Assessing pre- and post-zygotic barriers between North Atlantic eels (Anguilla anguilla and A. rostrata)

Elucidating barriers to gene flow is important for understanding the dynamics of speciation. Here we investigate pre- and post-zygotic mechanisms acting between the two hybridizing species of Atlantic eels: Anguilla anguilla and A. rostrata. Temporally varying hybridization was examined by analyzing 85 species-diagnostic single-nucleotide polymorphisms (SNPs; FST 0.95) in eel larvae sampled in the spawning region in the Sargasso Sea in 2007 (N=92) and 2014 (N=460). We further investigated whether genotypes at these SNPs were nonrandomly distributed in post-F1 hybrids, indicating selection. Finally, we sequenced the mitochondrial ATP6 and nuclear ATP5c1 genes in 19 hybrids, identified using SNP and restriction site associated DNA (RAD) sequencing data, to test a previously proposed hypothesis of cytonuclear incompatibility leading to adenosine triphosphate (ATP) synthase dysfunction and selection against hybrids. No F1 hybrids but only later backcrosses were observed in the Sargasso Sea in 2007 and 2014. This suggests that interbreeding between the two species only occurs in some years, possibly controlled by environmental conditions at the spawning grounds, or that interbreeding has diminished through time as a result of a declining number of spawners. Moreover, potential selection was found at the nuclear and the cytonuclear levels. Nonetheless, one glass eel individual showed a mismatch, involving an American ATP6 haplotype and European ATP5c1 alleles. This contradicted the presence of cytonuclear incompatibility but may be explained by that (1) cytonuclear incompatibility is incomplete, (2) selection acts at a later life stage or (3) other genes are important for protein function. In total, the study demonstrates the utility of genomic data when examining pre- and post-zygotic barriers in natural hybrids. Heredity advance online publication, 9 November 2016; doi:10.1038/hdy.2016.96.
Temperature effects on gene expression and morphological development of European eel, Anguilla anguilla larvae

Temperature is important for optimization of rearing conditions in aquaculture, especially during the critical early life history stages of fish. Here, we experimentally investigated the impact of temperature (16, 18, 20, 22 and 24°C) on thermally induced phenotypic variability, from larval hatch to first-feeding, and the linked expression of targeted genes [heat shock proteins (hsp), growth hormone (gh) and insulin-like growth factors (igf)] associated to larval performance of European eel, Anguilla anguilla. Temperature effects on larval morphology and gene expression were investigated throughout early larval development (in real time from 0 to 18 days post hatch) and at specific developmental stages (hatch, jaw/teeth formation, and first-feeding). Results showed that hatch success, yolk utilization efficiency, survival, deformities, yolk utilization, and growth rates were all significantly affected by temperature. In real time, increasing temperature from 16 to 22°C accelerated larval development, while larval gene expression patterns (hsp70, hsp90, gh and igf) were delayed at cold temperatures (16°C) or accelerated at warm temperatures (20-22°C). All targeted genes (hsp70, hsp90, gh, igf-1, igf-2a, igf-2b) were differentially expressed during larval development. Moreover, expression of gh was highest at 16°C during the jaw/teeth formation, and the first-feeding developmental stages, while expression of hsp90 was highest at 22°C, suggesting thermal stress. Furthermore, 24°C was shown to be deleterious (resulting in 100% mortality), while 16°C and 22°C (~50 and 90% deformities respectively) represent the lower and upper thermal tolerance limits. In conclusion, the high survival, lowest incidence of deformities at hatch, high yolk utilization efficiency, high gh and low hsp expression, suggest 18°C as the optimal temperature for offspring of European eel. Furthermore, our results suggest that the still enigmatic early life history stages of European eel may inhabit the deeper layer of the Sargasso Sea and indicate
vulnerability of this critically endangered species to increasing ocean temperature.
Effects of salinity and sea salt type on egg activation, fertilization, buoyancy and early embryology of European eel, *Anguilla anguilla*

Improper activation and swelling of in vitro produced eggs of European eel, *Anguilla anguilla*, has been shown to negatively affect embryonic development and hatching. We investigated this phenomenon by examining the effects of salinity and sea salt type on egg dimensions, cell cleavage patterns and egg buoyancy. Egg diameter after activation, using natural seawater adjusted to different salinities, varied among female eels, but no consistent pattern emerged. Activation salinities between 30–40 practical salinity unit (psu) produced higher quality eggs and generally larger egg diameters. Chorion diameters reached maximal values of 1642 ± 8 μm at 35 psu. A positive relationship was found between egg neutral buoyancy and activation salinity. Nine salt types were investigated as activation and incubation media. Five of these types induced a substantial perivitelline space (PVS), leading to large egg sizes, while the remaining four salt types resulted in smaller eggs. All salt types except NaCl treatments led to high fertilization rates and had no effect on fertilization success as well as egg neutral buoyancies at 7 h post-fertilization. The study points to the importance of considering ionic composition of the media when rearing fish eggs and further studies are encouraged.

