

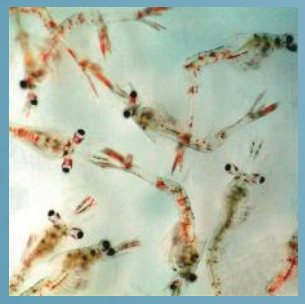
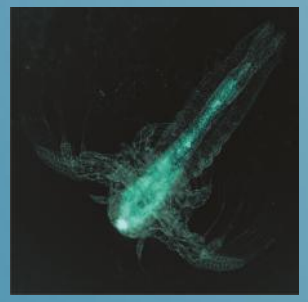
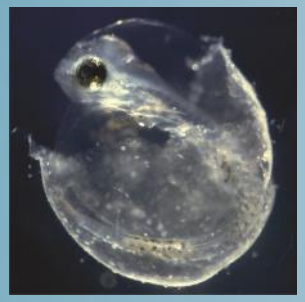

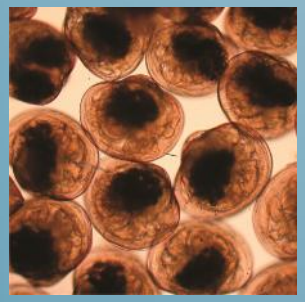

larvi 2013

6th fish & shellfish larviculture symposium

Bacterial community assembly in developing cod larvae (*Gadus morhua*)

Ingrid Bakke

ghent university, belgium, 2-5 september 2013

Bacterial community assembly in developing cod larvae (*Gadus morhua*)

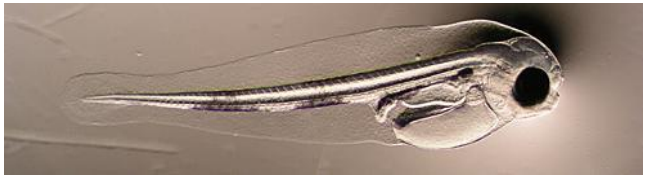

Photo: Akvaplan-Niva AS

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Introduction

Photo: LarvalBase

- After hatching and mouth opening, the intestinal system of a fish larva is colonized
- This colonization is important to host health and development
- Conserved responses to colonization of the gut system (mouse and zebra fish):
  - Stimulation of epithelial cell proliferation
  - Promotion of nutrient metabolism
  - Development of the mucosal immune system
- Microbiota associated with cultivated marine fish larvae
  - The larvae shear the rearing water with bacteria
  - The digestive and immune systems are immature
  - Vulnerable to opportunistic bacteria
- Possible to steer the larval microbiota?

Background

- Need for knowledge:
  - Sources for the larval microbiota?
  - **Determinants for the composition of the gastrointestinal microbiota?**

Results for cod larvae in PROMICROBE at NTNU/SINTEF in Trondheim:

- Study I: Effect of different live feed diets on larval microbiota
- Study II: Effect of different rearing water systems on larval microbiota
- Study III: A deep sequencing approach to characterize variation in larval microbiota between individuals and with time

Photo: Nofima

Study I Effect of live feed diet

Experimental tanks at SINTEF Fisheries and Aquaculture

Experimental design

- First feeding experiment with cod larvae
- Three different live feed diets from 3 to 22 dph:
  - Diet **COP**: Copepods cultured on microalgae *R. baltica*
  - Diet **RR**: Rotifers cultured on microalgae *R. baltica*
  - Diet **CR**: Rotifers cultured on Bakers yeast and MarolE
- For each diet: Three tanks, totally 9 tanks (100 l)
- From 18 – 36 dph: Artemia to all tanks
- Microbial communities investigated for individual larvae, water and live feed samples by PCR/DGGE

environmental microbiology reports

Environmental Microbiology Reports (2013) 5(4), 537–548 doi:10.1111/1758-2229.12042

