New species for EU aquaculture

**Deliverable Report**

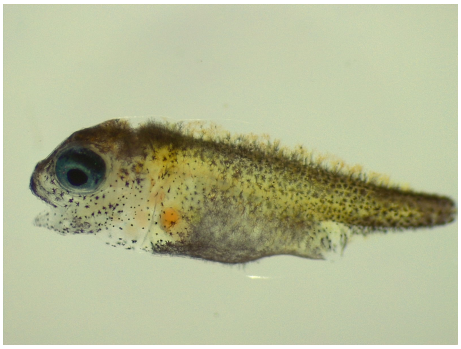
| | | | | |
|---|---|---|---|---|
| **Deliverable No:** | D18.3 | | **Delivery Month:** | 61 |
| **Deliverable Title** | Develop a feeding protocol for wreckfish larvae | | | |
| **WP No:** | 18 | | **WP Lead beneficiary:** | P8. IEO |
| **WP Title:** | Larval husbandry -wreckfish | | | |
| **Task No:** | 18.1 | | **Task Lead beneficiary:** | P1. HCMR |
| **Task Title:** | Development of feeding methodology. | | | |
| **Other beneficiaries:** | P8. IEO | P32. MC2 | P19. CMRM | |
| **Status:** | Delivered | | **Expected month:** | 36 |
| …….. | | | | |

**Lead Scientist preparing the Deliverable:** Nikos Papandroulakis (HCMR), Blanca Álvarez-Blázquez (IEO),

**Other Scientists participating:** Rodríguez Villanueva, J.L., Linares, F., (CMRM), Vilar, A., (MC2), Pérez Rial, E., Lluch, N., Gómez, C. (IEO)

**Objective:**

A feeding protocol will be developed based on studies of the different stages of the larval development after hatching; the yolk sac consumption, acceptance of exogenous food and duration of larval development and growth until the acceptance of inert food (weaning). The sequence of live food consumption will be determined as a function of age and the time of weaning. Results on survival and viability will also be provided, as well as morphometric determinations during larval culture. The deliverable described the results of these studies with the aim of improving the feeding protocol.



# Contents

# 1. Introduction

Wreckfish aquaculture is an interesting option in order to further diversify the European industry. The fast growth of wreckfish, *Polyprion americanus* (Kentouri et al., 1995; Papandroulakis et al., 1997, Rodríguez Villanueva et al., 2014), its easy handling in captivity and the relative high market price, are making wreckfish a possible candidate for specific diversification of marine aquaculture.

Following the first reported natural spawns in captivity and the detailed description of embryonic and larvae development until mouth opening (Papandroulakis et al, 2004), the work of Peleteiro et al. (2011) described viable spawns by artificial fertilization with sperm and oocytes from Aquarium Finisterrae. Additionally, a Spanish private company with facilities for wreckfish broodstock in Valdoviño (A Coruña) produced the first juvenile (one individual).

As in all species, larval rearing is considered as one of the major bottlenecks for commercial production. During DIVERSIFY, several attempts were made towards the definition of the optimum parameters for the rearing of the species. Although with limited success, recently 25 juveniles were obtained at the facilities of the Instituto Galego de Formación en Acuicultura (IGAFA, CMRM) as a result of larval rearing from eggs obtained from IEO and MC2. A summary of the trials performed by the partners during the course of the project is presented.

# 2. Material and methods:

## 2.1 HCMR trials.

During the project, three trials were organized at HCMR in 2015, 2016 and 2018. As the breeders at HCMR spawned only once with low quality eggs, the only source of eggs was from the breeders of IEO and MC2. Two batches of eggs were sent and used at HCMR hatcheries.

- From MC2, 2000 larvae were transported in May 2015, with 5 l seawater enclosed in polystyrene boxes (**Figure 2.1.1**). The duration of the transport was 24 hours. When arrived (19.5˚C, 19.5 mg l-1 DO, 8 pH) the larvae were incubated in a 500 l tanks.
- From the broodstock of HCMR, in June 2016, following a spawning induction. Approximately 4000 eggs were incubated in a 2000 l tank at 16.5˚C, 7.2 mg $l^{-1}$ DO and 8.2 pH.
- From IEO a shipment was organized in March 2018. The eggs arrived at 16$^{o}$C, the pH was at 6.5 and the DO >15 mg/l (salinity at 36 psu). The stage was just before hatching. The eggs were split in two groups: (a) 18,500 viable and 5,500 dead eggs and (b) 15,500 and 2,500 respectively. They were incubated at 15.5°C in the Mesocosm facility of the institute.

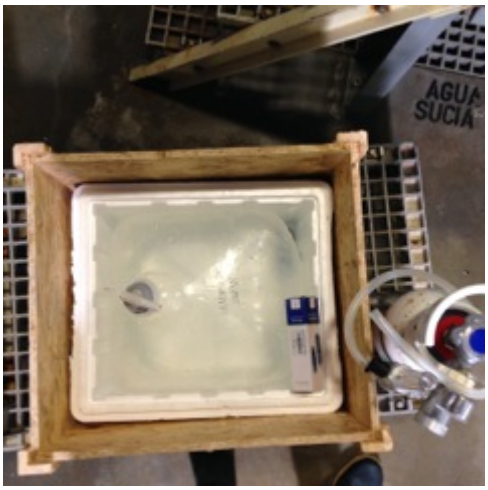



**Figure 2.1.1.** Transportation of eggs from MC2.

Trials were performed only during 2016. The batch of 2015 from MC2 did not survive beyond the autotrophic stage. The same occurred with the batch of 2018 as all eggs died before hatching.

During 2016, tanks were organised as closed water recirculating systems. The tanks used were of 2,000 l with depth of 2 m (**Figure 2.1.2**).

**Figure 2.1.2** Rearing tanks of 2000 l.

After incubation and during the autotrophic stage, temperature was maintained at 16˚C and was gradually increased afterwards at 17.5 (**Figure 2.1.3**). First feeding was at 10 dph when larvae opened their mouth and developed their eyes, obtaining the characteristic black color.

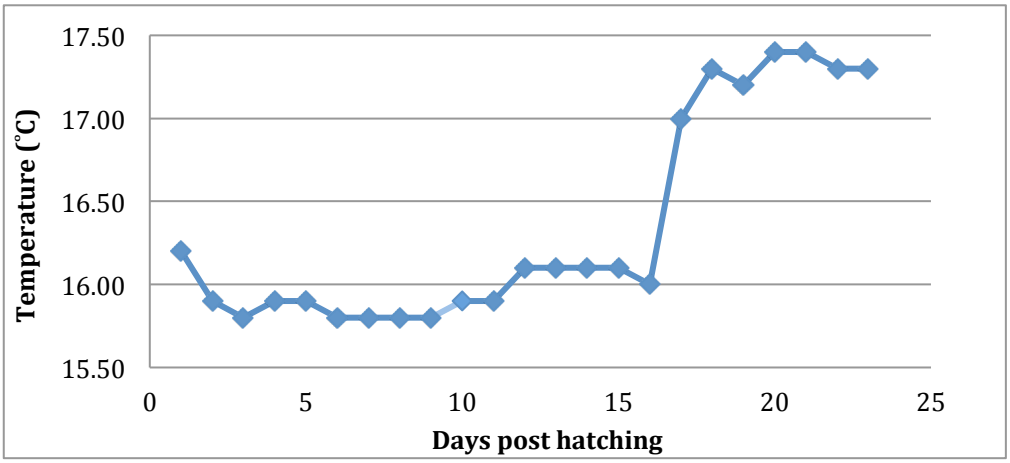



**Figure 2.1.3.** Temperature profile during the rearing.

Feeding was based on enriched rotifers, *Artemia* AF (since 13 dph) and *Artemia* EG (since 24 dph).