**General information**

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography
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BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.503 SNIP 0.536
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First-feeding by European eel larvae: A step towards closing the life cycle in captivity
First evidence of first-feeding European eel larvae that have been reared in captivity•Up to 50% of larvae ingested a diet composed of concentrated rotifer paste, with or without natural feeding stimulants•Documentation of a significant increase in feeding success under higher light intensities•Results move us a step closer towards understanding an undisclosed phase in the European eel life cycle
First production of larvae using cryopreserved sperm: Effects of preservation temperature and cryopreservation on European eel sperm fertilization capacity

Sperm cryopreservation is a useful tool in captive fish reproduction management, that is to synchronize gamete production, especially in the case of species as the European eel, where the time of female spawning readiness is unpredictable. Several protocols to cryopreserve sperm of this species have been described, but until recently fertilization trials were not feasible. This study evaluated the effect of cold storage of diluted sperm prior to fertilizations and tested whether a previously defined protocol for European eel sperm cryopreservation can be successfully applied in fertilization trials to produce viable offspring. In our experiment, the sperm motility was evaluated after the extraction and the best samples were selected and pooled. Until stripping of eggs and fertilization, diluted sperm samples were maintained at either 4 or 20°C, or cryopreserved, following existing protocols. Fertilization of two egg batches was attempted. Diluted sperm caused a similar percentage of fertilized eggs and a similar number of embryos and larvae, independently of storage temperature (4 or 20°C). The cryopreserved sperm resulted in a lower percentage of fertilized eggs, but embryos developed and a few larvae (cryolarvae) were obtained 55 h after fertilization in one of the two egg batches. This result evidences that the tested cryopreservation protocol is applicable for eel reproduction management, although improvements will be required to enhance fertilization success.

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Universidad Politécnica de Valencia, Billund Aquakulturservice A/S
Authors: Asturiano, J. (Ekstern), Sørensen, S. R. (Intern), Perez, L. (Ekstern), Lauesen, P. (Ekstern), Tomkiewicz, J. (Intern)
Ontogeny and growth of early life stages of captive-bred European eel

Captive breeding of European eel, Anguilla anguilla is challenged by the complex hormonal control of Anguillid eel reproduction and the distinctive ontogeny of the leptocephalus larvae that are unique to the Elopomorph superorder. Recent experimental research has succeeded in the production of viable eggs and larvae of European eel, providing the basis for studies on early life stages of this species in captivity. In this study, we describe and illustrate morphological characteristics of eggs, embryos, and larvae from fertilization to termination.
of the yolk sac stage and provide a comparison with additional commercially important eel species. Furthermore, we model growth during the critical first phase in larval ontogeny, i.e. the yolk sac stage, and test for maternal effects. The eggs of A. anguilla typically have numerous oil droplets that coalesce into a single large oil droplet, while the zygote forms a large perivitelline space, reaching an egg diameter of 1.45 ± 0.12 mm at 3.0 to 3.5 h post fertilization. Embryonic development from fertilization to larval hatch lasted ~46–48 h at 20 °C with the larvae emerging in a relatively undeveloped stage with a protuberant yolk sac. During the period of yolk and oil absorption, the larvae underwent significant changes in head and body morphology. At the completion of yolk sac absorption, the largely transparent larvae had a set of protruding teeth, pigmented eyes and tail, and a simple alimentary tract. Larvae appeared capable of feeding at ~12 days post hatch at 20 °C, and were able to survive another ~10 days without feeding. Larval length approached and asymptotic maximum of 6.8 mm a round day 10 in non-fed larvae. Larval batches from different maternal origins varied in yolk sac size and the extent of yolk sac resources influenced larval size at the end of the yolk sac stage. The ontogenetic description presented here fills a gap in knowledge about the yet undiscovered early life stages of native European eel, which can provide a framework of reference for the development of hatchery technology. Such progress is urgently needed for a self-sustained aquaculture of this high-value and critically endangered species. Statement of relevance: European eel is a high-value species in aquaculture, however, production is presently hampered by reliance on wild caught fry. Captive production of glass eels will reopen markets in Europe and Asia, benefiting European eel producers. The results presented here document recent progress within assisted reproduction and larval culture of this species in aquaculture and aid establishing hatchery technology of this species.

**General information**

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Section for Marine Living Resources, Billund Aquakulturservice A/S, Danish Aquaculture Organisation
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Understanding the processes behind fish stock dynamics: Where are we?

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Organisations: National Institute of Aquatic Resources, Institute Management, Section for Ecosystem based Marine Management, Section for Marine Ecology and Oceanography
Authors: Köster, F. (Intern), Eero, M. (Intern), Sørensen, H. (Intern), Huwer, B. (Intern), Sørensen, S. R. (Intern)
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Reaching out: Communicating the Danish Eel Expedition 2014

General information
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Organisations: National Institute of Aquatic Resources, Institute Management, Section for Ecosystem based Marine Management, Section for Marine Ecology and Oceanography
Authors: Reeh, L. (Intern), Christoffersen, M. (Intern), Sørensen, S. R. (Intern), Nielsen, T. G. (Intern), Munk, P. (Intern)
Publication date: 2015
Event: Poster session presented at 18. Danske Havforskermøde, Copenhagen, Denmark.
Main Research Area: Technical/natural sciences
Publication: Communication › Poster – Annual report year: 2015
Does the 'snot' of the oceans matter? Engaging with the public on gelatinous zooplankton. Lessons learned from The Danish Eel Expedition 2014

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Organisations: National Institute of Aquatic Resources, Institute Management, Centre for Ocean Life, Section for Marine Ecology and Oceanography, Section for Ecosystem based Marine Management
Authors: Reeh, L. (Intern), Jaspers, C. (Intern), Sørensen, S. R. (Intern), Christoffersen, M. (Intern), Nielsen, T. G. (Intern), Munk, P. (Intern)
Publication date: 2014
Main Research Area: Technical/natural sciences
Publication: Research › Conference abstract for conference – Annual report year: 2014

