Fertilization strategies for Sea Bass Dicentrarchus labrax (Linnaeus, 1758): effects of pre-incubation and duration of egg receptivity in seawater

Studying gamete biology can provide important information about a species fertilization strategy as well as their reproductive ecology. Currently, there is a lack of knowledge about how long sea bass Dicentrarchus labrax eggs can remain viable after being activated in seawater. The objectives of this study were to understand the effects of pre-incubation of fresh and overripe sea bass eggs in seawater and to determine the duration of egg receptivity. Pooled eggs (fresh and overripe) from four females were pre-incubated in seawater for 0 min (control), 0.5 min, 1 min, 3 min, 10 min and 30 min and then fertilized by pooled sperm from four males. The fresh eggs had a higher fertilization success than overripe eggs. Our results revealed a significant effect of pre-incubation time for both the fresh (P < 0.01) and overripe eggs (P < 0.01). Fertilization success of eggs significantly declined for both these treatments after 3 min of pre-incubation, which clearly indicates that sea bass eggs are able to be fertilized by sperm for up to 3 min after release into seawater. This study has particular importance for understanding fertilization strategies, reproductive potential, as well as reproductive ecology of sea bass.
Mærkningsmetode for små fisk: Et casestudie med kysttobis (Ammodytes tobianus)

General information
State: Published
Organisations: Section for Marine Ecology and Oceanography, National Institute of Aquatic Resources, Section for Marine Living Resources, Den Blå Planet
Authors: Jørgensen, M. G. P. (Intern), Deurs, M. V. (Intern), Butts, I. (Intern), Jørgensen, K. (Ekstern), Behrens, J. (Intern)
Publication date: 2017
Event: Abstract from Dansk Havforskermøde, Helsingør, Denmark.
Main Research Area: Technical/natural sciences

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.

**General information**

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, University of South Bohemia, University of Warmia and Mazury in Olsztyn, University of Windsor
Authors: Siddique, M. A. M. (Ekstern), Linhart, O. (Ekstern), Krejszeff, S. (Ekstern), Zarski, D. (Ekstern), Pitcher, T. E. (Ekstern), Politis, S. N. (Intern), Butts, I. (Intern)
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- Web of Science (2017): Indexed yes
- BFI (2016): BFI-level 1
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- Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 1
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- Web of Science (2015): Indexed yes
- BFI (2014): BFI-level 1
- Scopus rating (2014): SJR 1.109 SNIP 0.951 CiteScore 2.51
- BFI (2013): BFI-level 1
- Scopus rating (2013): SJR 1.192 SNIP 1.242 CiteScore 2.96
- ISI indexed (2013): ISI indexed yes
- BFI (2012): BFI-level 1
- Scopus rating (2012): SJR 1.062 SNIP 1.183 CiteScore 3.02
- ISI indexed (2012): ISI indexed yes
- Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 1
- Scopus rating (2011): SJR 1.262 SNIP 1.117 CiteScore 3.07
- ISI indexed (2011): ISI indexed yes
- BFI (2010): BFI-level 1
- Scopus rating (2010): SJR 1.05 SNIP 0.978
- BFI (2009): BFI-level 1
- Scopus rating (2009): SJR 1.095 SNIP 0.948
- BFI (2008): BFI-level 1
- Scopus rating (2008): SJR 0.925 SNIP 1.03
- Scopus rating (2007): SJR 0.85 SNIP 0.947
- Scopus rating (2006): SJR 0.858 SNIP 1.07
- Scopus rating (2005): SJR 0.978 SNIP 1.007
- Scopus rating (2004): SJR 0.637 SNIP 0.835
- Scopus rating (2003): SJR 0.676 SNIP 1.079
PIT-tagging method for small fishes: A case study using sandeel (Ammodytes tobianus)

Passive integrated transponder (PIT) tags are commonly used to assess fish movement for use in fisheries management. Here, we investigated physiological and behavioral effects of tagging on sandeels (Ammodytes tobianus) using PIT tags constituting 2.1 ± 0.9% of their body weight. Swimming stamina (RSS), calculated as time spent swimming against the current relative to total swimming time, and tail beat frequency were compared between tagged and untagged fish as was blood hematocrit levels at 7, 14, and 42 d post-tagging. Survival and tag retention were also documented at 14, 42, and 84 d (via x-rays and dissections). RSS was not different between tagged and untagged fish with means (± SD) of 60 ± 9% and 61 ± 12%. Tail beat frequency was not different between tagged and untagged fish at 2.8 ± 0.3 and 3.0 ± 0.4 beats s⁻¹ for tagged and untagged fish, respectively. Likewise, hematocrit was not affected by tagging and levels were between 21–26% for both groups. Survival rates were high and did not differ between groups (96% for tagged and 99% untagged fish). Tag retention was 100%. X-rays and dissections did not reveal any signs of tag movement at 14–84 d, and there was no difference between relative positions of the tags. None of the tags were encapsulated in the body cavity after 14 d, whereas 40 and 56% of the tags were encapsulated in a thin tissue membrane between the intestine and kidney after 42 and 84 d, respectively. After 14 d all incisions had healed with only minor or no signs of the tag insertion site. Collectively, these data provide substantial evidence for the possibility of conducting large-scale tagging studies on this species in the field.

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Organisations: Section for Marine Ecology and Oceanography, National Institute of Aquatic Resources, Section for Marine Living Resources, Auburn University, National Aquarium of Denmark
Authors: Jørgensen, M. G. P. (Intern), Deurs, M. V. (Intern), Butts, I. (Intern), Jørgensen, K. (Ekstern), Behrens, J. W. (Intern)
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Scopus rating (2016): CiteScore 2.21 SJR 1.12 SNIP 1.136
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.067 SNIP 1.133 CiteScore 2.01
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.105 SNIP 1.312 CiteScore 2.17
Web of Science (2014): Indexed yes
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Tagging method for small fishes: A case study using lesser sandeel (Ammodytes tobianus)

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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Section for Marine Living Resources, Den Blå Planet
Authors: Jørgensen, M. G. P. (Intern), Deurs, M. V. (Intern), Butts, I. (Intern), Jørgensen, K. (Ekstern), Behrens, J. (Intern)
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Event: Abstract from Dansk Havforskermøde, Helsingør, Denmark.
Main Research Area: Technical/natural sciences
Publication: Research › Conference abstract for conference – Annual report year: 2017
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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Organisations: Section for Marine Ecology and Oceanography, National Institute of Aquatic Resources, Section for Marine Living Resources, IFREMER, GEOMAR - Helmholtz Centre for Ocean Research Kiel
Authors: Politis, S. N. (Intern), Servili, A. (Ekstern), Mazurais, D. (Ekstern), Zambonino-Infante, J. (Ekstern), Miest, J. J. (Ekstern), Tomkiewicz, J. (Intern), Butts, I. A. E. (Intern)
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Scopus rating (2016): CiteScore 2.55 SJR 1.056 SNIP 0.924
Web of Science (2016): Indexed yes
Abundance of specific mRNA transcripts impacts hatching success in European eel, Anguilla anguilla L.

Maternal mRNA governs early embryonic development in fish and variation in abundance of maternal transcripts may contribute to variation in embryonic survival and hatch success in European eel, Anguilla anguilla. Previous studies have shown that quantities of the maternal gene products β-tubulin, insulin-like growth factor 2 (igf2), nucleoplasmmin (npm2), prohibitin 2 (phb2), phosphatidylinositol glycan biosynthesis class F protein 5 (pigf5), and carnitine O-palmitoyltransferase liver isoform-like 1 (cpt1) are associated with embryonic developmental competence in other teleosts. Here, the relations between relative mRNA abundance of these genes in eggs and/or embryos and egg quality, was studied and analyzed. We compared egg quality of the two groups: i) batches with hatching and ii) batches with no hatching. Results showed no significant differences in relative mRNA abundance between the hatch and no hatching groups for any of the selected genes at 0, 2.5, and 5 HPF. However, at 30 HPF the hatch group showed significantly higher abundance of cpt1a, cpt1b, β-tubulin, phb2, and pigf5 transcripts than the no hatch group. Therefore, these results indicate that up-regulation of the transcription of these genes in European eel after the mid-blastula transition, may be needed to sustain embryonic development and hatching success.

