New developments in microbial management in larviculture

Olav Vadstein

Norwegian University of Science and Technology
«Analysis and Control of Microbial Systems» Group

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Introduction

Outline:

- Status for the hypothesis of detrimental larvae-microbe interactions as a may cause for poor performance
- What determines the composition of the microbiota of larvae
- Composition versus activity of microbiota
- Microbial Management strategies and our toolbox
  - Overview
  - Status
- Prospects & Conclusions

The detrimental larvae-microbe interactions hypothesis

A substantial number of studies have accumulated during the last 25 years

Multiple experimental approaches have been used

Evidence during the last 25 years

- Use of antibiotics (Vadstein et al., 1993a; Munro et al., 1994 and 1999; Verner-Jeffreys et al., 2004; Sørensen et al., 2014)
- Surface disinfection of eggs (Salvesen and Vadstein 1995; Salvesen et al., 1997; Grotmol and Totland, 2000)
- Bacteria free larvae (Munro et al., 1995; Dieckens et al., 2009; Forberg et al., 2011)
- Manipulation of the microbiota in the environment (Forberg et al., 2011; Makridis et al., 2000a and b)
- Manipulation of the microbiota of live feed (Munro et al., 1999; Olsen et al., 2000)
- Correlations at the individual level between composition of the microbiota and growth rate of larvae (Forberg et al., 2016; Trinh et al. 2017)

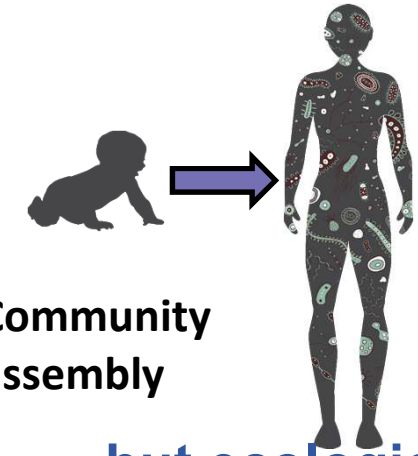
It is fair to conclude: Hypothesis is supported

The conclusion => Questions

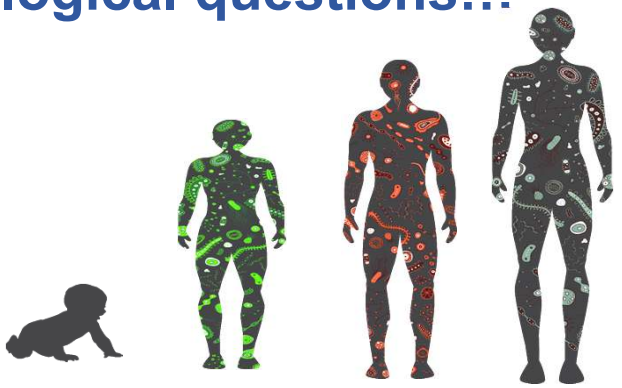
- How is the microbial environment of the rearing system influencing the colonisation process of reared fish larvae?
- How large is the variation in the microbiome between individuals?
- What is the relationship between structure and function
- Does factors influencing microbial community assembly change with development?
- Is it possible to steer the microbiota of larval?

We should not solve this by trial and error

These are essentially ecological questions…

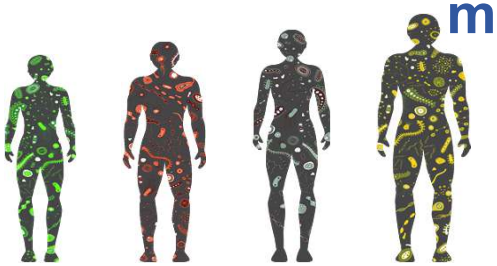


Community assembly

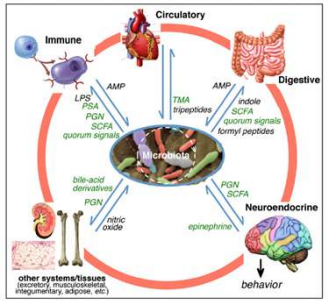


Succession

…but ecological thinking is not yet common in microbiome research.