Ichthyodinium identified in the eggs of European eel (Anguilla anguilla) spawned in captivity
A presumed parasitic protozoan was found in the eggs of European eel obtained from an experiment on captive breeding of eel, Anguilla anguilla, based on silver eels from a freshwater lake in the northern part of Denmark. Gross morphology of the organism was comparable to that of early stages of Ichthyodinium, a syndinian dinoflagellate parasite found in pelagic eggs of various marine fish species. Sequences of genes coding for small subunit ribosomal RNA confirmed that the organism was an Ichthyodinium species, and molecular phylogenetic analysis demonstrated the presence of two Ichthyodinium genotypes: one occurring in the Atlantic Ocean and adjacent coastal waters and one in the Pacific Ocean area. The inclusion of several GenBank-derived environmental gene sequences, from the Caribbean Sea, revealed to represent Ichthyodinium, suggesting that this parasite genus is ubiquitous in the World's oceans

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, University of Copenhagen
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Improving biophysical rearing conditions during early life stages of European eel

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Authors: Sørensen, S. R. (Intern)
Publication date: 2014
Event: Abstract from DAFINET and Targetfish FP7 Workshop –Fish models in Research, Copenhagen, Denmark.
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Kunstig befrugtning og babyboom i ålens verden

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography
Authors: Tomkiewicz, J. (Intern), Sørensen, S. R. (Intern)
Publication date: 2014
Event: Abstract from Dansk Selskab for Marinbiologi, Fyraftensmøde, København, Denmark.
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Larval production and survival during the early larval stage in European eel

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Billund Aquaculture Service Aps, Danish Aquaculture Organisation
Authors: Tomkiewicz, J. (Intern), Butts, I. (Intern), Sørensen, S. R. (Intern), Politis, S. N. (Intern), Lauesen, P. (Ekstern), Krüger-Johnsen, M. (Intern), Graver, C. (Ekstern)
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Microbial interference and potential control in culture of European eel (Anguilla anguilla) embryos and larvae

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Section for Aquaculture, Billund Aquaculture Service Aps, Ghent University
Authors: Sørensen, S. R. (Intern), Skov, P. V. (Intern), Lauesen, P. (Ekstern), Tomkiewicz, J. (Intern), Bossier, P. (Ekstern), Schryver, D. (Ekstern)
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Muscle development in European eel Anguilla anguilla yolksac larvae and effects of egg incubation temperature

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Norwegian University of Science and Technology
Authors: Kjørsvik, E. (Ekstern), Wold, P. A. (Ekstern), Bardal, T. (Ekstern), Davidsen, M. (Ekstern), da Silva, F. (Intern), Tomkiewicz, J. (Intern), Sørensen, S. R. (Intern)
Publication date: 2014
Main Research Area: Technical/natural sciences
Publication: Research › Paper – Annual report year: 2014

On the way to successful European eel larval rearing: Impact of biophysical conditions and gamete quality

The European eel is a widely distributed fish species of economic and cultural importance. It inhabits both coastal and freshwater systems, and is targeted by fisheries and treasured as food item. Although eels are reared in aquaculture, this industry relies solely of wild-caught juvenile glass eels that arrive to the European coasts after a 6000 km journey from the Sargasso Sea, where they were hatched. The adolescent eels start their long migration from the European continent back to their spawning area in the Sargasso Sea in late autumn as silver eels. As long as the eels are within the European continent, they are in an immature stage, and they do not start migration and maturation until the silvering stage. This stage is however tightly controlled by brain and pituitary hormones, preventing maturation of gonads remote from their natural breeding area. This hormonal inhibition of maturation is the main reason why it is difficult to reproduce European eel in captivity. Although, attempted since 1930ies, utilizing maturational hormones primarily from other fish species, we only recently succeeded in refining reproduction protocols that enable rich quantities of viable gametes from this species. In view of these obstacles, the last decade’s research has shown substantial progress. This PhD has contributed to this progress through new knowledge and development of procedures for successful egg activation and fertilization as well as incubation and larvae culture. My PhD work addressed biophysical determinants fundamental to producing healthy eggs and larvae. One of my aims was to improve methods and results of in vitro fertilization. This research included
characterisation of sperm density, "optimal" sperm to egg ratios and gamete mixing. Eel gametes are activated by salt water and incubated in a marine aquatic environment. In this regard, my aim was to identify suited salinities and seawater sources, supporting a good embryonic development. Embryonic development lasts two days from fertilization to hatch. During this time, as well as in early larval stages, mortality is high. Here, my aim was to assess effects of temperature and microbial interference during incubation and larval rearing on order to reduce this mortality in cultures. The results have provided valuable new insights, contributing to progress of in vitro fertilization methods and reduced mortality in egg and larval culture. Our fertilisation procedures initially applied spermatocrit as for sperm quantification technique to standardise sperm:egg ratio. Although being a practical method, it featured moderate precision. Spectrophotometry in contrast, showed high precision in addition to being a fast and practical and subsequently supported experiments that identified optimal sperm:egg ratio. Egg activation and swelling are among the processes often seen to fail in experiments. Activation salinity was found to be a determinant of egg fertilisation, buoyancy, and egg size although egg size effects differed among individual females. Fertilization percent was typically high in the range 30 and 40 ppt, while rate of un-activated and dead eggs rose in higher salinities. Egg swelling could be optimized using certain artificial salt types and impeded using others. During egg incubation, microbial interference was found to be a major obstacle for hatch, rather caused by microbial activity than presence. Larval mortality was highly dependent on whether antimicrobial conditions were bacteriostatic of bactericidal. This calls for future technology and microbial management, e.g. by matured water integrated in RAS technology. The results obtained through these studies have added to Danish progress within artificial reproduction in European eel by improved fertilization protocols and identification of important parameters during the early life stages. Such progress has led to present focus on eel larval culture and feeding, which has brought attention to eel as a potential “new species” in aquaculture.

