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Performance thresholds of hatchery produced European eel larvae reared at different salinity regimes

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Abstract

The future of European eel aquaculture depends on closing the life cycle in captivity. Present focus is on developing suitable larval rearing technology. This study explored new salinity reduction applications to elucidate performance thresholds of European eel larvae produced under realistic hatchery conditions, using Kreisel tanks and recirculating aquaculture systems for larval culture. The study links eel larval survival and biometrics to expression of genes related to underlying molecular mechanisms by taking parental effects into account. Larvae from different families were reared either at constant salinity of 36 psu (Control) or subjected to salinity reduction (36 to 18 psu) initiated 3 days post hatch (dph) and at a rate of 4 psu/day, occurring either within 1 h (Fast) or 24 h (Slow). An extreme scenario, reducing salinity directly from 36 to 18 psu within 1 h on 6 dph (Drastic) was also tested. Early and gradual salinity reduction (Slow or Fast) led to increased growth rate and larger larvae, while influencing the expression of *dio3* (deiodination mechanism and thyroid endocrine system). Expression of *atp6* and *cox1* (energy metabolism) was constant, indicating that energy metabolism was stable and independent of salinity, while expression of *nkcc1a* (ion regulation) was upregulated in the Control, suggesting an upregulation of active Na^+, K^+, and Cl^- transport and thus increased cellular energy consumption. This explained that eel larvae experiencing an early and progressive salinity reduction, used their energy reserves more efficiently, leading to improved growth and survival. However, salinity reduction caused heart edema. Expression patterns of 12 genes [stress/repair (*hsp90*), immune response (*mhc2*), neurogenesis (*neurod4*), deiodination (*dio2*), thyroid metabolism (*thαa*, *thαb*, *thβb*), energy metabolism (*atp6*), skeletogenesis (*bmp2b*, *bmp5*), growth (*igf2b*), ion regulation (*nkcc2b*)] on 6 dph and 5 genes [water transport (*aqp3*), immune response

(*il1β*), thyroid metabolism (*thβb*), skeletogenesis (*bmp5*), heart development (*nppb*)] on 12 dph were driven by genotype (family) × environment (salinity) interactions, revealing batch specific phenotypic plasticity and describing a genetic programming of molecular mechanisms and intrinsic sensitivity to environmental drivers that need to be considered in future eel aquaculture. In conclusion, early and progressive salinity reduction (Fast or Slow) benefits larval eel growth and survival, but emerging implications regarding heart edema need to be addressed in future studies. On the other hand, we show that biotechnical difficulties for introducing salinity reductions, can be circumvented by directly moving larvae from seawater to iso-osmotic conditions, but suited application timing needs to be explored.

Key words: *Anguilla anguilla*; fish; aquaculture, environmental biology; molecular ontogeny; gene expression

Introduction

The European eel, *Anguilla anguilla* is a critically endangered species (Pike et al., 2020) and it's future in aquaculture relies on closing the life cycle in captivity. In this regard, increased scientific inquiry has steadily advanced the development of assisted reproduction technology for a stable production of viable gametes and culture of offspring (Mordenti et al., 2019; Tomkiewicz et al., 2019). However, as natural conditions cannot be accurately consulted, lack of information regarding preferred environmental conditions during the earliest life stages (i.e. egg to first-feeding), challenge the development of hatchery techniques and technologies. Consequently, targeted experimental eel research efforts aim at identifying optimal offspring rearing conditions, such as temperature (Okamura et al., 2007; Ahn et al., 2012; Politis et al., 2017), light (Politis et al., 2014 a, Butts et al., 2016), and salinity (Okamura et al., 2009; Ahn et al., 2015; Sørensen et al., 2016, Politis et al., 2018a), as well as exploring first-feeding options (Kagawa et al., 2005; Okamura et al., 2013; Butts et al., 2016; Politis et al., 2018b). Together, this has provided substantiated insights into larval ontogeny and led to significant progress towards closing the life cycle of anguillid eels.

Regarding salinity, as eel offspring naturally occur in the ocean (Castonguay and McCleave, 1987), they should be biologically equipped to develop in hyper-osmotic seawater environments. Interestingly though, it has been suggested to reduce salinity towards iso-osmotic conditions during early life history in the hatchery for Japanese eel, *A. japonica* (Okamura et al., 2009). A similar rearing strategy is suggested in other fish species such as the gilthead seabream (*Sparus aurata*) resulting in better growth and survival (Tandler et al., 1995). This benefit of salinity reduction during larviculture has so far been attributed to an energy surplus associated to lower

resource allocation for osmoregulation, which can then be utilized more efficiently to ease development and survival (Okamura et al., 2016). On this basis, application techniques and timing of such a salinity reduction are targeted for efficient European eel larval culture. A first study on European eel offspring, targeted this issue (Politis et al., 2018) exploring species specific sensitivity to this extrinsic factor. Here, larvae were reared under controlled conditions, in experimental beakers and with the addition of antibiotics in order to minimize noise of other parameters. Applying the suggested salinity regime under hatchery conditions however, revealed technical challenges.

