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Microbial maturation of intake water at different carrying capacities affects microbial control in rearing tanks for marine fish larvae

Kari J.K. Attramadal [a,*], Giusi Minniti [a], Gunvor Øie [b], Elin Kjørsvik [a], Mari-Ann Østensen [a], Ingrid Bakke [c], Olav Vadstein [c]

[a] Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
[b] SINTEF Fisheries and Aquaculture, Trondheim, Norway
[c] Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

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ABSTRACT

The selection pressure can be used to reduce the opportunities for proliferation of potentially harmful bacteria. Hypothetically the chance of proliferation of opportunistic bacteria in the fish tank can be minimized by microbial maturation of the incoming water at a microbial carrying capacity similar to that in the rearing tanks. In a start feeding experiment with Ballan wrasse (*Labrus bergylta*) we compared the bacterial environment in two flow through systems: a microbially matured system (MMS), where the water presented to the fish was matured in a biofilter, and a fed microbially matured system (F-MMS), where the biofilter was fed organic matter to increase the microbial carrying capacity. As predicted, the MMS showed a more variable and often high microbial growth potential in the tank water. The microbial community composition of the tank water was more stable, diverse and species rich in the F-MMS than in the MMS. The results are promising for controlling the microbiota of the rearing water by competent use of water treatment and selection regimes.

Statement of relevance: The experiment shows that small changes in management (organic load and maturation of water) of water treatment give significant different microbiota in fish tanks.

The experiment also shows that by increasing the microbial carrying capacity in a maturation unit to the level of the tank microbial carrying capacity, the microbial community in the fish tank becomes more stable and less open for opportunistic proliferation.

This work reveals promising possibilities for controlling the microbiota of the rearing water in land based aquaculture by competent use of water treatment and selection regimes.

Improved control and understanding of microbial control is very relevant for the aquaculture industry.

1. Introduction

High mortality of larvae and low reproducibility between replicate fish tanks are common in aquaculture hatcheries. These problems are often attributed to infections from opportunistic bacteria (Vadstein et al., 2004). The selection pressure can be used to reduce the opportunities for proliferation of potentially harmful bacteria by filling the niches of the rearing water with harmless bacteria that outcompetes the opportunists, so called microbial maturation (Skjermo et al., 1997). The carrying capacity (CC) is the maximum number of bacteria that can be sustained in the system over time. According to the ecological theory of r/K-selection (MacArthur and Wilson, 1967), selective pressures drive succession in one of two generalized directions: r-selection occurs in unpredictable environments where the ability to reproduce quickly is crucial, whereas K-selection occurs in stable environments where the ability to compete successfully for limited resources (e.g. dissolved organic matter, DOM) is important, i.e. in a community close to the CC. Because microbial maturation in flow through systems (FTS) is related to the relatively low microbial CC of intake water, the transition to significantly higher substrate levels in the rearing tanks represents a change to r-selection and a potential opening for proliferation of opportunists. Hypothetically, the chance of opportunistic proliferation in the fish tank can be minimized by microbial maturation of the incoming water at a CC similar to that in the rearing tanks (Salvesen et al., 1999; Attramadal et al., 2012a, 2012b, 2014). By maturing the water and eliminating the gap in CC between production steps, the fish farmer can gain microbial control. It is hypothesised that a flow through system with microbial maturation (microbially matured system, MMS) offers less microbial control of the rearing water compared to a similar system where organic matter is added to the maturing unit (fed microbially matured system, F-MMS) to increase the CC of the microbially matured water going to the tanks. The F-MMS is predicted to give a more stable and mature microbial community with a lower and more stable net microbial growth potential than the MMS. Further,

* Corresponding author.
E-mail address: Kari.Attramadal@ntnu.no (K.J.K. Attramadal).

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the microbiota of fish reared in the two different systems is expected to be distinguishable.

2. Materials and methods

2.1. Water treatment systems

Two systems for water treatment were compared: one system where the water was microbially matured in a biofilter (MMS) and a similar system where the biofilter was fed with fish feed to increase microbial carrying capacity (F-MMS). Intake water from Trondheimsfjorden (70 m depth) was sand filtered. In the conventional microbially matured system (MMS), the water was matured in a 6 m^3 aerated reservoir (minimum 12 h hydraulic retention time, HRT) with biofilter media (1 m^3 KMT3, Kaldnes Miljøteknologi AS, Norway) before it was introduced to the fish tanks. In the fed microbially matured system (F-MMS), the water was matured in a 450 L aerated biofilter with 150 L KMT Kaldnes K1 Media. The specific biofilm surface of the two different biofilm carriers was the same: 500 m^2 m^{-3} in bulk (Rusten et al., 2006). The water exchange rates in the two maturing units were set to give the same hydraulic retention time per biofilm carrier surface area.

The F-MMS biofilter was conditioned by addition of 30 g d^{-1} (in several doses) pulverized formulated fish feed (Gemma Micro Diamond 300, Skretting, Norway) for 10 days before larvae were transferred to tanks. From 4 days post-hatch (dph) pulverized fish feed (80% Gemma Micro Diamond 300 and 20% AgloNorse 5 (Tromsø Fiskeindustri, Norway), totally 40 g 4–14 dph and 60 g 14–27 dph) was added in two doses to the F-MMS biofilter each day.

