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**Benini, Elisa; Politis, Sebastian Nikitas; Kottmann, Johanna Sarah; Butts, Ian A. E.; Sørensen, Sune Riis; Tomkiewicz, Jonna**

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MR. SEBASTIAN NIKITAS POLITIS (Orcid ID : 0000-0002-2022-2095)
MR. SUNE RIIS SØRENSEN (Orcid ID : 0000-0001-5981-1383)

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Authorship Statement:
Conceptualization: All authors
Data curation: Elisa Benini, Sebastian N. Politis, Ian A. E. Butts.
Formal analysis: Elisa Benini, Sebastian N. Politis, Ian A. E. Butts.
Funding acquisition: Jonna Tomkiewicz, Ian A. E. Butts.
Investigation: Elisa Benini, Sebastian N. Politis, Johanna S. Kottmann, Sune R. Sørensen.
Methodology: Sebastian N. Politis, Sune R. Sørensen, Jonna Tomkiewicz, Ian A. E. Butts.
Project administration: Jonna Tomkiewicz, Ian A. E. Butts.
Resources: Jonna Tomkiewicz, Ian A. E. Butts.
Software: Jonna Tomkiewicz, Ian A. E. Butts.
Supervision: Sebastian N. Politis, Sune R. Sørensen, Jonna Tomkiewicz, Ian A. E. Butts.
Validation: Sebastian N. Politis, Ian A. E. Butts.
Visualization: Sebastian N. Politis, Sune R. Sørensen.
Writing ± original draft: Elisa Benini, Sebastian N. Politis.
Writing ± review & editing: All authors

# Effect of parental origin on early life history traits of European eel

Elisa Benini[1#], Sebastian N. Politis[2#*], Johanna S. Kottmann[2], Ian A.E. Butts[3], Sune R. Sørensen[2,4], Jonna Tomkiewicz[2]

[1] STMI Aqua Systems, Copenhagen, Denmark

[2] National Institute of Aquatic Resources, Technical University of Denmark

[3] Auburn University, School of Fisheries, Aquaculture and Aquatic Sciences, USA

[4] Billund Aquaculture Service, Billund, Denmark

[#] Both authors equally contributed to this work

* Corresponding author: snpo@aqua.dtu.dk

**Contents**

Establishment of European eel (*Anguilla anguilla*) hatchery production will rely on selectively bred individuals that produce progeny with the best traits in successive generations. As such, this study used a quantitative genetic breeding design, between 4 females and 9 males (4 wild-caught and 5 cultured), to investigate the effect of paternal origin (wild-caught *vs*. cultured) and quantify the relative importance of parental effects, including genetic compatibility, on early life history (ELH) performance traits (i.e. fertilization success, embryonic survival at 32 hours post-fertilization, hatch success, and larval deformities at 2 days post-hatch) of European eel. Wild-caught males had higher (56%) spermatocrit values than cultured males (45%), while fertilization success, embryonic survival, hatch success, and larval deformities were not significantly impacted by paternal origin. This demonstrates that short term domestication of male eels does not negatively affect offspring quality and enables the consideration of cultured male broodstock in future breeding programs. Moreover, paternity significantly explained 9.5% of the variability in embryonic survival, providing further evidence that paternal effects need to be taken into consideration in assisted reproduction protocols. Furthermore, maternity significantly explained 54.8% of the variation for fertilization success, 61.7% for embryonic survival, 88.1% for hatching success, and 62.8% for larval deformities, validating that maternity is a major factor influencing these "critical" ELH traits. Lastly, the parental interaction explained 12.8% of the variation for fertilization success, 8.3% for embryonic survival, 4.5% for hatch success, and 20.5% for larval deformities. Thus, we conclude that eggs of one female can develop more successfully when crossed with a compatible male, highlighting the importance of mate choice for successful propagation of high-quality offspring.

Together, this knowledge will improve early offspring performance, leading to future breeding programs for this endangered and economically important eel species.

**Keywords:** *Anguilla anguilla*, fish embryo, larvae, paternity, maternity

## 1. Introduction

Self-sustainable aquaculture and conservation efforts are needed to restore the critically endangered European eel, *Anguilla anguilla* stock (Jacoby and Gollock, 2014). The life cycle of this catadromous fish species has not been closed in captivity. As such, aquaculture and restocking programs depend on wild-caught glass eels. At present, improved assisted reproductive technologies and *in-vitro* fertilization techniques are implemented to produce viable eggs and hatched larvae (Mordenti et al., 2013; Butts et al., 2014; Müller et al., 2016). Additionally, controlled laboratory studies have identified optimal environmental conditions for improved early offspring performance, i.e. light (Politis et al., 2014a), salinity (Sørensen et al., 2016a), and temperature (Politis et al., 2017; Politis et al., 2018). Together, this new innovative technology has enabled the production of copious amounts of offspring reaching the first-feeding stage (Butts et al., 2016; Sørensen et al., 2016b). On the contrary, little is known about parental factors, via genetic selection or breeding, and if or how they impact early life history (ELH) performance traits.

The main objective of genetic selection or selective breeding is to find parental combinations and/or stocks that produce progeny with the best traits in successive generations, including among others, growth, survival, reproductive success, and disease resistance (Gjedrem, 2010; Migaud et al., 2013). Generally, parental effects originating from the mother, termed maternal effects, are predominant compared to paternal effects (Chambers and Leggett, 1996) and are considered the major source of phenotypic variation within a population (Green, 2008). This variability arises from both genetic (Rozenfeld et al., 2016; Bobe and Labbé, 2010) and non-genetic constituents from the egg yolk (Kamler, 2005). These yolk reserves are developed during oogenesis and maturation of eggs (e.g., vitellogenesis), and have been shown to influence larval size at hatch (Buckley et al., 1991), larval size and age at first-feeding (Gisbert et al., 2000), as well as survival rates during the transition from the endogenous to the exogenous feeding stage (Rideout et al., 2005).

On the contrary, contributions originating from the father, are often overlooked due to the common practice of pooling milt from multiple males for seed production in aquaculture. Males contribute only genetic material, *via* the spermatozoon, to the embryo (Rideout et al., 2004). Nevertheless, paternal effects have been shown to impact offspring variability during the “critical”

ELH stages for several economically important aquaculture species (reviewed by Butts and Litvak, 2007a). Additionally, it has been shown that an egg batch from a particular female can develop more successfully when sired by a specific "compatible male", but develop less successfully when sired by an "incompatible male" (Neff and Pitcher, 2005; Politis et al., 2014b; Siddique et al., 2017). Thus, the choice of compatible parents (selective breeding) can improve offspring performance and increase aquaculture production yields (Probst et al., 2006).

