Paternal identity impacts embryonic development for two species of freshwater fish

Paternal, compared to maternal, contributions were believed to have only a limited influence on embryonic development and larval fitness traits in fishes. Therefore, the perspective of male influence on early life history traits has come under scrutiny. This study was conducted to determine parental effects on the rate of eyed embryos of Ide (Leuciscus idus) and Northern pike (Esox lucius). Five sires and five dams from each species were crossed using a quantitative genetic breeding design and the resulting 25 sib groups of each species were reared to the embryonic eyed stage. We then partition variation in embryonic phenotypic performance to maternal, paternal, and parental interactions using the Restricted Maximum Likelihood (REML) model. Results showed that paternal, maternal, and the paternal × maternal interaction terms were highly significant for both species; clearly demonstrating that certain family combinations were more compatible than others. Paternal effects explained 20.24% of the total variance, which was 2-fold higher than the maternal effects (10.73%) in Ide, while paternal effects explained 18.9% of the total variance, which was 15-fold higher than the maternal effects (1.3%) in Northern pike. Together, these results indicate that male effects are of major importance during embryonic development for these species. Furthermore, this study demonstrates that genetic compatibility between sires and dams plays an important role and needs to be taken into consideration for reproduction of these and likely other economically important fish species.
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-1) 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.
Temperature induced variation in gene expression of thyroid hormone receptors and deiodinases of European eel (Anguilla anguilla) larvae

Thyroid hormones (THs) are key regulators of growth, development, and metabolism in vertebrates and influence early life development of fish. TH is produced in the thyroid gland (or thyroid follicles) mainly as T4 (thyroxine), which is metabolized to T3 (3,5,3'-triiodothyronine) and T2 (3,5-diiodothyronine) by deiodinase (DIO) enzymes in peripheral tissues. The action of these hormones is mostly exerted by binding to a specific nuclear thyroid hormone receptor (THR). In this study, we i) cloned and characterized thr sequences, ii) investigated the expression pattern of the different subtypes of thrs and dios, and iii) studied how temperature affects the expression of those genes in artificially produced early life history stages of European eel (Anguilla anguilla), reared in different thermal regimes (16, 18, 20 and 22°C) from hatch until first-feeding. We identified 2 subtypes of thr (thrα and thrβ) with 2 isoforms each (thrαA, thrαB, thrβA, thrβB) and 3 subtypes of deiodinases (dio1, dio2, dio3). All thr genes identified showed high similarity to the closely related Japanese eel (Anguilla japonica). We found that all genes investigated in this study were affected by larval age (in real time or at specific developmental stages), temperature, and/or their interaction. More specifically, the warmer the temperature the earlier the expression response of a specific target gene. In real time, the expression profiles appeared very similar and only shifted with temperature. In developmental time, gene expression of all genes differed across selected developmental stages, such as at hatch, during teeth formation or at first-feeding. Thus, we demonstrate that the expression of thrs and dios show sensitivity to temperature and are involved in and during early life development of European eel.

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Fathers modify thermal reaction norms for hatching success in Atlantic cod, Gadus morhua

Climate-driven warming is altering marine ecosystems at an unprecedented rate and evolutionary adaptation may represent the last resort for many ectothermic organisms to avoid local extinction. The first step to elucidate the potential for adaptation to unfavorable thermal conditions is to assess the degree of genotype-based variation in thermal reaction norms of vital fitness traits. Marine broadcast spawning fishes experience extremely high rates of mortality during early life stages. Paternally derived (genetic) variation underlying offspring fitness in adverse environmental conditions may therefore hold important implications for resilience. This study examined how males differ in their ability to sire viable offspring and whether the paternal contribution modified thermal reaction norms for hatching success in two replicated trials with cod Gadus morhua from the Northwest Atlantic (trial 1) and Baltic Sea (trial 2). Each trial included five temperature treatments (2.0, 4.0, 6.0, 8.0, 10.0 °C in trial 1, and 6.5, 8.0, 9.5, 11.0, 12.5 °C in trial 2) encompassing optimum conditions as well as the amount of warming projected in various future pathways for the year 2100. In both trials, mean hatching success significantly decreased towards thermal extremes. However, half-sibling families varied in their response to different incubation temperatures as indicated by significant paternity × temperature interactions and crossing of reaction norms. The influence of paternity itself was highly significant and explained 56% and 44% of the observed variation in hatching success in trials 1 and 2, respectively. Early embryogenesis represented the most crucial developmental period in terms of thermal tolerance and paternally mediated variation in hatching success. High variation in daily embryo survival among half-sibling families and temperature treatments was observed during blastula and gastrulation stages (until 100% epiboly), while almost no mortality occurred during subsequent development and throughout the hatching period. The observed magnitude of genetic variation underlying thermal reaction norms for embryo viability represents a relevant resource for adaptive responses (favorable selection) of cod populations exposed to environmental variability and/or directional changes, such as ongoing ocean warming.
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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