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Standardization of fertilization protocols for the European eel, Anguilla anguilla
Standardization of artificial fertilization protocols for the European eel, Anguilla anguilla, is a prerequisite for optimizing the use of available gametes in hatchery facilities and for conserving sperm from high quality males, which is either cryopreserved or in living gene banks. The objectives of this research were to provide a rapid, accurate and precise method to quantify sperm density by examining the relationship between sperm density and absorbance by use of a spectrophotometer, determine the optimal number of sperm required to fertilize eggs in a controlled setting, and explore how long eggs are receptive to fertilization post-stripping. Mean sperm density and absorbance at 350nm were 1.54e+10±4.95e+9 sperm/mL and 1.91±0.22 nm, respectively. Regression analysis demonstrated a highly significant positive relationship between sperm density and absorbance using a spectrophotometer at 350nm (R²=0.94, p<0.001, y=2.273e+10x-2.805e+10); significant but slightly weaker relationships were also detected at 400, 500, and 600nm (R²≤0.93, p<0.001). Fertilization success using sperm to egg ratios ranging from 1.3e+3 to 1.0e+6 sperm per egg increased from 37.5 to 68.1%, respectively. Sperm to egg ratio had a significant effect on fertilization success (p<0.0001), where fertilization success increased from 1.3e+3 to 2.5e+4 sperm per egg; adding greater than 2.5e+4 sperm per egg had no significant effect. Furthermore, the duration of time post-stripping had a significant effect on egg fertilization success (p<0.0001), such that between 0 and 10min post-stripping 57.4 to 78.2% of the eggs were fertilized while at 15min post-stripping a significant decrease in fertilization success was detected (47.5%). For all statistical models, the female variance component was significant for fertilization success (p<0.0001) and explained ≤84% of the models variance. In conclusion, European eel eggs should be fertilized within 10min post-stripping using 2.5e+4 sperm per egg. Together, these findings will contribute to the development of European eel breeding technology and further our understanding on sperm biology and reproductive biology in fishes.

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, University of Windsor
Authors: Butts, I. (Intern), Sørensen, S. R. (Intern), Politis, S. N. (Intern), Pitcher, T. (Ekstern), Tomkiewicz, J. (Intern)
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Development of techniques and technology for embryonic and larval rearing of the European eel

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Effect of preservation temperature and cryopreservation on European eel sperm fertilization capacity. First production of larvae using cryopreserved sperm

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography
Authors: Asturiano, J. (Ekstern), Sørensen, S. R. (Intern), Pérez, L. (Ekstern), Lauesen, P. (Intern), Tomkiewicz, J. (Intern)
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Event: Poster session presented at 4th International Workshop on Biology of Fish Gametes, Faro, Portugal.
Main Research Area: Technical/natural sciences
Publication: Research › Poster – Annual report year: 2014

Evaluation of methods to determine sperm density for the European eel, Anguilla anguilla

European eel, Anguilla anguilla, is a target species for future captive breeding, yet best methodology to estimate sperm density for application in in vitro fertilization is not established. Thus, our objectives were to evaluate methods to estimate European eel sperm density including spermatocrit, computer-assisted sperm analysis (CASA) and flow cytometry (FCM), using Neubauer Improved haemocytometer as benchmark. Initially, relationships between spermatocrit, haemocytometer counts and sperm motility were analysed, as well as the effect of sperm dilution on haemocytometer counts. Furthermore, accuracy and precision of spermatocrit, applying a range of G-forces, were tested and the best G-force used in method comparisons. We found no effect of dilution on haemocytometer sperm density estimates, whereas motility associated positively with haemocytometer counts, but not with spermatocrit. Results from all techniques, spermatocrit, CASA and FCM, showed significant positive correlations with haemocytometer counts. The best correlation between spermatocrit and haemocytometer counts was obtained at 6000 × g (r = 0.68). Of two CASA variants, one or three photographic fields (CASA-1 and CASA-2), CASA-2 showed a very high accuracy to haemocytometer counts (r = 0.93), but low precision (CV: CASA-2 = 28.4%). CASA was tested with and without microfluorospheres (FCM-1 and FCM-2), and relationships to haemocytometer counts were highly accurate (FCM-1: r = 0.94; FCM-2: r = 0.88) and precise (CV: FCM-1 = 2.5; FCM-2 = 2.7%). Overall, CASA-2 and FCM-1 feature reliable methods for quantification of European eel sperm, but FCM-1 has a clear advantage featuring highest precision and accuracy. Together, these results provide a useful basis for gamete management in fertilization protocols.

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Universidad Politecnica de Valencia
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Evaluation of methods to determine sperm density for the European eel, Anguilla anguilla

**General information**

**State:** Published  
**Organisations:** National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography  
**Authors:** Sørensen, S. R. (Intern), Gallego, V. (Ekstern), Pérez, L. (Ekstern), Butts, I. (Intern), Tomkiewicz, J. (Intern), Asturiano, J. (Ekstern)  
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Microbial interference and potential control in the production of European eel Anguilla anguilla larvae

General information
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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Billund Aquaculture Service Aps
Authors: Schryver, P. D. (Ekstern), Sørensen, S. R. (Intern), Skov, P. V. (Intern), Lauesen, P. (Ekstern), Tomkiewicz, J. (Intern)
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Microbial interference with hatch and survival of European eel larvae
Recent research has significantly improved our knowledge and capabilities in the field of in vitro production of yolk sac larvae from European eel (Anguilla anguilla). Female broodstock European eels are matured by weekly administration of pituitary extract and male eels with hCG (human chorionic gonadotropin), which afford gametes for in vitro fertilization studies. The maturing process may lead to mass hatchings of up to ½ million larvae of which some survive the entire yolk sac phase. However, the rearing of larvae suffers from high larval mortalities, and water quality might be a crucial factor for larval survival in rearing systems. By applying antibiotic treatment as a research tool, it was possible to determine the extent of microbial interference in the production of high numbers of good quality larvae. By controlling microbiota during egg and larval incubation, the egg hatching success and larval longevity more than doubled. Using scanning electron microscopic analysis it was observed that microbe inhibiting treatments reduced bacterial colonization of the eggs surface, which possibly cause reduced gas and ionic exchange across chorionic membrane. These results suggest that future eel larviculture should not only focus on optimizing physical incubation conditions, but certainly also on the control over microbial interference.