The present study thus explored different salinity reduction scenarios and performance thresholds in captive-produced European eel larvae reared under realistic hatchery settings. Yolk sac larvae from different families were reared in four different salinity regimes, using Kreisel tanks connected to independent recirculating aquaculture systems (RAS). By taking parentage into account, effects of salinity treatments on larval survival and biometrics were investigated and linked to expression patterns of selected genes driving underlying mechanisms and molecular processes related to salinity and early ontogeny. In one of the treatments (Control), salinity was kept constant at 36 psu, which closely resembles the salinity conditions encountered in the assumed spawning area in the Sargasso Sea. In another treatment (Fast), starting on day 3, salinity was successively reduced to 18 psu, in steps of 4 psu per day (within 1 h). This treatment applies one of the previously suggested reduction regimes for European eel larvae (Politis et al., 2018a). In the next treatment (Slow), starting on day 3, salinity was reduced to 18 psu, in successive steps of 4 psu per day (within 24 h), representing a more gradual and gentle salinity reduction. In the last treatment (Drastic), the salinity reduction from 36 to 18 psu was applied on

6 dph, with the change occurring within 1 h, requiring only two RAS at the desired salinity levels.

Material and Methods

Ethics statement

All fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 86/609/EEC). Experimental protocols were approved by the Animal Experiments Inspectorate (AEI), Danish Ministry of Food, Agriculture and Fisheries (permit number: 2015-15-0201-00696). Briefly, adult eels were anesthetized using ethyl p-aminobenzoate (benzocaine) before tagging and for females, before ovarian biopsies and stripping. Yolk-sac larvae were anesthetized prior to imaging and euthanized prior to sampling using tricaine methanesulfonate (MS-222).

Broodstock management

Female broodstock were wild-caught from Saltbæk Vig, Denmark (55°44'51.1"N 11°08'28.3"E), while males were raised from wild-caught glass-eels at a commercial eel farm (Royal Danish Fish, Hanstholm, Denmark). After collection, broodstock were transferred to an experimental facility of the Technical University of Denmark, where they were maintained in ~1250 L polyethylene tanks integrated into a closed recirculating system, under a continuous flow rate of ~10-15 L min^{-1} per tank, low intensity light (~20 lux), and 12 h light/12 h dark

photoperiod. Acclimatization took place over two weeks, in order to reach a salinity of ~36 psu and a temperature of ~20°C. As eels naturally undergo a fasting period from the onset of the pre-pubertal silvering stage, they were not fed during this period. Prior to experimentation, the broodstock were anaesthetized (ethyl p-aminobenzoate, 20 mg L^{-1}; Sigma-Aldrich Chemie, Steinheim, Germany), tagged with a passive integrated transponder, and length and weight recorded.

Gamete production and embryonic incubation

To induce vitellogenesis, female eels received weekly injections of salmon pituitary extract (Argent Chemical Laboratories, Washington, USA) at 20 mg kg^{-1} (initial) body weight. To stimulate follicular maturation and induce ovulation, female eels received an additional injection of 17α,20ß-dihydroxy-4-pregnen-3-one (Sigma-Aldrich, St. Louis, MO, USA) at 2.0 mg kg^{-1} body weight (Ohta et al., 1996; Kottmann et al., 2020). Then, within 12–15 h, females were strip-spawned. Males received weekly injections of human chorionic gonadotropin (hCG, Sigma Aldrich Chemie, Steinheim, Germany) at 150 IU/fish. Prior to fertilization, they were given an additional injection and milt was collected ~12 h thereafter. Milt samples were pipetted into an immobilizing medium (Peñaranda et al., 2010), while spermatocrit was assessed according to Sørensen et al. (2013).

