New species for EU aquaculture

Deliverable Report

Deliverable No:	D17.2	**Delivery Month:**	57
Deliverable Title	Determine if RAS is a more effective protocol than FT for Atlantic halibut larvae		
WP No:	11	**WP Lead beneficiary:**	P7. IMR
WP Title:	Larval husbandry – Atlantic halibut		
Task No:	17.1	**Task Lead beneficiary:**	P7. IMR
Task Title:	Recirculation (RAS) vs Flow through (FT) systems during yolk sac and first feeding stages and the effects on larval survival, quality and growth.		
Other beneficiaries:	P22. SWH		
Status:	**Delivered**	**Expected month:**	54
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Other Scientists participating: Norberg, B., A. Nerland and Ø. Bergh (IMR)

Objective: The objective of this deliverable was to determine if RAS is a more effective protocol than FT for Atlantic halibut (*Hippoglossus hippoglossus*) larvae rearing.

Introduction

The commercial production of Atlantic halibut (*Hippoglossus hippoglossus*) 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. The yolk sac and first feeding stages in Atlantic halibut are performed in different rearing systems. RAS systems for both these stages are presented here. The first test of a RAS system for the yolk sac stage was done in 2015 and a second, modified trial, was conducted in 2017. Both experiments were done without replicate silos. For the first feeding stage, experiments were conducted in 2016 and 2017. These experiments were done using triplicate tanks both for the RAS system and control tanks. The protocol is based on the second trial for both yolk sac and first feeding stages.

Yolk sac stage:

Standard incubation procedure: The yolk sac stage lasts for 43 days at 6°C in Atlantic halibut. Fertilised eggs are transferred to the silos approximately 3 days prior to hatch. At this time, a salinity gradient has been established in the upper part of the silo by use of freshwater. Hatching is synchronized by use of light, which arrests hatching, and thereafter darkness to induce hatching. The salinity gradient is present during hatching and for one or two more days, depending of the buoyancy of the larvae. Recirculation is not used in this period. The silos used for water treatment and for larval rearing, are 5000 litres in volume (**Fig. 17.2.1**). Approximately 1 to 2 litres of eggs (40 000-80 000 eggs) are normally incubated in one silo, depending on the size of the egg batch. There is no feeding or any addition of organic material during this period.

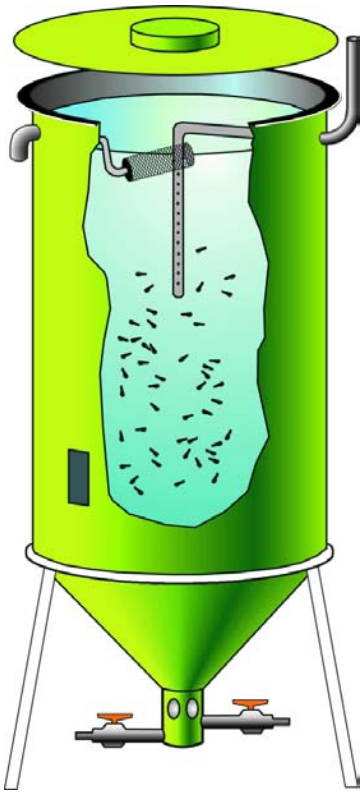

Figure 17.2.1. Schematic illustration of yolk sac silo.

Materials and methods:

Two trials were conducted before the protocol was finalized. In the first trial, water temperature was adjusted between the RAS silo (without larvae) and the silo inoculated with larvae. In the second trial this temperature adjustment was done within the RAS silo, resulting in a more even temperature profile (**Fig. 17.2.2**).

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Figure 17.2.2. Illustration of the RAS used for yolk sac larvae. A= silo with larvae, B=water treatment, C= water pump including flowmeter. D= water cooler.

Results:

Only small differences in survival were observed during yolk sac incubation between FT and RAS both in 2015 and 2017 (**Fig. 17.2.3**). Proportion of jaw deformed larvae (**Fig. 17.2.4**) was 14% in 2015 and 11% in 2017 for the RAS larvae and 9% in 2015 and 17% in 2017 for the FT larvae.

