Reproduction of European eel and larval culture
State of the art
Jonna Tomkiewicz

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Reproduction of European eel and larval culture - State of the art

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CHALLENGES FOR EEL AQUACULTURE

- Eel aquaculture relies on wild caught glass eels
- Severe decline in eel stocks renders present aquaculture production unsustainable
- Captive breeding is needed to sustain eel aquaculture

Challenges for hatchery production of glass eels

Complex life cycle

Hormonal control and inhibition of maturation during silvering

Lack of knowledge about wild eel reproduction and early life stages
PRO-EEL OBJECTIVES

- Increase knowledge about eel physiology and hormonal control of reproduction
  → *Improvement of broodstock nutrition and hormonal treatments for induction of maturation*

- Uncover mechanisms of final oocyte maturation, sperm function and fertilisation capability
  → *Establishment of a stable production of viable eggs and embryos*

- Access requirements for successful egg and larval development and identify suitable larval feeds
  → *Establishment of rearing technology and first feeding in culture of European eel larvae*
PRO-EEL CONCEPT

Link basic research to application and technology development to fill gaps in knowledge and methodology.

Establishment of a knowledgebase:
- Neuro-endocrine function and hormonal control mechanisms
- Liver function and energy allocation
- Male maturation and sperm quality
- Female maturation, ovulation and egg quality
- Development and functional anatomy of embryos and larvae

Application development:
- Broodstock nutrition and selection criteria
- Controlled maturation and gamete production
- Fertilisation procedures and incubation methods
- Larval culture and feeding

Protocols for eel reproduction and larval culture

Full scale experiments
FULL SCALE EXPERIMENTS

An integrated approach

Broodstock selection and nutrition

Hormonal treatments and responsiveness

Gamete production of females and males

Larval production and culture

Fertilisation and incubation

Full scale experiments
### FULL SCALE EXPERIMENTS

**Comparison of reproductive success of farmed and wild broodstocks**

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Results</th>
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<tbody>
<tr>
<td>Broodstock maturation – Wild and farmed: different diets and hormonal treatment</td>
<td>Broodstock response Stripping success</td>
</tr>
<tr>
<td>Fertilisation (4-5 males per female) and incubation</td>
<td>Fertilisation success Embryonic developmental success</td>
</tr>
<tr>
<td>Hatching and larval rearing improving gradually technology</td>
<td>Larval production success</td>
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<td>Larval quality and longevity</td>
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</tbody>
</table>
 Female response to hormonal treatment varies

Response of females as weight change over time classified by ovarian histology at sacrifice
Enhanced farmed broodstock nutrition optimising egg & larvae quality

- Comparing farmed and wild eels
- Formulated diets

**Essential FA in different diets**

<table>
<thead>
<tr>
<th></th>
<th>DAN-EX</th>
<th>JD1</th>
<th>JD2</th>
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<tbody>
<tr>
<td>Protein %</td>
<td>48.1</td>
<td>47.3</td>
<td>47.4</td>
</tr>
<tr>
<td>Fat %</td>
<td>25.7</td>
<td>22.9</td>
<td>24.6</td>
</tr>
<tr>
<td>EPA g/kg</td>
<td>19.4</td>
<td>8.1</td>
<td>11.9</td>
</tr>
<tr>
<td>DHA g/kg</td>
<td>24.4</td>
<td>17.2</td>
<td>20.5</td>
</tr>
<tr>
<td>ARA g/kg</td>
<td>1.08</td>
<td>4.51</td>
<td>3.12</td>
</tr>
<tr>
<td>Vit. C ppm</td>
<td>257</td>
<td>257</td>
<td>400</td>
</tr>
<tr>
<td>Vit. E ppm</td>
<td>163</td>
<td>163</td>
<td>400</td>
</tr>
</tbody>
</table>

**Farmed broodstock on different diets**
FEMALE NUTRITION AND REPRODUCTIVE SUCCESS

Comparing performance of farmed and wild broodstocks

Does feed matter – Yes!
First cleavages 3h
Cells over yolk-mass 14t
Evident embryo 24t
Apparent segments, tail is free 40 t

Embryonic stage duration app. 48 h until hatch
DEVELOPMENT OF YOLKSAC LARVAE

2-3 hours

6-7 hours

10-11 hours

500 µm
DEVELOPMENT OF YOLKSAC LARVAE

- 2 days
- 5 days
- 9 days
- 14 days

1 mm
QUALITY OF YOLKSAC LARVAE

Measurements of morphological characteristics and general developmental success

Amount and use of yolk

Length increase
Measuring development in general morphology, amount and use of yolk and length increase.
QUALITY OF YOLKSAC LARVAE

Differences in larval quality between farmed and wild broodstock

Differences in larval quality

Farmed:
Series: A and C

Wild:
Series B and D
**Standardisation of fertilisation procedures**

Importance of sperm to egg ratio and time post-stripping

- Egg fertilisation capacity in relation to time post-stripping
- Optimal sperm # and time post-stripping
  - 25,000 sperm per egg
  - Eggs fertilized within 10 min

Butts et al., Larvi 2013, poster
Establishment of biotechnical conditions for egg incubation and larval rearing

- Water quality management
- Recirculation, flow-through
- Mesocosmos etc.

Transport and export

- Transport of eggs by flight from Denmark to Norway
- Successful parallel experiments
Incubation: Test of egg disinfection treatments

Treatments:
- Filtered autoclaved Seawater
- Glutaraldehyde
- Hydrogen peroxide
  - low 2 g L$^{-1}$
  - high 6 g L$^{-1}$
Larval survival in relation to disinfection treatment
QUALITY OF YOLKSAC LARVAE

Measurements of morphological characteristics and general developmental success

Amount and use of yolk

Length increase
- Large yolk syncytium at 6 DPH,
- Relatively immature liver/pancreas at 12 DPH
Digestive enzymes in eel larvae day 3-14

Very high activities in lipase-like enzymes after hatching

- Similar development in expression of the enzymes’ corresponding coding genes
  - lipase, amylase, trypsin and aminopeptidase N

Data suggest that yolksac eel larvae have an elevated nutritional requirement for lipids

Important for development of new strategies for feeding eel larvae?
LARVAL FEEDING STAGE AND FEED?

European eel larvae 14-15 DPH

What can they eat?
Analysis of larval head morphology
Study of larval behaviour
CONCLUSION

Successful results of PRO-EEL

- Enhanced broodstock feeds for high quality eggs and larvae comparable to wild broodstocks
- Optimal fertilization protocols
- Stable production of viable eggs and larvae
- Culture of yolksac larvae
- Insight into larval nutritional requirements

Future goals
- Identification of suitable feed, feeding larval culture and ongrowing