Deliverable Report

Deliverable No:	D17.4	**Delivery Month:**	36
Deliverable Title	Comparison of feeding on-grown *Artemia* versus *Artemia* nauplii on Atlantic halibut larval performance		
WP No:	17	**WP Lead beneficiary:**	P7. IMR
WP Title:	Larval husbandry – Atlantic halibut		
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Other Scientists participating: Birgitta Norberg (IMR)

Objective: The objective of this Deliverable was to compare use of on-grown *Artemia* versus *Artemia* nauplii on Atlantic halibut (*Hippoglossus hippoglossus*) larval performance

Introduction

A possible strategy to alleviate the slow growth in the later larval stages of Atlantic halibut and improve juvenile quality is to use on-grown *Artemia*. On-grown *Artemia* are larger, contain more protein and phospholipids and have a different micronutrient status from *Artemia* nauplii (Hamre and Harboe, unpublished; Task 11.2). They also have a lower shell to nutrient content. Olsen et al., (1999) found that Atlantic halibut larvae fed on-grown *Artemia* develop into juveniles with better pigmentation and eye migration than Atlantic halibut fed *Artemia* nauplii. This was verified in a feeding experiment performed in 2005 (K. Hamre, P17. NIFES and T. Harboe, unpublished). The industry is considering implementing this knowledge in their production line, but will need further documentation.

A holistic understanding of feeding and digestive functions is important for designing diets for fish larvae and the adaptation of rearing conditions to meet requirements for the best presentation of prey and microdiets, and their optimal ingestion, digestion and absorption (Ronnestad et al., 2013). In this sense, it is obvious that a better knowledge of larval digestive ontogeny and its physiology when using different scientific approaches and techniques (e.g. feeding Atlantic halibut larvae with *Artemia* nauplii or on-grown *Artemia*) will contribute to the optimization of diets and rearing conditions. The analysis of the main digestive enzymes under these two different rearing strategies may help to understand the plasticity of the processing capacity of the digestive system in species such as Atlantic halibut, to deliver nutrients to the rapidly growing larval tissues under variable feeding and environmental conditions.

Materials and methods

Atlantic halibut larvae from one single egg batch were hatched and further incubated in two 5-m^3 silos until 260 day-degrees post hatch. They were then transferred to 6 first feeding tanks and stocked at ca. 5000 larvae $tank^{-1}$. The first feeding tanks were 1.5 m in diameter and 0.8 m in height. The tanks had continuous water supply entering near the surface and an outlet sieve in the middle of the tank (**Fig. 1**)

Figure 1. First feeding tank for Atlantic halibut larvae

Each tank had a fluorescent light above its center and was equipped with a shadow frame, to reduce light reflections from the tank wall, which can attract the larvae. The tanks also had central aeration from near the bottom and an automatic cleaning system consisting of a cleaning arm (car windshield wiper) that rotates slowly at the bottom by use of an electric motor (**Fig. 2**).

Figure 2. Automatic tank cleaning system at the bottom of the first feeding tank for Atlantic halibut larvae

The temperature of the rearing water was 12 ± 0.2°C. Water flow started at 1 l min^{-1} at the start of incubation and increased within the next 4 days to 5 l min^{-1}, where it was held for the remainder of the experiment. Dissolved clay (30 g morning and 30 g evening) was added to each tank daily to keep turbidity high during the live feed period. Live feed was added three times a day at 10.00, 15.00 and 21.00. Light was on from 07.00 to 24.00.

Artemia cysts (EG, INVE Aquaculture) were hatched in a separate tank, incubated for 24 hours and then transferred to either short-term enrichment or on-growing tanks. Conical 300 l fiberglass tanks were used for hatching, short-term enrichment and on-grown *Artemia*. All tanks were equipped with temperature (500W, Carlo Gavazzi 600+ temperature regulator) and oxygen control systems (Ocea, Norway). Hatching and short-term enrichment was performed in stagnant conditions, while on-growing tanks had a flow-through system (see ***Deliverable report 17.1 Production protocol of on-grown Artemia***).