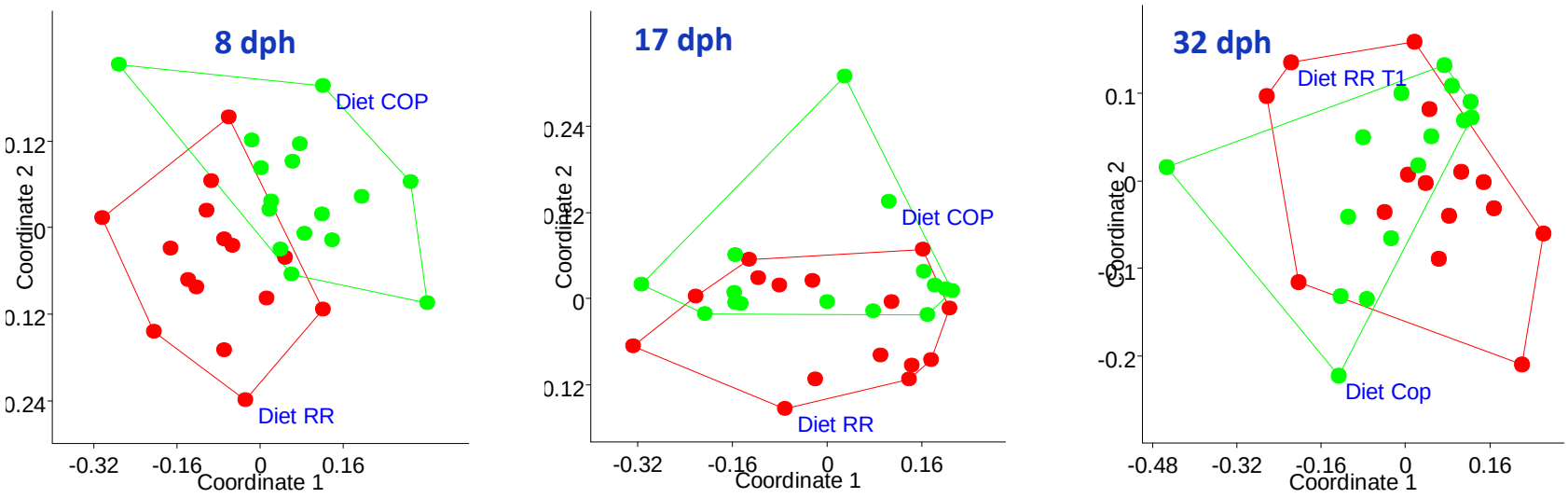
Live feed is not a major determinant of the microbiota associated with cod larvae (*Gadus morhua*)

Ingrid Bakke,[1*] Jorunn Skjermo,[3] Tu Anh Vo[2] and Olav Vadstein[1]

Study I Results

- The live feed diets represented different microbiotas
- Small, but significant differences in larval microbiota between all 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



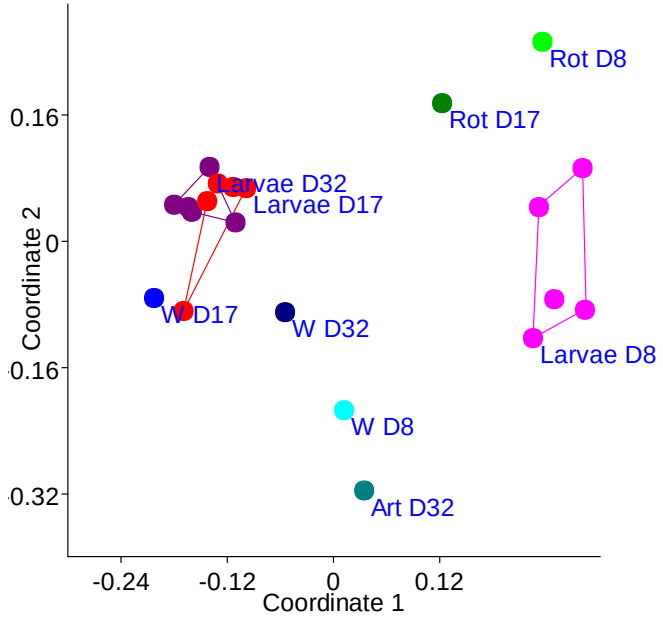
Study I Results

- At 17 and 32 dph:
  No differences in larval microbiota due to rearing with different diets
  Differences in larval microbiota due to rearing in different tanks

