions were synthesized from heterotrophically produced and

commercially available eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA), and AA-phospholipid oils (Martek Biosciences, Columbia, MD). Olive oil was used as a negative control in order to keep emulsions isolipidic (Table I).

In the first experiment, summer flounder eggs were obtained from conditioned broodstock held at the University of Rhode Island's Narragansett Bay Campus. Hatched larvae were transferred to five 75-l aquaria (one unfed control) and reared in green water, where they were fed enriched rotifers (*Brachionus plicatilis*) from 3-7 days after hatch (dah). Rotifers were cultured on baker's yeast and *Tetraselmis* sp., and harvested fractions were enriched with each treatment emulsion as described by Koven et al. (2001) and fed twice daily at 5 rotifers.ml^{-1}. At 8dah, larvae were taken from the five treatment tanks and randomized into 2-l bowls at 50 larvae.l^{-1}, with five replicates per treatment. The smaller bowl size allowed for greater control of prey density, ensuring larvae were not feeding on unenriched prey. Daily water exchanges (80%) and algal additions were performed. Larvae were sampled on 18dah for survival, growth, and tolerance to a salinity stress test (70ppt) as described by Dhert et al. (1992). All parameters were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's HSD *post hoc* test where necessary.

In the second experiment, the role of AA in late larval development was examined as a function of pre-enrichment at the rotifer stage. Hatched larvae were reared through the rotifer stage in 75-l aquaria, with half of the larvae receiving AA0 and the other half receiving the best level of AA as determined in the first experiment. Larvae were then fed a combination of unenriched 24 and 48h *Artemia* for a period of 8d, and then each group was randomized into 2-l bowls where larvae received 72h *Artemia* enriched with each of the five AA treatment emulsions as described by Koven et al. (2001). The experiment was performed in triplicate; enriched *Artemia* were fed twice daily with densities maintained at 1 nauplius.ml^{-1}, and daily water exchanges (80%) were performed. Larvae were sampled at 47dah for survival, growth, and tolerance to a salinity stress test (80ppt) as previously mentioned. Parameters were analyzed via a between-subjects factorial ANOVA (2×5), followed by Tukey's HSD *post hoc* test for significant main effects and simple effects tests for significant interaction effects. Within each experiment, enriched rotifers, *Artemia*, and larvae were sampled in triplicate for fatty acid methyl ester (FAME) analysis via gas chromatography.

Results and discussion

There were no significant differences in the growth or survival of larvae fed enriched rotifers in the first experiment. However, larvae fed AA6-enriched rotifers showed significantly greater survival in a salinity stress test at 18dah

than larvae fed AA0- and AA3-enriched rotifers (Table II). This AA-stimulated enhancement of stress tolerance may result from the direct stimulation of the HPI axis or through the mediation of osmoregulatory function via metabolites of AA, such as prostaglandins.

Table II. Mean values (± SE) of survival (%), individual weight (mg), and survival in the salinity stress test (%) of larvae fed rotifers enriched with the AA emulsions (18dah). Values in the same column with a common superscript are not significantly different at $P<0.05$ (Tukey HSD).

Diet	Survival	Weight	Survival in stress test
AA0	66.8 ± 12.0^{a}	0.14 ± 0.013^{a}	44.0 ± 6.0^{a}
AA3	62.4 ± 12.0^{a}	0.18 ± 0.020^{a}	67.5 ± 5.6^{b}
AA6	62.4 ± 7.9^{a}	0.21 ± 0.029^{a}	92.0 ± 5.8^{c}
AA9	64.4 ± 7.1^{a}	0.18 ± 0.015^{a}	88.0 ± 3.7^{bc}
AA12	72.4 ± 4.5^{a}	0.24 ± 0.015^{a}	88.0 ± 3.7^{bc}

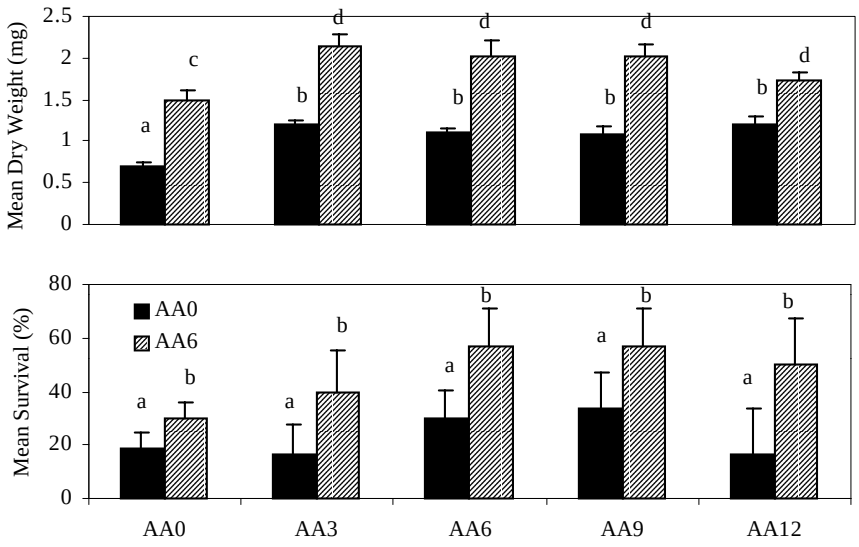

Fig. 1. Mean dry weight (top) and survival (bottom) in a salinity stress test of larvae fed *Artemia* enriched with various AA emulsions after receiving either AA0 or AA6-enriched rotifers early in larval development. Error bars represent standard error of the means. Values with the same letter are not significantly different at $P<0.05$ (Tukey HSD).

