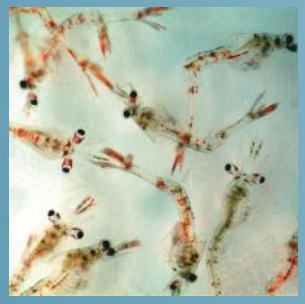
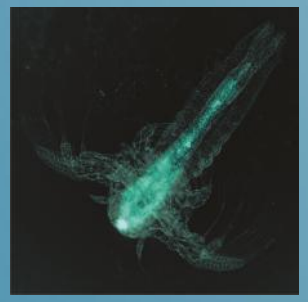
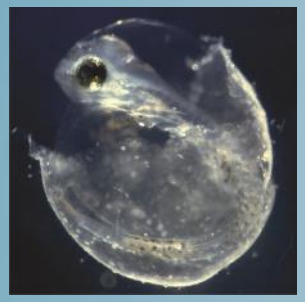
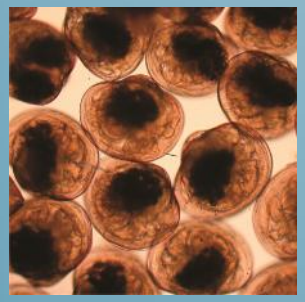




larvi 2013

6th fish & shellfish larviculture symposium

Reproduction of European eel and larval culture
State of the art

Jonna Tomkiewicz

ghent university, belgium, 2-5 september 2013

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

Jonna Tomkiewicz, Technical University of Denmark

Ian A.E. Butts, Sune Riis Sørensen, Sebastian N. Politis, Josianne Støttrup, Peter Munk & Maria Krüger-Johnsen, DTU, Denmark

Peter De Schryver & Mathias Bouillart, Ghent University, Belgium

José Zambonino-Infante, Geneviève Corraze & Sachi Kaushik, INRA, France

Sylvie Dufour & Anne-Gaëlle Lafont, CNRS, France

Juan F. Asturiano, ICTA-UPV, Spain

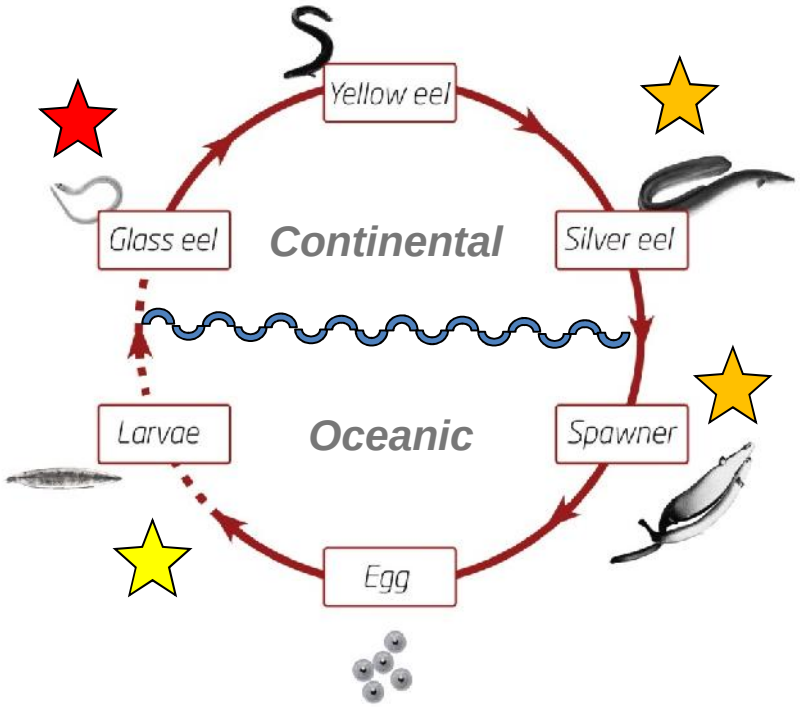
Elin Kjørsvik, NTNU, Norway

Terje van der Meeren, IMR, Norway

Peter Lauesen, Billund Aquaculture Service, Denmark

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**

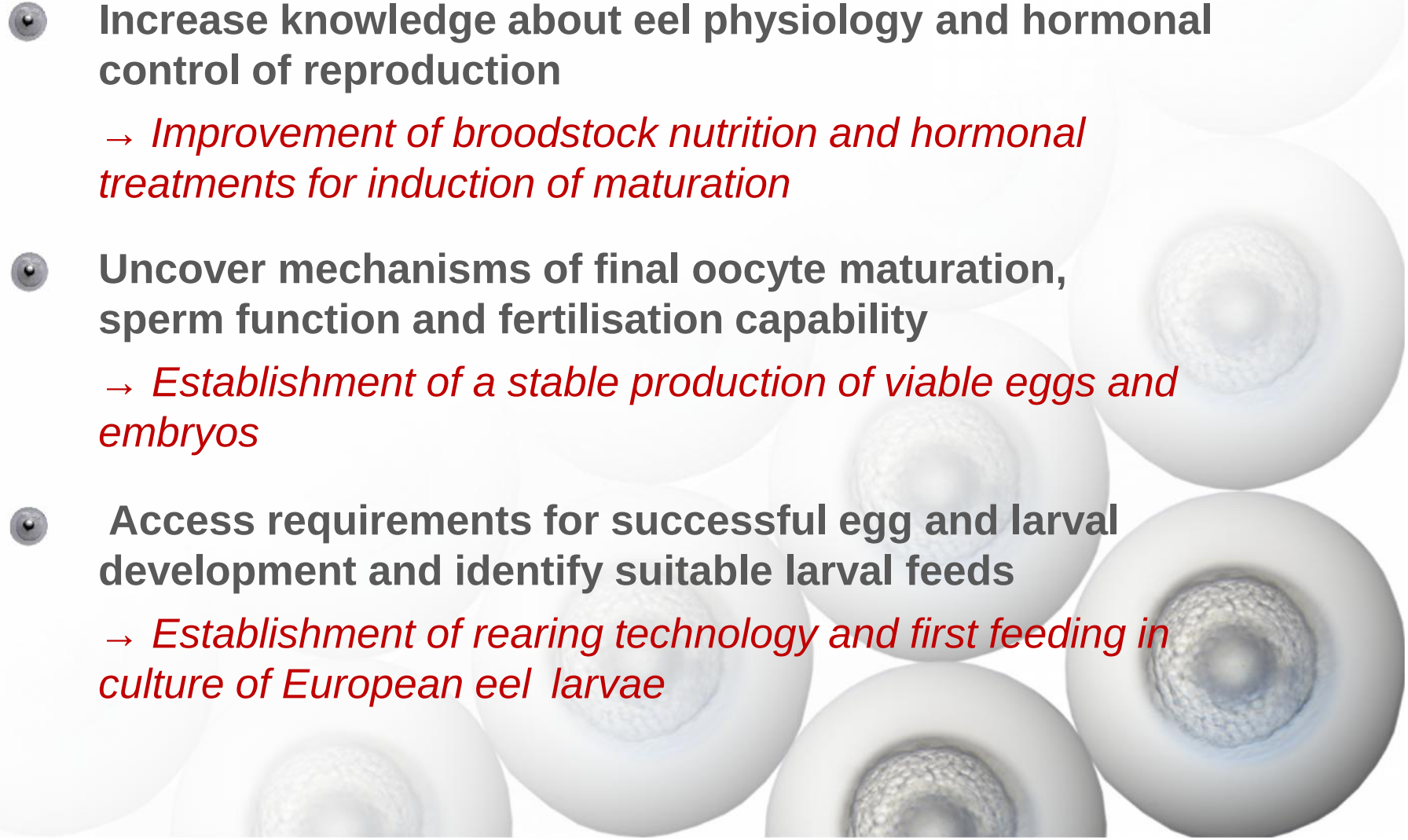
  → Improvement of broodstock nutrition and hormonal treatments for induction of maturation

- **Uncover mechanisms of final oocyte maturation, sperm function and fertilisation capability**

