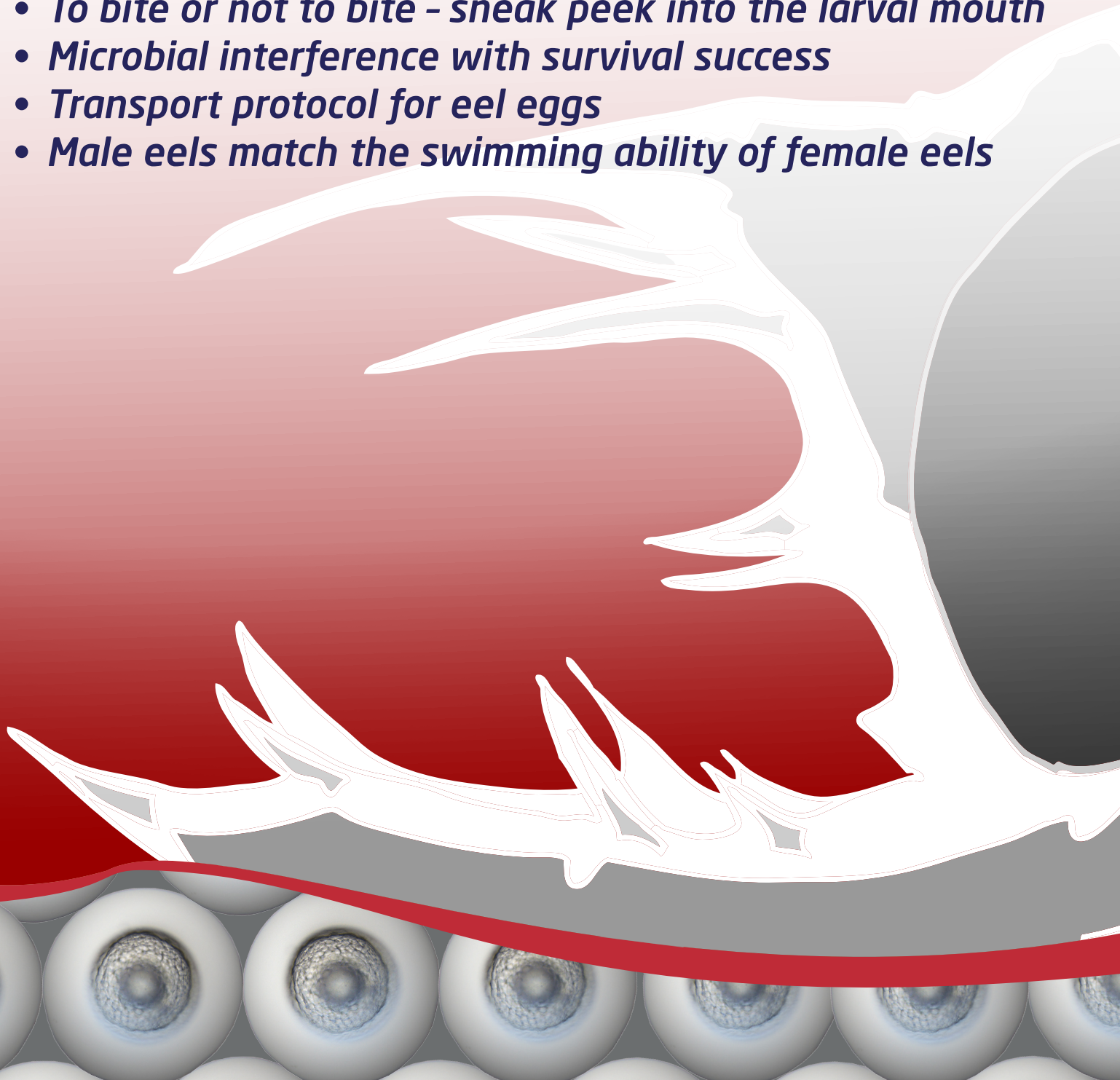


Contents

- ***A leap towards captive breeding of European eel***
- ***Events and project activities***
- ***Unique muscle development in eel larvae***
- ***To bite or not to bite - sneak peek into the larval mouth***
- ***Microbial interference with survival success***
- ***Transport protocol for eel eggs***
- ***Male eels match the swimming ability of female eels***



A leap towards captive breeding of European eel

by Project Coordinator, Jonna Tomkiewicz, Technical University of Denmark

PRO-EEL set out three years ago with the aim to enhance the knowledge base on European eel reproduction and develop the technology needed to produce viable offspring and rear larvae to the first feeding stage.