Subsequently, the use of genetically improved stocks can lead to a more efficient and cost-effective hatchery production, which has important implications for current and future aquaculture development (Gjedrem, 2000). However, common domestication procedures (intentionally or not) can cause a sharp modification of the genetic heritage between cultured and wild animals (Skaala et al., 1990; Gjedrem and Kolstad, 2012). The unconscious selection following selective breeding, can drive the selection of traits away from requirements under natural conditions and thus alienates individuals from the wild population (Skaala et al., 1990; Huntingford, 2004). In this regard, differences between wild and cultured fish are well documented in several fish species (Huntingford, 2004), especially for reproductive performance (Fleming et al., 1996, Reisenbichler and McIntyre, 1977; Arechavala-Lopez et al., 2012). From an aquaculture perspective, dietary regime, photoperiod, and husbandry conditions are the major sources of variability in gamete quality (Bobe and Labbé, 2010). Egg and sperm quality have been consistently shown to be different in wild-caught and cultured stocks (Bobe and Labbé, 2010; Migaud et al., 2013), where gametes produced by wild-caught fish often show higher quality (Ochokwu et al., 2015). Thus, selective breeding programs need to be implemented in order to improve cultured fish, via enhanced offspring performance, and to alleviate pressures on wild stocks.

This study used a quantitative genetic breeding design, between 4 females and 9 males from two different origins (4 wild-caught and 5 cultured from the glass eel stage), resulting in 36 individual parentage crosses of European eel. Offspring were reared from fertilization until 2 days post hatch (DPH) to investigate the effect of paternal origin (wild-caught *vs.* cultured) and to quantify the relative importance of maternal, paternal, and parental interactive effects ("genetic compatibility") on fertilization success, embryonic survival at 32 hours post-fertilization (HPF), hatch success, and larval deformities at 2 DPH.

## 2. Material and Methods

### 2.1. Broodstock husbandry

Broodstock were caught in Lake Vandet, Denmark, during fall 2016. Mean (± SD) length and body weight of the 4 wild-caught females were 67 ± 5.8 cm and 655.5 ± 199.3 g (Table 1), respectively, while the 4 wild-caught males were 39.4 ± 2.9 cm and 93.5 ± 18.8 g, respectively (Table 2). The 5 cultured males were reared in a commercial eel farm (Stensgård Eel Farm A/S) and fed on a standard diet (DAN-EX 2848, BioMar A/S, Brande, Denmark) (Støttrup et al., 2013). Their mean (± SD) length and weight were 37.8 ± 1.3 cm and 108 ± 13.1 g, respectively (Table 2). Broodstock used in this study were randomly selected for fertilization and experimentation. Eels were transported and acclimatized for two weeks at the EEL-HATCH facility of the Technical University of Denmark. Females were kept in ~2000 L and males in ~500 L tanks, equipped with a recirculation system. Aeration and continuous water flow (~100 L $min^{-1}$) was applied. Salinity and temperature were kept constant at 36 psu and 20 ± 1 °C, respectively. While length and initial weight were recorded, the broodstock were anaesthetized (ethyl p-aminobenzoate, 20 mg $L^{-1}$; Sigma-Aldrich Chemie, Steinheim, Germany) and a passive integrated transponder was implanted in the dorsal muscle.

**2.2. Gamete maturation and milt collection**

Females were matured by weekly injections of salmon pituitary hormone (Argent Chemical Laboratories, Washington, USA) at 18.75 mg $kg^{-1}$ body weight and males by weekly injections of human chorionic gonadotropin (hCG, Sigma Aldrich Chemie, Steinheim, Germany) at 150 IU / fish (Gallego et al., 2012). An additional injection of 17α,20 -dihydroxy-4-pregnen-3-one (Sigma-Aldrich, St. Louis, MO, USA) at 2.0 mg $kg^{-1}$ body weight was given to stimulate follicular maturation and induce ovulation (Ohta et al., 1996), after which spawning occurred between 12-14 h. Biopsies were taken to categorize oocyte stage, and all females were induced to spawn in the same oocyte developmental stage (stage 5-6; see Palstra et al., 2005).

Males were given an extra injection of hCG and milt was collected after ~12 h. For every spawning female, milt was freshly collected and sperm quality evaluated. Milt was collected by applying slight pressure along the abdominal region. Within 10 s, 100 µL of milt from each male was pipetted into 10 mL of immobilizing medium (Peñaranda et al., 2009). For each male, sperm density was counted (see section 2.5 for Neubauer haemocytometer methods) and then adjusted with immobilizing medium in order to reach $25 \times 10^3$ sperm cells per egg (Butts et al., 2014), in 0.4 mL of milt-immobilizing medium, for subsequent *in vitro* fertilization trials.

The percentage of buoyant eggs was measured for all females using a volumetric column.

Here, 3.5 g of eggs were added to a 25 mL graduated cylinder and filled with 25 mL of saltwater at 18 °C and 36 psu. After 30 min, a clear distinction between floating and sinking eggs was visible and the amount of floating eggs was quantified. At 3 HPF, ~100 eggs per female were sampled and imaged using a Nikon Eclipse 55i microscope (Nikon Corporation, Tokyo, Japan). From these pictures, the diameter of 25 randomly selected eggs per female was measured.

### 2.3. Experimental procedure

For each female, 0.5 mL of eggs (3 × replicates) were separately fertilized in weight boats with 0.4 mL of pre-adjusted milt-immobilizing medium, resulting in 36 crosses [4 females × 9 males (4 wild-caught and 5 cultured) × 3 replicates]. Gametes from each replicate were then activated using 11.7 mL of activation media, which consisted of reverse osmosis water salted to 36 psu using Red Sea salt (Red Sea International, Eilat, Israel) at 20 °C. After 5 min of gamete contact time, the eggs/embryos were transferred into 200 mL beakers for incubation at 36 psu and 18 °C (Politis et al., 2017). After 4 h of incubation, embryos from each treatment were transferred into new beakers with filtered UV seawater at 36 psu, supplemented with 50 ppm ampicillin and rifampicin (Sigma Aldrich Chemie, Steinheim, Germany; Sørensen et al., 2014) and kept in a thermal incubator at 18 °C (Politis et al., 2017; Politis et al., 2018).

### 2.4. Data collection

*Sperm density*

Diluted sperm (10 µL) was counted using a Neubauer haemocytometer and observed using a compound microscope (Nikon Eclipse 55i, Nikon Corporation, Tokyo, Japan) at 400× magnification. The number of sperm was counted in five (the four corners and central square) of the 25 squares. The mean was multiplied by 25 and then by the dilution ratio to estimate sperm density. Sperm densities are expressed as the total number of sperm per mL of a male's ejaculate (Butts et al., 2014).

