Development of the gut microbiota in Atlantic cod *Gadus morhua* L. larvae fed copepods and rotifers

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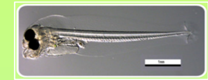


INTRODUCTION

In Norway the interest for Atlantic cod culture has been comprehensive during the last 10 years, and much effort from the industry and the R&D-institutions has brought the cultivation technology from an extensive, small scale using harvested plankton as start feed, to an intensive, industrial scale using rotifers and commercial cultivation diets. Variability in the quality of larvae and juveniles produced by the intensive technology may however suggest that rotifers are not satisfactory for optimal development and growth of the larvae in the earliest stages. On the other hand, high quality of cod juveniles is normally obtained under extensive culture conditions, where the larvae are fed harvested natural copepods during the first weeks after hatching. Development of copepod cultivation technology has therefore gained interest the last years. Enhanced larval quality can be attributed to several factors, such as differences in prey size and movement, nutritional factors and possibly differences in the microbiology of the live feed.

THE GUT FLORA OF COD LARVAE

There are large variations between the gut flora of individual larvae from the same rearing conditions (Fjellheim, 2007). The gut microbiota is important for the function of the gut, and in fish larvae the establishment of this flora depends on bacteria introduced from the fish eggs, water and feed, but also on nutritional factors in the feed and the development of the digestive tract.

OBJECTIVES

1. To compare the effects of the diets – the cultivated live feed organisms *Acartia tonsa* (copepode) and *Brachionus plicatilis* (rotifers) - on the development of the microbial community (MC) in the gut of cod larvae from hatching until day 60.
2. To study the relationship between the diversity of the gut MC and the size of the larvae.

EXPERIMENTAL SETUP

Cod larvae were hatched and reared under conditions described below (Fig.1 and Tab 1). All treatments were run in triplicates.
The first feeding diets were:

1) Copepods *Acartia tonsa* cultivated on the microalgae *Rhodomonas* (Feed P)
2) Rotifers *Brachionus plicatilis* Nevada cultivated on *Rhodomonas* (Feed R)
3) Rotifers *Brachionus plicatilis* Nevada fed a standard diet (Feed C).

Figure 1. Tanks used for first feeding of cod.

Table 1. Rearing conditions during the experiment.

DAYS (p. hatching): 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 45 61

DAYS (p. hatching)	Conditions (in order along the days)
Temp(°C)	8; 9; 10; 11; 12°C
Light	Dark; Light (40w), continuous
Aeration	Weak; Increased aeration
Water exchange	1 pr day; 2 pr day; 4 pr day; 6 pr day; 8 pr day; 10 pr day
Algae	Nannochloropsis oculata (paste)
Feed P	Copepods Acartia tonsa (Rhodomonas); Artemia (MarolE); Gemma Micro
Feed R	Rotifers *B. plicatilis* (Nevada) (*Rhodomonas*); Artemia (MarolE); Gemma Micro
Feed C	Rotifers B. plicatilis (Nevada) (Rotifer Diet + Pavlova paste); Artemia (MarolE); Gemma Micro

From day 25 all larvae got only Artemia and from day 33 only formulated feed, Gemma Micro (Skretting). Sampling of 10 single fish (whole larvae or gut from juveniles), water from rearing tanks and live feed organisms was performed on day 4, 17, 32, 45, and 61 after hatching. The gut was dissected from the fish on the two last sampling days. Standard length, dry weights and MC of the gut of 10 individual larvae were analysed. The MC of water and live feed organisms were also analysed on the same days.

ANALYSIS OF MICROBIAL COMMUNITY (MC)

Samples of fish, water and live feed organisms were stored at -20°C before processing. DNA was extracted from the microorganisms in the individual larvae. PCR and nested PCR was used to amplification of 16Sr DNA using the following primers:

PCR primers	
8f	5`-AGA GTT TGA TC(AC) TGG CTC AG – 3`
984r	5`-GTAAGGTCTTCGCGTT– 3`
Nested PCR primers	
338fGC	5'-cgcccgccgcgcgcggcgggcggggcgggggcacgggg ACTCCTACGGGAGGCAGCAGCAG–3'
518r	5'-ATTACCGCGGCTGCTGG - 3'

PCR products were analyzed by DGGE and the DGGE profiles were analyzed with the GEL 2k program (Fig. 2), developed by Svein Nordland (University of Bergen, Norway). The numerical data were used to calculate the similarity with the Bray-Curtis dissimilarity indexes and ANOSIM to test whether there was a significant difference between two or more groups of sampling units. The larvae groups are different in bacterial species composition *if* the compositional dissimilarities *between* the group are greater than those *within* the groups.

