

PROJECT FINAL REPORT

Grant Agreement number: 245257

Project acronym: PRO-EEL

Project title: Project title: Reproduction of European Eel: Towards a Self-sustained Aquaculture

Funding Scheme: Collaborative project

Period covered: from 2010-04-01 to 2014-07-31

Name of the scientific representative of the project's co-ordinator, Title and Organisation:

Jonna Tomkiewicz, Dr.

Technical University of Denmark, National Institute of Aquatic Resources

Tel: +45 35883408

E-mail: jt@aqua.dtu.dk

Project website address: www.pro-eel.eu

Table of Contents

1	Final publishable summary report	3
	1.1 Executive summary	3
	1.2 Project context and the main objectives	4
	1.3 Scientific and technological results and foregrounds	7
	1.4 The potential impact and the main dissemination activities and exploitation of results	31
	1.5 Project public website and contact information	34
2	Dissemination and use of foreground	35
	2.1 A - Dissemination (public)	35
	A1 List of scientific, peer reviewed publications	35
	A2 List of other international dissemination activities (book chapters, international conference contributions, other international meeting contributions)	38
	2.2 B - Patents and exploitable foreground	54
	B1 List of applications for patents, trademarks, registered designs, etc. &	54
	B2 exploitable foreground	54
	2.3 C - Education and young researcher training	54
	C1 List of education and young researcher training during PRO-EEL	54
3	Report on societal implications	60
4	PRO-EEL partners and contacts	66

1 Final publishable summary report

1.1 Executive summary

Aquaculture of European eel, which relies on wild-caught glass eels, has been challenged by a severe decline in the stock since the late 1970s. This decline has rendered the stock severely depleted and listed the species as “critically endangered”. Thus, today’s eel aquaculture critically needs the technology to produce glass eels in captivity as the basis for a self-sustained production to be able to meet the high market demand. In this context, PRO-EEL represents a collaborative effort among European research institutes and aquaculture industry with the common objective to substantiate progress in controlled reproduction and larval culture technology of the European eel.

The aim of PRO-EEL has been to expand the knowledge base on European eel reproduction and develop standardised protocols for controlled production of viable offspring and culture of larvae. The objectives were to: i) acquire knowledge on eel physiology and hormonal control of reproduction, improve broodstock nutrition, selection, and conditioning, as well as to develop suitable methods to induce maturation in female and male eels, ii) advance and test protocols to facilitate a stable production of high quality eggs and sperm, and establish standardised fertilisation procedures for a sustained offspring production, and iii) establish incubation techniques, larval culture technology, and test potential larval feeds aiming at first feeding culture of larvae.

PRO-EEL has significantly substantiated knowledge about hormonal control and physiology of eel reproduction. Early in the project period, the European eel genome was sequenced, which allowed the consortium to perform a large variety of gene expression studies. Thus, genomic data have been extensively used to describe gene expression in the brain, pituitary, ovaries, and liver, gonadotropin receptors were cloned and sequenced, and bioassays developed and applied in studies on their distribution and regulation of gene expression. This cutting edge research has led to a new understanding of eel hormonal control mechanisms, and initiated the development of novel treatments. Thus, bioactive recombinant European eel gonadotropins were successfully produced for development of improved therapeutic alternatives to the traditionally used pituitary extracts from salmon or carp.

Broodstock nutritional requirements and energy allocation were studied in feeding trials and reproduction experiments to enhance gamete quality from farmed broodstock. A benchmark analysis that compared farmed and wild-caught female eels from various locations in Europe showed a distinct difference in composition of minerals, fatty acids, and persistent organic pollutants. In particular, the composition of essential fatty acids in farmed and wild-caught broodstock differed. Thus, diets developed within the project for farmed broodstock specifically focused on long chain polyunsaturated fatty acids and antioxidants. Diets with higher arachidonic acid (ARA) levels and balanced eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) ratios yielded promising results in terms of increased egg quality and viable offspring production. Similarly, dietary fatty acid composition was found to affect sperm volume and different sperm quality characteristics.

Broodstock physiological responses to treatments were studied and tested experimentally using traditional and novel hormonal treatments and conditioning methods to induce gamete production as well as manipulation of temperature regime during induced maturation. Results included enhanced procedures for induction of spermatogenesis, oogenesis and final maturation thereby improving gamete quality and management in egg fertilisation procedures. Application in standardised protocols significantly increased the production of viable offspring from wild-caught and farmed broodstock. Together, enhanced fertilisation success and zygote development increased embryonic survival and quality and contributed to a substantial increase in the production of yolk-sac larvae.

Developmental characteristics and biophysical requirements of embryos and larvae were outlined from morphological, physiological and behavioural observations of offspring obtained from full-scale experiments. New insight was used to improve and standardise of methods and culture techniques. Physical and biological culture conditions for embryos and larvae in terms of e.g. temperature, salinity, light regime, and turbidity were studied and different tank systems tested to enhance larval survival. A major step forward was achieved in studies of the effects of microorganisms on egg and larval performance. Based on this information, recirculation systems were applied for increased microbial control, which proved successful in maintaining substantial numbers of larvae that survived into the first feeding stage. Hence during the project, larval performance and longevity was enhanced significantly, which allowed us to perform the first feeding trials with live and inert larval feeds.

Thus, the PRO-EEL consortium has extensively enhanced the knowledge base within eel physiology and morphology in different life stages and established protocols for successful breeding and larval culture enduring into the first feeding stage. Together, these results and achievements of the PRO-EEL project represent a major breakthrough in European eel research and an important and promising step towards successful breeding and larval rearing for a sustainable aquaculture of this species.

1.2 Project context and the main objectives

Project context

European eel (*Anguilla anguilla*) is a species that has puzzled mankind for centuries due to its unusual life cycle with a continental growth phase from “glass eels” to “yellow eels” and an oceanic phase, where “silver eels” migrate towards the Sargasso Sea to spawn and this is where their offspring originate. So far neither maturing nor spawning eels have been observed in nature and it is the occurrence of eel larvae that has identified the spawning area. Here, the early-stage larvae develop into the unusual leptocephalus larval stage and their route to Europe has been mapped, however, little is known about their diet and ambient environment during the journey until their transformation into glass eels in coastal areas.

The eel is particularly vulnerable as a species, because it reproduces only once during its lifetime, but at the same time is fished in all its immature, continental life stages, including glass eels, yellow eels and migrating silver eels. The market demand of all the targeted life stages of eel is high, resulting in high market values. Yellow and silver eels have been heavily exploited by the fisheries and their habitats deteriorated, e.g. passage to and from rivers and lakes impeded by dams and hydropower stations, and increasing contamination of continental waters by man-made pollutants. Glass eels are exploited primarily as basis of eel aquaculture. More than 80 % of eels consumed in the world are based on aquaculture of glass eels caught in nature; however, in return the capture-based aquaculture delivers a substantial amount of juvenile eels for restocking purposes.

The present eel aquaculture practice, relying on wild-caught glass eels, however, has been challenged by a severe decline in the European eel stock and its recruitment since the late 1970s, which has rendered the stock severely depleted. Thus, today’s eel aquaculture critically needs the technology to produce glass eels in captivity as basis for the existing, efficient technology for on-growing of glass eels using recirculation aquaculture systems. Therefore, the establishment of captive breeding methods and hatchery technology for an eel aquaculture self-sustained in glass eels has become a target for producers, managers, and researchers.

In order for the eel aquaculture industry to become sustainable, the life cycle needs to be closed in captivity. Research on captive reproduction of eels has previously been attempted, but has proved difficult due to the complexity of the eel's reproductive physiology involving inhibition of maturation at a prepubertal stage. Even though research in Japan has progressed with assisted reproduction of the Japanese eel (*Anguilla japonica*), large-scale breeding of eel is still not realised for any eel species and both aquaculture and restocking continue to rely on capture of wild-caught glass eels. Future sustainability of eel aquaculture will depend on continuous controlled reproduction, i.e. reproduction achieved from generations bred and maintained in captivity, thereby removing the dependence on a permanent supply of glass eels or breeders from the wild. This will require production of high quality offspring based on knowledge on how to maximise gamete quality and egg fertilisation success as well as on what are the optimal incubation and rearing conditions for eggs and larvae, respectively. The PRO-EEL project represents a collaborative effort among European research institutes and the aquaculture industry with the common objective to substantiate progress in controlled reproduction and larval culture technology of the European eel.

Main objectives

The ambition of PRO-EEL has been to substantiate the knowledge base on European eel reproduction and develop standardised protocols for controlled production of high quality gametes, embryos, and larvae. The aim was to overcome primary bottlenecks in controlled reproduction of European eel in captivity, which concerns deficiencies in knowledge and methodology to induce gametogenesis and obtain viable offspring from captive broodstock in a regular and predictable manner. The ultimate goal was to significantly enhance offspring production and establish larval culture technology, leading to the feeding larval stage.

A particular bottleneck in the captive reproduction of eel species is a dopaminergic inhibition of sexual maturation that commences during the silvering stage, which necessitates hormonal induction of gametogenesis. However, poor gamete quality and embryonic development competence has hampered previous attempts to produce viable offspring using assisted reproduction technology. Thus, understanding physiological requirements that influence processes that drive gamete development, maturation and fertilisation have been key research areas within PRO-EEL in order to enhance gamete quality, fertilisation success and sustain embryonic development. Here, characterisation of the eel genome and sequencing of genes provided new research tools, enabling insights into functional genomics and characterisation of specific gene expression relevant to reproduction physiology.

An additional bottleneck concerns development of the rearing technology and methods needed to sustain embryonic and larval development, accomplishing the critical early life history stages. Therefore, knowledge about zygotes, embryos, and larvae in different stages of development has been another focus area. As a rule, stringent requirements in terms of the physical, chemical, and microbial environment as well as nutrition must be met to ensure proper development and high survival rates during the early life history stages. In this context, the distinctive leptocephalus larvae of eels, which differ from any other larvae of cultured fish species, adds further complex challenges to research on suitable hatchery practices. In particular, significant gaps in knowledge about the natural habitats and prey hamper development. Thus, new insight into larval ontogeny, functional morphology and nutritional requirements were needed for innovative solutions to culture technology and feeding practices.

The main objectives of PRO-EEL were accordingly to:

- Acquire specific knowledge on eel physiology and hormonal control of reproduction in order to improve broodstock nutrition, selection and conditioning as well as to develop suitable methods for induction of maturation in female and male eels.

- Advance and test standardised protocols to facilitate a stable production of high quality eggs and sperm, and establish optimised fertilisation procedures to ensure healthy embryonic development for a sustained production of viable offspring.
- Develop incubation and larval culture technology and test suitable larval feeds aiming at feeding culture of larvae.

To meet these objectives, research and technology development has systematically progressed from substantiating basic knowledge using *in vivo* and *in vitro* studies, via application development including small- to medium-scale tests to ascertain and improve techniques, towards full-scale tests of developed protocols ranging from broodstock establishment to larval culture in order to enhance reproductive success and larval production (Fig. 1). Basic research studies have focused on neuro-endocrine function and hormonal control mechanisms; broodstock nutritional requirements and energy allocation; spermatogenesis, sperm quality and storage; oogenesis, final follicular maturation and egg quality; requirements for successful fertilisation; and development, physiology and functional anatomy of embryos and larvae. Application development considered broodstock diets, feeding schemes, and selection criteria; hormonal treatments to induce gametogenesis, gamete maturation and assessment of quality; gamete management, fertilisation procedures and egg incubation techniques; and larval culture technology and larval diets.

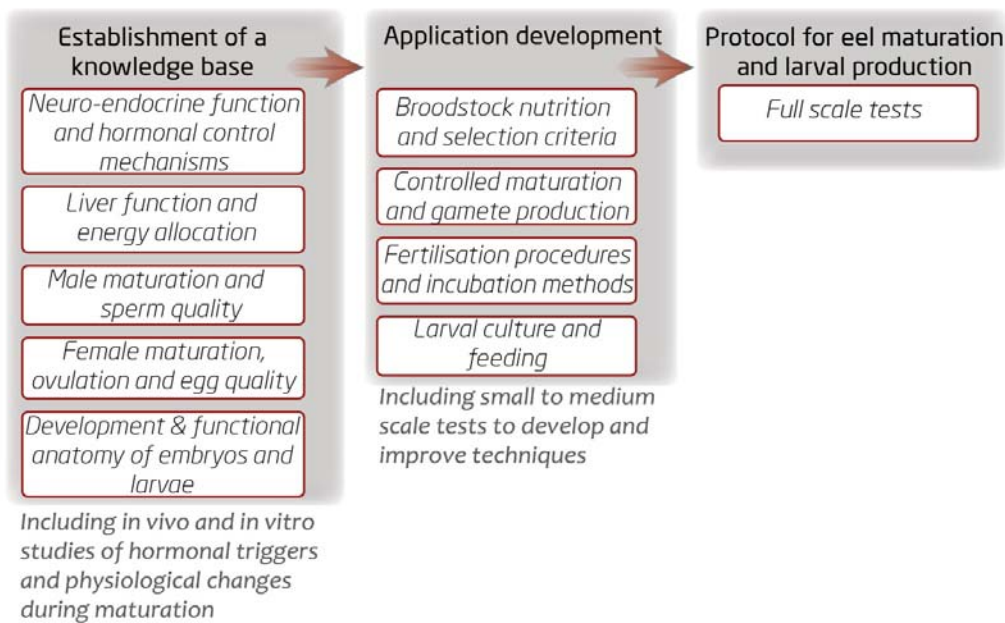


Figure 1 Project concept and work strategy.

Protocols integrating developed methods, techniques and technology were tested in seven successive series of full-scale experiments during the project period. These full-scale experiments that have formed the backbone of the project and the basis for the progression towards larval production and culture, ranged from establishment of broodstock from wild-caught and farmed eels, induction of gametogenesis and final follicular maturation, to embryonic and larval culture. As egg and larval production succeeded in providing increased numbers of viable offspring, the ontogeny and functional anatomy of early larval stages became a focus area to establish criteria for healthy development. Finally, experiments to adapt rearing conditions to meet larval requirements and decrease mortality became feasible; thus, approaching the ultimate goal to supply a sufficient quantity of high quality larvae for controlled feeding trails.

1.3 Scientific and technological results and foregrounds

Organisation of the project

The research and technology development of the PRO-EEL project was organised into nine work packages (WP) each dealing with a specific topic as well as two work packages dealing with scientific coordination and management. The organisation of work-packages by disciplines has taken advantage of the unique expertise provided by the project partners. The nine work packages focusing on research and technology development (RTD) are described below and their interdependencies are illustrated in Figure 2.

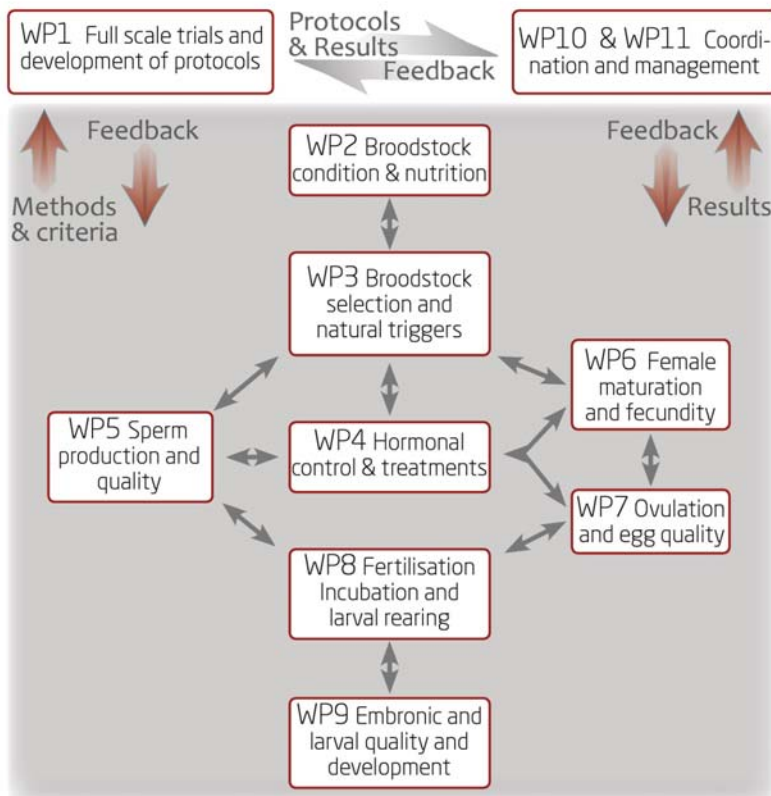


Figure 2 Project work package structure and linkages. WP1 acted as full-scale tests that received input from each of the work packages (WP2 to WP9) in order to develop improved protocols for broodstock breeding and offspring culture technology. WP10 and WP11 coordinated and managed project activities, communication, and dissemination.

WP1 “Full-scale test of protocols to produce feeding larvae” aimed at establishing standardised and functional protocols for successful reproduction of European eel in captivity and developing methods and technology for larval culture in close collaboration with all project partners. This WP was coordinated by the Technical University of Denmark (DTU). WP2 “Broodstock condition and nutrition” had the purpose to assess broodstock nutritional requirements and develop suitable broodstock diets to enhance eel gamete production/quality and offspring viability. This WP was led by the National Institute for Agronomic Research (INRA). WP3 “Broodstock selection and natural triggers for maturation” had to establish criteria for broodstock selection of farmed and wild-caught eels, to improve methods for induction of maturation through conditioning and identification of natural triggers. WP3 was led by Leiden University (LU). WP4 “Neuro-endocrine function and novel treatments for maturation” aimed at increasing the knowledge base on neuro-endocrine function and developed novel treatments for maturation in order to replace current protocols based on repeated injections of heterologous gonadotropins, i.e. human chorionic gonadotropin (hCG) in male eel; carp or salmon pituitary extracts (CPE/SPE) in female eel. This work package was led by the National Center for Scientific Research (CNRS). WP5 “Spermatogenesis and sperm production, selection and storage” had the purpose to optimise protocols to induce spermatogenesis and obtain a stable

production of high quality sperm, validate sperm quality criteria, and establish protocols for fertilisation to standardise the sperm concentration in fertilisation experiments. This WP was led by Institute for Animal Science and Technology, Polytechnic University of Valencia (ICTA-UPV). WP6 "Vitellogenesis and re-allocation of body stores" aimed at elaborating upon existing and novel methods to induce vitellogenesis and survey the ovarian development during treatment with the purpose to provide protocols for successful development of oocytes. WP6 was led by DTU. WP7 "Final oocyte maturation and ovulation" had the purpose to enhance ovulation success, egg quality, and embryonic developmental competence by improving methods to induce final oocyte maturation and ovulation. WP7 was led by NOFIMA A/S. WP8 "Fertilisation, incubation and larval rearing techniques" focused on increasing fertilisation rates, embryonic and larval survival and development of a suitable start feeds to obtain feeding cultures of larvae. This included establishment of adequate fertilisation procedures and development of incubation and rearing methods and technology. This WP was led by the University of Ghent (UGent). The last RTD work package WP9 "Development and quality assessment of embryos and larvae" had the purpose to investigate and describe embryonic and larval development with reference to behaviour, morphological changes, and somatic growth and to provide evaluation criteria for successful development of eggs and larvae. This WP was led by DTU.

An additional two work packages, WP10 and WP11, ensured the scientific coordination, integrating research activities and synthesis, discussion and evaluation and project management and communication. These work packages were addressed by DTU, being the project coordinator.

Main results and achievements

The following sections describe science and technology development with focus on main results and achievements within the nine scientific work packages.

Full-scale tests of protocols to reproduce eels and culture larvae (WP1)

The aim of this work package was to establish standardised and functional protocols for successful reproduction of European eel in captivity, including development of methods and technology for larval culture. Methods, criteria, and technologies developed by WP2-9 for specific parts of the eel reproductive cycle were verified experimentally through full-scale tests ranging from selection of broodstock to rearing of larvae. The development of methods, criteria, and technologies are described in detail in each of the following work packages.

Full-scale experiments comprised the entire sequence from broodstock selection to larval culture. Female broodstock included wild-caught silver eels and farmed eels fed a commercial diet or seven different enhanced broodstock diets developed in the project. All male eels used in the full-scale experiments received the commercial diet. Wild-caught female broodstock and farmed female eels on standard diet were used to benchmark the results on egg quality and reproductive success obtained from farmed female eels fed enhanced diets. The experiments applied a statistical design including groups of 10 to 15 female or male eels to evaluate effects of different broodstock origin, nutrition, and maturation protocols on gamete quality and offspring production under controlled conditions. This included tests of broodstock diets, conditioning and treatments to induce gametogenesis in female and male eels, respectively. In addition, different treatments and methods used for final oocyte maturation and ovulation, and harvest of eggs were tested, as well as methods to harvest, store and dose sperm. Once the gametes were harvested, fertilisation methods and procedures were evaluated, and developed methods and technology for incubation and culture of embryos and larvae tested. Finally, inert feed for larvae was tested in relation to development of rearing conditions.

In total, seven full-scale experimental series were conducted in collaboration among partners. These included three experimental series using wild-caught female broodstock, all originating from the Danish lake “Vandet”. Another four experimental series tested farmed female broodstock on commercial (DAN-EX BioMar A/S) or enhanced diets (JD1, JD2, PRO-EEL1 - PRO-EEL5). Male eels received the commercial diet (DAN-EX). Farmed eels were reared on the experimental diets on a commercial farm prior to transfer to the experimental facility of DTU. Standard experimental conditions used artificial saltwater at 36 PSU and a temperature at 20 °C. All full-scale experimental series using farmed female broodstock comprised tests comparing different groups using a factorial design, as well as additional groups that were dedicated to WP-specific experiments.

Full-scale experimental protocols for female hormonal treatments used weekly injections of salmon pituitary extract (SPE) to induce eel oogenesis and injection of progestagen (i.e. dihydroxy progesterone, DHP) towards the end of the female treatment in order to induce final oocyte maturation and ovulation. Male eels received injections of human chorionic gonadotropin (hCG) to induce spermatogenesis. Manipulation of rearing conditions included tests of a cooling period prior to hormonal treatment. Different hormonal schemes to induce gametogenesis and final oocyte maturation were tested using fertilisation success, embryonic development and hatch success as criteria to improve gamete production, oocyte maturational competence, and offspring quality.

Fertilisation protocols were enhanced through improvement of gamete harvesting and management including application of a sperm storage medium, standardised sperm:egg ratios as well as activation conditions and procedures. Similarly, incubation procedures were gradually enhanced considering salinity, salt type, temperature as well as physical conditions and water exchange. Methods to monitor fertilisation percent, embryonic survival and hatch percent were implemented throughout the full-scale series and revealed a significant increase in the production of viable embryos and hatching larvae over time.

Furthermore, larval culture technology and techniques progressed greatly during the course of the project and significantly increased larval survival. Experimental work on larval culture included microbial control and water quality management, flow-through versus recirculation technology and size of tanks as well as temperature, salinity, and light, etc. During the last part of the project, enhanced larval culture techniques increased survival during the yolk-sac stage and tests of inert larval feeds were initiated. Quantification methods were developed and implemented alongside the technology development and documented progress in larval production, survival, and longevity.

The ultimate results increased the number of responders versus no or slow responders to 57% and 90% of these females were successfully stripped. The mean amount of eggs produced by females was 424 g ± 139 g. All egg batches were successfully fertilised with a mean fertilisation rate of 40% ± 30 STD of all eggs stripped. Thus, the overall mean fertilised egg production was in the range of 250,000 eggs per female. In total, 50% of the fertilised egg batches produced embryos and larvae. Mean larval hatch was 54% of the eggs from the floating layer, resulting in an estimated average larval production per female in the range 170,000 larvae ranging from 1800 to 890,000 larvae. Larval survival from eggs stocked into the recirculation system showed a moderate decline from day 1 to 3 post hatch, followed by a substantial decline from 4 to 8 days post hatch. The fully developed feeding larval stage was reached at ~12 days post hatch at 20°C. Larval longevity averaged 14.9 days with a maximum of 22 days, which is well into the feeding stage. Addition of instant algae and inert feeds were tested and promoted survival. Next steps in successful on-growing include targeting suited feeds and enhancement of rearing technology to sustain early larval stages.

Throughout the seven experimental series an intensive sampling program was performed providing WP2-9 with samples and data from tests of specific methods, techniques, and systems. Feeds, male and female eels were sampled at different time points prior to and during treatment, including brain, pituitary, blood plasma, and tissue samples for e.g. molecular, physiological, histological, lipid,

mineral, and contamination studies as well as morphometric data. These samples and data were extensively applied in endocrinological studies, characterisation of gametogenesis and development of enhanced treatments. Similarly, gametes were sampled for quality assessment, and fertilised eggs, embryos and larvae were sampled at various stages of development for analysis of morphology and physiology with the aim to document healthy offspring development, test culture conditions and develop or identify suited diets that can be used in attempts to establish suited culture conditions and feed for larvae. In total, the database included 75,000 data records related to sampling during the full-scale experiments. Furthermore, a significant amount of gametes, embryos and larvae entered targeted experiments to improve fertilisation, incubation, and larval rearing procedures. Furthermore, an egg transportation protocol allowed successful transfer of eggs/embryos to other locations for experimentation on larval culture.

Together, these progressive experiments and their results have led to development of “state-of-the-art” standardised protocols for European eel broodstock establishment and breeding protocols as well as larval culture technology.

