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PRO-EEL

Enhancing European eel culture

PRO-EEL

Breeding the European eel

The **PRO-EEL** project is striving towards a self-sustained aquaculture for European eel with fry production under controlled conditions, work which will help to reduce the demand for wild glass eels

The European eel, *Anguilla Anguilla*, is a critically endangered species which was recently placed on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. This species has long been of significant economic value and has served as a delicacy across the Western European seaboard. However, the stock has dramatically declined in recent decades and attempts to maintain a sustainable aquaculture of the eels has been hampered by the reducing recruitment of glass eels. This has led to a call for more effective management of the species and a self-sustained aquaculture, which are indicated goals of the European Commission.

WILD SARGASSO SEA

PRO-EEL, an ambitious European Seventh Framework Programme (FP7) project, seeks to develop standardised protocols for the production of high quality viable eggs and feeding larvae of European eel as a prerequisite for captive breeding. However, progress has been hindered by limited knowledge about their reproduction in the wild.

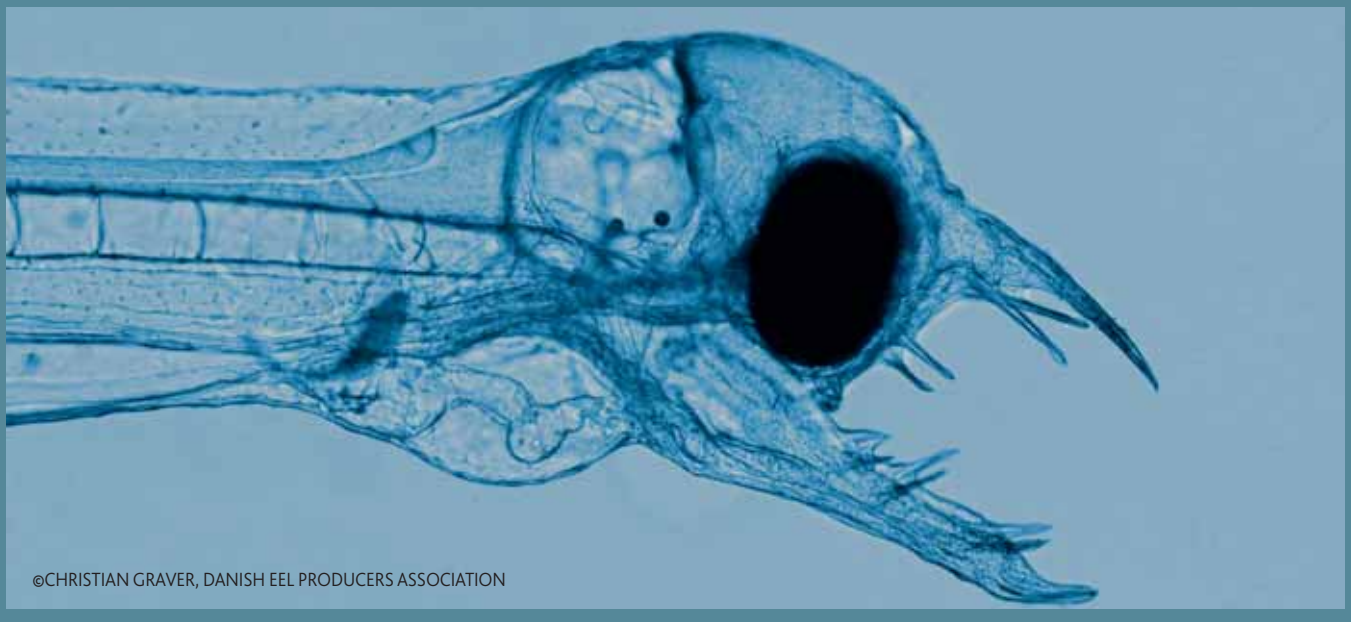
While little is known about the breeding habits of the European eel, it is generally accepted that they spawn in the Sargasso Sea. The larvae then begin a 300 day migration, using ocean currents to drift toward Europe whereupon the larvae transform into a juvenile stage, the glass eel. Reaching freshwater they become elvers, slowly growing into yellow eels. After five to 20 years in freshwater or coastal waters they commence the silvering process at the onset of sexual maturation and become known as silver eels, at which point they begin the migration back to the Sargasso Sea.

During silvering, gonadal development becomes inhibited by complex hormonal control mechanisms. This inhibition needs to be released to continue the gonadal development when the migrating eels approach the spawning area, but the timing and mechanisms responsible for cessation of the inhibition are uncertain.

It is this gradual process of maturation during silvering and migration that researchers have not been able to replicate in captivity for European eel, an obvious impediment to successful aquaculture applications. The PRO-EEL team are now utilising new gene technology and molecular tools to obtain knowledge about the hormonal mechanisms which control maturation. One such tool is ELISA testing, which detects active hormones, another is analysis of gene expression. The information gathered will then be used to develop novel treatments and to test responsiveness to maturation control, providing a basis for regular and predictable production of viable eggs and larvae. This will be facilitated through the development of a code of best practice which will ensure the stable production of high quality eggs and semen to guarantee healthy embryonic development for the sustained production of yolksac larvae.