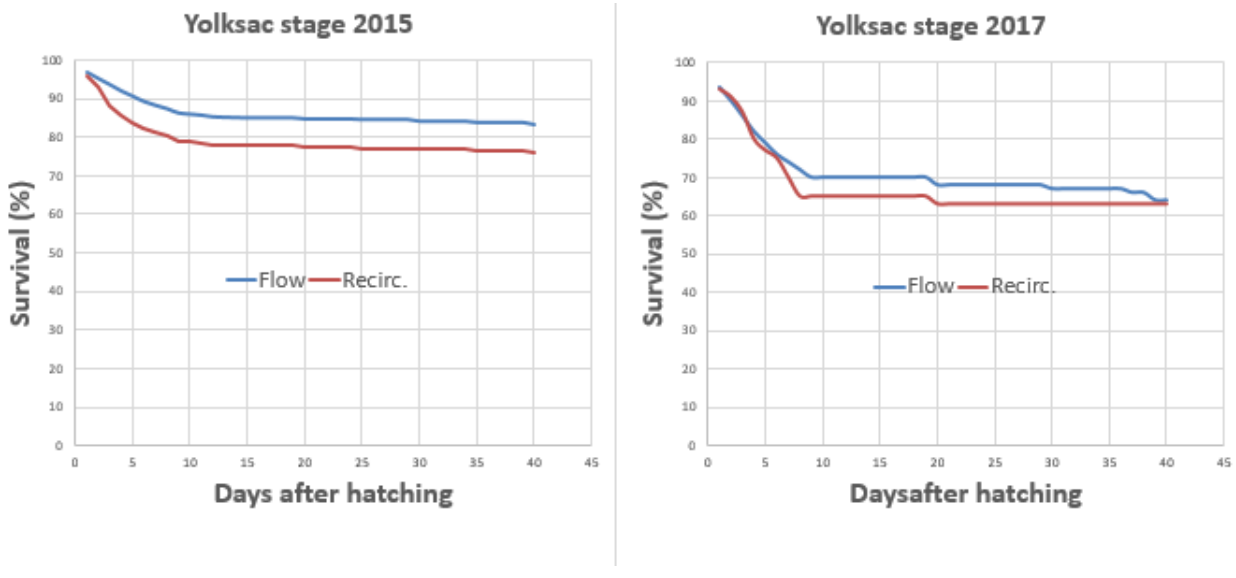

Figure 17.2.3. Larval survival (%) in 2015 and 2017.

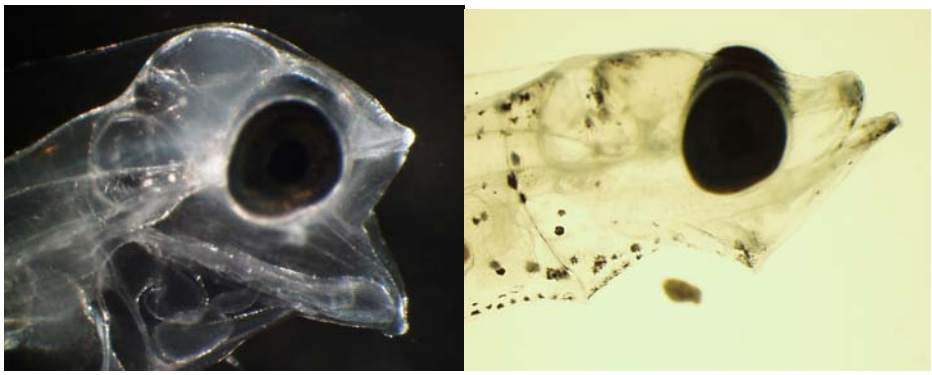

Figure 17.2.4. Atlantic halibut larvae with (left) and without (right) jaw deformity.