- Larval microbiota at 17 and 32 dph remarkable similar despite change of diet

- Larval microbiota generally more similar to water microbiota than to live feed microbiota

RR Tank 3: Larval, water and live feed microbiota



Study II Effect of rearing water

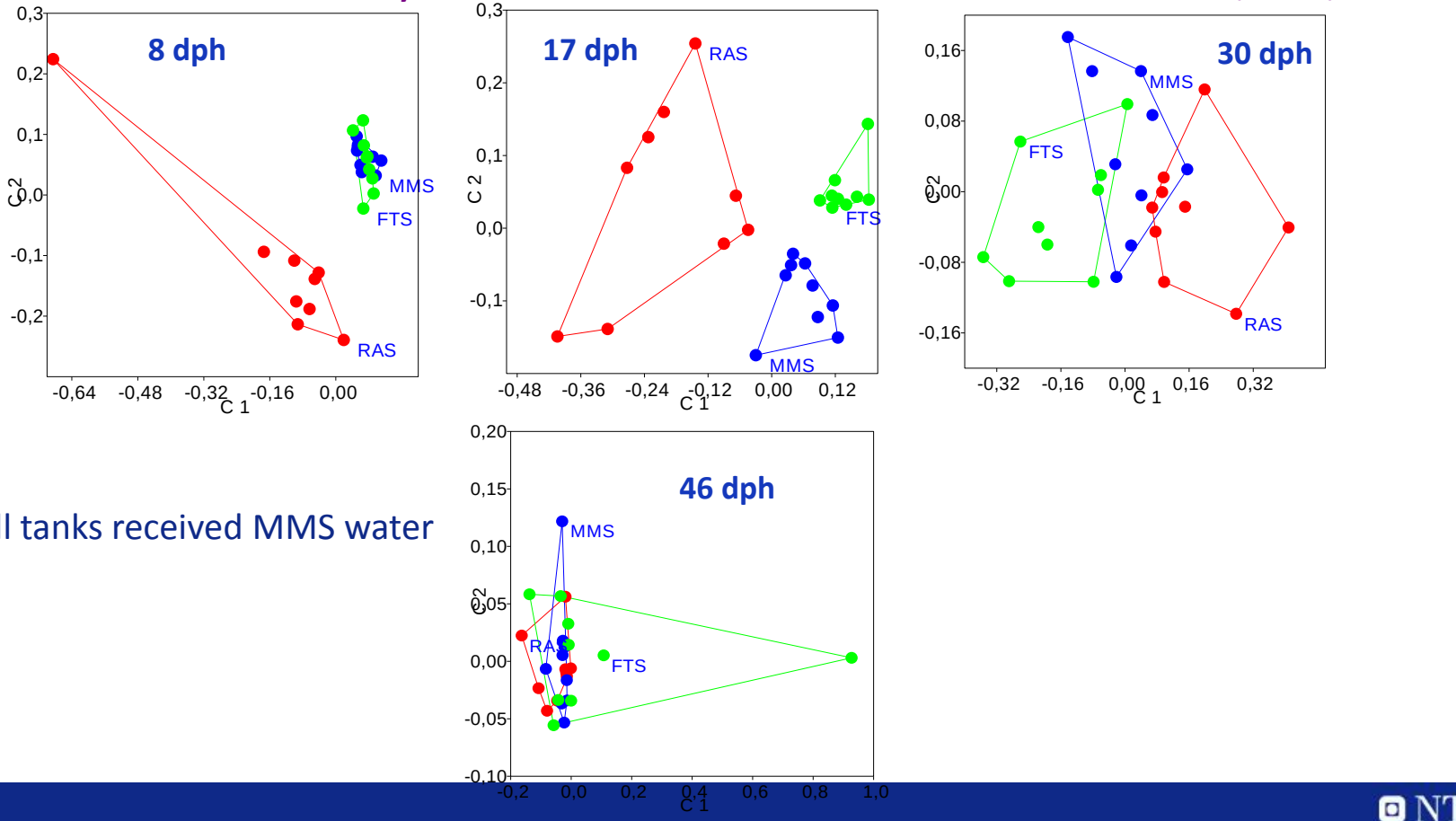
Experimental design

- First feeding experiment with cod larvae
- Three different rearing water systems until 30 dph:
  - **FTS**: Conventional flow-through system
  - **MMS**: Microbially matured water (with biofilter) in a flow-through system
  - **RAS**: Recirculating aquaculture system without disinfection
- For rearing water system: Three tanks, totally 9 tanks (100 l)
- After 30 dph: All tanks received MMS water
- Microbial communities investigated for individual larvae, water and live feed samples by PCR/DGGE

Study II Results

- Significant differences in larval microbiota between different water rearing systems
- Change of identical water (MMS) to all tanks → similar larval microbiota between tanks

Non-metric MDS based on Bray-Curtis similarities for cod larval microbiota reared with FTS, MMS, and RAS