Seawater was pumped from 160 m depth. For hatching and short-term enrichment, the water was treated with chlorine and thereafter thiosulphate for at least 18 hours. For the on-growing tanks water pumped from 160 m was filtered (5 µm) before entering the tanks. Flow rate was 15 l h^{-1} for the entire period. The disinfectant Sanocare ACE (100 g; INVE Aquaculture, Belgium) was mixed with one l of freshwater using a blender (Hamilton Beach commercial) for 2 minutes and added to the tanks daily. ORI-GO from Skretting AS (Stavanger, Norway) was used for grow-out of *Artemia* nauplii. LARVIVA MULTIGAIN (Biomar, Denmark) was used for short-term enrichment of both nauplii and on-grown *Artemia,* using the manufacturer's standard procedure for short term enrichment of *Artemia*. Enrichment period was 12 hours and density of *Artemia* was 200 ind·ml^{-1}.

The larvae in all six tanks were fed *Artemia* nauplii from 1 until 14 days post first feeding (dpff). Then, one group of larvae was fed *Artemia* nauplii, and another group on-grown *Artemia* (2 out of 3 meals) in triplicate tanks until 28 dpff. The amount of *Artemia* fed each meal was based on the clearance rate of *Artemia* in each larvae tank. This was done by examining 100 ml of rearing water from each tank for *Artemia* content, which should be zero at least one hour before the next meal. Parameters such as the remaining number of *Artemia*, water flow, temperature and number of *Artemia* fed the larvae were recorded daily.

Results and discussion

Artemia.
The number of *Artemia* nauplii and on-grown *Artemia* given each tank increased from day 1 until day 12 after first feeding (**Fig. 3**). Thereafter the daily amount of *Artemia* nauplii given the three control tanks did not increase for the rest of the experimental period. For the tanks given on-grown *Artemia*, the number increased during this period (day 14 to 28). A fraction of *Artemia* was washed out from the tanks due to water exchange. *Artemia* lost by water exchange however, declined as the larvae became more effective in catching prey. This could explain that number of *Artemia* fed each tank did not increase in accordance with larval growth (**Fig. 4**). Larval mortality was highest from day 4 to 7 dpff and thereafter very low for the rest of the period.

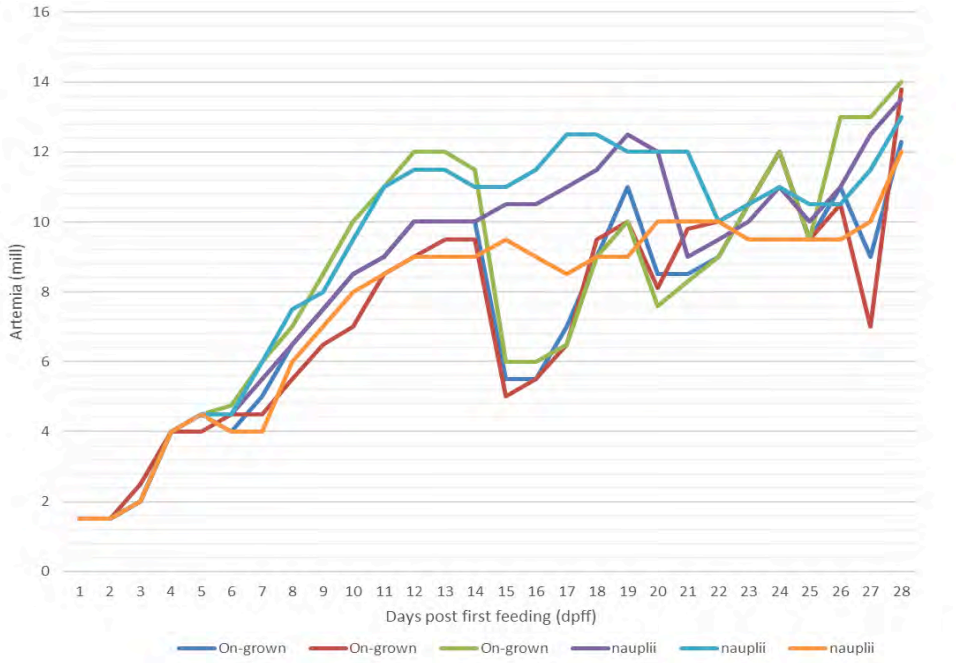

Figure 3. The number of *Artemia* (million per day) given to the six experimental tanks.