General information
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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Billund Aquaculture Service Aps, Ghent University
Authors: Sørensen, S. R. (Intern), Lauesen (Ekstern), Tomkiewicz, J. (Intern), de Schryver, P. (Ekstern)
Number of pages: 1
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Reproduction of European eel and larval culture: state of the art

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Billund Aquaculture Service Aps, Ghent University, Billund Aquaculture Service Aps
Authors: Tomkiewicz, J. (Intern), Stettrup, J. (Intern), Corraze, G. (Ekstern), Kausik, S. (Ekstern), Holst, L. (Ekstern), McEvoy, F. (Ekstern), Dufour, S. (Ekstern), Lafont, A. (Ekstern), Asturiano, J. (Ekstern), Sørensen, S. R. (Intern), Tveiten, H. (Ekstern), de Schryver, P. (Ekstern), Butts, I. (Intern), Munk, P. (Intern), Zambonino-Infante, J. (Ekstern), Politis, S. N. (Intern), Krüger-Johnsen, M. (Intern), Lauesen, P. (Intern)
Publication date: 2013
Main Research Area: Technical/natural sciences
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Techniques for rearing European eel during early life history

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Billund Aquakulturservice A/S
Authors: Butts, I. (Intern), Sørensen, S. R. (Intern), Politis, S. N. (Intern), Lauesen, P. (Ekstern), Tomkiewicz, J. (Intern)
Publication date: 2013
Main Research Area: Technical/natural sciences
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Publication: Research › Paper – Annual report year: 2014

Reproduction of European Eel in Aquaculture (REEL): Consolidation and new production methods
Project aim: Enhance methods and technology applied to produce and culture European eel larvae as basis for the development of a future self-sustained eel aquaculture.
Background: The severe decline of the European eel stock calls for conservation measures including national eel management plans and establishment of a self-sustained eel aquaculture. In 2005, the National Institute of Aquatic Resources at the Technical University of Denmark (DTU Aqua), the Faculty of Life Sciences at Copenhagen University (KU-Life) and the eel aquaculture industry started to build up a research and technology platform for the development of methods to reproduce European eel in aquaculture. Two major projects: Artificial Reproduction of Eels II and III (ROE II and III) succeeded during 2005-2008 to produce viable eggs and larvae that lived up to 12 days. The larvae thereby accomplished the yolk-sac stage and became ready to start feeding. The results were in particular promising because they evidenced that methods successfully applied to Japanese eel has a potential for application also to the European eel. ROE II and III were supported by the Ministry of Food, Agriculture and Fisheries and the European Commission through the Financial Instrument for Fisheries Guidance (FIFG) and the Danish Food Research Program 2006, respectively.
Results: The REEL project accomplished through three series of experiments to consolidate previous results. The longevity of larvae was extended from 12 to 20 days after hatch in first feeding experiments thereby entering the leptocephalus phase. Maturation potential and methods to induce maturation were further tested, and farmed and wild eel broodstocks as well as different treatments were compared. In particular, fertilisation procedures to produce fertilised eggs and embryos and monitoring techniques were enhanced. The technology needed to culture embryos and larvae was substantially improved. The potential for new hormonal treatments was explored and recombinant eel hormones have been produced. New broodstock diets were developed with focus on the lipid composition essential for development and survival of fish larvae. In addition, the experimental facility established by DTU Aqua at Lyksvad Fish Farm was enhanced by improving the experimental and laboratory facilities. The REEL project provided the basis for the establishment of an EU collaborative research project: Reproduction of European Eel: Towards a Self-sustained Aquaculture (PRO-EEL) coordinated by DTU Aqua. REEL included the partners DTU Aqua, KU-Life, Danish Eel Farmers Association (DEFA), Billund Aquaculture Service (BA), BioMar, and Bioneer of which four are integrated in the PRO-EEL project that in total has 15 international partners.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Population Ecology and Genetics, Section for Coastal Ecology, Section for Ocean Ecology and Climate, National Food Institute, Division of Industrial Food Research, Section for Aquaculture
Authors: Tomkiewicz, J. (Intern), Tybjerg, L. (Intern), Støttrup, J. (Intern), McEvoy, F. (Ekstern), Ravn, P. (Ekstern), Sørensen, S. R. (Intern), Lauesen, P. (Intern), Graver, C. (Intern), Munk, P. (Intern), Holst, L. K. (Ekstern), Vestbø, B. (Ekstern), Svalastoga, E. (Ekstern), Jacobsen, C. (Intern), Holst, B. (Ekstern), Steenfeldt, S. J. (Intern), Buelund, L. (Ekstern), Hornum, T. (Intern), Kofoed, T. (Intern)
Number of pages: 47
Publication date: 2012

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Main Research Area: Technical/natural sciences
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Produktion af torskelarver til udsætning i den østlige Østersø – RESTOCK

General information
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Organisations: Section for Coastal Ecology, National Institute of Aquatic Resources, Section for Population- and Ecosystem Dynamics, Section for Aquaculture, Section for Fish Diseases
Number of pages: 143
Publication date: 2009