General information
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Organisations: Section for Ecosystem based Marine Management, National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, IFREMER
Authors: Rozenfeld, C. (Intern), Butts, I. A. (Intern), Tomkiewicz, J. (Intern), Zambonino-Infante, J. (Ekstern), Mazurais, D. (Ekstern)
Composition of seminal plasma and ovarian fluid in Ide Leuciscus idus and Northern pike Esox lucius

General information
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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, University of South Bohemia, University of Warmia and Mazury
Authors: Siddique, M. (Ekstern), Linhart, O. (Ekstern), Kujawa, R. (Ekstern), Krejszeff, S. (Ekstern), Butts, I. (Intern)
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BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.38 SJR 0.551 SNIP 0.924
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.639 SNIP 0.999 CiteScore 1.39
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.695 SNIP 0.916 CiteScore 1.55
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.66 SNIP 0.937 CiteScore 1.23
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.621 SNIP 1.207 CiteScore 1.57
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.776 SNIP 0.978 CiteScore 1.26
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.661 SNIP 0.856
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.648 SNIP 0.898
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 0.633 SNIP 0.859
Scopus rating (2007): SJR 0.551 SNIP 0.878
Scopus rating (2006): SJR 0.632 SNIP 0.946
Scopus rating (2005): SJR 0.683 SNIP 0.995
Scopus rating (2004): SJR 0.542 SNIP 1.01
Scopus rating (2003): SJR 0.255 SNIP 0.596
Scopus rating (2002): SJR 0.234 SNIP 0.562
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.179 SNIP 0.33
Development of a broodstock diet to improve developmental competence of embryos in European eel, Anguilla anguilla

We examined the effect of dietary arachidonic acid (ARA) and eicosapentaenoic acid (EPA) on the production of embryos and hatched larvae in the European eel, Anguilla anguilla. Two diets with high and intermediate levels of ARA and low and intermediate levels of EPA (Feed 1: ARA 1.9%, EPA 4.2%; Feed 2: ARA 1.2%, EPA 5.1% of total fatty acids) were tested against a commercial diet (DE: ARA: 0.5%, EPA: 8.2% of total fatty acids). After 24 weeks of feeding, ARA levels in the muscles and ovaries increased to 0.9% and 1.3% of total fatty acids, respectively, in Feed 1 and were significantly higher than in Feed 2 and DE. Female broodstock was not fed during hormonal treatment to induce vitellogenesis and ovulation. EPA levels in females fed the test diets decreased in the both muscle and ovary and were significantly lower in eggs from females fed Feed 1. The highest percentage of stripped females, producing viable eggs and larvae, were those females fed the highest dietary ARA levels (Feed 1). The level of lipid peroxidation products in eggs was similar among treatment, indicating that the lowest dietary levels of vitamin C and vitamin E were sufficient. In the unfertilized eggs, ARA levels were also highest (1.1% of total fatty acids) in the diet with highest ARA levels (Feed 1).

General information
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Authors: Støttrup, J. G. (Intern), Tomkiewicz, J. (Intern), Jacobsen, C. (Intern), Butts, I. (Intern), Holst, L. (Ekstern), Krüger-Johnsen, M. (Intern), Graver, C. (Ekstern), Lauesen, P. (Ekstern), Fontagné-Dicharry, S. (Ekstern), Heinsbroek, L. (Ekstern), Corraze, G. (Ekstern), Kaushik, S. (Ekstern)
Pages: 725-737
Publication date: 2016
Main Research Area: Technical/natural sciences
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
Authors: Sørensen, S. R. (Intern), Butts, I. (Intern), Munk, P. (Intern), Tomkiewicz, J. (Intern)
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Main Research Area: Technical/natural sciences

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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.
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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Authors: Butts, I. (Intern), Sørensen, S. R. (Intern), Politis, S. N. (Intern), Tomkiewicz, J. (Intern)
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
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 undertook 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 at 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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Authors: Sørensen, S. R. (Intern), Tomkiewicz, J. (Intern), Munk, P. (Intern), Butts, I. A. (Intern), Nielsen, A. (Intern), Lauesen, P. (Ekstern), Graver, C. (Ekstern)

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- Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524 Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 2
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- BFI (2013): BFI-level 1
- Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18 ISI indexed (2013): ISI indexed yes
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- BFI (2011): BFI-level 1
- Web of Science (2011): Indexed yes
- BFI (2010): BFI-level 1
- BFI (2009): BFI-level 1
- Scopus rating (2009): SJR 0.941 SNIP 1.263 Web of Science (2009): Indexed yes
- BFI (2008): BFI-level 2
Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish

Di-(2-ethylhexyl) phthalate (DEHP) interferes with male reproductive endocrine system in mammals, however its effects on fish reproduction are largely unknown. We evaluated sperm quality and investigated reproductive endocrine system in mature goldfish (Carassius auratus) exposed to nominal 1, 10, and 100μg/L DEHP. To examine DEHP estrogenic activity, one group of goldfish was exposed to 17β-estradiol (5μg/L E2) for comparison. Following 30d of exposure, sperm production was decreased and suppressed in DEHP and E2 treated goldfish, respectively. Sperm motility and velocity were decreased in goldfish exposed to 100 and 10μg/L DEHP at 15s post-sperm activation, respectively. Compared to control, 11-ketotestosterone (11-KT) levels were decreased at 10 and 1μg/L DEHP at day 15 and 30, respectively. In E2 treated goldfish, 11-KT levels were decreased compared to control during the period of exposure. E2 levels were increased in goldfish exposed to E2, but remained unchanged in DEHP treated goldfish during the period of exposure. StAR mRNA levels encoding regulator of cholesterol transfer to steroidogenesis were decreased in DEHP and E2 treated goldfish following 15 and 30d of exposure, respectively. Luteinizing hormone (LH) levels were decreased in DEHP and E2 treated goldfish following 15 and 30d of exposure, respectively. In DEHP treated goldfish, gnrh3, kiss1 and its receptor (gpr54) mRNA levels did not change during the experimental period. In E2 treated goldfish, gnrh3 mRNA levels were decreased at day 7, but kiss1 and gpr54 mRNA levels were increased at day 30 of exposure. The mRNA levels of genes encoding testicular LH and androgen receptors remained unchanged in DEHP and E2 treated goldfish. In contrast to E2 treated goldfish, vitellogenin production was not induced in DEHP treated goldfish and mRNA levels of genes with products mediating estrogenic effects remained unchanged or decreased. In conclusion, DEHP interferes with testis and pituitary hormonal functions to reduce sperm quality in goldfish and does not exhibit estrogenic activity.
Effects of pre-incubation of eggs in fresh water and varying sperm concentration on fertilization rate in sterlet sturgeon, *Acipenser ruthenus*

Standardization of fertilization protocols for sterlet Acipenser ruthenus is crucial for improving reproductive techniques and for conservation purposes. Our objectives were to determine the number of sperm (tested 430,000:1, 43,000:1, 4300:1, 430:1 sperm to egg) required to fertilize eggs and explore how pre-incubation of eggs in freshwater for 0. min, 0.5. min, 1. min, and 10. min interacts with different sperm ratios. Fertilization success ranged from 29.7% at 430:1 to 84.2% at 430,000:1. Pre-incubation time had no effect on fertilization success at 430,000:1 and 43,000:1 sperm to egg ratios, while it was significant at the 4300:1 and 430:1 ratios. The use of adequate experimental suboptimal sperm to egg ratio revealed...
a positive effect of pre-incubation time, such that at the 430:1 ratio, 0.5 min pre-incubation increased the fertilization rate
than 10. min. At 0. min pre-incubation the proportion of fertilized eggs increased at the 430,000:1 ratio, while at 1. min
fertilization increased at the 4300:1 ratio. At the 10. min pre-incubation time, fertilization increased at the 43,000:1 ratio.
Moreover, at the 0.5. min pre-incubation time, the 43,000:1 ratio increased the fertilization rate than the 430:1 ratio.
Generally, for 430:1 ratio, the fertilization rate is lower than in control. Transmission electron microscopy showed that pre-
incubation of eggs in water for <10. min does not trigger a cortical reaction or the formation of a perivitelline space.
Results suggest that with a low sperm to egg ratio 0.5 to 1. min pre-incubation of eggs in freshwater prior to fertilization
can enhance fertilization rate of sterlet
Impact of dietary fatty acids on muscle composition, liver lipids, milt composition and sperm performance in European eel

In order for European eel aquaculture to be sustainable, the life cycle should be completed in captivity. Development of broodstock diets may improve the species' reproductive success in captivity, through the production of high-quality gametes. Here, our aim was to evaluate the influence of dietary regime on muscle composition, and liver lipids prior to induced maturation, and the resulting sperm composition and performance. To accomplish this fish were reared on three "enhanced" diets and one commercial diet, each with different levels of fatty acids, arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Neutral lipids from the muscle and liver incorporated the majority of the fatty acid profile, while phospholipids incorporated only certain fatty acids. Diet had an effect on the majority of sperm fatty acids, on the total volume of extractable milt, and on the percentage of motile sperm. Here, our results suggest that the total volume of extractable milt is a DHA-dependent process, as we found the diets with the highest DHA levels induced the most milt while the diet with the lowest DHA level induced the least amount of milt. The diet with the highest level of ARA induced medium milt volumes but had the highest sperm motility. EPA also seems important for sperm quality parameters since diets with higher EPA percentages had a higher volume of milt and higher sperm motility. In conclusion, dietary fatty acids had an influence on fatty acids in the tissues of male eel and this impacted sperm performance.
Receptivity of winter flounder larvae to artificial diet from the yolk-sac stage to metamorphosis

In the period from 4 days post-hatching to metamorphosis, winter flounder that were naïve to artificial feed were exposed to an artificial diet and allowed to forage for 8 min. The presence or absence of artificial diet in the gut was used as an indicator of acceptance. The relationship between diet acceptance and age was found to be a dome-shaped function (incidence of ingested food = −0.0233 day^2 + 1.101 day − 4.9428, r^2 = 0.85). Here, our analysis suggests that winter flounder should be exposed to artificial diets by 26 days post-hatch at 10 °C, after this time receptivity declines. This age corresponds to larvae that are in the flexion developmental stage and have a standard length between 5.95 and 6.23 mm.
The effects of inbreeding on sperm quality traits in captive-bred lake trout, Salvelinus namaycush (Walbaum, 1972)

