RECIRCULATION (RAS) VS FLOW THROUGH (FT) SYSTEMS DURING YOLK SAC AND FIRST FEEDING STAGES: EFFECTS ON REARING SYSTEM BACTERIOLOGY, AND SURVIVAL, QUALITY AND GROWTH OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*) LARVAE.

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Introduction

The commercial production of halibut fry is currently carried out in flow through systems (FT), while there is a growing consensus that a recirculation system (RAS) would offer more stable environmental and chemical water parameters that would lead to improved larval performance. At the Institute of Marine Research (IMR) it is standard practice to treat the larvae with antibiotics the first three days of the start feeding period, in case of dropping appetite during this period. To avoid use of antibiotics, and potentially decrease mortality, use of RAS was tested during yolk sac incubation and first feeding.

Addition of probiotics is a way of improving survival of fish larvae, which is presently gaining increased interest. It is not clear whether the intestinal microflora of halibut larvae is determined by the feed or by water quality parameters (see Bergh et al., 1994; Attramadal, 2011).

Materials and Methods

A RAS system for halibut yolk sac incubators was constructed by connecting two identical silos (B and C): one silo for larvae and one as a bio-filter (C). Silo A had normal water flow through (FT) with seawater taken from 160 metres depth, filtered through 15um filter and temperature regulated (heat pump). Between the two silos a water cooling system was mounted. Water from the silo incubated with halibut larvae was passively led from the outlet (top of silo) into the silo functioning as a bio-filter and thereafter pumped further back to the bottom of the silo incubated with larvae. The RAS silo was filled with seawater from the same water source as the FT silo and run for two weeks prior to incubation of halibut eggs. Halibut eggs were administered to silo A and B, three days prior to hatching. From incubation until hatching was completed, both silos were run as flow through incubators. This is due to addition of freshwater during this period to create a density gradient in the upper part of the silos (prevents the newly hatched larvae from contact with the outlet sieve). After completion of hatching, the RAS silo was connected to the bio-filter for the entire yolk sac period (43 days).
At an age of 265 day-degrees, halibut larvae were transferred from a flow-through yolk sac incubator to 6 first feeding tanks. Numbers of larvae were approximately 5000 in each tank. Three of the tanks were connected to a RAS system (Tropical Marine Center). The three other tanks were supplied with a standard flow-through water system with water coming from 160m depth. The tanks had a volume of 1400 l and the water flow was 5 l $\cdot$ minute^{-1}. Water temperature was 12 ± 0.3°C during the whole period. The larvae were fed enriched *Artemia* nauplii three times a day and the amount of remaining *Artemia* in the tanks was estimated before each feeding to give an estimate of feeding activity in each tank. Samples of water and larvae were taken weekly for analysis of nitrogen compounds (NH_3 and NO) and larval growth. To achieve feeding behavior and feed ingestion it is necessary to have turbid water. This is commonly made by addition of microalgae to the water. At IMR we have substituted the algae with dissolved clay, which was added to the first feeding tanks morning and evening (Attramadal et al., 2011).

The experiments were conducted both in 2016 and repeated with some adjustments in 2017. The water cooling system during the yolk sac stage was changed from being between the bio-filter silo and larvae silo to being held within the bio-filter silo. The TMC 5000 recirculation unit was started one month prior to larval incubation in 2017, and was constantly conditioned by supplement of a daily amount of 1.7 g NH_4Cl.

Results and discussion

There were only small differences in survival during the yolk sac stage in 2016. However, the number of larvae suffering from jaw deformities was higher in the recirculation incubator. In 2017, survival was high in both silos but a higher proportion of jaw deformed larvae was found in the flow-through incubator. This indicates that other factors than recirculation are accountable for jaw deformities.

In 2016, there were no significant differences in survival during the start-feeding stage between flow-through and recirculating tanks. However, larval growth was significantly depressed in the recirculating tanks. The concentration of un-ionized ammonia was much higher (0,025 mg/l) in the recirculating tanks compared to the flow through tanks (0,001 mg/l). First-feeding trials in 2017 are in progress and results will be presented and discussed.

Bacteriological samples are taken from yolk-sac and first-feeding larvae, and from all incubation and first-feeding systems through the experiemntal periods, and sequence analyses will be carried out for microbiome characterization.

References

Attramadal, K.J.K. (2011) Water treatment as an approach to increase microbial control in the culture of cold water marine larvae. Doctoral thesis at NTNU, Norwegian University of Science and Technology. NTNU, 2011:231. ISBN 978-82-471-3021-6 (printed ver.); ISBN 978-82-471-3023-0 (electronic. ver); ISSN 1503-8181

Attramadal, K.J.K., Tøndel, B., Salvesen, I., Øie, G., Vadstein, O., and Olsen, Y. (2011). Ceramic clay reduces the load of organic matter and bacteria in marine fish larval culture tanks. Aquacultural Engineering 49 (2012) 23– 34.

Bergh, Ø., Naas, K.E**.,** and Harboe, T. (1994)**.** Shift in the intestinal microflora of Atlantic halibut (*Hippoglossus hippoglossus*) larvae during first feeding. Can. J. Fish. Aquat. Sci. 51:1899-1903.

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