  → Establishment of a stable production of viable eggs and embryos

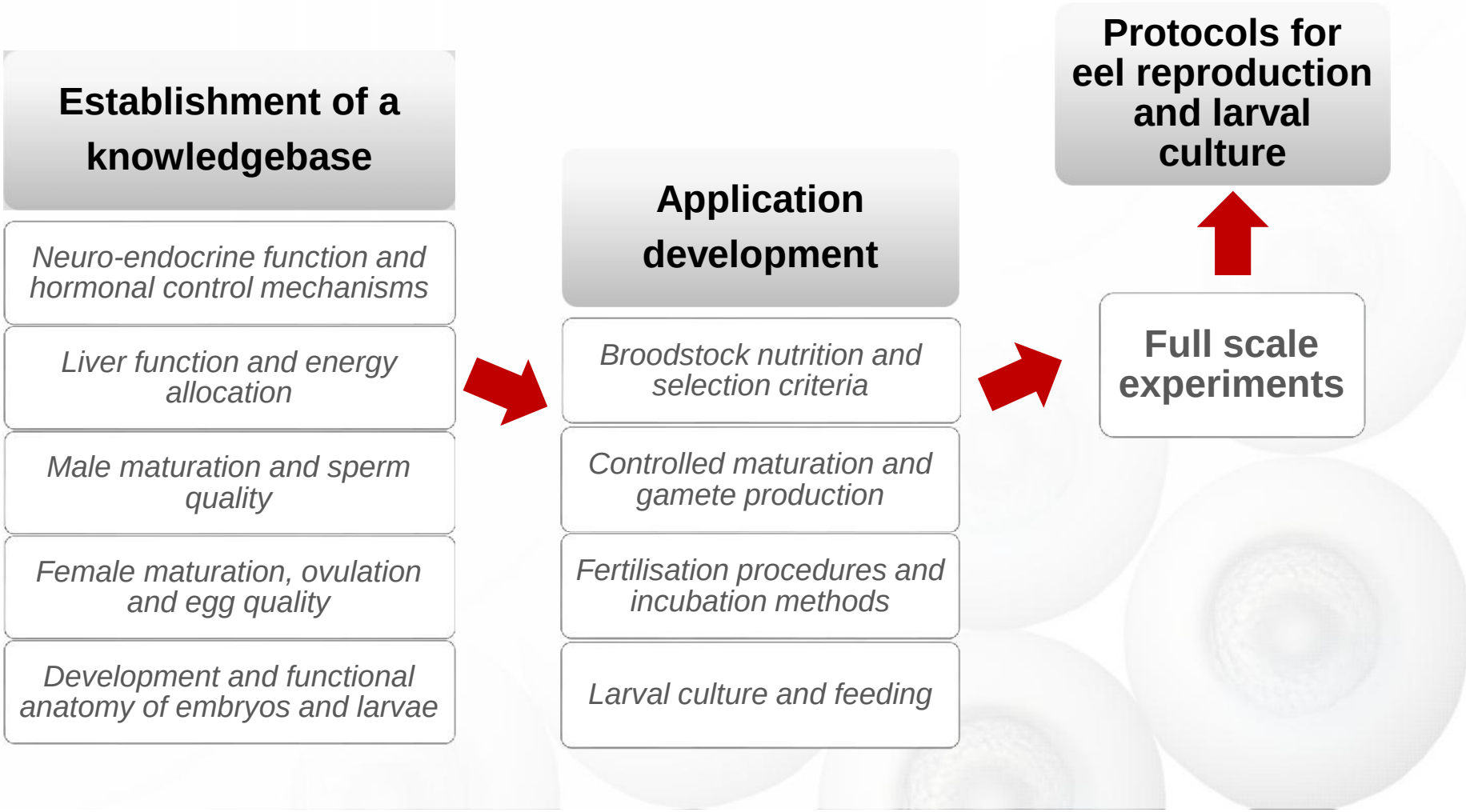
- **Access requirements for successful egg and larval development and identify suitable larval feeds**

  → Establishment of rearing technology and first feeding in culture of European eel larvae