NEW DEVELOPMENTS

Initial studies into the successful hatching of European eels were conducted in the mid-1980s, with the offspring surviving up to three and a half days. A recent project, managed by the Technical University of Denmark (DTU), produced viable larvae a number of times, with the captive eel living for up to 21 days. This study had a profound effect on the formation of PRO-EEL, as project leader Dr Jonna Tomkiewicz describes: "The results and multidisciplinary approach of the recent DTU projects form the basis of the PRO-EEL project. Internationally leading scientists from different disciplines additionally provide results and complementing expertise which will contribute to establish the knowledge base forming the foundation of the PRO-EEL concept. In collaboration we will develop novel methods to be tested in full scale common experiments, which are essential to the success of our project".



LARVAE OF EUROPEAN EEL 12 DAYS OLD AND READY TO FEED

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DEVELOPING EMBRYOS OF EUROPEAN EELS ABOUT 36 HOURS AFTER FERTILISATION

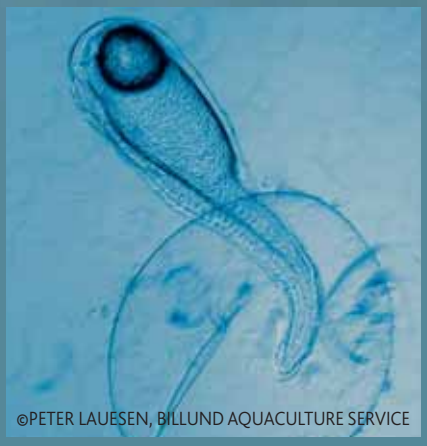
The PRO-EEL consortium thus represents a multidisciplinary approach, with leading experts in eel reproduction complemented by partners filling gaps in knowledge and technologies. In total, the consortium is comprised of 15 partners from European research institutes, industry partners and an international collaboration partner country (ICPC). They believe that the full utilisation of modern technology, research expertise and industry know-how processed by the team will directly translate into a more reliable production of viable egg and larvae, subsequently extending the lifespan of the captive breed eel larvae in feeding cultures. Tomkiewicz believes that this is an early but important development in future eel aquaculture production: "Establishing first feeding cultures of larvae will constitute a key breakthrough. First feeding is a difficult step in aquaculture and besides reproduction it is the key objective of PRO-EEL. Still, the eel larval phase lasts about one year," she continues, "and eel production will require added research on proper feed and culture techniques to ensure larval development and the transformation from larvae to glass eels".

A NOVEL APPROACH

Research with a similar goal, focused on the Japanese eel aquaculture, has already demonstrated the ability to breed eels in captivity through the use of injected hormones extracted from Salmon pituitaries to induce maturation and ovulation, an approach also used in previous reproductive experiments on European eel. However, this methodology is highly problematic as even small deviations in quality, dosage or timing could result in poor egg production reducing the chances of viable embryos.

Tomkiewicz believes that the multidisciplinary and methodical approach adopted by PRO-EEL will enable the team to overcome these issues and provide a sound basis for future treatments: "Gene technology and molecular methods will help obtain knowledge about the hormonal mechanisms of European eel, which can then be used to develop novel treatments, test responsiveness and release maturational control, thereby optimising gamete and larval quality".

HATCHING LARVAE OF EUROPEAN EEL



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CLOSE COLLABORATION

The PRO-EEL initiative integrates industry partners to provide expert knowledge related to eel aquaculture, which, in the long term, may lead to a self-sustained eel aquaculture. Reproduction of European eels has become a particularly important research area due to the decrease of the stock and sharp price increases in the cost of glass eels. This has led to the integration of industrial partners in the PRO-EEL consortium, which is particularly useful as researchers and industry share a common goal and the collaboration brings valuable expertise and knowledge.

PRO-EEL

REPRODUCTION OF EUROPEAN EEL: TOWARDS A SELF-SUSTAINED AQUACULTURE

OBJECTIVES

PRO-EEL aims to: acquire specific knowledge on hormonal control and physiology of European eel reproduction; develop and test new standardised protocols to facilitate stable production of high quality eggs and semen and establish standardised fertilisation procedures to ensure healthy embryonic development; and develop suitable and environmentally friendly larval feeds.