Project participants strive to meet these objectives, covering basic research, application development and establishment of production technology. Their research addresses hormonal regulation of reproduction, optimisation of broodstock diets for farmed broodstock, enhancement of broodstock fertility via assisted reproductive technology, standardisation of fertilisation protocols, and establishment of larval culture techniques.

A wide range of methodologies have been applied to reach these goals and not least developed molecular tools have enhanced knowledge about eel reproductive responsiveness and lar-



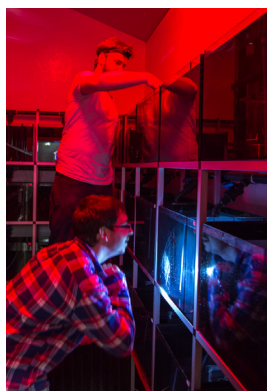
European eel larva 14 days post hatch in feeding experiments. The body is transparent and 8 mm long. Photo Sune Riis Sørensen

val physiology.

During this 3-year period, our knowledge about European eel has significantly increased and application development has progressed with focus on overcoming bottlenecks in breed-

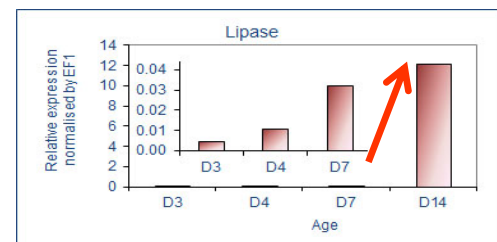
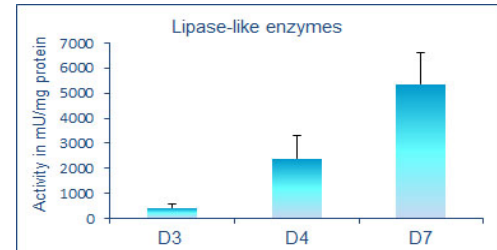
ing technology of this species. Thus, studies on the hormonal regulation under different conditions that receive particular attention due to the inhibition of maturation in eels in our waters, have provided new insight into the mechanisms responsible for reproductive responsiveness and success. This includes among other, first evidence of a regulatory hormonal mechanism, i.e. a kisspeptin system in eel, which may play a direct inhibitory role on pituitary expression. Such understanding of the eel hormonal regulation is of great value to future development of novel treatments for better maturation success.

In an application context, enhanced broodstock diets to improve egg and larval quality have been developed. Experiments comparing wild female silver eels and farmed female broodstocks on enhanced diets have shown that the fatty acid profile of farmed female eel can be altered, provided a long feeding period, and that such enhanced diets can improve fertilisation and hatch success of farmed broodstock. Improvement of assisted



Established larval culture systems for rearing of yolk sac larvae. Photo Sune Riis Sørensen

reproduction technology and standardised fertilisation methods has enabled regular mass production of viable eggs and larvae, allowing experimental work



Results on biochemical, histological and molecular study of digestive tract development in European eel larvae prior to exogenous feeding won best poster award at AE2013 (Mazurais et al.). This included analyses of digestive enzyme activity and gene expression, showing very high activities in lipase-like enzymes after hatching and similar development in expression of the enzymes' corresponding coding gene.

on larval culture technology. This includes studies of temperature, light, and microbial control in rearing systems, providing first definitions of parameters for inclusion in larval rearing protocols. Latest progress include, successful culture yolk sac larvae to the feeding stage, first feeding trials and pioneering work on the morphology of the feeding apparatus and digestive system to gain insight into the digestive capabilities of European eel larvae.

These advances in larval culture are particular focus of this Newsletter.