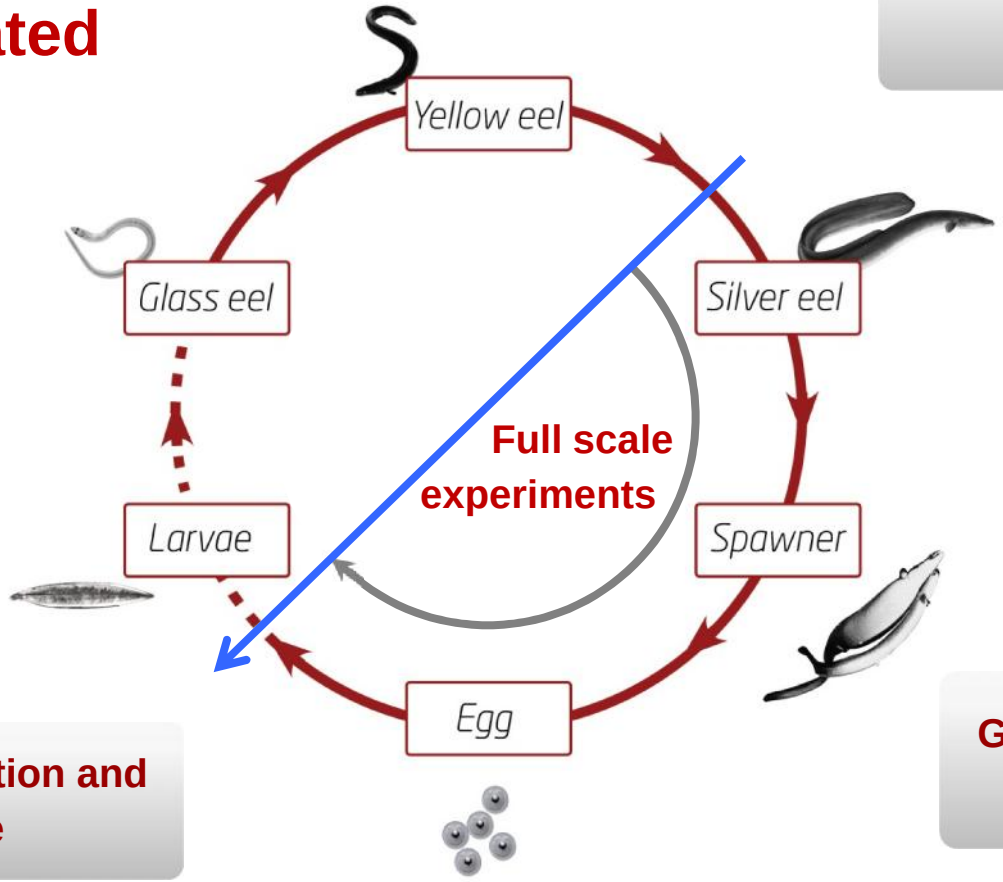
PRO-EEL CONCEPT

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



FULL SCALE EXPERIMENTS

An integrated approach



Broodstock selection and nutrition

Hormonal treatments and responsiveness

Gamete production of females and males

Fertilisation and incubation

Larval production and culture

FULL SCALE EXPERIMENTS

Comparison of reproductive success of farmed and wild broodstocks

Protocols

Broodstock maturation – Wild and farmed: different diets and hormonal treatment

Fertilisation (4-5 males per female) and incubation

Hatching and larval rearing improving gradually technology

Results

Broodstock response
Stripping success

Fertilisation success
Embryonic developmental success

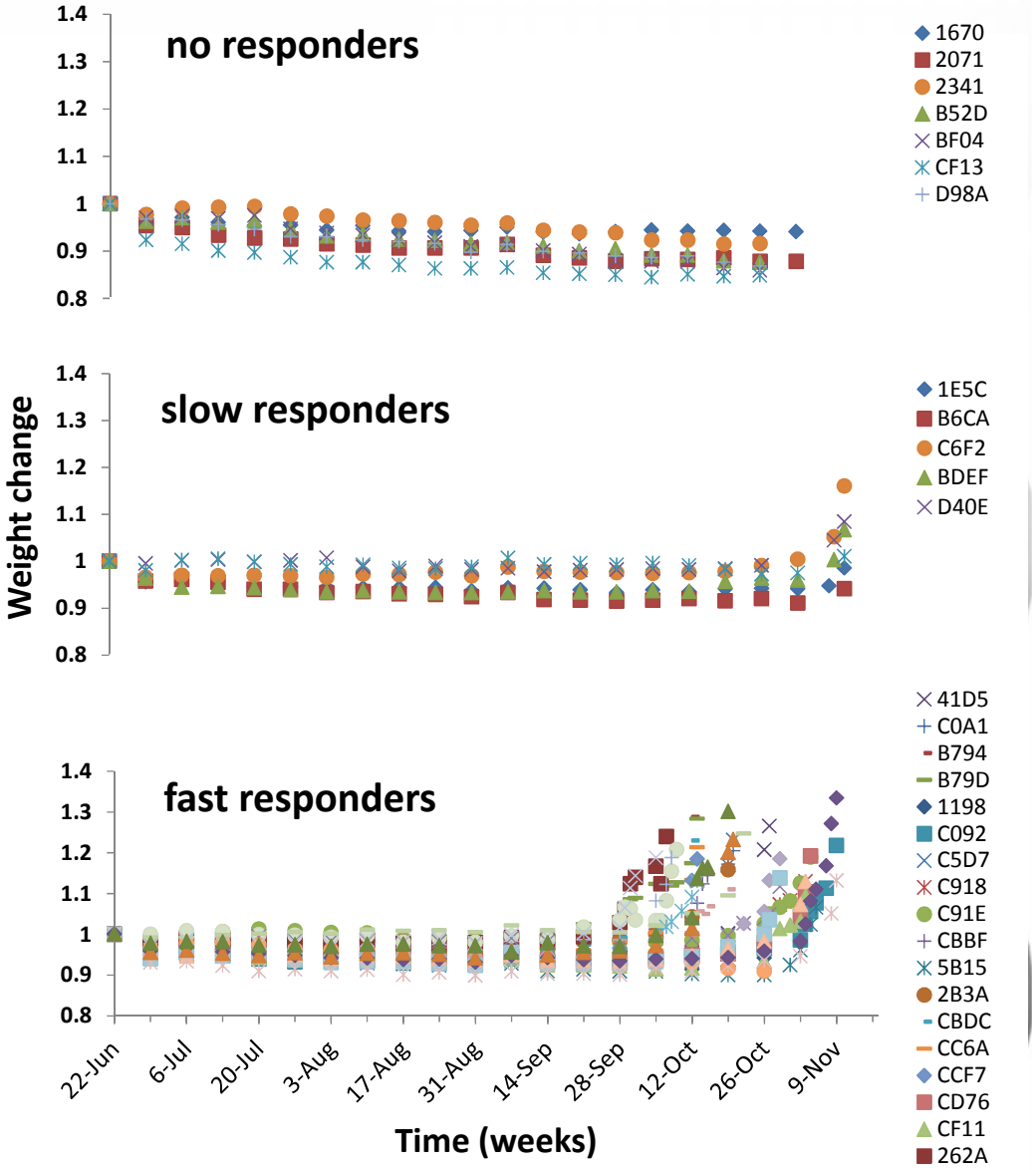
Larval production success

Larval quality and longevity

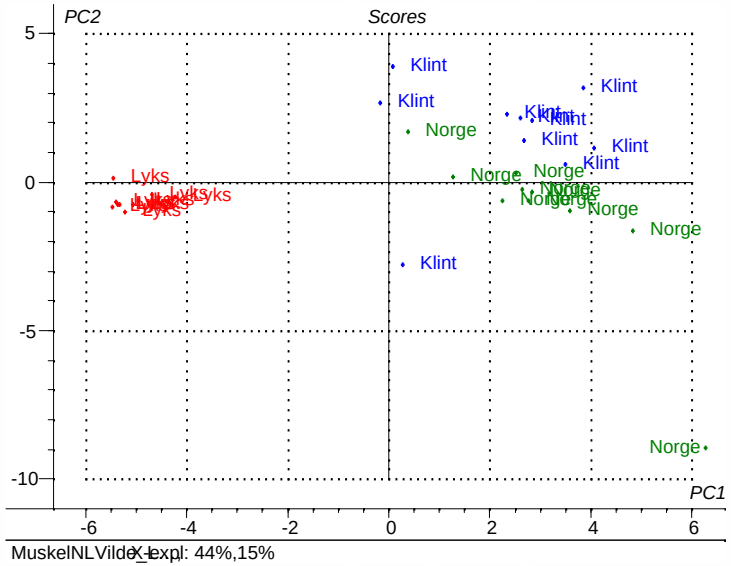
BROODSTOCK RESPONSE

Female response to hormonal treatment varies

Response of females as weight change over time classified by ovarian histology at sacrifice



FEMALE BROODSTOCK NUTRITION

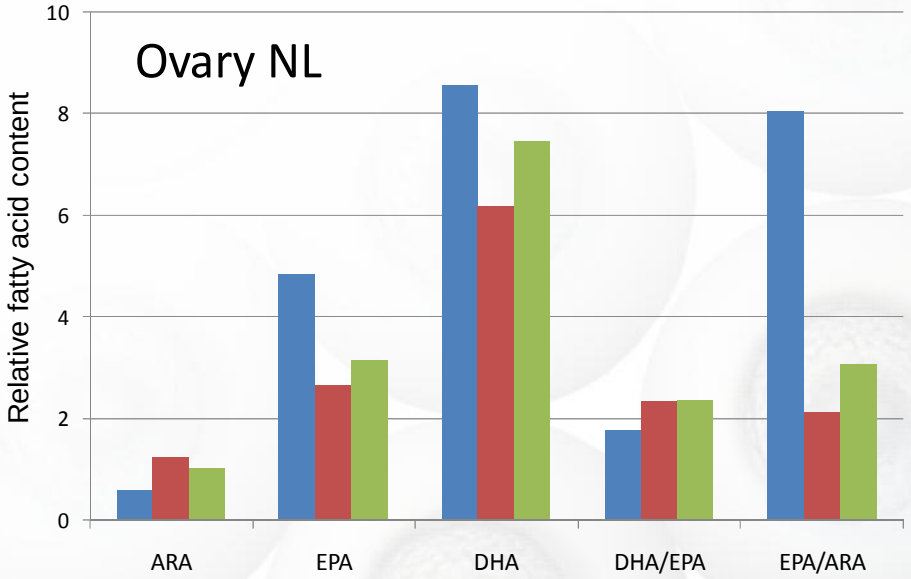


	DAN-EX	JD1	JD2
Protein %	48.1	47.3	47.4
Fat %	25.7	22.9	24.6
EPA g/kg	**19.4**	**8.1**	**11.9**
DHA g/kg	**24.4**	**17.2**	**20.5**
ARA g/kg	**1.08**	**4.51**	**3.12**
Vit. C ppm	257	257	400
Vit. E ppm	163	163	400

