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The effects of moderate ozonation or high intensity UV-irradiation on the microbial environment in RAS for marine larvae

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ABSTRACT

Marine fish larvae are sensitive to infections by opportunistic bacteria. Procedures like disinfection and pulse feeding may destabilise the microbial community and promote r-selection and proliferation of opportunists in intensive rearing tanks. Recirculation aquaculture systems (RAS) have been hypothesised to stabilise and mature the microbial community, creating a more beneficial environment for the larvae during the live feed period. Two marine RAS with Atlantic cod larvae (*Gadus morhua* L.) with either moderate ozonation (RAS O_3) or high intensity UV-irradiation (RAS UV) were compared with a flow-through system (FTS). The two RAS developed a different and more stable composition of the microbial community than the FTS. The RAS O_3 had a more mature and stable microbial community than the RAS UV. The density and the activity of bacteria were higher in the rearing tanks than in the in-flowing water in the RAS UV system, whereas for the RAS O_3 system densities and activity of bacteria were similar, indicating low disinfection efficiency with moderate ozonation. Atlantic cod larvae reared in the RAS O_3 showed the best survival and growth, whereas the RAS UV larvae performed equally well or better than their siblings in the FTS. This was in spite of the fact that the physicochemical water quality of the two RAS was inferior to that of the FTS. The agar-based method used to quantify opportunists may be too general to capture important differences in the microbial community of the rearing water. For the future, molecular methods could be used to identify which functional groups or species of bacteria are contributing to the observed RAS effect. Our results support the hypothesis of RAS as a microbial control strategy during first feeding of larvae. However, a RAS for marine larvae should probably not include strong disinfection because it leads to a reduction in bacterial numbers, which is likely to result in a destabilization of the microbial community.

1. Introduction

Pelagic marine fish larvae hatch at an early developmental stage. The first weeks after hatching they depend on the general immune system, and larvae are sensitive to infections. Most infections in young marine larvae are assumed to be caused by opportunistic bacteria becoming pathogenic when the resistance of the host is lowered by environmental stress. A high fraction of opportunistic bacteria in the rearing water has been shown to reduce the performance of marine fish larvae (Salvesen et al., 1999; Skjermo and Vadstein, 1999; Skjermo et al., 1997; Vadstein et al., 1993). Efficient strategies to increase microbial control may help to increase and stabilise the production of marine juveniles. The use of recirculating aquaculture systems (RAS) has been suggested to stabilise and improve the microbial environment for marine larvae during first feeding, but little is known about how disinfection doses and different disinfection methods like ozonation and UV-irradiation affect the development of the microbial community in RAS (Attramadal et al., in press).

The abundance of bacteria and the composition of the microbial community of the rearing water depend on the supply of bacteria and organic matter, together with selective forces in the tank and in the sources (Vadstein et al., 1993, 2004). The main contributors of bacteria to rearing tanks in marine hatcheries are live feed (Blancheton and Canaguier, 1995; Olsen et al., 1999; Skjermo and Vadstein, 1993), algae (Salvesen et al., 2000) and intake water, as eggs are commonly surface disinfected (Salvesen and Vadstein, 1995). According to the ecological r/K-theory (MacArthur and Wilson, 1967), r-selected opportunistic species have the ability to grow fast and are typically favoured in perturbed or unpredictable environments with little competition (e.g. a high substrate supply per capita). K-strategists, on the other hand, are specialists that compete better for limited resources, and are favoured in stable environments with communities close to the carrying capacity (CC). The CC is

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the maximum number of bacteria that can be sustained in a system for an extended period of time. CC is defined by density dependent restrictions like availability of resources, which typically is supply of dissolved organic matter (DOM) for the heterotrophic bacteria.

Procedures like disinfection and pulse feeding may destabilise the microbial community in intensive marine hatcheries (Salvesen, 1999; Salvesen et al., 2000). Following a perturbation, opportunistic pioneer species of bacteria are the first to colonise the free niches that are created due to increased supply of resources or decimation in the number of competitors (Hess-Erga et al., 2010). As the population increases to CC and the resources become limited, the r-strategists are gradually out-competed by K-selected species. This succession in the microbial community takes about one week in sea water at 5–18 °C (Salvesen, 1999).

The host/microbe interactions in the rearing water may be improved by promoting K-selection to reduce the fraction of opportunists (Vadstein et al., 1993). K-selection may be created by maintaining low substrate availability per bacteria (Salvesen et al., 1999; Skjermo et al., 1997; Vadstein et al., 1993). This strategy involves controlling two important factors: 1. the supply of substrate and 2. the number of bacteria competing for the substrate. Ideally, the supply of dissolved organic matter should be stable and the microbial population should be close to CC to obtain a low fraction of opportunists. RAS may provide a sufficiently long retention time of water to allow the microbial succession to take place at a relatively stable level of organic matter which secures K-selection, and are therefore hypothesised to mature and stabilise the microbial community of the rearing water compared to flow through systems (FTS) (Attramadal et al., in press).