In the second experiment, larvae receiving AA6-enriched rotifers showed significantly greater growth (mean length and dry weight) over larvae fed AA0-enriched rotifers prior to being fed the enriched 72h *Artemia*. Large variation

may have masked this significance in the previous experiment. Pre-enrichment with AA6 rotifers proved to be a significant main effect in the survival, growth, and stress tolerance of larvae fed enriched *Artemia*. An optimal level of AA could not be significantly defined over any of the parameters, although larvae fed AA0-enriched *Artemia* showed significantly decreased growth over any other treatment (Fig. 1). Thus the importance of AA in early larval nutrition is evident, although its role may not be equally effective throughout larval development.

The analysis of FAME samples is still ongoing, although data suggest (Koven et al., 2001) that rotifer, *Artemia*, and larval fatty acid levels and ratios should reflect those contained in the emulsions.

Conclusions

The incorporation of AA at a level of 6% into the enrichment protocols of rotifers may serve to increase stress tolerance in early summer flounder larval development, while increasing survival, growth, and stress tolerance in late larval development.

References

Abou-Samra A.B., K.J. Catt, G. Aguilera. 1986. Role of arachidonic acid in the regulation of adrenocorticotropin release from rat anterior pituitary cell cultures. Endocrinol. 119:1427-1431.

Bernardini R., A. Chiarenza, A.E. Calogero, P.W. Gold, and G.P. Chrousos. 1989. Arachidonic acid metabolites modulate rat hypothalamic corticotropin-releasing hormone secretion in vitro. Neuroendocrinol. 50:708-715.

Copeman L.A., C.C. Parrish, J.A. Brown, M. Harel, and J. Parsons. 1999. Bull. Aquacul. Assoc. Canada 99-4:19-21.

Dhert P., P. Lavens, and P. Sorgeloos. 1992. Stress evaluation: a tool for quality control of hatchery-produced shrimp and fish fry. Aquacult. Eur. 17:6-10.

Estevez A., L.A. McEvoy, J.G. Bell, and J.R. Sargent. 1999. Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live-prey enriched in arachidonic and eicosapentaenoic acids. Aquaculture 180:321-340.

Koven W., Y. Barr, S. Lutzky, I. Ben-Atia, R. Weiss, M. Harel, P. Behrens, and A. Tandler. 2001. The effect of dietary arachidonic acid (20:4n-6) on growth, survival, and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. Aquaculture 193:107-122.

LARVI '01 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
European Aquaculture Society, Special Publication No. 30, Oostende, Belgium, 2001

ULTRAHIGH-DENSITY CULTURE SYSTEM OF THE ROTIFER, *BRACHIONUS ROTUNDIFORMIS*

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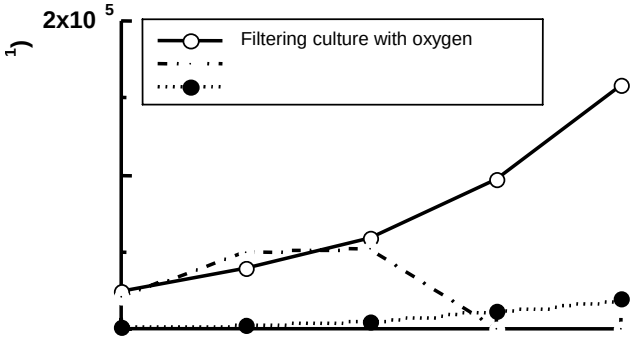
We developed a high-density mass-culture system of marine rotifers using commercial condensed freshwater *Chlorella*, oxygen-gas feeding, and pH control. This culture system enabled stable cultures at the several-ten-thousand-rotifer.ml^{-1} level. The system has been gradually prevailing in the larviculture field in Japan. Recently, we further developed a novel culture system designed for ultrahigh-density production.

In the novel culture system, a sophisticated water exchange unit was incorporated in a culture tank (40 l) and its production performance was tested in the laboratory. The water exchange unit comprised a microfiltration membrane unit (0.4-µm pore size) and pumps for filtration of culture water and seawater supply. Feeding of oxygen gas (95% purity, 0.075-0.125 vvm) was essential for maintaining high permeability of the filter. The culture broth was filtered out with a time interval of



5-s suction and 10-s pause. Freshwater *Chlorella* paste (*C. vulgaris*, PV: 600ml.l^{-1}) was fed continuously into the culture water using a peristaltic feeding pump at a feed rate of 3.8-8.0 l.tank^{-1}.day. So-called S-type rotifers, *Brachionus rotundiformis*, were cultured for 4 days in batch using the system with or without filtration and oxygen supply. The filtration unit was washed daily in NaClO solution (1% active ingredient) for 30min and then reused.