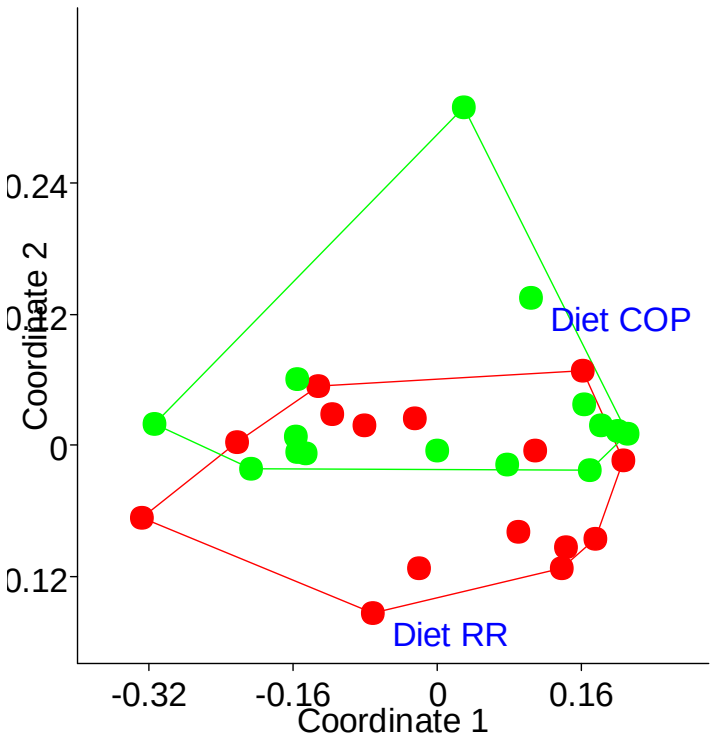


All tanks received MMS water

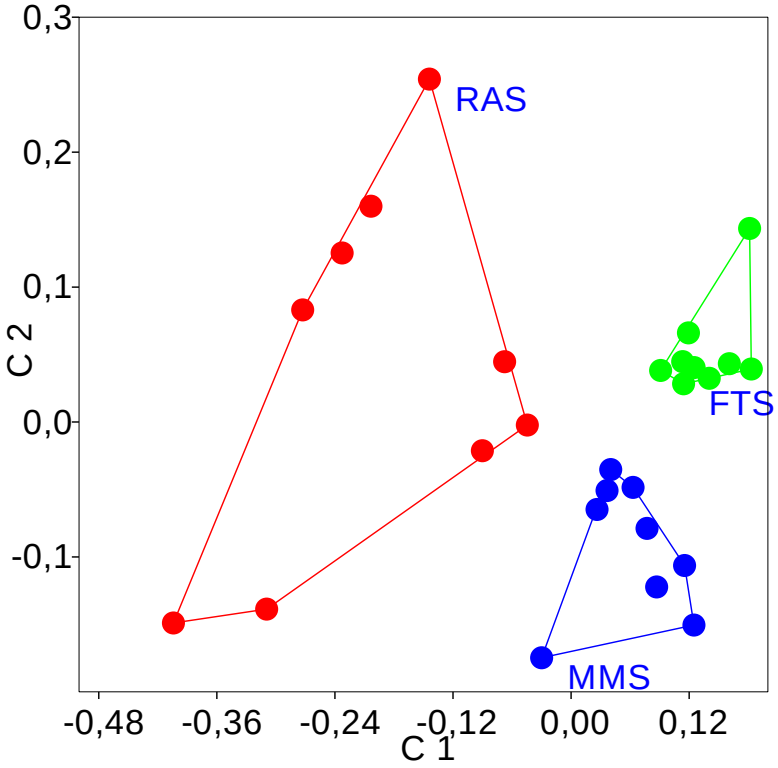
Study I versus Study II

Larval microbiota 17 dph:

Reared with different diets



Reared with different water systems



Study III

Deep seq. of microbiota associated with developing cod larvae

Experimental design

- Microbial communities characterized by amplicon pyrosequencing (“deep sequencing”)