PARTNERS

Technical University of Denmark, DTU, Denmark • **Foundation for Agriculture Research, DLO,** Netherlands • **University of Leiden, LU,** Netherlands • **National Center for Scientific Research, CNRS,** France • **Institute for Animal Science and Technology, Polytechnic University of Valencia, ICTA-UPV,** Spain • **Nofima Akvaforsk – Fiskeriforskning A/S, NOFIMA,** Norway • **Ghent University, UGent,** Belgium • **University of Copenhagen, KU,** Denmark • **National Institute for Agronomic Research, INRA,** France • **Billund Aquaculture Service Aps, BAS,** Denmark • **Wageningen University, WU,** Netherlands • **National Institute of Sciences and Technologies of the Sea, INSTM,** Tunisia • **Institute for Marine Research, IMR,** Norway • **Norwegian University of Science and Technology, NTNU,** Norway • **BioMar A/S , BIOMAR,** Denmark

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DR JONNA TOMKIEWICZ is senior researcher at the Technical University of Denmark (DTU) and originally trained in population ecology. She has participated in several international multidisciplinary projects covering aspects of fish reproduction and life history. She is principal investigator of a series of recent project directed towards the reproduction of eels in captivity.

PRO-EEL

Enhancing European eel culture

European eel are declining in numbers and recent efforts to breed them in captivity challenge their complex hormonal control of the maturation process. **Dr Jonna Tomkiewicz** outlines innovative research work which aims to reproduce this species of eel for future aquaculture

Can you begin by outlining the project's objectives?

The PRO-EEL project aims to increase our knowledge on European eel reproductive physiology and enable successful maturation and offspring production. This includes choosing appropriate broodstocks, enhancing eggs and sperm quality, improving fertilisation methods and developing culture conditions favourable for the production of viable eggs and larvae. A particular issue is the definition of suitable larval rearing conditions and initial feed for the larvae which is crucial for their healthy development and growth.

Many researchers have attempted to reproduce European eels, but none have yet succeeded. Why is this?

In wild eels, the development of ovaries and testes becomes inhibited by complex hormonal control mechanisms at the onset of their long spawning migration. When the eels approach the spawning area in the Sargasso Sea this inhibition must be released, however, the mechanisms responsible for cessation of the inhibition and continuation of the development are uncertain.

Due to this specific inhibition of development prior to migration, the eels do not mature naturally in European waters and in captivity. Therefore the eels receive hormonal treatment to develop gametes and fertilisation is made *in vitro*. This method that has been applied for Japanese eels with promising results, however, seems difficult to apply for European eel.

What major challenges and obstacles have you faced in the project and in what ways have they been addressed, mitigated, or surmounted?

The primary bottleneck in a controlled reproduction of eels concerns deficiencies in knowledge about their reproductive physiology and a challenge will be to enhance methods applied to induce and finalise gamete development. The PRO-EEL team aims at introducing novel methods to meet these challenges, and in particular the insight of the partners CNRS, ICTA-UPV and NOFIMA in hormonal control will be useful in development of maturation protocols with DTU, KU and BAS.

A self-sustained aquaculture builds on a broodstock from farmed production, which implies that the nutritional requirements of the mother fish producing healthy eggs and larvae need attention. In PRO-EEL, fish nutritional specialists at INRA, BIOMAR, DTU and WU cooperate in the development of feed for the eels. Eel from different regions and traditions are basis of a comparative analyses of wild eels and broodstock eels ranging from northern Norway to Tunisia in the south where the ICPC partner INSTM supplements data and information.

Other challenges are the identification of suitable larval rearing conditions and initial feed for the larvae. A particularly critical issue in aquaculture is the step where larvae need to start feeding on their own. Determining suitable feed for the eels and establishing feeding cultures of larvae will be particularly challenging, because little is known about the feed of larvae in nature. This challenge is met by specialists from UGent, IMR, NTNU, INRA, BIOMAR, DTU and BAS.

How significant are the recent results gained from series of projects led by the Technical University of Denmark, in which larvae of European eel were produced and lived for up to 21 days? Can you highlight the project's most significant achievements to date?

During recent years the DTU research team have succeeded in producing viable eggs and culture larvae of captive European eel for up to 21 days. These results were a breakthrough, because they proved that viable offspring of European eel can be obtained through methods to induce maturation and ovulation hormonally and artificial fertilisation using stripped gametes.

During a period of 12 days from hatching, the larvae completed the period, where they rely on the nutrition from the egg and reached the stage where they are ready to start feeding. Repeated production of large amounts of larvae, ie. up to several hundred thousand, is promising; however, improvement of methodology is needed in order to obtain a stable and reliable production of offspring.

These results were obtained in a professional collaboration among national partners with different expertise related to aquaculture. The interdisciplinary approach of the Danish projects has been extended in PRO-EEL, which brings together internationally leading researchers in eel reproduction complemented by excellence in disciplines filling gaps in knowledge and technology. The integration of the aquaculture industry promotes the applicability of the developed technology.

FEMALE EEL RECEIVING THE WEEKLY HORMONE INJECTION

What do you anticipate to be the long-term benefits of the project in terms of preserving the European eel?

The real solution to a sustainable aquaculture is to propagate and breed eels in captivity. In this way, measures can be taken to protect and restore the wild eel population. The domesticated animals can then be used for consumption.

The PRO-EEL project is a first step towards a self-sustained aquaculture. If successful, genetically safe programmes can be developed for stock enhancement purposes.