Broodstock management and spawning induction of greater amberjack, *Seriola dumerili*


Hellenic Center for Marine Research (HCMR) Crete, GREECE
Reproduction in captivity

objectives of the project:

- Study the reproductive cycle of captive vs wild fish and identify potential dysfunctions
- Establish broodstock management procedures in tanks and sea cages
- Induce spawning using hormonal therapies
- Collect eggs in sea cages
Reproductive cycle studies
mid May - late July
19-26°C

Purse seine, Lampedousa Island, Italy

Sea cages, Salamina Island, Greece
Reproduction in captivity

Handling during early gametogenesis:

Arrested vitellogenesis (smaller oocytes)

Low or lack of sperm production

Rearing in captivity affects spermatogenesis and sperm quality in greater amberjack, Seriola dumerili (Risso, 1810)
Reproduction in captivity

objectives of the project:

- Study the reproductive cycle of captive vs wild fish and identify potential dysfunctions
- Establish broodstock management procedures in tanks and sea cages
- Induce spawning using hormonal therapies
- Collect eggs in sea cages
### Available broodstocks

<table>
<thead>
<tr>
<th>Stocks</th>
<th>Rearing method</th>
<th>Number of individuals</th>
<th>Size (kg)</th>
<th>Feeding (pellets &gt;2 yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARGO</td>
<td>sea-cages</td>
<td>49</td>
<td>7.1-16.0</td>
<td>raw fish</td>
</tr>
<tr>
<td>GMF</td>
<td>sea-cages</td>
<td>28</td>
<td>6.3-15-6</td>
<td>live fish</td>
</tr>
<tr>
<td>SOUDA</td>
<td>sea-cages</td>
<td>12</td>
<td>7.4-14.8</td>
<td>moist pellet</td>
</tr>
<tr>
<td>AQUALABS</td>
<td>land-based</td>
<td>27</td>
<td>6.5-23.8</td>
<td>raw fish</td>
</tr>
<tr>
<td>ARGO</td>
<td>land-based</td>
<td>9</td>
<td>8.1-11.1</td>
<td>live, raw fish</td>
</tr>
<tr>
<td>FORKYS</td>
<td>land-based</td>
<td>22</td>
<td>7.7-10.3</td>
<td>raw fish, squid</td>
</tr>
</tbody>
</table>

**Spawning induction period:**
- mid May - late July
- 20-26°C
Initial design - two approaches

- Fish in tanks during the year
- spawning induced at the expected spawning season
- egg collection

- Fish in sea cages during the year
- spawning induced at the expected spawning season
- egg collection in cages
Broodstocks in tanks

- Inconsistent-incomplete gametogenesis (even w/out handling)
- Low or no sperm production
- Almost no production, or very poor egg quality

![Graph showing oocyte diameter in land-based and sea-cages rearing conditions with P < 0.0001](image)

![Microscope image](image)
Broodstocks in sea cages

- Consistent and complete gametogenesis (also maturation),
- Still relatively low sperm production and quality,
- Limited egg collection when spawning in the sea, but good egg quality.
New approach

- Fish in sea cages during the year
- Spawning induced at the expected spawning season
- Transfer immediately to land-based tanks
- Egg collection in tanks
## First cage-to-tank results

### Daily fecundity (eggs)

<table>
<thead>
<tr>
<th>Days post first treatment</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>22</th>
<th>24</th>
<th>26</th>
<th>28</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>tank 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tank 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Fertilization (%)

<table>
<thead>
<tr>
<th>Year</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**GnRHa**
Sperm quality of amberjack

<table>
<thead>
<tr>
<th>Year / Place / Sampling time</th>
<th>Motility (%)</th>
<th>Duration (min)</th>
<th>Density (10^9 szoa ml⁻¹)</th>
<th>Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-Jun Aqualabs</td>
<td>72±6</td>
<td>2.84±0.35*</td>
<td>28.4±1.5</td>
<td>6±1*</td>
</tr>
<tr>
<td>18-Jun GMF</td>
<td>50±2</td>
<td>3.13±0.45</td>
<td>29.3±1.6</td>
<td>7±1*</td>
</tr>
<tr>
<td>23-Jun Souda</td>
<td>60±3</td>
<td>4.13±0.30**</td>
<td>30.3±2.8</td>
<td>9±1**</td>
</tr>
<tr>
<td>1-Jul Aqualabs</td>
<td>70±4</td>
<td>2.94±0.56</td>
<td>28.4±1.5</td>
<td>6±1*</td>
</tr>
<tr>
<td>29-Jun Souda</td>
<td>60±5</td>
<td>3.13±0.45</td>
<td>29.3±1.6</td>
<td>7±1*</td>
</tr>
<tr>
<td>23-Jun Aqualabs</td>
<td>60±3</td>
<td>4.13±0.30**</td>
<td>30.3±2.8</td>
<td>9±1**</td>
</tr>
<tr>
<td>17-Jul Souda</td>
<td>70±4</td>
<td>2.94±0.56</td>
<td>28.4±1.5</td>
<td>6±1*</td>
</tr>
<tr>
<td>5-Jul Aqualabs</td>
<td>80±6</td>
<td>3.56±0.45</td>
<td>30.3±2.8</td>
<td>9±1**</td>
</tr>
<tr>
<td>7-Jul ARGO</td>
<td>70±4</td>
<td>2.94±0.56</td>
<td>28.4±1.5</td>
<td>6±1*</td>
</tr>
</tbody>
</table>
Optimization of spawning induction method