## 2.2 IEO trials

During **2015**, 10 natural spawns were obtained at MC2 and IEO with fecundity between 62 and 97%, hatching rates between 4 and 56% and survival until 22 days post hatching (DPH), as shown in **Table 2.2.1.**

**Table 2.2.1.** Parameters of the different spawns at the MC2 and IEO y larval culture.

| SPAWN TYPE | STOCK | DATE | FEC (%) | HATCH (%) | LARVAE (nº) | LARVAL DENSITY (nº Larv/l ) | MEAN Tª | FEED | SURVIVAL (dph) | WATER SYSTEM |
|---|---|---|---|---|---|---|---|---|---|---|
| I/STRIPPINC | IEO | 10-04-15 | 62-24 | 0,4 | 110 | 0,2 | 17,4 | Enrich rot | 17 | CC UNTIL 10 DPH |
| 2/NAT | MC2(IEO) | 27-05-15 | 86 | 22 | 100 | 0,2 | 19,1 | | 10 | |
| 3/NAT | MC2(IEO) | 05-06-15 | 90 | 30 | 1000 | 2,0 | 18,4 | | 10 | |
| 4/NAT | MC2 | 18-05-15 | 97 | 0,02 | 20 | 0,2 | 15,1 | Enrich rot+copépods | 10 | WATER REN. |
| 5/NAT | MC2 | 22-05-15 | 81 | 8,6 | 2600 | 52,0 | 14,7 | | 18 | |
| 6/NAT | MC2 | 27-05-15 | 86 | 22 | 10600 | 12,4 | 14,5 | | 18 | |
| 7/NAT | MC2 | 01-06-15 | 95 | 56 | 180000 | 24,3 | 14,4 | | 22 | |
| 8/NAT | MC2 | 05-06-15 | 84 | 30 | 18500 | 15,6 | 14,7 | | 17 | |
| 9/NAT | MC2 | 08-06-15 | 75 | 3 | 500 | 10,0 | 15,3 | | 18 | |
| 10/INDUCED | HCMR | 04-05-15 | 86 | 22 | 4000 | 2 | 16,3 | Enrich rot +copépods | 22 | RAS |
| 11/NAT | MC2 (HCMR) | 27-05-15 | 84 | 30 | 2000 | 2 | 16,3 | | 22 | |

Larvae from natural spawns from MC2)were cultured in 50-l tanks, with rotifers enriched with phytoplankton and copepods in flow-through circuit. On the fifth day larvae were transferred to 83 l polycarbonate tanks with natural light (water flow of 30 l / h). They were kept in those conditions until mouth is developed (**Fig. 2.2.1**). At that time a formalin prophylactic treatment was made. The group of larvae was fed with copepods, mainly *Tisbe battagliari*.

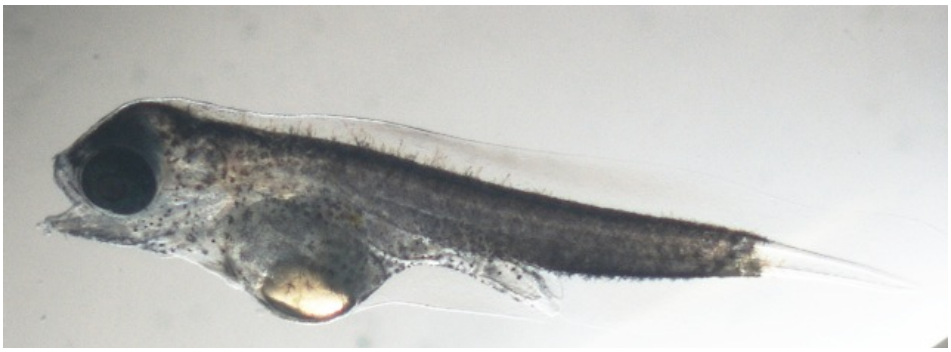


**Figure 2.2.1.** Wreckfish larvae at 8 dph.

Larvae at the IEO were from "*in vitro*" spawning, with oocytes and sperm coming from the same stock. They were incubated in 500 l tanks in a closed circuit system until 10 dph, using the "Green Water" system with rotifers enriched with phytoplankton until 14 dph. Larval density was 0.2 (IEO) and 52 (MC2) larvae/liter, with natural photoperiod during endogenous feeding. After the mouth opening and the consumption of all yolk sac, artificial light (410 Lux) was used for 12 h per day until the end of the rearing period. Larval, yolk sac and lipid droplet length was measured and photos of all developmental stages were taken**.**

During **2016**, the spawn quality at MC2 improved considerably, and the stock at the IEO started to have good quality spawns as well (**Table 2.2.2)**. Larvae were fed on rotifers and *Artemia* enriched with T-Iso until 27 dph (Álvarez-Blázquez et al., 2016).

**Table 2.2.2**. Parameters of different spawns at the MC2 and IEO facilities.

| SPAWN TYPE | STOCK | DATE | FEC (%) | HATCH (%) | LARVAE (nº) | LARVAL DENSITY (nº Larv/l ) | MEAN Tª | FEED | SURVIVAL (dph) | WATER SYSTEM |
|---|---|---|---|---|---|---|---|---|---|---|
| 1/NAT | IEO | 13-05-16 | 70 | 3,25 | 3921 | 7,8 | 19,8 | Enrich rot + ARTEMIA AF | 20 | CC UNTIL 12 DPH |
| 2/NAT | IEO | 18-05-16 | 75 | 21,2 | 7793 | 8,7 | 20,7 | Enrich rot + ARTEMIA AF | 20 | CC UNTIL 9 DPH |
| | | | 75 | 7,1 | 2560 | 2,8 | 16,2 | Enrich rot + Artemia AF + EG | 27 | |
| 3/NAT | IEO | 20-05-16 | 88 | 7,3 | 2200 | 9,2 | 17,1 | NONE | 4 | WATER REN. |
| 4/NAT | MC2(IEO) | 13-06-16 | 100 | 36 | 2500 | 2,8 | 18,3 | Enrich rot + ARTEMIA AF | 20 | |
| 5/INDUCED | MC2(IEO) | 12-07-16 | 85 | 65 | 1495 | 3,3 | 16,7 | Enrich rot + ARTEMIA AF | 23 | |
| | | | | | 3937 (tr) | 8,8 | 16,8 | Enrich rot + ARTEMIA AF | 25 | |
| 6/NAT | MC2 | 26-05-16 | 98 | 2 | 1600 | 0,2 | 16,5 | Copepod Tisbe + Acartia | 22 | CC |
| 6/NAT | MC2 | 26-05-16 | | | 1000 | 1,0 | 17,5 | | 11 | WATER REN. |
| 7/NAT | MC2 | 13-06-16 | 100 | 35 | 6000 | 10,0 | 17,5 | | 10 | |
| 8/INDUCED* | MC2 | 08-07-16 | 49 | 13 | 600 | 0,1 | 15,5 | | 3 | CC |
| 9/INDUCED* | MC2 | 12-07-16 | 85 | 65 | 6000 | 0,8 | 15,5 | | 23 | |
| 10/INDUCED* | MC2 | 16-07-16 | 75 | 2 | 100 | 2,5 | 17,5 | NONE | 4 | WATER REN. |
| 11/INDUCED* | MC2 | 20-07-16 | 60 | 11 | 2500 | 62,5 | 17,5 | Copepod Tisbe + Acartia | 15 | |

In MC2, in order to check if the low survival was caused by prophylactics treatments contrary to previous years, no formalin or other chemicals were used neither during eggs incubation nor larvae development. Hatching occurred after 5 days of incubation at a temperature of 14-16ºC. High larval deformities occurred even without prophylactic treatment. After 8-9 dph some larvae were transferred to 8,000 l tank with green water with daily addition of microalgae 30 l and self-stable production of *Acartia tonsa* + *Tisbe battagliai*.