Biogeography



Host selection

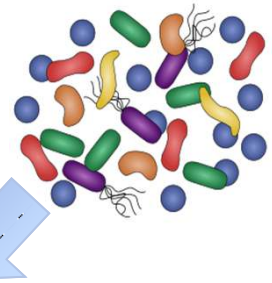
Some of the slides are provided by Brendan Bohannan

What determine community composition

- In ecological terms: community assembly and succession
- Important to understand as it sets the limits to management
- Traditionally niche based theory, and theory was a mess

- Stephen P. Hubbell proposed a neutral theory
  - Fitness differences are neglectable, dispersal and stochasticity decide
- Mark Vellend tried to create order – inspired by genetics
  - Four overarching processes: selection among species, drift, dispersal, and speciation

Hosts as “island” ecosystems in a community ecology context



“mainland” Source

Dispersal:
movement of organisms

“Neutral”

Drift:
Random changes in abundance

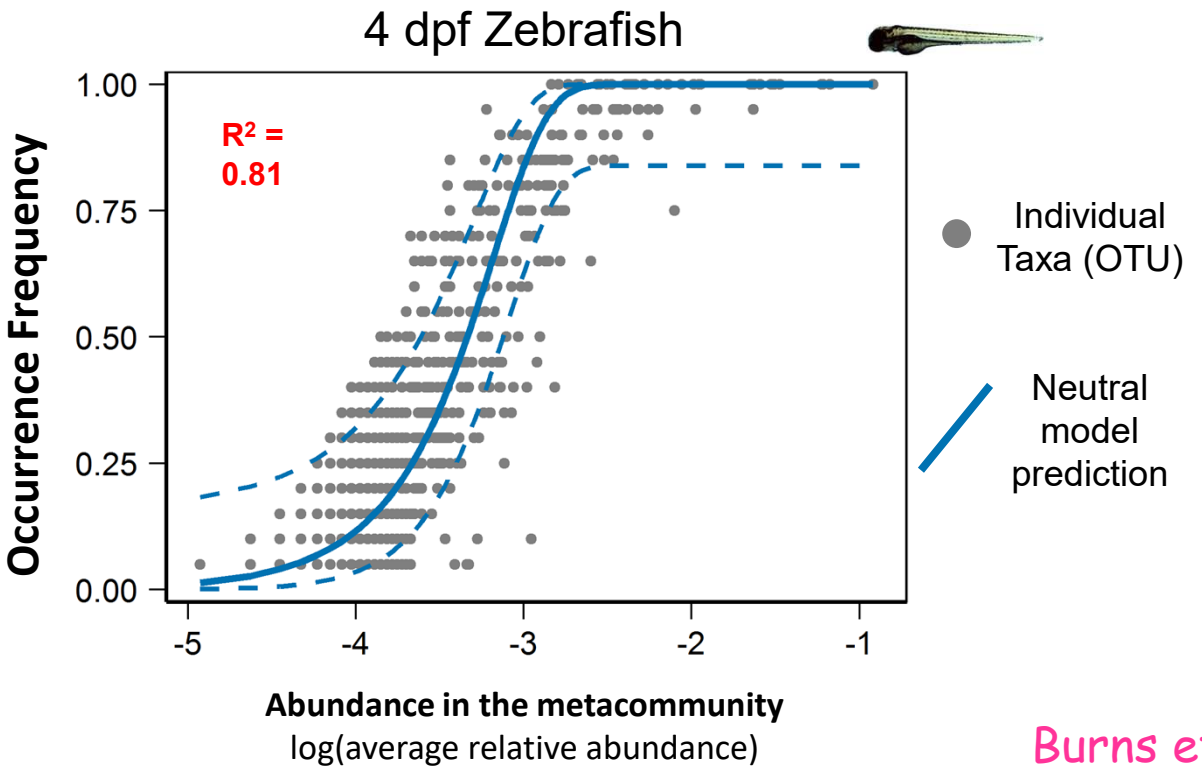
Host = “island” of habitat

Selection:
“sorts” species based on fitness

“Non-neutral”

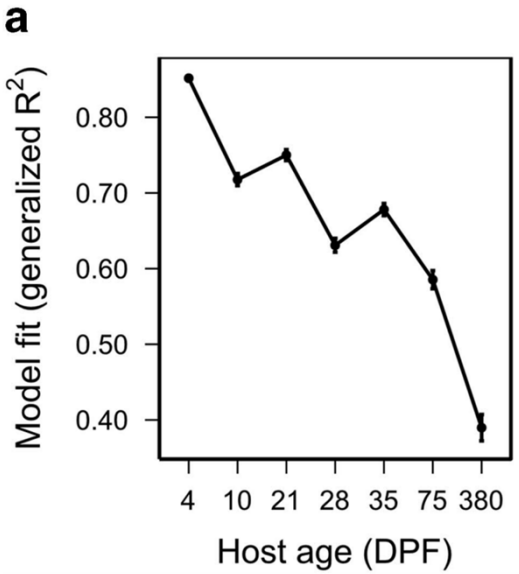
A local *community* assembles in host from a “*regional*” pool