The effects of inbreeding in both captive and wild-caught species and populations have been reported to affect a wide variety of life history traits. Recently, the effects of inbreeding on reproductive traits such as sperm quality have become a subject of particular interest for conservation biology, evolutionary ecology, and management of captive populations. This study investigated the effects of inbreeding on sperm quality in a captive population of experimentally inbred and outbred lake trout, Salvelinus namaycush. It was found for moderately to highly inbred males (males with half-sib and full-sib parents, respectively), that sperm quality traits (velocity, motility, linearity, longevity, spermatocrit and morphology) showed no apparent inbreeding depression. The apparent lack of inbreeding effects on sperm quality traits may be due to several factors including (i) no inbreeding depression in the studied population, due to purging, low levels of inbreeding or lack of detection at the gametic level, or (ii) relaxed selective pressures due to benign hatchery conditions. The present study provides significant insight into the effects of inbreeding on sperm quality in a captive-bred salmonid population, and has important implications for hatchery rehabilitation programs for this species.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Trent University, University of Windsor
Authors: Johnson, K. (Ekstern), Butts, I. A. E. (Intern), Smith, J. L. (Ekstern), Wilson, C. C. (Ekstern), Pitcher, T. E. (Ekstern)
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Web of Science (2016): Indexed yes
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Scopus rating (2015): CiteScore 0.84
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.06
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 0.99
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 0.99
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
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Scopus rating (2011): CiteScore 1.04
Thermal effects on early life history stages of European eel Anguilla anguilla

General information
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Organisations: National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography
Authors: Politis, S. N. (Intern), Miest, J. (Ekstern), Adamek, M. (Ekstern), Servili, A. (Ekstern), Mazurais, D. (Ekstern), Zambonino, J. (Ekstern), Tomkiewicz, J. (Intern), Butts, I. (Intern)
Publication date: 2015
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Main Research Area: Technical/natural sciences
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Effects of reproduction on growth and survival in Atlantic cod, Gadus morhua, assessed by comparison to triploids

Despite increasing interest in optimal life history theory and the associated physiological, ecological and evolutionary processes, little information exists on gonad-soma tradeoffs and longevity of individuals over long time periods. We examined somatic and survival costs of reproduction in captive iteroparous, batch-spawning Atlantic cod (Gadus morhua), utilizing diploids and triploids, knowing that triploid females invest little to no energy into gametogenesis. Based on annual specific growth rate, there was no evidence for a somatic cost of reproduction at ages 2 (virgin year) and 4. years, but there was at age 3. years. At age 2. years, low investment in reproduction likely accounted for the lack of a somatic cost of reproduction, whereas at age 4 the absence was associated with heightened growth post-spawning enabling mature fish to catch up to immature fish. At age 3, compensatory growth during post-spawning was below that of immature fish. Survival represented a significant component of the cost of reproduction. Laboratory experiments examining the cost of reproduction have traditionally focused on shorter time periods, commonly spanning several months, whereas ours spanned nearly four years. Although previously done for bivalves, to our knowledge, this is the first time the cost of reproduction has been evaluated using triploid fish as a comparator.

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Authors: Trippel, E. A. (Ekstern), Butts, I. (Intern), Babin, A. (Ekstern), Neil, S. R. (Ekstern), Feindel, N. J. (Ekstern), Benfey, T. J. (Ekstern)
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BFI (2016): BFI-level 1
Larval production and survival during the early larval stage in European eel

General information
State: Published
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 (19 ± 8%), larvae reared in the 12:12 h photoperiod had higher survival (13 ± 8%) than those reared in the 24:0 h light/dark photoperiod (11 ± 8%), and larvae reared in red light (11 ± 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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Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.186 SNIP 1.021 CiteScore 2.27
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.067 SNIP 1.007 CiteScore 2.14
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.239 SNIP 1.017
Web of Science (2010): Indexed yes
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Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.26 SNIP 1.134
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.214 SNIP 1.308
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.262 SNIP 1.247
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.164 SNIP 1.134
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.091 SNIP 1.121
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.351 SNIP 1.341
Scopus rating (2002): SJR 1.385 SNIP 1.323
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.363 SNIP 1.269
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.349 SNIP 1.245
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.562 SNIP 1.12

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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.
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 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.
Development of a sperm cryopreservation protocol for redside dace, Clinostomus elongatus: implications for genome resource banking

Populations of Redside Dace Clinostomus elongatus have declined in many areas across the species' North American range. Therefore, the development of sperm cryopreservation technology would provide an invaluable means of preserving genetic diversity in populations that are in imminent danger of extirpation. We developed cryopreservation protocols by testing the effects of diluent (buffered sperm motility-inhibiting saline solution [BSMIS]; BSMIS + glycine;...
sucrose; and Hanks' balanced salt solution [HBSS]), cryoprotectant (dimethyl sulfoxide [DMSO]; propylene glycol [PG]; N,N-dimethylacetamide [DMA]; and methanol), freezing rate (1, 5, and 10 · C/min), and male-to-male variation on sperm quality. Incubating sperm in extenders affected motility; BSMIS + glycine + methanol, BSMIS + glycine + PG, and HBSS + methanol were the only treatments for which motility was not significantly different from that of fresh sperm. Sperm frozen with sucrose had higher motility than sperm frozen with BSMIS + glycine, and sperm frozen with DMSO had higher motility than sperm frozen with methanol. Freezing rates were evaluated for BSMIS + glycine, HBSS, and sucrose; all diluents were frozen with DMSO. The effect of freezing rate was not significant for BSMIS + glycine or for HBSS, but an effect was detected for sucrose, with sperm frozen at 5 · C/min or 10 · C/min having higher motility than sperm frozen at 1 · C/min. The effect of extender was not significant at 1 · C/min or 5 · C/min, but an effect was detected at 10 · C/min such that sperm frozen with sucrose had the highest motility. Male-to-male variability was evaluated by using sucrose + DMSO and a freezing rate of 10 · C/min. For these males, the sperm motility recovery index ranged from 6.67% to 79.27%, and the sperm velocity recovery index ranged from 21.37% to 57.33%. Our findings demonstrate that cryopreservation of Redside Dace sperm in a sucrose + DMSO extender at a freezing rate of 10 · C/min is adequate for preserving genetic diversity via sperm banks.

**General information**
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Organisations: National Institute of Aquatic Resources, Section for Population Ecology and Genetics
Authors: Butts, I. A. (Intern), Mokdad, A. (Ekstern), Trippel, E. (Ekstern), Pitcher, T. (Ekstern)
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Scopus rating (2014): SJR 0.932 SNIP 1.073 CiteScore 1.78
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ISI indexed (2013): ISI indexed yes
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Scopus rating (2012): SJR 1.1 SNIP 1.181 CiteScore 1.66
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Scopus rating (2011): SJR 0.973 SNIP 0.966 CiteScore 1.33
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Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.203 SNIP 1.057
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.952 SNIP 0.891
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 1.148 SNIP 1.184
Scopus rating (2007): SJR 1.19 SNIP 1.218
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Development of techniques and technology for embryonic and larval rearing of the European eel

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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
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%). FCM 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 Politécnica de Valencia
Authors: Sørensen, S. R. (Intern), Gallego, V. (Ekstern), Pérez, L. (Ekstern), Butts, I. (Intern), Tomkiewicz, J. (Intern), Asturiano, J. (Ekstern)
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Evaluation of methods to determine sperm density for the European eel, Anguilla anguilla

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Authors: Sørensen, S. R. (Intern), Gallego, V. (Ekstern), Pérez, L. (Ekstern), Butts, I. (Intern), Tomkiewicz, J. (Intern), Asturiano, J. (Ekstern)
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**Ovarian fluid influences sperm performance in lake trout, Salvelinus namaycush**

The objectives of this study were to determine whether (i) the presence and concentration of ovarian fluid (OF) affects sperm performance traits, and (ii) variation in sperm performance traits is due to male identity, female identity, and/or male×female interactions in lake trout, Salvelinus namaycush. Spermatozoa from four males were activated in river water and OF from four females at two concentrations (10 and 15%). Presence of ovarian fluid influenced sperm traits; no differences were detected between 10 and 15% OF. Sperm traits varied depending on parental identity, such that sperm
of some males perform better in the OF of all females and that in OF of some females sperm traits are higher for all males.