In **2017**, only larvae from 40% of the spawns were reared, due to technical problems during the incubation eggs and also by the negligent death of part of the stock of MC2 females. In IEO and CMRM, a total of five trials of larval rearing were done (**Table 2.2.3**). Larvae feeding rotifers enriched with *Isochrysis* survived at maximum 29 dph in IEO facilities, with a mean seawater temperature of 16ºC and larval density of 6.8 larvae.ml$^{-1}$. Trials were focus in egg incubation and larvae culture at different sea water temperatures (see Deliverable 18.2) and also in trials with different enrichments for live food (see Deliverable 12.1). Length, yolk sac and droplet were measure, obtaining curves of growth in length and yolk sac and droplet consumption.

**Table 2.2.3.** Parameters of the different spawns at the MC2 and IEO and larval culture.

| SPAWN TYPE | STOCK | DATE | FEC (%) | HATCH (%) | LARVAE (nº) | LARVAL DENSITY (nº Larv/l ) | MEAN Tª | FEED | SURVIVAL (dph) | WATER SYSTEM |
|---|---|---|---|---|---|---|---|---|---|---|
| 1/NAT | IEO | 13-04-17 | 88 | 10,9 | 2049 | 6,8 | 15-17 | Enrich rot | 29 | WATER REN. |
| | | | | | 2049 | 6,8 | 19-21 | Enrich rot | 24 | |
| 2/NAT | IEO | 22-04-17 | 98 | 25,6 | 15400 | 4,4 | 16-19 | Enrich rot | 22 -26 | WATER REN. |
| 3/NAT | CMRM | 05-04-17 | 100 | 45,7 | 18400 | .. | 14,1-15,8 | Enrich rot | 13 | WATER REN. |
| 4/NAT | CMRM | 10-04-17 | 100 | 19,6 | 8533 | .. | 15-16,3 | Enrich rot | 5 | WATER REN. |
| 5/NAT | CMRM | 18-04-17 | 100 | 4,04 | 1050 | 14 | 14-17 | Enrich rot | 11 | WATER REN. |
| 6/NAT | CMRM | 26-04-17 | | 1,29 | 164 | 1,88-4,68 | 14-17,4 | Enrich rot | 11 | WATER REN. |

During **2018** more advances in larval husbandry were made due to the fact that fertilized eggs were obtained in adequate quantity and quality (**Table 2.2.4**) in the three stocks in Galicia (Spain).

**Table 2.2.4.** Trials with different larvae batches at MC2 facilities during 2018.

| SPAWN TYPE | STOCK | DATE | FEC (%) | HATCH (%) | LARVAE (nº) | LARVAL DENSITY (nº Larv/l ) | MEAN Tª (ºC) | FEED | SURVIVAL (dph) | WATER SYSTEM |
|---|---|---|---|---|---|---|---|---|---|---|
| 1/NAT | IEO(MC2) | 27-02-18 | 60 | 20,4 | 10000 | 10,0 | 16,8 | ENRICH ROT (ARA) + ARTEMIA | 23 | CC UNTIL 12 DPH |
| 2/NAT | IEO(MC2) | 15-03-18 | ¿? | 10 | 11000 | 15,1 | 18 | ENRICH ROT (ARA) + ACARTIA | 20 | WATER REN. |
| 3/NAT | IEO(MC2) | 24-03-18 | ¿? | 23,3 | 14000 | 15,0 | 17,5 | ENRICH ROT (ARA) | 22 | WATER REN. |
| 4/NAT | MC2 | 28-03-18 | 97,5 | 37 | 60000 | 30,0 | 17,5 | ENRICH ROT (ARA) + ACARTIA | 20 | WATER REN. |
| 5/NAT | MC2 | 02-04-18 | 90 | 9 | 10000 | 25,5 | 18 | ENRICH ROT (ARA) + ACARTIA | 12 | WATER REN. |
| 6/NAT | MC2 | 07-04-18 | 97,8 | 0,1 | 200 | 6,0 | 18 | ENRICH ROT (ARA) + ACARTIA | 17 | WATER REN. |
| 7/NAT | IEO(MC2) | 16-04-18 | 89 | 49,8 | 11000 | 11,0 | 18 | ENRICH ROT (ARA) + ACARTIA + TISBE | 37 | WATER REN. |
| 8/NAT | MC2 | 19-04-18 | 97,1 | 4 | 9000 | 8,0 | 18,5 | ENRICH ROT (ARA) + ACARTIA + TISBE | 35 | WATER REN. |
| 9/NAT | MC2 | 24-04-18 | 91,3 | 42 | 38000 | 20,0 | 18,5 | ENRICH ROT (ARA) | 15 | WATER REN. |
| 10/NAT | MC2 | 30-04-18 | 93,9 | 10 | 6000 | 25,0 | 18,8 | ENRICH ROT (ARA) + ARTEMIA + ACARTIA + TISBE | 19 | WATER REN. |
| 11/NAT | MC2 | 05-05-18 | 89,4 | 9 | 15000 | 15,0 | 18,8 | ENRICH ROT (ARA) | ¿? | WATER REN. |
| 12/NAT | MC2 | 10-05-18 | 88,2 | 19 | 100000 | 15,0 | 18 | ENRICH ROT (ARA) + ACARTIA + TISBE | 15 | WATER REN. |
| 13/NAT | MC2 | 15-05-18 | 89,9 | 31 | 60000 | 15,0 | 18,7 | ENRICH ROT (ARA) + ACARTIA + TISBE | 11 | WATER REN. |
| 14/NAT | MC2 | 20-05-18 | 97,6 | 13 | 15000 | 10,0 | 18,4 | ENRICH ROT (ARA) + ACARTIA + TISBE | 18 | WATER REN. |

At MC2, larvae rearing was performed with water temperature between 17.5 and 18.8ºC and live food based on rotifer and *Acartia* since 6 to 22 dph. Larval density was between 0.2 and 11.1 larvae.l$^{-1}$. On the fifth day a larvae trap was placed to collect larvae from the incubator and collected larvae were transferred to 30 l tanks with natural light and 30 l.h$^{-1}$ turn over. They were kept in those conditions until mouth is developed. Following this, they were feed on enriched *rotifer* and *Acartia tonsa* cultured with multi microalgae complex *Rhodomona/Nannocloropsis/Tetraselmis*. On 9 dph many of the larvae were transferred to cylindrical 1-m$^3$ tanks with color of black/ bleu or white. Best result were obtained in 1 m$^3$ tank with white walls and a kind of green water with a stable culture of *Acartia tonsa* at 18-20 ºC, a daily addition of 20 l microalgae mixture of *Rhodonona/Nanocloropsis/ Tetraselmis*. Light was with a Day light 36 W Osram lamp at 24 h photophase. A central slow big air bubble was also present in the tank and the water flow was at 3 l min$^{-1}$.