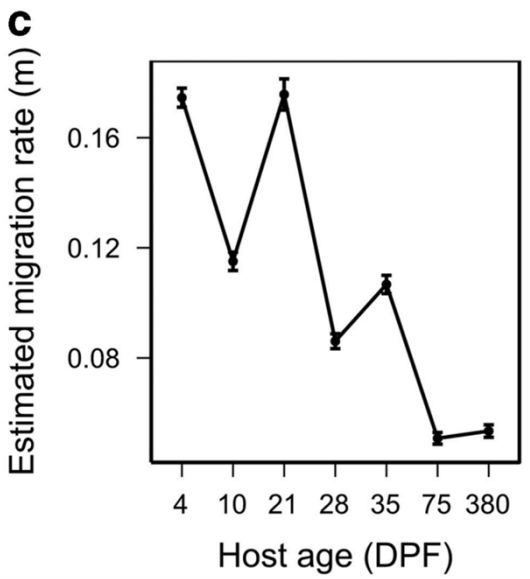
Neutral models explain substantial variation



Burns et al. 2016

Model fit and dispersal varies with age



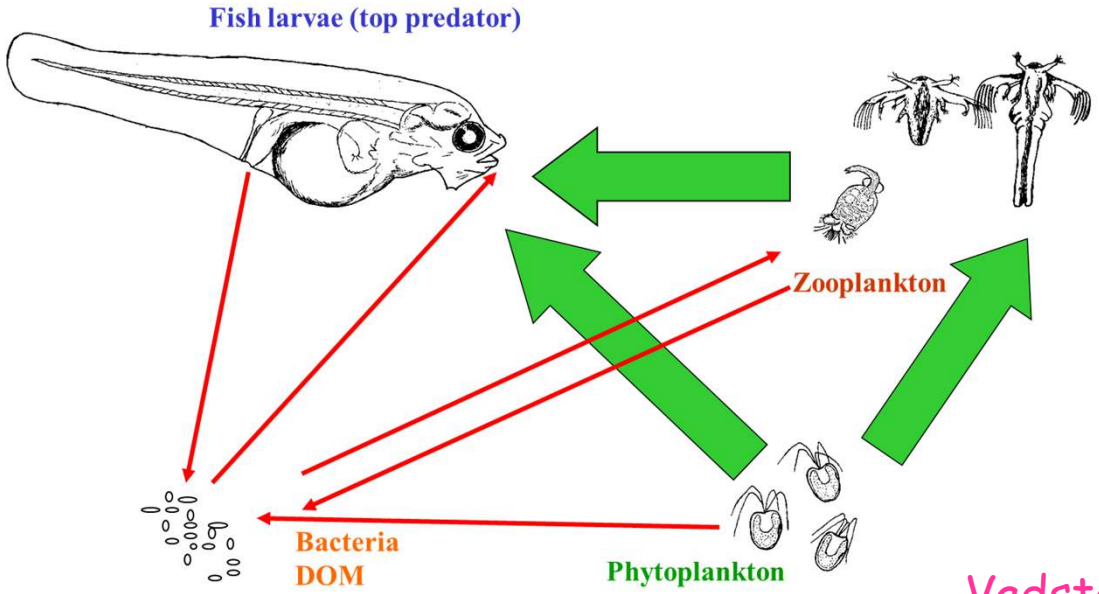


m = fraction of recruits coming from the source pool into the local community

Host selection increases with age?

Burns et al. 2016

What if we also include water and feed microbiota?