Broodstock condition and nutrition (WP2)

The aim of WP2 has been to assess broodstock nutritional requirements and develop suitable broodstock diets to enhance eel gamete production and offspring viability from farmed European eel broodstock.

Farmed and wild-caught female eels were sampled from various locations in Europe to provide a benchmark analysis. The fish were measured to provide various indices and different tissues were analysed for biochemical composition, proximate and mineral, lipid and fatty acid, and persistent organic pollutants (POP). The different eel groups showed a distinct difference in tissue composition of minerals, fatty acids, and POP. For example, the levels of As and Cd were higher in farmed eels, although these levels were also high in polluted areas of the Netherlands. Levels of Hg and Pb in turn were mostly higher in wild-caught eels. Of interest was also that the level of Ni was exceptionally high in eels from the Danish lake “Vandet”, from is where the wild-caught broodstock in the full-scale experiments originated. Furthermore, two relatively ‘clean’ populations of wild eels had quite high levels of Hg.

Differences in tissue lipid composition between wild-caught and farmed female eels and also between locations were highlighted. Wild-caught eels had lower muscle lipid content and higher plasma triglycerides levels after spawning compared to farmed eels. The wild-caught eels had also a different fatty acid composition compared to that in farmed eels. In the gonads, the most notable difference was the higher relative level of arachidonic acid (ARA) in wild-caught eels (2.6 to 5.9%) compared to farmed (0.6 to 0.9%). Also levels of several n-3 PUFAs were higher. Eicosapentaenoic acid (EPA) levels were lower in wild-caught fish (1.4 to 3.9%) compared to farmed fish (4.9 to 5.5%), resulting in much lower EPA/ARA ratios in the wild-caught fish (0.5 to 1.9%) as compared to the farmed fish (6-7.6%). Docosahexaenoic acid (DHA) levels were lower in wild-caught eels (3.4 to 6.0%) compared to farmed eel (8.7 to 9.4%). The DHA:EPA ratio was only slightly lower but much more variable in the wild-caught fish (0.8 to 3.8%) compared to the farmed fish (1.7 to 1.8%).

With regard to the contamination, in particular POP’s, the muscle of farmed eels contained low levels of PCB’s, some industrial contaminants, and pesticides. For wild-caught eel this was also observed in one area of the Netherlands “Friesland” and especially in wild-caught female eels from the Danish lake “Vandet”. The concentrations in these eels were very low; it is likely that the only source of contamination in these cases were atmospheric deposition. The extremely high levels in the “lower rivers” of the Netherlands have been a reason to shut down the eel fishery for human consumption from this area. PCB’s were also relatively high in Lake Albufera, Spain, but in this sample also DDT (residues) possibly from the rice culture near the lake are reasons for concern.

In total, seven broodstock diets were formulated, produced and tested during the project and using the commercial diet DAN-EX 2848 (BIOMAR A/S) as a reference diet in some of the experiments. With regard to body lipid stores, the results showed that lipogenesis in liver is in the same range as in other fish species and it is not or little affected by dietary treatments. Surprisingly, lipogenesis in muscle was very low in muscle indicating that lipids deposited in muscle mainly originate from dietary lipids. As regard to plasma lipids, we showed a graded increase in triglycerides and a decrease in cholesterol during maturation and no marked changes in phospholipids. These variations were similar in all the experiments conducted, independent of the dietary treatments tested. Muscle lipid content was very high (32 to 38%) and similar independent of the diets tested. Taking into account muscle mass, we showed that 49% of the lipids stored in the muscle are mobilised during the maturation process. No differences related to diets were found in lipid peroxidation products in muscle. Egg lipid content was around 6.8% and cholesterol represented 4% of total lipids, with no significant differences induced by the dietary treatments tested. Lipid peroxidation products in eggs (conjugated dienes, anisidine value and lipid-soluble fluorescent products) were also in the same range irrespective of the broodstock diets. These results suggested that dietary lipid content (22 vs. 26%) did not affect body lipid stores (muscle and eggs) and lipid reserves that can be used for energy sources and reproductive investment throughout the development. Moreover, the differences observed in reproductive performances were not linked to the quality of lipid reserves and/or lipid peroxidation status.

In particular, the effect of dietary ARA, EPA, and DHA on the production of embryos and hatched larvae of European eel in the project was examined and the results showed that it was important to achieve a correct balance of these essential fatty acids in the diet to improve reproductive success. The dietary levels of vitamin C and E were tested and the lowest levels provided were found to be sufficient.

Overall the results showed that higher levels of ARA in the diet were incorporated in the tissues of European eel, but ample feeding duration must be ensured to secure full incorporation of dietary ARA in the body tissues prior to hormonal treatment of broodstock female eel. The diets with the higher ARA levels and low EPA:ARA ratios gave the most promising results in terms of reproductive output and offspring survival.

Broodstock selection and natural triggers for maturation (WP 3)

Complete artificial maturation of female European silver eels has been possible for more than a decade using hormonal induction of gametogenesis; however, the procedure is labour intensive and expensive, including weekly injections of pituitary extracts for up to half a year. The success rate of successful ovulation varies from less than 10% to more than 90% with a variable proportion of particularly the female broodstock being insensitive to hormonal stimulation. A high percentage of non-responders reduce efficiency and increase costs, and fully sustainable farming of European eels would benefit from (1) replacement of the extended treatment with gonadotropins (at least partially) by induction of maturation via natural triggers and (2) discrimination of female eels that are susceptible to hormone treatment, so-called responders, from non-responders via a minimally invasive method at an early stage in the artificial maturation protocol.

(1) Two environmental parameters were tested in detail, namely the effect of endurance swimming on male European eels (Fig. 3) and the effect of reduced temperature on female European eels. We found that farmed male European eels can swim continuously for a total distance of almost 7,000 km within 6 months. During this simulated migration the males lost less than 30 g of their body weight. This showed that eels have an extremely low energy cost of swimming. Since swimming in schools may provide additional energetic advantage, the energy consumption of individual males was compared with that of groups of seven males. Although the optimal swimming speeds were not significantly different, the cost of transport at the optimal swimming speed of group-wise swimming

males was more than 30% lower than that of the individually swimming males. Whether eels also migrate in groups during their journey to the Sargasso Sea is not known. However, in contrast to our expectation, endurance swimming did not induce sexual maturation in farmed male eels.

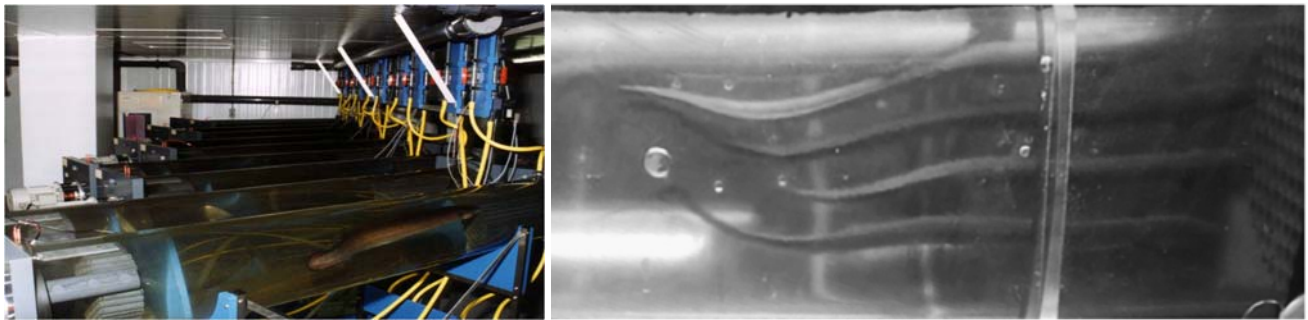


Figure 3 Left figure: Blazka-type swimming tunnels at LU used to examine the effect of endurance swimming on metabolism and sexual maturation. Right figure: top view of a group of male eels in a Blazka-type swimming tunnel showing anguilliform swimming motion in a parallel swimming formation at 0.40 m s⁻¹. (Ron Dirks, LU).

In many fish species, a prolonged cold period is a powerful natural trigger for sexual maturation. Therefore, we compared the effect of 5-month incubation of farmed female European eels at low (15°C) or high (21°C) temperature on induction of sexual maturation and on responsiveness to subsequent treatment with pituitary extract. We found no significant stimulatory effect of the cold period on sexual maturation based on e.g. eye index, gonad weight, and liver weight. In conclusion, the use of natural triggers to induce sexual maturation of European eels is a complicated challenge and may require a more integrated approach where multiple parameters (temperature, light, endurance swimming, and salinity change) are combined at the same time.

(2) The first draft genome sequence of an organism has a major impact on research in multiple fields, including molecular biology and physiology. Publication of the complete genome sequences of the European and Japanese eel in 2012 has opened up the way for highly detailed molecular analysis of all aspects of the artificial maturation process, e.g. via microarray, Illumina RNAseq, and large-scale qRT-PCR techniques. Ovarian, pituitary, and liver transcriptomes from eels at different maturation stages (yellow, silver, spawned) were mapped and used to annotate the majority of protein coding genes in the European eel genome. This has resulted in unprecedented new insight into the molecular mechanisms of sexual maturation of the European eel.

We discovered that up-regulated ovarian expression of genes coding for some key steroidogenic enzymes were good indicators of sexual maturation. This has resulted in a new, minimally invasive method to discriminate between responders and non-responders based on the fold-increase in blood-plasma estradiol level after four weekly gonadotropin injections. In addition, several molecular markers were identified in transcriptomes derived from pectoral fins that can be used to monitor hormone-induced sexual maturation of female European eel (Table 1). These newly identified fin markers also provided important new insights into several fundamental processes in eel biology.

Table 1 Selection of genes that were differentially up- or down-regulated in pectoral fin of farmed female European eels, being responsive (resp) or non-responsive (nonresp) to treatment with carp pituitary extracts. FC: fold-change (R. Dirks, LU).

up-regulated genes			
Gene description	FC-nonresp	FC-resp	FC-resp/FC-nonresp
Golgi-associated plant pathogenesis-related protein 1	4.1	1433.9	350.5
mepirin A subunit beta-like	0.5	312.8	579.8
lactose-binding lectin I-2	1.1	59.9	52.5
calpain-1 catalytic subunit-like isoform X2	1.5	34.0	22.4
mucin-22-like	5.6	547.5	97.7
DDB1- and CUL4-associated factor 6	1.4	27.1	18.8
keratin	4.6	298.2	64.9
L-dopachrome tautomerase-like	1.0	72.2	75.7
GTP cyclohydrolase 1	4.2	210.1	50.3
fibroblast growth factor-binding protein 1 precursor	1.4	35.5	25.1
fatty acyl-CoA hydrolase precursor, medium chain-like isoform X1	1.5	66.5	45.0
beta-1,4 N-acetylgalactos-aminyltransferase 2-like	0.3	9.4	29.9
prostaglandin G/H synthase 1-like	2.6	41.8	15.9
vesicular monoamine transporter 2	1.2	39.8	33.8
four and a half LIM domains protein 3	1.1	16.4	15.3
transmembrane protease serine 13-like	0.6	17.0	29.4
growth regulation by estrogen in breast cancer-like	2.0	28.3	14.4
down-regulated genes			
Gene description	FC-nonresp	FC-resp	FC-resp/FC-nonresp
NXPE family member 3-like	0.8	769.1	954.0
tetranectin-like	0.4	101.7	271.4
C-type lectin 1	16.9	300.8	17.8
hemoglobin anodic subunit alpha	1.2	20.7	17.9
hemoglobin anodic subunit beta	1.2	16.4	13.5
hemoglobin cathodic subunit alpha	0.7	11.1	15.8
hemoglobin cathodic subunit beta	0.7	11.3	15.3
retinol-binding protein 2 precursor	0.3	9.8	37.9
selenium-binding protein 1 isoform X1	1.4	53.6	38.3
SAM domain and HD domain-containing protein 1-like	1.5	19.4	12.9
sulfotransferase family 3, cytosolic sulfotransferase 3	0.5	5.3	10.1

For example, expression of the serine protease gene "Golgi-associated plant pathogenesis-related protein 1" increased more than 1400-fold in responders, whereas its expression barely changed in the non-responders. The expression of several lectin, mucin and keratin genes changed dramatically (both up- and down-regulation) in the pectoral fin of responsive eels, but not those of non-responsive eels, which was in accordance with the knowledge that the skin of a responsive eel is significantly reorganised during maturation.

Also genes associated with steroid signalling (estradiol response) and tyrosine/dopamine metabolism (pigmentation) were strongly up-regulated in responders and not in non-responders. Surprisingly, the expression of all four genes coding for haemoglobin subunits and of several other blood marker genes were strongly reduced in the pectoral fin of responders compared with non-responders. Whether this is caused by active down-regulation of these genes during sexual maturation or is a result of a reduced hematocrit value remains to be determined.

In addition to the blood plasma estradiol-based method and the pectoral fin maturation marker-based method, a new ultrasound method was developed that can, at an early stage of hormone treatment, discriminate between responders and non-responders based on signals in the ovarian texture.

In conclusion, it is now possible to discriminate between responders and non-responders via (a) simple ELISA analysis of blood samples, (b) qRT-PCR analysis of pectoral fin samples, or (c) ultrasound analysis of ovarian texture.

Neuro-endocrine function and novel treatments for maturation (WP 4)

All eel species remain sexually immature for most of their life, being blocked at a pre-pubertal stage as long as they do not perform their oceanic reproductive migration. This phenomenon prevents spontaneous sexual maturation and reproduction in captivity and represents a crucial limiting factor for the development of eel aquaculture.

In teleost fishes as in mammals, reproduction is under the neuroendocrine control of the gonadotropic axis: brain gonadotropin releasing hormone (GnRH) stimulates pituitary gonadotropins (luteinising hormone, LH, and follicle stimulating hormone, FSH) synthesis and release, which stimulate gonadal activity (steroidogenesis and gametogenesis). Work by M. Fontaine and co-workers, at the National Museum of Natural History, France, demonstrated that eel sexual immaturity was due to a low production of endogenous pituitary gonadotropins. Current protocols for inducing eel gametogenesis are still based on these discoveries, using repeated injections of human chorionic gonadotropin (hCG) to induce spermatogenesis in males, and repeated injections of carp or salmon pituitary extracts (CPE or SPE) to induce vitellogenesis in females. In addition, injection of progestagen (i.e. dihydroxy progesterone, DHP) at the end of the female treatment is now used to induce final oocyte maturation and ovulation. However, even if such treatments led to recent success in the reproduction of the Japanese eel, low egg quality and poor larval viability are still a major problem. Furthermore, this protocol has proven less successful in the European eel. This is likely due to a stronger blockade of sexual maturation in this species, related to its most distant spawning ground. Recent studies from PRO-EEL partners indicated that the low production of pituitary gonadotropins in the eel is due to a lack of stimulation by GnRH and also to a strong inhibitory control by brain dopamine (DA).

The PRO-EEL project aimed at improving current protocols and developing new strategies to induce more physiological eel maturation. The objectives included either to produce and administer homologous recombinant European eel gonadotropins or to trigger the production and release of eel endogenous gonadotropins, based on increased knowledge on eel neuroendocrine control of reproduction.

A substantial proportion of the project consisted in developing new tools to investigate and manipulate the neuroendocrine control of eel sexual maturation. This included identification and characterisation of various brain, pituitary, and peripheral neuromediators, hormones and receptors, production of homologous recombinant hormones, establishment of antibodies and homologous immunoassays, screening of specific agonists and antagonists. Experimental investigations were mainly performed *in vitro*, in order to minimise *in vivo* animal experimentation and reduce the number of animals sacrificed. To that aim, we used and developed mammalian and fish cell lines, primary cultures of eel pituitary cells, and eel brain explants.

The following major achievements were attained during the PRO-EEL project: Bioactive homologous recombinant European eel gonadotropins LH and FSH, each constituted by two subunits, have been successfully produced. This opens the ways to novel homologous treatments avoiding the possible immune reactions to heterologous hormones present in fish pituitary extracts. This also allows the use of controlled doses of hormones, in contrast to unknown content in pituitary extracts. In order to avoid the stress of repeated injections, a new strategy based on fish cell implants producing eel recombinant gonadotropins was also explored. This strategy revealed limitations due to short lasting of the hormone produced, and possible immune reaction to the implant itself. Future strategies may involve the administration of homologous recombinant European eel gonadotropins

via sustained-releasing capsules, and/or molecular engineering of long half-life recombinant eel gonadotropins.

Homologous immunoassays have been set-up for European eel gonadotropins. Competitive ELISAs for European eel LH and FSH have been developed. They are functional and able to recognise the recombinant European eel LH or FSH, and the native European eel LH or FSH, respectively. These ELISA tools provide new methods to follow and improve the purification of recombinant European eel gonadotropins, as well as to investigate, *in vivo* or *in vitro*, the regulatory mechanisms of gonadotropin production in the European eel.

Characterisation of European eel gonadotropin receptors and development of bioassays: Gonadal LH and FSH receptors are essential for mediating the stimulatory effects of gonadotropins on eel sexual maturation. European eel LH and FSH receptors have been characterised, and specific molecular assays developed to investigate eel gonad receptivity to gonadotropin treatments. Cutting-edge eel-specific bioassays, based on HEK cell lines expressing recombinant eel LH or FSH receptors, were also developed as new tools to measure eel native or recombinant gonadotropin (LH or FSH) levels and bioactivities.

Characterisation of European eel GnRH receptors: Brain GnRH stimulates the production of pituitary gonadotropins through interaction with its specific receptor. Three GnRH receptors (GnRH-R) were identified in the European eel, and specific molecular assays developed to investigate eel pituitary receptivity to GnRH. The expression of one of them (GnRH-R2) was shown to increase during experimental sexual maturation, revealing a raise in pituitary receptivity. *In vitro* experiments demonstrated that testosterone and estradiol selectively and strongly increased GnRH-R2 expression by eel pituitary cells. This suggests that sex steroids, which are produced during sexual maturation, may be responsible for the increasing pituitary receptivity in matured eels. From an applied point of view, the increase in the pituitary sensitivity to GnRH in matured eels further supports the development of novel protocols using GnRH agonist administration, together with dopamine antagonist, to induce endogenous LH ovulatory peak in matured eels. The objective is to induce more physiological final oocyte maturation and ovulation, by triggering the release of endogenous LH, which induces endogenous DHP production. This protocol would replace the current use of the final exogenous DHP injection.

Characterisation of European eel dopamine D2 receptors: Dopamine (DA) exerts, via binding to DA-D2-type receptor, a strong direct inhibition on the production of eel pituitary gonadotropins. Two dopamine D2 receptors (DA-D2A and DA-D2B) have been identified in the European eel, while a single one is present in mammals. Molecular histology of native DA-D2A and DA-D2B receptors revealed that eel pituitary LH and FSH cells express both receptors, with a predominance of the DA-D2B subtype in LH cells. Properties of eel recombinant DA-D2A and DA-D2B were analysed in HEK cell lines. Both receptors are functional and able to activate intracellular pathways in presence of dopamine or dopamine D2 type agonists. We could select *in vitro*, on recombinant receptors in HEK cells, and on native receptors in eel pituitary cells, DA-D2 antagonists able to potently counteract DA inhibition of gonadotropins, to be tested *in vivo*. *In vivo* trials performed with one of the selected DA-D2 antagonists, domperidone, revealed promising results. Future trials will test *in vivo* the novel mammalian DA-D2 antagonist, eticlopride, which we showed to be also highly efficient *in vitro* on eel DA-D2 receptors. These results support the relevance for developing novel protocols, including a double treatment with GnRH agonist and DA-D2 antagonist in sexually matured eels, in order to induce an endogenous LH ovulatory peak.

Several novel neurohormonal systems (ligand and receptors) were characterised in the European eel. Kisspeptin neuropeptide (kiss) and its receptor (kissR) are recently discovered brain actors that activate puberty in mammals. While one single kiss and one single kissR are present in human, two kiss genes and three kissR genes were identified in the European eel, representing the largest gene

number in teleosts. Specific molecular assays were developed to investigate their expression and regulation during sexual maturation. Homologous European eel kiss peptides were synthesised *de novo* and tested *in vitro* on eel pituitary cell primary cultures. Homologous Kiss exerted a paradoxical inhibition of LH expression by eel pituitary cells, revealing for the first time an inhibitory effect of kiss on reproductive functions. Another neuropeptide belonging to the same RF-amid superfamily as kiss, Gonadotropin inhibitory hormone (GnIH), was recently discovered in birds for its inhibitory effect on reproduction. This peptide was shown to have stimulatory or inhibitory effects in teleosts. We identified the presence of a single GnIH gene in the European eel, coding for two different GnIH peptides. Eel GnIH peptides were synthesised and tested for their effects *in vitro* on eel pituitary cells. Homologous GnIH inhibited eel LH expression. From an applied point of view, our findings of the negative effects of Kiss and GnIH in the eel prevent to propose novel treatments based on these neuropeptides to induce sexual maturation in the eel.

Several novel peripheral hormone systems (sex steroids, corticosteroids, gonadal peptides, metabolic hormones, and their receptors) were characterised in the European eel. Specific molecular assays were developed to investigate their expression and regulation during sexual maturation. Their effects were tested *in vitro* on eel pituitary cell cultures. We further assessed that a gonadal peptide, activin, is so far the best hormone for stimulating eel FSH production, but exerts an inhibitory effect on LH. From an applied point of view, these opposite effects of activin, positive on FSH but negative on LH, are limiting the potential use of activin to develop novel protocols for inducing eel sexual maturation. Activin treatments could still be used as a tool to induce the production of eel native FSH. A metabolic hormone, insulin-like growth factor (IGF), had a moderate stimulatory effect on both LH and FSH expression, providing a potential stimulatory tool in the eel. Sex steroids strongly increased GnRH receptor (GnRH-R2) expression by eel pituitary cells *in vitro*. From an applied point of view, a preliminary treatment with sex steroid may be considered to further increase pituitary responsiveness to GnRH.

Lastly, a successful protocol was set up for *in vitro* culture of eel brain parts, a system that maintains both structures and connections of neurones. This new *in vitro* system will allow investigating brain regulatory control in eel reproduction, while avoiding *in vivo* animal experimentation and reducing the number of experimental eels, as compared to *in vivo* studies.

Spermatogenesis and sperm production, selection and storage (WP 5)

Spermatogenesis is the process of male gamete formation, meaning the final production of spermatozoa. The main purposes of this work package were to optimise protocols to induce spermatogenesis and obtain a stable production of high quality sperm, validate sperm quality criteria and establish protocols for fertilisation to standardise the sperm concentration in fertilisation experiments. New knowledge about the mechanisms controlling the testis development and factors determining spermatogenesis and production of high quality sperm were obtained, and sperm fertilisation capacity methods were evaluated for enhanced application in controlled experiments.

In a first experiment, European male eel maturation was induced using standard hormonal treatment with human chorionic gonadotropin (hCG) under three different thermic regimes to test the influence of temperature on the induction of maturation in eel males. Sperm characteristics (volume, density, motility, morphology, percentage of viability) from each sample were evaluated. Samples of blood, brain, pituitary, muscle, liver and testis were taken from every sacrificed male to be used in the different analyses. The results demonstrated that the onset and progression of spermiation are strongly influenced by seawater temperature. A constant temperature of 20 °C demonstrated the best results in all the sperm parameters, becoming a reliable and productive method for inducing eel spermiation.

A second experiment aimed at testing the efficacy of standard treatment (hCG) versus two alternative hormonal treatments (recombinant hCG and PMSG), using constant 20 °C as seawater temperature. In this case, the onset and progression of spermiation were influenced by the hormone used: hCG_{rec} produced the best results in all the sperm parameters, thus becoming an effective alternative treatment to the standard hCG treatment. Moreover, hCG_{rec} gave rise to the best economical profitability, making it possible to obtain good quality sperm samples at a lower price than by using the other two hormonal treatment.

Histological analysis of testis samples from both experiments was carried out to classify the samples considering the development stage. An improved method for classifying the stages of testis development (S1–S5) during progressive spermatogenesis was created, considering the predominant cell types to characterise every stage of testis development. Using this new classification highlights the changes occurred and their relationship with the sex steroids effect and the maturation timing. The analysis of the testis samples showed how the evolution of appearance of the different stages can be faster under higher temperature and hCG_{rec} treatment, and showed evidence of the presence of several subpopulations of spermatozoa in the eel sperm. Using sperm samples a standardised method was established for the evaluation of the eel sperm quality testing different procedural settings (models of chambers, microscopy magnification, software recording conditions) during the measurement of the sperm motility by CASA (computer-assisted sperm analyzer). The plasma levels of some steroids having a regulatory role during the eel spermatogenesis were determined and correlated with the stages of testis development assessed by histological observations. The highest plasma levels of 17 β -estradiol, testosterone and 11-ketotestosterone were found in S1, when spermatogonial proliferation occurs. A correlation was found between 17 α -20 β -dihydroxy-4-pregnen-3-one (DHP) levels and some fatty acids during the proliferation and growing phases (S1-2), suggesting that DHP might modulate lipid metabolism in the liver during early spermatogenesis. The DHP levels increased significantly during the growing phase (S2) and remained at high levels throughout the subsequent development stages (S3-S5).