At CMRM, two batches of larvae were cultured, one from IEO natural spawn and one from MC2 (**Table 2.2.5**). Water temperature was 17.6 and 17.9ºC, and the tanks volume was 400 l. No aeration was provided and natural photoperiod was applied since 7 and 9 dph. Life food was based on rotifer, *Artemia* nauplii (Instar I) enriched metanauplii (Instar II). Rotifers and Artemia were enriched with ARA enrichment products as it was described in Deliverable 12.1.

**Table 2.2.5.** Trials in larvae culture at IGAFA (HCMR) during 2018

| SPAWN TYPE | STOCK | DATE | FEC (%) | HATCH (%) | LARVAE (nº) | LARVAL DENSITY (nº Larv/l ) | MEAN Tª (ºC) | FEED | SURVIVAL (dph) | WATER SYSTEM |
|---|---|---|---|---|---|---|---|---|---|---|
| 1/NAT | IEO(CMRM) | 27-02-18 | 60 | 20,4 | 5000 | 10 | 16,3 | COPEPODS | 23 | CC |
| 2/NAT | IEO(CMRM) | 04-04-18 | 80 | 2,6 | 7000 | 13 | 16,9 | COPEPODS + ARTEMIA | 21 | CC |
| | | | | | 6500 | 12,5 | 17,6 | ENRICH ROT (ARA) + ARTEMIA + FEED | >150 | RAS + NO AIR |
| 3/NAT | CMRM | 22-03-18 | 100 | 7 | 2000 | 10 | 15,6 | NONE | 11 | CC |
| 4/NAT | CMRM | 27-03-18 | 100 | 33,9 | 8900 | 11 | 15,1 | NONE | 3 | RAS + NO AIR |
| 5/NAT | IEO(CMRM) | 12-04-18 | 97,5 | 14 | 5000 | 10 | 15,6 | ENRICH ROT (ARA) + COPEPODS | 25 | RAS + NO AIR |
| 6/NAT | CMRM | 08-05-18 | 100 | 18,5 | 1800 | 9 | 19 | NONE | 4 | CC |
| 7/NAT | MC2(CMRM) | 10-05-18 | 88,2 | 19 | 6500 | 12,5 | 17,9 | ENRICH ROT (ARA) + ARTEMIA + FEED | >120 | RAS + NO AIR |

More than 50 trials of larval culture have been carried out in the IEO facilities (**Table 2.2.6**). Several rearing conditions such as photoperiod, water circuit, use of aeration or sequence of feeding used were tested. Experiments were carried out in closed circuit up to 6-12 dph, with photoperiod of 12L:12D and dissolved oxygen was between 7.2-8.4 mg.l-1. Moderate aeration was applied in the center of the tank, green water from day zero and the temperature varied between 15-18ºC. Larvae density ranged from 2 to 30 larvae l$^{-1}$. A feeding sequence with rotifers (10-25 dph) and *Artemia nauplii* (11-30 dph) was tested.

Additional tests were carried out and some of the rearing parameters were readjusted. Larvae of 1 dph were introduced at densities between 6-20 larvae l$^{-1}$ in the rearing tanks and kept in darkness until 7 dph. Temperature ranges between 17-20ºC, open circuit (2.5 renewals d$^{-1}$) and no aeration was also tested. A photoperiod of 10L:14D and green water culture conditions were applied since 7dph. A feeding sequence for rotifer (7-13 dph), *Artemia* nauplii (8-11 dph) and enriched metanauplii (10-29 dph) was supplied. In all cases survivals between 8 and 30 dph were reported.

A final set of trials was performed with some variables modified. The culture density was maintained between 5-15 larvae l$^{-1}$, the temperature was controlled at 17.9 ± 1ºC, the dissolved oxygen between 7.0 and 8.4 mg.l-1, the light intensity was adjusted to 400 lux on the surface of the

tank and a new feeding sequence was applied: rotifer (7-18 dph), *Artemia nauplii* (12-19 dph) and enriched *metanauplii* (17-30 dph). The rest of the culture conditions were the same as those described in the previous paragraph. Survivals between 18 and 34 dph were observed.

For all live prey, concentrations were adjusted for rotifer, *Artemia nauplii* and *metanauplii*, between 3-10, 0.5-1 and 0.5-2 individuals $ml^{-1}$ respectively.