*Spermatocrit*

Spermatocrit was evaluated according to Sørensen et al., (2013). Here, fresh milt from each male was drawn into three microhaematocrit tubes, 75 mm long, with a 1.1–1.2 mm opening and

sealed using Vitrex™ Sigillum wax. Tubes were centrifuged (Haematokrit 210, Andreas Hettich GmbH & Co.KG, Tuttlingen Germany) for 10 min at 13 000 g. Spermatocrit was determined using a digital calliper (0.05 mm).

*Sperm motility*

Sperm motility was estimated by adding ~0.2 µL of milt to a microscope slide, situated on a microscope (Nikon Eclipse 55i, Nikon Corporation, Tokyo, Japan) at room temperature. Sperm were activated by adding 200 µL of North Sea seawater and adjusted to 36 ppt with artificial Red Sea salt. No coverslip was added for sperm activation. Motility was assessed at 400× magnification, within 10 s after activation, using an arbitrary scale where 0: represents no motile sperm; while I: 25%; II: 25–50%; III: 50–75%; IV: 75–90%; and V: 90–100% motile sperm (Pérez et al., 2009). All samples were performed in triplicate and analyzed by the same trained observer.

*Fertilization success, embryonic survival, and hatch success*

At 4 HPF, embryos were imaged using a Nikon Eclipse 55i microscope (Nikon Corporation, Tokyo, Japan) at 20× magnification and categorized as fertilized when it was possible to observe >4 cleavages (Sørensen at al., 2016b). Fertilization success was calculated as the percentage of fertilized eggs divided by the total number of eggs (Butts et al., 2014). To evaluate embryonic survival, further images were taken at 32 HPF (Fig. 1). Eggs were considered alive when the first somite segmentation was visible. Oversized, dark, discoloured eggs/embryos, or those with abnormities in the cytoplasm were considered dead (Sørensen et al., 2016a). Eggs/embryos were mixed for 3 s before sampling three aliquot samples. Hatching rate was calculated as the percentage of total number of hatched larvae divided by the sum of unhatched eggs, embryos, and larvae (Rozenfeld et al., 2016).

*Larval deformities*

At 2 DPH, all larvae were anaesthetized using MS-222 (Sigma Aldrich Chemie, Steinheim, Germany) at ~250 ppm and digitally imaged. Larvae were evaluated as deformed when head, body, yolk-sac, and/or tail region was abnormal and/or malformed compared to normal development (Sørensen et al., 2016b).

231 **2.5. Ethics**

232

233 Fish were handled in accordance with the European Union regulations concerning the
234 protection of experimental animals (Dir 86/609/EEC). Eel experimental protocols were approved by
235 the Animal Experiments Inspectorate (AEI), Danish Ministry of Food, Agriculture and Fisheries.

236

237 **2.6. Statistical Analysis**

238

239 All data were analyzed using SAS statistical software (version 9.1; SAS Institute Inc., Cary,
240 North Carolina). Residuals were tested for normality using the Shapiro Wilk test and homogeneity of
241 variances was tested using a plot of residuals versus fit values (PROC GLOT, SAS Institute 2003).
242 Data were $\log_{10}$ or arcsine square-root-transformed when data deviated from normality and/or
243 homoscedasticity (Zar, 1996). A mixed model ANOVA was applied were paternal origin (wild-
244 caught vs. cultured) was considered fixed and female, male, and male × female were considered
245 random. For all random effects, variance components (VC) were calculated. Alpha was set at 0.05.

246

247 **3. Results**

248

249 The percentage of floating eggs ranged from 60% to 99%, while egg size ranged from 1131±
250 152.4 to 1449 ± 102.9 μm (Table 1). Sperm motility for both wild-caught and cultured males ranged
251 between 50 and 75% (category III), while wild-caught males had significantly ($P < 0.05$) higher
252 spermatocrit values than the cultured males; 56 ± 2.6% vs 44.6 ± 4.3%, respectively (Table 2).

253 Mean fertilization success was 51.1 ± 5% for the cultured males and 52.3 ± 5% for the wild-
254 caught males (Fig. 2A). Paternal origin did not significantly impact fertilization success, however,
255 mean fertilization success was highly variable among the parental crosses (Fig. 3C), where it ranged
256 from 32.3 ± 0.6 (wild-caught male 4 × female 3) to 67.5 ± 3.9% (wild-caught male 4 × female 2).
257 The maternal VC was significant for fertilization success and explained the largest portion of the
258 variance (58.8%) in the model (Fig. 3A). Additionally, the maternal × paternal VC was significant
259 and explained 12.8% of the variance (Fig. 3C), while the paternal VC was non-significant (Fig. 3B).

260 At 32 HPF mean embryonic survival was 31.9 ± 7% for the cultured males and 28.8 ± 7% for
261 the wild-caught males. Embryonic survival was not impacted by paternal origin (Fig. 2B). The
262 majority of the model's variance ($P < 0.01$; VC = 61.7%), for embryonic survival, was attributed to

maternity (Fig. 3D), while the paternal (Fig. 3E), and maternal × paternal VCs (Fig. 3F) contributed 9.5% ($P < 0.05$) and 8.30% ($P < 0.01$), respectively.

Male origin did not significantly affect hatching success (Fig. 2C), but was highly variable amongst the parental crosses (Fig. 3I), where it ranged from 1.7 ± 0.03 (wild-caught male 4 × female 3) to 37.4 ± 4.07% (wild-caught male 4 × female 1). Again, the majority of the variance ($P < 0.001$; VC = 88.1%) was attributed to maternity (Fig. 3G), while the maternal × paternal VC ($P < 0.001$) explained 4.5% of the variance (Fig. 3I), and the paternal VC was non-significant.

Percentage of larval deformities did not differ significantly between the wild-caught and cultured males (Fig. 2D). Among the parental crosses, the paternal VC was non-significant (Fig. 3K), while the maternal VC significantly ($P < 0.05$) accounted for 62.8% of the variance (Fig. 3J). Moreover, the maternal × paternal VC was significant ($P < 0.001$) and accounted for 20.5% of the variance (Fig. 3L), such that deformities ranged from 20.8 ± 2.4 (cultured male 2 × female 3) to 96 ± 2.2 % (cultured male 3 × female 2).