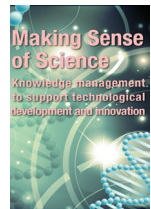
EVENTS AND PROJECT ACTIVITIES

LARVI2013 GHENT, BELGIUM

The 6th Fish and Shellfish Larviculture Symposium was held September 2nd to 5th 2013 in Ghent, Belgium, organised by the Laboratory of Aquaculture & Artemia Reference Center, Ghent University. The conference addressed larviculture challenges, considering a large variety of research fields, including broodstock management, maturation and spawning, larval development and deformities, larval nutrition, larviculture at commercial scale, and microbial management. The PRO-EEL project was represented by a talk entitled "Reproduction of European eel and larval culture: State of the art" by the coordinator Jonna Tomkiewicz, who overviewed PRO-EEL research progress with focus on captive offspring production and larvi-culture. In addition, Mathias Bouilliart captured the audience with his intriguing research on functional morphology of the feeding apparatus of European eel larvae. Additionally, posters presented PRO-EEL results at the conference. The conference provided good opportunities for discussions on larval rearing technology, including eel!



Attendees at the 6th International Fish & Shellfish Larviculture Symposium - larvi'13 held 2-5 September 2013 in Ghent, Belgium. Photo: Laboratory of Aquaculture & ARC, Ghent University



AE2013 TRONDHEIM, NORWAY

Aquaculture Europe 9-12th August 2013 organised by the European Aquaculture Society (EAS) was held at the Norwegian University of Science and Technology (NTNU) in Trondheim. The conference featured a dedicated session on eel research chaired by Jonna Tomkiewicz, DTU, Denmark and Peter De Schryver, Ghent University, Belgium. This session, hosted by PRO-EEL, focused on recent advances in captive breeding of European eel. Eight presentations and three posters covered a broad range of project results such as female fecundity, improvement of broodstock diets and egg quality, optimised fertilisation protocols, microbial interference in egg and larvae culture, rearing techniques, and new insight into the larval mouth structure and feeding capability. This recent progress in breeding technology development brought attention to eel as a potential "new species" in aquaculture.



PRO-EEL contributors to the AE2013 session: *Eel Focus - Aquaculture Development*, held in Trondheim, 9-12th August 2013

PRO-EEL COLLABORATIVE WORKSHOP

The 1st PRO-EEL Collaborative Workshop was held 27th of March at the Polytechnic University of Valencia in Spain. Project presentations summarised ongoing research and an open poster session framed the workshop. About 100 motivated attendees including project participants, interested scientist and stakeholders contributed to inspiring discussions of results and future synergetic research. The Workshop was organised by Grupo de Acuicultura Y Biodiversidad (GAB), Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de Valencia (ICTA UPV) and the Technical University of Denmark (DTU). Billund Aquaculture Service, BioMar, GAB and DTU sponsored the Workshop.



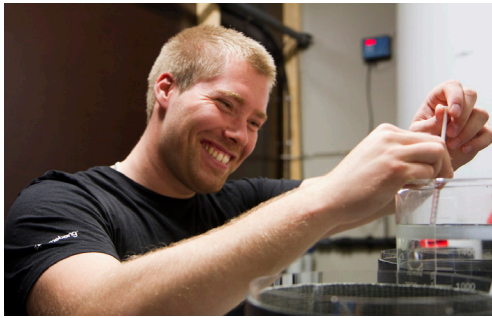
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PRO-EEL team at the 1st PRO-EEL Collaborative Workshop held in Valencia 27th March 2012

Axial muscle development in yolk-sac larvae of eels, a preparation for the leptocephalus transition?

by Martin Davidsen, Norwegian University of Science and Technology, Norway, Trine Galloway, SINTEF Fisheries and Aquaculture, Norway, and Elin Kjørsvik, Norwegian University of Science and Technology, Norway

In European eel yolk-sac larvae, muscle morphology and onset of muscle differentiation follow a unique pattern. This became clear from an MSc study performed by Martin Davidsen, NTNU, as an integrated part of PRO-EEL experiments on larval development. Martin sampled larvae for histological studies of the larval muscle development from they hatch to 6



Martin Davidsen, NTNU, working on his study at DTU's experimental facility, Lykssvad, in Denmark. Photo: Sune Riis Sørensen, DTU Aqua days after hatch. The study indicates that eel larvae from quite early on prepare for the rather special larval development stage characteristic for the eel genus, called the leptocephalus larva, in which the larva grow into an elongate, flattened leaf-like larva. The European eel larva is around 3

