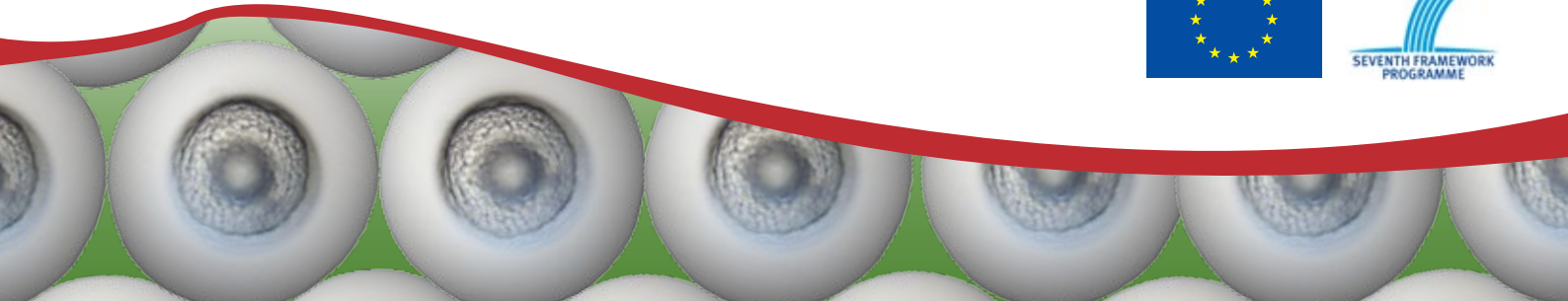
PRO-EEL ACHIEVEMENTS AND HIGHLIGHTS

The past year featured successful experiments, sampling and analyses enabling targeted work on the project objectives. This newsletter provides information about recent achievements and highlights. PRO-EEL researchers will present results at the upcoming Aquaculture Europe Conference in Rhodes, Greece. Meet PRO-EEL participants at AE2011!

PRO-EEL 3rd NEWSLETTER

October 2011

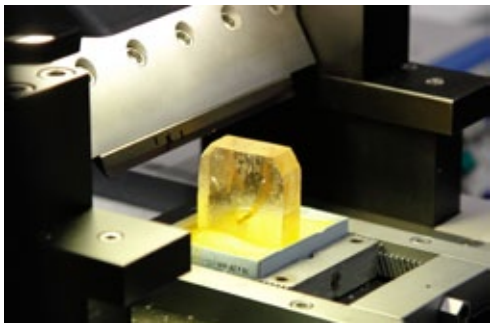


Bits of eel ontogeny

by Professor Dr. Dominique Adriaens
Evolutionary Morphology of Vertebrates & Zoology Museum., University of Gent,
Email: dominique.adriaens@UGent.be

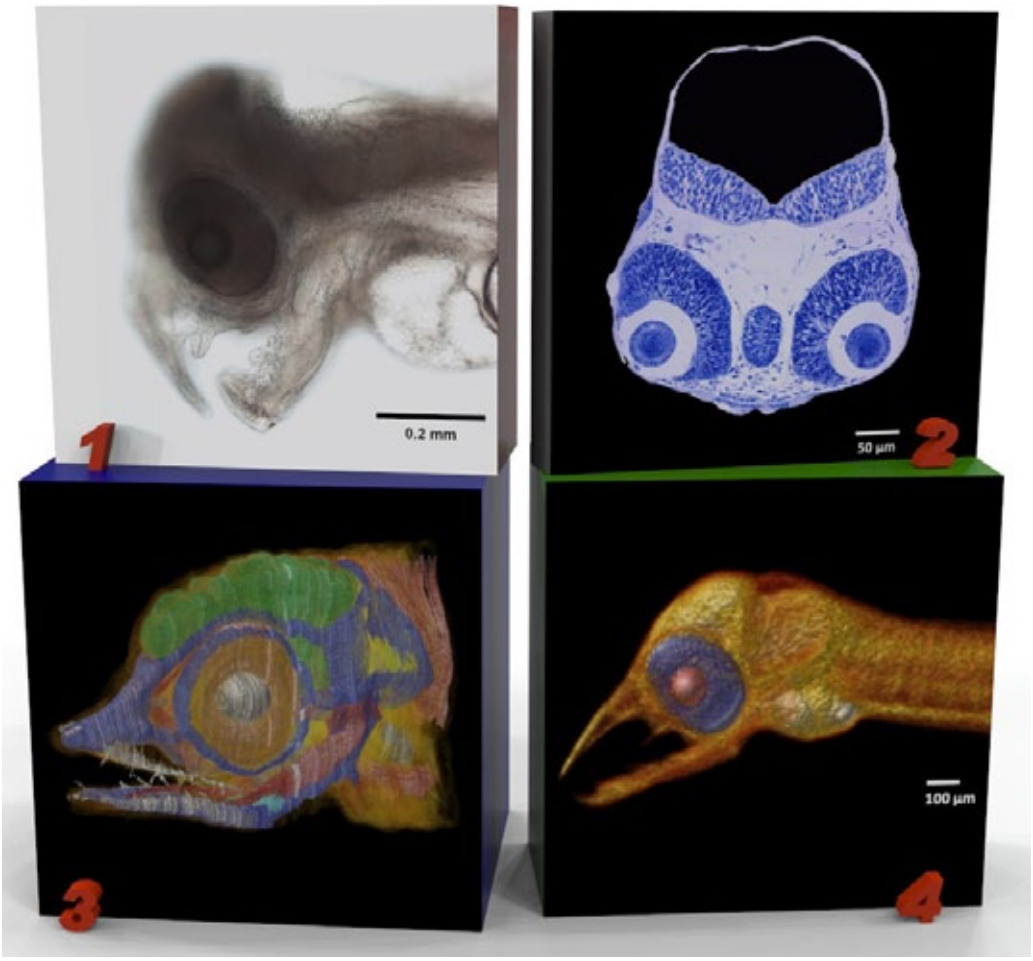
As interesting as the understanding of how larval European eel deals with food may be, as challenging it is to obtain relevant information on the feeding apparatus in fish as small as just a few millimeters. Still, looking at the early preleptocephalus larvae of European eel, it is quite astonishing how the head, including the feeding apparatus, becomes modified into a fierce-looking face of a killer fish, equipped with fang-like teeth as seen here!