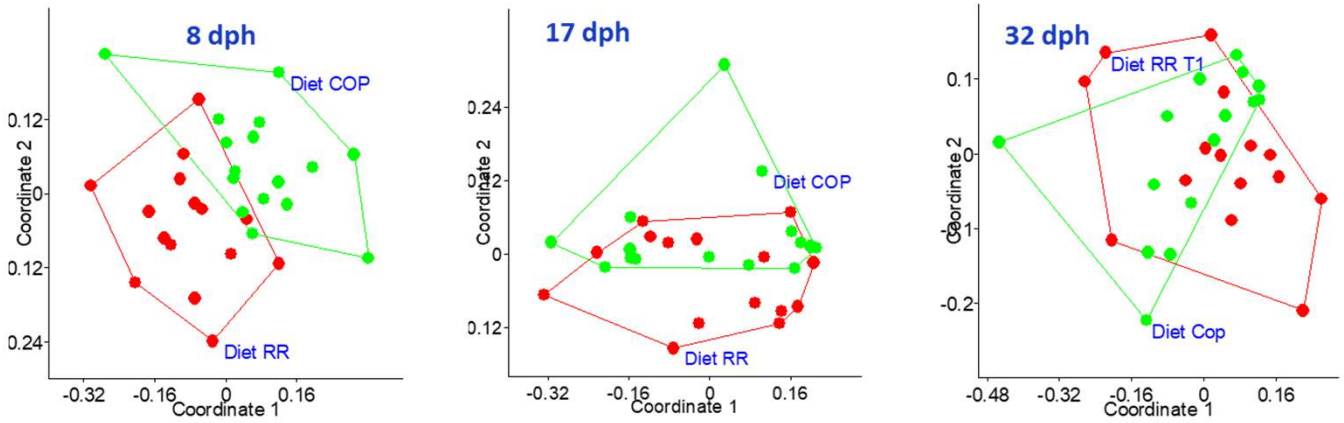


Vadstein et al. 2018a

Cod larvae fed rotifers or copepods

- The live feed diets represented different composition of microbiota
- Small, but significant differences in larval microbiota between diets at 8 dph
- No significant differences in larval microbiota between diets at 17 and 32 dph

Non-metric MDS based on Bray-Curtis similarities for cod larval microbiota reared with RR and COP diets