2.2. Rearing regime

Ballan wrasse (*Labrus bergylta*) larvae (3 dph) from Marine Harvest Labrus (Øygarden, Norway) were incubated at 12.5 °C in water from the MMS to 4 dph, when they were transferred to the experimental tanks (160 L, black, coned bottom) at a final density of 60 individuals L^{-1}. Larvae were maintained in darkness the first 2 days, then with continuous light. Water exchange rates were increased stepwise from 2 to 4 and then 6 × tank volume d^{-1}. Clay (Vingerling K148, WBB Fucs GmbH, Germany) was distributed to the fish tanks (0.1 g L^{-1} d^{-1}) from 2 dph by a feeding robot (Storvik, Norway). The clay was dissolved in water with a hand blender and was kept in suspension by strong aeration. Each system included three fish tanks. Tank outlets were central cylinders covered with nylon net (400 μm in the rotifer period, thereafter 800 μm) and with weak aeration. The rearing regime is presented in Table 1.

The rotifers (*Brachionus* 'Cayman') were cultured in a semi-continuously harvested flow through system and fed DHA *Chlorella* algae paste (2.5 μg $rotifer^{-1}$ d^{-1}, Chlorella Industry Co., Ltd., Japan) and short time enriched (2 h) with Multigain (Biomar, Norway). *Artemia nauplii* were hatched from INVE EG (Belgium) cysts and short time enriched (24 h) with Multigain before they were fed to the fish (from 23 dph). Live feed organisms were washed on a sieve with sea water and transferred to a 250 L reservoir tank before they were fed to the larval tanks by the feeding robot 4 to 6 times a day, each meal amounting to a tank concentration of 12000 rotifers L^{-1} or 3000 *Artemia* L^{-1}, respectively. From 6 dph the tank bottoms were siphoned for debris three times a week.

2.3. Microbial community analyses

The total numbers of bacteria in water were determined by flow cytometry (FAC-Scan Becton Dickinson, UK). Samples (50 mL) were fixated with glutaric dialdehyde (1% final concentration) and stored in darkness at 4°C. The net microbial growth potential was calculated from the total number of bacteria in water samples that were left for 3 days in open bottles at the same temperature as the fish tank water before they were fixated and was related to the total number of bacteria in samples withdrawn at the same time, but fixated immediately.

Samples for characterization of the composition of the microbial communities in water were collected in 60 mL sterile syringes and filtered through sterile 0.2 μm 2.5 cm^2 hollow fibre syringe filter for aqueous solutions (DynaGard, Microgon Inc., California) which were stored at −20°C.

Samples for characterization of the microbial community composition of larvae were obtained by collecting 6 random individuals from each tank. To avoid larvae with guts filled with live feed, sampling took place right before feeding, and the sampled larvae were left in beakers with no feed for 1 h before an overdose of tricaine methanesulfonate (MS222) was added. Larvae were rinsed in two steps with milliQ water before they were stored in micro centrifuge tubes at −20°C.

To ensure complete DNA extraction, the frozen larvae were homogenized individually in a TissueLyzer (25 Hz for 2 min, Qiagen) with lysis buffer and a 5 mm stainless steel bead added. DNA from both water samples and larvae was extracted using a DNeasy tissue kit from Qiagen following the protocol for Gramme positive bacteria with some minor modifications in the homogenization and the lysis steps (Bakke et al., 2013). Total DNA was quantified with a NanoDrop spectrophotometer (ND-1000 Spectrophotometer, NanoDrop Technologies). The DNA extracts were stored at -20°C. An approximately 200 bp fragment encompassing the v3 region of the 16S rRNA gene was amplified using a nested PCR protocol to avoid amplification of Eukaryotic DNA (Bakke et al., 2011). The primers used for the internal PCR reaction was 338F (5′-actcctacgggaggcagcag-3′) and 518R (5′-attaccgcggctgctgg-3′). PCR products were analysed by DGGE as described by Muyzer et al. (1993). DGGE was performed with the INGENYphorU DGGE system (Ingeny), using 8% acrylamide gels with a denaturing gradient of 35–55% (where 100% correspond to 7 M urea and 40% formamide), 0.5 times TAE electrophoresis buffer, at 100 V and 60°C for approximately 18 h. After electrophoresis, DGGE gels were stained with Sybr Gold (1:10 000 dilution, Molecular Probes) for a minimum of 1 h, rinsed with MilliQ water, and visualized and photographed in a GenBox geldoc system (Syngene).

The DGGE gel images were analysed with the software programme Gel2K (Svein Norland, Dept. of Biology, University of Bergen, Norway), which converts band profiles to histograms. Peak areas, reflecting the intensities of the DGGE bands, were exported and used for further calculations (Table S1).

Table 1
The rearing regime of Ballan wrasse larvae maintained in 160 L tanks at a density of 60 individuals L^{-1}.

Days post-hatch	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
Temperature (°C)	12	12	12	13	13	13	13	13	13	14	14	16	16	15	16	15	15	15	16	16	16	16	16	16	15	15	15
Clay added (g $tank^{-1}$)	16	16	16	16	16	16	16	16	16	16	16	16	16	16	32	32	32	32	32	32	32	32	48	48	48	48	48
Exchange rate (tank volume d^{-1})	2	2	2	2	2	2	2	2	2	2	2	2	2	4	4	4	4	4	4	4	4	4	6	6	6	6	6
Feed type (rotifers/*Artemia*)	n	n	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r/a	r/a	r/a	r/a	r/a
Meals d^{-1}	0	0	1	2	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4/2	4/2	4/3	4/3	4/3