Understanding how these larvae can use these teeth and their jaws to grab and manipulate food items, requires a proper understanding of the 3D-topography and physical interactions of especially muscles and skeletal elements within the head. This can be achieved by generating series of hundreds of 1 µm thick histological sections of of the complete head.



The slicing of eel larvae embedded in resin. Photo by Sune Riis Sørensen DTU

As such, any distinction can be made between all structures, comprised of all kinds of different tissues (dermal versus chondral bones, muscles, tendons, ligaments, joints, cartilages, etc.), and how they act upon each other (see picture 2 on figure shown here). When putting all these together again, a 3D computer model of all relevant components of the feeding apparatus can be reconstructed (see picture 3). Even current skills in micro-CT-scanning with counter staining allow 3D visualization of something as small as these larvae (see picture 4). This will ultimately allow an evaluation of changes in functional performance of active feeding in eel larvae as they switch from yolk sac feeding to feeding on prey items … something crucial both in the wild and in rearing tanks.



Professor Dominique Adriaens at University of Gent is sectioning larvae for morphological studies and as can be seen on the steps below and described in the text, he is sectioning larvae head in 1 micro meter thick (0.001 mm) slices and combine these again in a virtual copy in 3D. Figure by Dominique Adriaens, Ugent

Provisional results from Persistent Organic Contaminant (POPs) analysis in female, spawning eels.

by Dr. Michiel Kotterman, IMARES, Wageningen UR (University & Research centre)
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Silver eels, previously used in successful spawning experiments in 2010 at DTU were analysed at IMARES. Eight individual eels, including good spawners and poor spawners, were analysed for lipid and POP content. Eggs retrieved from these eels were also analysed for lipid and POP content. This experiment gave insight in POP levels in the eels and maternal transfer from mother to egg.

A small amount of lipid, normally enough for analysis of POPs in eels, was taken into analysis. As these silver eels under induced maturation do not feed for nearly half a year, lipid metabolism could have resulted in high POP levels. However, POP levels were, compared to other data of European eels, extremely low.

Only a few PCB congeners could be quantified properly in the eels and eggs (one egg sample was even consistently below quantification limit). The average of PCB153, an indicator PCB which is almost always present in the highest concentrations in biota, was 10 micrograms per kg eel. This is a very low concentration and other POPs were not even detected in this analysis, showing that the lake the eels originate from is indeed very clean.

When extrapolating PCB levels to total-TEQ (Dutch studies show a fair correlation between PCB levels and total-TEQ levels), this may indicate that total-TEQ levels in these eels could be as low as 1 ng/kg. These levels are lower than any effect of TEQ observed on reproduction in fish species in proper dose-effect studies.

PCB levels did vary between individuals, without any correlation with good or bad spawning results but given the overall low levels found, this was as would be expected.