Fig. 1 shows the comparison of rotifer productions between filtering and ordinary cultures in 4 days. The ordinary batch culture without filtration crashed on the third day. The filtration culture without oxygen reached 20 000 rotifer.ml^{-1} in 4 days when they were inoculated at 800 rotifer.ml^{-1}. The filtering culture tank with oxygen supply showed stable rotifer growth and reached to 159 000 ind.ml^{-1} in 4 days when the culture was started at the rotifer density of 24 000 ind.ml^{-1}. The gross and net rotifer production obtained in this novel system (40 l) in 4 days was 6.36×10^{9} and 5.39×10^{9} rotifers, respectively.

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DELIVERING BIOACTIVE COMPOUNDS TO FISH LARVAE USING MICROCAPSULATED DIETS

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Some compounds, such as nutrients and hormones, have special relevance to the research and development of rearing technologies for larval and juvenile fish. One main goal when working with these substances is to find an effective method of administration into the body of small aquatic animals. The usual method has been to include them in the food. When using inert food, the food particles need to be water-stable but digestible by the poorly developed gut of early larval fish. In this study, three different compounds (amino acid, hormone, and vitamin) were incorporated into a protein-walled microencapsulated diet. The usefulness of this kind of particle for larval fish nutrition was evaluated. Specifically, this microencapsulated diet was examined for (i) absorption and leaching patterns of the free amino acids, (ii) the kinetics of incorporation of estradiol in *Sparus aurata* larvae, and (iii) growth results in relation to the supplementation of vitamin C in larvae of *S. aurata* and *Solea senegalensis*.

All three compounds were incorporated into the microcapsules. The efficiency of inclusion, however, can be low, but the capsules were able to retain the compounds when immersed in water and successfully deliver them into the digestive tract of the larvae. These results indicate the applicability of this type of microencapsulation in nutritional studies in small aquatic animals. Replacement of live prey in the rearing of larval fish and the role of the present studies using specific ingredients in microdiets are discussed.

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European Aquaculture Society, Special Publication No. 30, Oostende, Belgium, 2001

EFFICIENCY OF THE INCLUSION OF PROTEASES IN MICROCAPSULES USED IN LARVAL FEEDING OF MARINE FISH

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Introduction

Substitution of live feed during the larval rearing of fish until weaning onto commercial compound feeds is one of the main challenges of current marine fish culture. It is widely accepted that larval food digestion during the first days of life is acutely limited by their lack of a well-developed enzymatic system, and this point is overcome by the exogenous delivery of enzymes supplied by the ingestion of live prey. Although recent research has demonstrated that larvae of some species are able to produce enough proteases to digest a compound food (Moyano et al., 1996), an important limitation remains during the two or three days after mouth opening. At this early larval stage, the gut is still unable to produce enough enzymes to ensure digestion of an inert food, thus the presence of exogenous enzymes in feeds may determine its partial autohydrolysis, enhancing its further digestive utilization by larvae. In fact, inclusion of proteases in larval feeds has been correlated with a better nutritive utilization and growth (Kolkowski et al., 1993). Nevertheless, some technical issues, like the selection of the enzyme source or stability of enzyme after processing, must be considered as a basis for further biological evaluation. In the present paper, three different enzyme sources were tested in order to determine their potential application as additives in the formulation of microcapsules used in feeding of sea bream (*Sparus aurata*) larvae.

Materials and methods

Microcapsules (MC) were manufactured as described by Yúfera et al. (1999) by interfacial polymerization of the dietary protein. The diameter of the particles ranged from 50-350μm and their gross energy content was similar to that of live prey. Three different enzyme sources were selected for microcapsule protease supplementation: bovine pancreatin (BP; Sigma P7545), cod trypsin (CT; Sigma T9906), and a semi-purified extract obtained from the intestine of adult sea

bream (SE). The level of inclusion of each enzyme source was calculated after determination of its specific activity, in order to reach an equivalent activity in the microcapsules.

The presence and level of protease activity finally retained by the MC and the permanence of such activity after immersion in seawater for a 2-h period was tested using a standard protease assay, as described in Martinez et al., (1999). The autohydrolysis of microcapsules, including enzymes, was tested *in vitro* using an automated pH-stat device (718 STAT TITRINO; Methrom, Ltd.) and expressed as ml of NaOH needed to compensate the variation of pH due to protein hydrolysis.

The MC were used to feed different groups of sea bream larvae, and the growth and survival was recorded in each case. The larvae were maintained in 300-l tanks provided with a continuous supply of water, being fed only on microcapsules from first feeding. Each type of protease-supplemented MC was tested separately. Two groups of larvae, one fed only on live prey and the other fed only on microcapsules not including proteases, served as positive and negative controls, respectively. Each experiment was performed in triplicate. During the course of the experiment, larvae were sampled and the activity of their digestive proteases was tested using the methodology described in Martínez et al. (1999).