## 4. Discussion

Only a limited number of studies have examined how stock origin (wild *vs.* cultured) impacts ELH stages in fishes (Lanes et al., 2012) and just a few have focused-on males (Butts and Litvak, 2007a), despite its potential relevance for aquaculture. In this regard, differences between wild-caught and cultured males, in terms of sperm quality and their ability to fertilize eggs, were described for species such as Atlantic cod, *Gadus morhua* (Skjæraasen et al., 2009; Butts et al., 2011), where cultured males showed lower sperm quality compared to wild ones. Furthermore, sperm quality and reproductive success can be influenced by rearing conditions such as stocking density, nutrition, photoperiod, temperature, and water quality (Asturiano et al., 2001, Skjæraasen et al., 2009; Palstra et al., 2010, Gallego et al.,2012). In our study, wild-caught males had higher spermatocrit values than cultured males, while fertilization success, embryonic survival, hatch success and larval deformities were not significantly different between the paternal origins. Therefore, short term domestication (i.e. cultured males were reared from the glass eel stage, while wild-caught males were kept at our facility for 10 weeks before spawning) appears to not hinder the reproductive performance of European eel males, as we provide evidence that cultured and wild-caught males can produce embryos and larvae of comparable quality and thus can be used in future breeding programs. This is a promising result regarding future assisted reproduction programs, as cultured males are accessible all-year-round. However, issues such as sufficient genetic variability need to be taken into consideration for future selective breeding in order to avoid long-term

297 domestication problems, such as inbreeding.

298 Moreover, we observed that for all the traits considered in this study, maternal effects were
299 greater than paternal and maternal × paternal interaction effects. As such, our results provide further
300 support that females undoubtedly play a major role during ELH stages of fish, especially considering
301 that they contribute nuclear genetic, extra-nuclear genetic and non-genetic material to offspring.
302 Among the latter, egg yolk and lipid reserves depend on environmental conditions experienced by
303 the female (Rideout et al., 2004). Differences in fertilization success, embryonic survival, hatch
304 success, and larval deformities can thus be explained by differences in nutrition and environmental
305 conditions experienced by the female parents during their life in nature (Brooks et al., 1997, Kamler
306 2005). In particular, a number of studies have focused on the correlation between the inclusion of
307 fatty acids in broodstock diets and the performance of early life stages. For instance, Bruce et al.,
308 (1999) showed that inclusion of highly unsaturated fatty acids (HUFA) in female broodstock diets
309 can improve offspring survival at early stages and hatching rate. Inclusion of polyunsaturated fatty
310 acids (PUFA) into male broodstock diet has been proven to influence reproductive success, in
311 particular fish fed with diets containing high levels of PUFA showed a longer spermiation period and
312 higher sperm density (Asturiano et al., 2000, Asturiano et al., 2001). For European eel, fertilization
313 success and larval production have been correlated to particular essential fatty acids and lipids in the
314 female (Støttrup et al., 2013) and male broodstock diet (Baeza et al., 2015). Based on our results, it
315 appears that egg buoyancy may be considered a possible method to quickly predict egg quality, as F3
316 produced the lowest amount of buoyant eggs with the lowest fertility and hatch success. Further
317 research is needed to quantify additional egg quality indicators.

318 Overall, the role of paternity has been neglected due to the common practice of pooling
319 sperm from different males during egg quality studies. Unfortunately, this practice can obscure
320 potential paternal effects on variations in early development of embryos and larvae (Green and
321 McCormick, 2005; Ottesen and Babiak, 2007). In this study, paternity significantly affected
322 embryonic survival at 32 HPF but no significant effect was observed for fertilization success, hatch
323 success or larval deformities. The significant paternal effects we witnessed during embryonic
324 development could potentially demonstrate that only after the mid-blastula stage, the zygotes started
325 to transcribe their own genes and thus expressed the genetic paternal contribution (Bobe and Labbé,
326 2010; Kekäläinen et al., 2010). For other traits, paternal effects were revealed only through the
327 interaction with a respective female. Similar results were found for Baltic cod (*Gadus morhua*
328 *callarias*) and winter flounder (*Pseudopleuronectes americanus*), where paternal effects were
329 observed through significant male × female interactions, suggesting that the relative contribution to

embryos and larvae for each male depends on the female with which the male is crossed (Butts and Litvak, 2007b; Trippel et al., 2005).

In addition, we demonstrate that the parental interaction is influencing early development of European eel. Evidence of compatible mate "choice" influencing offspring viability, has previously been reported for Artic charr, *Salvelinus alpinus* (Janhunen et al., 2010; Kekäläinen et al., 2010), Alpine whitefish, *Coregonus sp.* (Wedekind et al., 2001) Atlantic and Baltic cod (Politis et al., 2014b; Dahlke et al., 2016) as well as Ide and Northern pike (Siddique et al., 2017). In those studies, the optimal parental combination and their genetic compatibility increased fertilization and hatch success as well as decreased larval deformities. In our study, we observed that some (compatible) parental combinations produced offspring of higher quality than other (less or incompatible) parental combinations. For instance, depending on the male × female compatibility, we observed that hatching success ranged from 1.7 to 37.4%, while larval deformities ranged from 20.8 to 96.2%. Similarly, a recent parentage assessment study regarding the closely related Japanese eel (*Anguilla japonica*), showed that larvae originating from specific parental combinations had higher survival than others (Sudo et al., 2018). Thus, our findings are in accordance with previous studies and further support the "genetic (in)compatibility hypothesis" stating that some parental crosses are more genetically compatible than others, resulting in higher offspring quality (Neff and Pitcher, 2005).

In conclusion, we did not observe a major influence of paternal stock origin on development of European eel during ELH. This information is crucial for aquaculture purposes, as short-term domestication does not seem to negatively affect offspring quality and thus enables the consideration of cultured male broodstock for future breeding programs. Moreover, paternity affected embryonic survival, further supporting the increasing evidence that paternal effects need to be taken into consideration in assisted reproduction protocols. Maternity was found to be the main factor influencing offspring development, as it strongly affected all parameters considered in this study. Lastly, we demonstrate that eggs of one female can develop more successfully when crossed with a compatible male, highlighting the importance of mate choice for successful production of high-quality offspring. As such, we strongly encourage further research on mate choice and parental combinations, especially towards understanding the underlying mechanisms of genetic compatibility. Together, this knowledge will improve offspring quality and lead to future breeding programs towards a sustainable aquaculture of economically important species such as the European eel.

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## 6. Conflict of interest statement

The authors declare that no competing interests exist.