Eggs from each female were fertilized with a pool of milt from 3-5 males to create different crosses (n = 3 families). Eggs from each female were stripped into dry plastic containers. Then, milt was added in a standardized sperm/egg ratio and gametes were swirled together, while all gametes were used for fertilization within 4 h of collection (Butts et al., 2014; Benini et al.,

2018). Artificial seawater (20°C), prepared using reverse osmosis filtration (Vertex Puratek 100 gpd RO/DI, Vertex Technologies Inc., CA, USA) and salted to 36 psu (Aquaforest Reef Salt, Brzesko, Poland), was added for a gamete contact time of 5 min (Butts et al., 2014; Sørensen et al., 2016a, 2016b). Eggs/embryos were then moved into 15 L containers filled with the above described artificial seawater to separate the buoyant viable from the sinking unviable eggs. After 2h, the floating layer was transferred to 60 L black conical incubators, supplied with conditioned filtered seawater (Politis et al., 2018b) at a flow through rate of ~350 mL min^{-1}. Gentle aeration was added after ~10 hpf, while temperature was lowered to 18 ± 0.5°C for better embryonic development (Politis et al., 2017). Light was kept at a low intensity of ~10 lux (Politis et al., 2014 a) and sinking dead eggs were purged from the bottom valve of each incubator. At ~48 hpf, aeration was stopped, and larvae hatched at ~56 hpf.

Experimental design and conditions

The experiment was repeated three times, each time using a different family cross (in total: *3 family crosses × 4 salinity treatments × 3 replicates = 36 experimental units*). After hatch, larvae (~1000 individuals per replicate) were randomly distributed into replicated 8 L acrylic Kreisel tanks (n = 3) for each family and each treatment. Eel larvae were reared throughout the endogenous feeding stage (from 0 to 12 dph). The Kreisel tanks of each treatment were connected to a separate RAS unit (Fig 1), where flow rates of conditioned filtered seawater were kept at ~500 mL min^{-1}. Each RAS unit had a sump reservoir of ~1 m^3, from where water entered a 1 m^3 biofilter (RK elements, 750 m^2 per 1 m^3, RK BioElements, Skive, Denmark), followed by a similar sized trickle filter (BioBlock 200, 200 m^2 per 1 m^3, Expo-Net Denmark,

Hjørring, Denmark), and then re-entered the bottom reservoir. Here, a protein skimmer (Turboflotor 5000 single 6.0, Aqua Medic GmbH, Bissendorf, Germany) was included for removal of waste protein. A UV lamp was also included to treat the newly filtered water (11W, JBL ProCristal, Neuhofen, Germany).

Larvae were subjected to four different salinity treatments (Fig 2). In the Control treatment, salinity was kept constant at 36 psu. In the Fast treatment, starting on day 3, salinity was reduced in steps of 4 psu per day, by replacing 100 L of seawater with reverse osmosis water (JG Wasseraufbereitung 600, 2500L/d, WaterQuality® and RoHS certified, Germany) within 1 h. In the Slow treatment, starting on day 3, salinity was reduced in steps of 4 psu per day, by drip-wise replacing 100 L of seawater with reverse osmosis water within 24 h. Lastly, in the Drastic treatment, larvae were originally connected to the Control RAS at 36 psu and moved on day 6 to the 50% salinity reduced RAS of the Fast treatment. Throughout the experiment, temperature was maintained at 18 ± 0.5°C and pH at 8.1 ± 0.5.

Sampling and data collection

Survival

Larval survival was monitored daily through assessment of mortality, i.e. counting and removing dead larvae from all experimental units. Additionally, all larvae at the end of the experiment as well as those sampled from each experimental unit were enumerated and recorded. Larval survival was then calculated as a percentage from hatch until 12 dph.

Biometrics

In order to follow larval development (biometry) from hatch until the feeding stage, larvae were sampled at 0, 6, and 12 dph. From each replicate (n = 3), family (n = 3), and treatment (n = 4) ~10 larvae were randomly sampled and imaged using a digital camera (Digital Sight DS-Fi2, Nikon Corporation, Japan) attached to a zoom stereomicroscope (SMZ1270i, Nikon Corporation, Japan). All larvae were anesthetized using tricaine methanesulfonate (MS-222; Sigma-Aldrich, Missouri, USA) prior to digital imaging. Subsequently, NIS-Elements-D analysis software (Version 3.2) was used to analyze the larval images (Nikon Corporation, Japan), where total body area (BA) and oil-drop area (ODA) were measured for each larva. Larval growth [$(BA_{(day12)} - BA_{(day0)}) / Age_{(12\ days)}$] and oil-drop utilization [$(ODA_{(day0)} - ODA_{(day12)}) / Age_{(12\ days)}$] rate were measured from the change in body and oil-drop area, respectively. Growth efficiency was then measured by dividing the increase in body area by the corresponding decrease in oil-drop area (Politis et al., 2014 b). Moreover, the severity of pericardial edema was calculated using the ratio between edema/neurocranium heights (Okamura et al., 2009).