Specific analyses (rtPCRs) were developed and/or used to evaluate the expression of key-genes of the brain-pituitary-gonad axis (aromatase, androgen receptors, GnRH receptors and steroidogenic enzymes) controlling the gametogenesis of males and females. Our results evidenced their respective roles during the gonad maturation and provided new insights into the role of steroids. Specific rtPCRs to measure the expression of several steroidogenic enzymes were carried out and the results of gene expression evaluation proved that the temperature effect on the testis maturation is regulated through the modulation of the expression of steroidogenic enzymes. A differential activation of genes at the testis depending on the environment temperature could be the regulatory system used by the male eels to regulate their maturation process during their travel to the spawning areas in the West Atlantic.

The analyses of fat and fatty acids in muscle, liver and gonads provided information on three issues related with fatty acids and male eel reproductive performance. First, the dynamics of fatty acids during male maturation at different stages of testis development was explored; secondly, the relationship between fatty acids and steroid hormones was studied; and finally the effect of fatty acids on sperm quality parameters was quantified. The obtained results confirmed the importance of the fatty acid profile on broodstock feeding, as well as the importance of specific dietary fatty acids during the development of the spermatogenesis (as potential modulators of the synthesis of steroid hormones) and for final sperm quality, and in particular how some fatty acids had a key role in, not only sperm production, but also in sperm velocity. Figure 4A shows the main fatty acid mobilisation from the liver to the gonad during eel spermatogenesis, and 4B shows the main correlations between some fatty acids from muscle, liver and gonad and some sperm parameters. Moreover, four different diets were tested to assess the impact of dietary fatty acids on muscle, liver and milt composition, as well as sperm production and motility. Dietary fatty acids had an influence on fatty acids in the

tissues of male eel, and this impacted sperm performance traits (total sperm volume, percentage of motile cells).

Together, this information is important to formulate broodstock diets improving sperm quality and consequently the production of eel larvae.

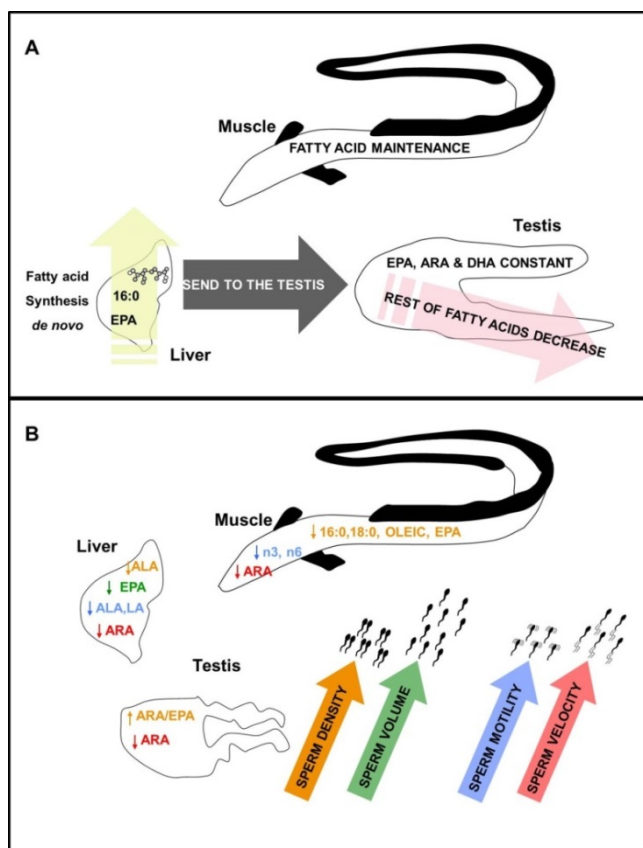


Figure 4 A Main fatty acid mobilisation during eel spermatogenesis. B Summary of the main correlations between the most important fatty acids and sperm quality parameters. (R. Baeza, ICTA-UPV).

Techniques were developed to precisely quantify sperm density and methods to preserve sperm before fertilisation to be later used for controlled fertilisation studies. Moreover, first eel larvae were obtained using cryopreserved sperm, confirming the usefulness of a previously designed cryopreservation protocol. Some experiments were developed comparing our protocol with an alternative one, having standardisation as a target.

The major proteins in the eel seminal plasma, as well as their changes through the spermatogenesis and their relationship with sperm quality parameters were determined. The ionic composition of sperm dilution and freezing media as well as the intracellular variations in the main ions involved in eel sperm activation were studied.

Standardised fertilisation methods, including gamete handling procedures, environmental conditions, determination of sperm density, optimised sperm to egg ratio and eggs receptiveness post-stripping, activation salinity and salt composition, were developed and can now be incorporated into routine hatchery and experimental protocols.

In conclusion, the obtained results have improved our understanding of the physiological mechanisms controlling male eel reproduction, the systems for sperm production, evaluation, preservation and handling for fertilisation, and have substantially enhanced protocols to induce spermatogenesis, sperm storage and fertilisation procedures.

Vitellogenesis and re-allocation of body stores (WP 6)

The aim of this WP was to elaborate upon existing and novel methods to induce vitellogenesis and survey the ovarian development during treatment with the purpose to provide protocols for successful development of oocytes. Experiments primarily used traditional treatments to induce oogenesis i.e. repeated injections of carp or salmon pituitary extracts (CPE or SPE), but varying e.g. hormone dose and temperature regimes. The knowledge base was enhanced through examination of oogenesis and studies of factors determining oogenesis, including ovarian function and development, mechanisms for regulation of hepatic vitellogenesis, oocyte membrane development and function, and mobilisation of body stores and reproduction requirements. Results were applied to optimise protocols to secure functionality and relevant composition of oocytes.

Oogenesis and ovarian morphology: Morphological changes during development from primary growth oocytes in immature specimens via vitellogenesis and final oocyte maturation to ovulation were analysed using histology. In total, seven successive follicular stages were characterised, classifying ovarian development from the immature to postovulatory state. These included first growth phase oocytes (divided into four previtellogenic sub-stages), three progressive vitellogenic stages, final maturation, hydration, and postovulatory follicles. Furthermore, immature ovaries were rich in adipocytes. During development, lipids from these were reallocated to the developing oocytes that accumulated of both yolk and lipid droplets. The oocytes in developing ovaries were organised in successive cohorts, indicating a batch spawning strategy. Realised batch fecundity was assessed and the batch strategy was confirmed by production of viable offspring from a 2nd batch. Batch fecundity ranged from ~250,000 to 1,250,000 eggs per female.

Ovarian function and development: A crucial step in ovarian development is the onset of vitellogenesis. This process is coupled to increased sensitivity to the gonadotropins, luteinising hormone (LH) and follicle stimulating hormone (FSH), through increasing numbers of receptors (LH-R and FSH-R), the production of estradiol (aromatase), and the incorporation of vitellogenin (VTG) into the developing oocytes through increasing numbers of VTG-R. One FSH receptor (FSH-R) and two LH receptors (LH-R1 and LH-R2) were characterised, and LH and FSH expression was measured in control females sacrificed before the maturation process and in stripped females from full-scale experiments. A positive feedback of the steroids on LH pituitary expression was observed, as well as a negative feedback of the steroids on FSH pituitary expression, in all the treated females. A qPCR was developed for the vitellogenin receptor VTG-R, and tissue distribution demonstrated that the highest expression of the VTG-R is in the eel ovary, but it is also present in other tissues. VTG-R mRNA was found to be expressed already in the yellow eel ovary and not further up-regulated during sexual maturation. This reveals that VTG-R mRNA expression is not the limiting factor in the control of VTG ovarian incorporation in the eel. Furthermore, three nuclear (ERa, ERb1, ERb2) and two membrane (GPER1, GPER2) estradiol receptors were characterised in the eel; all were expressed in the ovary. Specific measurement of aromatase, ARA, ARb, LHRa, LHRb and FSHR showed that all these genes are expressed in the eel ovaries. Also, the regulation of the leptin system and gonadotropin receptors in the ovary during maturation was studied, including two leptin genes (Leptin1, Leptin2), and two leptin receptors (LEPR1, LEPR2) were categorised, which is the first time in vertebrates. The ovarian expression of both Leptin and Leptin receptor systems were differentially regulated during experimental maturation. The leptin system appears to be differentially involved in the eel maturation process and potentially in the inhibitory mechanisms with a potential function of Leptin1 and LPER1 in the ovary.

Mechanisms for regulation of hepatic vitellogenesis include the regulation of the various steroid receptors (membrane and nuclear receptors) and vitellogenin expression in the liver. A new and rapid protocol of cell dispersion was successfully developed for primary cultures of European eel hepatocytes. Several test experiments demonstrated that the culture's hepatocytes were able to

express vitellogenin in response to estradiol treatment in a dose and time dependant manner. These new methods of European eel hepatocyte primary cell culture enabled screening of a large number of potential regulatory factors as well as to investigate the cellular and molecular mechanisms of their actions. Several tests showed an up-regulation of the liver vitellogenin expression in hormonally treated females. Studies of estradiol receptors in the liver including analysis of the expression of the membrane estradiol receptors (GPER1, GPER2) showed that all five estradiol receptors were differentially regulated, indicating that the five eel estradiol receptors may be differentially involved in the liver functions during induced maturation. Further analyses included Insulin-like growth factor 1 receptor (IGF1R), growth hormone receptor (GHR), cortisol, and estradiol.

Oocyte membrane development and function: Zona pellucida (ZP) proteins are major constituents of the fish egg envelope and play an important role in binding and recognition of the sperm cells and in early development of the embryo. Deep sequencing analysis of ovarian transcriptomes of the European eel revealed that the ZP genes are among the most abundantly expressed genes in this organ. Automatic gene prediction and annotation based on the recently published draft genome sequence of European eel already resulted in an unusually high number of 19 zona pellucida sperm-binding protein/glycoprotein genes. Further multiple TBLASTN analyses, using identified amino acid sequences of the ZP genes, resulted in the identification of several true ZP genes that belonged to a separate group. In conclusion, a total of 33 true ZP genes were identified. To determine the expression levels of the newly identified ZP genes in ovary and liver, Illumina RNAseq reads derived from ovary and liver RNA samples were mapped against the ZP gene reference file. The majority of ZP genes were expressed at very high levels in all gonad samples and at much lower levels in most of the liver samples.

Mobilisation of body stores and reproduction requirements: During development, lipid stored in adipose tissue in the ovary as well as lipids reallocated from other body stores contributes to oocyte growth and accumulation of yolk and lipid. The lipid composition of stripped eggs, in particular essential fatty acid contents, was found to differ between wild-caught and farmed female broodstock and for farmed eels in relation to feed type. Computer tomography (CT) was applied to study mobilisation of body stores. Voxel bases analysis of CT scan showed that female eels treated with different hormone doses followed the same overall organ developmental pattern, oogenesis (classified according to histological characteristics), and energy allocation. Thus, the gross morphological changes in the body during maturation were equivalent for all fish, but the regime of hormone treatment affected the duration of oocyte and ovarian development. CT scan as well as ultrasound scanning was found to be a useful and non-invasive method to monitor female responsiveness and ovarian development.

Evaluation of conditioning, traditional treatments and novel methods and development of protocols to induce vitellogenesis included conditioning using temperature and swimming as natural triggers, application of different doses of hormone, and hormone producing implants. Temperature and swimming prior to onset of hormonal treatment did not work as a trigger of natural maturation, although swimming had a slightly positive response in wild silver eels compared to farmed female eels. Cold temperature conditioning prior to induction of vitellogenesis tended to have a negative effect on female responsiveness and reproductive success. Subjecting farmed female European eels to endurance swimming prior to treatment neither triggered maturation. However, the maturation process of wild-caught female silver eels was partially stimulated by swimming, suggesting that the initial maturational status of the female eel determines whether it is sensitive to exercise-induced sexual maturation. The use of natural triggers to induce sexual maturation of European eels may require a more integrated approach where multiple parameters (temperature, light, exercise, salinity) are simultaneously changed.

Experiments investigating temperature effects during the maturation process showed that temperature modulated the progression of vitellogenesis. Results indicated that low temperatures (10 to 15°C) may favour the early vitellogenic process, as indicated by higher steroid plasma levels and higher ovarian aromatase expression, but high temperatures (18 to 20°C) caused an acceleration of the last vitellogenic steps. It is suggested that the use of low and variable temperatures during early vitellogenesis may provide better egg quality in terms of buoyancy and embryonic development.

Implant technology as a potential novel treatment to induce vitellogenesis was evaluated in medium-scale experiments. Multiple consecutive injections with eel LH-producing implants induced a significant increase in eye index and blood plasma estradiol levels and also induced a significant increase in gonadosomatic index (GSI); however, the increase in GSI was much lower than in eels treated with carp pituitary extract and the implant treatment did not result in full maturation and ovulation. The half-life of the implant-derived eel LH in the eel's blood circulation was too short. Full maturation probably requires continuous high blood plasma levels of LH; above a threshold that could not be reached with the implants, even after repeated injections.

The standard hormonal treatment using weekly injection of CPE and SPE still appears to be the most efficient treatments to induce vitellogenesis in female European eel. Both SPE and CPE are able to induce vitellogenesis and produce viable eggs. In full-scale experiments, a constant, weekly dose of SPE yielded higher response rates and reproduction success than an increasing dose. In latter, lower SPE level at onset of maturation delayed the maturation process and the increase in dose after eight weeks did not remedy this effect. Using a constant low dosage led to full maturation but increased the duration of the vitellogenesis and the number of non-responders. Although both treatments lead to final maturation in a number of females, the response rate and reproductive success using the constant dose was repeatedly found to be higher.

Final oocyte maturation and ovulation (WP 7)

The purpose of WP7 was to enhance ovulation success, egg quality and embryonic developmental competence by improving methods to induce final oocyte maturation and ovulation.

After the oocyte completes its growth, it becomes ready for the next phase of oogenesis, i.e. the resumption of meiosis, which is accompanied by several maturational processes in the nucleus and cytoplasm of the oocyte. This process, called oocyte maturation, occurs prior to ovulation and is a prerequisite for successful fertilisation. It consists of breakdown of the germinal vesicle (GVBD), chromosome condensation, and assembly of the first polar body. It is established that oocyte maturation in fish is regulated by three major mediators; gonadotropin, maturation-inducing hormone (MIH), and maturation-promoting factor (MPF), which function sequentially at the level of the ovarian follicle cells, the oocyte surface, and oocyte cytoplasm. In many teleosts, blood luteinising hormone (LH) levels begin to rise at or near the time when vitellogenic growth of the ovarian follicle is completed. LH binds to its receptor on granulosa cells of the follicle and stimulates a sequence of events that includes acquisition of oocyte maturational competence, production of MIH, and MIH-dependent resumption of meiosis (nuclear maturation) and cytoplasmic maturation.

During the first part of the project, samples of blood and ovaries were obtained for studies of the physiological regulation of final stages of eel oocyte maturation. Attainment of individual and detailed plasma sex steroid profiles during final stages of oocyte development revealed that DHP, assumed to be the maturation inducing hormone (MIH) in eel, occurs at very high plasma concentrations, but also that this steroid is subject to rapid changes throughout the process (Fig. 5).

It is also apparent that injection of DHP influences the level of other sex steroids like testosterone, 11-ketotestosterone, and oestradiol-17 β .

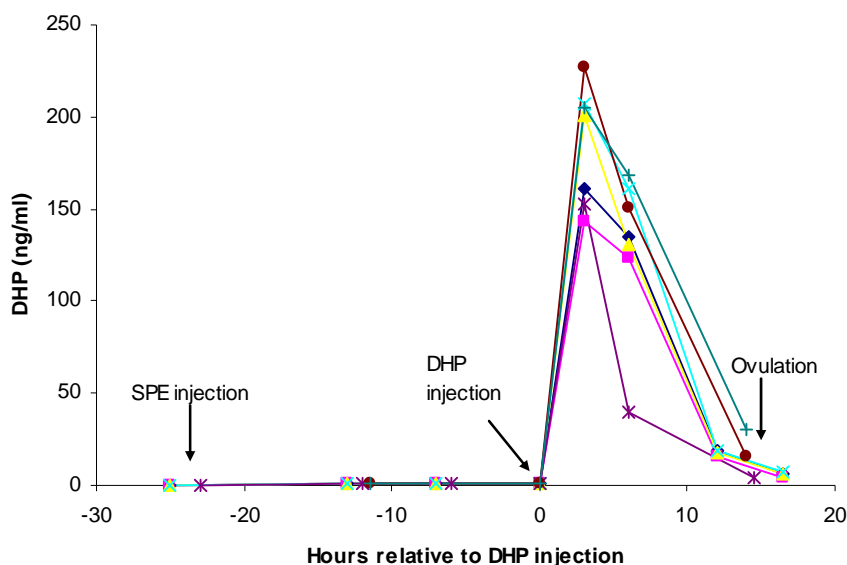


Figure 5 Temporal changes in plasma DHP concentrations during induced female European eel maturation. Standard protocols for SPE and DHP induced maturation were applied (H. Tveiten, NOFIMA).

The establishment of an *in vitro* protocol both for sex steroid synthesis and final oocyte maturation has created a valuable tool for clarifying the role of steroid hormones in oocyte maturation and ovulation. Results from *in vitro* induced oocyte maturation indicate that oocytes can respond to DHP both at an earlier stage and at a lower hormone concentration than those applied in protocols for induced maturation *in vivo* (Fig. 6).

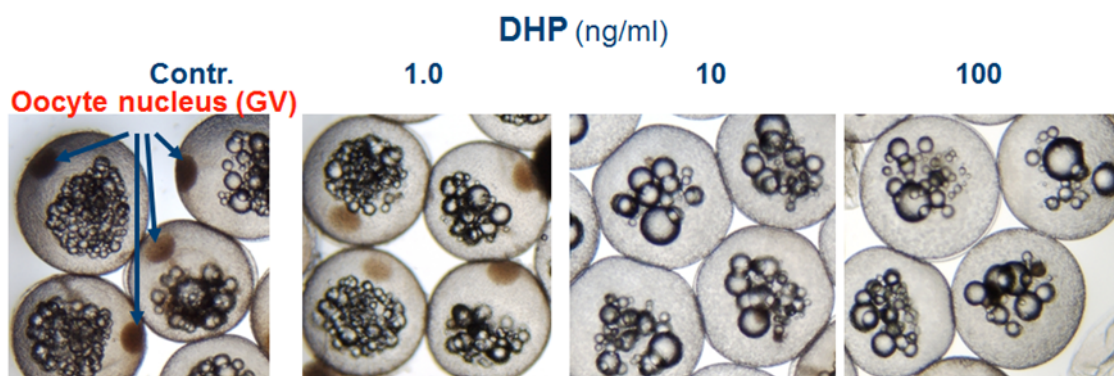


Figure 6 Effects of 17,20b-dihydroxy-4-pregnen-3-one (DHP) on *in vitro* germinal vesicle breakdown (GVBD) in European eel oocytes (H. Tveiten, NOFIMA).

From several experiments, it was concluded that DHP can induce oocyte maturation *in vitro* at concentrations higher than 10 ng/ml, once oocytes have entered the migratory nucleus stage. DHP concentrations above 10 ng/ml did not increase the capacity of DHP to induce maturation. The studies have, however, revealed a discrepancy between DHP concentrations necessary to induce maturation *in vitro* (10 ng/ml) with those necessary to induce maturation *in vivo* (150 to 200 ng/ml) (Fig. 6).

The observed discrepancy between the ability of DHP to induce maturation *in vitro* and *in vivo* (see above) may be related to steroid metabolism and, thereby, loss of its biological function. Analyses revealed that DHP was the main steroid produced in mature eel ovaries *in vitro*, but also that most of

the DHP is metabolised to a DHP-sulfate conjugate. Together, these results indicate that the follicles are able to produce DHP in reasonable amounts, but that the steroid is rapidly metabolised to a biologically inactive compound. Consequently, a protocol to indirectly change *in vivo* DHP concentrations during maturation was developed. The rationale of this protocol was to stimulate DHP synthesis by gradually increasing SPE concentrations and the precursor (17P) for DHP synthesis. Thus, DHP would likely appear at more physiological relevant concentrations. Females were treated with 3 subsequent injections with a combination of SPE and 17P spaced by about 12 hours. Treatment resulted in female ovulation and progress in oocyte development. A new protocol to directly influence DHP concentrations was also designed. To change the plasma levels of DHP during maturation, timing, dose and steroid delivery vehicle were altered compared to the standard induction protocol. DHP (0.3 mg/kg) was injected in molten coconut butter at the time of SPE injection. The new protocol transiently elevated DHP to physiological relevant concentrations (c.15 ng/ml) and was able to induce ovulation, but not oocyte maturation.

A DHP receptor (ePR1) qRT-PCR assay has also been established. DHP-receptor expression was relatively stable during the priming period (after SPE injection) of maturation but decreased rapidly after DHP exposure and was low at the time ovulation.

Other strategies to induce maturation were also investigated. Attempts to induce final oocyte maturation through stimulation of pituitary LH release by Gonadotropin Releasing Hormone Analogue (GnRHa) in combination with dopamine (DA) antagonists were undertaken. Females at different stages of development were injected with two successive injections of GnRHa and a DA antagonist (domperidone, DOM). GnRHa and DOM treatment did result in a rapid change in body weight index (BWI) but no ovulation was recorded. GnRHa+DOM also resulted in a small and transient increase in plasma DHP level, but this elevation in DHP was apparently not sufficient to induce OM/ovulation. Plasma sex steroid responses to GnRH treatment differed substantially between individuals and the steroid under investigation. Our data indicates that the steroid responses were inversely related to initial plasma E2 concentration.

Injection of GnRHa induced a two-fold increase in the expression of both pituitary gonadotropins. This increase was significant for LH-beta, but not for FSH-beta. Injection of domperidone also induced a significant increase in the expression of LH-beta, but no increase of FSH-beta. These results indicate that pituitary LH expression in matured female eels can positively respond to GnRH stimulation and to the blockage of dopamine inhibitory control. Injection of a GnRH agonist induced an increase in the expression of the three GnRH receptors subtypes, however only significant for GnRHR2. In contrast, domperidone had no effect on the expression of GnRH receptors. These results suggest an auto up-regulation by GnRH of its own receptors.

An experimental trial was undertaken to investigate the potential effect of matured male pheromones on matured females. Exposure to spermiating males induced significant increases in expression of both pituitary gonadotropins. Concerning GnRH receptors, exposure to spermiating males induced a selective significant increase in the expression of GnRHR1a, with no change in the expression of the other two subtypes. These results suggest that the exposure to males, possibly via pheromones, may trigger an increase in female pituitary sensitivity to GnRH and pituitary gonadotropin expression. Also, experiments have shown that during final stages of development females release large quantities of possible pheromonal cues (DHP and DHP-sulphate) to the water. Both these compounds are known to have pheromonal effects in other species.

Progress has also been made in the studies of gonadotropin and sex steroid receptor expression in the ovary during final stages of maturation. SPE treatment appears to transiently up-regulate the Estradiol nuclear receptor alpha (ERa), before the expression drops rapidly before ovulation. The expression of the gonadotropin receptors, FSHR and LHR2, did not respond to SPE and generally

decreased throughout oocyte maturation (OM). An inverse relationship between plasma E2 concentrations and LHR2 expression was recorded.

Expression of various hormone receptors was studied at different stages of maturation on biopsies from females producing “good” and “poor” quality eggs. Relationships between oocyte/egg gene expression and developmental competence were established. At SPE injection, ovaries resulting in good eggs had generally a higher expression of all the investigated genes, compared to that of the poor quality eggs. Ovarian expression of LHR2 at the time of SPE injection explained about 45% of the variation and in hatching rate of the resulting eggs/embryos.

In conclusion, improved protocols for induction of final maturation and ovulation have been developed during the course of the project. The results obtained indicate that the timing and dose of the injected DHP are critical factors to obtain good quality eggs. Also, since GnRHa and dopamine antagonist treatment, so far, did not give the desired effect on DHP synthesis and release, future focus should be on systematically changing the timing and dose of the DHP. The obtained data have also provided integrated analyses of female characteristics, treatments (SPE and DHP), fertilisation success and embryonic development competence. Improved methodologies have been applied to investigate the state of maturation of oocytes obtained from biopsies in order to evaluate the timing of priming and induction of final maturation. Development of methods for nuclear and follicular staining has provided useful information about GVM, GVBD, and ovulation success of stripped eggs. Results obtained using these methodologies to assess final maturation and ovulation success has been integrated in new induction protocols.

Fertilisation, incubation and larval rearing techniques (WP8)

The aim of WP8 was to improve fertilisation rates, embryonic and larval survival and to develop suitable feed for larval culture. This covers establishment of adequate fertilisation procedures and development of incubation and rearing methods and technology.

The work performed can be divided into the following major categories:

- 1) Optimisation of egg fertilisation and incubation procedures to maximise the production of viable larvae
- 2) Enhancement of larval incubation conditions to maximise the longevity of the larvae and produce adequate amounts of larvae at first feeding
- 3) Perform first feedings trials using several types of live and inert diets

1) Optimisation of egg fertilisation and incubation procedures to maximise the production of viable larvae. A substantial amount of work was invested into the development of a standardised procedure for egg fertilisation and egg incubation. Of primordial importance was the development of an egg classification system that allowed us to estimate the quality of the eggs during embryonic development, as well as to characterise the type of abnormality in the case of egg development failure. The classification system for developing eggs was in first instance based on the cleavage pattern regularity at the 4 to 64-cell stage using standard microscopic monitoring procedures. The evaluation focused on a relatively simple split into two types of cleavages: type 1a) regular cell divisions with cells of equal size and shape (and high expectancy of hatching), and type 1b) irregular cell division (and variable expectancy of hatching). Several other egg type classifications were based on the abnormality during development that resulted in non-hatching of the egg.