We also noted relatively high lipid contents which was not expected in these non-feeding eels. This also resulted in low POP levels in relation to lipid weight. The initial lipid content of these eels is not known, but the high levels observed i.e. from 21 to 33.5 %, and on average of 28% indicate a very high initial lipid. As these eels had not swum 6000 km to the Sargasso Sea, this high residual lipid percentage could have served as the energy source for the long migration.

The amount of PCBs in the eggs was correlated with the amount in the flesh of the mother. When expressed on lipid base the relation between PCB congener in the flesh and in the eggs was good.

Exceptionally clean eels used as wild broodstock in DTU Aqua experiments originate from Lake Vandet in northern Jutland,

The first movies on eel larvae ready for feeding

by Prof. Peter Munk, PRO-EEL
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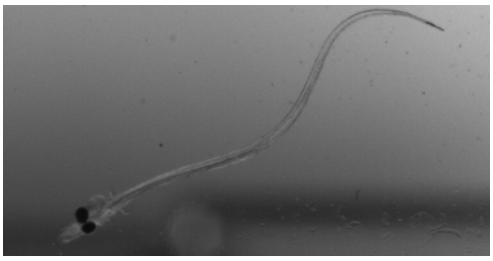
During the second full scale experimental series B at DTU, we succeeded in keeping reasonable numbers of larvae alive until the end of their yolk sac stage. We could therefore investigate their more advanced behavior at this stage when they change from quite passive vertical positions to higher activity and horizontal "eel-like" swimming.

A series of visual observations of behavior were made directly in rearing tanks of 10 liters, where they swam around (relaxed!?) using slow undulations of the body. First attempts were made to define and register behavioral elements. The larvae were inspecting the walls of the tank, while they unfortunately didn't go for the different kinds of inert food they were offered.

Larval behavior was further investigated in a smaller container of 0.1 liter, using a high speed video camera to resolve details of their behavior. Some "shots" of larval swimming were obtained, but when the eel larvae were transferred to this smaller chamber, they changed to a frantic behavior "sticking" to the bottom of the container. This made it difficult to make good registrations. Hence, coming studies will apply a much wider range of chamber sizes and illumination possibilities, in order to find the right combination where both the larvae and the researcher are satisfied.



European eel larva 12 day post hatch able to make frantic burst to the bottom. Photo by Peter Munk, DTU

Longer spermiation time, higher sperm volume, better quality

by Professor Juan F. Asturiano and Luz Perez, ICTA-UPV, Instituto de Ciencia y Tecnología Animal, Polytechnic University of Valencia, Spain.
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Sperm obtained in production and quality experiments conducted by Juan F. Asturiano by research group at the Polytechnical University of Valencia.

Experiments conducted at Polytechnic University of Valencia ICTA-UPV revieled that 20 °C is the best temperature for successfully inducing maturation in eel males. Now new hormonal treatments to induce male maturation have been assayed at the Polytechnic University of Valencia (Spain), obtaining nice results in terms of sperm production: longer spermiation period, higher sperm volume, higher spermatozoa density and better quality (spermatozoa movement capacity) in comparison with previously used treatments.

Fertilization assays are the next step. Coming experiments will check the fertilization capacity of eel sperm in fresh vs. after cryopreservation. The use of frozen sperm could guarantee the synchronization of gamete production, independent of the female spawning time.

First demonstration of an inhibitory action of kisspeptin on reproduction in eels

by Dr. Sylvie Dufour and her team
Centre National de la Recherche Scientifique. Paris National Natural History Museum
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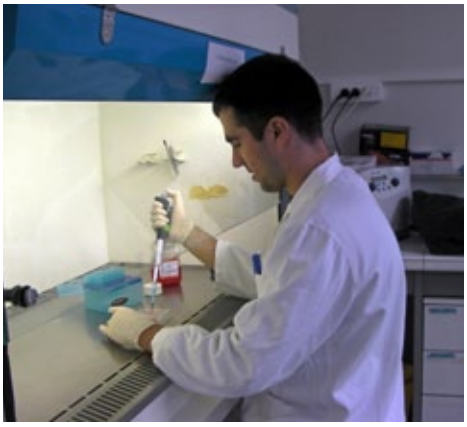
Kisspeptin is a peptide, discovered first for its anti-metastasic function, then later highlighted for its potential key-role in brain stimulatory control of puberty in mammals. Puberty is a crucial step in the eel, since sexual maturation is arrested at a prebubertal stage as long as the oceanic reproductive migration is not achieved. This blockade results from a too low production of pituitary hormones, gonadotropins (LH and FSH). Current protocols for inducing female eel maturation are therefore based on treatments with pituitary extract, which provides the eel with necessary exogenous hormones. The CNRS partner of the PRO-EEL project is focusing on deciphering the brain mechanisms controlling the blockade of puberty, and recently initiated the study of kisspeptin in the European eel. As a first step, we demonstrated the presence of a kisspeptin system (peptide and its receptor) in the eel. We then looked for kisspeptin effect on pituitary gonadotropin expression, using eel pituitary cell cultures. Unexpectedly, we found that kisspeptin exerts an inhibitory effect on eel gonadotropin (LH) expression. This is the first demonstration of an inhibitory action of kisspeptin on reproduction. This further strengthens the uniqueness of eel regulatory processes likely related to its remarkable life cycle.