**General information**

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Organisations: University of Windsor, Ontario Ministry of Natural Resources
Authors: Galvano, P. M. (Ekstern), Johnson, K. (Ekstern), Wilson, C. C. (Ekstern), Pitcher, T. E. (Ekstern), Butts, I. A. (Intern)
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Web of Science (2017): Indexed Yes
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ISI indexed (2012): ISI indexed yes
Scopus rating (2011): SJR 0.568 SNIP 0.863 CiteScore 1.75
ISI indexed (2011): ISI indexed no
Scopus rating (2010): SJR 0.503 SNIP 1.091
Scopus rating (2009): SJR 0.353 SNIP 0.781
Scopus rating (2008): SJR 0.403
Scopus rating (2007): SJR 0.37
Scopus rating (2006): SJR 0.477
Scopus rating (2005): SJR 0.255
Scopus rating (2004): SJR 0.144
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**Paternal effects on early life history traits in Northwest Atlantic cod, Gadus morhua**

It is important to understand parental effects on early life history of fish as manifested, for example, in individual fitness of offspring. Immediately after fertilization, parental contributions (both genetic and non-genetic) to embryos will affect larval ontogeny, physiology, morphology and survival. In marine fish, rates of natural mortality are highest during early life and are negatively correlated with rates of growth and body size. In these early life stages (eggs, larvae, young juveniles) subtle differences in mortality can cause large differences in recruitment and year-class success. Therefore, it is particularly critical to understand factors that contribute to variability in mortality during early life. This study focuses on evaluating the potential influence of paternity on rates of mortality and development in eggs and larvae of Northwest Atlantic cod, Gadus morhua. To accomplish this 12 males and two females were crossed using a full-factorial breeding design. Paternity had a strong influence on fertilization success, hatching success, cumulative embryonic mortality, larval standard length, eye diameter, yolk-sac area, and cumulative larval mortality. Female 1 showed an overall ‘weaker’ performance of offspring than Female 2, indicating that deviations can stem from differences in female quality. Nevertheless, paternal contributions to embryonic and larval development were still evident despite differences in female
Physiological functions of osmolality and calcium ions on the initiation of sperm motility and swimming performance in redside dace, Clinostomus elongatus
Reproductive potential of fish stocks is critically dependent on sperm performance in an aquatic environment. The aim of this study is to test hypotheses, which govern the initiation of sperm motility and swimming performance, through physiological functions of osmolality and Ca2+ ion, in a threatened species of freshwater fish, the redside dace, Clinostomus elongatus. Spermatozoa motility was activated in either ionic or non-ionic media spanning a range of osmolalities. The role of Ca2+ channels on induction of spermatozoa motility and velocity was experimentally investigated by diluting sperm in media that contain various Ca2+ channel blockers. Results show that initiation of spermatozoa motility is a hypo-osmolality dependent mechanism. Inhibitors for L-type Ca2+ channels partially prohibited initiation of spermatozoa motility, while velocity was significantly reduced in both L-type and T-type Ca2+ channel blockers. Examination using W-7, an inhibitor for Ca2+-dependent calmodulin, showed significant decreases in spermatozoa motility and velocity. Involvement for Ca2+ in axonemal beating was confirmed by significant increases in velocity after adding Ca2+ into the activation media, while motility remained unchanged in Ca2+ supplemented activation media. Together, these findings suggest the involvement of Ca2+ in hypo-osmolality-dependent initiation of spermatozoa motility mediated by activation of Ca2+ binding protein in the axoneme of a freshwater fish sperm. Blocking Ca2+ exchange through L- or T-type Ca2+ channel influences flagellar beating force and leads to decrease in spermatozoa velocity. (C) 2013 Elsevier Inc.
Reproduction of European eel and larval culture: state of the art

General information

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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)
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Reproductive investment patterns, sperm characteristics, and seminal plasma physiology in alternative reproductive tactics of Chinook salmon (Oncorhynchus tshawytscha)

Although alternative reproductive tactics (ARTs) are common across a range of taxa, little is known about whether the different tactics have adapted to sperm competition risk. Chinook salmon, Oncorhynchus tshawytscha, have two ARTs: large males that participate in dominance-based hierarchies for access to spawning females, known as hooknoses,
and small males that attempt to sneak fertilizations during spawning events from peripheral positions, known as jacks. Jacks continually face sperm competition risk because they always spawn in the presence of another male, whereas hooknoses face relatively low sperm competition risk because other males are not always present during spawning events. Based on the sneak-guard model of sperm competition this asymmetry in sperm competition risk predicts that jacks ought to invest significantly more into sperm-related traits important for sperm competition success relative to hooknoses. In the present study we report on reproductive investment patterns, sperm characteristics, and seminal plasma physiology of males that exhibit ARTs in Chinook salmon. We found that jacks invest significantly more of their somatic tissue into gonads compared with hooknoses. Sperm velocity also varied significantly between the ARTs, with jacks having significantly faster sperm than hooknoses. No significant differences in seminal plasma physiology metrics related to sperm quality were detected between the ARTs. We interpret these sperm investment patterns in light of the sneak-guard model of sperm competition that is based on differences in sperm competition risk and alternative investment possibilities among ARTs.

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Sperm quality of hatchery-reared lake trout Salvelinus namaycush throughout the spawning season

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Scopus rating (2011): SJR 0.442 SNIP 0.868 CiteScore 0.8
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Techniques for rearing European eel during early life history

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https://www.was.org/easonline/Mobile/Paper.aspx?i=2037
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Effects of phthalate on reproduction in male broodfish

Di-(2-ethylhexyl)-phthalate (DEHP) is the most commonly used plasticizer in PVC formulation for a wide variety of applications including medical devices, construction products, clothing and car products. DEHP is also used in non-polymer materials such as lacquers and paints, adhesives and printing inks and cosmetics. Public concerns on phthalates distributions in the environment have been increasing since they can cause liver cancer, structural abnormalities and alternations in male reproductive system. In fish, although there are a few studies that show alternations in testicular steroidogenesis, but information about its effects on sperm quality is rare. Moreover, the molecular mechanisms of DEHP on testicular functions leading to reduction of sperm quality are still unknown.

In the present study, alternations of reproductive function in mature male goldfish were studied following 1 month exposure to 1, 10 and 100 DEHP. Two groups of males were exposed to acetone (solvent control) and to 17-estradiol (E2, estrogenic positive control). Modulations of androgen and estrogen mediating genes as well as vitellogenin mRNA expression were investigated in the samples of brain, liver and testis to study their transcriptomic response to DEHP using a quantitative real-time PCR. Samples of blood plasma were collected to investigate alternations in sex steroid levels using ELISA. Sperm quality including sperm volume, motility and velocity were evaluated to understand whether alternations in testicular functions lead to reduction in sperm quality.

E2 level was unchanged in DEHP-treated group compared to the solvent control, but it was significantly increased in E2-treated group. 11-ketotestosterone level was significantly decreased in either DEHP-treated groups or E2-treated group. No sperm was produced in E2-treated group. Sperm volume was significantly lower in all DEHP-treated groups compared to that of solvent control. Sperm motility and velocity evaluated at 15 s post-sperm activation was significantly decreased at 100 and 10 DEHP, respectively. Transcriptomics response of testis showed significant alternations in AR (decreased in E2-treated group), StAR (decreased in E2-treated group and DEHP-treated group at 1 ERa (increased in E2-treated group and DEHP-treated group at 1 and increased at 10 and 100 and CYP19a (increased in E2-treated group). In brain, significant increase of ERα and CYP19a mRNA expression were observed in E2-treated group, while they were unchanged in DEHP-treated groups. In liver, expression of Vtg and CYP19a mRNA were only increased in E2-treated group.

In conclusion, the present study suggest anti-androgenic DEHP mode of on reproductive function in male broodfish that leads to decrease of sperm quality. The mechanism of action is dose-dependent via alternations in sex steroidogenesis mediating genes

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Authors: Hatef, A. (Ekstern), Alavi, S. (Ekstern), Milla, S. (Ekstern), Butts, I. (Intern), Carnevali, O. (Ekstern), Fontaine, P. (Ekstern), Linhart, O. (Ekstern)
Fertilization strategies for winter flounder: effects of spermatozoa density and the duration of gamete receptivity
Winter flounder is one of the most commonly used models for studying fish biology in North America; however little is known about their reproductive ecology, especially during the spawning event. The objectives of this research were to determine the optimal number of spermatozoa required to fertilize eggs and to explore how long spermatozoa (30 to 240 s post-activation) and eggs (30 to 7680 s post-activation) are receptive to fertilization after exposure to seawater. We conducted experiments using gametes from wild-caught fish and measured fertilization success by examining eggs at 5 to 6 d post-fertilization. On average 34 038 sperm cells per egg were required to fertilize 81.3% of the eggs. Duration after spermatozoa activation had an effect on the proportion of eggs fertilized (F(3,F-6.69 = 338.38; p <0.0001; mixed-model ANOVA). At 30 s post-spermatozoa activation, 98% of the eggs were fertilized. After 60 s, a significant decrease in fertilization success was detected. Duration after egg exposure to seawater had an effect on the proportion of eggs fertilized (F(8,F-16 = 19.89; p <0.0001; mixed-model ANOVA). For all trials (30 to 1920 s), the percentage of eggs fertilized ranged from 61 to 90%. A significant decrease to 11% occurred at 3840 s after egg exposure. This area of research has particular importance for our understanding of reproductive strategies, evolutionary challenges, reproductive potential and recruitment. In addition, examining sperm-egg interactions provides information important to management of living and frozen-thawed gene banks.
Ovarian fluid enhances sperm velocity based on relatedness in lake trout, Salvelinus namaycush

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Primary and secondary sexual characters in alternative reproductive tactics of Chinook salmon: Associations with androgens and the maturation-inducing steroid