**Table 2.2.6.** Trials with different larvae batches at IEO facilities during 2018.

| SPAWN TYPE | STOCK | DATE | FEC (%) | HATCH (%) | LARVAE (nº) | LARVAL DENSITY (nº Larv/l ) | MEAN Tª (ºC) | FEED | SURVIVAL (dph) | WATER SYSTEM |
|---|---|---|---|---|---|---|---|---|---|---|
| 1/NAT | IEO | 16-02-18 | 30 | 2 | 3429 | 7 | 18,3 | ROT ENRIQ. ARA 12-18 DPH | 18 | CC UNTIL 14 DPH |
| 2/NAT | IEO | 21-02-18 | 98 | 8,5 | 14100 | 13 | 17,9 | ROT ARA 11- 24DPH. A0 15-24 DPH | 24 | CC UNTIL 11 DPH |
| 3/NAT | IEO | 26-02-18 | 84 | 3 | 1971 | 2 | 15,9 | ROT. ARA 13-28 DPH. A0 14-28 DPH | 28 | CC UNTIL 6 DPH |
| | | | | | | 2,3 | 17,6 | ROT ARA 7-26 DPH. A0 14-26 DPH | 26 | CC UNTIL 6 DPH |
| 4/NAT | IEO | 27-02-18 | 60 | 20,4 | 73260 | 36 | 16,8 | ROT ARA 10-19 dph. A0 13-23 dph | 23 | CC UNTIL 12 DPH |
| 5/NAT | IEO | 04-03-18 | 80 | 2,6 | 7282 | 1,8 | 15,4 | ROT ARA 12-27 DPH. A0 20-27 DPH | 27 | CC UNTIL 11 DPH |
| | | | | | | 77 | 15,4 | ROT ARA 12-30 DPH. A0 15-30 DPH | 30 | CC UNTIL 9 DPH |
| 6/NAT | IEO | 09-03-18 | 75 | 82 | 171921 | 21 | 15,2 | ROT 10----DPH. A0 11-....DPH | 29 | WATER REN. |
| | | | | | | 28 | 15,3-21,4 | ROT 10-22 DPH. A0 15-22 DPH | 22 | WATER REN. |
| 7/NAT | IEO | 10-03-18 | 92 | 37,5 | 37844 | 18,9 | 15,6 | ROT 11-25 DPH. A0 19-27 DPH | 27 | WATER REN. |
| 8/NAT | IEO | 15-03-18 | ¿? | 10 | 16964 | 10 | 16-18 | DIED AT 5 DPH | 5 | WATER REN. |
| | | | | | | 10 | 19-21 | | 5 | WATER REN. |
| 9/NAT | IEO | 19-03-18 | 100 | 24,6 | 102570 | 39 | 16 | ROT ARA 10-20 DPH. A0-A1: 12-24 DPH | 24 | WATER REN. |
| | | | | | | 26 | 15,9 | ROT 10-18 DPH. A0 11-18 DPH | 19 | CC UNTIL 9 DPH |
| | | | | | | 30 | 15,9 | | 4 | |
| | | | | | | 36 | 15,7 | | 4 | |
| | | | | | | 23 | 15,6 | ROT ARA 10-12 DPH. A0 11-12 DPH | 13 | CC UNTIL 9 DPH |
| | | | | | | 10 | 16-18 | | 12 | WATER REN. |
| | | | | | | 10 | 19-21 | | 12 | WATER REN. |
| 10/NAT | IEO | 24-03-18 | 91 | 7 | 23320 | 10 | 16,5 | ROT CONTROL 9-17 DPH. A0 12-20 DPH | 21 | WATER REN. |
| | | | | | | 18,2 | 16,5 | ROT ARA 9-15 DPH. A0 12-20 DPH | 21 | WATER REN. |
| 11/NAT | IEO | 24-03-18 | ¿? | 11,7 | 21888 | 4,5 | 17,6 | ROT CONTROL 9-14 DPH. A0 11-13. A-1 T-ISO 13-22 DPH. PIENSO <0,2 MM 12-22 DPH | 22 | WATER REN. |
| 12/NAT | IEO | 29-03-18 | 96 | 16,2 | 82694 | 46 | 17,1 | ROT 8-15 DPH. A0 11-14, A-1 T-ISO 15-22 DPH | 22 | WATER REN. |
| 13/NAT | IEO | 29-03-18 | 97 | 26 | 45182 | | | | | |
| | | | | | 47456 | 24 | 16,8 | ROT ORIGREEN 7-15 DPH. A0 10-14, A1 ORIGREEN 14-19 DPH | 19 | WATER REN. |
| 14/NAT | IEO | 02-04-18 | 89 | 17,9 | 110740 | 7,1 | 17,2 | ROT ORIGREEN 7-14 DPH. A0 11-13, A-1 ORIGREEN 14-17 DPH | 17 | WATER REN. |
| | | | | | | 11 | 17,1 | ROT T-ISO 7-14 DPH. A0 11-12, A-1 ISO 13-17 DPH | 18 | WATER REN. + NO AIR |
| | | | | | | 11,3 | 17,1 | | 17 | |
| | | | | | | 11,3 | 17,1 | | 17 | |
| 15/NAT | IEO | 07-04-18 | 97 | 32,7 | 134802 | 5,5 | 15,1 | ROT 7-10 DPH. A0 8-11 DPH. | 11 | WATER REN. |
| | | | | | | 20,4 | 18,4 | ROT 7-10 DPH. A0 8-10, A1 10-18 DPH | 18 | CC |
| | | | | | | 14,9 | 18,2 | ROT 8-10 DPH. A0 9-10, A1 11-16 DPH. | 18 | |
| | | | | | | 16 | 19,3 | ROT 6-11 DPH, A0 7-10, A1 11-16 DPH | 16 | WATER REN. |
| | | | | | | 16,8 | 19,2 | ROT 6-11 DPH, A1 7-13 DPH | 13 | WATER REN. |
| | | | | | | 16,1 | 19,2 | ROT6-11 DPH. A0 7-10, A1 11-24, A2 25-28. PIENSO SKRETTING <0,2 MM TAMIZADO23-34 DPH DPH | 34 | WATER REN. |
| 16/NAT | IEO | 12-04-18 | 97,5 | 14 | 54116 | 12,8 | 17,6 | ROT 7-14 DPH. A0 7-9, A1 10-20 DPH. | 20 | WATER REN. |
| | | | | | | 19,6 | 18,6 | ROT 7-12 DPH. A0 8-9, A1 10-15 DPH | 16 | WATER REN. |
| | | | | | | 12 | 19,9 | ROT ARA 6-7 DPH. A0 7-9, A1 9-15 DPH | 15 | WATER REN. |
| | | | | | | 12 | 19,9 | ROT CONTROL 6-7 DPH. A0 7-9, A1 9-15 DPH | 15 | WATER REN. |
| 17/NAT | IEO | 12-04-18 | 95,2 | 34,2 | 69284 | 50,7 | 17,5 | ROT 7-12 DPH, A0 7-9, A1 10-12 DPH | 12 | WATER REN. |
| | | | | | | 99,7 | 18 | ROT 7-9 DPH. A0 7-9, A1 10-22 DPH | 22 | WATER REN. |
| | | | | | | 15,8 | 18,4 | ROT 7-12 DPH. A0 8-9, A1 1022 DPH | 22 | WATER REN. |
| | | | | | | 16,6 | 17,9 | ROT 7-10 DPH. A0 8-9, A1 10-14 DPH | 14 | WATER REN. |
| 18/NAT | IEO | 16-04-18 | 89 | 35,2 | 67428 | 14 | 18,7 | ROT 6-11 DPH. A0 7-9, A1 10-20 DPH | 23 | WATER REN. |
| | | | | | | 18,6 | 19,1 | ROT 6-11 DPH. A0 6-9, A1 10-20 DPH | 21 | WATER REN. |
| | | | | | | 16,4 | 18,8 | ROT 6-13 DPH, A0 6-9, A1 10-17 DPH | 17 | WATER REN. |
| | | | | | | 20,4 | 18,4 | ROT 6-11 DPH, A0 6-9 DPH, A1 10-17 DPH | 17 | WATER REN. |
| 19/NAT | IEO | 17-04-18 | 96 | 26,5 | 23258 | 2,4 | 18 | ROT 6-16 DPH, A0 7-9, A1 10-24 DPH | 24 | WATER REN. |
| 20/NAT | IEO | 25-04-18 | 82 | 24 | 39798 | 5,7 | 19,2 | ROT ARA 6-7 DPH | 8 | WATER REN. |
| | | | | | | 5,7 | 19,2 | ROT CONTROL 6-7 DPH | 8 | WATER REN. |
| | | | | | | 19,2 | 19,1 | ROT T-ISO 5-8 DPH, A0 9-10, A1 11-22 DPH | 22 | WATER REN. |
| | | | | | | 18,3 | 18,9 | ROT T-ISO 5-8 DPH, A0 9-10, A1 18 DPH | 18 | WATER REN. |
| | | | | | | 12,9 | 18,9 | ROT 5-7 DPH, A0 9, A1 10-AA DPH | 11 DPH | CC |
| | | | | | | 10 | 18,9 | ROT 5-7 DPH | 8 | CC |
| | | | | | | 10 | 18,9 | ROT 5-7 DPH | 8 | CC |

# 3. Results

## 3.1 HCMR Trials

The batch from HCMR survived until 24 days post hatching. The growth performance of the larvae is presented in **Figure 3.1.1**