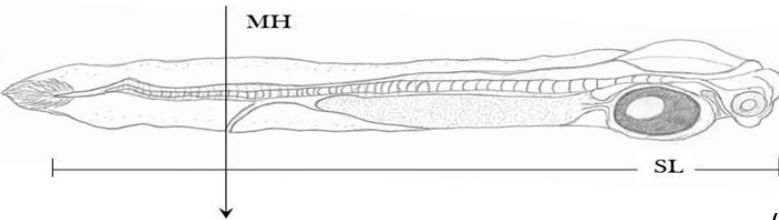


Figure 1. Schematic drawing of a European eel larvae three days after hatch, indicating start and end points for standard length (SL) measurements and the reference point behind the anus, where histological transverse sections were cut (arrow MH) for studies of muscle development. Drawing by Martin Davidsen, NTNU.

mm (SL) long at hatch and shows several layers of undifferentiated precursor cells destined to become muscles. As the larva grow, the muscle mass behind the anus (see Fig. 1) becomes more developed, with multinucleated muscle cells containing myofilaments organised into bundles of myofibrils. The early stage undifferentiated cells developed into regularly arranged muscle cells, and at Day 3 it was possible to distinguish between red and white muscle fibers. Muscle growth was observed both in numbers and size of fibers, and from hatch to Day 6, the larvae grew to double length (SL ~see Fig. 1).

The European eel larvae show a peculiar arrangement likely unique for this species. The elongated muscle fibers are stacked along the sides as seen in the transverse section of a larva below (Fig. 2a) and the absence of horizontal septum (see Fig. 3) and the stratified orientation, seems to be unique for eel larvae. We see this unique muscle fiber orientation as a step in the transformation towards the peculiar leaf-like leptocephalus larvae, where muscle

cells form a thin layer outside a jelly-like inner mass, consisting of glycosaminoglycans (see Figure 2b). This stage is strikingly different from the develop-

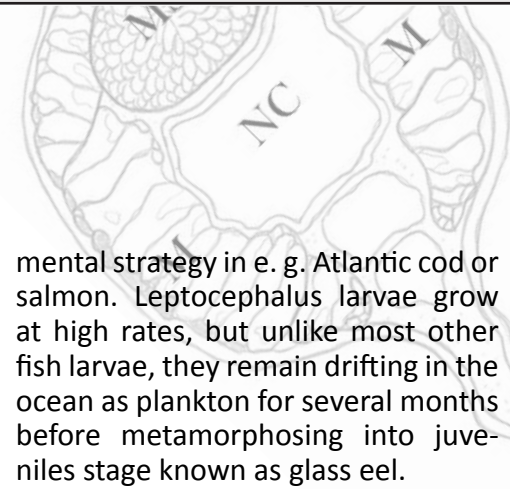


Figure 2. Schematic drawings showing transverse sections of (a) an 6 days old European eel larva at yolk-sac stage (6 mm SL), and (b) Japanese eel leptocephalus (20-24 mm SL). a. M, axial muscle; NC, notochord; MS, medulla spinalis; SN, spinal nerve; D, dermis. Illustrations by Martin Davidsen, NTNU.

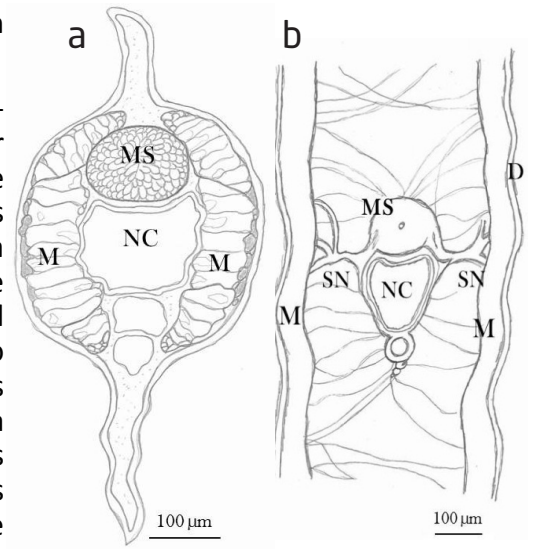


Figure 3. Cross section of generalised fish larvae with indication of vertical and horizontal septa.