Results and discussion

Preliminary tests showed that the ratio of protease activity among CT, BP, and SE was 5:4:1. This result was used to include each enzyme in a sufficient amount to ensure the same initial level of protease activity in the MC. Nevertheless, after elaboration the remaining activity was 1.61, 2.19, and 2.49mU.mg^{-1} for MC including BP, CT, and SE, respectively (Fig 1). This confirmed a different efficiency for each protease source, which should be affected in a different manner by the chemicals used in the process of microencapsulation. After immersion in seawater for 2h – an ample time interval to ensure ingestion by larvae – MC retained a significant amount of the initial activity (70-80% during the first hour and 50-60% after the second hour). In the case of MC containing SE, no significant activity loss was recorded (Fig 1). Those results show the suitability of this microencapsulation process for delivering active enzymes to the larvae. The presence of other compounds (i.e., proteins, lipids, etc.) in the semi-purified extracts of sea bream probably offered a better protection against the chemicals used in the elaboration, as well as a better cross-linking with the casein. This resulted in both a higher remaining activity and a better stability of such enzyme activity when compared to purified enzymes. The autohydrolysis of MC under controlled conditions (pH 8.5, 25°C), simulating the intestine of larvae in absence of any other source of enzyme,

showed that MC containing enzymes were degraded from two to three times more quickly than those not containing enzymes.

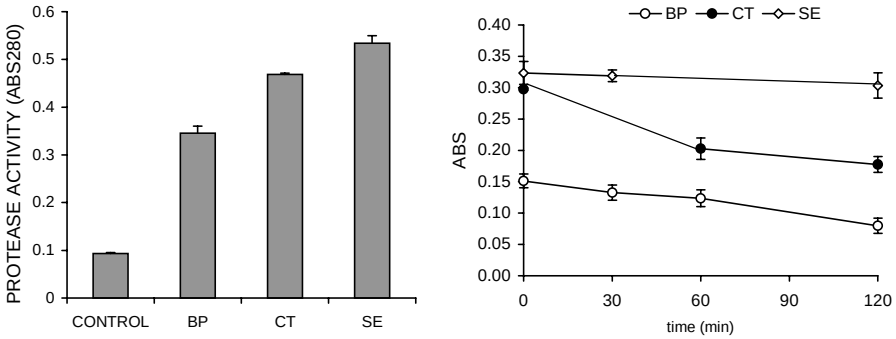

Fig. 1. (Left) Protease activity retained in each type of microcapsule. (Right) Protease activity retained in microcapsules after submersion in seawater.

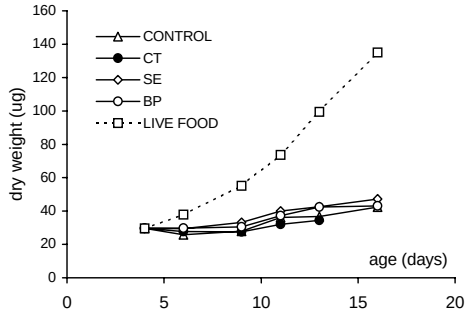

Fig. 2. Growth of larvae fed on the different experimental diets.

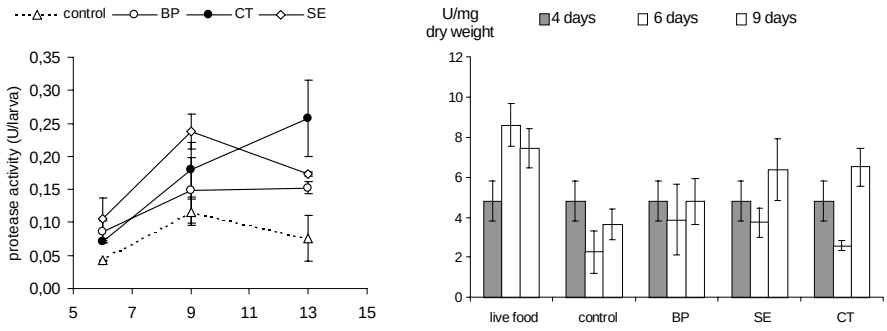

Fig. 3. (Left) Protease activity in larvae fed on the different types of microcapsules. (Right) Protease activity measured at different ages in larvae fed on live feed or the experimental microcapsules.

Larvae fed on artificial food duplicated their biomass during the experimental period, although their final weight represented only about one third of that reached by larvae fed on live food (Fig 2). Nevertheless, no significant differences were found in the growth and survival of the different groups of larvae fed on MC. Total protease activity measured in larvae is shown in Fig. 3. In all cases, larvae fed on MC containing enzymes showed a greater protease activity than those fed on enzyme-free MC. When activity was expressed as U mg per dry weight (Fig 3), significant differences were obtained when comparing protease activity in larvae fed on MC not including enzymes and those including CT or SE.

Taking into account those results, the possibility of including active enzymes in the MC exists, considering that the type and source of enzyme greatly influences its further activity. On the other hand, feeding of larvae with MC containing enzymes seems to positively affect their ability to digest protein, through a significant increase in the amount of active enzyme present in their digestive tract.

The optimization of the formulation of MC to ensure a better delivery of enzymes is the subject of experiments currently in progress.

Acknowledgements

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References

Kolkowski S., A. Tandler, W. Kissil, and A. Gertler. 1993. The effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata*, Sparidae, Linnaeus) larvae. Fish Physiol. Biochem. 12:203-209.

Martínez I., F.J. Moyano, C. Fernández-Díaz, and M. Yúfera. 1999. Digestive enzyme activity during larval development of the Senegal sole (*Solea senegalensis*) Fish Physiol. Biochem. 21:317-323.

Moyano F.J., M. Díaz, F.J. Alarcón, and M.C. Sarasquete. 1996. Characterization of digestive enzyme activity during larval development of gilthed seabream (*Sparus aurata*). Fish Physiol. Biochem. 15:121-130.