- Two tanks from Study I (the “diet study”): diets from 3 to 22 dph:
  Tank 1 (T1) Diet **COP**: Copepods cultured on microalgae *R. baltica*
  Tank 2 (T2) Diet **RR**: Rotifers cultured on microalgae *R. baltica*
- **Target sequence: Variable region 4 of the bacterial 16S rRNA gene**

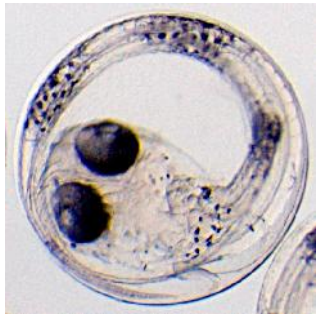
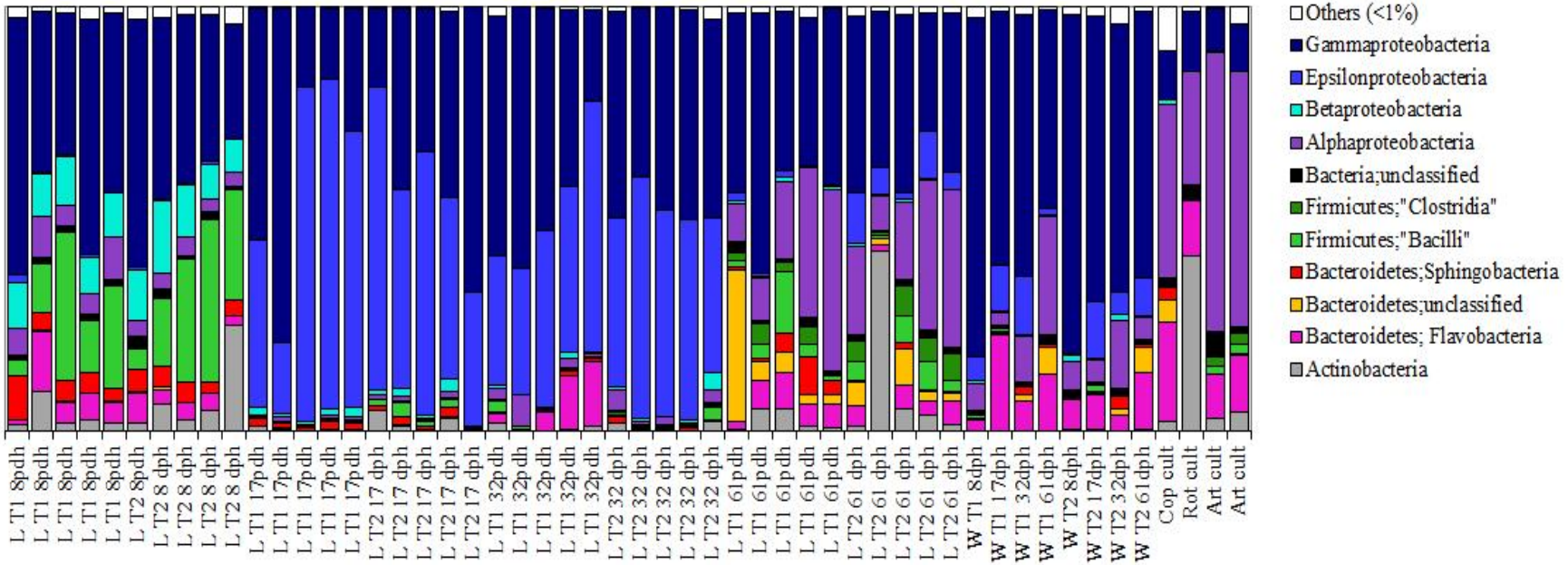