The egg classification system was applied during a large variety of experiments. A first series of experiments focused on optimising egg activation and fertilisation. The final suggested protocol included the storage of milt from males following extrusion in an immobilisation solution similar to the seminal plasma (to prevent premature activation). This procedure was done directly prior to extrusion of the eggs from the female. It was found that high fertilisation success was achieved

when a sperm cell to egg ratio of 25,000:1 was applied in combination with fertilisation within 10 minutes after stripping of the eggs. Next, the incubation of eggs at a temperature of 20°C was found to be optimal in achieving maximised embryonic development and hatching success (as compared to temperatures of 12, 16, and 24°C). Besides temperature also salt type and salt concentration in the egg activation and incubation medium were found important. The optimal salinity was egg batch dependent but always ranged from 30 to 40 psu. Salt type was of high importance for proper egg activation and 3 salt types were identified, of which two were artificial sea salts, to optimise the standardisation of the activation and incubation medium. Finally, it was suggested to incubate the eggs in complete darkness as light was found to significantly decrease egg hatch success.

In addition to physico-chemical parameters, a series of experiments showed that the composition of the microbial community in the incubation water of the eel eggs was of high importance for egg embryonic development. Uncontrolled microbial activity was responsible for more than 50% of the hatch success and especially in the last half of egg incubation, bacteria induced detrimental effects. The hypothesis was formulated that microbial management (i.e. controlling which bacteria are allowed in the presence of the eggs and thus to grow on the egg chorion) is important during egg incubation to maximise hatch success and embryonic development.

2) Optimisation of larval incubation conditions to maximise the longevity of the larvae and produce adequate amounts of larvae of first feeding age. As the work on egg fertilisation and egg incubation resulted in the efficient production of egg batches producing high numbers of larvae, the next challenge was to optimise larval incubation conditions to maximise longevity. Work focused on the identification of optimal biological and physico-chemical conditions, as well as the development of the most suited incubation systems. To allow research on larval incubation to be performed at the facilities of different partners, a long distance transportation protocol was developed that allowed to efficiently transport eggs at 30 to 32 h post fertilisation within 15 h to a partner that then incubated eggs prior to hatching. The protocol did not affect the hatching success of the eggs, or the longevity of the larvae.

What concerns the actual incubation of the larvae seemed to be the light regime that was applied in the larval rearing system. European eel larvae were found to be highly negatively phototactic, a feature which develops in the period from 10 h to 30 h post hatching. Incubation under dark conditions or the use of red low intensity light in a 12 h photoperiod could be suggested as optimal for the culturing of European eel larvae at the pre-leptocephalus stage. The fact that the larvae seemed negatively phototactic corresponds to the idea that they live at greater depths with limited light during the earliest life stages. These observations were also supported by the findings that the creation of turbid water (by addition of clay or algae) could also considerably increase larval culture success, although it remains to be elucidated whether this was a mere physical effect or may be related, for example, to the stimulation of the larval digestive system. In addition to light, the temperature during larval incubation seemed to strongly affect larval development. Normal tissue formation in yolk-sac stage larvae seemed to be highest and the number of malformations seemed to be lowest at a temperature of 20°C. Incubation at 12, 16, and 24° C had a negative effect on survival rates, indicating that these temperatures were suboptimal for egg incubation. The optimum temperature of 20°C is in accordance with the findings that have been concluded for the Japanese eel, and with the temperature conditions in the Sargasso Sea, where European eel larvae originate in nature.

In addition to individual parameters, several types of larvae incubation systems were developed and evaluated throughout the PRO-EEL project. These ranged from small-scale (15 L) to large-scale (5000 L). A system at IMR that resulted in high and long survival was the large-scale silo system. This system consists of a steep cone, upwelling, and salinity-separated top and bottom water layer, avoiding larvae to be drained in the outlet at the surface or minimising contact with the bottom. A

salinity gradient from full-saline seawater (35‰) to about 30‰ was established by means of freshwater entering the upper 1.5 m of the water column through a vertical gradient tube (Fig. 7). The gradient created this way was continuous. This proved successful in maintaining substantial numbers of larvae surviving up to the period of expected first feeding.

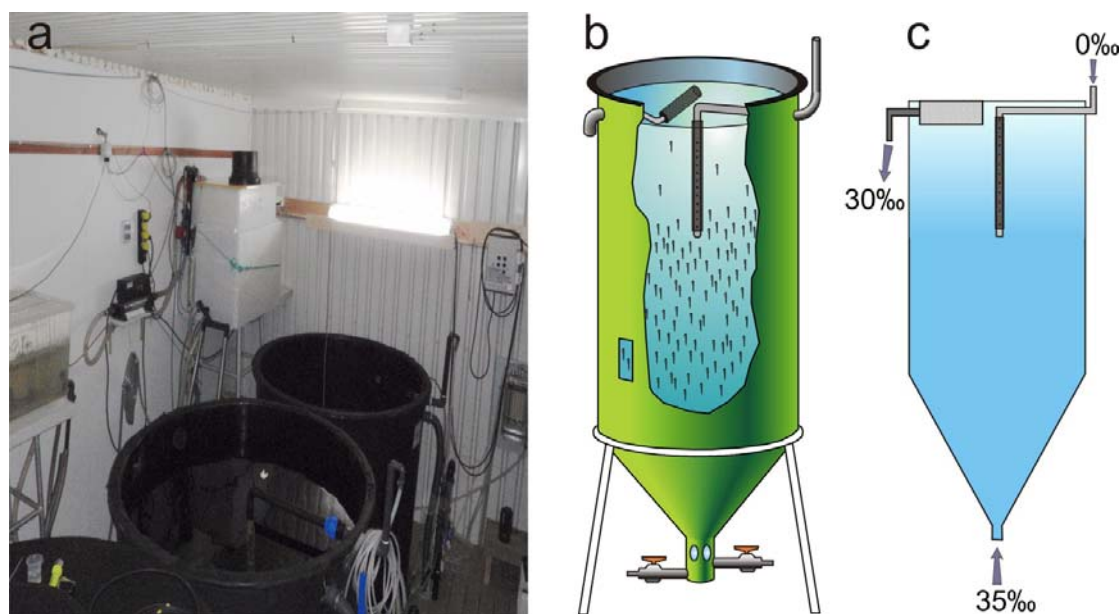


Figure 7 C5000 culture system at IMR based on two 5000 l fibreglass silos with a salinity gradient in the upper 1/3 of the volume. **a)** Photograph of the silos with header tank for freshwater or brackish water on the wall to the left, header tank for full saline seawater in the middle (covered with insulating material), titanium heater (between the two header tanks), temperature logger (on the wall above the titanium heater), and a dimmable light tube system (on the wall to the right). **b)** Schematic outline of a silo with larvae inside. Gradient tube and outlet sieve are seen at the top, and valves for tending and inlet of full saline seawater are at the bottom. Inspection windows of acrylic are indicated on the silo wall and at the bottom for control of tending. **c)** Schematic outline of salinity gradient and water flow directions in a silo (van der Meeren, IMR).

A step forward was achieved in characterising the influence that micro-organisms have on the larval culture performance. Bacteria not only negatively affected egg hatching success and embryonic development, but also had an influence on the longevity of larvae. For this reason, the application of small-scale recirculation aquaculture systems (RAS) for microbial maturation of the larval culture water was initiated. By the maturation of the culture water, the aim was to lower the ratio of opportunistic (harmful) over commensal (beneficial) bacteria in the larval incubation water. The use of the RAS was performed with success for maintaining substantial numbers of larvae surviving up to the period of expected first feeding reaching a maximal longevity of 22 days post hatch.

3) Perform first feedings trials using several types of live and inert diets. The availability of high numbers of larvae at first feeding stage through optimisation of larval incubation conditions offered the opportunity to perform first feeding trials that included formulated diets and live feed. Different feed types and feed compositions were tested. Inert feeds were either formulated based on information obtained from digestive enzyme analyses in larvae at first feeding age, which yielded insight into larval nutritional requirements (high requirement of lipids) or were based on knowledge gained from experience with other marine fish species. The use of the inert diets did not result in increased survival of the larvae and ingestion of these inert diets could be observed in only a low number of larvae. Despite the fact that the inert diets did not seem optimal, behavioural studies

showed that the sensory system of the larvae was triggered by the presence of the feed in the water. It thus offers opportunities for future development of inert feeds.

Several types of live feed were tested as well. These included plankton from a zooplankton mesocosm production system, rotifers (*Brachionus* sp.) and *Artemia*. All of these feed types were applied in the C5000 silo system for larval development. Within this environment a few larvae ingested rotifers. Even though ingestion implicates active swallowing, it is far from certain that the uptake was an active choice by the larvae. Based on information obtained from analyses in WP9 concerning potential prey size and larval biting forces in combination with literature information on suggested live prey for Japanese eel, it seems that a smaller type of prey will be needed for European eel larvae.

Development and quality assessment of embryos and larvae (WP9)

The purpose of this WP was to delineate major trends in embryonic and larval development with reference to behaviour, morphological changes and somatic growth in order to provide relevant evaluation criteria for successful development of eggs and larvae.

Several of the experimental series resulted in successful egg development and hatching of viable larvae. The development of embryos and larvae was followed intensively. Larval quality was evaluated based on developmental, phenotypic plasticity, and behavioural performance, with the main objective to provide feedback to other WPs for improving larval quality and rearing conditions. Larvae of the European eel have only been reared in a few earlier studies, and none have presented the amount of detailed information on the early life stages as assembled throughout the present work. Hence a range of important achievements were achieved.

1) Basic components of somatic growth were outlined and we showed a major maternal influence related to the amount of yolk in the egg. The work on morphological analyses of embryos and larvae was extensive and developmental rates were analysed. This work outlined larval growth patterns based on several series of egg and larvae batches from the full-scale experimental series. During the period of absorption of yolk and the oil droplet, the larvae undertake drastic changes in body and head morphology. At full yolk-sac absorption larvae (approximately 12 days post hatch at 20 °C) have a functional digestive tract, pigmented eyes and large protruding teeth. Morphological changes during early ontogeny are illustrated in Figure 8. We found a marked relationship between larval nutritional resources cf. yolk-sac volume and enhanced growth during the yolk-sac stage, and thus a strong maternal influence on larval size, development, and growth. This influence was apparent already in the egg stage and reflected in larval sizes at hatching.

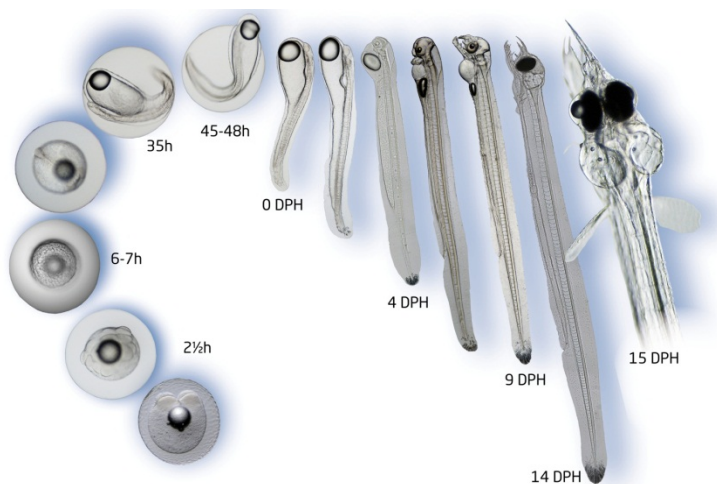


Figure 8 Summary of the embryonic and larval development of European eel bred and reared in captivity (S.R. Sørensen, DTU).

2) From a poor muscle development at hatch, a rapid muscle differentiation takes place coinciding with development of other major organs. Analysis and histological sectioning of the larval body was carried out focussing on muscle and organ development and linked to the rearing temperature. The muscle development was related to growth of other major organs, and implications for locomotion activities were evaluated. Newly hatched larvae had very poor muscle development with mainly immature precursor cells at the anus reference point, indicating a rather poor ability to swim by anguilliform movements, as only their anterior body part would have some functional muscle tissue. Immediately after hatching, these larvae were also mostly passive, hanging vertically in the water column. During the first three days after hatching, the observed rapid muscle differentiation coincided with development of other major organs such as otic vesicles and pericardium. At the same time, the number of myotomes was increasing rapidly, muscle tissue was growing and became more mature and differentiated (Fig. 9). The development up to 12 days of age showed a progressive muscle growth of a tall and slender one cell wide white muscle layer, surrounded by a one cell wide red muscle layer. During this later yolk-sac stage, at about 10 days of age, the larvae began swimming horizontally by undulating body movements or by high frequency beating of the posterior body part and tail.

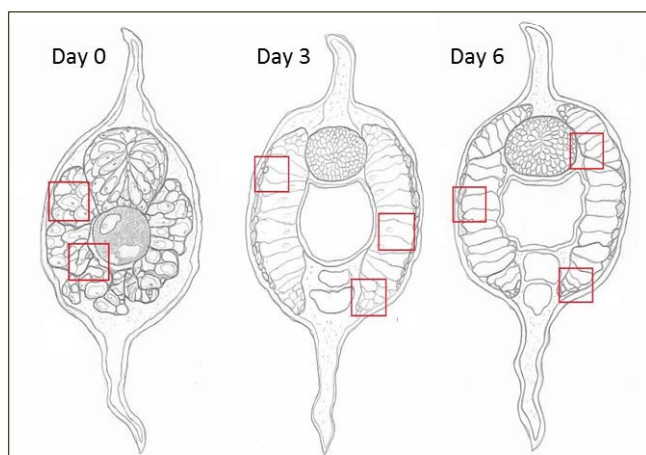


Figure 9 Cross sections of European eel yolk-sac larvae 0 to 6 days post hatch, just behind the anus, demonstrating the muscle development. The red boxes indicate areas where electron micrographs were taken (E. Kjørsvik, NTNU).

The one layer muscle cell structure was clearly adapted to the leptocephalus type of development, and is quite different from muscle organisation found in elvers and adult eels, or in other teleost larvae. Larvae may start feeding around 12 days after hatching (20 °C), based on our studies of larval development of digestive functions. How start-feeding is affecting further muscle growth and development, and how the muscle growth of feeding larvae is developing in relation to the glycosaminoglycan layer in the older leptocephali stages, is still largely unknown.

3) Larvae have peculiar teeth morphology and orientations; a very weak biting force indicates very soft natural food particles. Another important question taken up in the larval investigations was larval feeding capacity, analysing the ontogeny of the musculoskeletal system of the feeding apparatus. By this we wanted to assess the efficiency of the feeding apparatus to deal with inert food and different types of live feed. Histological sections were photographed and these digital images were uploaded and re-aligned. All cartilaginous and bony elements, as well as ligaments, tendons and muscles, were traced and identified (Fig. 10). To obtain an overall idea of the morphology of the larva, bigger anatomical structures (eyes, brain, heart, otic vesicle etc.) were reconstructed as well. Most striking

features are the peculiar teeth morphology and orientations, the presence of branchial arches (no gills), a non-rigid operculum and the ligament between the hyoid and the lower jaw. Using this reconstruction, an estimation of both the bite force and the kinematic efficiency of the present hyoid four-bar mouth opening mechanism were done. To further analyse the functionality of the system, a preliminary kinematic analysis on *in vivo* video-recordings of larvae was additionally performed in relation to the full-scale experiments, which focused on the jaw mechanism. As a combined result, a rather small bite force ($\pm 50 \mu\text{N}$) and a rather small maximum gape angle of the lower jaw ($\pm 25^\circ$) were obtained.

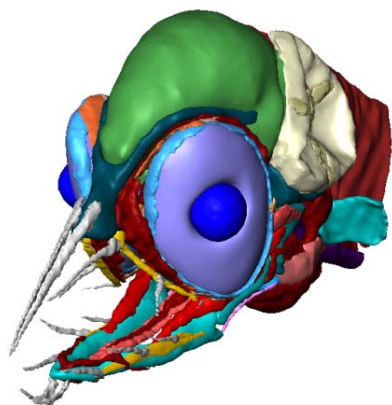


Figure 10 3D-reconstruction of European eel larvae 15 DPF obtained by P1 in WP1 experiments (M. Bouilliart, UGent).

The morphological evaluation of the teeth makes it doubtful that they are used for actual biting, as their peculiar positions suggest them to be highly susceptible to rotation/bending during biting. Still, the distinct attachment sites of the first pair of teeth in both upper and lower jaw may indicate that these teeth are involved in biting. This indicates that the natural feed for the larvae are soft organisms/structures, which they disintegrate and swallow.

4) The enzyme activity in the digestive track showed unusual characteristics and indicated an important nutritional need in neutral lipids. The ontogeny of the digestive system of the feeding apparatus is evaluated through biochemical, histological, and molecular tools from hatching up to exogenously feeding larvae, in order to assess the efficiency and the features of the digestive system and to develop more adequate dry-feed formulations. We were able to trace the presence of digestive enzymes in the pancreas and gut for larvae of different ages. One day after hatching, the digestive tract was poorly developed, but at day 6 after hatch, the digestive tract appeared as a straight tube with a tight lumen.

The activity and mRNA of the most common pancreatic enzymes (trypsin, amylase and lipase) could be detected as early as at day 4 during the eel larval development. At day 7 the activity and the mRNA expression of these enzymes sharply increased suggesting that this developmental stage is crucial for the settlement of a functional pancreas. It is noteworthy that very high lipase activities were detected immediately after hatching, and such enzymatic pattern revealed a specific and important nutritional need in neutral lipids for eel larvae. Our results showed that trypsin and amylase exhibited a similar sharp increase in activity and expression around day 7 post hatch. On the contrary, lipase expression sharply decreased during the same period, and paradoxically its enzyme activity reached very high levels up to 500 times higher than that usually observed in marine fish larvae

5) Lateral and cranial neuromasts are developed in the first few days after hatching, which likely makes them very sensitive to water movement early in their development. The ontogeny of the

sensory apparatus in eel larvae was studied during the days following hatching. The purpose was to assess the timing of their ability to register and process sensory cues required for the onset of feeding, to provide morphological indications of sensitivity to water movement and evaluate the sensitivity of larvae to handling. The approach was to create a time-line for the occurrence of the neuromasts of the lateral line system in eel larvae, and subsequently identifying the types of occurring neuromasts (Fig. 11).

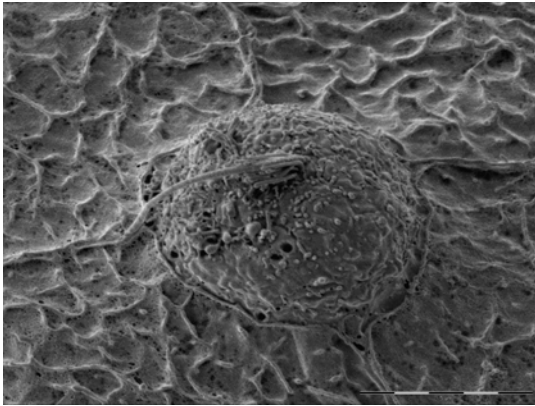


Figure 11 Scanning electron micrograph of a Type II ordinary superficial neuromast from a 4-day old larva (P. Skov, DTU).

For this purpose, scanning electron microscopy was used to identify spatial and temporal occurrence of neuromasts along the lateral line and the cephalic region. The results show that the development of lateral and cranial neuromasts occurs in the first few days after hatching. This suggests that larvae are sensitive to water movement early in their development, but the extent to which this may have implications for their initial feeding behaviour or how they orient themselves in water currents (e.g. drifting behaviour) remains to be determined. The developmental state of the visual system prior to the time considered to be first feeding suggests that eel larvae are visual feeders at that time, while the contribution from olfactory or gustatory senses is unknown.

6) Larval escape speed is extremely fast, potentially injuring them in culture; larval activity and feeding responses are stimulated by the presence of both live and inert feeding items. Behavioural elements were investigated and registered during experiments in different physical, chemical, and biotic environments using video-based observations. Studies on early yolk-sac larvae activity bursts, as well as fast escape bursts have been documented by video-recording (type duration, speed). The escape bursts were very abrupt; the change from passive, vertically oriented position to escape swimming could be measured to only 0.2 s. The speed of the escaping larvae was of a magnitude that they could be injured (broken teeth) if meeting the tank wall. Observations showed that yolk-sac larvae are strongly photophobic; hence they should be reared in very dim light.

For investigation of potential feeding responses we observed four modal action patterns of larvae that received small algae or an inert feed. Observations indicated that presence of algae and inert diet stimulated activity. We also observed that the frequency of s-bends and frequency of attacks changed when potential prey was present. Together, these findings suggest that eel larvae are triggered by the presence of potential food items such as the microalgae and/or inert diet particles during the late pre-feeding period. These will also stimulate the larval foraging behaviour.

In conclusion, observations and analysis performed have enabled us to puzzle together a picture of larval development and behaviour and the range of variability in developmental traits. The detailed knowledge gained in this WP has proven important for our understanding developmental processes of this exceptional organism and our ability to assess larval quality and control the rearing process.

It has become apparent that the eel *leptocephalus* larvae are very different from larvae of all other fish currently used in aquaculture, having the special morphology of the Elopomorphs and being exceptionally sensible to the artificial environmental conditions in aquaculture. As the early ontogeny of European eel, including embryonic development and yolk-sac larvae, remains unknown in nature, this research has provided novel insight into the biology of eel.

1.4 The potential impact and the main dissemination activities and exploitation of results

Potential impact

The PRO-EEL project addresses the European eel, which is a high value species of considerable commercial importance in aquaculture with a substantial market potential. Capture-based eel aquaculture is well-established with efficient technology that applies recirculation aquaculture systems for on-growing from glass eels to market size. However, the present production is critically hampered by the dependence on wild-caught glass eels due to the severe decline of the European eel stock. In 1999, the International Council for Exploration of the Sea (ICES) revealed long-term declines in the abundance of all continental life stages (glass eel, yellow eel, and silver eel) and current advice considers the European eel stock to be severely depleted. Hence, wide-ranging management measures were needed, as the European eel constitutes a single stock, which is shared by over 30 countries.

In 2007, the European Commission initiated Europe-wide eel management plans (Council Regulation (EC) 1100/2007) with the objective to establish measures for the recovery of the European eel stock for implementation by member states in 2009. In 2009, also the inclusion of the species in the Annex II of the Convention on International Trade in Endangered Species (CITES) was enforced, prohibiting international trade of European eel into and out of the EU. Furthermore, European eel was added to the red list of the International Union for Conservation of Nature and Natural Resources (IUCN) in 2010, and its present status is considered as being critically endangered. Although, a recent upward trend in the recruitment of the European eel is a positive sign, the decline of the stock causes severe concern about the future of European eel as a commercial exploitable species in capture-based aquaculture. Therefore, the establishment of captive breeding methods and hatchery technology for a self-sustained eel aquaculture has become a target for producers, managers, and researchers.

In this context, the aim of PRO-EEL has been to advance research-based methods for a production of viable offspring from wild-caught and farmed broodstock and develop techniques for culture of early life stages for a future self-sustained aquaculture of European eel. The project has succeeded in enhancing breeding technology as well as in establishment of larval culture to the first feeding stage. This will not immediately lead to production of glass eels due to the long duration of the larval stage and limited knowledge about larval feed, physiology, and essential life requirements. Thus, further research targeting suitable feeds, feeding regimes, and culture systems will be needed to enhance larval performance for an efficient hatchery practice. Also breeding practices including hormonal treatment and improved reproductive performance of farmed eel broodstock needs further attention. However, with the significantly expanded knowledge base and technology, it is expected that novel treatment for breeding and rearing techniques for feeding larval culture will be established in the near future. The results and achievements of the PRO-EEL project represent a major breakthrough in European eel research and a very important and promising step towards continued controlled reproduction and a sustainable aquaculture of European eel.

An eel aquaculture being self-sustained with glass eels will improve competitiveness in the industry and contribute directly to ensuring the sustainability of aquaculture for this valuable species. The global demand and markets for eel are substantial and establishment of a captive glass eel production would enable the aquaculture industry to access these profitable markets. Targeted markets include not only European markets but also significant Asian markets, e.g. markets in Japan, China, and Korea. The Asian markets are presently inaccessible due to European eel trade restrictions (CITES), but would be restored by captive glass eel production. Furthermore, it is anticipated that a sustainable eel aquaculture will diminish the current pressure on the wild eel stock, thereby contributing to established measures for the conservation and recovery of the stock of European eel as targeted by the Council Regulation (EC No. 1100/2007).

Future captive breeding and hatchery technology will generate a new commercial activity that ultimately can re-establish the highly profitable eel market for the European aquaculture industry. This includes suppliers of fish feed and aquaculture systems, as well as retail supply chains and food processing activities (e.g. eel smoking), thereby adding value to the sector and society. In addition to its commercial value, the eel has substantial cultural value, being target for fisheries for thousands of years and an integral part of traditional food culture in many European countries as well as a species known to most people due to its intriguing life cycle.

A particular strength of the PRO-EEL project has been its interdisciplinary approach and the unique expertise of the consortium. The PRO-EEL consortium has brought together leading institutes and experts in European eel reproduction complemented by excellence in disciplines filling gaps in scientific knowledge and technology. Furthermore, a tight collaboration with the aquaculture industry has promoted the applicability of developed protocols and technology, which will benefit future initiatives to substantiate progress in commercial eel breeding and hatchery technology.