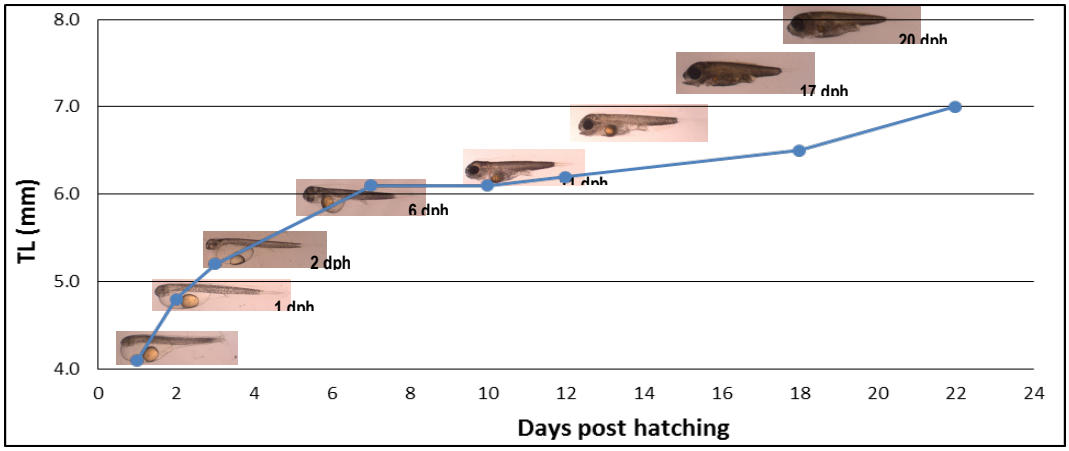



**Figure 3.1.1.** Growth performance of the larvae.

During the rearing some malformed individuals were observed (**Figure 3.1.2**)

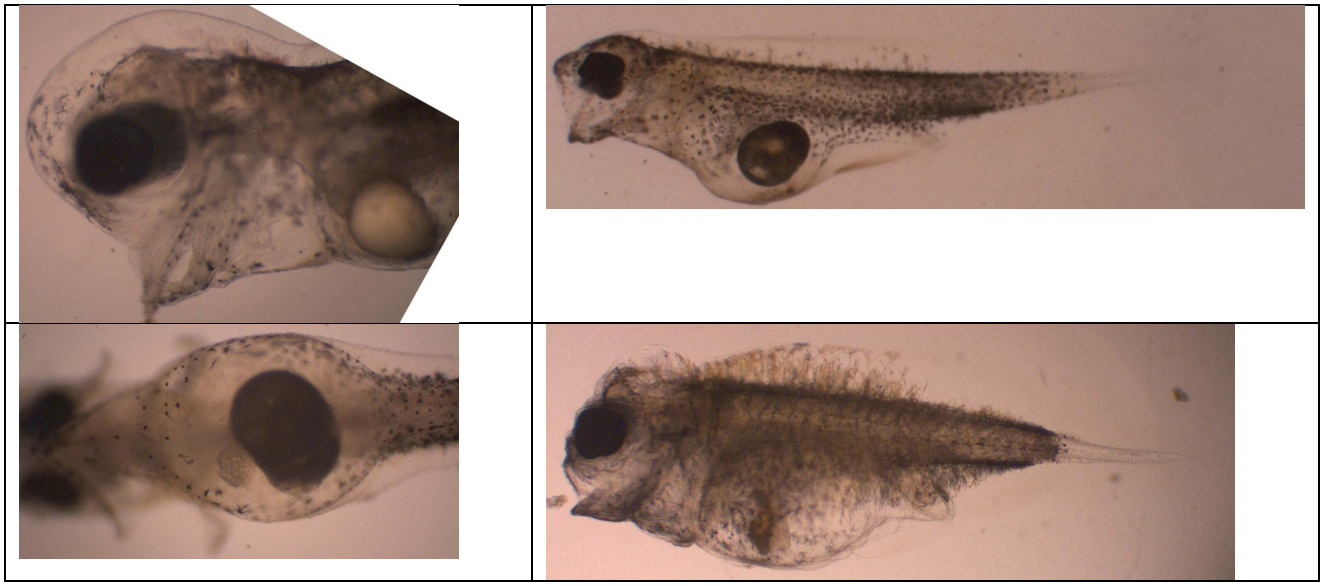


**Figure 3.1.2** Malformed individuals observed during the rearing.

The problem was identified as similar to a syndrome related to swollen yolk sac (SYSS) described in Murray cod, (freshwater fish in Australia) that it is related to inadequate nutrition of the broodstock (Gunasekera et al., 1998). Furthermore, similar appearance has been also described in the Blue Sac Disease – (BSD) that is common in trout (Brzuzan et al., 2007). Several reasons suggested; most common toxicity from Nitrogen compounds such as ammonia, oxidative stress plays significant role. Although it seems that for the wreckfish the Swollen Yolk Sac Syndrome seems to be the case, further studies are required.

### 3.2 IEO Trials

In **2015,** most of the larvae from the MC2 spawns reached 18 dph and only two groups survived until 23 dph although individuals were not healthy presenting several deformities. Larval, yolk sac and lipid droplet length was measured (**Fig. 3.2.1**) and photos of all developmental stages were taken (**Fig. 3.2.2**).

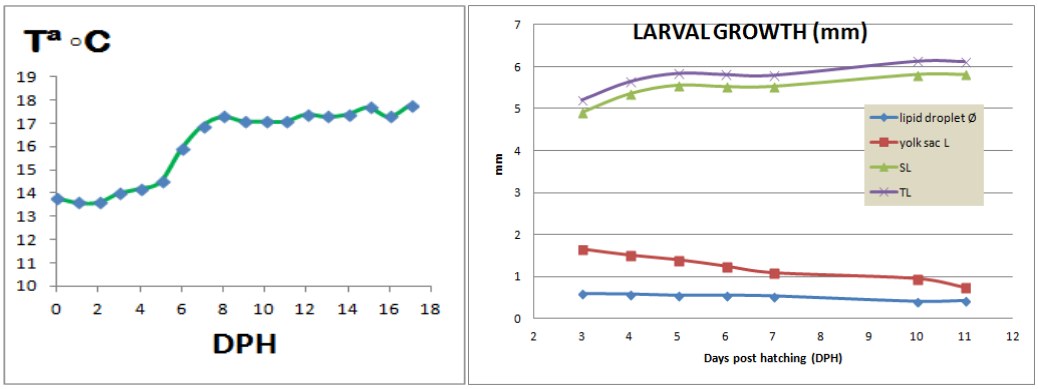



**Figure 3.2.1.** Water temperature (13.8-17.9ºC) and larval growth until 11 DPH. Changes in yolk sac (mm) and in the oil droplet (mm)

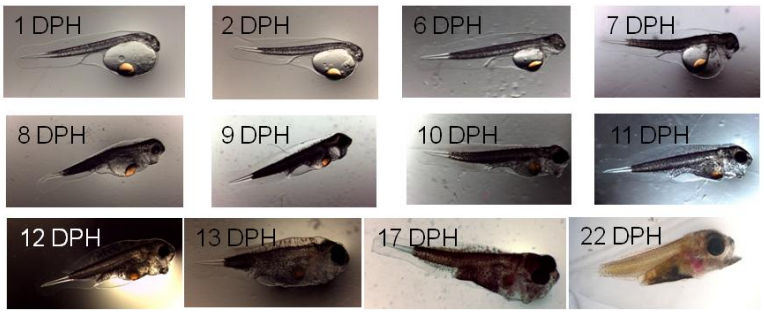



**Figure 3.2.2**. Larval Growth until 22 days post-hatching (DPH).

Malformed individuals observed during the rearing in the IEO facilities **(Fig. 3.2.3)**.