Photo: Tora Bardal

Study III Results

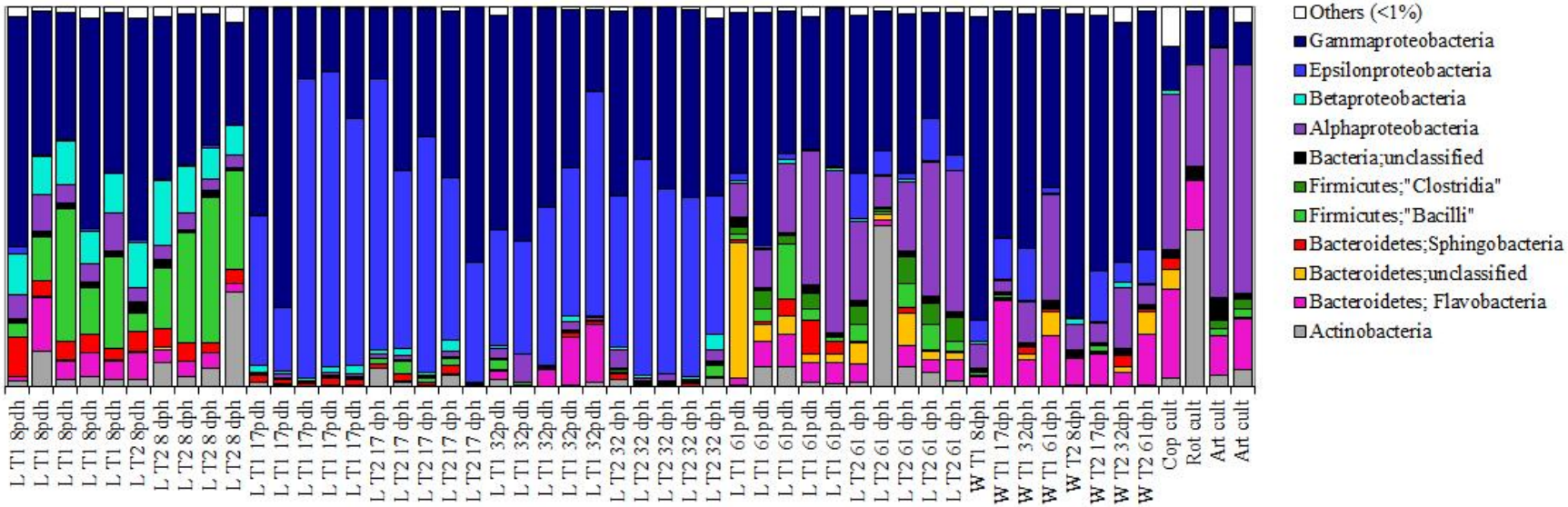
- Large number of high quality DNA sequences were obtained
  Average number of sequences ("after trimming") per sample:
  - Larva: 6248 ± 1196
  - Rearing water: 8424 ±514
  - Live feed: 8580 ±649