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To bite or not to bite?

by Mathias Bouilliart and Dominique Adriaens
University of Ghent,

The European eel larva's mouth parts in the early preleptocephalus stage are peculiar, in particular the long, forward pointing teeth. Their needle-like form and unique position not only question the efficiency of this feeding apparatus, but make researchers wonder what their function in feeding actually is.

In order to uncover the true functionality of this feeding apparatus, two additional aspects must be taken into account, namely how these creatures are able to control the up- and downward movements of their lower jaw and, additionally, how big

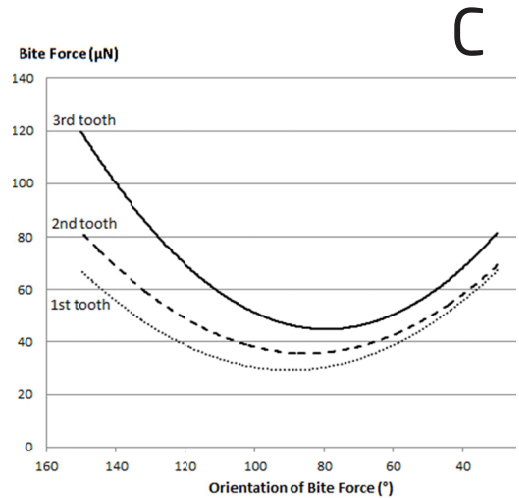
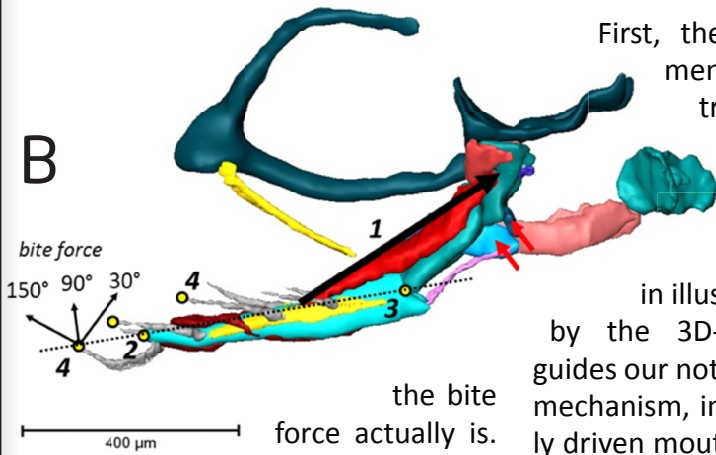
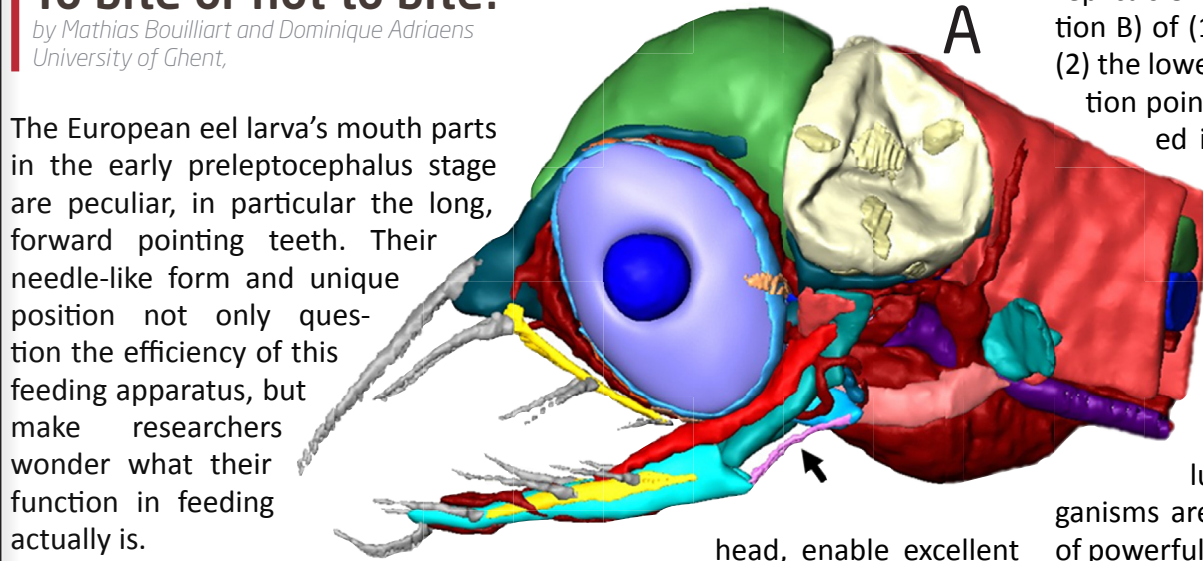
the bite force actually is. Given the larvae's extremely small size (the head region is less than 1mm in length), performing any type of measurement directly on the organism itself, is out of question. Modern 3D-reconstruction, however, using histological sections of the