Essential FA in different diets

Enhanced farmed broodstock nutrition optimising egg & larvae quality

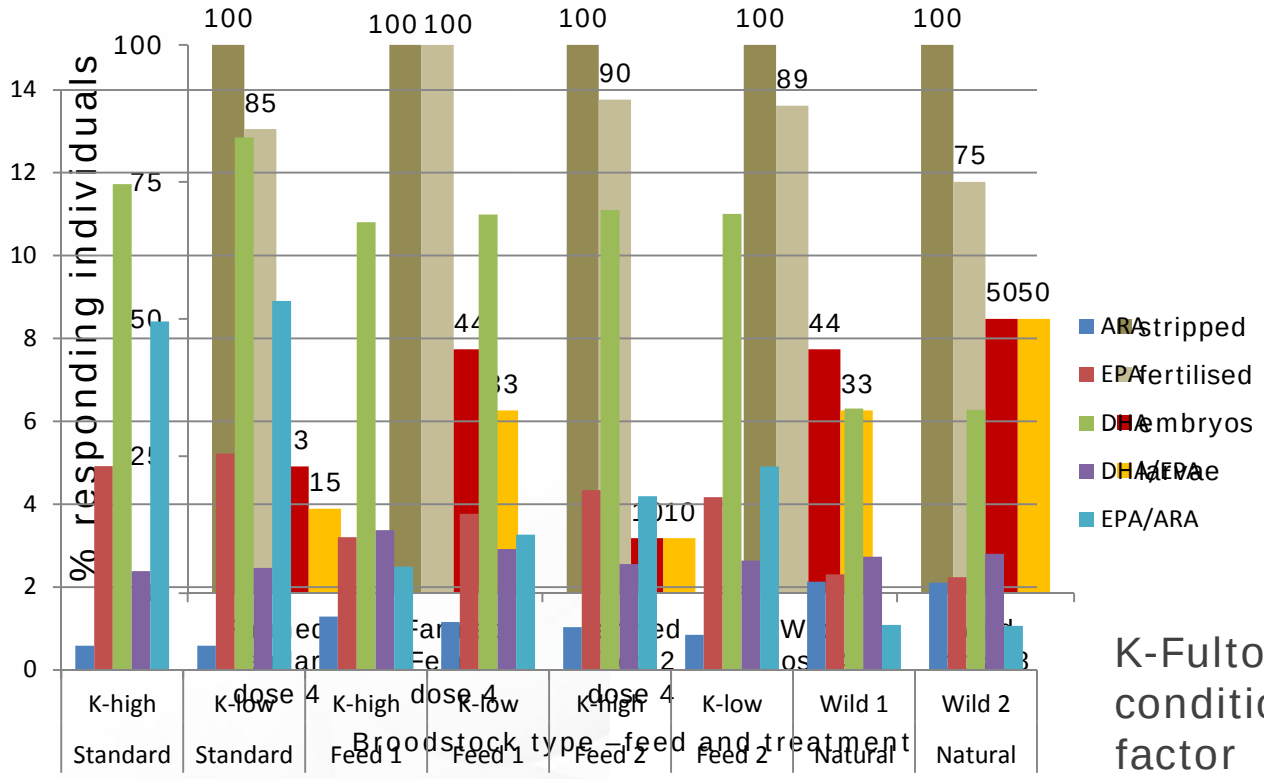
- Comparing farmed and wild eels
- Formulated diets



Farmed broodstock on different diets

FEMALE NUTRITION AND REPRODUCTIVE SUCCESS

Comparing performance of farmed and wild broodstocks



K-Fulton's condition factor

Does feed matter – Yes!