Main dissemination activities

The PRO-EEL dissemination strategy has focused on publication in peer reviewed scientific journals and presentations at targeted international scientific conferences, including aquaculture conferences and physiology/endocrinology symposia. A high dissemination level and the targeted fora have contributed significantly to the extensive outreach the PRO-EEL has had to scientists and stakeholders, and to the positive feedback that the project has received. Furthermore, an active project website, leaflets and two networks, a scientific and a stakeholder network, have disseminated results in a popular way to a broader audience including the aquaculture industry. Press releases, Newsletters and conferences have raised the awareness of international and national press and media and resulted in newspaper articles, interviews and popular science publications. Together these dissemination activities have brought substantial attention to the results and progress of the PRO-EEL project.

Collaborative research has been carried out among PRO-EEL partners in joint experiments conducted at different locations and samples have been analysed in cooperation. As a result many publications and presentations comprise an array of project partners, making the cross cutting approach of PRO-EEL and excellent international collaboration visible. During the project period, more than 40 publications in high-ranking international journals plus three book chapters have been published and another approximately 20 manuscripts are *In Preparation*. Most publications are easily available either via “open access journals” (e.g. PlosOne) or are available in repositories (e.g. ResearchGate).

The dissemination of results has met many fora around the world and PRO-EEL results were presented at a wide range of international symposia and workshops. Thus, results have been presented as more than 85 oral or poster contributions at international scientific conferences and

another 84 presentations were given at other international meetings. This has contributed significantly to the wide outreach of the PRO-EEL project and broadened the general interest in eel reproduction research and applied breeding technology.

Three project workshops and an international symposium were part of the dissemination activities of the PRO-EEL project. The consortium has organised or taken active part in organising the following workshops and conferences:

- PRO-EEL Collaborative Workshop 2012, Valencia, Spain
- Aquaculture Europe 2013, Trondheim, Norway
Session: Eel Focus – Aquaculture development (EU PRO-EEL Project)
- The 3rd Eel Genome Symposium 2014, Leiden, The Netherlands
- Aquaculture Europe 2014, San Sebastian, Spain
Session: Progresses in Eel Aquaculture Research

The first PRO-EEL collaborative workshop was held in Valencia hosted by ICTA-UPV and included interaction with two other EU research projects “SELFDOTT” and “LIFECYCLE” as well as a COST Action “Larvanet”. The two following workshops were invited and organised as specific sessions at Aquaculture Europe, which provided the opportunity to reach a broad aquaculture forum. These sessions were very well attended and rewarded. As these workshops were targeting the aquaculture society and stakeholders, a specific symposium related to eel physiology and molecular studies was held in 2014 to disseminate specifically these areas of the project. This symposium in particular focused on scientific progress resulting from the mapping of the European eel genome (and other anguillid eels). The combination of the workshops and the symposium made it possible to enhance the outreach to different groups of researchers and stakeholders, and at the same time promote contact and collaboration among PRO-EEL researchers and researchers in Asia, North America, Australia, and New Zealand that all have eel aquaculture research.

Exchange of staff inclusive researchers, PhD and MSc students has benefitted the researchers and students as well as the PRO-EEL project. In this context, PRO-EEL has been successful in attracting a considerable number of young researchers and thereby supporting education and young researcher training. This includes 10 BSc students, 10 MSc students, 15 PhD students, and 8 Post-Docs. Accordingly, an array of scientific theses has been elaborated and these young researchers have significantly added to achievements and dissemination of results.

Innovation and exploitation of results

The efficient dissemination of generated results and foreground knowledge that includes basic research, application development and aquaculture techniques provides the opportunity to use these new insights to further substantiate progress in controlled reproduction and larvi-culture technology of eels. The knowledge base and developed methods and techniques, however, are not only relevant for eels; new insight into different aspects of genomics, physiology, morphology, etc. also can be of relevance to other (new) species in aquaculture and to other research areas.

Novel results and insights obtained in different research disciplines in PRO-EEL have advanced the field including innovative techniques and technology. Further research, application development and fine tuning of innovative biotechnologies, culture systems and larval diets may lead to future exploitable foreground and products.

Concluding remarks and perspectives

In conclusion, the establishment of captive breeding methods and hatchery technology for a self-sustained eel aquaculture is critically needed in order to produce glass eels in captivity for a self-

sustained and sustainable aquaculture. The PRO-EEL consortium set out objectives to advance current knowledge and develop research-based methods for a production of viable offspring from wild-caught and farmed broodstock and develop techniques for culture of early life stages. The project has succeeded in enhancing breeding technology and in establishing larval culture to the first feeding stage, which is promising for a future self-sustained aquaculture of European eel. These results and their dissemination via Aquaculture Europe Conferences and the international press have brought interest to eel as a potential new species in aquaculture breeding and hatchery technology. Hereby, it appears that PRO-EEL, during the course of the project, has accomplished to turn the general opinion of eel as a species characterised by reproductive failure in captivity to a potential new species in captive breeding and hatchery technology for a sustainable eel aquaculture.

1.5 Project public website and contact information

PRO-EEL public website www.pro-eel.eu

The official PRO-EEL website was launched in July 2010. The website is and will continue to be updated regularly for at least the next three years. The website presents project objectives, partners, activities, results, and dissemination of the project. In particular, the latter will continue to be updated.

Contact information:

Project scientific coordinator

Jonna Tomkiewicz, Dr.

Technical University of Denmark, National Institute of Aquatic Resources

Tel: +45 35883408

E-mail: jt@aqua.dtu.dk

Project manager

Ole Henrik Haslund.

Technical University of Denmark, National Institute of Aquatic Resources

Tel: +45 35883393

E-mail: ohh@aqua.dtu.dk

2 Dissemination and use of foreground

2.1 A - Dissemination (public)

This section lists peer reviewed articles published, accepted or in review in international scientific journals. In addition to these publications a substantial number of scientific publications are in preparation.

A1 List of scientific, peer reviewed publications

- 2011: Pasquier J., Lafont A.G., Leprince J., Vaudry H., Rousseau K., Dufour S. 2011. First evidence for a direct inhibitory effect of kisspeptins on LH expression in the eel, *Anguilla anguilla*. *Gen. Comp. Endocrinol.* 173, 216-225.
- Burgerhout E, Brittijn SA, Kurwie T, Decker P, Dirks RP, Palstra AP, Spaink HP, van den Thillart GEEJM. BMC. 2011. First artificial hybrid of the eel species *Anguilla australis* and *Anguilla anguilla*. *Developmental Biology* 2011, 11:16
- Pérez, L., Peñaranda, D.S., Dufour, S., Baloche, S., Palstra, A.P., van den Thillart, G.E.E.J.M, Asturiano, J.F. 2011. Influence of temperature regime on endocrine parameters and vitellogenesis during experimental maturation of European eel (*Anguilla anguilla*) females. *Gen. Comp. Endocrinol.* 174, 51-59.
- Tomkiewicz, J., Kofoed, T.M.N. and Pedersen, J.S. 2011. Assessment of testes development during induced spermatogenesis in European eel *Anguilla anguilla*. *Marine and Coastal Fisheries: Dynamics, Management and Ecosystem Science* 3, 106-118.
- 2012: Gallego, V., Mazzeo, I., Vilchez, M.C., Peñaranda, D.S., Carneiro, P.C.F., Pérez, L., Asturiano, J.F., 2012. Study of the effects of thermal regime and alternative hormonal treatments on the reproductive performance of European eel males (*Anguilla anguilla*) during induced sexual maturation. *Aquaculture* 354-355, 7-16.
- Henkel CV, Burgerhout E, de Wijze DL, Dirks RD, Minegishi Y, Jansen HJ, Spaink HP, Dufour S, Weltzien FA, Tsukamoto K, van den Thillart GEEJM, 2012. Primitive Duplicate Hox Clusters in the European Eel's Genome. *PLoS ONE* 7(2)
- Henkel C.V., Dirks R.P., de Wijze D.L., Minegishi Y., Aoyama J., Jansen H.J., Turner B., Knudsen B., Martin M., Kenneth Hvam K., Boetzer M., Pirovano W., Weltzien F.A., Dufour S., Tsukamoto K., Spaink H.P., van den Thillart GEEJM. First draft genome of the Japanese eel, *Anguilla japonica*, constructed via a versatile de novo assembly pipeline. *Gene* 511, 195-201.
- Mazzeo, I., Peñaranda, D.S., Gallego, V., Hildahl, J., Nourizadeh-Lillabadi, R., Asturiano, J.F., Pérez, L., Weltzien, F.A., 2012. Variations in the gene expression of zona pellucida proteins, *zpb* and *zpc*, in female European eel (*Anguilla anguilla*) during induced sexual maturation. *General and Comparative Endocrinology* 178(2)
- Minegishi, Y., Dirks, R.P., de Wijze, D.L., Brittijn, S.A., Burgerhout, S.A., Spaink, H.P., van den Thillart, G.E.E.J.M., 2012. Quantitative bioassays for measuring biologically functional gonadotropins based on eel gonadotropic receptors. *Gen. Comp. Endocrinol.* 178, 145–152.

Minegishi Y, Henkel CV, Dirks RP, van den Thillart GEEJM, 2012. Genomics in Eels — Towards Aquaculture and Biology. *Marine Biotechnology* ;DOI 10.1007/s10126-012-9444-5

Pasquier, J., Lafont, A.G., Jeng, S.R., Morini, M., Dirks, R.P., van den Thillart, G., Tomkiewicz, J., Chang, C.F., Rousseau, K., Dufour, S., 2012. Multiple kisspeptin receptors in early osteichthyans provide new insights into the evolution of this receptor family. *PLoS ONE* 7(11): e48931. doi:10.1371/journal.pone.0048931

Pasquier J., Lafont A-G, Tostivint H., Vaudry H., Rousseau K., Dufour S., 2012. Comparative evolutionary histories of kisspeptins and kisspeptin receptors reveal both parallel and divergent features. *Frontiers in Endocrinology*. doi: 10.3389/fendo.2012.00173.

2013: Støttrup, J.G., Jacobsen, C., Tomkiewicz, J., Jarlbæk, H. 2013. Modification of essential fatty acid composition in broodstock of cultured European eel *Anguilla anguilla* L. *Aquaculture Nutrition*, 19(2), pp 172–185

Heinsbroek L., Støttrup J., Jacobsen C., Corraze G., Kraiem M., Holst L.K., Tomkiewicz J., Kaushik S., 2013. A review on broodstock nutrition of marine pelagic spawners: The curious case of the freshwater eels (*Anguilla spp.*). *Aquaculture Nutrition*. *Aquaculture Nutrition* 19, 1-24.

Sørensen, S.R., Gallego, V., Pérez, L., Butts, I.A.E.; Tomkiewicz, J., Asturiano, J. 2013. Evaluation of methods to determine sperm density for the European eel, *Anguilla anguilla*. *Reproduction in Domestic Animals*, 48 (6), 936-944.

Burgerhout, E., Tudorache, C., Brittijn, S.A., Palstra, A.P., Dirks, R.P., van den Thillart, G.E.E.J.M., 2013. Schooling reduces energy consumption in swimming male European eels, *Anguilla anguilla* L. *J. Exp. Mar. Biol. Ecol.* 448, 66–71.

Burgerhout E., Brittijn, S.A., Tudorache, C., de Wijze, D.L., Dirks, R.P., van den Thillart, G.E. 2013. Male European eels are highly efficient long distance swimmers: effects of endurance swimming on maturation. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 166, 522-527.

Peñaranda, D.S., Mazzeo, I., Hildahl, J., Gallego, V., Nourizadeh-Lillabadi, R., Pérez, L., Asturiano, J.F., Weltzien, F.-A., 2013. Molecular characterization of three GnRH receptor paralogs in the European eel, *Anguilla anguilla*: Tissue-distribution and changes in transcript abundance during artificially induced sexual development. *Mol. Cell. Endocrinol.* 369, 1–14.

Gallego, V., Carneiro, P.C.F., Mazzeo, I., Vilchez, M.C., Peñaranda, D.S., Soler, C., Pérez, L., Asturiano, J.F., 2013. Standardization of European eel (*Anguilla anguilla*) sperm motility evaluation by CASA software. *Theriogenology* 79, 1034–1040.

2014: Baeza, R., Mazzeo, I., Vilchez, M.C., Gallego, V., Peñaranda, D.S., Pérez, L., 2014. Asturiano, J.F. Effect of thermal regime on fatty acid dynamics in male European eels (*Anguilla anguilla*) during hormonally-induced spermatogenesis. *Aquaculture*, 430, 86-97.

Butts, I. A.E., Sørensen, S. R., Politis, S. N., Pitcher, T. E., & Tomkiewicz, J. (2014). Standardization of fertilization protocols for the European eel, *Anguilla anguilla*. *Aquaculture*, 426, 9-13.

Dirks, R.P., Burgerhout, E., Brittijn, S.A., de Wijze, D.L., Ozupek, H., Tuinhof-Koelma, N., Minegishi, Y., Jong-Raadsen, S.A., Spaink, H.P., van den Thillart, G.E. 2014. Identification of molecular markers in pectoral fin to predict artificial maturation of female

European eels (*Anguilla anguilla*). Gen. Comp. Endocrinol. 204C, 267-276.

Gallego, V., Martínez-Pastor, F., Mazzeo, I., Peñaranda, D.S., Herráez, M.P., Asturiano, J.F., Pérez, L., 2014. Intracellular changes in Ca²⁺, K⁺ and pH after sperm motility activation in the European eel (*Anguilla anguilla*): Preliminary results. Aquaculture 418–419, 155–158.

Gallego, V., Vilchez, M.C., Peñaranda, D.S., Pérez, L., Herráez, M.P., Asturiano, J.F., Martínez-Pastor, F., 2014. The subpopulation pattern of eel sperm is affected by post-activation time, hormonal treatment and thermal regime. Reproduction, Fertility and Development. Available On-line.

Mazzeo, I., Peñaranda, D.S., Gallego, V., Baloché, S., Nourizadeh-Lillabadi, R., Tveiten, H., Dufour, S., Asturiano, J.F., Weltzien, F.A., Pérez, L., 2014. Temperature modulates the progression of vitellogenesis in the European eel. Aquaculture, Volume 434, 20 October 2014, Pages 38-47

Peñaranda, D.S., Mazzeo, I., Gallego, V., Hildahl, J., Nourizadeh-Lillabadi, R., Pérez, L., Weltzien, F.A., Asturiano, J.F., 2014. The regulation of aromatase and androgen receptor expression during gonad development in male and female European eel. Reproduction in Domestic Animals, 49(3), 512-521.

Politis, S.N., Butts, I.A.E., Tomkiewicz, J. 2014 Light impacts embryonic and early larval development of the Europeaneel, *Anguilla anguilla*. Journal of Experimental Marine Biology and Ecology 461, 407–415.

Sørensen, S.R., Skov, P.V., Lauesen, P., Tomkiewicz, J., Bossier, P., Schryver, D.S., 2014. Microbial interference and potential control in culture of European eel (*Anguilla anguilla*) embryos and larvae. Aquaculture 426, 1-8.

Sørensen, S. R., Tomkiewicz, J., & Skovgaard, A. (2014). *Ichthyodinium* identified in the eggs of European eel (*Anguilla anguilla*) spawned in captivity. Aquaculture, 426, 197-203.

van der Meeren, T., Karlsen, Ø., Liebig, P. L., & Mangor-Jensen, A., 2014. Copepod production in a saltwater pond system: A reliable method for achievement of natural prey in start-feeding of marine fish larvae. Aquacultural Engineering 62, 17-27

2015: Baeza, R., Peñaranda, D.S., Vilchez, M.C., Tveiten, H., Pérez, L., Asturiano, J.F. 2015. Exploring correlations between sex steroids and fatty acids and their potential roles in the induced maturation of the male European eel. Aquaculture 435, 328-335.

Baeza, R., Mazzeo, I., Vilchez, M. C., Gallego, V., Peñaranda, D.S., Pérez, L., Asturiano, J.F. Relationship between sperm quality parameters and the fatty acid composition of the muscle, liver and testis of European eel. Submitted to Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology in November 2014.

Sørensen, S.R., Butts, I.A.E, Munk, P., Tomkiewicz, J. Effects of salinity and sea salt type on egg activation, fertilisation, buoyancy and early embryology of European eel, *Anguilla anguilla*. Zygote. In press.

Josianne G. Støttrup, Jonna Tomkiewicz, Charlotte Jacobsen, Ian A.E. Butts, Lars K. Holst, Maria Krüger-Johnsen, Christian Graver, Peter Lauesen, Geneviève Corraze, Sadasivam Kaushik. Development of a broodstock diet to improve developmental competence of embryos in European eel, *Anguilla anguilla*. Aquaculture Nutrition. Accepted.

Submitted papers:

Asturiano, J.F., Sørensen, S.R., Pérez, L., Lauesen, P., Tomkiewicz, J. Effect of preservation temperature and cryopreservation on European eel sperm fertilization capacity. First production of larvae using cryopreserved sperm. *Aquaculture*, in review.

Butts, I.A.E., Baeza, R., Støttrup, J.G., Krüger-Johnsen, M., Jacobsen, C., Pérez, L., Asturiano, J.F., & Tomkiewicz, J. Under Review. Impact of dietary fatty acids on muscle composition, liver lipids, milt composition and sperm performance in male European eel. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*. in review.

Baeza, R., Mazzeo, I., Vilchez, M.C., Gallego, V., Peñaranda, D.S., Pérez, L., Asturiano, J.F. Relationship between sperm quality parameters and fatty acid composition of the muscle, liver and testis of European eel. *Theriogenology*, in review.

Bouilliart, M., Tomkiewicz, J., Lauesen, P., Adriaens, D. Musculoskeletal anatomy and feeding performance of pre-feeding engyodontic larvae of the European eel (*Anguilla anguilla*). *Journal of Anatomy*. In review.

Mazzeo, I., Giorgini, E., Gioacchini, G., Maradonna, F., Vilchez, M.C., Dufour S., Pérez, L., Carnevali, O., Asturiano, J.F. Molecular building of developing European eel (*Anguilla anguilla*) oocytes: histological, biochemical and spectroscopic studies. *Journal of Fish Biology* in review.

Morini, M, Pasquier, J, Dirks, R, van den Thillart, G., Tomkiewicz, J., Rousseau. K., Dufour, S., and Lafont, A-G. First evidence of duplicated Leptin Receptors in a vertebrate, the eel, brings new insights into the evolutionary history of the leptin system. *Plos-One*. in review.

A2 List of other international dissemination activities (book chapters, international conference contributions, other international meeting contributions)

For further information please the PRO-EEL public web site, including press releases, flyers, articles published in the popular press, videos, media briefings, interviews, films, TV clips, newspaper articles etc.

For theses developed and published, please see Section C Education and training of young researchers.

Book chapters

2010-14: I. Mazzeo, D.S. Peñaranda; V. Gallego; R. Nourizadeh-Lillabadi; H. Tveiten; F.A. Weltzien; J.F. Asturiano; L. Pérez, 2012. Temperature effect on expression of P450 aromatase, zona pellucida proteins and androgen receptors, and plasma levels of E2 in female European eel during induced sexual maturation. *In Avances en Endocrinología Comparada*, Vol. VI. ISBN: 978-84-96703-42-1 Edited by: Asociación Ibérica de Endocrinología Comparada (M.J. Delgado, Á.L. Alonso, N. de Pedro & E. Isorna, eds.) Pages: 125-128

D.S. Peñaranda, I. Mazzeo; V. Gallego; R. Nourizadeh-Lillabadi; F.A. Weltzien; L. Pérez; J.F. Asturiano, 2012. Regulation of gene expression of CYP19A, GnRH receptors I and II,

and androgen receptors α and β in male European eel (*Anguilla anguilla*) during induced sexual maturation. In *Avances en Endocrinología Comparada*, Vol. VI. ISBN: 978-84-96703-42-1 Edited by: Asociación Ibérica de Endocrinología Comparada (M.J. Delgado, Á.L. Alonso, N. de Pedro & E. Isorna, eds.). Pages: 222-224

Rindom, S., Tomkiewicz, J., Munk, P., Aarestrup, K., Als, T.D., Pedersen, M.I., Graver, C., Anderberg, C. 2014 Eels in culture, fisheries and science in Denmark. In *Eels and Humans* (Eds. Tsukamoto, K. and Kuroki, M.). Springer, Tokyo, p. 41-61 (Humanity and the Sea).

International conference contributions (presenting author in bold)

2011: **Burgerhout E**, Brittijn SA, Kurwie T, Decker P, Dirks RP, Palstra AP, Spaik HP & Van den Thillart GEEJM. Artificial reproduction of the New Zealand short-finned eel and hybridization with the European eel. Oral presentation at European Aquaculture Conference EA2011, Rhodes 18-21 October 2011 (Greece).

Cabrita, E., Martínez-Páramo, S., Engrola, S., Dinis, M.T., Asturiano, J.F., Sarasquete, C., 2011. Sperm cryopreservation from grouper, eel, bass and other fish species: advances and applications. Oral presentation at the World Aquaculture Society 2011 meeting, 7-10 June 2011, Natal, Brazil. Book of Abstracts, p. 194.

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, European Aquaculture Conference EA2011, 18-21 October 2011, Rhodes, Greece.

Gallego, V., Carneiro, P.C.F., Mazzeo, I., Vilchez, M.C., Baeza, R., Peñaranda, D.S., Pérez, L., Asturiano, J.F., 2011. Standardization of European eel sperm motility evaluation by CASA software. Poster at the 3rd International Workshop on Biology of Fish Gametes, 7-9 September 2011, Budapest, Hungary. Book of abstracts. Pp.: 144-145.

Gallego, V., Martínez-Pastor, F., Mazzeo, I., Peñaranda, D.S., Herráez, M.P., Asturiano, J.F., Pérez, L., 2011. Role of intracellular changes in calcium, potassium and pH in the initiation of European eel sperm motility. Oral presentation at the 3rd International Workshop on Biology of Fish Gametes, 7-9 September 2011, Budapest Hungary. Book of abstracts. Pp.: 73-74.

Gallego, V., **Mazzeo, I.**, Carneiro, P.C., Baeza, R., Vilchez, M.C., Peñaranda, D.S., Pérez, L., Asturiano, J.F., 2011. Study of thermal regime effect on European eel (*Anguilla anguilla*) males reproductive performance during induced sexual maturation. Poster at the 9th International Symposium on Reproductive Biology of Fish, 9-14 August 2011, Kochin, India. Book of abstracts. Pp.: 221-222.

Guido E.E.J.M. van den Thillart, Danielle L. de Wijze, Ron P. Dirks, Yuki Minegishi, Herman P. Spaik. Eel-specific cellular implants and bioassays toward artificial maturation of the European eel. Oral presentation at European Aquaculture Conference EA2011, Rhodes 18-21 October 2011 (Greece).

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 at European Aquaculture Conference EA2011, Rhodes 18-21 October 2011 (Greece)

Mazzeo, I., Peñaranda, D.S., Gallego, V., Nourizadeh-Lillabadi, R., Tveiten, H., Weltzien, F.A., Asturiano, J.F., Pérez, L., 2011. Effect of thermal regime on the expression of sex-related genes in female European eel. Poster at the 9th International Symposium on Reproductive Biology of Fish, 9-14 August 2011, Kochin India. Book of abstracts. Pp.: 36-37.

Mazzeo, I., Peñaranda, D.S., Gallego, V., Nourizadeh-Lillabadi, R., Tveiten, H., Weltzien, F.A., Asturiano, L. Pérez, L., 2011. Temperature effect on expression of P450 aromatase, zona radiata proteins, and androgen receptors, and plasma levels of E2 in female European eel during induced sexual maturation. Oral presentation at the 8^o Congreso Asociación Ibérica de Endocrinología Comparada, 5-7 September 2011, Madrid, Spain.

Minegishi Y., Henkel CV., de Wijze D.L., Dirks R.P., Burgerhout E., Jansen H.J., Spaink H.P., Dufour S., Weltzien F.A., Tsukamoto K., van den Thillart G.E.E.J.M. (2011). The european eel genome provides powerful new tools for optimizing artificial maturation protocols. GIA,13-16 September 2011, Heraklion, Greece.

Pasquier J., Lafont A.G., Rousseau K., Dufour S., 2011. Investigation of the kisspeptin system in the European eel. Conference of the PhD school of the Marine Science Center, University Pierre & Marie Curie, 14-15 September 2011, Paris, France.

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 at European Aquaculture Conference EA2011, 18-21 October 2011, Rhodes, Greece.

Peñaranda, D.S., Mazzeo, I., Gallego, V., Nourizadeh-Lillabadi, R., Weltzien, F.A., Pérez, L., Asturiano, J.F., 2011. Gene expression regulation of P450 aromatase, GnRH and androgen receptors in European eel (*Anguilla anguilla*) males during induced sexual maturation. Poster at the 8^o Congreso Asociación Ibérica de Endocrinología Comparada, 5-7 September 2011, Madrid, Spain.

Pérez L., Vílchez M.C., Gallego V., Mazzeo I., Peñaranda D.S., Weltzien F.-A., Dufour S., Asturiano J.F., 2012. Trying to reproduce the European eel (*Anguilla anguilla*) under captivity: experiments with females. Oral presentation at Domestication in Finfish Aquaculture. 23-25 October 2012, Olsztyn (Poland).

Tomkiewicz, J. Reproduction of European eel: Towards a self-sustained aquaculture (PRO-EEL). Oral presentation at European Aquaculture Conference EA2011, Rhodes 18-21 October 2011, Greece.

