Deliverable Report

Deliverable No:	D17.1	**Delivery Month:**	24
Deliverable Title	Production protocol of on-grown *Artemia*		
WP No:	11	**WP Lead beneficiary:**	P7. IMR
WP Title:	Larval husbandry – Atlantic halibut		
Task No:	17.3	**Task Lead beneficiary:**	P7. IMR
Task Title:	Production of on-grown *Artemia*		
Other beneficiaries:	P22. SWH		
Status:	**Delivered**	**Expected month:**	24
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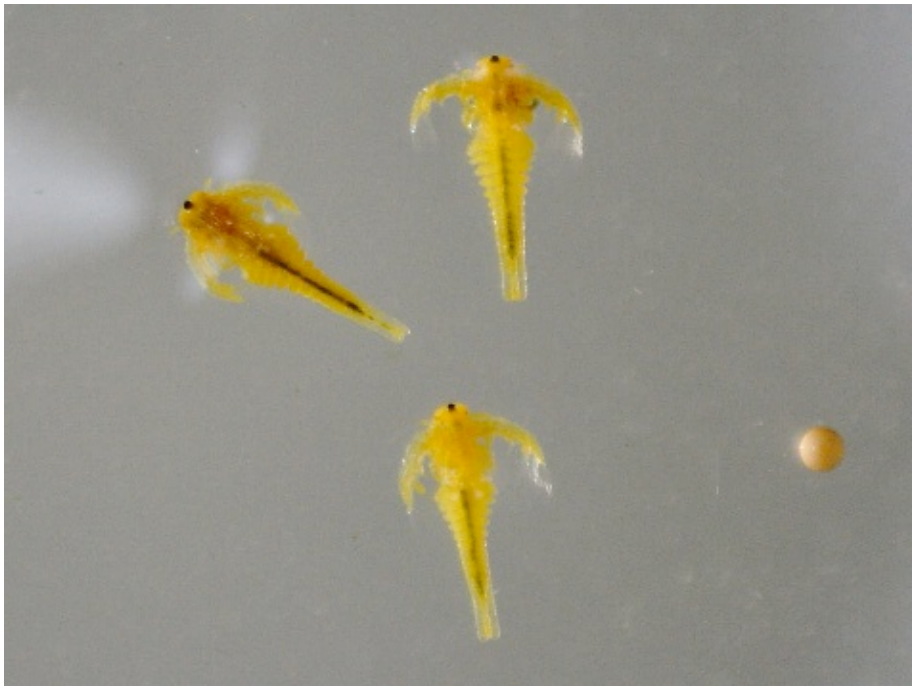
Other Scientists participating: Norberg, B. (IMR), Erstad, B. (SWH)

Objective: The objective of this Deliverable was to develop a production protocol for on-grown *Artemia*.



Introduction

At present, Atlantic halibut (*Hippoglossus hippoglossus*) larvae are fed *Artemia* nauplii through the whole first feeding period. An observed reduction in growth rate during the later phases of first feeding indicates that this feed is insufficient to maintain high growth. A larger prey size, with a higher nutrient content may

be a more appropriate choice for those stages. Therefore, a production protocol, based on Olsen et al., 1999, for on-grown *Artemia* was further developed, where water renewal and quality are crucial parameters. This protocol includes feeding, washing and disinfection of the *Artemia*, and has been tested both at an experimental (P7. IMR) and commercial scale (P22. SWH). The experiments that led to this protocol were followed by analyses of biochemical profiles of macro- and micronutrients of the on-grown *Artemia* that are presented in ***Deliverable D11.1 Report on the nutrient profile of Artemia nauplii and on-grown Artemia*** . The current deliverable includes the description of a production protocol of on-grown *Artemia*.

Materials and methods

A total of five trial runs were conducted before the reported protocol was finalized. Adjustments were made as described in the following section.

Artemia cysts (EG, INVE Aquaculture) were hatched in a separate tank, held for 24 hours from incubation, and then transferred to on-growing tanks. Conical fiberglass tanks, with a volume of 300 l, were used both for hatching and on-grown *Artemia*. All tanks were equipped with temperature (500W, and Carlo Gavazzi 600+ temperature regulator) and oxygen control systems (Ocea). The on-growing tanks had a flow-through system (**Fig. 1**).