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Scopus rating (2010): SJR 1.151 SNIP 1.394
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Ultrasonographic predictors of response of European eels (Anguilla anguilla) to hormonal treatment for induction of ovarian development

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Light impacts embryonic and early larval development of the European eel, Anguilla anguilla

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Ontogeny of the immune response during early life history of European eel and its temperature dependence

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Thermal effects on early life history stages of European eel Anguilla anguilla

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Larval production and survival during the early larval stage in European eel

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Light impacts embryonic and early larval development of the European eel, Anguilla anguilla

Little is known about the natural ecology of European eel during early life history. We extend our understandings on the ecology of this species by studying how early life stages perform under various light regimes. We assessed the effects of intensity, photoperiod (12:12 and 24:0 h light/dark) and spectral composition on embryonic survival, hatch success, larval morphology and survival at 5 days post-hatch. Treatments consisted of low intensity white (full spectrum, 2.2 μmol m-2 s-1), blue (~470 nm, 0.7 μmol m-2 s-1), green (~530 nm, 0.4 μmol m-2 s-1), red (~690 nm, 0.2 μmol m-2 s-1) and high intensity white (full spectrum, 10.5 μmol m-2 s-1), blue (~470 nm, 3.9 μmol m-2 s-1), green (~530 nm, 1.5 μmol m-2 s-1), and red light (~690 nm, 1.1 μmol m-2 s-1). Additionally, offspring were reared in continuous darkness (0:24 h light/dark). Results showed that light critically influenced early life stages. In particular, for the 12:12 h photoperiod, embryonic survival, until 26 h post-fertilization was significantly higher when reared under low (62 ± 13%) than those reared under high intensity light (42 ± 13%). Furthermore, embryos reared in low light had a higher hatch success (16 ± 7%) than those in high intensity light (12 ± 7%). Larval yolk-sac area was significantly affected by photoperiod and body area was significantly affected by the interaction between intensity × photoperiod. The highest incidence of deformities (75%) occurred when embryos were reared in high intensity white light under a 24:0 h light/dark photoperiod. Larval survival was significantly affected by light regime, such that larvae reared in low light intensity had higher survival (20±8%) than those reared in high intensity (11±8%), larvae reared in the 12:12 h photoperiod had higher survival (19 ± 8%) than those reared in the 24:0 h light/dark photoperiod (13 ± 8%), and larvae reared in red light (22 ± 8%) had higher survival than those reared in green (14 ± 8%) or white light (11 ± 8%). Under continuous darkness, development and survival of offspring was as high as the best intensity-photoperiod-spectral composition regime. For all early life history traits, a strong maternal effect was evident, such that offspring of ‘poorer’ quality showed lower adaptability to extrinsic factors than offspring of higher quality. Together, these findings suggest a preference for no or low light during embryogenesis and no or 12:12 h low red light during the pre-leptocephalus stage.