2012: **Bouilliart, M.** J. Tomkiewicz, P. Lauesen, D. Adriaens. 2012. The feeding apparatus of first feeding European eel (*Anguilla anguilla*) larvae: a functional morphological approach. Oral presentation at 19th Benelux Congress of Zoology, Brussels, Belgium.

Bouilliart, M., Tomkiewicz, J., Lauesen, P., Adriaens, D. 2012. Functional morphology of the feeding apparatus in preleptocephalus larvae in European eel (*Anguilla anguilla*): getting ready towards feeding. Oral presentation at 14th European Congress of Ichthyology, Liège, Belgium.

Burgerhout, E., de Wijze, D., Henkel, C., Minegishi, Y., Brittijn, B., Dirks, R., Spaink, H. & van den Thillart, G., 2012. Tissue transcriptome analysis: applications for eel aquaculture. Oral presentation at European Aquaculture Society 2012; September 1-5, Prague, Czech Republic.

- Corraze G.**, Fontagné-Dicharry S., Stottrup J., Holst L.K., Larroquet L., Jacobsen C., Tybjerg L., Tomkiewicz J and Kaushik S., 2012. Impact of broodstock nutrition on the quality of tissue lipids and reproductive performance in European eel (*Anguilla anguilla*). Poster presentation at XV Int. Symp. Fish Nutrition and Feeding, 4-7 June 2012, Molde, Norway.
- Dufour, S.**, Rousseau, K., Neuroendocrinology of fish metamorphosis and puberty, Invited oral communication, Oral presentation at 7th International Symposium on Fish Endocrinology, 3-6 September 2012, Buenos Aires, Argentina.
- Dufour, S.**, Pasquier, J., Lafont, A.G., Maugars, G., Morini, M., Jolly, C, Fontaine, R., Rousseau, K., Pasqualini, C., 2012, Impact of genome duplication on fish neuroendocrine functions, Oral presentation (Invited speaker) at 38^{ème} colloque de la Société de Neuroendocrinologie, 19-21 September 2012, Banyuls-sur-mer, France.
- Heinsbroek L.**, Støttrup J.G., Jacobsen C., Corraze G., Kraiem M., Holst L.K., Tomkiewicz J., Kaushik S., 2012. Broodstock nutrition of European eel (*Anguilla anguilla* L.). Oral presentation at XV Int. Symp. Oral presentation at Fish Nutrition and Feeding, 4-7 June 2012, Molde, Norway.
- Heinsbroek L.**, Ulloa Rojas J.B., Verreth J.A.J., 2012. Effect of dietary lipid level and fatty acid composition on deposition, utilization and conversion of fatty acids in European eel *Anguilla anguilla* L. Oral presentation at AQUA2012, 2-6 September 2012, Prague, Czech Republic
- Lafont A.G.**, Morini M., Pasquier J., Rousseau K., Dufour S. 2012. Involvement of the duplicated leptin/leptin receptor system in the sexual maturation of the European eel, *Anguilla anguilla*. Poster at the 38^{ème} colloque de la Société de Neuroendocrinologie, 19-21 September 2012, Banyuls-sur-mer, France
- Lafont AG**, Morini M, Pasquier J, Rousseau K, Dufour S. 2012. Duplicated leptin/leptin receptor system in a basal teleost, the European eel. Poster presentation at 7th International Symposium on Fish Endocrinology, 3-6 September 2012, Buenos Aires, Argentina
- Mazzeo I.**, 2012. Molecular building of developing eel (*Anguilla anguilla*) oocytes: histological, biochemical and spectroscopic studies. Poster presentation at World Aquaculture Society-European Aquaculture Society annual meeting. September 2012, Prague (Czech Republic), Abstract book, p700.
- Mazzeo I.**, Baeza R., Gallego V., Vílchez M.C., Pérez L., Asturiano J.F., 2012. Study of thermal regime effect on fatty acid mobilization in European eel (*Anguilla anguilla*, L.) males during induced sexual maturation. Poster presentation at World Aquaculture Society-European Aquaculture Society annual meeting. September 2012, Prague (Czech Republic), Abstract book, p701.
- Minegishi Y**, Henkel C, de Wijze D, Dirks R., Burgerhout E, Jansen H, Spaik H, Dufour S, Weltzien FA, Tsukamoto K, van den Thillart G. 2012 The European eel genome provides the basics for optimization of artificial maturation protocols. Oral presentation at 6th World Fisheries Congress. Sustainable Fisheries in a Changing World, Edinburgh (Scotland), Abstract book p132
- Pasquier J**, Lafont AG, Rousseau K, Dufour S. 2012. Origine and evolution of the kisspeptin/kisspeptin receptor system in vertebrates, Poster at the 38^{ème} colloque de la Société de Neuroendocrinologie, 19-21 September 2012, Banyuls-sur-mer, France

Pasquier J., Lafont A.G., Rousseau K., Dufour S. 2012. Diversity and evolutionary history of the Kiss/Kissr system in vertebrates: new data from fish genomes, Oral presentation at the 7th International Symposium on Fish Endocrinology, 3-6 September 2012, Buenos Aires, Argentina.

Tomkiewicz J., Støttrup J., Sørensen S.R., Lauesen P., Munk P., Jacobsen C., Kaushik S., Corraze G., Tveiten H., Asturiano J.F., Pérez L., McEvoy F., Graver C., Krüger-Johnsen M., Dufour S., Lafont A.G., Kjørsvik E., van Delsen B., Holst L.K., 2012. Reproduction of European eel: towards a self-sustained aquaculture (PRO-EEL). Oral presentation at 6th World Fisheries Congress. Sustainable Fisheries in a Changing World, Edinburgh (Scotland), Abstract book p132

2013: **Asturiano, J.F.**, S.R. Sørensen, L. Pérez, P. Lauesen, J. Tomkiewicz, 2013. Effect of preservation temperature and cryopreservation on European eel sperm fertilization capacity. First production of larvae using cryopreserved sperm. Poster presentation at 4th International Workshop on Biology of Fish Gametes, 17-20 September 2013, Faro, Portugal.

Baeza, R., I. Mazzeo, M.C. Vilchez, V. Gallego, D.S. Peñaranda, L. Pérez, J.F. Asturiano, 2013. Effect of thermal regime on fatty acid mobilization in male European eels (*Anguilla anguilla*) during hormonally-induced spermatogenesis. Relationship with sperm quality parameters. Poster presentation at 17th International Congress of Comparative Endocrinology. ICCE 2013 15-19 July 2013 Barcelona, Spain.

Baeza, R., I. Mazzeo, M.C. Vilchez, V. Gallego, D.S. Peñaranda, L. Pérez, J.F. Asturiano, 2013. Fatty acid mobilization in male European eels during induced sexual maturation. Effect of thermal regime and relation with sperm quality parameters. Oral presentation at 4th International Workshop on Biology of Fish Gametes, 17-20 September 2013, Faro, Portugal.

Bouilliart M., Tomkiewicz J., Lauesen P., & Adriaens D. (2013). The feeding apparatus of first feeding European eel (*Anguilla anguilla*) larvae: a functional morphological approach. Oral presentation at the Society of Integrative and Comparative Biology Annual meeting, 3-7 January 2013, San Francisco, USA.

Bouilliart M., Tomkiewicz J., Lauesen P. & Adriaens D. (2013). Is the leptocephalus larva capable of biting? Analyzing the morphological limitations of the feeding apparatus in the European eel (*Anguilla anguilla*). Oral presentation at the 10th International Congress of Vertebrate Morphology, 8-12 July 2013, Barcelona, Spain.

Bouilliart M., Tomkiewicz J., Lauesen P. & Adriaens D. (2013). Exploring the feeding mystery of leptocephalus larvae: a mouth full of teeth. Oral presentation at the Aquaculture Europe Annual Meeting, 9-12 August 2013, Trondheim, Norway.

Bouilliart M., Tomkiewicz J., Lauesen P. & Adriaens D. (2013). Exploring the feeding mystery of leptocephalus larvae: a mouth full of teeth. Oral presentation at the 6th Fish & Shellfish Larviculture Symposium, 2-5 September 2013, Ghent, Belgium.

Butts, I.A.E., Pérez, L., Støttrup, J., Kruger-Johnsen, M., Tomkiewicz, J., & Asturiano, J.F. 2013. Dietary fatty acids influence male reproductive success in the European eel: Preliminary results. Oral presentation at 4th International Workshop on Biology of Fish Gametes 2013. Algarve, Portugal.

Butts, I.A.E., Sørensen, S.R., Politis, S.N., Lauesen, P., & Tomkiewicz, J. 2013. Techniques for rearing European eel during early life history. Aquaculture Europe 13. Oral pres. at Trondheim, Norway.

Butts, I.A.E., Sørensen, S.R., Politis, S.N., Lauesen, P., & Tomkiewicz, J. 2013. Development of techniques and technology for embryonic and larval rearing of the European eel. Poster pres. at Larvi2013 - 6th fish and shellfish larviculture symposium. Ghent, Belgium.

Corraze G., Støttrup J., **Larroquet L.**, Tomkiewicz J. and **Kaushik S.** Comparison of broodstock lipid stores in farmed and wild European eel (*Anguilla anguilla*) in link with reproductive performance. Poster presentation at Aquaculture Europe 13, August 9-12th 2013, Trondheim, Norway.

De Schryver, P., Sørensen, S.R., Skov, P.V., Lauesen, P., Tomkiewicz, J. (2013). Microbial interference and potential control in the production of European eel *Anguilla anguilla* larvae. Oral presentation at Aquaculture Europe 2013, August 9-12, Trondheim (Norway)

Gallego, V., M.C. Vílchez, D.S. Peñaranda, L. Pérez, M.P. Herráez, J.F. Asturiano, F. **Martínez-Pastor**, 2013. The hormonal treatment used to induce spermiation in European eel affects the motility subpopulation pattern. Poster presentation at 4th International Workshop on Biology of Fish Gametes, 17-20 September 2013, Faro, Portugal.

Mazurais, D., Kjørsvik, E., Wold, P.A., Politis, S.N., Cahu, C., Tomkiewicz, J. Zambonino-Infante, J., 2013. Biochemical, histological and molecular study of digestive tract development in European eel larvae (*Anguilla anguilla*) prior to exogenous feeding. Poster presentation at Aquaculture Europe 13. Trondheim, Norway.

Morini, M., D.S. Peñaranda, M.C. Vílchez, V. Gallego, R. Nourizadeh-Lillabadi, J.F. Asturiano, F.-A. Weltzien, L. Pérez, 2013. Evolution of DHP receptors expression in European eel testis through induced spermatogenesis. Poster presentation at 17th International Congress of Comparative Endocrinology. ICCE 2013 15-19 July 2013 Barcelona, Spain.

Morini, M., D.S. Peñaranda, M.C. Vílchez, V. Gallego, R. Nourizadeh-Lillabadi, J.F. Asturiano, F.-A. Weltzien, L. Pérez, 2013. Evolution of PLCZ1 expression in European eel testis through induced spermatogenesis. Poster presentation at 17th International Congress of Comparative Endocrinology. ICCE 2013 15-19 July 2013 Barcelona, Spain.

Morini, M., D.S. Peñaranda, M.C. Vílchez, V. Gallego, R. Nourizadeh-Lillabadi, J.F. Asturiano, F.-A. Weltzien, **L. Pérez**, 2013. Evolution of DHP receptors and PLCZ1 expression in European eel testis through spermatogenesis. Oral presentation at 4th International Workshop on Biology of Fish Gametes, 17-20 September 2013, Faro, Portugal.

Peñaranda, D.S., M. Morini, M.C. Vílchez, V. Gallego, I. Mazzeo, R.P. Dirks, G.E.E.J.M. van den Thillart, H. Tveiten, L. Pérez, **J.F. Asturiano**, 2013. Temperature affects the European eel spermiation modulating the testis steroidogenesis: physiological and migratory implications. Poster presentation at 17th International Congress of Comparative Endocrinology. ICCE 2013 15-19 July 2013 Barcelona, Spain.

Burgerhout, E., Brittijn, B., Tudorache, C., de Wijze, D., Dirks, R., van den Thillart, G. (2013). Effect of endurance swimming on maturation in farmed male European eels. Poster presentation at 17th International Congress of Comparative Endocrinology. ICCE 2013 15-19 July 2013 Barcelona, Spain.

Peñaranda, D.S., M. Morini, M.C. Vílchez, V. Gallego, I. Mazzeo, R.P. Dirks, G.E.E.J.M. van den Thillart, H. Tveit en, L. Pérez, J.F. Asturiano, 2013. Temperature affects the European eel spermiation modulating the testis steroidogenesis. Oral

presentation at 4th International Workshop on Biology of Fish Gametes, 17-20 September 2013, Faro, Portugal.

Pérez, L., I. Mazzeo, D.S. Peñaranda, M.C. Vilchez, V. Gallego, S. Dufour, F.-A. Weltzien, J.F. Asturiano, 2013. Modulatory effect of temperature on vitellogenesis and ovulation in the European eel. Poster presentation at 17th International Congress of Comparative Endocrinology. ICCE 2013 15-19 July 2013 Barcelona, Spain.

Pérez, L., M.C. Vilchez, V. Gallego, I. Mazzeo, D.S. Peñaranda, F.-A. Weltzien, S. Dufour, J.F. Asturiano, 2013. Trying to reproduce the European eel (*Anguilla anguilla*) under captivity: experiments with females. Planned oral presentation but cancelled due to medical reasons during Domestication in Finfish Aquaculture, 23-25 October 2012, Olsztyn (Poland). Publication: Domestication in Finfish Aquaculture (ISBN: 978-8362863-31-0) pp. 11-15.

Santangeli, S., F. Maradonna, G. Gioacchini, C. Verdenelli, M.C. Vilchez, V. Gallego, L. Pérez, O. Carnevali, J.F. Asturiano, 2013. Effect of the probiotic *Lactobacillus rhamnosus* on *Anguilla anguilla* testis. Poster presentation at 17th International Congress of Comparative Endocrinology. ICCE 2013 15-19 July 2013 Barcelona, Spain.

Santangeli, S., F. Maradonna, G. Gioacchini, C. Verdenelli, M.C. Vilchez, V. Gallego, L. Pérez, O. Carnevali, J.F. Asturiano. Effect of the probiotic *Lactobacillus rhamnosus* on the expression of genes involved in European eel spermatogenesis. Poster presentation at 4th International Workshop on Biology of Fish Gametes, 17-20 September 2013, Faro, Portugal.

Silva, F.F.G., Tomkiewicz, J., Kjørsvik, E., 2013. Oocyte development & fecundity estimation in European eel – preliminary results. Poster presentation at Aquaculture Europe 13. Trondheim, Norway

Silva, F.F.G., Tomkiewicz, J., Kjørsvik, E., 2013. Oocyte development & fecundity estimation in European eel – preliminary results. Poster presentation at Fishgametes2013. Algarve, Portugal.

Støttrup, J.G.¹, Tomkiewicz, J., Jacobsen, C., Krüger-Johnsen, M., Holst, L.K., & Lausen, P. 2013. Development of a broodstock diet to improve embryonic development competence in female European eel, *Anguilla anguilla*. Aquaculture Europe 13. Trondheim, Norway. ¹ **Butts, I.A.E.** presented on behalf of the authors.

Sørensen, S.R., V. Gallego, L. Pérez, I.A.E. Butts, J. Tomkiewicz, **J.F. Asturiano**, 2013. Evaluation of methods to determine sperm density for the European eel, *Anguilla anguilla*. Poster presentation at 4th International Workshop on Biology of Fish Gametes, 17-20 September 2013, Faro, Portugal.

Sørensen, S.R., Gallego, V., Pérez, L., **Butts, I.A.E.**, Tomkiewicz, J., & Asturiano, J.F. 2013. Quantitative methods to determine sperm density for the European eel, *Anguilla anguilla*. Oral presentation at 4th International Workshop on Biology of Fish Gametes 2013. Algarve, Portugal.

Tomkiewicz, J., Støttrup, J.G., Corraze, G., Kausik, S., Holst, L.K., McEvoy, F., Dufour, S., Lafont, A.G., Asturiano, J.F., Sørensen, S.R., Tveiten, H., De Schryver, P., Butts, I.A.E., Munk, P., Zambonino-Infante, J., Politis, S.N., Krüger-Johnsen, M., & Lauesen, P. 2013. Reproduction of European eel and larval culture. Oral pres. at Aquaculture Europe 13. Trondheim, Norway.

Tomkiewicz, J., Støttrup, J.G., Corraze, G., Kausik, S., Holst, L.K., McEvoy, F., Dufour, S., Lafont, A.G., Asturiano, J.F., Sørensen, S.R., Tveiten, H., De Schryver, P., Butts, I.A.E., Munk, P., Zambonino-Infante, J., Politis, S.N., Krüger-Johnsen, M., & Lauesen, P. 2013. Reproduction of European eel and larval culture: state of the art. Oral pres. at Aquaculture Europe 13. Trondheim, Norway. Larvi2013 - 6th fish and shellfish larviculture symposium. Ghent, Belgium.

Vílchez, M.C., I. Mazzeo, R. Baeza, V. Gallego, D.S. Peñaranda, J.F. Asturiano, L. Pérez, 2013. Effect of thermal regime on the quality of eggs and larval development of European eel. Oral presentation at 4th International Workshop on Biology of Fish Gametes, 17-20 September 2013, Faro, Portugal.

2014: **Asturiano J.F.**, Baeza R., Vilchez M.C., Gallego V., Peñaranda D.S., Tveiten H., Pérez L., 2014. Fatty acid dynamics during induced sexual maturation in male European eel. Relationship with steroids and sperm quality parameters. Poster presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Baeza, R., I. Mazzeo, M.C. Vilchez, V. Gallego, D.S. Peñaranda, H. Tveiten, L. Pérez, J.F. Asturiano. Water temperature effect on fatty acid mobilization in male European eels during sexual maturation and relationship between fatty acids, steroids and sperm quality parameters. Poster presentation at 10th International Symposium on Reproductive Physiology of Fish, 25-30 May 2014, Olhão, Portugal.

Butts, I.A.E., R. Baeza, J.G. Støttrup, M. Krüger-Johnsen, C. Jacobsen, L. Pérez, J. Tomkiewicz, J.F. Asturiano. Fatty acids influence male reproductive performance in European eel. Oral presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Kása, E., M.C. Vilchez, M. Morini, D.S. Peñaranda, L. Pérez, J.F. Asturiano, B. Urbányi, Á. Horváth. Vitrification of the sperm of European eel (*A. anguilla*): investigation of different protocols. Poster presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Kjørsvik E., Wold P.-A., Bardal T., Davidsen M., da Silva F.G., Tomkiewicz J., Sørensen S.R. 2014. Muscle development in European eel *Anguilla anguilla* yolksac larvae and effects of egg incubation temperature. Oral presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Martínez-Miguel, L., I. Rojo-Bartolomé, M.C. Vilchez, J.F. Asturiano, L. Pérez, I. Cancio. Molecular markers of oocyte differentiation and maturation in European eel (*Anguilla anguilla*) during artificially induced oogenesis. Oral presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Morini, M., M.C. Vilchez, A.G. Lafont, D.S. Peñaranda, V. Gallego, S. Dufour, J.F. Asturiano, L. Pérez. Influence of temperature regime on the vitellogenesis of the European eel (*Anguilla anguilla*). Poster presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Morini, M., Peñaranda D.S., Vilchez M.C., Gallego V., Nourizadeh-Lillabadi R., Asturiano J.F., Weltzien F.-A., Pérez L., 2014. Molecular characterization and expression of progesterin receptors and PLCZ1 in European eel through spermatogenesis. Oral presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Peñaranda, D.S., M. Morini, M.C. Vilchez, V. Gallego, I. Mazzeo, R.P. Dirks, G.E.E.J.M. van den Thillart, H. Tveiten, L. Pérez, J.F. Asturiano. Temperature as

modulator of the steroidogenic process in European eel: migratory implications. Oral presentation at 10th International Symposium on Reproductive Physiology of Fish, 25-30 May 2014, Olhão, Portugal

Peñaranda, D.S., M.C. Vílchez, Pla D., Gallego V., Sanz L., Pérez L., Asturiano J.F., Calvete J.J. 2014. Identification of major proteins from the seminal plasma of hormone induced sexual matured European eels *Anguilla anguilla* – Correlations with sperm quality. Oral presentation at Aquaculture Europe 2014 14-17 Oct. San Sebastian Spain.

Pérez L., Vílchez V., Gallego V., Herráez M.P., Morini M., Baeza R., Peñaranda D.S., **Asturiano J.F.**, 2014 Intracellular Potassium and sperm motility in the European eel. Oral presentation at Aquaculture Europe 2014 14-17 Oct. San Sebastián Spain.

Rozenfeld C., Butts I.A.E., Tomkiewicz J., Mazurais D., Zambonino J., 2014. Maternal mRNA expression in European eel eggs and embryos and its relationship to offspring development competence. Poster presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Santangeli, S., M.C. Vilchez, F. Maradonna, G. Gioacchini, C. Verdenelli, V. Gallego, D.S. Peñaranda, H. Tveiten, L. Pérez, O. Carnevali, J.F. Asturiano. Effect of the probiotic *Lactobacillus rhamnosus* on sperm quality and on the expression of genes involved in European eel (*Anguilla anguilla*) spermatogenesis. Poster presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Silva da F.F.G., Tveiten H., Lafont A.G., Dufour S., Støttrup J.G., Kjørsvik E., Tomkiewicz J., 2014. Final oocyte maturation and egg quality in European eel *Anguilla anguilla* induced *in vivo*. Poster presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Tomkiewicz, J., Butts, IAE, Sørensen SR, De Schryver P, Zambonino-Infante J, Mazurais D., Bouillart M, Adriaens D, Kjørsvik E, Politis SN, Støttrup JG, Kaushik S, Corraze G, Munk P, Krüger-Johnsen M. Reproductive success and survival of European eel larvae: Recent progress towards closing the life cycle in captivity. Annual meeting of the East Asia Eel Resource Consortium (EASEC), Gwangju, Korea, September 29-30, 2014. Invited presentation.

Tomkiewicz J., Butts I.A.E. Sørensen S.R., Politis S.N., Lauesen P., Krüger-Johnson M., Graver C., 2014. Production of European eel embryos and larvae in culture. Oral presentation at Aquaculture Europe 2014 14-17 Oct. San Sebastián Spain.

Vílchez, M.C., M. Morini, D.S. Peñaranda, L. Pérez, A. Depincé, E. Kása, C. Labbé, Á. Horváth, J.F. Asturiano. Comparison of two validated methods for cryopreserving European eel (*Anguilla anguilla*) sperm: standardization as target. Oral presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Vílchez, M.C., D. Pla, V. Gallego, L. Sanz, L. Pérez, J.F. Asturiano, J.J. Calvete, D.S. Peñaranda. Identification of major proteins from seminal plasma of hormone-induced sexual mature European eels (*Anguilla anguilla*). Correlation with sperm quality. Oral presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Vílchez, M.C., I. Mazzeo, R. Baeza, D.S. Peñaranda, V. Gallego, J.F. Asturiano, L. Pérez. Modulatory effect of temperature on ovulation and larval development in the European eel. oral presentation at Aquaculture Europe 14 (AE2014), 14-17 October 2014, San Sebastián, Spain.

Vílchez, M.C., I. Mazzeo, D.S. Peñaranda, V. Gallego, S. Dufour, F.-A. Weltzien, J.F. Asturiano, L. Pérez. Effect of thermal regime on vitellogenesis, ovulation and larval development of European eel. Poster presentation at 10th International Symposium on Reproductive Physiology of Fish, 25-30 May 2014, Olhão, Portugal.

Vílchez, M.C., M. Morini, D.S. Peñaranda, L. Pérez, A. Depincé, E. Kása, C. Labbé, Á. Horváth, J.F. Asturiano. Cryopreserving European eel (*A. anguilla*) sperm: comparison of two methods for standardization. Oral presentation at V Jornadas Ibéricas de Ictiología, 24-27 June 2014, Lisbon, Portugal.

Other conference and meeting contributions (Presenting author in bold)

2010: **Asturiano, J.F.** 2010. Últimos avances en la investigación en el control de la reproducción de anguilas en cautividad (*Last advances on the research on the control of eels reproduction under captivity*) Invited speaker: I JORNADAS ESPAÑOLAS DE LA ANGUILA, San Sebastian 28-30 September 2010.

Asturiano, J.F., 2010. Presentación del Grupo de Acuicultura y Biodiversidad (ICTA-UPV) + Avances en la investigación en el control de la reproducción de anguilas en cautividad (*Introduction to the Group of Aquaculture and Biodiversity (ICTA-UPV) + Advances on the research on the control of reproduction of eels under captivity*). Invited speaker during visit to EMBRAPA Tabuleiros Costeiros (Aracajú) Ministerio da Agricultura, Pecuária e Abastecimento, Brazil.

Tomkiewicz, J. 2010. PRO-EEL: Reproduction of European eel – towards a self-sustained aquaculture. Invited speaker: I JORNADAS ESPAÑOLAS DE LA ANGUILA, San Sebastian 28-30 September 2010.1

2011: Asturiano, J.F., **Mazzeo, I.**, Gallego, V., Ciccotti, E., Pérez, L., Capoccioni, F., 2011. Estimación de la edad en que las hembras de anguila (*A. anguilla*) de la Albufera de Valencia realizan su migración reproductiva. XIII Congreso Nacional de Acuicultura. Castelldefels, Barcelona (Spain), 21-24 November. (Spanish).