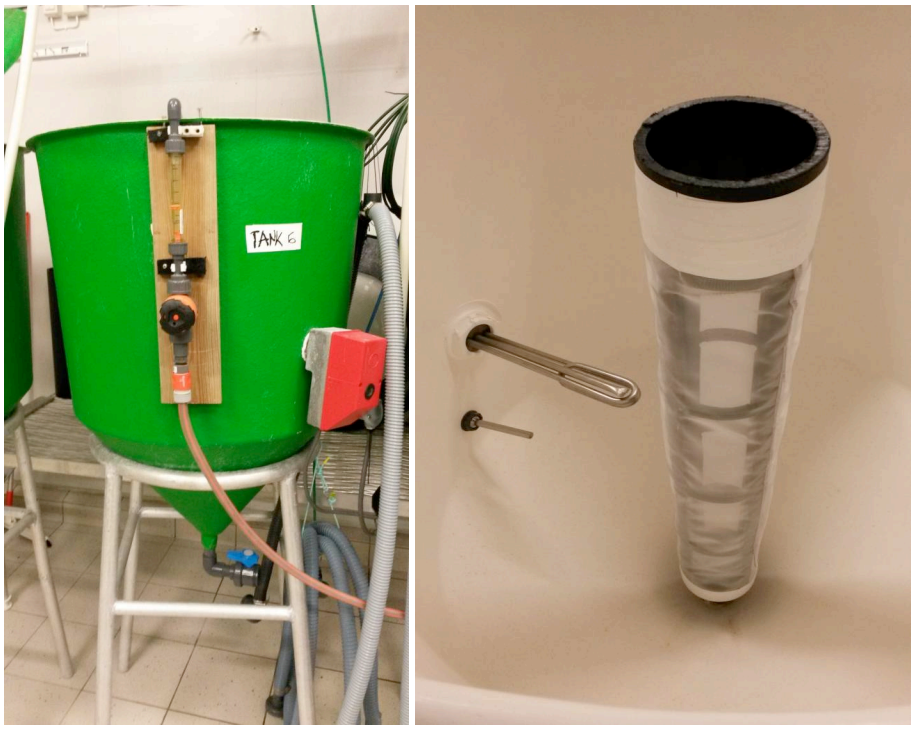


Figure 1. *Artemia* tank showing water supply and outlet sieve.

Seawater was pumped from 160 m of depth. For hatching of *Artemia* nauplii, the water was treated with chlorine and, thereafter, sodium thiosulphate for at least 18 hours. For the on-growing tanks, the water was filtered down to 5 µm before being supplied to the tanks. Flow rate was 15 l $\cdot h^{-1}$ from incubation and throughout the entire on-growing period. One hundred g of the disinfectant Sanocare ACE (INVE Aquaculture, Belgium) were mixed with 1 l of freshwater using a blender (Hamilton Beach commercial) for 2 min and added to the tanks daily.

The enrichment medium ORI-GO (Skretting AS; Stavanger, Norway) was used for grow-out of *Artemia* nauplii. At first, the feed was administered to the tanks using a belt feeder, but due to variation in how the feed dispersed in the *Artemia* on-growing tanks, the feed was mixed with 1 l of freshwater using a blender (Hamilton Beach commercial) for 2 min and was then added to the tanks twice a day. *Artemia* were fed 20 g of ORI-GO in each meal.

For the short-term enrichment of on-grown *Artemia*, LARVIVA MULTIGAIN (Biomar, Denmark) was used according to the manufacturer's standard procedure. Enrichment period was 2 h and density of *Artemia* was the same as in the on-growing tanks (100-110 individuals $\cdot$ ml^{-1}).

For measuring *Artemia* size and developmental stage, live *Artemia* were photographed using a dissecting microscope. For measuring number (density) and viability of *Artemia*, triplicate samples of 200 µl were taken daily and treated with buffodine. Treated samples were then counted using a dissecting microscope.

On day 3 the culture was transferred by a hose to an *Artemia* washer (**Fig. 2**) and concentrated from 280 l to approximately 70 l. The concentrate was then flushed (25 l $\cdot$ min^{-1}) under heavy aeration using 22°C seawater for 5 min, then washed with freshwater until the salinity reached less than 5 ppt and held there for 10 min. Thereafter the salinity was taken back to >31 ppt by flushing with seawater. The *Artemia* were then transferred to a holding tank before being fed to the larvae.

Figure 2. *Artemia* washer.

After the five tests were carried out (from nauplii to on-grown *Artemia*), with necessary adjustments, the following protocol was established and used in four identical tanks during a 15 day period. Growth and production data of the *Artemia* are presented in ***Deliverable 11.1 Report on the nutrient profile of Artemia nauplii and on-grown Artemia from IMR and SWH***.