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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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Temperature, paternity and asynchronous hatching influence early developmental characteristics of larval Atlantic cod, *Gadus morhua*

Offspring, especially during early development, are influenced by both intrinsic properties endowed to them by their parents, extrinsic environmental factors as well as the interplay between genes and the environment. We investigated the effects of paternity (P), temperature (T), and asynchronous hatching on larval traits of cod, *Gadus morhua* from the Atlantic Ocean and the Baltic Sea. Daily cohorts of 4 half-sib families of Atlantic larvae and 5 half-sib families of Baltic larvae were incubated and hatched at 5 temperatures (Atlantic 2.0-10.0°C, Baltic 6.5-12.5°C) and imaged for notochord length (LN), yolk-sac area (AY), and deformities. Larvae hatching on a given day were incubated at the same temperature and sampled at 4 days post-hatch (DPH) for growth, yolk utilization rate (YUR) and efficiency (YUE). The mean±SE duration of the hatching window decreased with increasing temperature in both Atlantic (5.4±0.1 to 2.6±0.3 days from 2.0 to 10.0°C) and Baltic larvae (6.2±0.4 to 5.0±0.6 days from 6.5 to 12.5°C) and LN increased and AY decreased for every subsequent day of hatch. Deformities increased with increasing T and P × T explained 52.3 and 26.8% of the variance for Atlantic and Baltic larvae, respectively. In Baltic larvae, size at peak hatch tended to decrease with increasing T and P × T explained 34.6% of the variance. In Atlantic larvae, growth, YUR and YUE were influenced by T while P alone explained 26.0% of the variance in YUE and up to 66.4% of the variance in morphological traits at 4 DPH. Asynchronous hatching significantly affected larval growth, YUR, and YUE with P explaining 37.1% of the variance in growth for Atlantic larvae. Temperature and asynchronous hatching interacted to produce larvae that were generally longer and had smaller AY if they were incubated at colder temperatures or if they hatched at the end of the hatching period at a specific temperature. Differences in larval morphometrics among temperatures for early hatching larvae decreased or even reversed for later hatching larvae. In light of anticipated global climate change, the present study on cod provides further insight in understanding the genotype-based variability and the adaptive potential to an ecologically changing environment.
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General information
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Organisations: Section for Population Ecology and Genetics, National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, IFREMER, Norwegian University of Science and Technology
Authors: Mazurais, D. (Ekstern), Kjørsvik, E. (Ekstern), World, P. (Ekstern), Politis, S. N. (Intern), Cahu, C. (Ekstern), Tomkiewicz, J. (Intern), Zambonino-Infante, J. (Ekstern)
Publication date: 2013
Event: Poster session presented at Aquaculture Europe 13, Trondheim, Norway.
Main Research Area: Technical/natural sciences

Development of techniques and technology for embryonic and larval rearing of the European eel

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography
Authors: Butts, I. (Intern), Sørensen, S. R. (Intern), Politis, S. N. (Intern), Lauesen, P. (Intern), Tomkiewicz, J. (Intern)
Publication date: 2013
Event: Poster session presented at Larvi 2013, Ghent, Belgium.
Main Research Area: Technical/natural sciences

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, Section for Ecosystem based Marine Management, Centre for Ocean Life
Authors: Tomkiewicz, J. (Intern), Støttrup, 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
Links:
https://www.was.org/easonline/Mobile/Paper.aspx?i=2052

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
Mortality and development during early life phases of Atlantic cod (Gadus morhua) in relation to paternity and water temperature

General information
State: Published
Organisations: University of Hamburg, Fisheries and Oceans Canada
Authors: Politis, S. N. (Intern), Dahlke, F. T. (Ekstern), Peck, M. A. (Ekstern), Trippel, E. (Ekstern)
Publication date: 2009
Main Research Area: Technical/natural sciences

Projects:

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 gonadotropin 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)  
Sørensen, Sune Riis (Intern)  
Kottmann, Johanna Sarah (Intern)  
Project Manager, organisational:  
Haslund, Ole Henrik (Intern)  
Phd Student:  
Politis, Sebastian Nikitas (Intern)  
Project Coordinator:  
Tomkiewicz, Jonna (Intern)  
Project  

European eel larval ontogeny and physiology  
National Institute of Aquatic Resources  
Period: 01/04/2014 → 12/03/2018  
Number of participants: 3  
Phd Student:  
Politis, Sebastian Nikitas (Intern)  
Supervisor:  
Butts, Ian (Intern)  
Main Supervisor:  
Tomkiewicz, Jonna (Intern)  

Financing sources  
Source: Internal funding (public)  
Name of research programme: Samfinansieret - Andet  
Project: PhD