The proximate mechanisms that underlie the evolution of within-sex variation in mating behavior, sexual characters and reproductive investment patterns are still poorly understood. Species exhibiting alternative reproductive tactics (ARTs) are ideal model systems to examine these mechanisms. Chinook salmon (Oncorhynchus tshawytscha) exhibits two distinct ARTs: hooknoses, which are large males that establish spawning dominance hierarchies via intense male–male competition and jacks, which are smaller precocious sneaking males that steal fertilizations via sperm competition. In this study, we examine plasma testosterone (T), 11-ketotestosterone (11-KT) and maturation-inducing steroid (MIS; 17α,20β-dihydroxy-4-pregnen-3-one) profiles of spawning hooknoses and jacks. Furthermore, we examine relationships between androgens and primary (gonad mass, gonadosomatic index and sperm traits) and secondary (total mass, body size, hump depth and kype length) sexual characters. Relationships between MIS and sperm traits are also examined. We found that hooknoses and jacks did not significantly differ in terms of plasma T, 11-KT or MIS concentrations. Moreover, we found significant positive relationships between levels of both androgens within each ART. There were no significant relationships between androgens, MIS and sperm traits. T and 11-KT concentrations co-varied positively with gonad investment and kype length in jacks. In hooknoses, 11-KT concentration was positively related to total mass, hump depth and condition factor. Overall, these findings suggest that there are differential androgen effects for each of the ARTs in Chinook salmon.
Primary and secondary sexual characters in alternative reproductive tactics of Chinook salmon: Associations with androgens and the maturation-inducing steroid

Automated sperm head morphology analyzer for open-source software

Sperm head morphology has been identified as a characteristic that can be used to predict a male’s semen quality. In the present study, we have developed an automated sperm head morphology analysis (ASMA) plug-in for open-source ImageJ software (http://rsbweb.nih.gov/ij/). We describe the plug-in's functionality, and confirm its validity for sperm head morphology analysis using fish sperm. Sperm head morphological measurements (length and width) made with the ASMA plug-in did not differ from manual measurements. Using the plug-in to measure sperm head-shaped objects of known size, the associated plug-in error rate was <0.5%. Brightness and contrast ratios influenced sperm head measurements, suggesting the need for standardized protocols. This plug-in was effective at measuring elliptical (i.e., Atlantic cod) as well as slightly irregular (i.e., Chinook salmon) shaped sperm heads. In conclusion, our ASMA plug-in represents a versatile alternative to costly sperm morphology software. (C) 2011 Elsevier Inc. All rights reserved.
Cryopreservation of Atlantic cod (Gadus morhua) sperm in large-volume straws: applications for commercial production and gene banking

In our study, we used a full factorial analysis of variance design to examine the effects of diluent [Mounib's sucrose-based diluent+hen's egg yolk (EY) and Hanks' balanced salt solution (HBSS)+EY], freezing rate (−2.5, −5.0 and −7.5 °C min−1) and thawing rate (2.5, 5.0 and 7.5 °C min−1) on motility and velocity of Atlantic cod sperm cryopreserved in 2.5 mL cryogenic straws. We found that post-thaw sperm performance was strongly influenced by the presence of higher-order interactions of the factors we tested. For all models broken down by diluent, the 2.5 °C min−1 thawing rate had the lowest sperm motility recovery index. Mounib's sucrose-based diluent+EY had the highest motility recovery index at all thawing rates. Mean per cent motility for fresh sperm (87.7±2.9%) was not significantly different than of sperm cryopreserved using Mounib's sucrose-based diluent+EY, frozen at −2.5 °C min−1 and thawed at 5.0 °C min−1 (77.1±2.9%). For Mounib's sucrose-based diluent+EY, velocity was significantly higher with sperm thawed at 7.5 °C min−1, than sperm thawed at 2.5 °C min−1, while thawing rate had no effect for HBSS+EY. Our findings have implications for cod mariculture and aiding in conservation efforts for a dominant marine fish species.

General information
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Organisations: Fisheries and Oceans Canada, Szent Istvan University
Authors: Butts, I. A. E. (Intern), Feindel, N. (Ekstern), Neil, S. (Ekstern), Kovács, É. (Ekstern), Urbányi, B. (Ekstern), Trippel, E. A. (Ekstern)
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Linking steroid hormones to primary and secondary sexual characters in alternative reproductive tactics of Chinook salmon

Mechanism of action of mercury on sperm morphology, adenosine triphosphate content, and motility in Perca fluviatilis (Percidae; Teleostei)

The main objectives of the present study were to investigate the performance of mercury chloride (HgCl₂) on sperm function and structure, identify sites of action of HgCl₂, and investigate the mechanism of action of HgCl₂ on fish (Perca fluviatilis L.) spermatozoa. Direct exposure of nonincubated sperm decreased sperm motility and velocity in a dose-dependent manner and was totally suppressed at 250μM HgCl₂. Adenosine-5'-triphosphate (ATP) content of sperm after activation in an activation medium (AM) containing more than 25μM HgCl₂ did not differ compared with nonactivated sperm. Motility and velocity of demembranated sperm decreased after activation in an AM containing 62μM HgCl₂, and was totally suppressed at 250μM HgCl₂. Incubation of sperm in an immobilizing medium (IM) containing HgCl₂ enhanced HgCl₂ effects after sperm activation in an AM containing HgCl₂. Sperm motility of incubated sperm in an IM without HgCl₂ was totally suppressed at 125μM HgCl₂ after 3h incubation. In case of incubated sperm in an IM containing HgCl₂, sperm motility was totally suppressed at 31μM HgCl₂. Adenosine-5'-triphosphate content of sperm was significantly lower in an IM containing HgCl₂ greater than 3μM compared with those of the control (no HgCl₂) and lower HgCl₂ concentrations. Damage to the plasma membrane and axoneme were observed in sperm incubated in an IM containing HgCl₂ compared with the control, when HgCl₂ concentration and incubation time increased. In conclusion, HgCl₂ acts on sperm through disruption of function of the plasma membrane, axoneme, and ATP content. © 2010 SETAC © 2011 SETAC.
Semen characteristics and their ability to predict sperm cryopreservation potential of Atlantic cod, Gadus morhua L.

There is a lack of biomarkers or indices that can be used to predict the quality of fish semen samples following the freezing and thawing cycle. In the present study, a series of semen indices were tested to assess if they could accurately forecast the cryopreservation potential of Atlantic cod (Gadus morhua) semen. Fresh and frozen-thawed sperm activity variables were compared, and relationships between frozen-thawed sperm activity and fertilization success were examined. In comparison with fresh sperm, activity variables of frozen-thawed spermatozoa were reduced. Of the 18 males examined, mean (± SEM) spermatocrit of fresh sperm was 40.72 ± 4.23%, osmolality of the seminal plasma 366.32 ± 4.95 mOsmol/kg, pH 8.32 ± 0.04, protein concentration 1.05 ± 0.08 mg/mL, anti-trypsin activity 153.83 ± 19.25 U/L, and total antioxidant capacity 0.15 ± 0.03 μmol Trolox equivalents/mL. Frozen-thawed fertilization success was highly variable among males with values ranging from 18.5 to 90.2%. Regressions yielded significant positive relationships between frozen-thawed motility, velocity, track crossing frequency, and subsequent fertilization success. Sequential multiple regressions explained up to 95% of the variation in frozen-thawed sperm activity. Spermatocrit and pH of fresh semen were negatively related, whereas osmolality and antioxidant capacity were positively related to frozen-thawed motility and velocity. Each of these indices can be measured within minutes of collecting a fresh sample of semen and are thus early indicators of the capacity of semen samples to withstand cryopreservation. These results have many benefits for conservation of wild stocks, aquaculture production, and for understanding semen biology and cryobiology of fishes.

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Authors: Butts, I. (Intern), Babiak, I. (Ekstern), Ciereszko, A. (Ekstern), Litvak, M. (Ekstern), Słowińska, M. (Ekstern), Soler, C. (Ekstern), Trippel, E. (Ekstern)
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Our objectives were to compare spermatozoa activity, morphology, and seminal plasma (SP) biochemistry between wild and cultivated Atlantic cod (Gadus morhua). Swimming velocities of wild cod spermatozoa were significantly faster than those of cultivated males. Wild males had a significantly larger spermatozoa head area, perimeter, and length, while cultivated males had more circular heads. Total monounsaturated fatty acids and the ratio of n−3/n−6 were significantly higher in sperm from wild males, while total n−6 from cultivated males was significantly higher than the wild males. Significantly higher concentrations of the fatty acids C14:0, C16:1n−7, C18:4n−3, C20:1n−11, C20:1n−9, C20:4n−3, C22:1n−11, and C22:6n−3 were observed in wild males, while significantly higher concentrations of C18:2n−6, C20:2n−6, and C22:5n−3 occurred in cultivated males. Osmolality, protein concentration, lactate dehydrogenase and superoxide dismutase activity of SP of wild males were significantly higher than the cultivated males. Antioxidant capacity of SP was significantly higher in cultivated males, while pH and anti-trypsin did not differ between fish origins. Four bands of anti-trypsin activity and nine protein bands were detected in SP. Performing a discriminant function analysis, on morphology and fatty acid data showed significant discrimination between wild and cultivated fish. Results are relevant to breeding programs and aquaculture development.
Sperm morphology, ATP content, and analysis of motility in Atlantic halibut (Hippoglossus hippoglossus)

Spermatozoon of Atlantic halibut (Hippoglossus hippoglossus (L., 1758)) parentheses around authority and year indicate that the binomen has changed from when the taxon was first identified. Names checked against the ITIS database (www.itis.gov). is uniflagellated, lacks an acrosome, and is differentiated into a head, midpiece, and flagellum. There are two to five mitochondria in the midpiece, as well as proximal and distal centrioles. The flagellum consisted of 9 + 2 microtubules surrounded by plasma membrane, which is extended at the proximal part of the flagellum owing to the presence of vacuoles. After sperm activation in seawater, sperm motility and velocity decreased from 98.4% ± 3.4% and
170.3 ± 8.9 µm·s⁻¹ at 15 s after sperm activation to 4.8% ± 4.7% and 9.2 ± 8.9 µm·s⁻¹ at 120 s after sperm activation, respectively. ATP content (nmol·L⁻¹ ATP per 108 spermatozoa) significantly decreased at 60 s after sperm activation (5.9 ± 1.5) compared with at 0 and 30 s after sperm activation (14.9 ± 1.5 and 14.5 ± 1.5, respectively). Beating waves propagated along the full length of the flagellum after sperm activation, whereas waves were restricted to the proximal section during the latter motility period. Wave amplitude significantly decreased at 45 s after sperm activation, but wavelength did not differ. The present study showed associations among sperm morphology, ATP content, flagellar wave parameters, and sperm velocity, which could be used in comparative spermatology.