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Source: orbit
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The production of Baltic cod larvae for restocking in the eastern Baltic. RESTOCK I. 2005-2007

General information
State: Published
Organisations: Section for Coastal Ecology, National Institute of Aquatic Resources, Section for Population- and Ecosystem Dynamics
Authors: Støttrup, J. (Intern), Overton, J. L. (Intern), Sørensen, S. R. (Intern)
Number of pages: 81
Publication date: 2008

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Publisher: DTU Aqua. Institut for Akvatiske Ressourcer
ISBN (Print): 87-74-81076-6
Original language: Danish
Series: DTU Aqua-rapport
Number: 189-08
Main Research Area: Technical/natural sciences
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Publication: Research › Report – Annual report year: 2008

Kystfodring og kystøkologi: Evaluering af reviefodring ud for Fjaltring

General information
State: Published
Organisations: Section for Coastal Ecology, National Institute of Aquatic Resources, Section for Shellfish, Section for Aquaculture
Authors: Støttrup, J. (Intern), Dolmer, P. (Intern), Røjbek, M. (Intern), Nielsen, E. (Intern), Ingvardsen, S. (Ekstern), Sørensen, P. (Ekstern), Sørensen, S. R. (Intern)
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Publication date: 2007

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Place of publication: Charlottenlund
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Original language: Danish
Series: DFU-rapport
Number: 171-07
Main Research Area: Technical/natural sciences
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Source: orbit
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Publication: Research › Report – Annual report year: 2007

Kystfodring og godt fiskeri: Undersøgelse af strandnær kystfodring ved Agger Tange

General information
State: Published
Organisations: Section for Coastal Ecology, National Institute of Aquatic Resources, Section for Shellfish, Section for Aquaculture
Authors: Støttrup, J. (Intern), Dolmer, P. (Intern), Røjbek, M. (Intern), Nielsen, E. (Intern), Ingvardsen, S. (Ekstern), Laustrup, C. (Ekstern), Sørensen, S. R. (Intern)
Number of pages: 52
Publication date: 2005
Eel hatchery technology for a sustainable aquaculture (EEL-HATCH) (39181)

Hatchery and rearing technology for commercial production of glass eels is fundamental to sustainable and profitable eel aquaculture. The vision is to enhance existing technology to rear European eel larvae to the glass eel stage, thereby closing the lifecycle in captivity. Pioneering research of the consortium has raised eel breeding from a state of reproductive failure to stable production of viable larvae.

Objectives include: Design “state of the art” hatchery facilities, optimize broodstock feeds, enhance assisted reproductive technology, and develop larval culture systems and diets. The main success criterion is achievement of large scale culture of larvae throughout the larval stage, leading to glass eel production. The establishment of sustainable aquaculture of this endangered species, presently relying on captive glass eel will rebuild the highly profitable market for eel aquaculture and suppliers as well as assist in conservation and stock management plans.

Results obtained during the half of the project period include the design and establishment of a dedicated research facility in relation to DTU Aqua in Hirtshals, involving several partners. The facility applies recirculation aquaculture systems with emphasis on matured water technology and microbial control. Scientific highlights include successful production of recombinant European eel gonadotropic hormones; enhanced reproduction, fertilization and incubation procedures; and optimized larval culture conditions, including e.g. temperature, salinity, and light regime. Larval diets have been developed and tested in first feeding and behavioral experiments, leading to the first published work on larval feeding for this species. Experiments on improved diets and optimized rearing tanks for larval growth are ongoing.

This project is coordinated by DTU Aqua.

The project is funded by Innovation Fund Denmark.

National Institute of Aquatic Resources
Section for Marine Living Resources
Billund Aquaculture Service Aps
BioMar A/S
North Sea Science Park
Bioneer A/S
STMI
Danish Aquaculture Association
Period: 01/04/2014 → 30/09/2017
Number of participants: 9
Research areas: Fish Biology & Aquaculture & Coastal Ecology
Project participant:
Butts, Ian (Intern)
Støttrup, Josianne Gatt (Intern)
Lund, Ivar (Intern)
Krüger-Johnsen, Maria (Intern)
The early life of eel in the Sargasso Sea – Influence of oceanography and climate (SARGASSO-EEL) (39107)

The recruitment of the European eel has been in dramatic decline during the last 30 years, and is at a severe low of only 3-5% of earlier magnitude. This change and its influence on the eel fishery have led to an intensified research in the oceanic phase of the European eel.

In order to contribute to further understanding of the life cycle of eel the Danish eel expedition set out in 2014 for the eel spawning grounds in the Sargasso Sea. Here a consortium of Danish scientists and international collaborators focused on the linkages between oceanography, biological production, eel spawning and the growth and drift of eel larvae.

During the expedition, a wide range of organisms was collected: From the smallest plankton of less than a millimeter to very large fish. A number of research groups are now working on samples and data from the expedition and assembling information on key processes in the early life of eels. Preliminary findings indicate that biological and physical changes have taken place in the spawning areas that may affect the eel larvae’s chances of survival and their journey to Europe.

The project was coordinated by DTU Aqua.

The project is funded by the Carlsberg Foundation and Danish Centre of Marine Research (cruise).