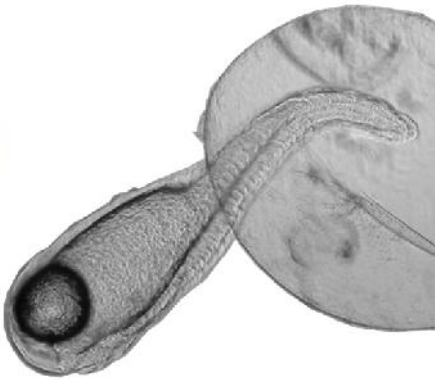
FWRTILISATION AND EMBRYONIC DEVELOPMENT

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

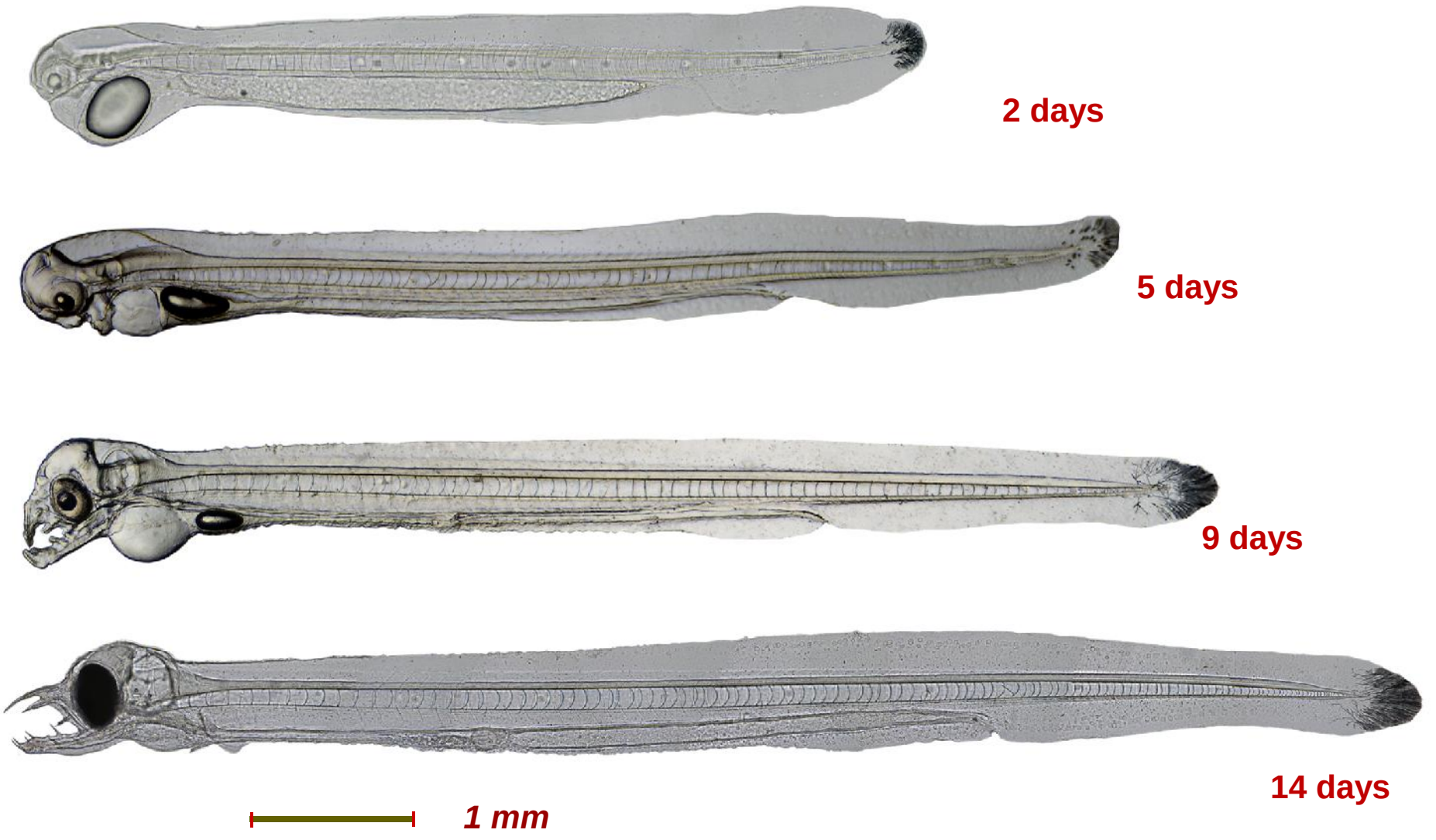
2-3 hours

6-7 hours

10-11 hours

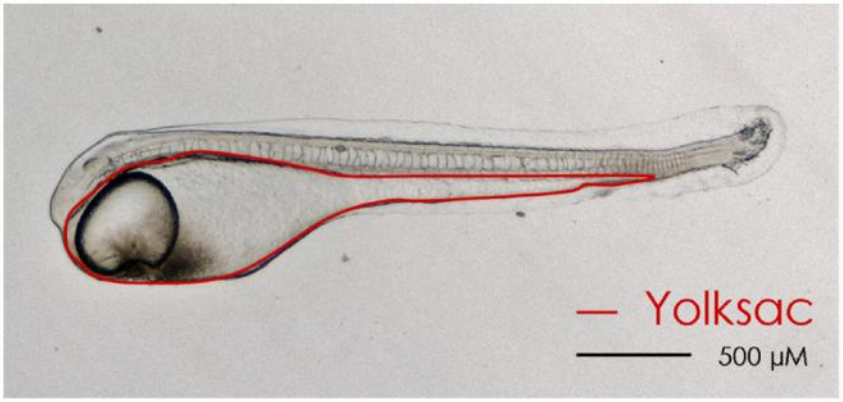
500 µm

DEVELOPMENT OF YOLKSAC LARVAE



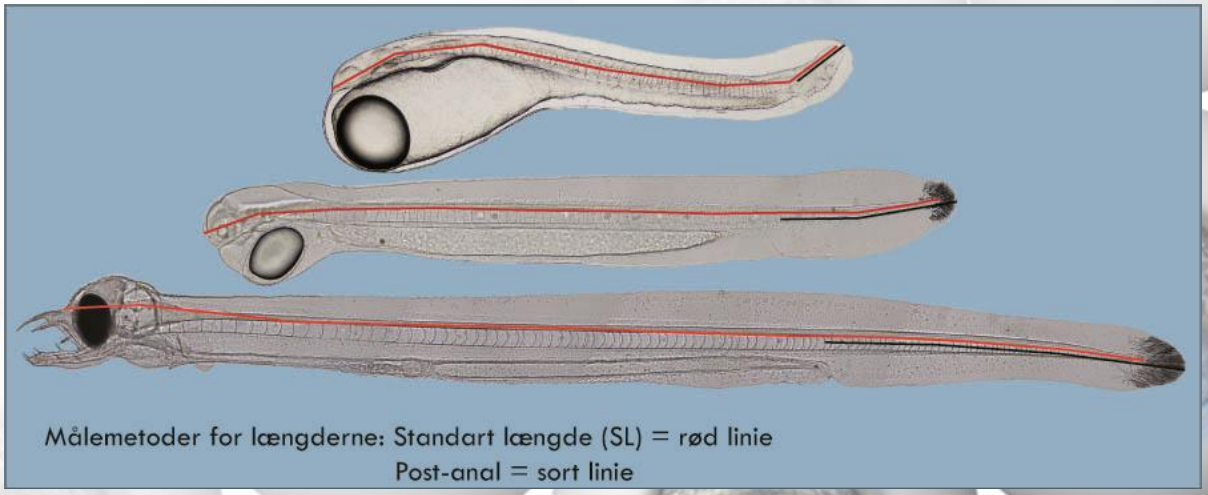
QUALITY OF YOLKSAC LARVAE

Measurments of morphological characteristics and general developmental success



Amount and use of yolk

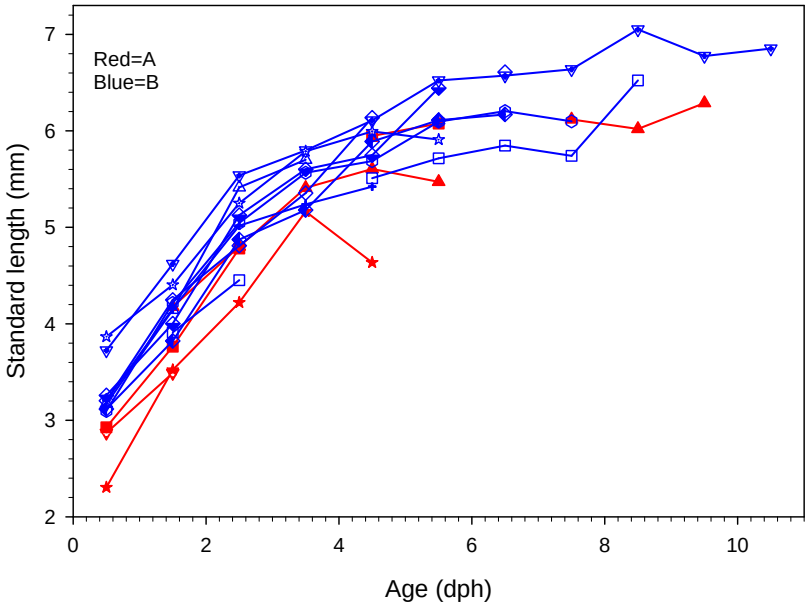
Length increase



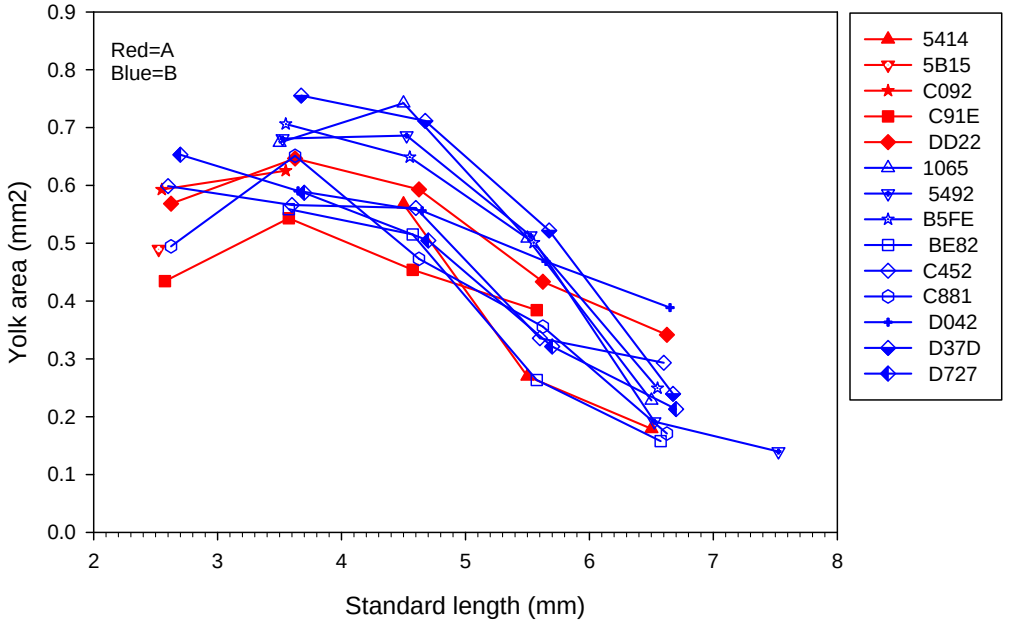
QUALITY OF YOLKSAC LARVAE

Measuring development in general morphology, amount and use of yolk and length increase