**General information**

State: Published
Organisations: University of South Bohemia, Fisheries and Oceans Canada, University of Nordland, Mount Allison University
Authors: Alavi, S. M. H. (Ekstern), Butts, I. A. (Intern), Hatef, A. (Ekstern), Mommens, M. (Ekstern), Trippel, E. A. (Ekstern), Litvak, M. K. (Ekstern), Babiak, I. (Ekstern)
Pages: 219-228
Publication date: 2011
Main Research Area: Technical/natural sciences

**Publication information**

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Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.27 SJR 0.677 SNIP 0.651
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.881 SNIP 0.734 CiteScore 1.38
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.849 SNIP 0.769 CiteScore 1.48
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.879 SNIP 0.886 CiteScore 1.66
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.729 SNIP 0.813 CiteScore 1.53
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.806 SNIP 0.729 CiteScore 1.37
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.806 SNIP 0.771
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.793 SNIP 0.742
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.958 SNIP 0.834
Scopus rating (2007): SJR 0.942 SNIP 0.85
Scopus rating (2006): SJR 0.887 SNIP 0.893
Scopus rating (2005): SJR 0.836 SNIP 0.865
Scopus rating (2004): SJR 0.842 SNIP 0.89
Scopus rating (2003): SJR 0.937 SNIP 0.945
Scopus rating (2002): SJR 0.996 SNIP 0.877
Cryopreservation of Atlantic cod Gadus morhua L. spermatozoa: Effects of extender composition and freezing rate on sperm motility, velocity and morphology

Broodstock selection programs are currently underway for Atlantic cod (Gadus morhua). To complement and further these selection programs we need to develop sperm cryopreservation procedures. This will allow genomic DNA from males from selected individuals or stocks to be frozen and conserved in perpetuity. In our study we used a full factorial ANOVA design to examine the effects of diluent (Mounib's sucrose-based diluent, Hanks' Balanced Salt Solution, Mounib's sucrose-based diluent+hen's egg yolk, and Hanks' Balanced Salt Solution+hen's egg yolk), cryoprotectant (propylene glycol, dimethyl sulphoxide, and glycerol), and freezing rate (−2.5, −5.0, −7.5, and −10.0°C/min) on motility of cod frozen–thawed sperm. Sperm velocity and morphometric analyses of sperm heads and flagella were also assessed. We found that sperm motility-recovery index was strongly influenced by the presence of higher-order interactions of the factors we tested. The best cryoprotection used diluents that contained hen's egg yolk. Generally, extenders containing propylene glycol yielded higher post-thaw sperm motilities than those with dimethyl sulphoxide or glycerol. In comparison to sperm from other frozen–thawed extenders, sperm from extenders supplemented with propylene glycol had significantly higher curvilinear velocity. Cryopreservation showed no impact on sperm head morphology parameters, however, considerable damage to frozen-thawed sperm flagella was observed. We believe that our experimental/statistical approach and our results add significantly new information to the study of semen biology/cryobiology in fishes. Our findings are also highly relevant to the development of cod mariculture and for aiding in conservation efforts of this very important marine species.

General information
State: Published
Organisations: University of South Bohemia, Fisheries and Oceans Canada, University of New Brunswick
Authors: Butts, I. (Intern), Litvak, M. (Ekstern), Kaspar, V. (Ekstern), Trippel, E. (Ekstern)
Pages: 174-181
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Cryobiology
Volume: 61
Issue number: 2
ISSN (Print): 0011-2240
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.701 SNIP 0.966 CiteScore 1.95
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.685 SNIP 0.978 CiteScore 1.77
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.584 SNIP 1.084 CiteScore 1.76
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.695 SNIP 1.222 CiteScore 2.1
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.797 SNIP 1.231 CiteScore 2.28
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.863 SNIP 1.23 CiteScore 2.21
Gamete storage: Tricks of the trade

General information
State: Published
Organisations: Fisheries and Oceans Canada
Authors: Butts, I. A. (Intern), Trippel, E. A. (Ekstern), Litvak, M. K. (Ekstern)
Publication date: 2010
Main Research Area: Technical/natural sciences
Links:
https://www.was.org/wasmeetings/meetings/ShowAbstract.aspx?id=19175
Publication: Research - peer-review › Journal article – Annual report year: 2010

Quantitative semen parameters of Atlantic cod (Gadus morhua) and their physiological relationships with sperm activity and morphology

Aspects of sperm motility and metabolism are important for understanding gamete quality. The objectives of this study were to investigate changes in adenosine 5'-triphosphate (ATP), and lactate dehydrogenase (LDH) of Atlantic cod sperm over time following activation, and to determine if initial semen parameters (ATP, LDH, Na+, K+, Ca2+, total protein, osmolality, pH, and spermatoctrit) are indicative of sperm quality. Analyses of ATP and LDH were also performed on sperm cells at 0.5, 2, and 90 min post-activation, while sperm activity was recorded at 0.5 and 2 min. Initial semen parameters did not appear to be strong indicators of sperm quality but some correlations were observed between pH, LDH, K+, and protein and motility parameters in the first two min post-activation. The lack of significant correlation may be attributed to the low variability of almost all parameters observed between males in this study. There was a significant effect of post-activation time on sperm ATP and LDH with both ATP and LDH decreasing with time after activation. The decrease in ATP levels during the course of sperm motility suggests that the metabolic activity is too low to substitute the energy consumed. Continued research on sperm motility and metabolism will lead to a better understanding of fertilization and gamete quality in this species.

General information
State: Published
Organisations: Fisheries and Oceans Canada, University of New Brunswick
Authors: Butts, I. (Intern), Rideout, R. M. (Ekstern), Burt, K. (Ekstern), Samuelsen, S. (Ekstern), Lush, L. (Ekstern), Litvak, M. K. (Ekstern), Trippel, E. A. (Ekstern), Hamoutene, D. (Ekstern)
Pages: 756-762
Publication date: 2010
Reproductive biology and spermatozoa cryogenics of Atlantic cod: Applications for aquaculture production and gene banking

General information
State: Published
Organisations: University of New Brunswick
Authors: Butts, I. (Intern)
Number of pages: 170
Publication date: 2010

Seasonal variations in seminal plasma and sperm characteristics of wild-caught and cultivated Atlantic cod, Gadus morhua
The objective was to investigate changes, throughout the spawning season, in body size attributes and quantitative semen characteristics of wild-caught and cultivated Atlantic cod, Gadus morhua L. Sperm velocity increased significantly throughout the spawning season of cod from both origins. Curvilinear velocity (VCL; 30sec post-activation) increased from 78.9±6.5 to 128.2±6.5μm/sec (mean±SEM) between the beginning and end of the spawning season, respectively, for wild-caught cod, whereas for cultivated fish, it increased from 26.6±2.4 to 48.9±3.1μm/sec between January and March. Spermatocrit did not undergo a significant seasonal change in wild-caught cod but did thicken for cultivated cod (24.6±4.2% in January to 40.5±4.4% in April; P

General information
Thermal reaction norms in sperm performance of Atlantic cod (Gadus morhua)

Phenotypic plasticity occurs when a genotype produces variable phenotypes under different environments; the shapes of such responses are known as norms of reaction. The genetic scale at which reaction norms can be determined is restricted by the experimental unit that can be exposed to variable environments. This has limited their description beyond the family level in higher organisms, thus hindering our understanding of differences in plasticity at the scale of the individual. Using a three-year common-garden experiment, we quantify reaction norms in sperm performance of individual genotypes within different families of Atlantic cod (Gadus morhua). Cod sperm showed phenotypic plasticity in swimming performance across temperatures (3, 6, 11, and 21 degrees C), but the pattern of the response depended upon how long sperm had been swimming (30, 60, 120, or 180 s), i.e., plasticity in plasticity. Sperm generally swam fastest at intermediate temperatures when first assessed at 30 s after activation. However, a significant genotype x environment interaction was present, indicating inter-individual differences in phenotypic plasticity. To our knowledge, this is the first study to describe variable sperm performance across environmental conditions as a reaction norm. The results have potential theoretical, conservation, and aquaculture implications.