Baeza, R., Mazzeo, I., Gallego, V., Vílchez, M.C., Pérez, L., Asturiano, J.F., 2011. Estudio del efecto del régimen térmico sobre la movilización de ácidos grasos en hembras de anguila europea (*Anguilla anguilla*, L.) durante la maduración sexual inducida. XIII Congreso Nacional de Acuicultura. Castelldefels, Barcelona (Spain), 21-24 November. (Spanish).

Gallego, V., Mazzeo, I., Carneiro, P., Vílchez, M.C., Baeza, R., Peñaranda, D.S., Barrera, R., Pérez, L., Asturiano, J.F., 2011. Producción de esperma de anguila europea (*Anguilla anguilla*) bajo tres regímenes térmicos y tres tratamientos hormonales. XIII Congreso Nacional de Acuicultura. Castelldefels, Barcelona (Spain), 21-24 November. (Spanish).

Vílchez, M.C., Mazzeo, I., Baeza, R., Gallego, V., Peñaranda, D.S., Asturiano, J.F., Pérez, L., 2011. Efecto del régimen térmico en la vitelogénesis de la anguila europea (*Anguilla anguilla*). XIII Congreso Nacional de Acuicultura. Castelldefels, Barcelona (Spain), 21-24 November. (Spanish).

2012: **Atkinson C.**, Pasquier J., Lafont A.G., Dufour S., 2012, Ongoing studies on eel GnIH systems. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

- Bouilliart M., De Kegel B., J. Christiaens, **Adriaens D.**, 2012. 3D Phenotypic model and functional feeding performance of larval European eel (15 DPF). Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)
- Asturiano J.F.** Pérez L., Gallego V., Peñaranda D.S., Mazzeo I., Baeza R., Vilchez C., Sørensen S.R., Tomkiewicz J., Støttrup J., Jacobsen C., Dufour S., Lafont A.G., Lauesen P., van den Thillart G., Tveiten H., 2012. Spermatogenesis and sperm production, selection and storage. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).
- Asturiano J.F.**, Mazzeo I., Gallego V., Ciccotti E., Heinsbroek L.T.N., Pérez L., Capoccioni F., 2012. Age determination of female European eels leaving the Albufera lagoon (Valencia, Spain) during its reproductive migration. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).
- Asturiano J.F.**, Pérez L., 2012. Intentando reproducir en cautividad a la anguila europea (*Anguilla anguilla*): experimentación con machos. Oral presentation at IV Jornadas Ibéricas de Ictiología. Situación actual y retos de futuro, July, Girona (Spain). Abstract book, p. 36
- Asturiano J.F.**, Sørensen S.R., Pérez L., Lauesen P., Tomkiewicz J. 2012. Effect of preservation temperature and cryopreservation on European eel sperm fertilization capacity. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Poster presentation).
- Baeza R.**, Mazzeo I., Moya J., Vilchez M.C., Gallego V., Pérez L., Asturiano J.F., 2012. Adapted method of fatty acid analysis in different tissues of European eel. 3rd Pro-Eel Project meeting (Valencia, Spain). (Oral presentation)
- Bouilliart, M., B. De Kegel, J. Christiaens, Lauesen, P., Tomkiewicz, J., **D. Adriaens.** 2012. 3D Phenotypic model and functional feeding performance of larval European eel (15 DPF). 3rd Pro-Eel Project meeting (Valencia, Spain). (*Oral presentation*).
- Burgerhout, E.**, Tudorache, C., Brittijn, S., Palstra, A., Dirks, R. & van den Thillart, G. (2012). Male silver eels swim more efficient in groups. 3rd Pro-Eel Project meeting (Valencia, Spain). (Oral presentation).
- Combarnous Y.**, Cahoreau C., 2012, Recombinant gonadotropin subunits and antibodies. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain
- Davidson M., Sørensen S.R., **Kjørsvik E.**, 2012. Early ontogeny and muscle development. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain
- Derouiche E.**, Hizem B., Elie P. & Kraiem M. Caractérisation de la fraction dévalante de l'anguille euro-méditerranéenne *Anguilla anguilla* (L. 1758) du littoral tunisien. Oral presentation at RIF - SFI (Paris 26-30 March 2012),
- Derouiche E.**, Pierre E. & Kraiem M., 2012. Anomalies morpho-anatomiques de l'anguille euro-méditerranéenne *Anguilla anguilla* (L., 1758) dans les lagunes de la Tunisie septentrionale (lac de Tunis, lac Ichkeul et lagune de Ghar El Melh) ATSMer. Poster presentation at Symposium (Sousse 19-22 December 2012)
- De Schryver P.**, Sørensen S.R., Tomkiewicz J., Zambonino J., Lauesen P., van der Meeren T., Jensen A.M., Kjørsvik E. (2012) Fertilization, incubation and larval rearing techniques of European eel. Oral presentation at 1st PRO-EEL Collaborative Workshop 2012, March 27th, Valencia, Spain
- de Wijze DL**, Dirks RP, Minegishi Y, Spaink HP, van den Thillart GEEJM. (2012). Eel-specific cellular implants and bioassays toward artificial maturation of the European eel . (2012) PRO-EEL Project meeting; March 28-30, Valencia, Spain.

de Wijze D.L., Burgerhout E., Minegishi Y., Jansen H., Brittijn S., Dirks R., Spaink H., van den Thillart G., 2012. Cellular implants as a new tool to stimulate sexual maturation in eels. 3rd Pro-Eel Project meeting (Valencia, Spain). (Oral presentation).

Dufour, S., Tomkiewicz, J., Krüger-Johnsen, M., van den Thillart, G., Lafont, A.G., Pasquier, J., Pérez, L., Asturiano, J.F., Tveiten, H., Lauesen, P., 2012, Neuro-endocrine function and novel treatments for maturation. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

Dufour, S., Pasquier, J., Lafont, A.G., Maugars, G., Morini, M., Jolly, C, Fontaine, R., Rousseau, K., Pasqualini, C., 2012, Impact of genome duplication on fish neuroendocrine functions, 38^{ème} colloque de la Société de Neuroendocrinologie, 19-21 September 2012, Banyuls-sur-mer, France (Invited oral communication)

Gallego V., Carneiro P.F.C., Mazzeo I., Vilchez M.C., Baeza R., Peñaranda D.S., Pérez L., Asturiano J.F. 2012. Standardization of European eel sperm motility evaluation by CASA software. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Poster presentation).

Gallego V., Martínez-Pastor F., Mazzeo I., Peñaranda D.S., Herráez M.P., Asturiano J.F., Pérez L. 2012. Ions involved in European eel sperm motility. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

Gallego V., Peñaranda D.S., Mazzeo I., Vilchez M.C., Carneiro P.F.C., Pérez L., Asturiano J.F., 2012. European eel (*Anguilla anguilla*) males reproductive performance during induced sexual maturation. Effect of thermal regime and hormonal treatment. 3rd Pro-Eel Project meeting (Valencia, Spain). (Oral presentation)

Hardman L., Lafont A.G., Pasquier J., Dufour S., 2012, Ongoing studies on eel corticosteroid receptors. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain.

Heinsbroek L., 2012. Broodstock nutritional requirements and benchmark analyses. Main Results. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain

Henkel C., Minegishi Y., Burgerhout E., de Wijze D., **Dirks R.**, Jansen H., Spaink H., Dufour S., Weltzien F.A., Tsukamoto K., van den Thillart G., 2012. Genome sequence of the European eel and its application in ovarian gene expression profiling. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Hjermitslev N.H., Tomkiewicz J., 2012. Production of larvae diets. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Holst L., Støttrup J., Corraze G., Heinsbroek L., Kausik S. 2012. Production of broodstock diets. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain

Jolly C., **Pasqualini C.**, Prézeau L., Dufour S., 2012, Two eel dopamine D2 receptors: which one is mediating the inhibitory control of reproduction? Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain

Kausik, S., Josianne Støttrup, Charlotte Jacobsen, Jonna Tomkiewicz, Michiel Kotterman, Genevieve Corraze, Peter Lauesen, Leon Heinsbroek, Lars Holst. Broodstock condition and nutrition. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

- Kjørsvik, E.** and Davidsen, M. 2012. Incubation temperature and embryonic development. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain
- Davidsen M., Sørensen S.R., **Kjørsvik E.**, 2012. Early ontogeny and muscle development. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain
- Kotterman M.**, Contaminants in eel; less healthy for humans or plain deadly for eel? Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain
- Lafont A.G.**, Morini M., Pasquier J., Rousseau K., Dufour S., 2012, Involvement of the duplicated leptin/leptin receptor system in the sexual maturation of the European eel, *Anguilla anguilla*. Poster presentation at the 38ème colloque de la Société de Neuroendocrinologie, 19-21 September 2012, Banyuls-sur-mer, France (Poster)
- Lafont A.G.**, Pasquier J., Rousseau K., Dufour S., 2012, Duplication du génome des téléostéens et récepteurs stéroïdiens chez l'anguille, Oral presentation at the scientific meeting "Journées scientifiques de l'UMR BOREA", 14-15 May 2012, Caen, France
- Lafont A.G.**, Tomkiewicz J., Dufour S., 2012, Multiple sex steroid receptors in the eel, with a special focus on the liver. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)
- Lund I.**, Steinfeldt S.J., Tomkiewicz J., Lauesen P. 2012. Larval rearing and feeding experiments. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)
- Mazurais D., **Zambonino J.L.**, 2012. New insights into digestive enzyme development in eel larvae. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)
- Mazzeo I.**, Baeza R., Gallego V., Vilchez M.C., Pérez L., Asturiano J.F., 2012. Thermal regime effect on fatty acid mobilization during vitellogenesis in European eel (*Anguilla anguilla*, L.). Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)
- Mazzeo I.**, Giorgini E., Gioacchini G., Maradonna F., Dufour S., Asturiano J.F., Carnevali O., Pérez L. 2012. Molecular building of developing eel oocytes: histological, biochemical and spectroscopic studies. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Poster presentation).
- Mazzeo I.**, Baeza R., Gallego V., Vilchez M.C., Pérez L., Asturiano J.F. 2012. Thermal regime effect on fatty acid mobilization during vitellogenesis in European eel (*Anguilla anguilla*, L.). Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)
- McEvoy F.**, Lene E. Buelund, Eiliv L. Svalastoga, Sebastian Politis, Maria Krüger-Johnsen, Jonna Tomkiewicz 2012. Imaging features of eels during artificially induced ovarian maturation. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)
- van der Meeren T.**, Jensen A.M. Harboe T., 2012. The silo gradient system as incubation unit for the pre-feeding stages of the European eel *Anguilla anguilla*. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)
- Morini M.**, Lafont A.G., Van den Thillart G., Dufour S., 2012, Eel leptin system in the European eel *Anguilla anguilla*, Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain

Munk P., Skov P.W., Sørensen S.R., Tomkiewicz J., Adrians D., Zambonino J., Kjørsvik E. 2012. Development and quality assessment of embryos and larvae. Oral presentation at the 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

Munk P., Tomkiewicz J., Sørensen S.R., Pargana A., Lauesen P., Graver C., 2012. Morphological and development differences among larvae from PRO-EEL Series A and B. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Müller T., Matsubara H., Kubara Y., Horváth Á., Urbányi B., Horváth L., **Asturiano J.F.**, Peñaranda D.S., Adachi S., Arai K., Yamaha E. 2012. Artificial hybridisation between Japanese eel females (*Anguilla japonica*) and European eel males (*A. anguilla*) by using cryopreserved sperm. Oral presentation at the 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

Pasquier J., Lafont AG, Rousseau K, Dufour S, 2012, Origine and evolution of the kisspeptin/kisspeptin receptor system in vertebrates. Poster presentation at the 38ème colloque de la Société de Neuroendocrinologie, 19-21 September 2012, Banyuls-sur-mer, France (Poster)

Pasquier J., Lafont A.G., Denis F., Van den Thillart G., Tomkiewicz J., Vaudry H., Rousseau K., Dufour S., 2012, Characterization of eel Kisspeptin systems and new insights on vertebrate evolution of kiss receptors, Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain

Pasquier J., Lafont A.G., Rousseau K., Dufour S., 2012, Le système kisspeptin chez l'anguille européenne: évolution et fonctionnalité, Oral presentation at the scientific meeting "Journées scientifiques de l'UMR BOREA", 14-15 May 2012, Caen, France.

Peñaranda D.S., Gallego V., Mazzeo I., Vilchez M.C., Carneiro P.F.C., Tveiten H., Dirks R.P., van den Thillart G.E.E.J.M., Pérez L., Asturiano J.F., 2012. Expression of steroidogenic enzymes in European eel males during induced sexual maturation under three thermal regimes, and relationship with sperm quality parameters. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Pérez L., Asturiano J.F., 2012. Intentando reproducir en cautividad a la anguila europea (*Anguilla anguilla*): experimentación con hembras. Oral presentation at IV Jornadas Ibéricas de Ictiología. Situación actual y retos de futuro, July, Girona (Spain). Abstract book, p. 36

Pérez L., Johnsen M.K., Dufour S., Lafont A.G., Mazzeo I., Tveiten H., McEvoy F., Buelund L., Svalastoga E., Lauesen P., Vitellogenesis and re-allocation of body stores. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

Pérez L., Mazzeo I., Peñaranda D.S., Gallego V., Nourizadeh-Lillabadi R., Tveiten H., Dufour S., Weltzien F.A., Asturiano J.F., 2012. Effect of thermal regime on the expression of key reproductive genes during hormonally-induced vitellogenesis in European eel females. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

De Schryver, P., Sørensen S.R., Tomkiewicz J., Zambonino J., Lauesen P., van der Meer T., Jensen A.M., Kjørsvik E. 2012. Fertilization, incubation and larval rearing techniques. Oral presentation at 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

Skov P.W., 2012. Neuromast development in larvae from captive broodstock. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Støttrup J., Jacobsen C., Tomkiewicz J., Holst L.K., Krüger-Johnsen M., Graver C., Lauesen P. 2012. Fatty acids in broodstock eel. Status for data and results with focus on Series A. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Sørensen S.R., Gallego V., Pérez L., Tomkiewicz J., Asturiano J.F., 2012. Evaluation of different methods for the quantification of European eel spermatozoa for in vitro fertilization assays. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Sørensen S.R., Lauesen P., Munk P., Tomkiewicz J., 2012. Quality criteria and biophysical characteristics of fertilized eggs. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Sørensen S.R., Munk P., Lauesen P. Tomkiewicz J., 2012. Effects of activation salinity on fertilisation rate and egg development in European eel (*Anguilla anguilla*) matured in captivity. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Tomkiewicz, J. 2012. PRO-EEL protocols to reproduce eels and culture larvae – halfway results and core challenges. Oral presentation at 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

Tomkiewicz J., Johnsen M.K., Graver G., Lauesen P. 2012. Overview over tasks and experiments – different series, broodstocks and results. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

Tomkiewicz, J., Støttrup, J., Lauesen, P., Sørensen, S.R., Tveiten, H., Asturiano, J.F., Jacobsen, C., Kaushik, S., Dufour, S., Munk, P., Lafont, A.-G., Perez, L., Corraze, G., Kjørsvik, E, De Schryver, P., Adriaens, D., Bouilliant, M, McEvoy, van der Thillart, G.,F., Krüger-Johnsen, M., Holst, L.K. 2012. Reproduction of European Eel: Towards a Self-Sustained Aquaculture (PRO-EEL). EuroTier, 13–16 November 2012, Hannover, Germany. (Invited oral presentation)

Tveiten H., Sørensen, S.R., Tomkiewicz, J., Van den Thillart G., Dufour S., Pérez L., Lauesen P., 2012. Final oocyte maturation and ovulation. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

Vílchez M.C., Mazzeo I., Peñaranda D.S., Gallego V., Nourizadeh-Lillabadi R., Tveiten H., Dufour S., Asturiano J.F., Weltzien F.A., Pérez L., 2012. Study of vitellogenesis in female European eel under different thermal regimes. Oral presentation at the 3rd PRO-EEL Project Meeting, 26-30 March 2012, Valencia, Spain. (Oral presentation)

van den Thillart Guido E.E.J.M. Dufour S., Lafont A.G., Pérez L., Asturiano J.F., Tveiten H. 2012. Broodstock selection and natural triggers for maturation. 1st PRO-EEL Collaborative Workshop (Valencia, Spain). (Oral presentation).

2013: **J.F. Asturiano**, D.S. Peñaranda, V. Gallego, R. Baeza, M.C. Vílchez, M. Morini, L. Pérez, 2013. Más cerca del reto de reproducir en cautividad a la anguila europea. Oral presentation at XIV Congreso Nacional de Acuicultura, 23-25 September 2013, Gijón (Spain)

R. Baeza, I. Mazzeo, M.C. Vilchez, V. Gallego, D.S. Peñaranda, L. Pérez, J.F. Asturiano, 2013. Movilización de ácidos grasos durante la maduración sexual inducida en machos de anguila europea. Efecto del régimen térmico y relación con parámetros de calidad espermática. Oral presentation at XIV Congreso Nacional de Acuicultura, 23-25 September 2013, Gijón (Spain)

J.F. Asturiano, 2013. Cryopreservation in aquatic species: the European eel as a case of study. Oral presentation at 1st AQUAGAMETE Training School: “Techniques for fish germline cryobanking”, 13-17 May 2013, Cádiz (Spain)

Kraiem, M., **Derouiche, E.**, 2013. Etude comparative de la composition en lipides totaux et acides gras du muscle, du foie et des gonades chez l’anguille euro-méditerranéenne *Anguilla anguilla* (L.1758) femelle argentée tunisienne. Poster presentation at XVèmes Journées Tunisiennes des Sciences de la Mer & 3ème Rencontre Tuniso-Française d’Ichtyologie. Mahdia - Tunesia 14th- 17thDec 2013

Sørensen, S.R., Lauesen, P., Tomkiewicz, J., De Schryver, P. (2013). Microbial Interference with Hatch and Survival of European Eel larvae. Dafinet Workshop – Diagnosis and Control of Fish Diseases, March, 9-11, Frederiksberg, Denmark

Tomkiewicz, J., Sørensen, S.R., 2013. Kunstig befrugtning og babyboom I ålens verden. Dansk Selskab for Marinbiologi, fyraftens møde. 18th June 2013

2014: **Burgerhout, E.**, de Wijze, D.L., Henkel, C.V., Brittij, S.A., Minegishi, Y., Dirks, R.P., Spaink, H.P. van den Thillart, G.E.E.J.M. 2014. Biomarkers for broodstock selection of farmed female European. Oral presentation at the Eel Genome Symposium 2014: new perspectives in evolution, reproduction and ecology, 16-17 January 2014, Leiden (The Netherlands).

Dirks, R.P., Burgerhout, E., de Wijze, D.L., Brittij, S.A., Ozupek, H., Tuinhof-Koelma, N., Spaink, H.P., van den Thillart, G.E.E.J.M. 2014. Molecular markers in pectoral fin to predict hormone-induced maturation of farmed female European eels (*Anguilla anguilla*). Oral presentation at the Eel Genome Symposium 2014: new perspectives in evolution, reproduction and ecology, 16-17 January 2014, Leiden (The Netherlands).

Kjørsvik, E., Mazurais, D., Wold, P.A., Bardal, T., Davidsen, M., Politis, S., Cahu, C., Tomkiewicz, J., and Zambonino-Infante, J. (2014). Funksjonell utvikling av muskel og fordøyelsessystem i plommesecklarver hos Atlantisk ål (*Anguilla anguilla*). Oral presentation at HAVBRUK 2014, Tromsø, Norway (Research Council of Norway, annual aquaculture conference).

M. Morini, D.S. Peñaranda, M.C. Vilchez, V. Gallego, R. Nourizadeh-Lillabadi, J.F. Asturiano, F.-A. Weltzien, L. Pérez, 2014. DHP receptors and PLCZ1 expression during the European eel spermatogenesis. Oral presentation at the Eel Genome Symposium 2014: new perspectives in evolution, reproduction and ecology, 16-17 January 2014, Leiden (The Netherlands)

M. Morini, A.G. Lafont, D.S. Peñaranda, M.C. Vilchez, V. Gallego, J.F. Asturiano, S. Dufour, L. Pérez, 2014. Characterization and expression of the Vitellogenin receptor in the European eel. Poster presentation at the Eel Genome Symposium 2014: new perspectives in evolution, reproduction and ecology, 16-17 January 2014, Leiden (The Netherlands) (Poster presentation)

Müller A.V., José M. Amigo, Fintan J. McEvoy, Sebastian N. Politis, Jonna Tomkiewicz 2014. Using ultrasound in monitoring induced ovarian maturation in the European eel

(*Anguilla anguilla*). Poster presentation at Danish Technical University - *Visionday* 2014 – May. Poster presentation

Peñaranda D.S., M. Morini, M.C. Vilchez, V. Gallego, I. Mazzeo, R.P. Dirks, G.E.E.J.M. van den Thillart, H. Tveiten, L. Pérez, J.F. Asturiano, 2014. Modulation of testis steroidogenic enzymes gene expression and steroid synthesis by temperature thermal regime during spermatogenesis in European eel. Oral presentation at the Eel Genome Symposium 2014: new perspectives in evolution, reproduction and ecology, 16-17 January 2014, Leiden (The Netherlands)

Sørensen S.R. 2014. Improving biophysical rearing conditions during early life stages of European eel. Oral presentation at Dafinet and Targetfish FP7 Workshop –Fish models in Research, 11-13 Nov University of Copenhagen, Denmark

Tomkiewicz J., 2014. European eel as experimental model I: Assisted reproduction technology and standardized fertilization methods for mass production of viability embryos and larvae. Oral presentation at Dafinet and Targetfish FP7 Workshop –Fish models in Research, 11-13 Nov. University of Copenhagen, Denmark

2.2 B - Patents and exploitable foreground

B1 List of applications for patents, trademarks, registered designs, etc. &

B2 exploitable foreground

No patent applications were elaborated/granted during the project period. Consequently B2-*Exploitable foreground* is not relevant.

2.3 C - Education and young researcher training

C1 List of education and young researcher training during PRO-EEL

The PRO-EEL project provides the basis of the education and training of bachelor, master and PhD students as well as early career options for Post-Docs. During the 2nd project period, studies commencing during the 1st project period were completed and new studies initiated. Studies specified below include: Bachelor, Master, PhD, Post-Doc, and other. Some of these were partly funded by the PRO-EEL project while others received funding from other sources.

Bachelor -accomplished:

Brondeel Carlien, Preparing to feed: combining functional morphology and kinematics in European eel larvae (*Anguilla anguilla*) (In Dutch, Original title: “Zich klaarmaken om te voeden: het samenspel van functionele morfologie en kinematica in Europese palinglarven (*Anguilla anguilla*)”) Ghent University, 2013, 2 months, Supervisors: D. Adriaens (UGent), M. Bouilliar (UGent).

Charlotte Colle, First characterization of GnIH receptors in the European eel. Université Pierre & Marie Curie, 2011, 3 months, Supervisors: J. Pasquier (UPMC), S. Dufour (CNRS).

Florian Denis, Kisspeptin regulation in the European eel. Ingeneer Supbiotech Paris, 2012, 3 months, Supervisors: J. Pasquier (UPMC), S. Dufour (CNRS).

María Elena Candalija Delgado, Expresión del gen codificador de la aromatasa en anguilas europeas sometidas a distintos regímenes térmicos durante el tratamiento hormonal inductor de la maduración sexual (“Expression of the gene codifying aromatase in European eels submitted to different thermal regimes during the hormonal treatment inducing the sexual maturation”). High Technical School of Agronomy Engineering and Natural Environment. Bachelor in Biotechnology. Universitat Politècnica de València, 2011. Supervisor: J.F. Asturiano (ICTA-UPV).

M^a Carmen Vílchez Olivencia, Efecto del régimen térmico en la vitelogenesis de la anguila europea (*Anguilla anguilla*) (“Effect of the thermal regime on the vitellogenesis of the European eel (*Anguilla anguilla*)”). High Technical School of Agronomy Engineering and Natural Environment. Bachelor in Engineering Agronomy. Universitat Politècnica de València, 2011. Supervisor: L. Pérez (ICTA-UPV).

Eladio Balbín Andújar, Efecto del régimen térmico en el desarrollo ovárico de hembras de anguila europea (*Anguilla anguilla*). (“Effect of the thermal regime on the ovarian development of the female European eels (*Anguilla anguilla*)”). High Technical School of Agronomy Engineering and Natural Environment. Bachelor in Technical Engineering in Agriculture. Universitat Politècnica de València, 2012. Supervisor: L. Pérez (ICTA-UPV).

Víctor García Herranz, Regulación de la expresión génica de enzimas esteroideogénicas en anguila europea (*Anguilla anguilla*) durante la maduración sexual inducida bajo tres regímenes térmicos y su relación con la calidad del esperma (“Regulation of the gene expression of steroidogenic enzymes in European eel (*Anguilla anguilla*) during the sexual maturation induced under three thermic regimes and its relation with the sperm quality”). High Technical School of Agronomy Engineering and Natural Environment. Bachelor in Biotechnology. Universitat Politècnica de València, 2013. Supervisors: J.F. Asturiano and D.S. Peñaranda (ICTA-UPV).

Morten Rose. Final oocyte maturation and egg/oocyte quality in European eel. BSc Thesis, Copenhagen University, Denmark. Supervisors: Jonna Tomkiewicz (DTU), Anders Priemé (KU).