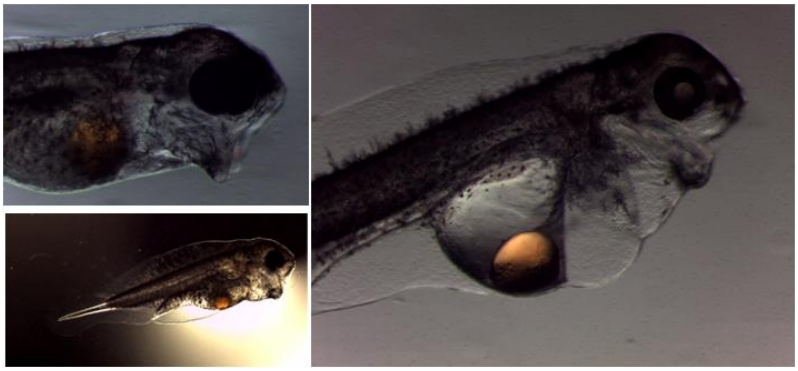


**Figure 3.2.3.** Malformed observed during larval rearing in the IEO.

In **2016**, the maximum healthy larval survival registered was 23 dph in 8,000-culture tank. *Pseudomona* spp and *Vibrio pelagius* were detected on larvae squash culture causing low survival rate. Larval length was 4.70±0.27 at 1 dph, yolk sac consumption was at 11dph at 17-20°C. The moment of mouth opening was at 4 dph at 17-20°C (Álvarez-Blázquez, 2017). There was no evidence that the food ingested by the larvae in this trials was digested (**Fig. 3.2.4)**, because the growth of larvae was negative, i.e. both length and dry weight decreased (**Fig. 3.2.5**), indicating that the surviving larvae were not properly fed while some of them presented also deformities, but the fact of to ingest and to accept the food was considered like a progress for the preliminary trials.

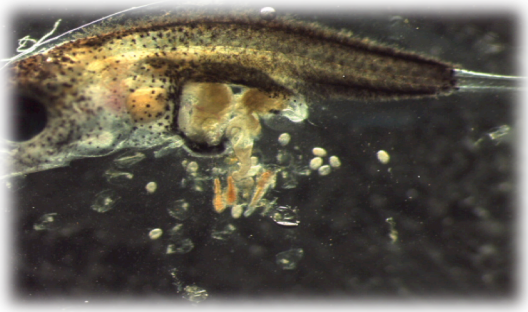


**Figure 3.2.4.** Larvae at 18 dph. Stomach content with *Artemia* and rotifer eggs.

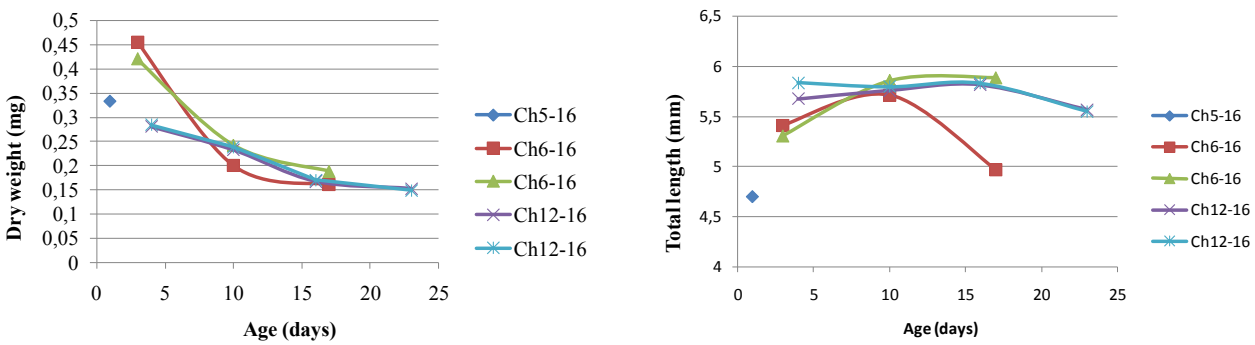



**Figure 3.2.5**. Dry weight (left) and total length (right) in different larvae culture batches.

Results of **2017** trials in terms of larvae growth are presented in **Figure 3.2.6.**

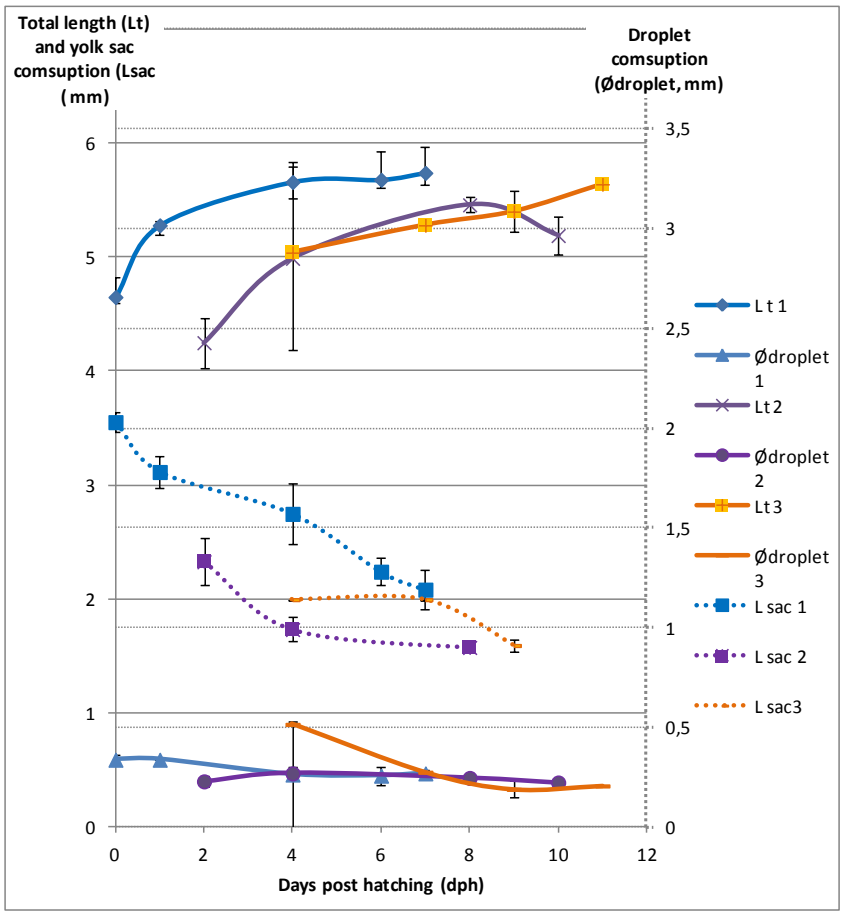



**Figure 3.2.6.** Length (top), yolk sac consumed (center) and lipid droplet consume (bottom) of larval batches during reared at the CMRM facilities.