Yúfera M., E. Pascual, and C. Fernández. 1999. A highly efficient microencapsulated food for the rearing early larvae of marine fish. Aquaculture 177:249-256.

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RAW AND UNPROCESSED NURSERY DIET FOR *PENAEUS MONODON* POSTLARVAE

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Introduction

Advantages of nursery rearing have been acknowledged for crustacean aquaculture and include increased control, efficiency, predictability, and profit in both phases of production (Aquacop, 1985a; Pretto, 1983). Aquacop (1985b) cited the need for a nursery phase between the hatchery and final production to provide a better assessment of stocked shrimp. Stocking juvenile shrimp, as opposed to direct stocking of postlarvae, enables managers to more accurately predict survival, standing crop, feeding rates, and production levels in growout ponds.

Among all management parameters, food and feeding is one of the indispensable portions. The nutritional quality of the diets provides the foundation of shrimp larval nursing and can largely determine the success or failure of nursing. The food must provide the larvae's basic nutritional requirements. Penaflorida (1989) successfully studied the growth evaluation of *P. monodon* fry by using different unprocessed raw ingredients as nursery diet and he found view, the raw unprocessed diets are cheaper than that shrimp meal and squid meal gave better results. From an economic point of any commercially produced nursery diet. In Bangladesh, information regarding the nutritional requirements of *P. monodon* larvae at the nursery phase is lacking. The present paper discusses the possibility of using different unprocessed raw diets as nursery diets for *P. monodon* postlarvae.

Materials and methods

The feeding experiment was conducted in 10 glass aquaria for 30 days. *P. monodon* postlarvae were placed in each aquarium at the density of 25 larvae.l^{-1}. All the experimental diets were assigned randomly and in duplicate among the 10 aquaria so that 5 different treatment groups were made. Daily rations were calculated on the basis of body weight. All the larvae were fed 3 times a day. Uneaten food was removed from the aquaria every day before the first feeding.

During the experimental period, salinity, dissolved oxygen, temperature, pH, and nitrite were measured daily. At least 30% of the water from each aquarium was replaced by new water every day.

Five different diets were used in each experiment (Table I).

Table I. Composition of diets used in the two experiments.

Raw diets used in experiment 1	Dry diets used in experiment 2
Squid (*Loligo* sp.)	Squid (*Loligo* sp.)
Hilsa fish muscle (*Tenualosa ilisha*)	Hilsa fish muscle (*Tenualosa ilisha*)
Shrimp head (*P. monodon*)	Shrimp head (*P. monodon*)
Mixture of hilsa muscle and shrimp head (1:1)	Mixture of hilsa muscle and shrimp head (1:1)
Dry nursery diet (commercially produced)	Dry nursery diet (commercially produced)

Each diet was finely ground and sieved through the appropriate mesh size. Particle size of the diet was adjusted according to the growth of the larvae

At the start of the experiment, 20 larvae from the initial population were measured for weight and length to calculate initial weight and length of the population. At 5-day intervals, 20 larvae from each treatment group were sampled during the experimental period for measurement. Following the same procedure, experiment 2 was conducted, but dry diets were used instead. Raw diets were sun-dried and ground to the appropriate particle size.

Data were subjected to one-way analysis of variance (ANOVA). Duncan's multiple range test was used to compare the means at the $P<0.05$ level.

Results and discussion

At the end of 30 days nursing period, the highest final mean weight (2.55±0.24g) and final mean length (3.03±0.08cm) were recorded for the group fed on raw squid in experiment 1. The highest final mean weight (0.12±0.0006) and final mean length (2.05±0.1cm) were recorded for the group fed on dry squid in experiment 2. The second highest final mean weight (4.42±0.3g) and mean length (2.99±0.08cm) were observed for the group fed on raw hilsa fish muscle in experiment 1, and in experiment 2, the second highest final weight (0.02±0.0007g) and final length (1.96±0.09cm) were recorded for group fed on dry hilsa fish muscle. Poor growth occurred in the group fed shrimp head, either raw or dry. A comparative study shows that growth in the group fed on squid or hilsa fish muscle (either raw or dried) was higher than the group fed a locally

manufactured commercial nursery diet (SABINCO). Better growth of *P. monodon* postlarvae, fed with squid meal as an animal protein source, has also been reported by Lim et al. (1979). The reason of better growth with squid meal might be due to the high protein content as well as essential amino acid content (Penafloride, 1989; Akiyama et al., 1992) The second highest growth was observed in the group fed raw (experiment 1) and dry (experiment 2) hilsa fish muscle. Hilsa fish is a indigenous fatty fish containing an average of 7-8% lipid, and this lipid could be a very good source of essential fatty acids like 20:5ω3 and 22:6ω3, resulting in a good growth of the postlarvae. A poor growth was recorded for the group fed shrimp head. This may be due to the low protein level of shrimp head (Shigueno, 1985). Moreover, shrimp head having a large amount of hard particles, which might be difficult to digest by the young larvae. Food conversion ratio (FCR) was 5.27 for raw squid, 5.12 for raw hilsa fish muscle, and 5.39 for raw shrimp head diet in experiment 1. In experiment 2, FCR was 2.76, 2.72, and 2.62 for the group fed on dry squid, dry hilsa fish muscle, and dry shrimp head, respectively. In different treatment groups of both experiments, survival rates ranged from 70-84%. Kanazawa (1984), by using an artificial diet as a nursery feed for *P monodon* larvae, recorded a survival rate of 70-89% which coincides with the present findings.