Study III Results

Larval microbiota: Temporal trends

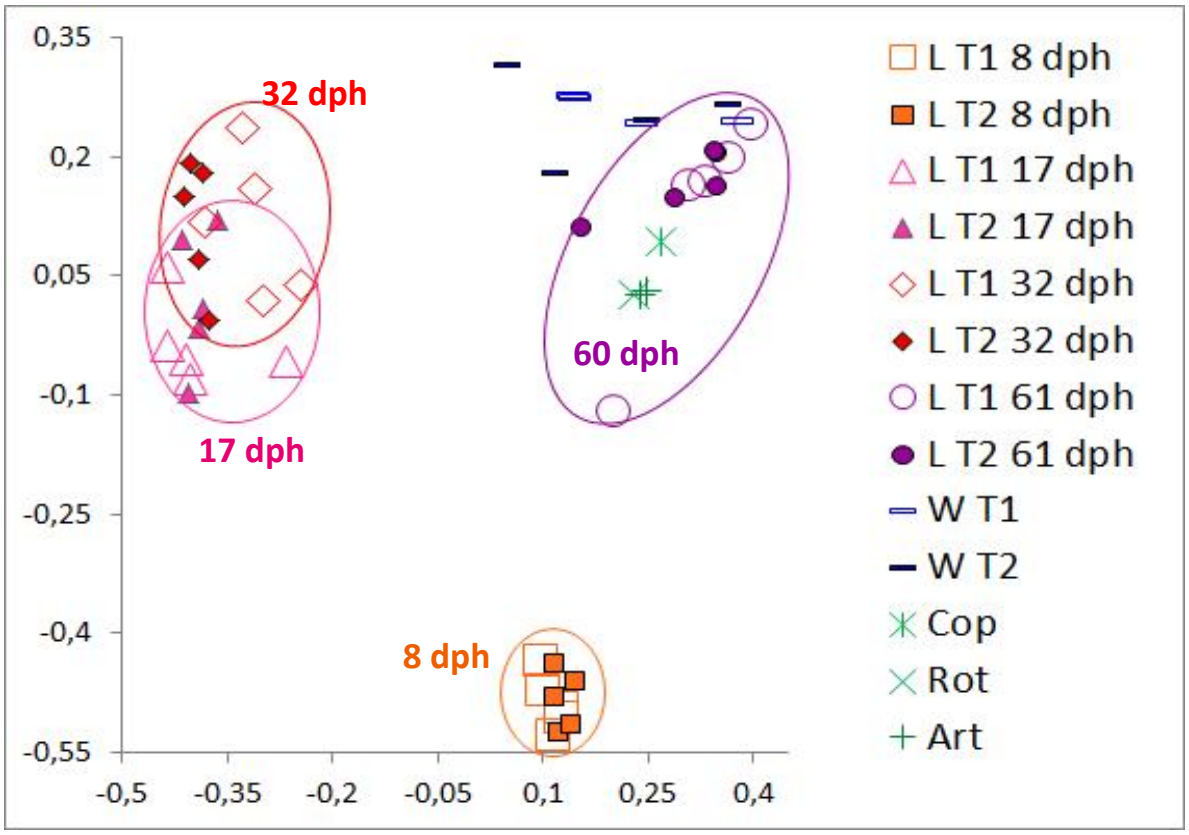
- The composition of the larval microbiota changes with age:
  - 8 dph: Pseudomonas, diverse β-proteobacteria, Bacilli is abundant
  - 17 dph and 32 dph: Low diversity, arcobacter (ε-proteobacteria) and γ-proteobacteria
  - 61 dph: High diversity, diverse γ-proteobacteria, high abundance of Rhodobacter