PRO-EEL Series A and B, Length at age

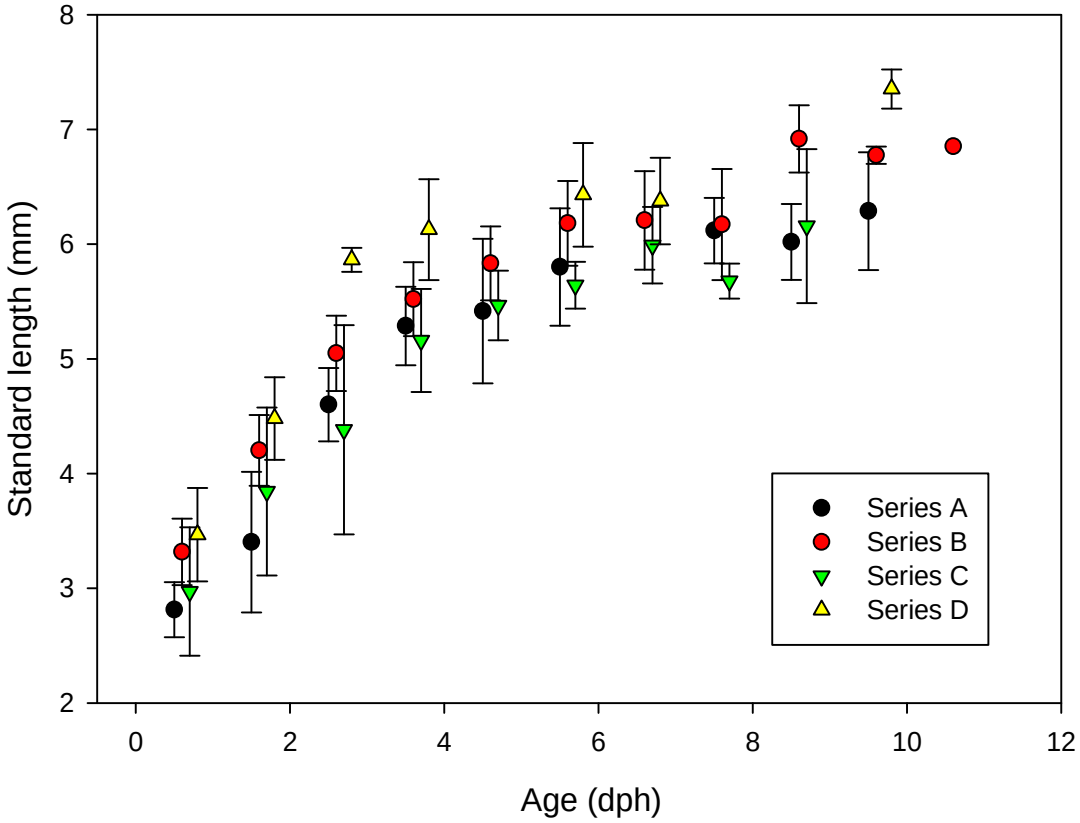


PRO-EEL Series A and B , Yolk at length



QUALITY OF YOLKSAC LARVAE

Differences in larval quality between farmed and wild broodstock



Differences in larval quality

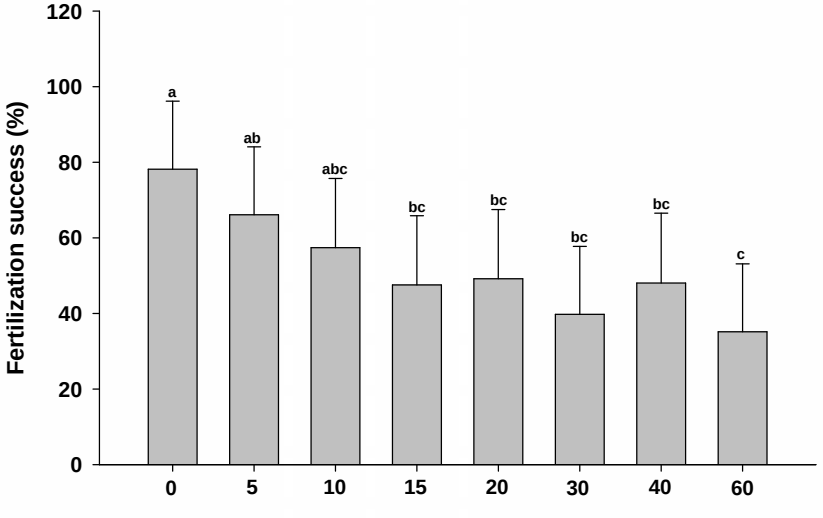
Farmed:

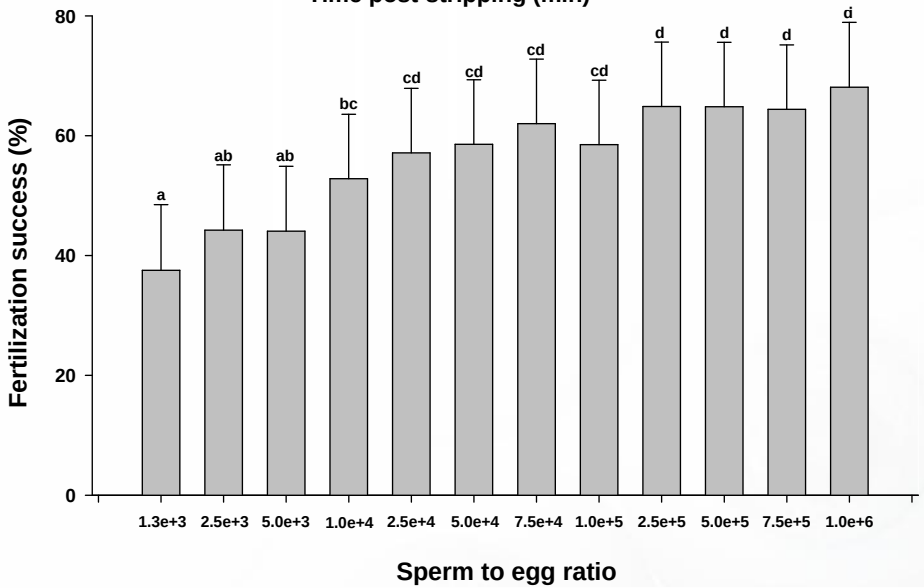
Series: A and C

Wild:

Series B and D

STANDARDISATION OF FERTILISATION PROCEDURES





Standardisation of fertilisation procedures

Importance of sperm to egg ratio and time post-stripping

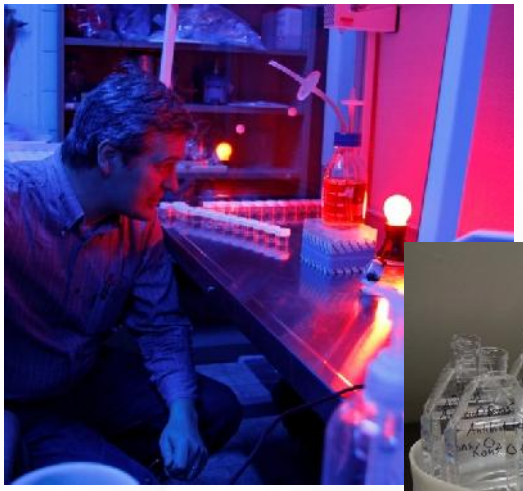
- Egg fertilisation capacity in relation to time post-stripping
- Optiomal sperm # and time post-stripping
  - –25,000 sperm per egg
  - –Eggs fertilized within 10 min

Butts et al., Larvi 2013, poster

EGG AND LARVAL REARING TECHNIQUES

Establishment of biotechnical conditions for egg incubation and larval rearing

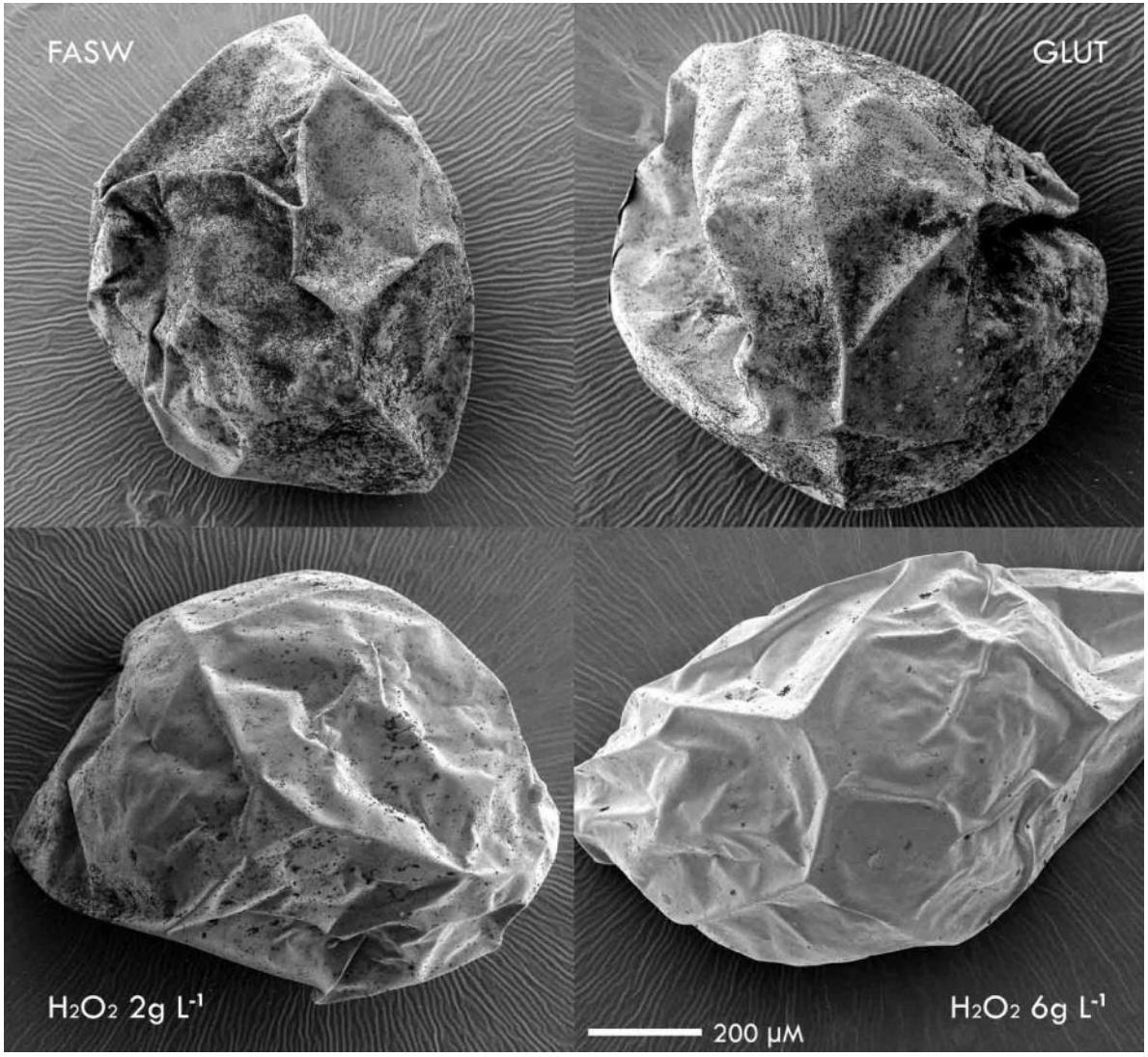
- Water quality management
- Recirculation, flow-through
- Mesocosmos etc.