## 7. References

Arechavala-Lopez, P., Sanchez-Jerez, P., Bayle-Sempere, J.T., Sfakianakis, D.G., Somarakis, S. (2012). Morphological differences between wild and farmed Mediterranean fish. Hydrobiologia. 679, 217-231 doi: 10.1007/s10750-011-0886-y

Asturiano, J.F., Sorbera, L.A., Zanuy, S. and Carrillo, M. (2000). Effects of polyunsaturated fatty acids and gonadotropin on prostaglandin series E production in a primary testis cell culture system for the European sea bass. Journal of Fish Biology. 57(6),1563-1574 *doi* 10.1111/j.1095-8649.2000.tb02232.x

Asturiano, J.F., Sorbera, L.A., Carrillo, M., Zanuy, S., Ramos, J., Navarro, J.C. and Bromage, N.(2001). Reproductive performance in male European sea bass (*Dicentrarchus labrax*, L.) fed two PUFA-enriched experimental diets: a comparison with males fed a wet diet. Aquaculture. 194(1),173-190. doi: 10.1016/S0044-8486(00)00515-9

Baeza, R., Mazzeo, I., Vílchez, M. C., Gallego, V., Peñaranda, D. S., Pérez, L., & Asturiano, J. F. (2015). Relationship between sperm quality parameters and the fatty acid composition of the muscle, liver and testis of European eel. Comparative Biochemistry and Physiology: Part A Molecular & Integrative Physiology. 181, 79-86. doi: 10.1016/j.cbpa.2014.11.022

Bobe, J., Labbé, C. (2010). Egg and sperm quality in fish. General and Comparative Endocrinology. 165, 535-548. doi: 10.1016/j.ygcen.2009.02.011

Brooks, S., Tyler, C. R., & Sumpter, J. P. (1997). Egg quality in fish: what makes a good egg? Reviews in Fish Biology and fisheries, 7(4), 387-416.

Bruce, M., Oyen, F., Bell, G., Asturiano, J.F., Farndale, B., Carrillo, M., Zanuy, S., Ramos, J. and Bromage, N. (1999). Development of broodstock diets for the European Sea Bass (*Dicentrarchus labrax*) with special emphasis on the importance of n−3 and n− 6 highly

unsaturated fatty acid to reproductive performance. Aquaculture. 177(1-4), 85-97 doi: 10.1016/S0044-8486(99)00071-X

Buckley, L.J., Smigielski, A.S., Halavik, T.A., Caldarone, E.M., Burns, B.R., Laurence, G.C. (1991). Winter flounder *Pseudopleuronectes americanus* reproductive success. I. Among-location variability in size and survival of larvae reared in the laboratory. Marine Ecology Progress Series. 74, 117-124. doi: 10.3354/meps074117

Butts, I.A.E., Litvak, M.K. (2007a). Parental and stock effects on larval growth and survival to metamorphosis in winter flounder (*Pseudopleuronectes americanus*). Aquaculture. 269(1), 339-348. doi: 10.1016/j.aquaculture.2007.04.012

Butts, I.A.E., Litvak, M.K. (2007b). Stock and parental effects on embryonic and early larval development of winter flounder *Pseudopleuronectes americanus* (Walbaum). Journal of Fish Biology. 70, 1070-1087. *doi*: 10.1111/j.1095-8649.2007.01369.x

Butts, I.A.E., Trippel, E.A., Ciereszko, A., Soler, C., Słowińska, M., Alavi, S.M.H., Litvak, M.K., Babiak, I. (2011). Seminal plasma biochemistry and spermatozoa characteristics of Atlantic cod (*Gadus morhua* L.) of wild and cultivated origin. Comparative Biochemistry and Physiology A: Molecular and Intergrative Physiology. 159, 16-24. *doi*: 10.1016/j.bpa.2011.01.014

Butts, I.A.E., Sørensen, S.R., Politis, S.N., Pitcher, T.E., Tomkiewicz, J. (2014). Standardization of fertilization protocols for the European eel. Aquaculture. 426-427, 9-13. *doi*: 10.1016/j.aquaculture.2014.01.020

Butts, I.A.E., Sørensen, S.R., Politis, S.N., Tomkiewicz, J. (2016). First-feeding by European eel larvae: A step towards closing the life cycle in captivity. Aquaculture. 464, 451-458. *doi*: 10.1016/j.aquaculture.2016.07.028

Chambers, R.C., Leggett, W.C. (1996). Maternal influences on variation in egg sizes in temperate marine fishes. Integrative and Comparative Biology. 36, 180-196. *doi*: 10.1093/icb/36.2.180

Dahlke, F.T., Politis, S.N., Butts, I.A.E., Trippel, E.A., Peck, M.A. (2016). Fathers modify thermal reaction norms for hatching success in Atlantic cod, *Gadus morhua*.Journal of Experimental Marine Biology and Ecology. 474, 148-155 *doi*: 10.1016/j.jembe.2015.10.008

Fleming I.A., Jonsson B., Gross M.R., Lamberg A. (1996). An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). Journal of Applied Ecology. 33(4), 893-905 *doi*:10.2307/2404934

Gallego, V., Mazzeo, I., Vílchez, M.C., Peñaranda, D.S., Carneiro, P.C.F., Pérez, L., Asturiano, J.F. (2012). Study of the effects of thermal regime and alternative hormonal treatments on the

reproductive performance of European eel males (*Anguilla anguilla*) during induced sexual maturation. Aquaculture. 354, 7-16 *doi*: 10.1016/j.aquaculture.2012.04.041

Gisbert, E., Williot, P., Castello-Orvay, F. (2000). Influence of egg size on growth and survival of early stages of Siberian sturgeon (*Acipenser baeri*) under small scale hatchery conditions. Aquaculture. 183, 83-94 *doi*: 10.1016/S0044-8486(99)00287-2

Gjedrem, T. (2000). Genetic improvement of cold-water fish species. Aquaculture Research. 31, 25-33 *doi*: 10.1046/j.1365-2109.2000.00389.x

Gjedrem, T. (2010). The first family-based breeding program in aquaculture. Reviews in Aquaculture. 2, 2-15 *doi*: 10.1111/j.1753-5131.2010.01011.x

Gjedrem, T. and Kolstad, K. (2012). Development of breeding programs for aquatic species should be given high priority. World Aquaculture Society. 43, 10-13

Green, B.S. and McCormick, M.I. (2005). Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Marine Ecology Progress Series. 289, 263-272

Green, B.S. (2008). Maternal effects in fish populations. Advances in Marine Biology. 54, 1-105 *doi*: 10.1016/S0065-2881(08)00001-1

Huntingford, F.A. (2004). Implications of domestication and rearing conditions for the behavior of cultivated fishes. Journal of Fish Biology. 65, 122-142 *doi*: 10.1111/j.0022-1112.2004.00562.x

Jacoby G. and Gollock M. (2014). *Anguilla anguilla*. The IUCN Red List of Threatened Species. T60344A45833138.