General information
State: Published
Organisations: University of New Brunswick
Authors: Purchase, C. F. (Ekstern), Butts, I. A. E. (Intern), Alonso-Fernandez, A. (Ekstern), Trippel, E. A. (Ekstern)
Pages: 498-510
Publication date: 2010
Main Research Area: Technical/natural sciences

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Journal: Canadian Journal of Fisheries and Aquatic Sciences
Volume: 67
Issue number: 3
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BFI (2018): BFI-level 2
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.56 SJR 1.322 SNIP 1.163
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.256 SNIP 1.051 CiteScore 2.22
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.443 SNIP 1.379 CiteScore 2.6
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.421 SNIP 1.081 CiteScore 2.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.324 SNIP 1.196 CiteScore 2.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.423 SNIP 1.09 CiteScore 2.13
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.425 SNIP 1.118
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Cryopreservation of winter flounder (*Pseudopleuronectes americanus*: CRC Marine Biology Series)

**General information**
State: Published
Organisations: University of New Brunswick
Authors: Rideout, R. M. (Ekstern), Butts, I. (Intern), Litvak, M. K. (Ekstern), Cabrita, E. (Ekstern), Robles, V. (Ekstern), Herraez, P. (Ekstern)
Publication date: 2009

**Host publication information**
Title of host publication: Methods in Reproductive Aquaculture
Publisher: CRC PRESS-TAYLOR & FRANCIS GROUP6000 BROKEN SOUND PARKWAY NW, STE 300, BOCA RATON, FL 33487-2742 USA
ISBN (Print): 9780849380532
Main Research Area: Technical/natural sciences
Source: dtu
Source-ID: n::oai:DTIC-ART:biosis/163624282::21913
Publication: Research - peer-review › Journal article – Annual report year: 2010

Male variability and sperm ATP content in Atlantic cod *Gadus morhua* L: Relationships with motility, velocity, head morphology, total protein, seminal fluid constituents, and fertilization success

**General information**
State: Published
Organisations: University of New Brunswick
Authors: Butts, I. (Intern), Rideout, R. (Ekstern), Burt, K. (Ekstern), Samuelson, S. (Ekstern), Lush, L. (Ekstern), Litvak, M. (Ekstern), Trippel, E. (Ekstern), Hamoutene, D. (Ekstern)
Publication date: 2009
Main Research Area: Technical/natural sciences
Publication: Research › Poster – Annual report year: 2009
The Atlantic Cod Genomics and Broodstock Development Project

The objective of the Atlantic Cod Genomics and Broodstock Development Project (CGP) is to develop breeding programs and fundamental genomics tools which will be used to supply the developing Atlantic cod aquaculture industry in Canada with improved broodstock (www.codgene.ca). The first three major spawning seasons have been completed, including rearing of fish in hatcheries through to transfer of juveniles to seacages. The fourth spawning season, which was the first to include elite broodstock as parent fish, is also completed. Evaluations of progeny have included assessment of family performance related to growth and overall health. In addition, investigations related to cod physiology and immunology have been conducted. Considerable variations among families have been observed. Heritability estimates suggest that the breeding programs will yield improved performance. The CGP has dramatically improved availability of genomic resources for cod. Approximately 158,000 sequences have been submitted to GenBank. A 20,000 element (20K) oligonucleotide microarray is being printed and tested. Development of gene-linked markers and a high density genetic map are ongoing. Marker identification has yielded >4,500 "predicted informative" single nucleotide polymorphisms (SNPs) and >140 microsatellite markers. In addition, social scientists have worked closely with other CGP scientists and industry partners to examine ethical, environmental, economic, legal and social issues related to the CGP. Resources developed by the CGP will enable marker assisted selection, and provide valuable tools for Atlantic cod research.

General information
State: Published
Organisations: Unknown
Pages: 71-73
Publication date: 2009
Main Research Area: Technical/natural sciences
The effect of sperm to egg ratio and gamete contact time on fertilization success in Atlantic cod Gadus morhua L.

Currently, Atlantic cod (Gadus morhua) is the primary finfish species being developed for aquaculture in North Atlantic waters. Despite the importance of this species, no research has been conducted to assess the effects of sperm density and gamete contact time on egg fertilization. In two separate experiments male and female gametes were crossed using nested factorial designs. For each male–female combination we tested sperm to egg ratios ranging from \(1\times10^3:1\) to \(5\times10^6:1\). We also tested two gamete contact times where sperm and eggs were held in contact with each other for 5 or 30 min. Mixed-model ANOVAs indicated that sperm density and gamete contact time had a significant effect on fertilization success. Below a sperm to egg ratio of \(1\times10^5:1\) fertilization success significantly decreased. Therefore, a standard sperm to egg ratio of \(1\times10^5\) sperm per egg is recommended for fertilization in Atlantic cod. At the \(1\times10^3:1\), \(5\times10^3:1\), and \(1\times10^4:1\) sperm to egg ratios maximum fertilization occurred after 30 min sperm to egg contact time. Gamete contact time was not significant at sperm to egg ratios of \(1\times10^5:1\) and \(1\times10^6:1\). Both the maternal and paternal variance components were significant for fertilization success. This information has important implications for optimizing family production in selective breeding programs, conserving sperm from superior pedigree in genome banks, maximizing the use of available gametes in hatchery or research facilities, and understanding mating success in the wild.

General information
State: Published
Organisations: Fisheries and Oceans Canada, University of New Brunswick
Authors: Butts, I. A. (Intern), Trippel, E. A. (Ekstern), Litvak, M. K. (Ekstern)
Pages: 89-94
Publication date: 2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Aquaculture
Volume: 286
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BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.294 SNIP 1.542 CiteScore 2.39
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.151 SNIP 1.394
Effects of spermatozoa density, longevity, and duration of receptivity on fertilization success of winter flounder (Pseudopleuronectes americanus)

General information
State: Published
Organisations: University of New Brunswick
Authors: Litvak, M. (Ekstern), Butts, I. A. (Intern), Roustaian, P. (Ekstern)
Publication date: 2008
Event: Abstract from 61st Canadian Conference for Fisheries Research, Halifax, Canada.
Main Research Area: Technical/natural sciences
Links: http://www.phys.ocean.dal.ca/ccffr/prog08.pdf
Publication: Research › Conference abstract for conference – Annual report year: 2008

Performance of cultivated northwest Atlantic cod (Gadus morhua) families: Initiation of a selective breeding program in eastern Canada
Declining wild populations of cod worldwide has resulted in a renewed interest in farming this renowned species, and was the impetus for the creation of the Atlantic cod genomics and broodstock development program (www.codgene.ca and www.genomecanada.ca). One of the aims of this program is to evaluate the potential for improvement of commercially important traits through selective breeding using family-based selection programs established in New Brunswick and Newfoundland. Wild broodstock were obtained from three locations in Canada (Bay of Fundy, Cape Sable Island, and Eastern Newfoundland) and one in the United States (New Hampshire coast). The broodfish were either artificially strip-spawned or pair-mated to establish full and half sibling families using the Berg and Henryon breeding design. In total, 429 crosses and 215 families have been generated at three marine fish hatcheries over the first two years of the four year project. Hormonal implants were used to facilitate timing and collection of gametes. Extended gamete contact time in seawater and optimal sperm:egg ratios were explored as methods to maximize fertilization rates. Each of these families was reared in individual tanks to PIT tagging at approximately 15 g. At hatch, individual families were reared in separate tanks and/or combined with other families and co-reared in larger tanks. Families were reared to PIT tagging at
approximately 15 g, transferred to sea cages for grow out and a pre-harvest performance evaluation made ~ 1 yr post-

stocking. Heritability estimates were high for body length and weight (h2 ~ 0.45 to 0.55) at juvenile and pre-harvest stages.

General information
State: Published
Organisations: Unknown
Authors: Trippel, E. (Ekstern), Garber, A. (Ekstern), Tosh, J. (Ekstern), Neil, S. (Ekstern), Fordham, S. (Ekstern),

Berlinsky, D. (Ekstern), Butts, I. A. (Intern), Litvak, M. (Ekstern), Powell, F. (Ekstern), Nardi, G. (Ekstern),

Puvanendran, V. (Ekstern), Lush, L. (Ekstern), Boyce, D. (Ekstern), Rise, M. (Ekstern), Symonds, J. (Ekstern),

Robinson, A. (Ekstern)
Publication date: 2008
Event: Abstract from 32nd Annual Larval Fish Conference, Kiel, Germany.
Main Research Area: Technical/natural sciences
Publication: Research › Conference abstract for conference – Annual report year: 2008

The effect of sperm to egg ratio and gamete contact time on fertilization success in Atlantic cod

General information
State: Published
Organisations: University of New Brunswick, Fisheries and Oceans Canada
Authors: Butts, I. (Intern), Trippel, E. A. (Ekstern), Litvak, M. K. (Ekstern)
Publication date: 2008
Event: Poster session presented at 61st Canadian Conference for Fisheries Research, Halifax, Canada.
Main Research Area: Technical/natural sciences
Publication: Research › Poster – Annual report year: 2008

Parental and stock effects on larval growth and survival to metamorphosis in winter flounder (Pseudopleuronectes

americanus)

Geographically separated winter flounder (Pseudopleuronectes americanus) populations in the northwest Atlantic Ocean
are both phenotypically and genetically distinct from one another. This has important implications for winter flounder
aquaculture with respect to broodstock selection; however, few studies have investigated the effect of population on larval
growth and survival in a hatchery setting. In this study, eggs from Passamaquoddy Bay females were fertilized with sperm
from Georges Bank and Passamaquoddy Bay males. Larvae were reared in common environmental conditions to
evaluate population and parental contributions to variations in growth, and survival during early life history. Mixed-model
nested ANOVAs revealed that larvae sired by Georges Bank males were significantly larger with respect to standard
length, eye diameter, head depth, and jaw length during certain stages in larval development. Maternal, paternal, and
parental interactions all contributed to morphological variation in developing larvae. Survival was strongly influenced by the
paternal variance component. These results have two major implications: 1) they provide further supporting evidence that
Georges Bank winter flounder are genetically selected for faster growth than larvae from inshore stocks, and 2) they
suggest that aquaculture operations should also account for paternal variation so that the best broodstock can be selected
for production.