National Institute of Aquatic Resources
Section for Marine Ecology and Oceanography
University of Copenhagen
Aarhus University
Pierre and Marie Curie University - University of Paris VI
Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin
Université de la Méditerranée
University of Alaska Fairbanks
University of Rhode Island
Sir Alister Hardy Foundation for Ocean Science (SAHFOS)
International Council for the Exploration of the Sea
Period: 01/08/2013 → 01/08/2016
Number of participants: 11
Research areas: Marine Populations and Ecosystem Dynamics & Fish Biology & Oceanography
Project participant:
Thomsen, Helge Abildhauge (Intern)
Sørensen, Sune Riis (Intern)
Bekkevold, Dorte (Intern)
Malanski, Evandro (Intern)
Jaspers, Cornelia (Intern)
Koski, Marja (Intern)
Christoffersen, Mads (Intern)
Hansen, Susanne (Intern)
PhD Student:
Ayala, Daniel Jiro (Intern)
Project Manager, academic:
Nielsen, Torkel Gissel (Intern)
Reproduction of European eel: Towards a self-sustained aquaculture (PRO-EEL) (38793)

Reproduction of European eel (Anguilla anguilla) in culture has become a research priority area due to a severe decline of natural stocks and an increasing interest to breed eels for a self-sustained aquaculture. As eels do not reproduce naturally in captivity, development of methodology and technology was needed for production of viable eggs and larvae from broodstock in a regular and predictable way.

Focus of PRO-EEL project was on the primary bottlenecks in a controlled reproduction of eels, which concern deficiencies in knowledge about eel reproductive physiology and methods applied to induce and finalize gamete development. During a 4-year period, the project significantly expanded current knowledge on the eel reproductive mechanisms and hormonal control of sexual maturation. The consortium developed standardized protocols for assisted production of high quality gametes (egg and sperm) and artificial fertilization, thereby obtaining a stable production of viable embryos. Furthermore, egg incubation procedures and culture of yolksac larvae were established for the first time for European eel, leading to the first feeding stage. The project disseminated novel literature on early life stages, including their ontogeny and requirements thereby describing egg and larval stages still unknown in nature and providing important information for future development of larval diets and rearing technology. Methodology and technology was established using small scale tests and validated in full scale experimental facilities managed by DTU.

The project was an international, EU-funded research project characterized by an integrative and multidisciplinary approach. The consortium brought together leading experts in eel reproduction complemented by expertise in disciplines filling gaps in knowledge and technology. The consortium included 15 partners, comprising European research institutes and industry partners as well as an international collaboration partner country (ICPC). Within DTU, the project involved DTU Food, Research Group for Bioactives – Analysis and Application, and several DTU Aqua research areas including Fish Biology, Aquaculture, Marine Populations and Ecosystem Dynamics, and Coastal Ecology.

The project was coordinated by DTU Aqua.

The project was funded by EU, Framework Programme 7.

National Food Institute
National Institute of Aquatic Resources
Section for Marine Ecology and Oceanography
Wageningen IMARES
Leiden University
National Centre for Scientific Research "Demokritos"
Polytechnic University of Valencia
NOFIMA
Ghent University
University of Copenhagen
National Institute for Agronomic Research
Billund Aquaculture Service Aps
National Institute of Sciences and Technologies of the Sea
Institute of Marine Research
Norwegian University of Science and Technology
BioMar A/S
Period: 01/01/2010 → 31/07/2014
Number of participants: 9
Research areas: Fish Biology & Aquaculture & Marine Populations and Ecosystem Dynamics & Coastal Ecology
Project participant:
Butts, Ian (Intern)
Støttrup, Josianne Gatt (Intern)
Eel Egg and Larval development in Relation to Bio-Physical Characteristics and Gamete Quality

National Institute of Aquatic Resources
Period: 01/07/2009 → 02/04/2014
Number of participants: 7
Phd Student: Sørensen, Sune Riis (Intern)
Supervisor: Bossier, Peter Georges Madeleine (Ekstern)
Main Supervisor: Tomkiewicz, Jonna (Intern)
Examiner: St. John, Michael (Intern)
Geffen, Audrey Jacheline (Ekstern)
Vadstein, Olav (Ekstern)

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Name of research programme: 1/3 DTU-stip, 2/3 FUR/andet
Project: PhD

Reproduction of European eel in aquaculture: Consolidation and new production methods (REEL) (38398)

Project aim: Enhance methods and technology applied to produce and culture European eel larvae as basis for the development of a future self-sustained eel aquaculture.

Background: The severe decline of the European eel stock calls for conservation measures including national eel management plans and establishment of a self-sustained eel aquaculture. In 2005, DTU Aqua, University of Copenhagen and the eel aquaculture industry started to build up a research and technology platform for the development of methods to reproduce European eel in aquaculture.

Two major projects: Artificial Reproduction of Eels II and III (ROE II and III) succeeded during 2005-2008 to produce viable eggs and larvae that lived up to 12 days. The larvae thereby accomplished the yolk sac stage and became ready to start feeding. The results were in particular promising because they evidenced that methods successfully applied to Japanese eel have a potential for application also to European eel. ROE II and III were supported by the Danish Ministry of Food, Agriculture and Fisheries and the Financial Instrument for Fisheries Guidance (FIFG) and RO III by the Danish Food Research Program 2006.

Results: The REEL project has accomplished through three series of experiments to consolidate previous results and extend the longevity of larvae from 12 to 20 days after hatch in first feeding experiments. Methods to induce maturation were further tested, and farmed and wild eel broodstocks and different treatments were compared. In particular, fertilization procedures to produce fertilized eggs and embryos and monitoring techniques were enhanced. The technology needed to culture embryos and larvae was substantially improved. The potential for new hormonal treatments was explored and recombinant eel hormones have been produced. New broodstock diets were developed with focus on the lipid composition essential for development and survival of fish larvae. In addition, the experimental facility established by DTU Aqua at Lyksvad Fishfarm was enhanced by improving the experimental and laboratory facilities. The REEL project has provided the basis for the establishment of an EU research project: Reproduction of European Eel: Towards a Self-sustained Aquaculture (PRO-EEL) (38793) coordinated by DTU Aqua. REEL included the partners DTU Aqua, the Danish Eel Producers Association, Billund Aquaculture, BioMar, Bioneer and Copenhagen University of which four are integrated in PRO-EEL.