Julie Josias Nielsen. European eel reproduction: Oogenesis and ovarian development. Bachelor thesis • Environmental Biology • Submission: 30. May 2014. Supervisors: Benni W. Hansen Roskilde University and Jonna Tomkiewicz, DTU.

Bachelor -ongoing:

Estefanía Esteller Sanchís, Comparativa de dos protocolos de congelación de esperma de anguila europea (*Anguilla anguilla*) con el objetivo de su estandarización (“Comparison of two protocols for the cryopreservation of European eel (*Anguilla anguilla*) sperm with the standardization as a target”). High Technical School of Agronomy Engineering and Natural Environment. Bachelor in Biotechnology. Universitat Politècnica de València, 2013. Supervisors: J.F. Asturiano and D.S. Peñaranda (ICTA-UPV).

Master -accomplished:

Alejandro B. Sastre Lapeyre. 2011. Estudio comparativo de la eficacia y rentabilidad económica de tres tratamientos hormonales en la espermiación de anguila europea.

(“Comparative study of the efficacy and economical profitability of three hormonal treatments to induce spermiation on the European eel”). Universitat Politècnica de València. MSc-thesis, Inter-university Aquaculture Master. Supervisor: J.F. Asturiano (ICTA-UPV).

Christian Atkinson. 2012. Characterization of the GnIH system in the European eel. University of Keele UK, Master Erasmus Program, 2012, 6 months. Supervisors: J. Pasquier (UPMC), S. Dufour (CNRS).

Leanne Hardman. 2012. Characterization of the corticosteroid receptors in the European eel. University of Keele UK, Master Erasmus Program, 2012, 6 months, Supervisors: AG Lafont (CNRS-PRO-EEL), S. Dufour (CNRS)

Marina Morini. 2012. Characterization of the leptin system in the European eel. Université Pierre & Marie Curie, Master Integrative Biology and Physiology, 2011-2012, 9 months, Supervisors: AG Lafont (CNRS-PRO-EEL), S. Dufour (CNRS).

Martin Davidsen. 2012. The effect of incubation temperature on embryonic development and muscle growth in yolk-sac larvae of the European eel (*Anguilla anguilla* L., 1758). Norwegian University of Science and Technology (NTNU), Dept. of Biology, MSc-thesis in aquaculture, International MSc-programme “Marine Coastal Development”. Supervisors: Elin Kjörsvik (NTNU), J. Tomkiewicz (DTU).

Mateo Ballester Moltó. 2011. Efectos del régimen térmico en el desarrollo sexual de machos de anguila europea (*Anguilla anguilla*). (“Effects of the thermic regime on the sexual development of male European eels (*Anguilla anguilla*)”). Universitat Politècnica de València. MSc-thesis, Inter-university Aquaculture Master. Supervisor: Luz Pérez (ICTA-UPV).

Rafael Darío Navarro Miranda. 2012. Efecto del régimen térmico en el desarrollo ovárico de hembras de anguila europea (*Anguilla anguilla*). (“Effect of the thermic regime on the ovarian development of female European eels (*Anguilla anguilla*)”). Universitat Politècnica de València. MSc-thesis, Inter-university Aquaculture Master. Supervisor: Luz Pérez (ICTA-UPV).

Virginia Calabuig. 2010. Efecto de la temperatura en el desarrollo gonadal de hembras de anguila inducidas hormonalmente. (“Effect of the temperature on the gonad development of female European eels hormonally induced”). Universitat Politècnica de València. MSc-thesis, Inter-university Aquaculture Master. Supervisor: Luz Pérez (ICTA-UPV).

María Carmen Vilchez Olivencia. 2013. Efecto del régimen térmico en la calidad de los huevos y desarrollo embrionario de la anguila europea (*Anguilla anguilla*) (“Effect of thermal regime on egg quality and embryo development in European eel”). Universitat Politècnica de València. MSc-thesis, Inter-university Aquaculture Master. Supervisor: Luz Pérez (ICTA-UPV).

Rozenfeld, C. 2014. mRNA expression impacts hatching success in European eel (*Anguilla anguilla* L.). MSc Thesis, Technical University of Denmark. Supervisors: Ian A.E. Butts, Jonna Tomkiewicz (DTU), Co-supervisors: José Zambonino and David Mazurias (IFREMER).

Master -ongoing:

Helene Rønquist Knutsen. Development of skeletal muscle and digestive system in wild European eel (*Anguilla anguilla* L.) larvae in relation to size. Norwegian University of Science and Technology (NTNU), Dept. of Biology, MSc-project in aquaculture, International MSc-programme “Marine Coastal Development”. Supervisors: Elin Kjörsvik (NTNU), J. Tomkiewicz (DTU).

PhD -accomplished:

Lene Buelund, 2011. Tracking tissue changes during hormonally induced maturation by means of computer tomography scans in female European Eel in Aspects of Quantification in Veterinary Imaging PhD Thesis, University of Copenhagen 2011. Supervisors FJ McEvoy and EL Svalastoga.

Víctor Gallego. 2013. Sperm physiology and quality in two marine teleosts: *Anguilla anguilla* & *Takifugu niphobles*. Departamento de Ciencia Animal. Universitat Politècnica de València. PhD fellowship from the Spanish Ministry of Economy and Competitiveness (MINECO; AGL2010-16009). 2009-2013. Supervisor: J.F. Asturiano (ICTA-UPV).

Erik Burgerhout. Tools and triggers for eel reproduction. PhD thesis, Leiden University (2013). Co-financed by PRO-EEL. 2012-2013. Supervisors HP Spaink, GEEJM van den Thillart, RP Dirks.

Sørensen, S.R. 2014. PhD Thesis: On the way to successful European eel larval rearing: Impact of biophysical conditions and gamete quality. PhD Thesis DTU Aqua. Supervisor: Jonna Tomkiewicz, Co-supervisors: Peter Munk, DTU Aqua, Peter Bossier ARC, University of Ghent, BE. Defended 25th of Feb. 2014 DTU Aqua.

PhD -ongoing:

Anna Wilhelmina Müller, 2012. Texture analysis of ultrasound imaging in the prediction of reproductive outcomes in female European Eel, in "Application of chemometric statistical techniques in veterinary imaging. PhD thesis, University of Copenhagen (2012). Supervisors FJ McEvoy and J Amigo.

Daniëlle de Wijze. Artificial reproduction of European eel: cell-based slow hormone-release systems and molecular maturation markers. Leiden University (LU), Dept. of Molecular Cell Biology, financed by PRO-EEL. 2010-2012 (stopped after two years). Supervisors: Guido van den Thillart (LU), Herman Spaink (LU), Ron Dirks (LU).

Emna Derouiche. Suivi biologique et dynamique des fractions de populations d'Anguille euro-méditerranéenne (*Anguilla anguilla*) dans différents hydrosystèmes tunisiens. Faculté des Sciences de Tunis. PhD fellowship from the Ministry of High Education and Scientific Research. 2009-2014, Supervisor: M. Kraïem (INSTM).

Filipa da Silva. Reproduction capacity of European eel in captivity: Fecundity, follicular maturation and developmental competence of embryos and larvae. Technical University of Denmark (DTU) & Norwegian University of Science and Technology (NTNU), PhD School Aquatic Sciences and Technology, PhD fellowship DTU, 2012-2014. Supervisors: J. Tomkiewicz (DTU), Elin Kjörsvik (NTNU) and Helge Tveiten (NOFIMA).

Ilaria Mazzeo. Effect of thermal regime on the expression of key reproductive genes during vitellogenesis hormonally induced in European eel females. Departamento de Ciencia Animal. Universitat Politècnica de València. PhD fellowship from Generalitat Valenciana. 2009-2014. Supervisors: J.F. Asturiano, D.S. Peñaranda (ICTA-UPV).

Jérémy Pasquier, 2012. Evolution of the neuroendocrine control of reproduction: origin and role of the kisspeptin system? Université Pierre & Marie Curie, PhD School Life Diversity, PhD fellowship from the Ministry of Research and Education. 2009-2012, Supervisors: S. Dufour (CNRS), K. Rousseau (MNHN), AG Lafont (CNRS-PRO-EEL).

María Carmen Vílchez. Factores ambientales y endocrinos implicados en la calidad de los gametos de la anguila europea. (“Environmental and endocrine factors conditioning the quality of the European eel gametes”). Departamento de Ciencia Animal. Universitat Politècnica de València. PhD fellowship co-funded by ICTA-UPV (Beca FPI Subprograma 2 UPV. 2011-S2-02-6521) and PRO-EEL. Supervisors: J.F. Asturiano, L. Pérez (ICTA-UPV). 2011-2014.

Marina Morini. Aspectos moleculares relacionados con el proceso reproductivo de la anguila europea. (“Molecular aspects related with the reproductive process of the European eel”). Departamento de Ciencia Animal. Universitat Politècnica de València. PhD fellowship funded by Programa Santiago Grisolia (Generalitat Valenciana. GRISOLIA/2012/006) as complementary help to PRO-EEL. 2011-2014. Supervisors: J.F. Asturiano, D.S. Peñaranda (ICTA-UPV).

Mathias Boulliart. Ontogenetic changes in feeding performance in Anguillidae leptocephalus larvae. Ghent University, PhD School Natural Sciences, PhD fellowship BOF, 2012-2016. Supervisor: Dominique Adriaens (Ghent University).

Rosa Baeza. Efecto de diferentes regímenes térmicos en la movilización de lípidos durante la maduración sexual de la anguila europea (*Anguilla anguilla*). (“Effect of different thermal regimes on the lipid mobilization during the sexual maturation of the European eel (*Anguilla anguilla*)”). Departamento de Ciencia Animal. Universitat Politècnica de València. PhD contract co-funded by ICTA-UPV and PRO-EEL. 2011-2014. Supervisor: J.F. Asturiano (ICTA-UPV).

Sebastian N. Politis. European eel ontogeny and physiology. Technical University of Denmark, PhD School Aquatic Sciences and Technology, PhD fellowship DTU, 2014-2017. Supervisors: Dr. Jonna Tomkiewicz (DTU), Ian Butts (DTU), and José Zambonino (Ifremer).

Post Doc -accomplished:

Cecile Jolly. 2010-2011. Neuroanatomical and functional study of the dopamine system involved in the control of reproduction in teleosts. CNRS. 2010-2011, ANR Grant. Supervisors: S. Dufour (CNRS), C. Pasqualini (CNRS)

Erik Burgerhout. Natural triggers of maturation, slow hormone-release systems and molecular maturation markers. Leiden University (LU), Dept. of Molecular Cell Biology, financed by PRO-EEL. 2013.

Gersende Maugars. 2011. Characterization of glycoprotein hormones and their receptors in the eel. MNHN Grant. Supervisor: S. Dufour (CNRS)

Yuki Minegishi. Eel-specific bioassays for gonadotropic hormones and molecular maturation markers. Leiden University (LU), Molecular Cell Biology, financed by PRO-EEL. 2010-2012.

Anne-Gaëlle Lafont. Study of the neuroendocrine systems in the European eel and role in the control

Post Doc -ongoing:

of reproduction. 2010-2013. PRO-EEL project CNRS. Supervisor: S. Dufour (CNRS)

Gersende Maugars. Study of glycoprotein hormones and their receptors in the eel. Evolution of glycoprotein hormones and their receptors in vertebrates. 2012-2013, CNRS Grant. Supervisor: S. Dufour (CNRS)

David Sánchez Peñaranda. Contract co-funded by the Spanish Ministry of Economy and Competitiveness (PTA2011-4948-I) and ICTA-UPV (including PRO-EEL support). Universitat Politècnica de València. 2012-2014. Supervisor: J.F. Asturiano (ICTA-UPV).

Sune Riis Sørensen. Microbial management and technology development to sustain embryo and larval culture of European eel. 2014-2016. Supervisor Jonna Tomkiewicz and Ian A.E. Butts DTU Aqua.

Other -accomplished:

David Sánchez Peñaranda. Research Stay (PRO-EEL experimentation). Norwegian School of Veterinary Science (NSVS) of Oslo (Norway). Co-funded by UPV, NSVS and PRO-EEL. 16 months between 2009 and 2011. Supervisor Dr. Finn-Arne Weltzien, NSVS, Norway.

Pégourié Gaïa (2012). Influence de l'apport alimentaire en acides gras long polyinsaturés sur les performances de reproduction et le métabolisme lipidique chez des géniteurs d'anguille Européenne (*Anguilla anguilla*). BTS Analyses Biologiques et Biotechnologiques, Centre de Formation des Apprentis Agricoles, Hasparren (64). Supervisor : Corraze Geneviève, INRA.

Christoffer Rozenfeld (August 2013 - December 2013): Technical University of Denmark. MSc. Thematic project title: Gene expression of digestive enzymes in larvae of European eel (*Anguilla anguilla*). Supervisor: Ian Butts (DTU) and Jonna Tomkiewicz (DTU).

3 Report on societal implications

A General Information (completed automatically when Grant Agreement number is entered.

Grant Agreement Number:

Title of Project:

Name and Title of Coordinator:

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)?

1. If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports?

No

Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 'Work Progress and Achievements'

2. Please indicate whether your project involved any of the following issues (tick box) :

YES

RESEARCH ON HUMANS

2. Did the project involve children?

No

3. Did the project involve patients?

No

4. Did the project involve persons not able to give consent?

No

5. Did the project involve adult healthy volunteers?

No

6. Did the project involve Human genetic material?

No

• Did the project involve Human biological samples?

No

• Did the project involve Human data collection?

No

RESEARCH ON HUMAN EMBRYO/FOETUS

7. Did the project involve Human Embryos?

No

8. Did the project involve Human Foetal Tissue / Cells?

No

9. Did the project involve Human Embryonic Stem Cells (hESCs)?

No

10. Did the project on human Embryonic Stem Cells involve cells in culture?

No

11. Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?

No

PRIVACY

12. Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?

13. Did the project involve tracking the location or observation of people?

No

RESEARCH ON ANIMALS

14. Did the project involve research on animals?

Yes

15. Were those animals transgenic small laboratory animals?

No

16. Were those animals transgenic farm animals?

No

17. Were those animals cloned farm animals?

No

18. Were those animals non-human primates?

No

RESEARCH INVOLVING DEVELOPING COUNTRIES

19. Did the project involve the use of local resources (genetic, animal, plant etc)?

No

20. Was the project of benefit to local community (capacity building, access to healthcare, education etc)?

No

DUAL USE

• Research having direct military use

No

21. Research having the potential for terrorist abuse

No

C Workforce Statistics		
3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).		
Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	0
Work package leaders	2	7
Experienced researchers (i.e. PhD holders)	16	30
PhD Students	7	10
Other	27	28
4. How many additional researchers (in companies and universities) were recruited specifically for this project?		5
Of which, indicate the number of men:		1
D Gender Aspects		
5. Did you carry out specific Gender Equality Actions under the project?		<input type="radio"/> Yes <input checked="" type="radio"/> No
6. Which of the following actions did you carry out and how effective were they?		
	Not at all effective	Very effective
<input type="checkbox"/> Design and implement an equal opportunity policy	○ ○ ○ ○ ○	○ ○ ○ ○ ○
<input type="checkbox"/> Set targets to achieve a gender balance in the workforce	○ ○ ○ ○ ○	○ ○ ○ ○ ○
<input type="checkbox"/> Organise conferences and workshops on gender	○ ○ ○ ○ ○	○ ○ ○ ○ ○
<input type="checkbox"/> Actions to improve work-life balance	○ ○ ○ ○ ○	○ ○ ○ ○ ○
<input type="radio"/> Other:	<input style="width: 100%;" type="text"/>	
7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?		
<input type="radio"/> Yes- please specify <input style="width: 150px;" type="text"/>		
<input checked="" type="radio"/> No		
E Synergies with Science Education		
8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?		
<input type="radio"/> Yes- please specify <input style="width: 150px;" type="text"/>		
<input checked="" type="radio"/> No		
9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?		
<input type="radio"/> Yes- please specify <input style="width: 150px;" type="text"/>		
<input checked="" type="radio"/> No		

F Interdisciplinarity				
10. Which disciplines (see list below) are involved in your project?				
<input checked="" type="checkbox"/>	Main discipline ¹ : 1.5			
<input checked="" type="checkbox"/>	Associated discipline ¹ : 2.3	<input type="checkbox"/>	Associated discipline ¹ : 4.1	
G Engaging with Civil society and policy makers				
11a Did your project engage with societal actors beyond the research community? (if 'No', go to Question 14)			<input checked="" type="checkbox"/> <input type="checkbox"/>	Yes No
11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?				
	<input type="checkbox"/> No			
	<input type="checkbox"/> Yes- in determining what research should be performed			
	<input type="checkbox"/> Yes - in implementing the research			
	<input checked="" type="checkbox"/> Yes, in communicating /disseminating / using the results of the project			
11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?			<input type="checkbox"/> <input checked="" type="checkbox"/>	Yes No
12. Did you engage with government / public bodies or policy makers (including international organisations)				
	<input type="checkbox"/> No			
	<input type="checkbox"/> Yes - in framing the research agenda			
	<input type="checkbox"/> Yes - in implementing the research agenda			
	<input checked="" type="checkbox"/> Yes, in communicating /disseminating / using the results of the project			
13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?				
	<input checked="" type="checkbox"/> Yes – as a primary objective (please indicate areas below- multiple answers possible)			
	<input checked="" type="checkbox"/> Yes – as a secondary objective (please indicate areas below - multiple answer possible)			
	<input type="checkbox"/> No			
13b If Yes, in which fields?				
Agriculture		Energy		Human rights
Audiovisual and Media		Enlargement		Information Society
Budget		Enterprise		Institutional affairs
Competition		Environment		Internal Market
Consumers		External Relations		Justice, freedom and security
Culture		External Trade		Public Health
Customs		Fisheries and Maritime Affairs	X	Regional Policy
Development Economic and Monetary Affairs		Food Safety		Research and Innovation
Education, Training, Youth		Foreign and Security Policy		Space
Employment and Social Affairs		Fraud		Taxation
		Humanitarian aid		Transport

¹ Insert number from list below (Frascati Manual).

13c If Yes, at which level? <input type="radio"/> Local / regional levels <input type="radio"/> National level <input type="radio"/> European level <input checked="" type="radio"/> International level		
H Use and dissemination		
14. How many Articles were published/accepted for publication in peer-reviewed journals?	>40	
To how many of these is open access² provided?		
How many of these are published in open access journals?	<10 (e.g. PlosOne)	
How many of these are published in open repositories?	>30 (e.g. ReseachGate)	
To how many of these is open access not provided?		
Hardly any		
Please check all applicable reasons for not providing open access:		
<input type="checkbox"/> publisher's licensing agreement would not permit publishing in a repository <input type="checkbox"/> no suitable repository available <input type="checkbox"/> no suitable open access journal available <input type="checkbox"/> no funds available to publish in an open access journal <input type="checkbox"/> lack of time and resources <input type="checkbox"/> lack of information on open access <input type="checkbox"/> other ³ :		
15. How many new patent applications ('priority filings') have been made? <i>("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).</i>	None	
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	None
	Registered design	None
	Other	None
17. How many spin-off companies were created / are planned as a direct result of the project?	None	
<i>Indicate the approximate number of additional jobs in these companies:</i>		
18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:		
<input type="checkbox"/> Increase in employment, or <input checked="" type="checkbox"/> Safeguard employment, or <input type="checkbox"/> Decrease in employment, <input type="checkbox"/> Difficult to estimate / not possible to quantify	<input checked="" type="checkbox"/> In small & medium-sized enterprises <input type="checkbox"/> In large companies <input type="checkbox"/> None of the above / not relevant to the project	
19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (FTE = one person working fulltime for a year) jobs:	<i>Indicate figure:</i>	

² Open Access is defined as free of charge access for anyone via Internet.

³ For instance: classification for security project.

Difficult to estimate / not possible to quantify	X
I Media and Communication to the general public	
20. As part of the project, were any of the beneficiaries professionals in communication or media relations?	
<input type="radio"/> Yes	<input checked="" type="radio"/> No
21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?	
<input checked="" type="radio"/> Yes	<input type="radio"/> No
22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?	
<input checked="" type="checkbox"/> Press Release	<input type="checkbox"/> Coverage in specialist press
<input checked="" type="checkbox"/> Media briefing	<input checked="" type="checkbox"/> Coverage in general (non-specialist) press
<input checked="" type="checkbox"/> TV coverage / report	<input checked="" type="checkbox"/> Coverage in national press
<input checked="" type="checkbox"/> Radio coverage / report	<input checked="" type="checkbox"/> Coverage in international press
<input checked="" type="checkbox"/> Brochures /posters / flyers	<input checked="" type="checkbox"/> Website for the general public / internet
<input type="checkbox"/> DVD /Film /Multimedia	<input checked="" type="checkbox"/> Event targeting general public (festival, conference, exhibition, science café)
23 In which languages are the information products for the general public produced?	
<input type="checkbox"/> Language of the coordinator	<input checked="" type="checkbox"/> English
<input type="checkbox"/> Other language(s)	

Question F-10: Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

- 1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]
- 1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)
- 1.3 Chemical sciences (chemistry, other allied subjects)
- 1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)
- 1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2. ENGINEERING AND TECHNOLOGY

- 2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)
- 2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]
- 2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised

technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

- 3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)
- 3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)
- 3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

- 4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)
- 4.2 Veterinary medicine

5. SOCIAL SCIENCES

- 5.1 Psychology
- 5.2 Economics
- 5.3 Educational sciences (education and training and other allied subjects)
- 5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary, methodological and historical SIT activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

- 6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)
- 6.2 Languages and literature (ancient and modern)
- 6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other SIT activities relating to the subjects in this group]

4 PRO-EEL partners and contacts

DTU

Technical University of Denmark,
National Institute of Aquatic Resources
Kavalerigården 6, Charlottenlund, 2920
Denmark
Contact person: Dr. Jonna Tomkiewicz
E-mail: jt@aqua.dtu.dk
Tel: +45 35883408

DLO

Foundation for Agriculture Research,
Institute for Marine Resources and Eco-
system Studies, Haringkade 1
1976 CP IJmuiden, The Netherlands
Contact person: Dr. Michiel Kotterman
E-mail: E-mail: Michiel.Kotterman@wur.nl
Tel: +31 317 487132

UL

University of Leiden, Kaiserstraat 63
2311 GP Leiden 2.05, Netherlands
Contact person: Dr. Guido van den
Thillart, E-mail:
g.van.den.thillart@biology.leidenuniv.nl
Tel: +31 (0)71 527 4363

CNRS

National Center for Scientific Research,
Rue Cuvier, CP- 7, Paris 75231/05
France
Contact person: Dr. Sylvie Dufour
E-mail: dufour@mnhn.fr
Tel: +33 (0)140793612

ICTA-UPV

Polytechnic University of Valencia. Insti-
tute for Animal Science and Technology
s/n, Camino de Vera, Valencia, 46022
Spain
Contact person: Dr. Juan F. Asturiano
E-mail: jfastu@dca.upv.es
Tel: +34 963879385

NOFIMA

Nofima Akvaforsk - Fiskeriforskning A/S
Muninbakken 9-13, Tromsø 9291, Nor-
way. Contact person: Dr. Helge Tveiten
E-mail: helge.tveiten@nofima.no
Tel: +47 77629000

UGENT

Ghent University, Rozier 44
Ghent 9000, Belgium
Contact persons: Prof. Peter Bossier
E-mail: peter.bossier@ugent.be
Tel: +32 92643759

KU

University of Copenhagen, Dyrhægevej
16, Frederiksberg C, 1870, Denmark
Contact person: Fintan McEvoy
E-mail: fme@life.ku.dk
Tel: +45 35332866

INRA

National Institute for Agronomic Re-
search, RD 918, Quartier ibarron, Saint
Pée sur Nivelle, 64310, France
Contact persons: Prof. Sadasivam Kausik
E-mail: kaushik@st-pee.inra.fr
Tel: +33 559515990

BA

Billund Aquaculture Service Aps., Kløver-
marken 27, Billund, 7190
Contact person: M. Dir. Bjarne Hald Olsen
E-mail: bjarne@billund-aqua.dk
Tel: +45 75338720

WU

Wageningen University, Marijkeweg 40
531/OA.060 Wageningen 6709PG
Netherlands
Contact person: Prof. Johan Verreth
E-mail: Johan.Verreth@wur.nl
Tel: +31 (0)317 48 3307

INSTM

National Institute of Sciences and Tech-
nologies of the Sea. Salammbô 28, Tunis
2025, Tunisia Contact person:
Prof. Mohammed Mejededdine Kraiem
E-mail: mejd.kraiem@instm.mrt.tn
Tel: +21 67170420

IMR

Institute for Marine Research, Austevoll
Marine Aquaculture Station, Storebø
5392, Norway
Contact person: Dr. Anders Mangor
Jensen, E-mail: AndersMJ@IMR.no
Tel: +47 56182263

NTNU

Norwegian University of Science and
Technology. Center of Fisheries and
Aquaculture, Battørkaia 17B,
Trondheim, 7010, Norway
Contact person: Prof. Elin Kjörsvik
E-mail: elin.kjorsvik@bio.ntnu.no
Tel: +47 73596313

BIOMAR

BioMar A/S, Mylius Erichsensvej 35
Brande, 7330, Denmark
Contact person: Mr. Lars Kristian Holst
E-mail: kh@biomar.dk
Tel: +45 96613107