Transport and export

- Transport of eggs by flight from Denmark to Norway
- Successful parallel experiments

EGG AND LARVAL REARING TECHNIQUES



Incubation:

Test of egg disinfection treatments

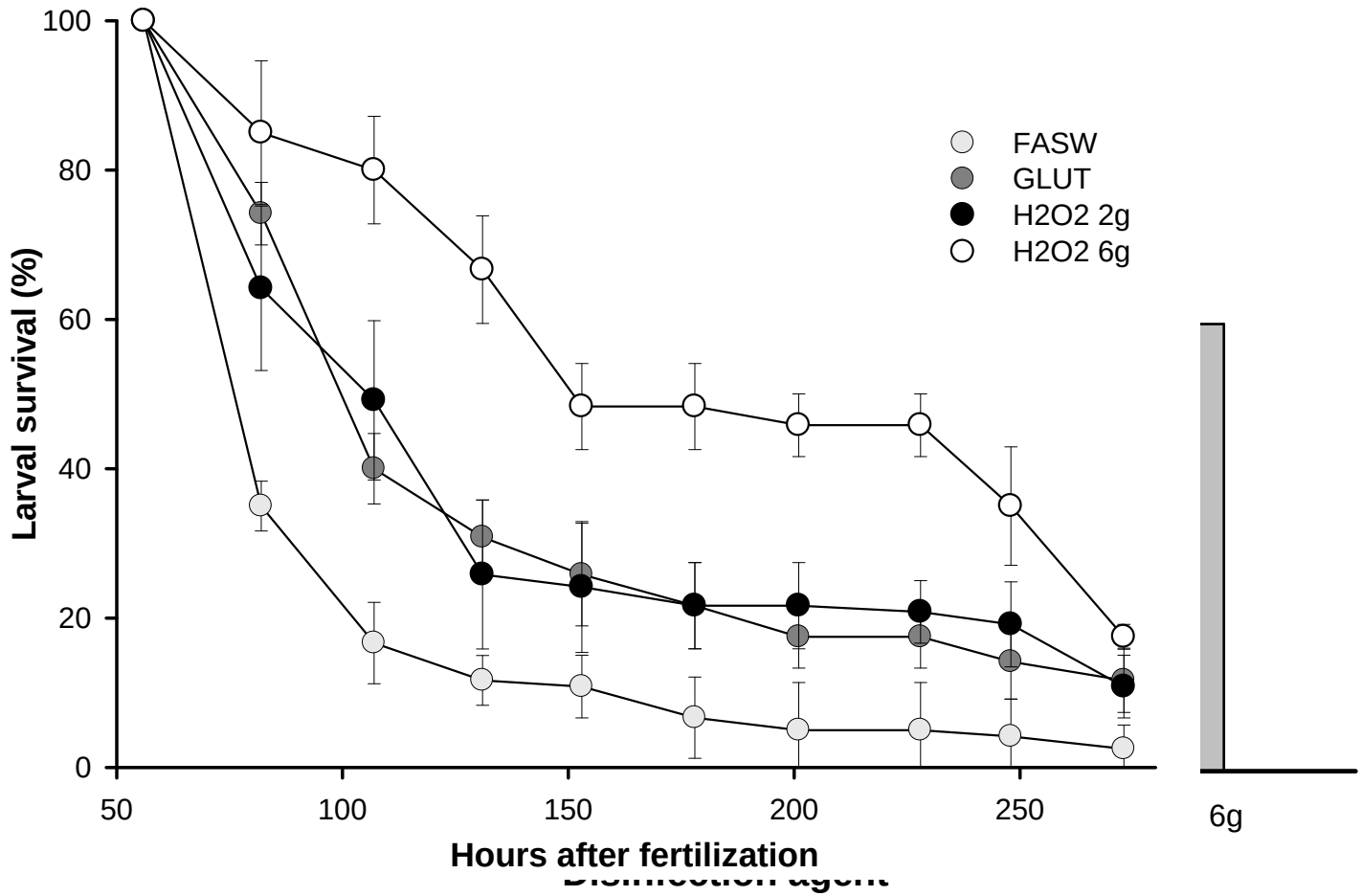
Treatments:

