

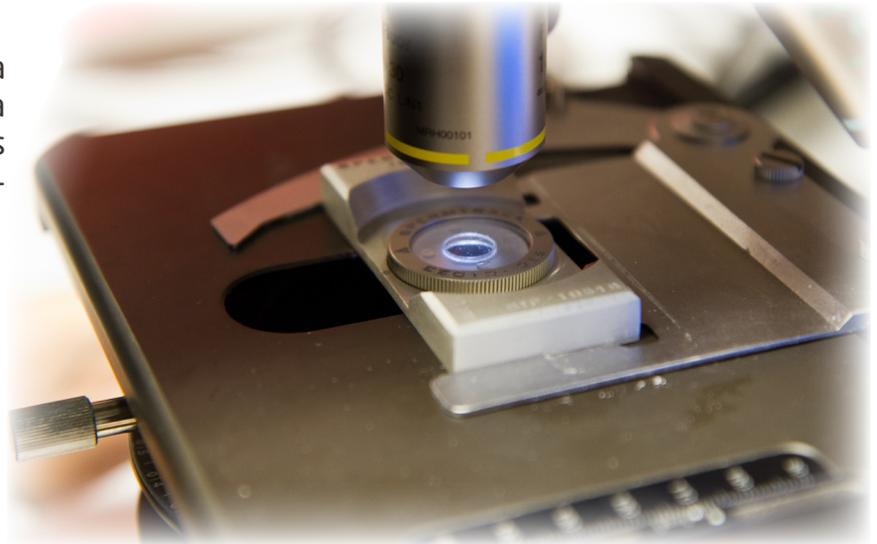
## **Newsletter content**

- *First European eel larvae obtained using cryopreserved sperm*
- *New record on larval longevity and feeding tests*
- *4<sup>th</sup> PRO-EEL steering meeting held in Brussels*
- *PRO-EEL workshop in Valencia, 27<sup>th</sup> March 2012*

## **JOINT PRO-EEL EXPERIMENTS ON EEL SPERM QUALITY AND FERTILIZATION CAPACITY**

In November-December, a series of PRO-EEL small scale experiments on evaluation of male eel sperm quality in fertilization experiments was carried out in collaboration between ICTA-UPV and DTU. A concurrent pilot experiment on larval production conducted by DTU and BA resulted in an extraordinary strong larval batch. The experiments took place at DTU's research facility "Lyksvad" in Denmark.

The PRO-EEL steering committee plans a project meeting in March 2012 in Valencia including a 1-day workshop for networks and interested parties. A first announcement is provided.



## First European eel larvae obtained using cryopreserved sperm

by Juan F. Asturiano and Luz Pérez, ICTA-UPV, Instituto de Ciencia y Tecnología Animal, Polytechnic University of Valencia, Spain, and Jonna Tomkiewicz and Sune Riis Sørensen, DTU Aqua, Technical University of Denmark

### Controlled female and male maturation

The experiments focused on tests of eel sperm quality and fertilization capacity with the purpose to enhance methods used in offspring production. During the period prior to the experiments, male eels were brought to spermiation through hormonal treatment with recombinant human chorionic gonadotropin (hCG). Similarly, female eels were ripened through weekly injections of salmon pituitary extract (SPE).



Hormone injections inducing maturation in female and male eels are applied in offspring production. Photo: Sune Riis Sørensen, DTU Aqua.

### Fertilization capability using cryopreserved sperm

Specific experiments tested the fertilization capability of sperm subjected to different treatment i.e. diluted fresh sperm and cryopreserved sperm from the same males. Stripped sperm was either diluted in a medium and stored at different temperatures or cryopreserved, i.e. semen was added a preserving medium and

frozen in liquid nitrogen. The study included evaluation of individual male sperm motility (for selection of suited males) and sperm density (to standardize concentrations in frozen/fresh samples) as well as estimation of fertilization and embryonic development success.