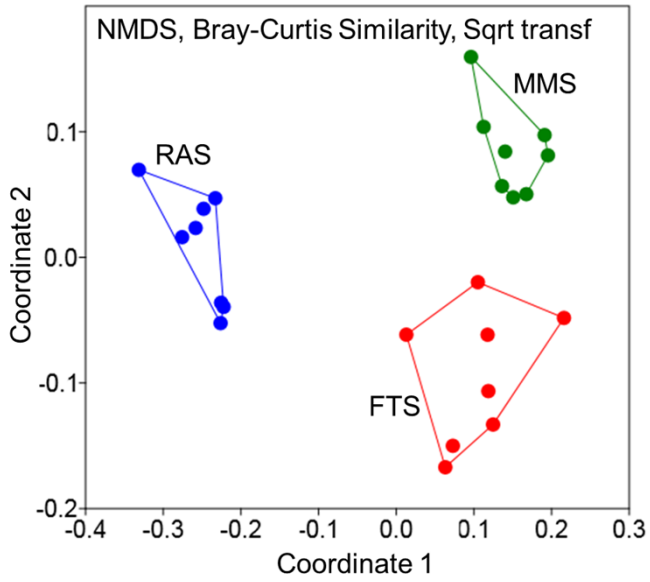


Based on data from Bakke et al. 2013

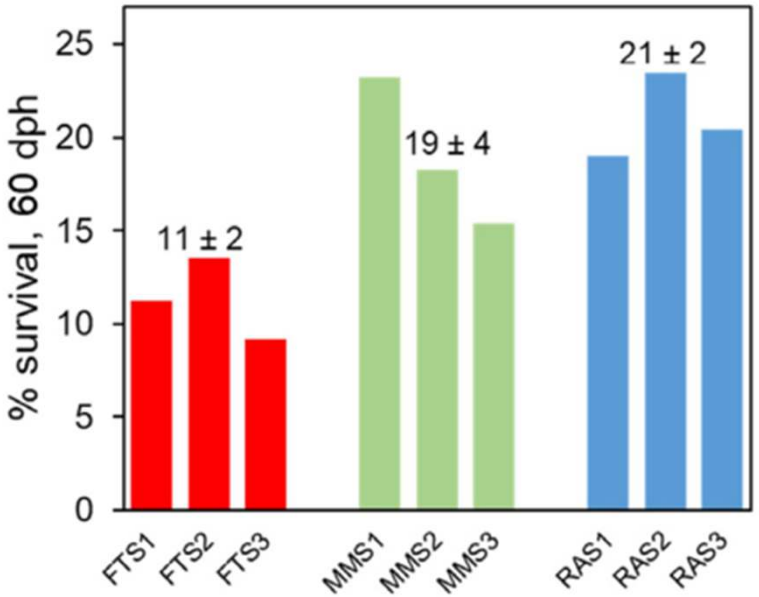
NTNU

Cod larvae: different water microbiota

Composition of water microbiota



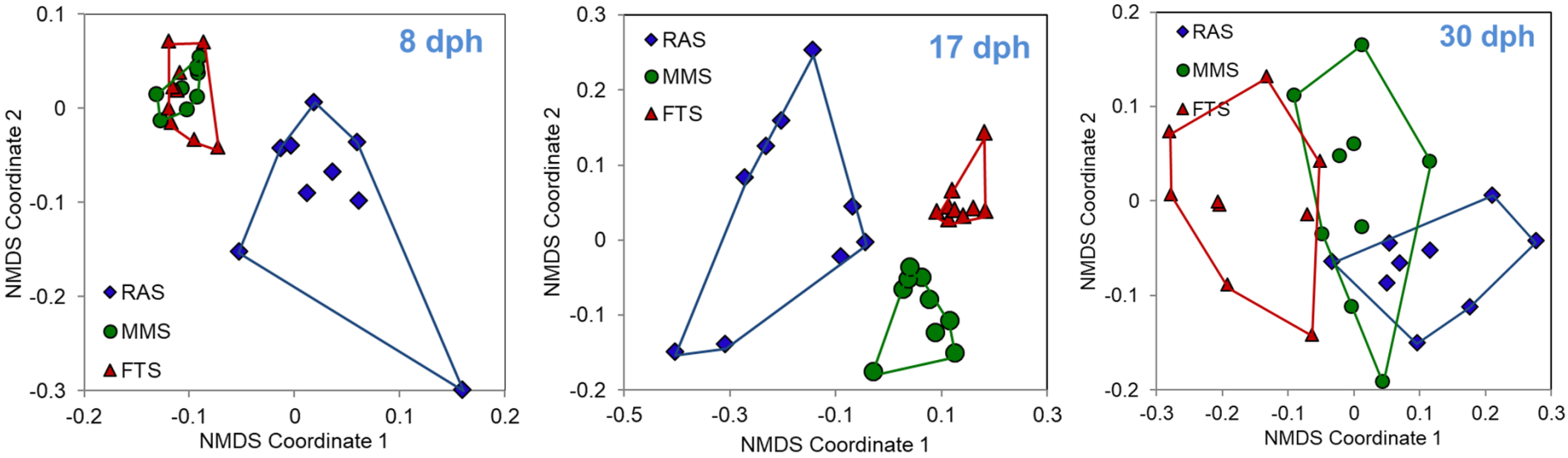
Survival of larvae at 60 dph



Data from Attramadal et al.
Vadstein et al. 2018b

Cod larvae: different water microbiota

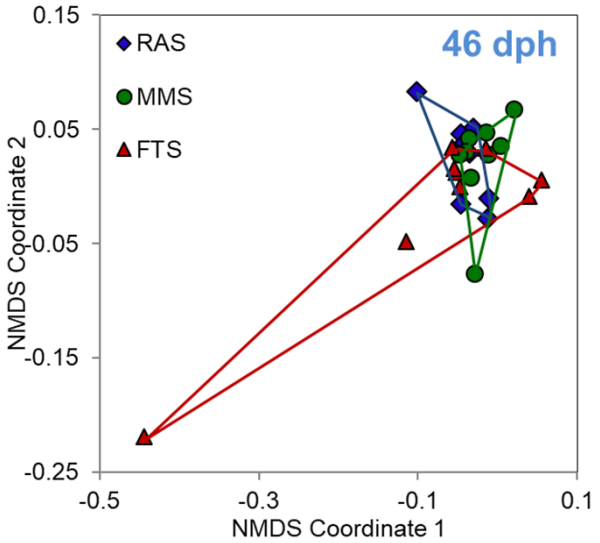
- Larval microbiota significantly different all days
  - (except FTS and RAS at 8 dph)