head, enable excellent 3-D replica (see illustration A). Such up-scaled replica allows accurate visualization of the musculoskeletal anatomy that includes tiny and slender structures as branchial elements and ligaments, and enables us a sneak peek into the mouth cavity.

First, the existence of a ligament (black arrow in illustration A) between the most posterior end of the lower jaw and the similar position on the hyoid arch (red arrows in illustration B) was revealed by the 3D-replica. Its presence guides our notion of an active feeding mechanism, involving a ligamentously driven mouth opening mechanism, in which an initial, muscle controlled, depression of the hyoid arch is translated into a simultaneous depression of the lower jaw.

Second, since only one muscle is responsible for the subsequent closing of the jaw. The hereby generated bite force can be estimated. Based on the

replica's 3D-coordinates (see illustration B) of (1) the jaw closing muscle, (2) the lower jaw, (3) the jaw articulation point and (4) the teeth inserted in this jaw, together with an estimation of the muscle's force based on its reconstructed volume and mean fiber length, a mean bite force of approximately 4×10^{-5} N (see graph C) is estimated. The latter illustrates that these tiny organisms are, anatomically, incapable of powerful biting ... an information that may help us understanding how these larvae can deal with food, both in a natural and artificial environment.



Graphical illustrations by Mathias Bouilliart, Ugent:
A) 3D-reconstruction of the head region of a 15dpf larva

B) Same reconstruction with indication of (i) the input-factors required to calculate the bite force and (ii) the obtained output

C) Graphical representation of the obtained bite forces at the first, second and third tooth at different angles.

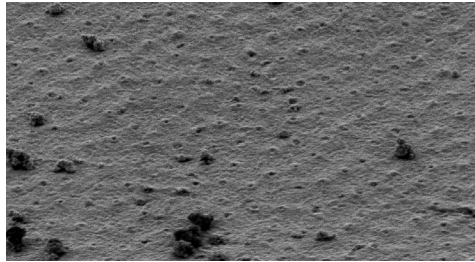
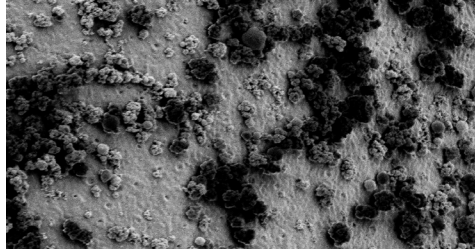
Microbial load interferes with the hatch and survival succes

by Peter de Schryver, University of Ghent, Belgium. and Sune Riis Sørensen, Technical University of Denmark

Ongoing work to improve water quality management has taken a leap towards establishment of suited culture conditions for eggs and larvae. Results obtained in a recent series of PRO-EEL experiments, showed severe impact of microbial infection on incubation and rearing success of eggs and yolk sac larvae. Therefore, it is of great importance to control



Cell culture bottles used in experimental incubation of eggs and hatch of larvae in microbial interference studies. Photo: Sune Riis Sørensen, DTU Aqua



Scanning Electron Microscopy (SEM) of the surface of an egg (magnified 5000X). The upper photo shows an egg with noticeable larger bacterial load than the lower picture. Surface area coverage by bacterial colonies, affecting survival and hatch success, is used to quantify bacterial load.