Protocol for on-grown *Artemia:*

Day 0:

Add ~25 $\cdot$ 10^{6} dry *Artemia* cysts to hatching tank using standard procedures.

Day 1:

Filter the tank and wash the concentrate with seawater for 10 min. Transfer the nauplii to the on-growing tank at a concentration of 100 to 110 ind. $\cdot$ ml^{-1}. Turn on water flow and set at 15 l $\cdot$ h^{-1}. Set temperature to 21°C. Add Sanocare and then first meal of ORI-GO at 09.00 and second meal at 15.00.

Day 2:

Keep temperature at 21°C. Add Sanocare and first and second meal of ORI-GO as in day one. Raise air-stone 20 cm from the bottom and remove 5 l of sediment using a siphone.

Day 3:

Repeat procedure of day 2.

Day 4 (morning):

Transfer the on-grown *Artemia* by a hose to an *Artemia* washer and concentrate from 280 l to approximately 70 l. Flush concentrate (25 $l \cdot min^{-1}$) under heavy aeration with 22 °C seawater for 5 minutes. Then concentrate further to approximately 20 L and wash with freshwater (25 $l \cdot min^{-1}$) until the salinity is less than 5 ppt and hold for 10 min. Increase salinity back to >31 ppt by flushing with seawater. Transfer *Artemia* to a holding tank before being fed to the larvae.

On- growing tanks: preparation:

- Install outlet sieve
- Fill tank with heated seawater (20-21°C).
- Turn on heater
- Turn on oxygen and aeration
- Add Sanocare
- Add first meal

Daily routines:

- Control temperature and oxygen.
- Adjust seawater flow.
- Check *Artemia* number and viability.
- Add OriGo and Sanocare.
- Remove sediment.
- Harvest.
- Enrich and wash the on-grown *Artema*.

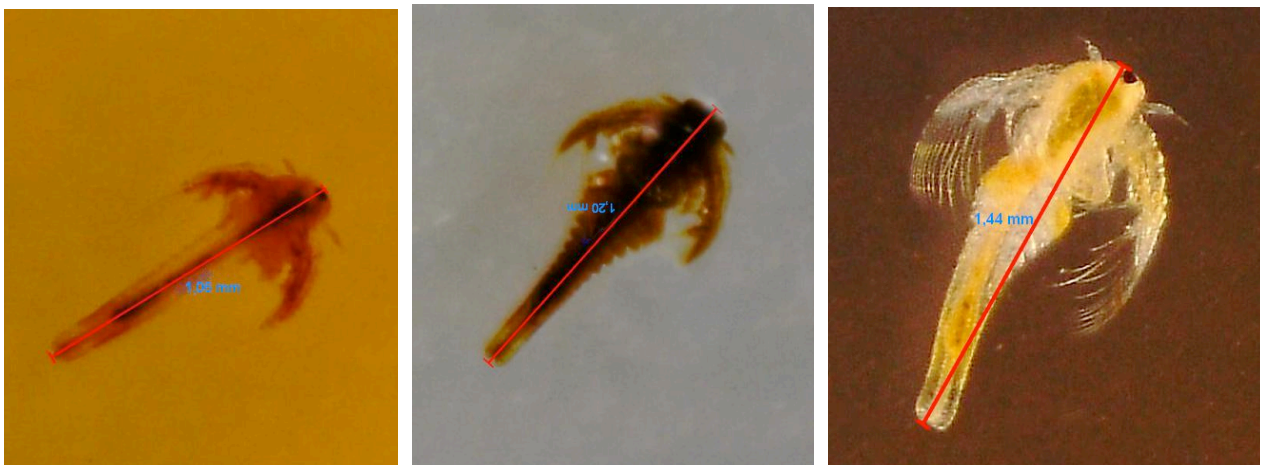


Figure 3. *Artemia* grown from nauplii for 2, 3 and 4 days (from left to right). Length: 1.06, 1.2 and 1.4 mm respectively.

References

Olsen, AI; Attramadal, Y; Jensen, A; Olsen, Y. (1999) Influence of size and nutritional value of *Artemia franciscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period. Aquaculture 179, 1-4: 475-487.

Deviations:

Samples were taken for analysis of bacterial density in the *Artemia* culture systems. The samples were cultured on broad-spectered marine agar plates in a dilution series. However, due to contamination the result was not possible to interpret. We decided not to repeat the trial, as subsequent experiments on first-feeding of larvae, with or without on-grown *Artemia* in triplicate, did not yield any significant differences in growth, larval quality or mortality.

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