- **Filtered autoclaved Seawater**
- **Glutar aldehyde**
- **Hydrogen peroxide**

  low 2 g L^{-1}

  high 6 g L^{-1}

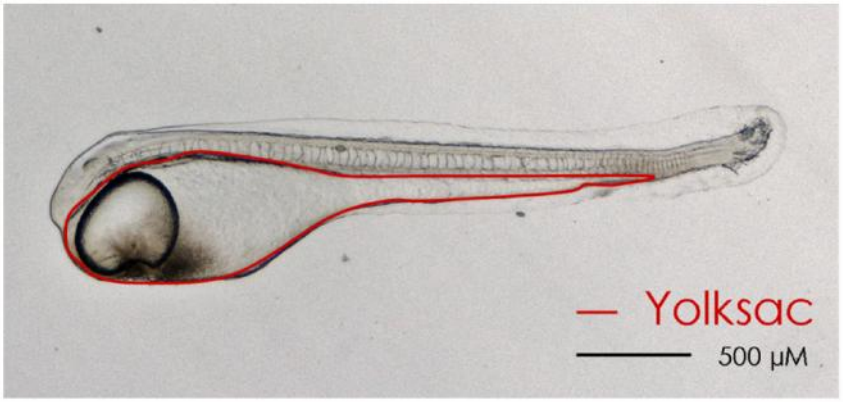
WATER MANAGEMENT AND MICROBIAL CONTROL



Larval survival in relation to disinfection treatment

QUALITY OF YOLKSAC LARVAE

Measurments of morphological characteristics and general developmental success



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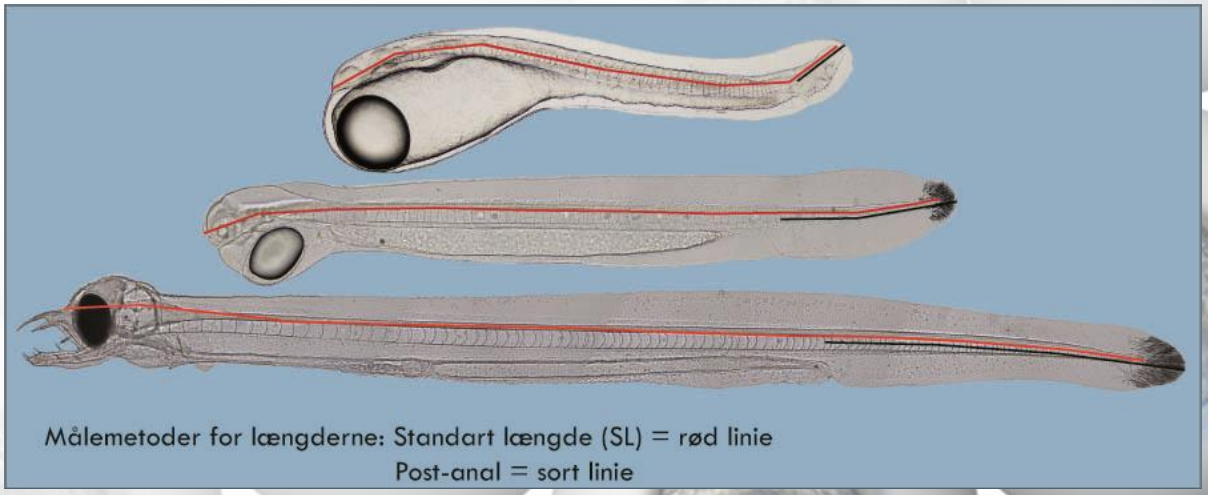
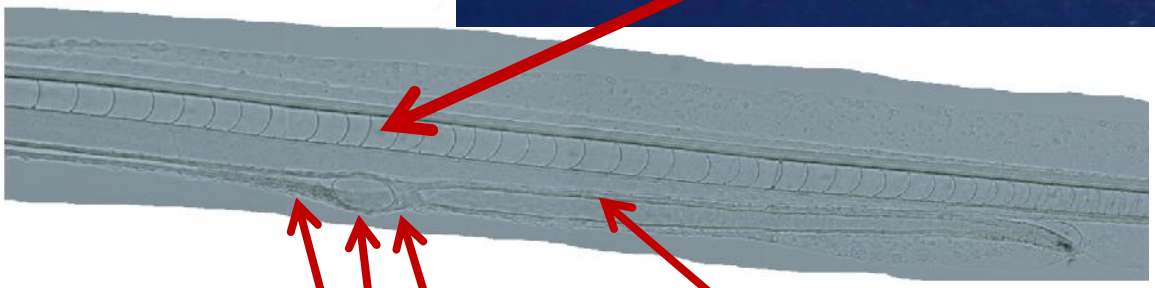
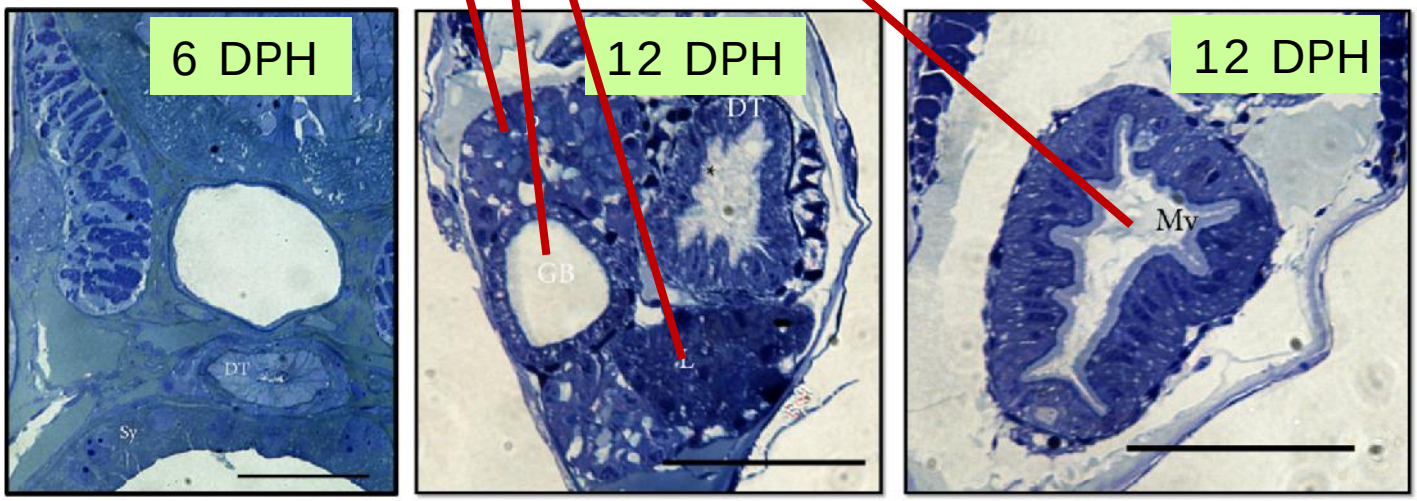


LARVAL DIGESTIVE SYSTEM

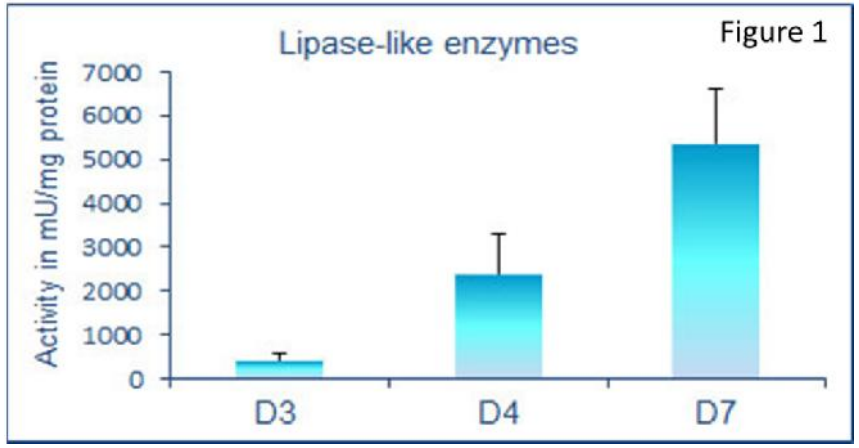
Digestive histology



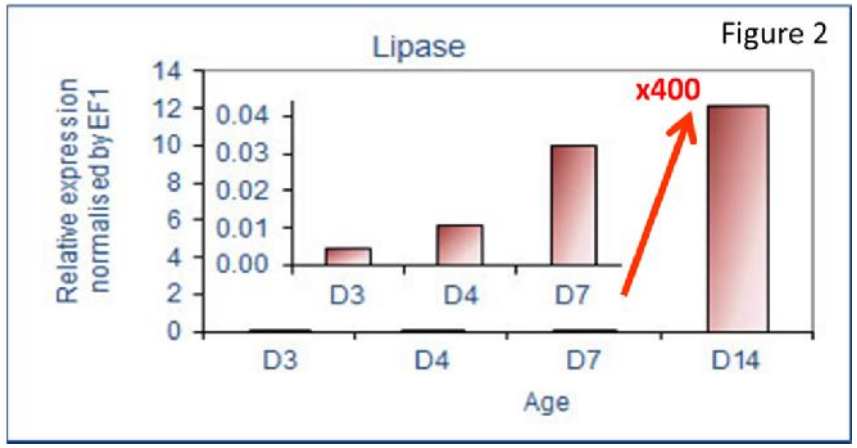


- Large yolk syncytium at 6 DPH,
- Relatively immature liver/pancreas at 12 DPH

LARVAL DIGESTIVE SYSTEM



Enzyme activity (biochemical study)



Enzyme gene expression

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?

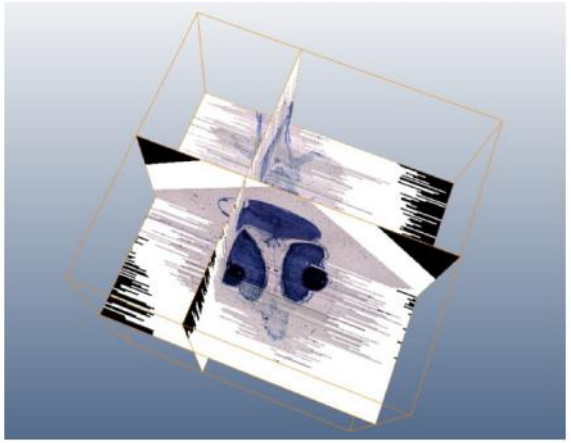
LARVAL FEEDING STAGE AND FEED?

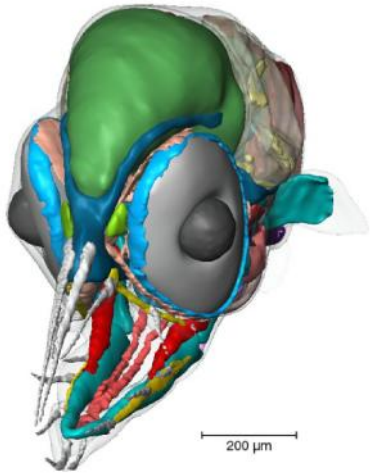
European eel larvae 14-15 DPH

What can they eat?

Analysis of larval head morphology

Study of larval behaviour





M. Bouillart et al., Lariv 2013

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 in to larval nutritional requirements

Future goals

- ➢ Identification of suitable feed, feeding larval culture and ongrowing