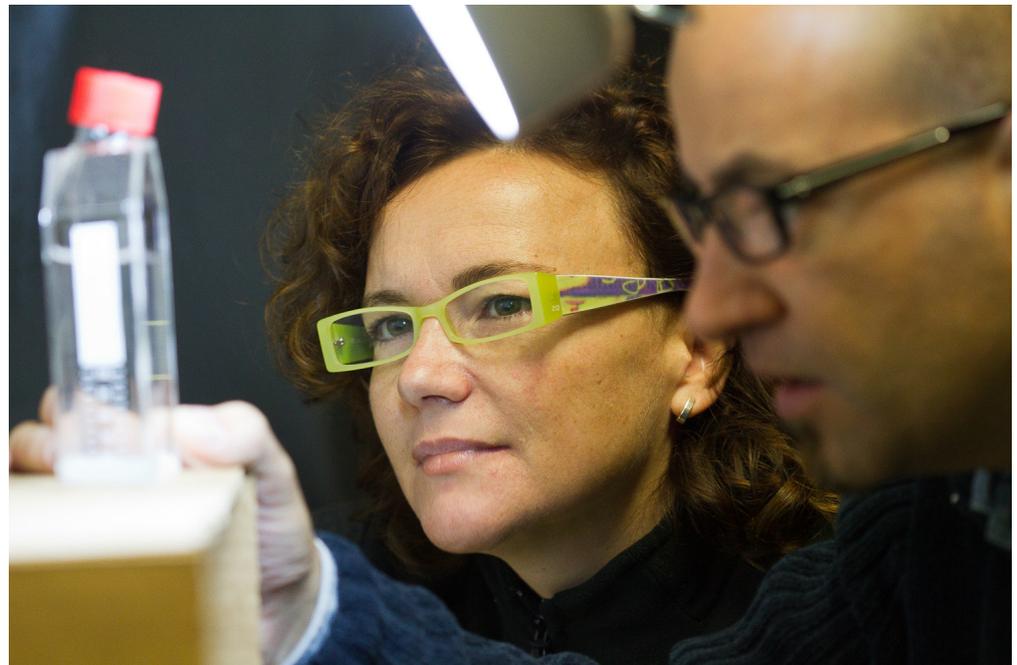
### Successful fertilization experiments

In a first fertilization experiment, we used thawed cryopreserved and diluted fresh sperm from several males and egg batches from two females. Procedures included determination of fertilization percent and photo documentation to describe development

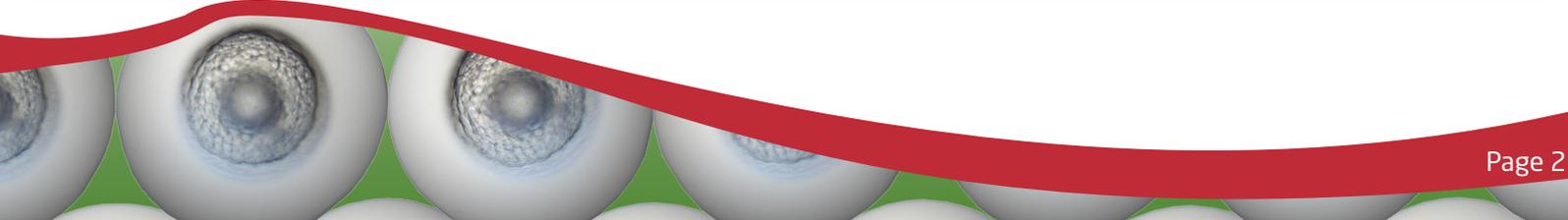
of embryos. Hatching percent was determined from hatch in cell culture bottles. In this experiment, we saw the first European eel eggs fertilized by thawed cryopreserved spermatozoa. A priori, the fertilization rates with thawed sperm were lower than from fresh sperm, however the experiment also identified shortcomings, which can be used to improve the cryopreservation protocol.



Juan F. Asturiano and Luz Pérez (ICTA-UPV) testing quality of sperm used in fertilization experiments of European eel. Photo: Sune Riis Sørensen, DTU Aqua.



Juan F. Asturiano and Luz Pérez (ICTA-UPV) at work counting viable embryos in the fertilization experiments. Photo: Sune Riis Sørensen, DTU Aqua.



## Hatch of viable larvae and prospects for cryopreservation of eel sperm

by Juan F. Asturiano and Luz Pérez, ICTA-UPV, Instituto de Ciencia y Tecnología Animal, Polytechnic University of Valencia, Spain, and Sune Riis Sørensen, DTU, Technical University of Denmark



Embryos developed eggs hatched and for the first time in history larvae were obtained using cryopreserved sperm to fertilise eggs of the European eel. This result opens a range of possibilities exploring application of sperm cryopreservation technology in larval production and experimental work.

Options for application in future offspring production includes sperm storage and transport facilitating timely availability of sperm and eggs in broodstock management, control

of genetic resources in production and potential stocking etc.



Cell culture bottles used for experimental incubation of egg and hatch of larvae in the fertilization experiments. Photo: Juan F. Asturiano.

## Does feed quality affect male reproductive performance?

by Jonna Tomkiewicz and Josianne G. Støttrup, DTU, Technical University of Denmark, Juan F. Asturiano and Luz Pérez, ICTA-UPV, Instituto de Ciencia y Tecnología Animal, Polytechnic University of Valencia, Spain

Experiments also included comparison of reproductive performance of males receiving four diets differing in fatty acid content and composition. The study included comparison of sperm density and motility in stripped semen from males on the different diets. First results indicate that diet affects both sperm density and motility.



The experiments are a first attempt to investigate dietary effects on reproductive performance of male eel. The development of eel broodstock feed is a collaboration among the PRO-EEL partners INRA (France), DTU (Denmark), BioMar (Denmark) and Wageningen University (Holland). Dietary experiments most often focus on female broodstocks and egg quality, however, these preliminary results may identify a potential for enhancing eel sperm quality through enhanced male broodstock culture.