Vadstein et al. 2018b

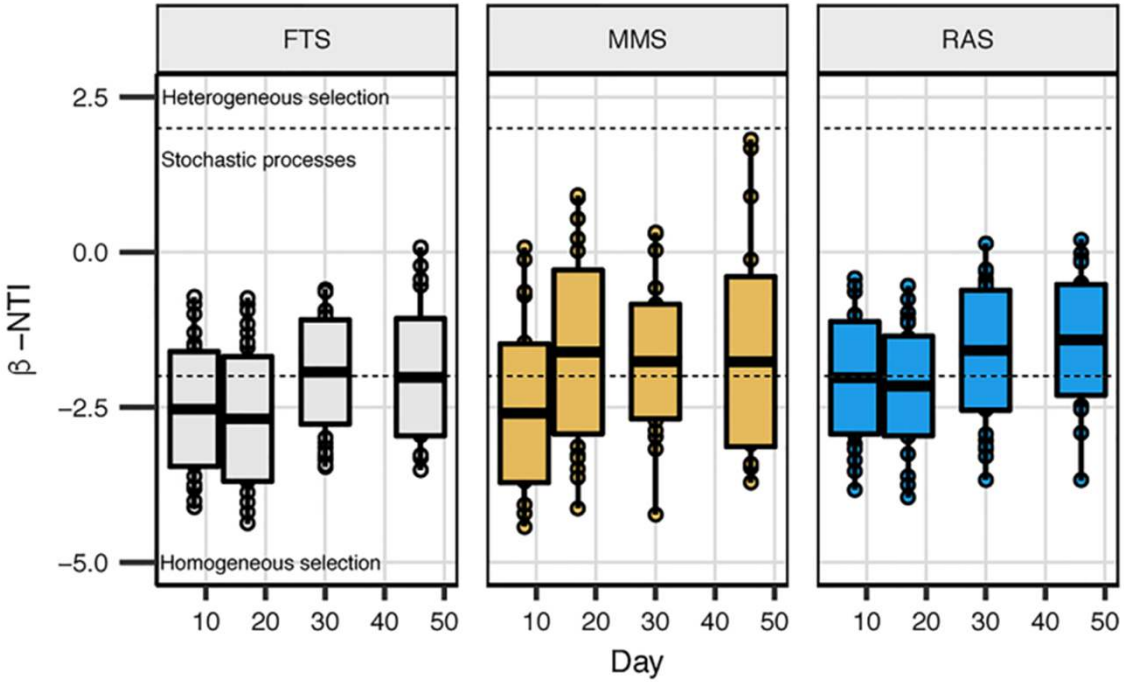
Cod larvae: different water microbiota

- Larval microbiota significantly different all days
  - (except FTS and RAS at 8 dph)
- Same water => same microbiota



Vadstein et al. 2018b

Total system and ecological processes

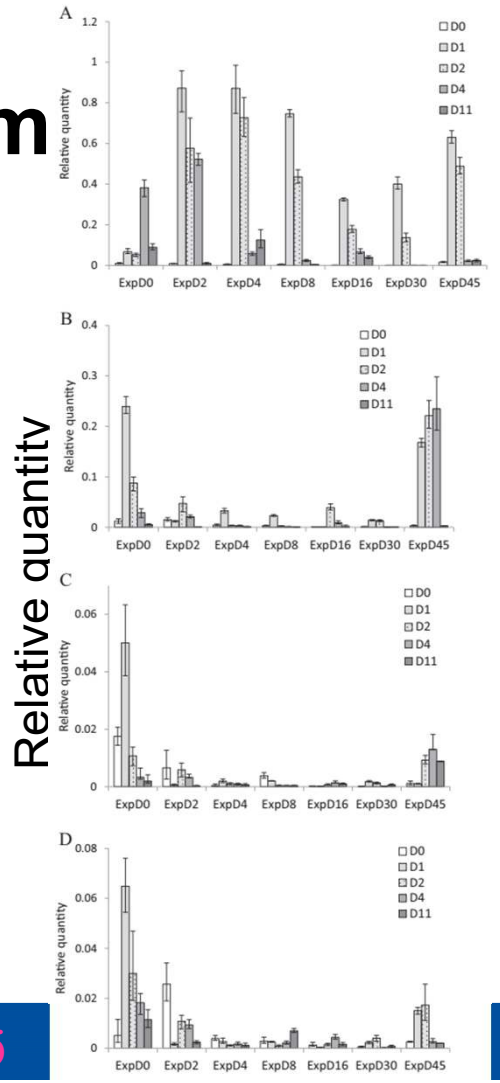


- Stochastic processes and selection are of similar importance at larval stages

Invasion of an established system

- Four probiotic candidates isolated from cod were tested through larval phase
- Most probiotic candidates were not clever invaders
- More resistance to invasion in established communities
- Conclusion: it is not easy to induce long term effects with probiotics

Is the probiotic concept trivial?