Ref: Pasquier et al. General and Comparative Endocrinology, 2011, 173, 216-225.



Research applying eel pituitary cell culture by which CNRS succeeded in demonstrating the existence of a Kisspeptide regulatory system in the eels and now also found an inhibitory effect of this peptide on the maturation process. Photo: Sylvie Dufour, CNRS

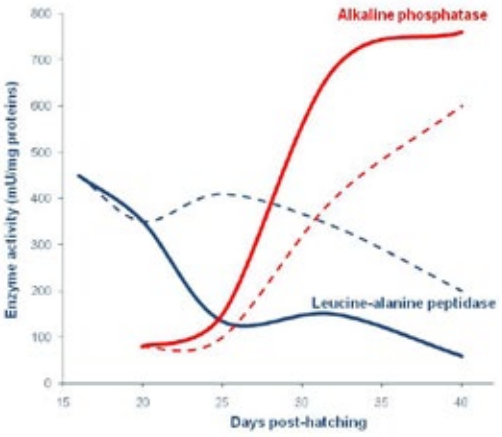
Digestive system of eel larvae explored using biochemical and molecular techniques

by Dr. Jose Zambonino and Dr. David Mazurais,,
IFREMER , French Research Institute for Exploitation of the Sea

The IFREMER group (Brest, France) will study the development of the eel larvae by examining the functionality of the digestive tract using a biochemical (assay of enzyme activities) and a molecular (gene expression) approach.

Indeed, the digestive tract of marine fish larvae undergoes a maturation process during which they progressively switch from a larval mode of digestion to an adult mode of digestion. This process can be positively or negatively influenced by many environmental or nutritional parameters.

Such knowledge on eel will allow us to identify the influence of the nutritional parameters that will be tested during the project.



The graphs show an example of normal development of intestinal cells (unbroken lines) and effects of an inadequate diet (broken lines) in marine fish larvae. Illustration by Dr. Jose Zambonino IFREMER, France.

PRO-EEL presentations and posters during the coming AE2011 conference

First results and achievements will also be presented at the coming European Aquaculture Conference EA2011, Rhodes 18-21 October 2011 including the following contributions:

Tomkiewicz, J. Reproduction of European eel: Towards a self-sustained aquaculture (PRO-EEL). Oral presentation.

Mazzeo, I., Baeza, R., Gallego, V., Vilchez, M.C., Pérez, L., Asturiano, J.F., 2011. Study of thermal regime effect on fatty acid mobilization in European eel (*Anguilla anguilla*, L.) females during induced sexual maturation. Oral presentation

Peñaranda, D.S., Herranz, V.G., Gallego, V., Mazzeo, I., Carneiro, P.C., Dirks, R.P., van den Thillart, G.E.E.J.M., Pérez, L., Asturiano, J.F., 2011. Gene expression regulation of steroidogenic enzymes in European eel (*Anguilla anguilla*, L.) males during induced sexual maturation under three thermal regimes, and relationship with sperm quality parameters. Poster presentation.

Corraze, G., Stoettrup, JG, Holst, L.K., Larroquet, L., Jacobsen, C, Tybjerg, L., Kaushik, S., and Tomkiewicz, J. Modulation of stored lipid reserves through broodstock nutrition and reproductive success in European eel (*Anguilla anguilla*). Poster presentation.

At AE2011 PRO-EEL opens the doors to inform and discuss project activities and experiments

The PRO-EEL team has organised an open house session for all interested conference participants and if you will the around, we hope this newsletter has encouraged you to meet the team and hear more from our progress towards reproduction of eels in captivity.

We will be at the conference center Room B, Friday the 21th of October from 3 to 5 PM.

Further information:

For further information, visit our homepage **www.pro-eel.eu**

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News and events of the project will regularly be updated to inform about progress of the project, and you can join the PRO-EEL networks via the project homepage to obtain our regular Newsletters and further relevant information. Next newsletter will become available in March 2012.

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