Janhunen, M., Piironen, J., Peuhkuri, N. (2010). Parental effects on embryonic viability and growth in Arctic char *Salvelinus alpinus* at two incubation temperatures. Journal of Fish Biology. 76, 2558-2570 *doi*: 10.1111/j.1095-8649.2010.02648.x

Kamler, E. (2005). Parent–egg–progeny relationships in teleost fishes: an energetics perspective. Reviews in Fish Biology and Fisheries. 15, 399-421 *doi*: 10.1007/s11160-006-0002-y

Kekäläinen J., Rudolfsen G., Janhunen M., Figenschou L., Peuhkuri N., Tamper N. (2010). Genetic and potential non-genetic benefits increase offspring fitness of polyandrous females in non-resource based mating system. BMC Evolutionary Biology. 10:20Lanes C.F.C., Bizuayehu T.T., Bolla S., Martins C., Fernandes J.M.O., Bianchini A., Kiron V., Babiak I. (2012) Biochemical composition and performance of Atlantic cod (*Gadus morhua* L.) eggs and larvae obtained from farmed and wild broodstocks. Aquaculture. 324-325: 267-275 *doi*: 10.1016/j.aquaculture.2011.10.036

Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., Herráez, M.P., Carrillo, M. (2013). Gamete quality and broodstock management in temperate fish. Review of Aquaculture. 5, 194-223 *doi*: 10.1111/raq.12025

Mordenti, O., Di Biase, A., Bastone, G., Sirri, R., Zaccaroni, A., Parmeggiani, A. (2013). Controlled reproduction in the wild European eel (*Anguilla anguilla*): two populations compared. Aquaculture International. 21 (5), 1045-1063 *doi*: 10.1007/s10499-012-9611-8

Müller, A.V., McEvoy, F.J., Tomkiewicz, J., Politis, S.N., Amigo, J.M. (2016). Ultrasonographic predictors of response of European eels (*Anguilla anguilla*) to hormonal treatment for induction of ovarian development. American Journal of Veterinary Research. 77, 5 *doi*: 10.2460/ajvr.77.5.478

Neff, B.D. and Pitcher, T.E. (2005). Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology. 14(1), 19-38 *doi*: 10.1111/j.1365-294X.2004.02395.x

Ochokwu, I.J., Apollos, T.G., Oshoke, J.O. (2015). Effect of egg and sperm quality in successful fish breeding. IOSR Journal of Agriculture and Veterinary Science. 8(8), 48-57

Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., Hirose, K. (1996). Changes in fertilization and hatching rates with time after ovulation induced by 17, 20β-dihydroxy-4-pregnen-3-one in the Japanese eel, *Anguilla japonica*. Aquaculture. 139(34), 291-301 *doi*: 10.1016/0044-8486(95)01167-6

Ottesen, O.H. and Babiak, I. (2007). Parental effects on fertilization and hatching success and development of Atlantic halibut (*Hippoglossus hippoglossus* L.) embryos and larvae. Theriogenology. 68(9), 1219-1227 *doi*: 10.1016/j.theriogenology.2007.08.015

Palstra, A. P., Cohen, E. G. H., Niemantsverdriet, P. R. W., Van Ginneken, V. J. T., & Van den Thillart, G. E. E. J. M. (2005). Artificial maturation and reproduction of European silver eel: development of oocytes during final maturation. Aquaculture. 249(1-4), 533-547.

Palstra, A. P. and van den Thillart G. (2010). Swimming physiology of European silver eels (*Anguilla anguilla* L.): energetic costs and effects on sexual maturation and reproduction. Fish Physiology and Biochemistry. 36, 297-322 *doi*: 10.1007/s10695-010-9397-4

Peñaranda, D.S., Pérez, L., Gallego, V., Barrera, R., Jover, M., Asturiano, J.F. (2009). European eel sperm diluent for short-term storage. Reproductive of Domestic Animal. 45 (3), 407-415 *doi*: 10.1111/j.1439-0531.2008.01206.x

Pérez, L., Peñaranda, D., Gallego, V., Asturiano, J. (2009). Testis development, sperm quality evaluation and cryopreservation in the European eel. In van den Thillart, G., et al. (eds.), Spawning Migration of the European Eel. Springer Netherlands. 333-362 *doi*: 10.1007/978-1-4020-9095-0_14

Politis, S.N., Butts, I.A.E., Tomkiewicz, J. (2014a). Light impacts embryonic and early larval development of the European eel, *Anguilla anguilla*. Journal of Experimental Marine Biology and Ecology 461, 407–415 *doi*: 10.1016/j.jembe.2014.09.014

Politis, S.N., Dahlke, F.T., Butts, I.A., Peck, M.A., Trippel, E.A. (2014b). Temperature, paternity and asynchronous hatching influence early developmental characteristics of larval Atlantic cod, *Gadus morhua.* Journal of Experimental Marine Biology and Ecology. 459, 70-79 *doi*: 10.1016/j.jembe. 2014.05.020.

Politis, S.N., Mazurais, D., Servili, A., Zambonino-Infante, J.L., Miest, J.J., Sørensen, S.R., Tomkiewicz, J., Butts, I.A.E. (2017). Temperature effects on gene expression and morphological development of European eel, *Anguilla anguilla* larvae. PloS one. *doi*: 10.1371/journal.pone.0182726

Politis, S.N., Servili, A., Mazurais, D., Zambonino-Infante, J.-L., Miest, J.J., Tomkiewicz, J., Butts, I.A.E. (2018). Temperature induced variation in gene expression of the thyroid hormone pathway of European eel larvae. General and comparative endocrinology. 259, 54-65 *doi*: 10.1016/j.ygcen.2017.11.003

Probst, N.W., Kraus, G., Rideout, R.M., Trippel, E.A. (2006). Parental effects on early life history traits of haddock *Melanogrammus aeglefinus*. ICES Journal of Marine Science. 63(2), 224-234 *doi*: 10.1016/j.icesjms.2005.11.015

Reisenbichler, R.R., McIntyre, J.D. (1977). Genetic Differences in Growth and Survival of Juvenile Hatchery and Wild Steelhead Trout, *Salmo gairdneri*. Journal of the Fisheries Research Board of Canada. 34(1), 123-128.

Rideout, R.M., Trippel, E.A., Litvak, M.K. (2004). Paternal effects on haddock early life history traits. Journal of Fish Biology. 64, 695-701.