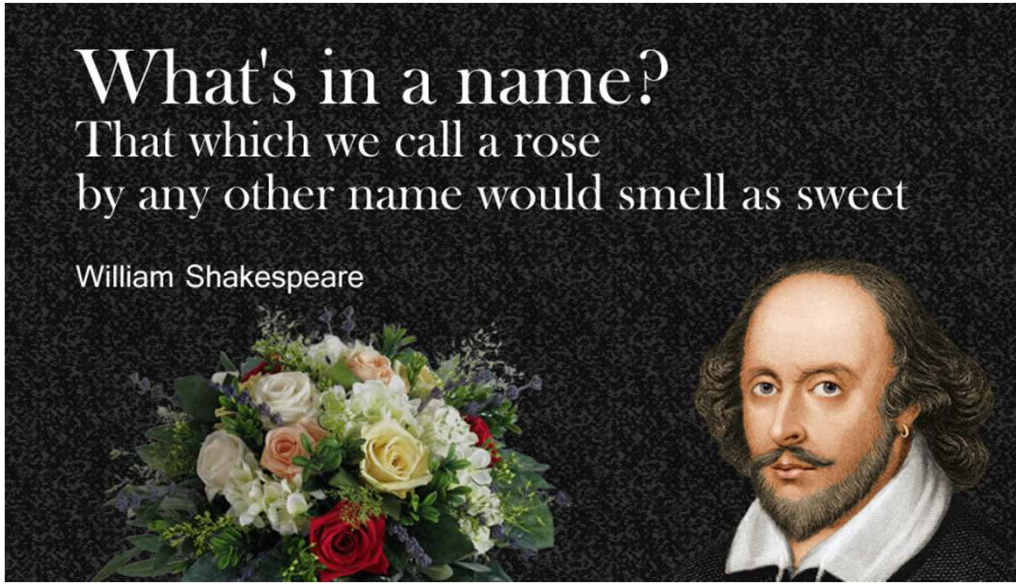
Skjermo et al. 2015

Overall conclusion on microbial community composition of larvae

- Huge progress in our understanding recently
- Both deterministic and stochastic processes are important
- The microbiota of larvae has a composition clearly different from the environmental microbiota.
- It is possible to control larval microbiota by controlling microbiota in water, but not using the feed
- ***
  This make microbial management possible, but …

Composition versus activity of microbiota

- Which functionalities has to be served by a mutualistic microbiota?



Main functions of gut microflora

- Metabolic - nutritional
  - Energy
  - Absorbable substrate
  - Vitamins
- Trophic
  - Influences cell growth and division
  - Influences host immune system – immune system modulation
- Protective – resistance against pathogenic bacteria
  - Competitive exclusion of opportunistic and pathogenic bacteria
  - Mechanisms proposed:
    - Competition for attachment sites
    - Competition for nutrients in available niches
    - Production/destruction of signal molecules with local effects

To determine function of microbiota

- What matter is production of active molecules
  - Digestive enzymes, bioactive compounds, i.e. proteomics and metabolomics
  - For proteome/metabolom it is has to separate bacteria and host

- What people use is metagenomics – or worse
  - i.e. the functional potential of the microbiota
- Transcriptomics would be better, but it is very hard to quantify the transcriptome of the gut microbiota

Overall conclusion on functionality of microbiota in larvae

- Very little is known for fish
- We have developed new experimental and analytical tools

- Hypothesis: If larvae are protected from detrimental microbes, they are able to select the functions they need