Conclusion

It may be concluded from the present findings that raw squid could be used as an optimal nursery diet for *P. monodon* postlarvae. Raw hilsa fish muscle could be a similar and alternative nursery diet, which gave comparable growth like squid. Both squid and hilsa fish muscle in the raw condition gave better growth than in the dry condition. This better growth might be due to easier digestion of the raw materials, or the raw diet itself might contain some extra growth promoting nutrients which cause better growth of the larvae. Such might be destroyed during drying of squid or hilsa fish muscle.

References

Akiyama D.M., W.G. Dominy, and A.L. Lawrence. 1992. Penaeid shrimp nutrition. p.535-567. In: Marine shrimp culture: principle and practice. A.W. Fast and I.J. Lester (Eds.) Elsevier.

Aquacop. 1985a. Overview of penaeid culture research : impact on commercial culture activity. p.3-10. In: Proceedings of the First International Conference on the Culture of Prawn/Shrimp. Y. Taki, J.H. Primavera, and J.A. Liobrera (Eds.). SEAFDEC, Philippines.

Aquacop. 1985b. A new approach in intensive nursery rearing of penaeids (abstract). In: Proceedings of the First International Conference on the Culture of Prawn/Shrimp. Y. Taki, J.H. Primavera, and J.A. Liobrera (Eeds.) SEAFDEC, Philippines. 169p.

Lim L., P. Suraniranat, and R. Platon. 1979. Evaluation of various protein sources for *Penaeus monodon* postlarvae. J. Biol. 8:29-36.

Penaflorida V. Dy. 1989. An evaluation of indigenous protein source as potential component in the diet formulation for tiger prawn, *Penaeus monodon*, using essential amino acid index (EAAI). Aquaculture 83:319-330.

Pretto R.M. 1983. Penaeus shrimp pond grow-out in Panama. p.169-178. In: J.P. McVey and J.R. Moore (Eds.) CRC Handbook of Mariculture, Vol I. Crustacean Aquaculture. CRC Press ,USA.

Shigueno K. 1985. Intensive culture and feed development in *Penaeus japonicus*. Proc. First Intl. Conf. Cult. prawn/shrimp. SEAFDEC, Philippines, 115-122.

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LIPID AND AMINO ACID METABOLISM DURING EARLY DEVELOPMENT OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*)

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Introduction

During the pre-feeding developmental stages of marine fish, all nutrients needed for development, growth, and homeostasis come from the yolk, and very small amounts of exogenous nutrients are ingested. Both lipids and amino acids can be catabolized as energy substrates during these endogenous feeding stages (Finn et al., 1991). Lipids and amino acids are also actively involved in biochemical and biophysical processes. Free amino acids are important in osmoregulation, while lipids are responsible for maintaining membrane properties and are precursors of bioactive molecules. These two compound classes are usually studied separately, but by studying them together around the time of hatching in halibut (*H. hippoglossus*), we show that there may be a direct metabolic connection between them.

Materials and methods

All Atlantic halibut eggs were obtained from captive broodstock that were held as described previously (Evans et al., 1996). Eggs were kept as before, and then about 1000 newly hatched larvae were transferred to five Petri dishes containing filtered seawater with penicillin and streptomycin. The Petri dishes were kept in a dark cold room at 5°C. All larvae used in this study were feeding endogenously.

Changes in the biochemical content of halibut eggs and larvae were determined over six developmental stages. At the unfertilized stage (Day 0) and at the fertilized stage (Day 0.3), 10-17 batches were sampled. The number sampled at the halfway to hatch stage (Day 7) was 5-12, at hatch (Day 14) it was 4-11, at 7 days post-hatch (Day 21) it was 3-8, and at 14 days post-hatch (Day 28) it was 2-4. For each batch, depending on the analysis, 1-3 samples of 15-30 individuals were collected randomly. Lipids, amino acids, and ammonia were determined according to Evans et al. (1996). Protein was determined as in Finn et al. (1991).

Results and discussion

Total lipid did not change (P>0.05) in the early embryonic stages, i.e., before Day 7 (Fig. 1a). Total lipid increased (P<0.05) about 25µg in larvae at hatch compared to eggs on Day 7 (Fig. 1a). All lipid classes investigated (phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, triacylglycerol, and sterol) increased by