Study III Results

- Similar larval microbiota at 17 and 32 dph
- No differences in larval microbiota between Tank 1 and Tank 2 (COP and RR diets)

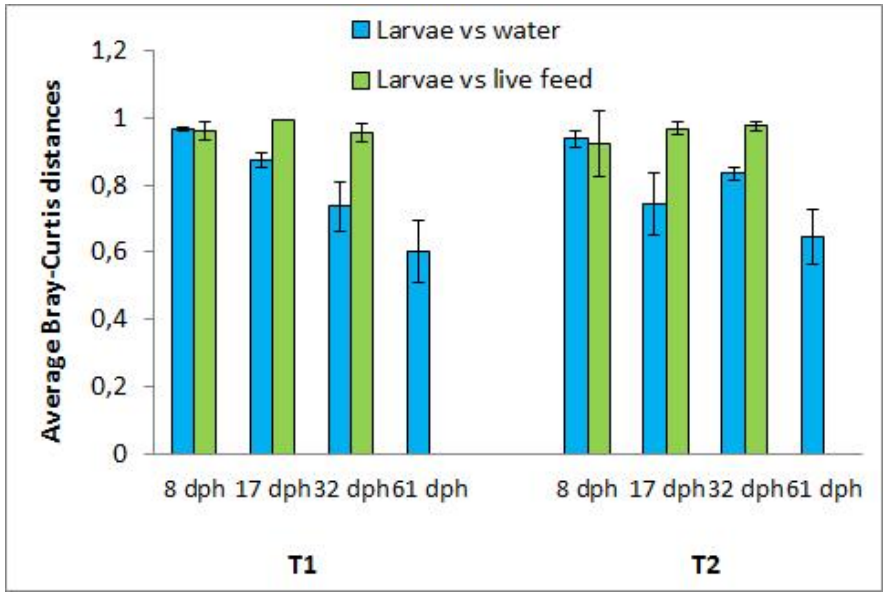
Pco plot based on Bray-Curtis distances for T1 (COP) and T2 (T2)



Study III Results Comparison of larval microbiota to water and live feed microbiota

- Larval microbiota highly dissimilar from live feed microbiota
- Larval microbiota highly dissimilar from water microbiota

Higher similarity with increasing age (excretion?)



- Where do the bacteria associated with the 8 dph larvae come from?
  - The most abundant larval OTUs are rare in the water and live feed microbiotas

OTU classification	% abundance Tank 1			% abundance Tank 2		
	Larvae	Water	COP Feed	Larvae	Water	Rot Feed
Pseudomonas	29.8	0.05	0.09	26.3	0.05	0.06
Bacillales	13.1	0.01	-	16.5	-	0.01
Microbacterium	3.6	0.18	0.30	6.4	0.38	40.5
Enterobacteriaceae	4.2	-	0.01	3.9	-	-

Summary

- Live feed diet seems to have little influence on the larval microbiota
- Rearing water affects the larval microbiota: Rearing water the major source for larval microbiota?
- Major changes in community structure during larval development
- The early larval microbiota very different from water and live feed microbiota: strong host selection?

Questions

- Determinants for community structure of the larval microbiota, what cause the changes in community structure?
  - Developmental changes in the digestive system?
  - Developmental changes in the immune system?
- Differences in early larval microbiota when rearing with different water systems:
  Which bacteria make these differences?
  Pyrosequencing of samples from Study II may provide an answer

Thank you for your attention!

This work was funded by The European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 227197 Promicrobe 'Microbes as positive actors for more sustainable aquaculture'.