## Record-breaking larval longevity and micro-injection of nutrients in feeding trials

by Svend Jørgen Steinfeldt, Ivar Lund, Sune Riis Sørensen, Peter Munk and Jonna Tomkiewicz, DTU, Technical University of Denmark, and Peter Lauesen, BA, Billund Aquaculture Service

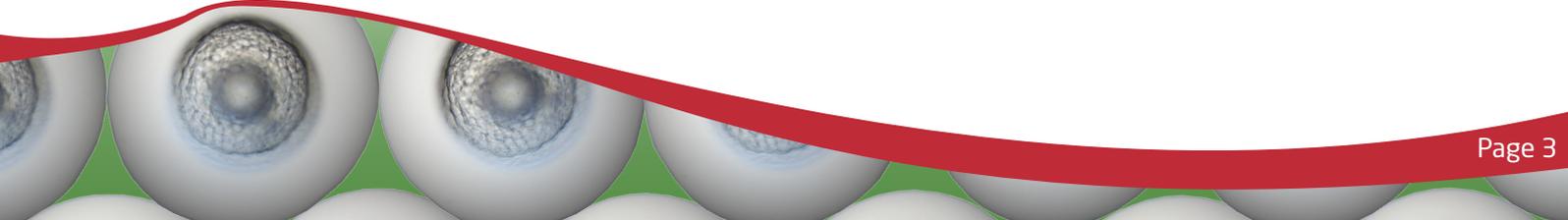
During the experimental series at Lykssvad, a batch of eggs were fertilised for larval production in a pilot study. The larvae were reared under different culture conditions at three locations including stagnant culture conditions, flow through systems and slightly aerated large volume tanks. All systems facilitated survival through the yolk sac stage with larval survival ranging from 21 to 25 days post hatch.

In particular the flow through system enabled high relative survival and allowed small scale feeding trials using formulated inert feeds. Also first trial on microinjection of specific nutrients into the gut of anesthetized

larvae was performed. Such complicated studies may add important information to our understanding of larval nutrient assimilation capabilities and larval feed requirements. The tests were quite successful in terms of larval tolerance to micro-injection and larval recovery after anaesthesia.

The next step will be to micro inject radio labelled amino acids and quantify larval assimilation of these to determine if the larval gut has the ability to utilize nutrients present in the gut.

Photo showing a 14 day old European eel larvae being feed using a micro-injection probe. Photo: Svend Jørgen Steinfeldt, DTU Aqua.



## 4<sup>th</sup> scientific steering meeting held in Brussels

Jonna Tomkiewicz and Sune Riis Sørensen, DTU, Technical University of Denmark

The PRO-EEL Steering Committee met 9<sup>th</sup> of November in Brussels at CreoDK. The Steering Committee includes representatives of the 15 project partners and in addition the project officer Dr. Stamatis Varsamos from the European Commission attended the meeting. Topics included future participation in conferences, workshops and organization of PRO-EEL meetings and events. Activities of the PRO-EEL team in 2012 of interest to the scientific and stakeholder networks:

- PRO-EEL workshop in Valencia, 27 March 2012
- Participation in the 6th World Fisheries Congress, Edinburgh 7 - 11 May 2012
- Aqua 2012, 1 - 5 September, Prague, Czech Republic



Participants in the 4<sup>th</sup> Scientific Steering Committee meeting in Brussels 9<sup>th</sup> November 2011. Photo: Sune Riis Sørensen, DTU Aqua.

## PRO-EEL workshop for stakeholders and scientists interested in eel research, Valencia, 27 March 2012

by Juan F. Asturiano

ICTA-UPV, Instituto de Ciencia y Tecnología Animal, Polytechnic University of Valencia, Spain.

A PRO-EEL project meeting is planned to take place in Valencia (Spain) from 27<sup>th</sup> to 30<sup>th</sup> of March 2012. The meeting includes an open day on March 27<sup>th</sup> for the PRO-EEL network and eel researchers in general. The meeting and workshop is hosted by Juan F. Asturiano, ICTA-UPV, Polytechnic University of Valencia, Spain.

The workshop will be opened by the local hosts and a presentation of the prospects of eel aquaculture in Spain. An overview over progress of the

PRO-EEL project will be presented by the scientific coordinator, Jonna Tomkiewicz, DTU Aqua. In following sessions project workpackage leaders will highlight the research achievements and main results of their WPs. One session will be dedicated to presentations by representatives of relevant EU research projects and network participants. The day ends with a poster session open to all PRO-EEL participants and network members providing the opportunity to show additional results and information.



Program and more detailed information about the workshop will become available in January.



*reproduction of european eel - towards a selfsustained aquaculture*

**PRO-EEL 4<sup>th</sup> NEWSLETTER**  
**DECEMBER 2011**



*All of us in PRO-EEL  
wish you*

**MERRY  
CHRISTMAS  
AND  
HAPPY NEW YEAR**