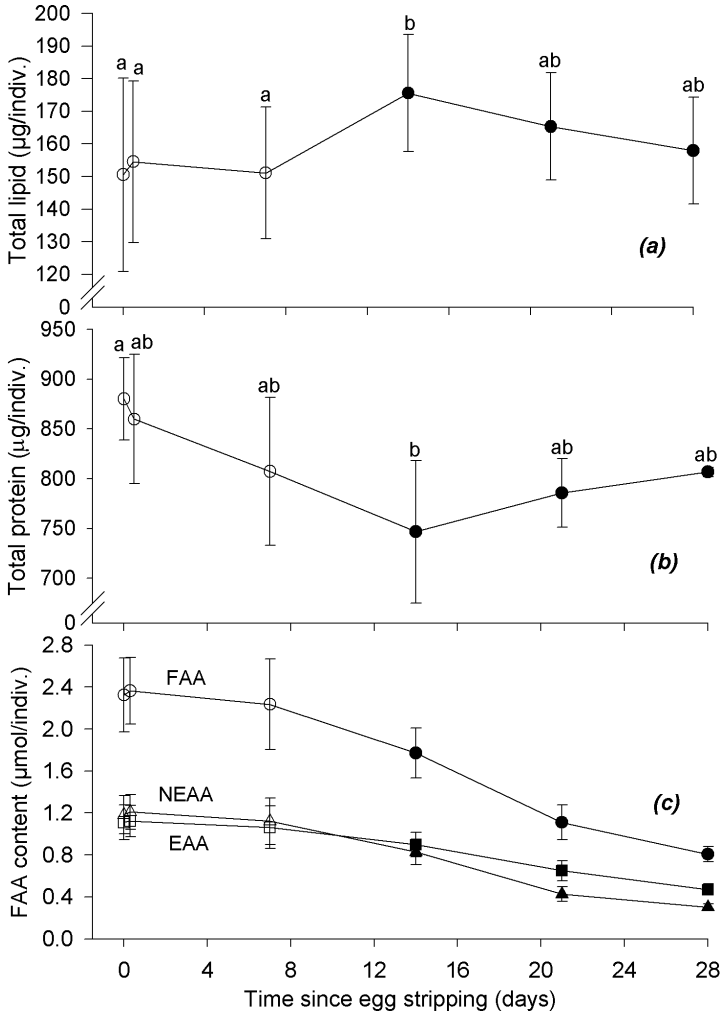

Fig. 1. Changes in (a) total lipid (b) total protein and (c) free amino acids in developing Atlantic halibut eggs and larvae. Open symbols represent egg stages; solid symbols represent larval stages. Squares are essential amino acids; triangles are nonessential amino acids. Data are mean±S.D. of up to 17 batches of eggs or larvae.

up to 16% at hatching. The observed increase of lipid at hatch is similar to the 18% on a dry weight basis, that can be calculated from the data of Whyte et al. (1993) for Pacific halibut. In winter flounder, lipid increased by as much as 78% at the time of hatching (Cetta and Capuzzo, 1982). After hatching, total lipid decreased (Fig. 1a), but not significantly.

Protein was the largest caloric component in eggs, accounting for 59% of total dry weight. Between Day 0 and Day 14, protein content declined (P<0.05) by 133µg (Fig. 1b). This value is very close to the chorion protein value of 155µg reported by Finn et al. (1991), especially considering that the average size of the egg samples used in this study (14µl) were smaller than eggs used in their study (15µl). After hatching, there was an increasing trend (Fig. 1b).

The average free amino acid (FAA) content in unfertilized eggs was 2323nmol.indiv.$^{-1}$ (Fig. 1c), similar to that reported by Finn et al. (1991: 2308nmol.indiv.$^{-1}$). Total FAA, total essential free amino acids (EAA), and total nonessential free amino acids (NEAA) were continuously depleted throughout the developmental stages (Fig. 1c). Total FAA decreased by 88nmol.egg^{-1} on Day 7 compared to Day 0. The average level of NH_3 /NH_4^+, which is believed to be the only N-end product in the embryonic stage (Finn et al., 1991), increased (P<0.05) from 45nmol.indiv.$^{-1}$ at Day 0 to 145nmol.indiv.$^{-1}$ at Day 7. The FAA pool decreased (P<0.05) on Day 14 compared to Day 7 (Fig. 1c). The decrease of 465nmol in FAA was accompanied by a 155nmol increase in NH_3/NH_4^+. Nearly 2/3 of the FAA at Day 0 disappeared by Day 28.

As shown in Fig. 1c, the FAA pool utilization is not evenly distributed between EAA and NEAA. The NEAA level is slightly higher than the EAA level during the embryonic stage, but since NEAA are utilized more than EAA, a reversal of the ratio is found during the larval stage. It is possible that EAA in the FAA pool are retained for protein synthesis. The higher depletion rate of NEAA was mainly due to the selective utilization of serine and alanine. Finn et al. (1995) found that in body protein and yolk protein of halibut larvae, EAA were always 2-14% more abundant than NEAA.

The depleted FAA can be used both as energy substrates and precursors for the synthesis of lipid, glucose, protein, and some other quantitatively unimportant N-rich substances (polyamines, nucleic acids, etc.). There was no net increase in protein before hatching (Fig. 1b), and total protein content decreased (P<0.05) at hatch. Therefore, the depletion of the FAA pool may not be a result of protein synthesis, although there is probably an active exchange of amino acids between FAA and protein pools. Whyte et al. (1993) reported total saccharides increased in Pacific halibut embryos as well. FAA is believed to be the precursor for gluconeogenesis. However, carbohydrate is a minor component in marine fish eggs,

accounting for <1% of dry weight in Pacific halibut eggs (Whyte et al., 1993). Therefore, it is unlikely that gluconeogenesis will utilize substantial amounts of FAA.