General information
State: Published
Organisations: University of New Brunswick
Authors: Butts, I. A. E. (Intern), Litvak, M. K. (Ekstern)
Pages: 339-348
Publication date: 2007
Main Research Area: Technical/natural sciences
Publication information
Journal: Aquaculture
Volume: 269
Issue number: 1-4
ISSN (Print): 0044-8486
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Web of Science (2018): Indexed yes
BFI (2017); BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016); BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015); BFI-level 2
Stock and parental effects on embryonic and early larval development of winter flounder Pseudopleuronectes americanus (Walbaum)

A hierarchical breeding design was used to determine if winter flounder Pseudopleuronectes americanus embryos and yolk-sac larvae sired by Georges Bank males developed and grew larger than fish sired by Passamaquoddy Bay males, and to examine parental contributions to variations in fertilization success, time to 50% hatch, hatch success and larval morphological development. Significant stock effects were detected for time to hatch and larval development. Eggs
fertilized by Passamaquoddy Bay males reached 50% hatch significantly earlier than eggs fertilized by Georges Bank males. Larvae sired by Georges Bank males were significantly larger during larval development for four of the six traits measured at 12 days post-hatch: head depth, jaw length, myotome height and body area. Embryo and larval development were strongly influenced by maternal contributions; there were significant maternal variance components for the majority of the variables measured. Paternal variance components were significant for fertilization success, time to hatch, larval jaw length and larval head depth, however, they acted principally through parental interactions. This information has important implications for the long-term sustainable development of winter flounder for aquaculture purposes as well as for understanding winter flounder genetic variation in the wild.
The effect of sperm to egg ratio and gamete contact time on fertilization success in Atlantic cod Gadus morhua L.

General information
State: Published
Organisations: University of New Brunswick, Fisheries and Oceans Canada
Authors: Butts, I. (Intern), Trippel, E. A. (Ekstern), Litvak, M. K. (Ekstern)
Publication date: 2007
Event: Poster session presented at First International Workshop on the Biology of Fish Sperm, Vodnany, Czech Republic.
Main Research Area: Technical/natural sciences
Publication: Research › Poster – Annual report year: 2007

Maternal, paternal and stock effects on the early life history of winter flounder (Pseudopleuronectes americanus): Implications for broodstock development

General information
State: Published
Organisations: University of New Brunswick
Authors: Butts, I. (Intern)
Number of pages: 102
Publication date: 2006

Publication information
Original language: English
Publisher: University of New Brunswick
Main Research Area: Technical/natural sciences
Publication: Research › Other contribution – Annual report year: 2006

Stock and parental effects on the early life history of winter flounder (Pseudopleuronectes americanus): Geographically separated winter flounder (Pseudopleuronectes americanus) populations in the northwest Atlantic Ocean are both phenotypically and genetically distinct. A hierarchical breeding design using eggs from Passamaquoddy Bay females was used to 1) determine if fish sired by Georges Bank males grew faster than fish sired by Passamaquoddy Bay males and 2) to examine parental contributions to variations in growth and performance during early life history stages. Mixed-model nested ANOVAs revealed that larvae sired by Georges Bank males grew faster than those sired by Passamaquoddy Bay males and that maternal, paternal, and parental interactions all contributed to growth and survival during early life history. Results will be discussed with reference to winter flounder genetic variation in the wild and development of this species for aquaculture
Development of winter flounder and other flatfish for land-based and inter-tidal aquaculture

General information
State: Published
Organisations: University of New Brunswick
Authors: Butts, I. (Intern), Litvak, M. (Ekstern)
Publication date: 2006
Event: Abstract from Flatfish Biology Conference, Westbrook, Canada.
Main Research Area: Technical/natural sciences
Publication: Research › Conference abstract for conference – Annual report year: 2006

Paternal and maternal contributions to the early life history stages of winter flounder (Pseudopleuronectes americanus) from Passamaquoddy Bay and Georges Bank

General information
State: Published
Organisations: Unknown
Authors: Kirkpatrick, M. (Ekstern), Butts, I. (Intern), Hammell, L. (Ekstern), Jardine, D. (Ekstern), Lall, S. (Ekstern), Litvak, M. (Ekstern), Rideout (Ekstern), Trippel, E. (Ekstern)
Pages: 39
Publication date: 2003
Event: Poster session presented at Aquaculture Canada, Victoria, British Columbia, Canada.
Main Research Area: Technical/natural sciences
Publication: Research › Poster – Annual report year: 2003

Paternal contributions on early life history stages of winter flounder (Pseudopleuronectes americanus) from Passamaquoddy Bay and Georges Bank

General information
State: Published
Organisations: Unknown
Authors: Butts, I. A. (Intern), Litvak, M. (Ekstern)
Pages: 38
Publication date: 2003
Event: Poster session presented at Aquanet, Vancouver, Canada.
Main Research Area: Technical/natural sciences
Projects:

**Egg quality and Offspring Performance in European Eel**
National Institute of Aquatic Resources  
Period: 15/12/2015 → 14/07/2019  
Number of participants: 3  
Phd Student:  
Kottmann, Johanna Sarah (Intern)  
Supervisor:  
Butts, Ian (Intern)  
Main Supervisor:  
Tomkiewicz, Jonna (Intern)

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Grundforskningsfonden  
Project: PhD

**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)
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)

Assessing and improving the quality of aquatic animal gametes to enhance aquatic resources – The need to harmonize and standardize evolving methodologies and improve transfer from academia to industry (AQUAGAMETE) (39130)

The aim of the AQUAGAMETE COST Action is to reach a consensus on protocols and guidelines (using internationally defined terminology, units of measurement and format of reporting) that permit the use of results in relational databanks for sound and common application in aquaculture research and commerce. There is an urgent need towards a universal scale to assess both the precise state of sexual maturation (for secure broodstock use) and related life history traits (gamete quality assessment, incubation of eggs) in teleost fish and other commercially important invertebrates used in either bioassays or aquaculture.

During the past six years, three international workshops on fish gametes demonstrated a rapid development of methodologies that encompass extensive opportunities for promising use in basic reproductive biology, genetic research, biotechnology and aquaculture practice. All of these can have far-reaching consequences on conservation of endangered species, assessment of anthropogenic and climatic impacts on aquatic species and application in aquaculture, as well as in fisheries management. In particular, it has been recognized that there are many highly diverting details in the practical application of these new methods used by most scientists and laboratories, which can cause highly variable if not contradicting results, even using the same species.

COST action management and scientific activities comprise meetings, congresses and workshops, training schools and short term training mission (STSM) program. The action has funded participation of delegates Jonna Tomkiewicz and Ian A.E. Butts in action management meetings and three AQUAGAMETE conferences, participation of two students in training schools, six short term missions (STSM) of MSC and PhD students performing work at the labs of international collaborators as well as their participation in AQUAGAMETE conferences. Exchange of students and collaboration has resulted in a series of publications enhanced through collaboration as well as enriched learning by students through international networking.

Other partners than DTU Aqua (countries and number of institutes): Austria (1), Belgium (1), Bulgaria (2), Croatia (1), Czech Republic (1), Finland (3), France (3), Macedonia (1), Germany (1), Greece (4), Hungary (1), Israel (2), Italy (2), Netherlands (1), Norway (1), Poland (2), Portugal (1), Serbia (1), Slovenia (2), Spain (10), Sweden (2), Turkey (2), UK (2), International Partner Countries (IPC): Brazil, Japan (1), Singapore, South Africa (1).

AQUAGAMETE is funded by COST, EU (European Cooperation in Science and Technology).

National Institute of Aquatic Resources
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.

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National Food Institute
National Institute of Aquatic Resources
Section for Marine Ecology and Oceanography
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Leiden University
National Centre for Scientific Research "Demokritos"
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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:
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  Støttrup, Josianne Gatt (Intern)
  Sørensen, Sune Riis (Intern)
  Skov, Peter Vilhelm (Intern)
  Steenfeldt, Svend Jørgen (Intern)
  Hornum, Inger (Intern)
Project Manager, academic:
  Tomkiewicz, Jonna (Intern)
  Munk, Peter (Intern)
  Krüger-Johnsen, Maria (Intern)