Rideout, R.M., Trippel, E.A., Litvak, M.K. (2005). Effects of egg size, food supply and spawning time on early life history success of haddock *Melanogrammus aeglefinus*. Marine Ecology Progress Series 285, 169-180 *doi:*10.3354/meps285169

Rozenfeld, C., Butts, I.A.E., Tomkiewicz, J., Zambonino-Infante, J.-L., Mazurais, D. (2016). Abundance of specific mRNA transcripts impacts hatching success in European eel, *Anguilla anguilla* L. Comparative of Biochemistry and Physiology: Part A: Physiology. 191, 59-65 *doi:*10.1016/j.cbpa.2015.09.011

Siddique, M.A.M., Linhart, O., Krejszeff, S., Żarski, D., Pitcher, T.E., Politis, S.N., Butts, I.A.E. (2017). Paternal identity impacts embryonic development for two species of freshwater fish. General and comparative endocrinology. 245, 30-35 *doi*: 10.1016/j.ygcen.2016.07.004

Skaala, Ø., Dahle, G., Jørstad, K.E., Nævdal, G. (1990). Interactions between natural and farmed fish populations: information from genetic markers. Journal of Fish Biology. 36(3), 449-460.

Skjæraasen, J.E., Mayer, I., Meager, J.J., Rudolfsen, G., Karlsen, O., Haugland, T., Kleven, O. (2009). Sperm characteristics and competitive ability in farmed and wild cod. Marine Ecology Progress Series. 375, 219-228 *doi*: 10.3354/meps07774

Sørensen, S.R., Gallego, V., Pérez, L., Butts, I.A.E., Tomkiewicz, J., Asturiano, J.F. (2013). Evaluation of methods to determine sperm density for the European eel, *Anguilla anguilla*. Reproduction of Domestic Animals. 48(6), 936-944 *doi*:10.1111/rda.12189

Sørensen, S.R., Skov, P.V., Lauesen, P., Tomkiewicz, J., Bossier, P., De Schryver, P., (2014). Microbial interference and potential control in culture of European eel (*Anguilla anguilla*) embryos and larvae. Aquaculture. 426-427, 1-8 *doi*:10.1016/j.aquaculture.2014.01.011

Sørensen, S.R., Butts, I.A.E., Munk, P., Tomkiewicz, J. (2016a). Effects of salinity and sea salt type on egg activation fertilization, buoyancy and early embryology of European eel, *Anguilla anguilla*. Zygote. 24, 121-138 *doi:*10.1017/S0967199414000811

Sørensen, S.R., Tomkiewicz, J., Munk, P., Butts, I.A.E., Nielsen, A., Lauesen, P., Graver, C. (2016b). Ontogeny and growth of early life stages of captive-bred European eel. Aquaculture. 456, 50-61

Støttrup, J.G., Jacobsen, C., Tomkiewicz, J., Jarlbaek, H. (2013). Modification of essential fatty acid composition in broodstock of cultured European eel *Anguilla anguilla* L. Aquaculture Nutrition. 19 (2), 172-185 *doi*: 10.1111/j.1365-2095.2012.00967.x

Sudo, R., Miyao, M., Uchino, T., Yamada, Y., Tsukamoto, K., Sakamoto, T. (2018). Parentage assignment of a hormonally induced mass spawning in Japanese eel (*Anguillla japonica*). Aquaculture. 484, 317-321 *doi:* 10.1016/j.aquaculture.2017.09.014

Trippel, E.A., Kraus, G., Köster, F.W, (2005). Maternal and paternal influences on early life history traits and processes of Baltic cod *Gadus morhua*. Marine Ecology Progress Series. 303, 259-267 *doi*: 10.3354/meps30325

Wedekind, C., Müller, R., Spicher, H. (2001). Potential genetic benefits of mate selection in whitefish. Journal of Evolutionary Biology. 14, 980-986 *doi*: 10.1046/j.1420-9101.2001.00349.x

Zar, J.H. (1996). Biostatistical Analysis. third ed. Prentice-Hall: New Jersey.

**Figure legends**

Table 1: Total length, weight, egg buoyancy, and egg size measures obtained from four European eel, *Anguilla anguilla*, female broodstock. Egg buoyancy is expressed as a percentage of floating eggs, while egg size values represent mean ± SD.

| Female | Date spawned (2017) | Length (cm) | Weight (kg) | Egg buoyancy (%) | Egg diameter (μm) |
|---|---|---|---|---|---|
| F1 | 18 February | 63 | 0.557 | 99 | 1131.8 ± 152.4 |
| F2 | 18 February | 64 | 0.542 | 98 | 1392.8 ± 139.2 |
| F3 | 04 March | 64 | 0.523 | 60 | 1276.5 ± 175.4 |
| F4 | 24 March | 77 | 1.000 | 91 | 1449.0 ± 102.9 |

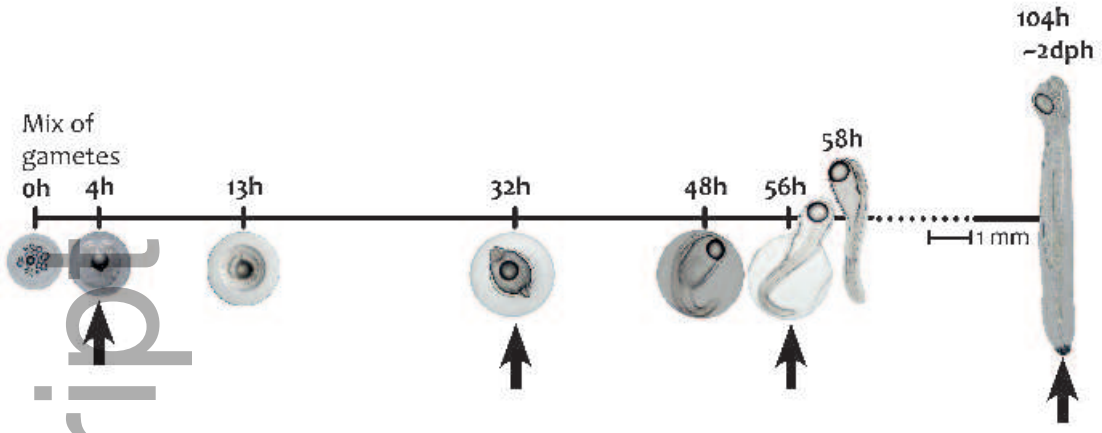
Table 2: Total length, weight, sperm density, and spermatocrit of the 4 wild-caught and 5 cultured European eel, *Anguilla anguilla,* male broodstock. Sperm density and spermatocrit values are expressed as mean ± SD.

