

RECIRCULATION AS A MICROBIAL CONTROL STRATEGY IN INTENSIVE AQUACULTURE OF MARINE LARVAE

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Background

Use of RAS technology may improve marine larvae production by microbial control of rearing water and in live feed production. Marine larvae production is increasing, but hatcheries still struggle with variable quality and survival. Low reproducibility has been attributed to detrimental microbial influences (Salvesen and Vadstein, 1995, Skjermo and Vadstein, 1999). Newly hatched marine larvae lack a functional specific immune system, and are vulnerable to general infections by opportunistic bacteria. In intensive cultivation several procedures destabilize the microbial community and favour proliferation of opportunistic and potentially harmful species. Destabilizing factors include disinfection, high and fluctuating concentrations of organic matter, and high densities of fish and prey with associated microflora. Stabilization and K-selection of the microbial community may counteract proliferation of opportunistic, r-strategic bacteria. Microbial maturation of intake water is a well working and documented strategy to control bacterial composition of intake water through K-selection, resulting in a more beneficial microbial environment for the fish larvae (Vadstein *et al.*, 1993). To mature intake water microbial succession is allowed in a reservoir with a large surface area for bacterial growth (Skjermo *et al.*, 1997). The method has a weak point however, as it is targeted at the relatively low microbial carrying capacity (CC), i.e. organic matter load, of intake water. The higher concentrations of organic matter in the fish tanks and live feed production tanks form new premises for selection and a potential opening for opportunistic proliferation. To create a stable and resistant K-selected microbial environment where the fish are reared, controlled selection should be carried out at a CC similar to that of the rearing tanks. Recirculation aquaculture systems (RAS) have certain properties that may contribute to improve and stabilize the microbial environment in marine hatcheries. Conceptually, RAS allow a long water maturation time at high microbial CC in the system. A large surface area is available for bacterial growth in the biofilter. The heterotrophic biofilter consumes organic matter from feed loss and faeces and supplies the fish tanks with bacteria.

Marine hatcheries and ongrowing systems are very different regarding biomass holding per water volume, organic and particulate loading, water consumption and fish sensitivity. In hatcheries biomass is low and the larvae sensitive to bacterial infections. Production of waste, oxygen consumption and organic loading is relatively low and particles are small. Whereas particle and waste product removal efficiency together with oxygen supply determine the success of production in ongrowing systems, microbial stability may be the main argument for using RAS in hatcheries.

Marine larval rearing

RAS are widely used to stabilize chemical water quality, reduce water consumption and control waste emission. Experiments were conducted to investigate the hypothesis that recirculation may also be used as a means for microbial management by increased maturation time and K-selection for desirable bacteria at a CC comparable to that in the fish tanks.

In experiment 1, the development of the microbial communities in a typical marine fish larval RAS for Atlantic cod (*Gadus morhua*), including ozonation was compared to a traditional flow through systems (FTS). It was evaluated whether the RAS microbial environment development was significantly different from the FTS and if the bacterial flora could be characterised as more stable, mature, and hence more beneficial for the larvae. The criteria used to define superior microbial water quality included a more stable microbial community composition over time, less variability between parallel fish tanks, and a more K-dominated microbial flora with higher diversity and a lower fraction of opportunists. Physiochemical water quality and fish performance was also evaluated.

In experiment 2, the physiochemical and microbial quality of culture water in two RAS with ozonation (O₃-RAS) or UV-irradiation (UV-RAS) was compared to that of a traditional FTS. The O₃-RAS was run with moderate ozonation to an oxidation reduction potential of 350 mV in a foam fractionator. This was not considered to represent disinfection (Hsieh *et al.*, 2002). Bacteria community development was different in the tanks of the two RAS, the UV-RAS being more similar to the microbial profile of the FTS. Fish performance was better and the microbial community development more stable and diverse in the O₃-RAS. The physiochemical environment was similar in the two RAS. There was no reduction in bacterial activity (incorporation of radioactive labelled leucine) measured following ozonation, confirming that the level of ozonation was too low to inactivate bacteria. Due to turbid water from algae addition the UV-RAS disinfection efficiency was low during the rotifer period. In the *Artemia* period, however, there was a clear reduction in bacterial activity following UV-irradiation. The different microbial development observed may be a result of the different disinfection efficiency or method.

In both experiments the RAS developed and maintained a significantly different and more diverse and stable microbial community as compared to the FTS. This was reflected in similar or better performance of RAS larvae, despite an apparent inferior physiochemical water quality, as judged by traditional criteria. From our results, recirculation seems to be an easy and efficient way to stabilize the microbial environment in marine hatcheries.

Live feed production

In aquaculture of marine fish larvae live feed is still necessary the first weeks after the yolk sac has been absorbed. Live feed production is labor intensive and may be unstable, which makes marine fish juvenile production time consuming and costly. Even when rinsed before feeding, live feed are commonly accompanied by high levels of opportunistic bacteria including *Vibrio* spp. and haemolytic types (Salvesen *et al.*, 1999, Olsen *et al.*, 2000). It is possible to mitigate the microbial composition of live feed, for example through algae (Makridis *et al.*, 2006). The bacterial flora of the live feed is shown to be directly transferred to first feeding larvae (Olsen *et al.*, 2000). A correlation has been found between bacterial counts on the live feed and larval mortality (Munro *et al.*, 1999). Bacterial production of RAS rearing tanks have been shown to depend on the concentration of live feed (Blancheton and Canaguier, 1995). Based on this, if the microbial composition of the live feed culture can be stabilized and improved by using RAS, there seem to be a chance this could also improve larval bacterial environment. Suantika and colleagues (2003) used RAS to successfully stabilize rotifer production. They found a stable bacterial load in the rotifer culture water after two and three weeks of RAS operation. Development of new technology at SINTEF and NTNU has made it possible to produce rotifers in semi continuous cultures in RAS for several months without the need to empty the production tank for cleaning.