Photo: Peter W. Skov, DTU Aqua

the microbial community. This work provides suggestions for egg disinfection and microbial control. The final series of PRO-EEL experiments will follow up results, addressing in particular microbial interference and water quality management in feeding larvae rearing culture.



Studies like this including sampling and replicates demand a lot of effort, counting and distributing eggs to bottles. Here, Peter de Schryver, UGent, inspects bottles during experiments at DTU's research facility Lyksvad in Denmark.

Photo: Sune Riis Sørensen, DTU Aqua

Successful protocol for transport of egg

by Sune Riis Sørensen, Technical University of Denmark

Peter de Schryver, University of Ghent, Belgium.

Peter Lauesen, Billund Aquaculture Service, Denmark

Terje van den Meerem, Institute for Marine Research, Austevoll, Norway

An aim of PRO-EEL was to establish a transport protocol to distribute fertilised eggs from the DTU research facility to partners abroad, testing specific larval culture conditions, feeds etc. Transport of viable eggs is common practice in aquaculture, but sensitivity of developing eel eggs to transport conditions was unknown. The tested and established protocol uses 3 L water-filled, zip-lock plastic bags topped with one third of oxygen. A monitoring program, recording survival of embryos and hatch of larvae, documented that survival and hatch rates transported batches equalled controls maintained at the

DTU facility. Transportation times up to 12 hours were applicable, using airplane, car, train and/or ferry. Such protocols allow unbiased, parallel experiments on rearing conditions at different places using offspring from the same female.



Resistance to transport is limited early in the embryonic development. However, 25 hours after fertilisation, successful shipment is feasible. Photo: Sune Riis Sørensen, DTU Aqua



Water-filled zip lock bags topped with oxygen and egg densities around 2000 eggs per litre proved successful. Photo: Sune Riis Sørensen, DTU Aqua

Swimming capacity of male eels similar to that of female eels

by Guido van den Thillart, Leiden University Netherlands

In fall, migrating European eels start their approx. 6000 km journey to their spawning area in the Sargasso Sea, which must be reached within about 6 months to enable spawning in the following spring. When the silver eels leave the European coasts, they are in an immature state, and migration is a potential trigger of sexual maturation. There are indications of swimming having such an effect on both male and female silver eels (Palstra & van den Thillart, 2010).

Previous studies on the swimming capacity of female eels showed that females are very efficient swimmers and able to swim the required distance within 4 months. Controlled swimming experiments with male eels were thus far not conducted.

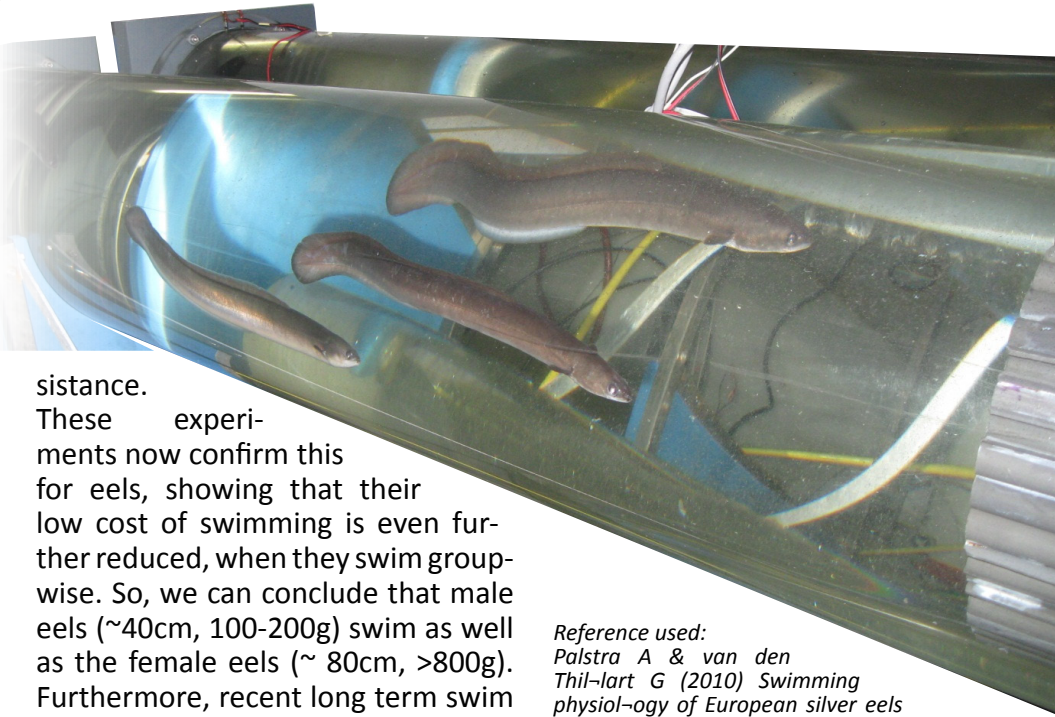
Knowledge on swimming capacity is, however required before long term swim trials can be carried out. According to accepted scaling relationships for all animals (Schmidt-Nielsen), energy cost of transport decreases with increasing body size. Therefore, the much smaller male eels were expected to have a lower optimal swimming speed and a higher cost of transport than female eels. So, this raised doubt whether male eels would be able to reach the Sargasso Sea within 6 months.