Larval growth.
There was no significant difference in growth at any time of the experiment. However, the standard deviations in this parameter at the end of the experiment were large for both experimental groups. Larval growth in this experiment did not differ from earlier growth studies on halibut larvae.

Larval survival.
Larval survival from first feeding until end of experiment at day 65 dpff was ca. 50% in all six tanks. An exact number is not possible to calculate as the initial number of larvae stocked was estimated. The first feeding tanks were tended daily by use of the cleaning system described above, and number of dead larvae was counted. Highest mortality (more than 90%) was observed from day 4 to 7 dpff, thereafter mortality was low throughout the rest of the experiment.

Eye migration and pigmentation.
More than 12.000 halibut fry were produced in this experiment and only two individuals were mal-pigmented. Degree of eye migration was evaluated on a scale from 0 to 3, where 3 is complete eye migration and 0 is no eye migration. In this experiment both groups scored higher than 2.5.

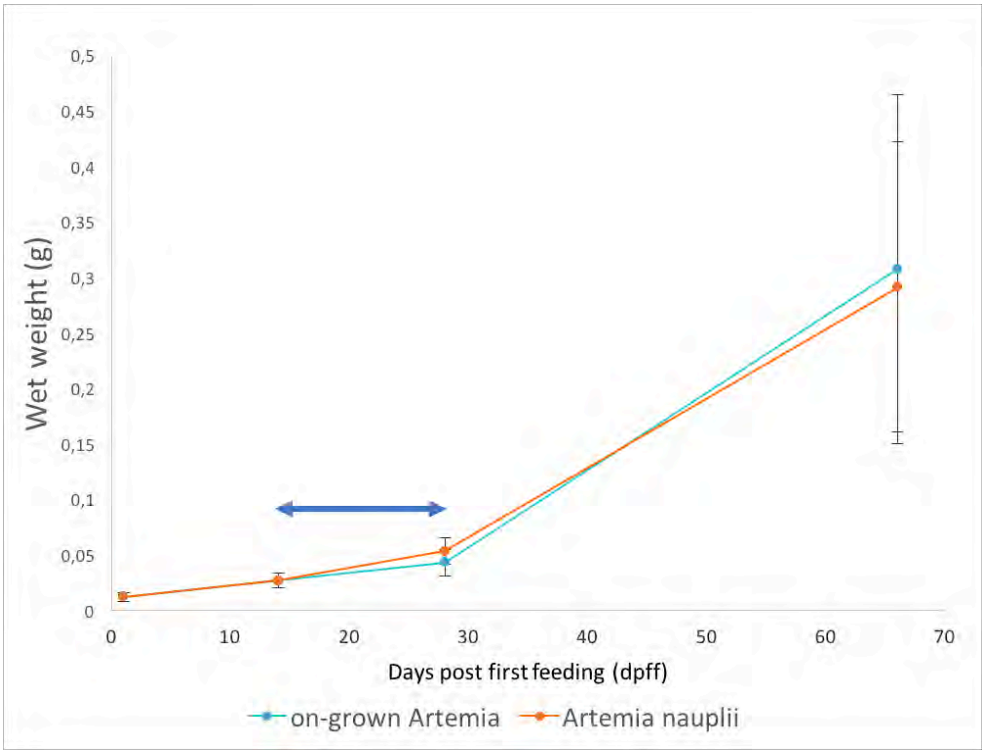

Figure 4. Atlantic halibut larval growth (wet weight) from first feeding until fry weaned to a dry diet. Blue arrow indicate period were larvae were fed on-grown *Artemia*.

Conclusions

Use of on- grown *Artemia* during the critical period of metamorphosis in Atlantic halibut larva did not differ from the use of *Artemia* nauplii with regard to growth, mortality and fry quality. In addition, the production of on-grown *Artemia* is labour-intensive, and high personnel costs may be prohibitive in implementation of this live feed source in commercial larviculture.

References

Rønnestad, I., Yufera, M., Ueberschar, B., Ribeiro, L., Sæle, O., and Boglione, C. (2013). Feeding behaviour and digestive physiology in larval fish: Current knowledge, and gaps and bottlenecks in research. Reviews in Aquaculture 5:S59-S98. DOI: 10.1111/raq.12010

Deviations: There were no deviations to the approved DOW.

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