In MC2, 20 ppm Iodine prophylactic treatment was given to eggs and larvae and we got survival of one healthy and hunting larvae feeding on copepods on 25 dph. On the other hand ,larvae coming from a spontaneous spawn were transferred to a 30 l Kreissel tank with natural light and 30 l/h turnover. Seven larvae survived at 20 dph. One larvae survived to 25 dph feeding on daily given copepods.

During **2018**, for the MC2 trials, on 34 dph in green water at least five healthy larvae were detected swimming well even in the water column and hunting preys. Also at IEO, survival varied between 4 and 30 dph. At the IGAFA trials, both larval batches survived over the weaning period and dry food was provided since 40 dph. At 48 dph larvae were completely weaned. Juveniles survived until 100 dph and were reared at ±18ºC of water temperature, in RAS condition achieving the first results in larval growth (see Deliverable 18.4). The growth in length of the wreckfish larvae survived until weaning, increased from 5.6 mm to 12.8 mm at 5 and 65 dph, respectively (**Fig. 3.2.7**)

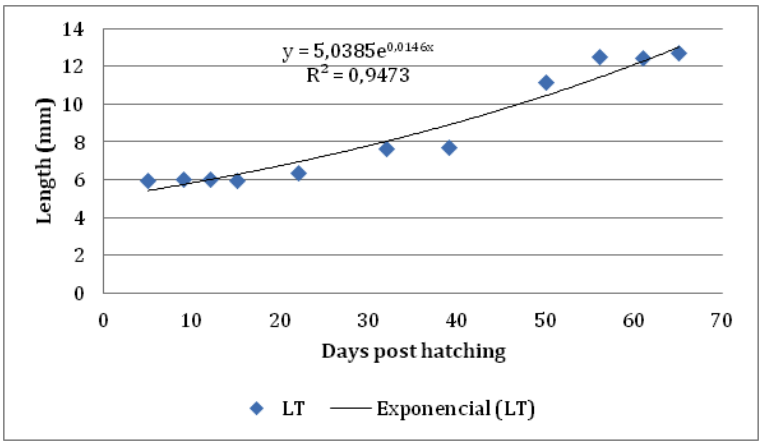



**Figure 3.2.7.** Wreckfish larvae length increasing (mm) since 5 to 65 dph.

The optimal rearing parameters applied for the best up to now result include. The larvae density was at 12.5 larvae.l$^{-1}$. The water temperature was kept at 17.5-18ºC and the dissolved oxygen between 7.2-8.4 mg.l$^{-1}$. The water salinity was at 36.4±1.7 psu. No aeration was provided in the tanks and the photoperiod was natural since 7-9 dph. The optimal feeding sequence for the larval culture of wreckfish in RAS is presented below (**Fig. 3.2.8**).

- Enriched rotifer (ARA enrichment product): 8-19 dph (4-6 rot.ml-2)
- Artemia nauplii: 15-23 dph (0.2-0.7 A0.ml-1)
- Artemia metanauplii (ARA enrichment product): 18-48 dph (0.2-0.7 ml$^{-1}$)
- Dry food: since 40 dph.

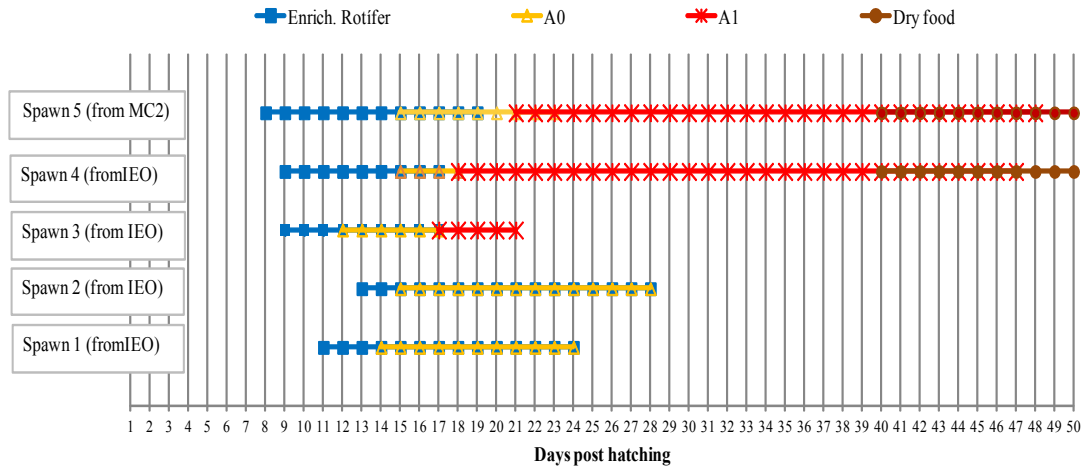



**Figure 3.2.8**. Sequence of live food consumption from CMRM facilities with to larvae culture of spawns from IEO and MC2 that achieving juvenile phase in RAS culture method.

## 4. Discussion

Very important results were achieved in larvae rearing of wreckfish. Although no industrial protocol was produced, the achieved results are of great significance as for the first time individuals survived the larval rearing phase and were weaned into artificial diets. It is worth mentioning that, in the CMRM facilities, there are 25 juveniles that reached 150 dph that were cultured at approximately 18ºC and represent a significant step forward in wreckfish larval culture and provides a basis for further studies. These data could be the starting point for future experiments towards the definitions of industrial conditions for the rearing of the species.

Concluding, the perspectives regarding wreckfish larval rearing are very encouraging. The increase of knowledge of this species in the larval and juvenile period could be very interesting for subsequent studies.

## 5. References

Álvarez-Blázquez, B., Rodríguez, J.L., Vilar, A., Mylonas, C., Papandroulakis, C., Pérez Rial, E., N. Lluch, G. Pazos and F. Linares**.** Progress in the wreckfish intensive culture. new candidate species for aquaculture. Aquaculture Europe 2017 (EAS), 17-20 October, Dubrovnik, Croatia

Álvarez-Blázquez, B., Vilar, Papandroulakis, C.A, Linares, F., Cal, R., Rodríguez, J.L., Lluch, N., Gómez, C., Pérez, E., and and Peleteiro J.B. (2016). First experiences of wreckfish (Polyprion americanus) larval husbandry in NW Atlantic and East Mediterranea. Aquaculture Europe 2016 (EAS), September 2016. Edimburg. United Kingdom.

Brzuzan, P., M Wozny, S Dobosz, H Kuzminski, M K Łuczynski and M Gora, 2007. Blue sac disease in larval whitefish, *Coregonus lavaretus* (L.): pathological changes in mRNA levels of CYP1A, ERa, and p53. Journal of Fish Diseases 2007, 30, 169–173

Gunasekera, R. M., G. J. Gooley, S. S. De Silva, 1998. Characterisation of 'swollen yolk-sac syndrome' in the Australian freshwater fish Murray cod, *Maccullochella peelii peelii*, and associated nutritional implications for large scale aquaculture. Aquaculture 169 _1998. 69–85

Peleteiro, J.B., Saavedra Penas, C., Pérez Rial, E., Álvarez-Blázquez, B., Soares, E.C., Vilar Perón, A., 2011. Diversificación de especies en acuicultura. Estudio preliminar de la reproducción de la cherna (*Polyprion americanus*) en cautividad, Congreso Nacional de Acuicultura, Castelldefels, Spain

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