We have now estimated the swimming capacity of male silver eels, swimming individually as well as in groups (Fig. 1). The results were remarkable (Fig. 2): We found that male eels are not only very efficient swimmers but they can swim at least as fast as the females. Furthermore they swim at a similar energy cost

of transport as the females, thereby contrasting the scaling theory of Schmidt-Nielsen. Group swimming is known to reduce the energy cost of transport due to lower re-

sistance. These experiments now confirm this for eels, showing that their low cost of swimming is even further reduced, when they swim group-wise. So, we can conclude that male eels (~40cm, 100-200g) swim as well as the female eels (~80cm, >800g). Furthermore, recent long term swim trial shows that male silver eels swim at an optimal speed of 0.5 m/s.

Figure 1. Swimming tunnel with male silver eels. Farmed male silver eels were swum in swimming tunnels at different speeds (0-1 m/s). Both individually and in groups, they can swim for months continuously when kept in a quiet, dark environment.



Reference used:
Palstra A & van den Thillart G (2010) Swimming physiology of European silver eels (*Anguilla anguilla* L.): energetic costs and effects on sexual maturation and reproduction. *Fish Physiol Biochem* (2010) 36:297-322

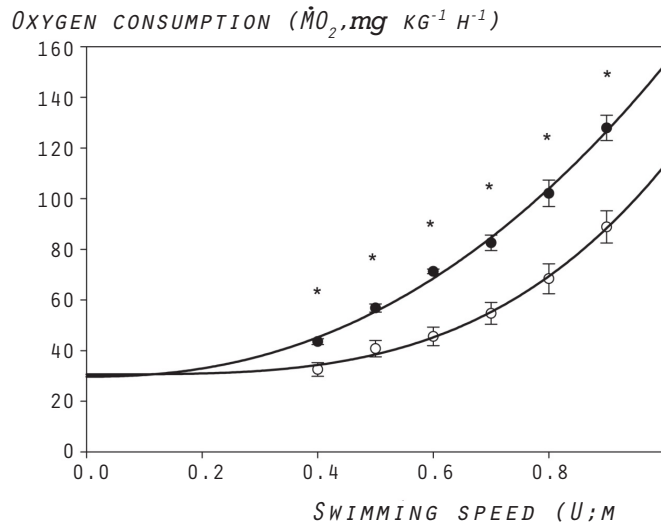


Figure 2. Swimming capacity of male silver eels - individually versus group-wise.

Figure 2. Swimming capacity of male silver eels - individually versus group-wise.

Oxygen consumption ($\dot{M}O_2$, $mg\ kg^{-1}\ h^{-1}$) as a function of swimming speed (U ; $m\ s^{-1}$); the curve is fitted to the formula = $SMR+aUb$.

Eels were swum individually (closed circles, $n=7$) and group-wise ($n=7$, 7 males per group, open circles). Significant differences, individuals versus groups, are indicated by asterisks (*); Mann-Whitney U test, $n=7$, $p<0.05$.



**PRO-EEL wishes
you A MERRY CHRISTMAS
AND A HAPPY
NEW YEAR**

*look forward to
our April Newsletter
presenting news from
experiments in
February-March*

*stay updated at
www.pro-eel.eu*