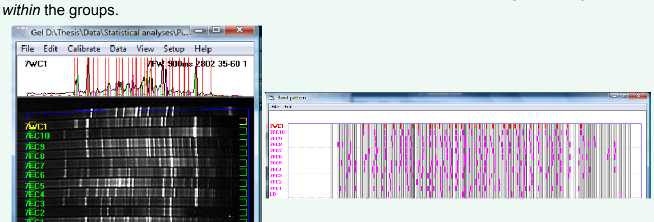
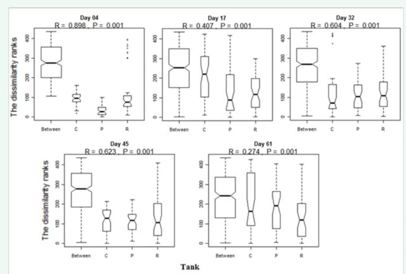


Figure 2. The DGGE profiles analyzed with GEL 2k. The lanes are normalized to contain the same total signal after background subtraction and the gel images are straightened and aligned to give a densitometric curve. The relative positions of the different peaks are grouped together and checked manually.

RESULTS

The dissimilarity was highest at day 17, after two weeks with different diets (Fig. 3). After the change to similar diets (from day 25) the gut flora became more similar between the groups. The diversity of the microbial community (MC) of the cod larvae increased significantly with increasing standard length of the larvae (Fig. 4). The gut flora changed considerably during the 60 days and became more complex (Fig.5). During the initial period with the three different diets larvae in Tank P had the highest stability, whereas rotifers (R and C) induced a shift of 75-80% of the flora. As the larvae developed and changed to new diets new bacterial species colonized the gut. Weaning to dry feed induced a shift of 50-60% of the flora.



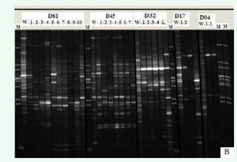


Figure 3. The differences in the MC between and within tanks compared on the different sampling days.

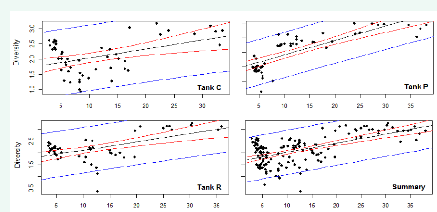


Figure 4. Relationship between diversity of the MC and the standard length of cod larvae in the three live feed regimes (Tank C, P and R) and summary of the three tanks (Summary).

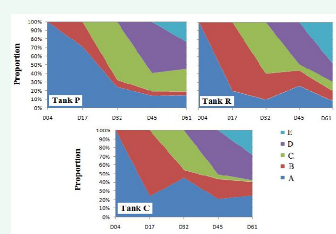


Figure 5. Increasing complexity of the MC in the gut of cod larvae of different age in the three feeding regimes (A=day 4, B=day17, C=day 32, D=day45, E=day 61 post hatching).

CONCLUSIONS

Between 10-20% of the bacterial strains stay in the gut from before first feeding until after the metamorphosis. Rotifers as first feed induce a stronger shift in the flora of cod larvae than copepods. The diversity of the MC increased with increasing size of the larvae, most for larvae fed copepods than for larvae fed rotifers.

LITERATURE

Fjellheim, A.J., Playfoot, K.J., Skjermo, J and Vadstein, O. 2007. *Vibrionaceae* dominates the microflora antagonistic towards *Listonella anguillarum* in the intestine of cultured Atlantic cod (*Gadus morhua* L.) larvae. Aquaculture 269 (2007) 98–106.

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