The project was coordinated by DTU Aqua.

National Food Institute
National Institute of Aquatic Resources
Artificial reproduction of eels: Phase III (ROE III) (38187)

The steady decline of the European eel stock has adverse consequences for the Danish eel aquaculture as all eel farming is at present capture based relying on wild caught glass eels. In 2005, DTU Aqua, University of Copenhagen and the eel aquaculture industry started to build up a research and technology platform for the development of methods to reproduce European eel in aquaculture.

The focus of ROE III was to follow up the pioneering work on artificial reproduction of European eels performed in the preceding pilot projects ROE I and II. The projects ROE II and III were a collaboration among DTU Aqua, University of Copenhagen and the eel aquaculture industry following up an initial survey ROE I of suited methodology lead by University of Copenhagen.

ROE III comprised the following activities:
(i) Experimental series with different treatment schemes and hormone dosage to improve the maturation process and optimize gamete quality;
(ii) Development of methods to monitor the maturation process on individual level using ultrasound scanning technology and ovary biopsy;
(iii) Analysis of broodstock fishes and improvement of the dietary fatty acid composition;
(iv) Investigation of parameters determining egg quality during incubation;
(v) First-feeding trials with eel larvae testing both artificial and live feed.

Three experimental series were completed focusing on methods for broodstock enhancement, maturation and fertilization plus culture of eggs and larvae. Already during the first experimental series, larvae accomplishing the entire yolk sac stage were achieved for the first in history for European eel. The yolksac larvae developed successfully during the period were they entirely depend on nutrition sources i.e yolk and lipid of maternal origin. The larvae were ready to start feeding day 12 post hatch. During the second experimental series, larval longevity was extended to 18 days during first feeding experiments. These recent results are a major breakthrough because they show for the first time that artificial hormone treatment can lead to viable offspring in European eel. Eggs and yolksac larvae were obtained from different hormonal treatments and mass hatchings were regularly obtained. Larval feeding using live and artificial larval feeds developed in collaboration with the food company BioMar were developed towards the end of the experiments and are ready for testing in new and coming projects.

The success of this project on improved methods, quality criteria and larval survival has led to form the basis of the project: Reproduction of European eel in aquaculture: Consolidation and new production methods and later (REEL) (38398) and later the EU FP project: Reproduction of European eel in Aquaculture: Towards a self-sustained aquaculture (PRO-EEL) (38793).

The project was coordinated by DTU Aqua.

National Institute of Aquatic Resources
RESTOCK (38566) (38400 pre-project)

The aim of the pre-project was to explore the potential for restocking the cod stock in the eastern Baltic. A theoretical
study was conducted to explore the potential for restocking bringing together scientists from the aquaculture sector,
fisheries managers, ecological scientists and scientists with a background in stock enhancement. The ecology, biology
and fisheries biology of the eastern Baltic was reviewed and provided the basis for the study. The results indicated a good
potential for restocking with first-feeding cod larvae (Støttrup et al. 2008). This was the first example of a study to examine
the potential for large-scale restocking prior to the release of fish. A 2- 3-month delay in the spawning period compared to
20-30 years ago has altered feeding conditions and predation susceptibility in a way that may have exacerbated the
delay in recruitment. Producing and releasing cod larvae during spring would mimic the spawning period recorded in
previous times and would coincide with the spring peak in copepod production. An evaluation of 3 different release
scenarios showed that a release of 474 million first-feeding larvae over 5 months (covering the historic and present day
spawning period) would enhance the average population of 2 year old by 10% and be biologically and economically the
most feasible scenario.

Three years of a six year follow up project (RESTOCK) to verify the theoretical findings was funded, but due to political
changes, funding for the final three years was not possible and the project was unable to empirically ascertain the
potential for restocking. During the three years, 3 cod broodstocks were established with different photoperiods and
subsequent spawning periods, together with the development of a technique to determine fish gender non-invasively
(McEvoy et al., 2009). Egg and larval incubation techniques were developed and several investigations on temperature,
salinity and food impacts on first feeding cod larvae to define the “window of opportunity” for release (i.e. time when the
larvae were ready to start feeding to when they began to be too poor in condition to feed) (Støttrup et al., 2008; Overton et
al. 2010; Meyer et al 2011a). A release strategy was developed and the first successful release of first-feeding fish larvae
at 23 m depth was conducted, but needed further adjustments (Støttrup et al., 2008). An extensive disease monitoring
program was established (Støttrup et al., 2008) and the presence of a protistan endoparasite generated a further study
(Skovgård et al., 2010). Studies were also conducted to determine explore marking techniques for identification of
released fish (Meyer et al., 2011b) and explore growth characteristics in cod larvae (Meyer et al., 2011a).

The project was coordinated by DTU Aqua.

National Institute of Aquatic Resources
Section for Ecosystem based Marine Management
National Veterinary Institute
Danish Fishermen's Association
University of Copenhagen
University of Hamburg
University of Caen
Period: 01/01/2005 → 31/12/2007
Number of participants: 9
Research area: Coastal Ecology
Project participant:
Sørensen, Sune Riis (Intern)
Røjbek, Maria (Intern)
Pedersen, Per Bovbjerg (Intern)
Tomkiewicz, Jonna (Intern)
Møllmann, Christian (Ekstern)
Sichlau, Morten (Ekstern)
Project Manager, academic:
Støttrup, Josianne Gatt (Intern)
Paulsen, Helge (Intern)
Dalsgaard, Inger (Intern)