First feeding stage:

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, to prevent appetite reduction during this period. To avoid use of antibiotics and to decrease mortalities use of a RAS was tested, in order to establish a stable microbial environment. It is not clear whether the intestinal microflora of Atlantic halibut larvae is determined by the feed or by water quality parameters (see Bergh et al., 1994; Attramadal, 2011). Short time enriched *Artemia* is most widely used for first feeding of Atlantic halibut larvae. The feeding period is normally 45 to 50 days before they are weaned to a dry diet.

Materials and methods:

A RAS system from Tropical Marine Centre (TMC) (**Fig. 17.2.5**) has been used by the IMR for research on several cold-water and warm-water marine species. In this set up three first feeding tanks were connected to the system (**Fig. 17.2.6**). The system consists of a reservoir (650 liter), filter bags, sand filter, re-gassing / trickling biofilter and a protein skimmer. We did not use the UV steriliser.

Figure 17.2.5. RAS system P5000P MARINE from Tropical Marine Centre.

Figure 17.2.6. First feeding tanks attached the RAS system.

The first-feeding tanks were flat bottomed, with a volume of 1100 l and a water flow of 5 l per minute. Water temperature was 12 ± 0.3°C during the whole period. The tanks had shadow frames to avoid illumination of the walls and fluorescent (daylight) light sources placed 70 cm above the water surface, giving a light intensity of approximately 400 lux at the surface. The tanks had central aeration near the bottom. The water outlet sieves were also in the center of the tanks, reaching from the bottom to the surface. Water inlets were placed near the tank wall approximately 10 cm below the surface. An automatic cleaning device (car wipers) were mounted in each tank and were run once a day. After one rotation, dead material was removed by a siphon. The water volume that was removed daily by siphoning represented the water exchange in the RAS system. The recirculating volume was calculated to 97%. Water turbidity was created by use of dissolved clay (Sibelco, Vingerling K148, white) to an initial turbidity of 2 NTU (Harboe and Reitan 2005). Approximately 10g of clay was dissolved in one liter of freshwater and added to each tank twice a day. Before the water returned to the RAS unit it was filtered to remove *Artemia* and part of the clay (**Fig. 17.2.7**). The reminding clay was left in the RAS unit, mostly in the reservoir.

Figure 17.2.7. Bag filter to remove access *Artemia* and sedimentation tank for clay.

Results:

Larval growth was significantly higher in the FT system compared to RAS in 2016. In 2017 the situation was opposite as the larval growth was significantly higher in the RAS group. There were no differences in growth between the FT group in 2016 and the RAS group in 2017 (**Fig. 17.2.8**).