UV-irradiation and ozonation are two common methods used for disinfection of intake water. The water treatment in a RAS may include UV-irradiation to reduce the abundance of bacteria. Ozonation in RAS, however, is in most cases motivated by improvement of the physicochemical water quality rather than disinfection (Summerfelt, 2003; Tango and Gagnon, 2003). Ozone is unsuitable as a disinfection method in marine RAS for several reasons. One is that RAS process water requires high amounts of ozone to inactivate bacteria, as the oxidative power of ozone and residual oxidants is consumed in reactions with organic matter and other components of the rearing water. Another reason is that several compounds formed when high doses of ozone are added to sea water are highly toxic to fish and live feed (Davis and Arnold, 1997; Grotmol and Totland, 2000; Ozawa et al., 1991), although some of the residual oxidants can be reduced by passing the water through activated carbon filters (Kobayashi et al., 1993; Ozawa et al., 1991).

The level of ozonation of culture water is commonly controlled by continuous measurements of the oxidation reduction potential (ORP), which is an indirect measure of the concentration of free radicals in a solution. Moderate ozonation to an ORP of about 300–350 mV is common and considered safe for marine fish in RAS, although some production of toxic bromate has been demonstrated at this level (Tango and Gagnon, 2003). Ozonation to 350 mV does not represent efficient disinfection in a marine RAS (Hsieh et al., 2002), but has been shown to improve the physicochemical water quality (Kobayashi et al., 1993; Tango and Gagnon, 2003) leading to increased fish survival and growth (Ozawa et al., 1991; Reid and Arnold, 1994).

The disinfection effects of UV-irradiation and ozonation are strongly influenced by turbidity and the presence of inorganic, as well as organic particles in the water that may protect microorganisms from inactivation (Hess-Erga et al., 2008). UV-transmittance is reduced by turbidity and may be blocked by particles, whereas the oxidative power of ozone and residual oxidants can be reduced due to degradation of disinfectant at the surface of suspended solids and the rate limited transport into particles (Perrins et al., 2006). During the rotifer period, addition of microalgae to the culture water has a beneficial influence on survival and growth of marine fish larvae (Howell, 1979; Lazo et al., 2000; Naas et al., 1992; Reitan et al., 1993; Salvesen et al., 1999). During the "green water" period, with high turbidity due to small suspended particles, the disinfection efficiency in a RAS is probably low for both UV-irradiation and ozonation.

UV-irradiation is a physical disinfection method, whereas ozone is a chemical agent. Most likely, the dose and mechanism of the hygienic barrier may influence microbial selection, stabilization, maturation and the development of the microbial community in a RAS over time. Because RAS are commonly operated at low or moderate levels of ozonation, but with high intensity UV, the two methods are likely to represent different levels of disinfection efficiency.

Here we present a study of the effects of UV-irradiation versus moderate ozonation on the level of maturation, K-selection and stability of the microbial community in the rearing water of two marine RAS with Atlantic cod (*Gadus morhua* L.), and we compare them with a FTS. The two RAS were identical apart from including either ozonation to 350 mV and two active carbon filters or UV-irradiation. Both the UV-irradiation and the ozonation happened before the water was biofiltered. The growth and survival of fish reared in the three systems were compared.

2. Materials and methods

2.1. Experimental setup

Fig. 1 shows a flow scheme of the systems. Intake water (70 m depth) from Trondheimsfjorden was pumped through a sand filter (Triton TR-140, Pentair Inc., USA) and a protein skimmer (Helgoland 500, Erwin Sander Elektroapparatebau GmbH, Germany) before it was led to a reservoir with a vacuum aerator and a heating/cooling system (Carrier Corp., USA).

Both RAS included a 150 L pump sump with a removable cross flow filter of 50 μm during the rotifer period and 150 μm thereafter

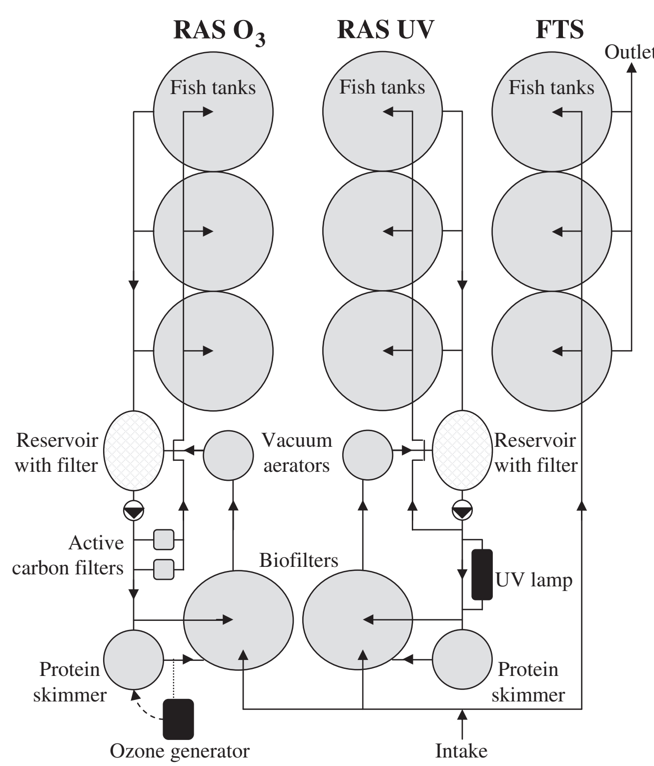

Fig. 1. Flow scheme of the two RAS and the FTS (not to scale).