We know very little – we can hope larvae knows

Microbial management strategies and our toolbox

The microbial management toolbox

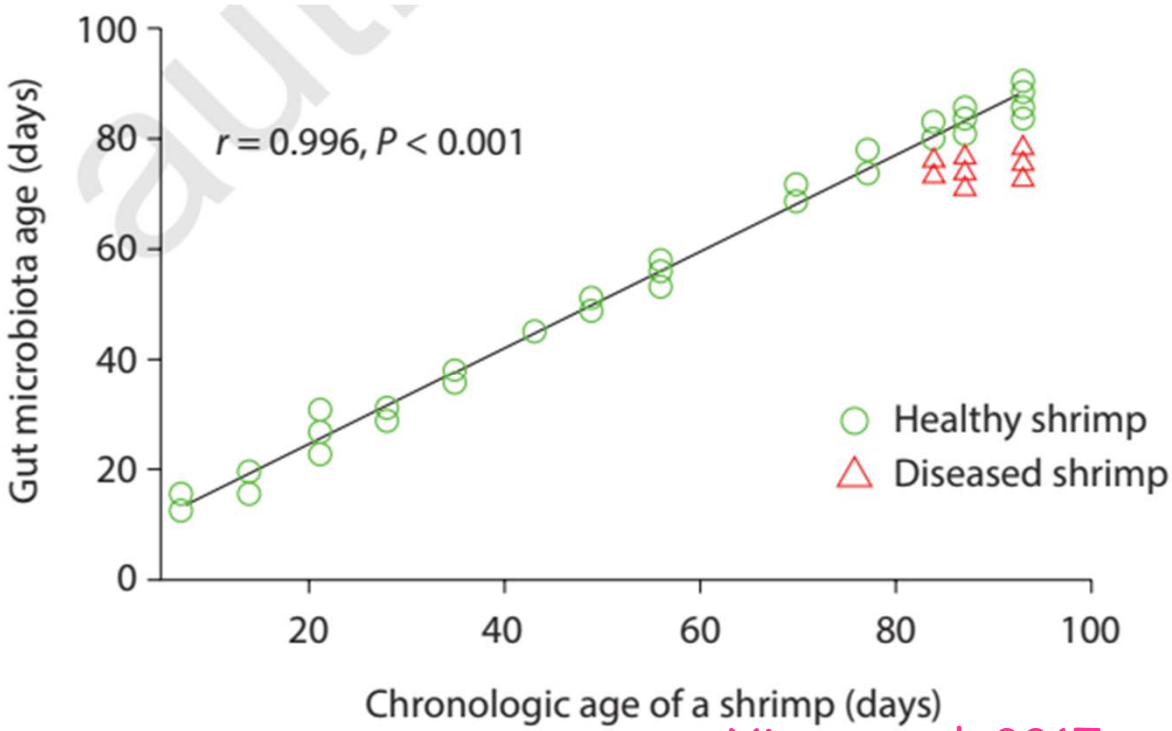
Non-selective reduction of microb

- Disinfection of eggs and wat
- Reduction in input of organic
- Removal of organic matter (p
- Grazer control of bacterial bi

Selective enhancement of microb

- Selection for desirable bacte
- Addition of selected bacteria
- Incorporation of selected bac
- Phage therapy – kill some ar

Improvement of resistance agains



Xiong et al. 2017

Modified from Vadstein et al. 2018a

The microbial management toolbox

Non-selective reduction of microbes:

- Disinfection of eggs and water (inc ozonation)
- Reduction in input of organic matter
- Removal of organic matter (per se, clay, biofilters, dilution)
- Grazer control of bacterial biomass

Selective enhancement of microbes: Composition and activity

- Selection for desirable bacteria (inc Maturation, RAS, prebiotics)
- Addition of selected bacteria to tanks (Probiotics)
- Incorporation of selected bacteria in feed (Probiotics)
- Phage therapy – kill some and enhance others

Improvement of resistance against microbes:

- Stimulation of general immune system
- Modulation of general and specific maternal immunity

Modified from Vadstein et al. 2018a

Status

- Recent work focus on probiotics and overload of organic matter
- There is some novel work on phage therapy
- Work on shrimp is a driver, with several interesting findings

Non-selective reduction of microbes:
- Disinfection of eggs and water (inc ozonation)
- Reduction in input of organic matter
- Removal of organic matter (per se, clay, biofilters, dilution)
- Grazer control of bacterial biomass

Selective enhancement of microbes: Composition and activity
- Selection for desirable bacteria (inc Maturation, RAS, prebiotics)
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Improvement of resistance against microbes:
- Stimulation of general immune system
- Modulation of general and specific maternal immunity

- “This current low richness and evenness in research hampers the progress” still holds (De Schryver and Vadstein 2014)

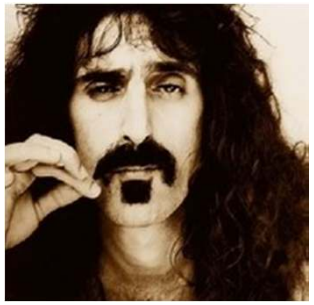
Prospects & Conclusion

- Conclusion: Microbial management in larval rearing is a prerequisite for sustainable development of aquaculture
- There is a need for a consorted action which include industry, academia and funding agencies
  - The goal must be a knowledge based development of general knowledge and managements tools

- Conclusion: The future is bright if we stand together and have the patients for a long term perspective

Thank you

Information is not knowledge.
Knowledge is not wisdom.
Wisdom is not truth.
Truth is not beauty.
Beauty is not love.
Love is not music.
Music is THE BEST.



Frank Zappa

Data is not information,
information is not knowledge,
knowledge is not understanding,
understanding is not wisdom.

Clifford Stoll