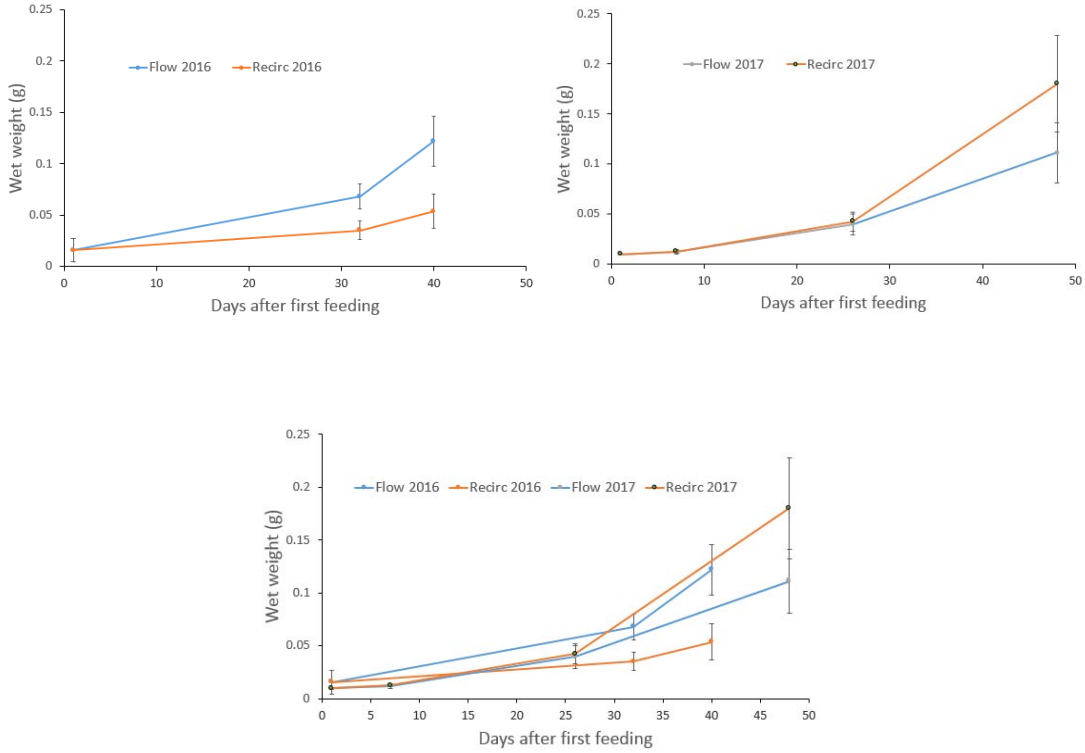

Figure 17.2.8. Larval weight development in 20016 (a) 2017 (b) and combined (c).

In 2016, the RAS system was started only a short time prior to larvae incubation. The concentration of un-ionized ammonia was significantly higher in the RAS tanks compared to the FT tanks. In 2017 the RAS system was started one month before larval incubation and fed daily with 1,7 g of NH_4CL. At the end of the experiments there was clay in the unit, but the concentration of un-ionized ammonia did not increase The concentration of un-ionized ammonia did not differ from the FT system in 2017 (**Fig. 17.2.9**).

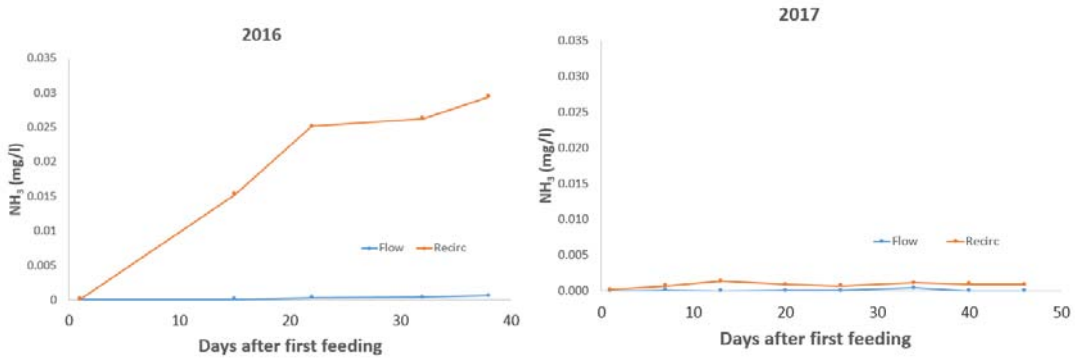

Figure 17.2.9. Concentration of unionized ammonia for the FT and RAS systems in 20016 (a) and 2017 (b).

There were no significant differences in survival through first feeding between FT and RAS tanks in 2016. In 2017, high mortality occurred in one of the RAS tanks (**Fig. 17.2.10**).