After deamination, the carbon skeleton of FAA can be the preferred carbon source for lipid synthesis in adult fish (Henderson and Tocher, 1987). In the FAA pool of unfertilized Atlantic halibut eggs, ketogenic amino acids accounted for 1/3 of the total FAA. These amino acids can produce acetyl-CoA after deamination. Acetyl-CoA can be used as a precursor of fatty acids and lipid synthesis, or it can enter the tricarboxylic acid cycle (TCA) to produce ATP by combining with oxaloacetate to form citrate. The calories associated with the lipid increase accounted for 73% of the total calories associated with the decrease of FAA from fertilization to hatching.

Under conditions of anoxia or hypoxia, amino acids can also be directed to fatty acid chain elongation (Evans et al., 2000). In the case of fish embryos under normal culture conditions, anoxia or hypoxia is unlikely to happen, but a hypoxic condition may result from the intense movement associated with hatching. Pelagic embryos such as those of halibut are susceptible to hypoxia since they are found naturally in well-oxygenated waters. Therefore, at hatch it is possible that parts of the embryo may experience hypoxia and stimulate the process of fatty acid chain elongation.

References

Cetta C.M. and J.M. Capuzzo. 1982. Physiological and biochemical aspects of embryonic and larval development of the winter flounder *Pseudopleuronectes americanus*. Mar. Biol. 71:327-337.

Evans R.P., C.C. Parrish, J.A. Brown, and P.J. Davis. 1996. Biochemical composition of eggs from repeat and first-time spawning captive Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 139:139-149.

Evans R.P., P. Zhu, C.C. Parrish, and J.A. Brown. 2000. Lipid and amino acid metabolism during early development of marine fish. p.477-493. In: Seafood in Health and Nutrition – Transformation in Fisheries and Aquaculture: Global Perspectives. F. Shahidi (Ed.). ScienceTech Publishing Company, St. John's, NF.

Finn R.N., H.J. Fyhn, and M.S. Evjen. 1991. Respiration and nitrogen metabolism of Atlantic halibut eggs (*Hippoglossus hippoglossus*). Mar. Biol. 108:11-19.

Finn R.N., I. Ronnestad, and H.J. Fyhn. 1995. Respiration, nitrogen and energy metabolism of developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*). Comp. Biochem. Physiol. 111A:647-671.

Henderson R.J. and D.R. Tocher. 1987. The lipid composition and biochemistry of freshwater fish. Progr. Lipid Res. 26:281-347.

Whyte J.N.C., W.C. Clarke, N.G. Ginther, and J.O.T. Jensen. 1993. Biochemical changes during embryogenesis of the Pacific halibut, *Hippoglossus stenolepis* (Schmidt). Aquacult. Fish. Manage. 24:193-201.

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THE APPLICATION OF BIOTECHNOLOGY IN LARVI- AND AQUACULTURE

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In order to meet the challenge of tripling production within the next 25 years, aquaculture must become a more intensive and cost-effective industry, while being fully compatible with the marine and coastal environments. The application of molecular and biotechnological tools will help surmount biological impediments to the development of sustainable aquaculture and augment performances of the farmed organisms.

In the area of reproduction and larval quality, manipulating the reproductive hormonal axis, together with the delivery of beneficial compounds into the developing gametes, will provide a year-round supply of high quality eggs and larvae. With the demonstration that many species of microalgae used in larviculture can grow heterotrophically, industrial fermentation technologies have been applied to mass-produce selected and nutrient-enriched microalgae to optimally feed early life stages of cultured marine organisms. The recent development of a microalgal transformation system has opened new avenues to bioengineer microalgae with selectable reporter genes for delivering advantageous compounds to larval stages. Quantitative and highly sensitive technologies to measure gene expression levels can be used as improved indicators of egg and larval quality.

In the area of larval and fish performance, peptide, protein, and genetic engineering will provide bioactive materials to enhance reproduction, growth, and development, and to design more efficient vaccines against viral and bacterial infections. Molecular diagnostics will be used for highly sensitive and specific detection of pathogens and pollutants. Genetic immunization will be implemented, together with novel DNA-delivery systems, for more efficient and simpler vaccination. Molecular genetics will be applied to accelerate genetic selection for desired traits. Gene cloning and transfer technologies will create organisms with multiple copies of tailored genes that can be up- or down-regulated (at the promoter level) or express molecules of interest (at the reporter

level), leading to improved phenotypes. Such genetically modified organisms will be engineered to reproduce predictably year round, to successfully survive through early life stages, to grow faster, to better convert feeds while producing nutritious flesh and less polluting waste materials, to optimally utilize energy in varying environments, and to generate enhanced immune protection against virulent diseases.

Genomics and gene mapping technologies are currently used to sequence the entire genome of several aquacultured shrimp and finfish species. Genomics, combined with bioinformatics, proteomics, and functional genomics approaches, will lead to the identification of genes and their regulatory mechanisms involved in various commercially important performance traits. This will open new avenues for enhancing the productivity and cost efficiency of aquaculture.

To protect the marine environment from both chemical and biological pollution, as well as to provide disease-free and optimal grow-out conditions, the use of contained aquaculture will intensify. Microbial and microalgal consortia will be engineered to fully remove waste products from water in recirculated aquaculture and mariculture operations.

This presentation will address recent advances and future directions in applying the tools of biotechnology to accelerate the development of an economically competitive and environmentally sustainable aquaculture industry in the 21st century.