1. Implants or multiple injections of GnRHa
2. Different doses of GnRHa
3. Timing of application within the season
Implants vs multiple injections

- Implants: mean weight 17±3 kg, sex ratio 1:1
- Injections:

  - 0 days
  - 7 days
  - 14 days
  - 21 days
Spawning kinetics

- **Fecundity (x1000 eggs)**
  - Implant
  - Injection

- **Fertilization (%)**

- **Days post first treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Implant</th>
<th>Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P = 0.012</strong></td>
<td>19 8 n/a</td>
<td>14 8 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GnRH treatment</th>
<th>P = 0.003</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Implant</th>
<th>Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P = 0.005</strong></td>
<td>a b</td>
<td>b</td>
</tr>
</tbody>
</table>

- **Total relative fecundity (eggs kg⁻¹ tank⁻¹)**

- **Daily relative fecundity (eggs kg⁻¹ day⁻¹)**
Oocyte diameter

![Graph showing oocyte diameter over days after first treatment.]

- **Days after first treatment**:
  - Day 0: 7a, 7b
  - Day 7: n/a
  - Day 14: 6ab, 6b, 6ab
  - Day 21: 3

- **Statistical significance**: P = 0.022

**Implants**
- Day 0: FOM, Vg
- Day 7: n/a
- Day 14: Ap, po, Vg
- Day 21: GVM, Vg

**Injections**
- Day 0: FOM, Vg
- Day 7: n/a
- Day 14: Ap, po, Vg
- Day 21: GVM, Vg
## Egg & larval quality

### Treatment

<table>
<thead>
<tr>
<th></th>
<th>Implant</th>
<th>Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>24 h embryos</strong> (%)</td>
<td>19 ± 8</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Hatching</strong> (%)</td>
<td>12 ± 4</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>5d larvae</strong> (%)</td>
<td>12 ± 4</td>
<td>n/a</td>
</tr>
</tbody>
</table>

### GnRHa treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h embryos (%)</td>
<td>36%</td>
<td>53%</td>
<td>70%</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Different doses of implants

- MEDIUM (50 µg GnRHa kg\(^{-1}\)) in previous year

- LOW (25 µg kg\(^{-1}\))
- HIGH (75 µg kg\(^{-1}\))

- Spawning kinetics
- Egg production and quality
- 5d larval survival
Different doses of implants

![Graph showing daily relative fecundity (eggs kg⁻¹) and fertilization (%) across different days post first treatment. The graph compares LOW and HIGH doses with error bars indicating variability. The x-axis represents days post first treatment, ranging from 0 to 25. The y-axis for daily relative fecundity ranges from 0 to 50,000, while the y-axis for fertilization (%) ranges from 0 to 100. The graph includes symbols for LOW and HIGH doses, with LOW doses represented by a triangle symbol and HIGH doses by a triangle symbol.]
Different doses of implants

Daily relative fecundity (eggs kg⁻¹)

Days post first treatment

Fertilization (%)
Timing of application within the reproductive season

- Spawning kinetics
- Egg production and quality
- 5d larval survival

4 different times in the reproductive season
Timing of application within the reproductive season

![Graph showing daily relative fecundity and fertilization percentage over days from start of experiment. The x-axis represents days from start of experiment with labels May 30 and July 18. The y-axis represents daily relative fecundity (eggs kg⁻¹). The graph includes four distinct application periods marked with arrows and labeled 1st, 2nd, 3rd, and 4th. The fertilization percentage is shown on the right y-axis ranging from 0 to 100%.](image-url)
Recommended protocol

- Fish in sea cages during the year with no handling after March-April!!
- Spawning induced at any time between late May - early July (20-25°C)
- Use a GnRHa implant at 50 $\mu$g kg$^{-1}$ every 2 weeks
- Transfer immediately to land-based tanks
- Return to cages after spawning
Broodstock management and spawning induction of greater amberjack, *Seriola dumerili*

Mylonas, C.C., Fakriadis, I., Raftopoulos, A., Iakovopouloos, G., Papandroulakis, N., Lisi, F., Sigelaki, I. and Papadaki, M.

Hellenic Center for Marine Research (HCMR)
Crete, GREECE

Co-funded by the Seventh Framework Programme of the European Union