Copepods are natural prey for cold water marine fish, and are known to have a beneficial nutrient composition, i.a. a relatively high HUFA content, for larval growth, survival and development. Experiments at SINTEF and NTNU show that cultured *Acartia tonsa* increase growth and survival of first feeding Atlantic cod larvae. Cultivation of copepods gives better control and biosecurity of the live feed compared to wild catch. Ongoing experiments are conducted to investigate production of *Acartia tonsa* in RAS. The microbial community development of culture water is studied.

Suspended particles removal and membrane integration

Small organic particles originating from algae, biofilter, live feed, faeces and dead larvae are hard to remove and tend to accumulate in hatcheries (Rueter and Johnson, 1995) and especially RAS (Chen *et al.*, 1993, Losordo *et al.*, 1998). Organic particles continuously dissolve to microbe substrate, so efficient removal may stabilize and reduce bacterial proliferation. In addition, the ammonia and carbondioxide contribution resulting from bacterial degradation of waste particles may be limited by efficient solids removal. The presence of live feed and other particles influences UV and ozone disinfection efficiency (Hess-Erga *et al.*, 2008). Traditional solids removal technology like microscreen filters combined with particle traps leave much of the dissolved, colloidal and fine fraction of the solids in the water. Membrane filtration may be a way to control accumulation of the colloidal and fine fraction of the solids and reduce the amount of organic material and particles in marine RAS hatcheries.

Membrane bioreactors MBR are commonly understood as the combination of biological treatment using activated sludge (AS) and membranes for filtration (AS-MBR, Leiknes and Ødegaard, 2007) to achieve an advanced level of organic, nutrient and suspended solids removal (Ivanovic *et al.*, 2008). Viadero and Noblet (2002) studied the use of AS-MBR in freshwater RAS and found membrane filtration useful as microscreen filters were not efficient in removing fine solids ($< 60 \mu\text{m}$). Being less cost effective than microscreen clarification these authors suggested membrane filtration for niche applications such as larval fish culture. Some work has already been conducted to test the potential of integrating the AS-MBR in marine RAS. This is not fully established and commercialized. However, there exist a number of commercial configurations of the AS-MBR system that treats municipal and industrial wastewater.

As with all membrane processes, membrane permeability in the AS-MBR will be reduced because of accumulation of materials on the surface of or within the membrane, which decreases the filtration flux. Membrane fouling is quite complicated and complex, but the main forms are solids deposition as cake layer, pore clogging by colloidal particles, adsorption of soluble compounds and biofouling (Leiknes and Ødegaard, 2007). It appears that suspended solids are only partially responsible for the resistance increase during filtration, the dissolved (such as extracellular polymeric substances, ESP) and colloidal fraction appears to be very important and quite extensive contributors as well (Wisniewski and Grasmick, 1998, Defrance *et al.*, 2000, Bouhabila *et al.*, 2001 Bae and Tak, 2005, Ahl *et al.*, 2006, Leiknes and Ødegaard, 2007).

All research concerning MBR in RAS water treatment systems to date have applied the active sludge process in combination with the membrane (AS-MBR). Research has recently been done to test the application of a new alternative wastewater treatment method combining a moving-bed-biofilm reactor (MBBR) and a submerged membrane reactor, forming a hybrid biofilm membrane bioreactor, BF-MBR. Separation of the suspended solids by BF-MBR in wastewater treatment is an alternative to the conventional AS-MBR. BF-MBR has the potential of utilizing the best characteristics of biofilm process and membrane separation resulting in a compact, efficient particle and nutrient removal system. BF-MBR may cause the reduction of membrane fouling by high biomass concentration and efficient removal of soluble organic matter, even during high particle loading rates (Leiknes and Ødegaard, 2007). BF-MBR has been tested for treatment of municipal wastewater from a combined sewer system (Leiknes *et al.*, 2006, Leiknes and Ødegaard, 2007) and oily wastewater from ships (Sun *et al.*, 2010), but it has apparently not yet been tested for marine aquaculture systems.

A stable nitrifying biofilm is harder to maintain in systems with cold and saline water (Watson, 1971, Wortman and Wheaton, 1991, Zhu and Chen, 2002). In addition, marine fish larval rearing systems are operating with low, but exponentially increasing levels of substrate as the fish grow, which further complicates the operation (Gutierrez-Wing and Malone, 2006). Nitrification efficiency increases in RAS biofilters when the C/N ratio of

the water decreases (Michaud *et al.*, 2006). Separation in two designated biofilters may increase efficiency of heterotrophic maturation and nitrification by securing optimal selection pressure for each process. Membrane filtration upstream to the nitrifying filter could minimize organic load and reduce competition with heterotrophs.

Conclusions

Marine hatcheries represent high value, low waste systems for larvae sensitive to general infections by opportunistic bacteria. In intensive larval rearing several procedures may destabilize the microbial community, creating ideal conditions for r-selection and favouring proliferation of opportunistic and potentially harmful microbes. Destabilizing factors include disinfection and high and fluctuating concentrations of organic matter. Live feed is essential in early stages of marine fish production, but represent a significant contribution of opportunistic bacteria. RAS have properties that may mature and stabilize the microbial community, creating a more benign bacterial flora in larval tanks. RAS could also be used to influence the microbial composition of rotifers and copepods. Integration of membrane filtration may be a way to reduce the accumulation of fine solids in marine hatchery RAS, and could be used to optimize nitrification by reducing heterotrophic competition.

Acknowledgements

This work was financed by the Norwegian Research Council, the Norwegian University of Science and Technology and SINTEF Fisheries and Aquaculture.

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