| Paternal origin | Length (cm) | Weight (kg) | Sperm density | Spermatocrit (%) |
|---|---|---|---|---|
| Wild-caught 1 | 38 | 0.077 | 157.1 ± 19.4 | 63.3 ± 6.7 |
| Wild-caught 2 | 41 | 0.108 | 130.7 ± 7.2 | 60.2 ± 1.5 |
| Wild-caught 3 | 35.5 | 0.073 | 131.4 ± 13 | 55.1 ± 8.3 |
| Wild-caught 4 | 38 | 0.098 | 125.3 ± 24.2 | 45.3 ± 1.4 |
| Cultured 1 | 39 | 0.125 | 135.7 ± 17.6 | 45.9 ± 6.3 |
| Cultured 2 | 37 | 0.099 | 110.9 ± 5.1 | 45.3 ± 1.9 |
| Cultured 3 | 39 | 0.116 | 62.7 ± 5.3 | 17.6 ± 1.7 |
| Cultured 4 | 36 | 0.092 | 154.9 ± 16.7 | 66.6 ± 2.6 |
| Cultured 5 | 37 | 0.099 | 168.2 ± 17.2 | 47.5 ± 4.8 |

Fig. 1: Embryonic development of European eel (*Anguilla anguilla*) from 0 hours post fertilization (HPF) until 2 days post hatch (DPH). The arrows indicate targeted developmental stages. Scale bar represents 1 mm.

Fig. 2: The effect of paternal origin (cultured or wild-caught) on European eel (*Anguilla anguilla*) fertilization success (A), embryonic survival at 32 hours post fertilization (B), hatch success (C), and larval deformities at 2 days post hatch (D) Values represent means ± SEM. Values with the same upper-case letter are not significantly different ($P > 0.05$).

Fig. 3: European eel (*Anguilla anguilla*) fertilization success, embryonic survival at 32 hours post fertilization (HPF), hatch success, and larval deformities, regarding the maternal (A, D, G, J), paternal (B, E, H, K), and the paternal × maternal interaction (C, F, I, L). Black bars represent cultured (C1-5) and white bars represent wild-caught (W1-4) males. For each section, a table is showing variance components and the associated p-values for each factor.

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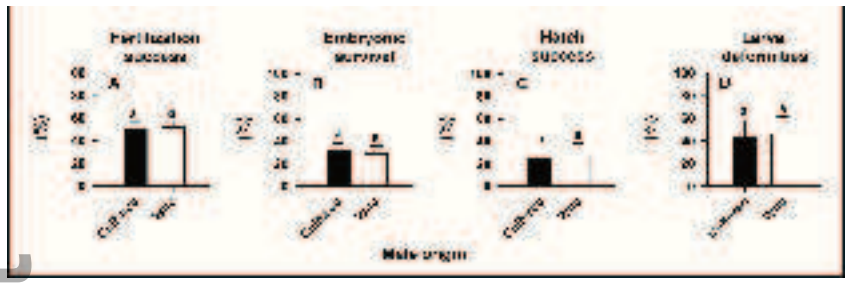

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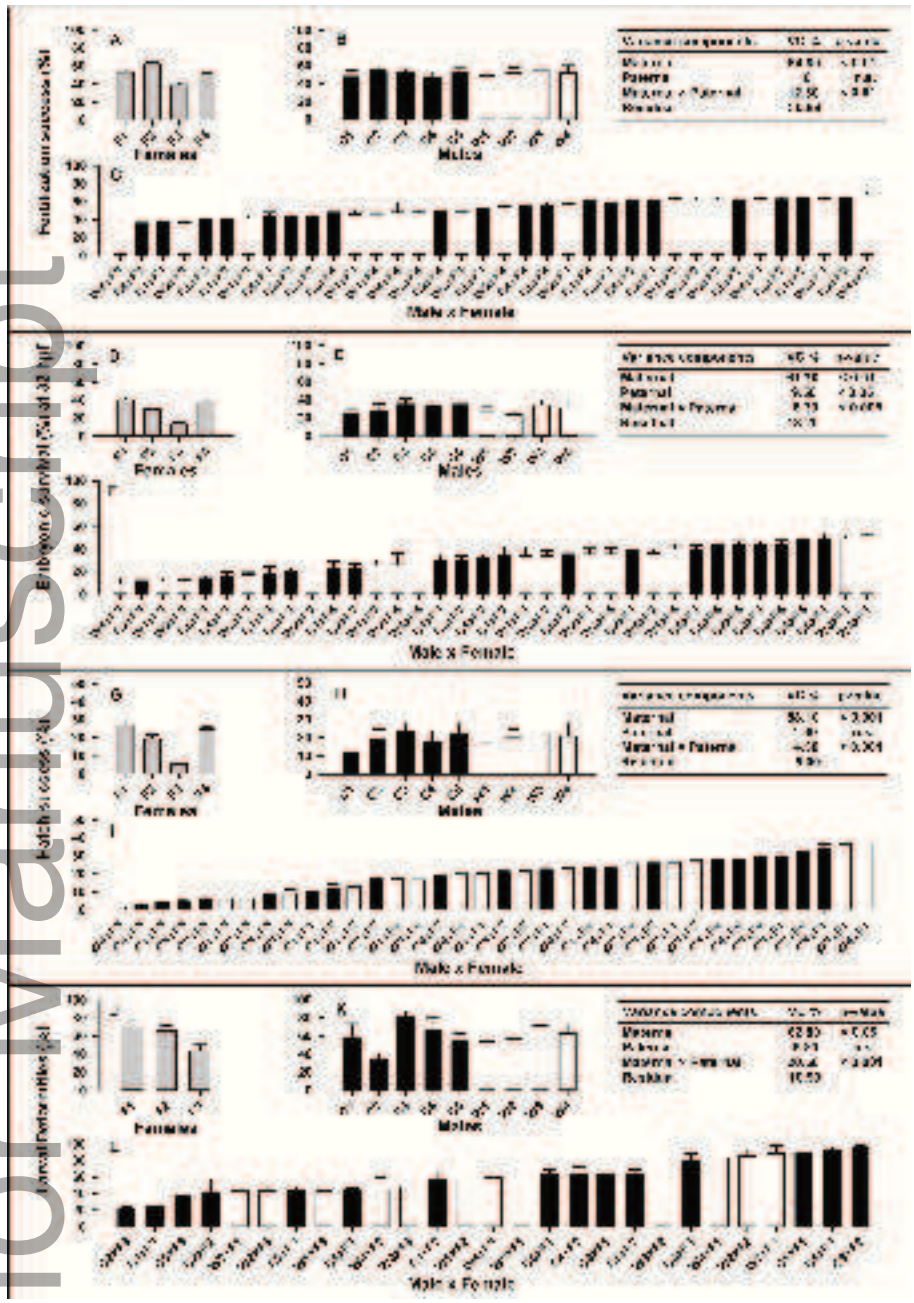

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