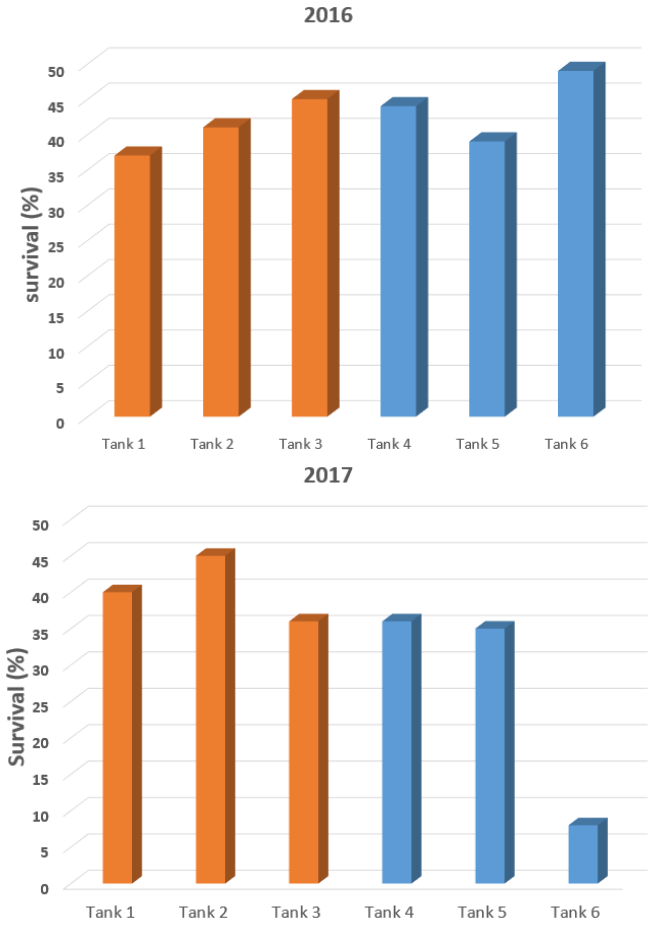

Figure 17.2.10. Survival (%) of larvae at end of the experiments. Tank 1, 2 and 3 =RAS. Tank 4, 5 and 6=FT. The survival is basted on estimated number of larvae incubated from start and counted larvae at the end of the experiment.

Discussion:

Atlantic halibut differs from other marine cold-water species by its comparatively long-lasting yolk sac stage. This stage has been a bottleneck in the production of fry, mainly due to the changes that appear in buoyancy from egg to larva. Small changes in temperature or salinity strongly influence the positioning of the larvae in the water column resulting in mortality and malformations like jaw deformity. We expected RAS to give lower variation in water density compared to an FT system, which would result in better production stability. Water temperature during this stage was 6°C and no organic material was added. There was therefore no need for a specific biofilter. The yolk sac incubators are large in volume and are normally incubated with 1 to 2 l of eggs, depending of the size of the egg batch. The experiment was repeated since it is not possible to have triplicate tanks. In both runs there were only small differences in survival between the treatments. Appearance of jaw deformities was larger in the RAS larvae then for the FT larvae in the first run. However, in the second run the case was the opposite. Both runs were within what we normally expect both when it comes to survival and proportion of jaw deformed larvae.

The first feeding tanks were smaller than the yolk sac incubators and contained a lower number of larvae per unit. Although larval survival in the RAS system was stable and high for both runs, larval growth was

significantly lower in the first run, both compared to the FT control and to RAS larvae in the second run. This was likely due to the high concentration of un-ionized ammonia, caused by insufficient priming of the RAS system in the first run. In the second run, the FT systems appeared less stable than the RAS system, as seen by both growth and mortality: growth was almost twice as high in the RAS system, and larval mortality increased in one FT tank in the second half of the period leading to a loss of >90% of the larvae in that tank. However, RAS larval growth in the second run did not differ from the growth of the FT larvae in the first run. Taken together, the results suggest that with adequate conditioning in the RAS system, a stable system is established where growth and survival of larvae is as good as, or better, than in FT systems with optimal conditions. Based on these two experiments we conclude that RAS was a more stable rearing system for Atlantic halibut larvae compared to the FT system.

References:

Bergh, Ø., Naas, K.E. & 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.

K. Attramadal (2011). Water treatment as an approach to increase microbial control in the culture of cold water marine larvae. Thesis for the degree of Philosophiae Doctor Trondheim, September 2011 Norwegian niversity of Science and Technology Faculty of Natural Sciences and Technology Department of Biology.

Harboe T. & Reitan K. I., (2005). Halibut fry production. Larvi '05 – Fish and shellfish larviculture symposium. Hendry C. I:, Van Stappen G., Wille M. & Sorgeloos P. (Eds.) *European Aquaculture society, Spesial publication No. 36, Oostende, Belgium.*