ADVANCES IN WRECKFISH LARVAE CULTURE

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The **main objectives** were to develop a larvae culture protocol and study the influence of different sea water temperatures.

### 2015

- Only from stock MC2 spawns was obtained, possibly due to the young age of the fish in the different stocks.
  - Two batches of eggs were used, one from the HCMR broodstock by transporting 2000 larvae from Galicia to Crete in polystyrene boxes, and another one from MC2.
  - The feeding protocol used normally in marine fish was applied.
  - Larvae survived until 24 dph in the HCMR.
    - Recirculated system.
    - 16°C graduated increased at 17.5°C.
    - First feeding was at 10 dph, and was based on enriched rotifers, *Artemia* AF (since 13 dph) and *Artemia* EG (since 24 dph).

During the rearing some malformed individuals were observed.
• Larvae survived until 22 dph in the **MC2**:  
  • 50 l tanks flow-through  
  • High larvae density (52 larvae/l)  
  • Rotifers enriched with microalgae  
  • Copepods.

• Larvae survived until 14 dph at the **IEO**:  
  • In vitro spawn  
  • Closed circuit until 10 dph,  
  • Low density in IEO (0.2 larvae/l)  
  • Rotifers enriched with T-Iso until 14 dph  
  • Natural photoperiod during endogenous feeding. After opening the mouth and consumption of the yolk sac, artificial light (410 Lux) was used for 12 h per day until the end of the culture period.  
  • Larvae, yolk sac and lipid droplet length were monitored and photos were taken.

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2015
Water temperature during larvae culture and larval growth, and droplet and yolk sac consumption.

Morphometric development until 22 days post-hatching (DPH).
MC2 spawns quality improved considerably. IEO had started to have good quality spawns as well.

- Larvae were fed with rotifers and *Artemia* enriched with T-Iso until 27 dph
- Growth performance of the larvae until 24 dph, with similar results for the Mediterranean and Atlantic stocks (MC2, CMRM and IEO)
- Larval length was 4.70 mm at one day post hatching and 7.2 mm at 22 dph.
- Yolk sac consumption was at 11 dph at 14-17°C sea water temperature and 8 dph at 17-20°C.
- The moment of mouth opening was at 7 dph at 14-17°C and 4 dph at 17-20°C.

Larval growth in Mediterranean and Atlantic.
Two PRELIMINARY trials were made with testing different incubation temperatures (14±0.5 °C and 17±0.5 °C) (IEO).

The optimal incubation temperature in this trials was shown 16±0.8°C. At this temperature range we obtained the best results regarding the normal embryonic development and the hatching rate of the eggs.

These preliminary results was used for setting an optimal range of incubation temperatures in future experiments, but not conclusive because the assays are insufficient.
Progress was made towards the optimization of the environmental parameters, taking advantage of the improved spawns and the availability of eggs, which allowed us to perform several trials testing different incubation and larval culture temperatures.

- Egg fertility rate between 40-100%
- Hatching rate until 65%
- Larval survival until 27 dph
Incubation experiment with 3 temperatures: 13-14°C, 16-17 and 19-20°C to extend and validate the results obtained during trials before.

- Embryonic development duration was different for each temperature:
  - 4 dpf at 19.5°C
  - 5 dpf at 16.6°C
  - 7 dpf at 13.7°C
- Critical days regarding the viability of embryogenesis
  - At 13.7°C eggs mortality was around 30% during the first three days of incubation.
  - At 16.6°C, the highest levels, around 75%, are concentrated in the first two days.
  - At 19.5°C the majority of the mortality, over 60%, was during the first day of incubation.

<table>
<thead>
<tr>
<th>$T^a$</th>
<th>Days of incubation</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>13,7</td>
<td></td>
<td>24% ± 9,7%</td>
<td>30,2% ± 9,5%</td>
<td>29,5% ± 10,9%</td>
</tr>
<tr>
<td>16,6</td>
<td></td>
<td>35,4% ± 23,8%</td>
<td>40,8% ± 20,9%</td>
<td>4,7% ± 3,4%</td>
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<tr>
<td>19,5</td>
<td></td>
<td>56,8% ± 8%</td>
<td>18,2% ± 7,5%</td>
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</tbody>
</table>

Percentage of eggs collected from the bottom (dead eggs) during the first three days of incubation with respect to the total of eggs that were incubated at first.
Significant differences were observed (p <0.05) in both hatching rate and deformed larvae between the temperatures of 13.7°C and 19.5°C, while the differences were not significant with respect to 16.6°C. This results suggest that low temperature promote very low hatching % and high larvae deformity.

<table>
<thead>
<tr>
<th></th>
<th>13,7 °C</th>
<th></th>
<th>16,6 °C</th>
<th></th>
<th>19,5 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Hatching</td>
<td>4,61 ± 3,9</td>
<td>% Hatching</td>
<td>12,11 ± 9,1</td>
<td>% Hatching</td>
<td>19,7 ± 7,5</td>
</tr>
<tr>
<td>% Deformed</td>
<td>76,25 ± 21,9</td>
<td>% Deformed</td>
<td>49,0 ± 23,9</td>
<td>% Deformed</td>
<td>28,8 ± 13,8</td>
</tr>
</tbody>
</table>
More advances in achieving natural spawns and in larval husbandry have been done in the three Galician wreckfish stocks:

- Very good larval hatching (42-82%) and in live larvae until 34-37 dph.

- At CMRM (IGAFA) two batches of larvae, one from a IEO natural spawn and the one from MC2, produced larvae alive until now (70-100 dph).

- This trial achieved important data on growth and increased our knowledge about the feeding protocol and the specific behavior and metamorphosis of wreckfish larvae.

- This was the first time in the project that we succeeded in producing juveniles weaned to inert food, and it signifies a milestone in the efforts to produce wreckfish under aquaculture conditions.
CONCLUSIONS

• Changes are being made in incubation, embryogenesis and larval husbandry that can be decisive to avoid the problem of malformed larval and achieve greater survival.

• During the first stages of egg development, vulnerability to external conditions is higher; nowadays the incubation parameters were adjusted and the facilities and systems were optimized.

• It has also advanced in the knowledge of the optimal incubation and larval culture temperature.

• The study of the technical conditions and the adequate parameters regarding the aeration, the circulation water, light and water parameters.

• Continue with the investigation of larval malformations that occur in a high percentage.

The achievements in larval husbandry and larval culture systems will be published on the DIVERSIFY website, www.diversifyfish.eu as soon as they are finalized.