Gene expression

To decipher effects of salinity regimes on key molecular processes and their timing during early eel ontogeny, the expression patterns of genes associated to water transport (*aqp1dup*, *aqp3*, *aqpe*), ion regulation (*nkcc1a*, *nkcc2a*, *nkcc2b*), thyroid metabolism (*thαa*, *thαb*, *thβb*, *dio1*, *dio2*, *dio3*), neurogenesis (*neurod4*, *ngn1*), growth (*gh*, *igf1*, *igf2b*), skeletogenesis (*admp*, *bmp2b*, *bmp5*), heart development (*nppa*, *nppb*, *npr1*, *npr2*), energy metabolism (*atp6*, *cox1*),

stress (*crfr1*, *crfr2*), stress/repair (*hsp70*, *hsp90*), and immune system ontogeny or immune response (*mhc2*, *il1β*, *tlr2*) were investigated (Table 1).

For this, ~30 larvae from each replicate, family cross, and treatment were randomly sampled at hatch and throughout the endogenous feeding stage (0, 6, and 12 dph). These larvae were recorded, euthanized using MS-222, preserved in RNAlater Stabilization Reagent, and kept at -20°C following the procedures suggested by the supplier (Qiagen, Hilden, Germany). In the laboratory, RNA was extracted using the NucleoSpin RNA Kit (Macherey-Nagel, Germany) following the manufacturer's instructions. RNA concentration and purity were determined by spectrophotometry using Nanodrop ND-1000 (Peqlab, Germany) and then transcribed using the qScriptTM cDNA Synthesis Kit (Quantabio, Germany) according to the manufacturer's instructions, including an additional gDNA wipe out step [PerfeCta DNase I Kit (Quantabio, Germany)]. The expression levels of target genes were determined by quantitative real-time PCR (RT-qPCR), using specific primers (Table 1). Primers were designed using Primer3 software v 0.4.0 (http://frodo.wi.mit.edu/primer3/) based on cDNA sequences available in GenBank Nucleotide, the European eel transcriptome database (EeelBase 2.0, http://compgen.bio.unipd.it/eelbase/) or the available European eel genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_013347855.1). All primers were designed for an amplification size ranging from 75 to 200 nucleotides and optimal Tm of 60°C. Expression of genes in each larval sample from 2 randomly selected replicates, from each family cross (n = 3), treatment, and larval age (0, 6, and 12 dph) were analysed in two technical replicates using the qPCR BiomarkTM HD system (Fluidigm) based on 96.96 dynamic arrays (GE chips). A pre-amplification step was performed with a 500 nM primer pool of all primers in TaqMan-PreAmp Master Mix (Applied Biosystems) and 1.3 µL cDNA per sample for 10 min at 95°C and then 14

cycles of 15 s at 95°C and 4 min at 60°C. Obtained PCR products were diluted 1:10 with low EDTA-TE buffer. The pre-amplified product was loaded onto the chip with SSoFast EvaGreen Supermix low Rox (Bio Rad) and DNA-Binding Dye Sample Loading Reagent (Fluidigm). Primers were loaded onto the chip at a concentration of 50 μM. The chip was run according to the Fluidigm 96.96 PCR protocol with a Tm of 60°C. The relative quantity of target gene transcripts was normalized (ΔCT) to the geometric mean of the 2 most stable (reference) genes. The *ef1a* and *npr3* genes were chosen as housekeeping genes, after qBase+ software revealed that these mRNA levels were stable throughout analyzed samples (M < 0.4); M gives the gene stability and M < 0.5 is typical for stably expressed reference genes (Hellemans et al., 2007). Coefficient of variation (CV) of technical replicates was calculated and checked. Further analysis of gene expression was carried out according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analysis

All data were analyzed using SAS statistical analysis software (v.9.1; SAS Institute Inc., Cary, NC, USA). Residuals were evaluated for normality (Shapiro–Wilk test) and homoscedasticity (plot of residuals vs. predicted valves) to ensure they met model assumptions. Data were log(10) or arcsine square root (percentage data) transformed to meet these assumptions when necessary. Alpha was set at 0.05 for testing main effects and interactions. Treatment means were contrasted using Tukey's Honest Significant Difference test. Body area, oil droplet area, growth rate, oil droplet utilization, growth efficiency, edema severity, and survival at 12 dph as well as gene expression (33 genes) at each age (6 and 12 dph) were analyzed using a series of mixed model factorial ANOVAs (PROC GLM). The main model

variables were salinity treatment (fixed effect), family (random effect), and the family × salinity treatment interaction (random effect), hereafter, referred to as genotype (family) × environment (salinity) interaction. Variance components (VC) for random effects were generated in PROC VARCOMP using the Restricted Maximum Likelihood (REML) estimation method and expressed as a percentage. The mean and standard error for each salinity treatment and family effect were calculated using PROC MEANS. Additionally, a series of mixed effects models were run to investigate gene expression changes over time and throughout early larval ontogeny for each salinity regime (Control, Fast, Slow, Drastic). These ANOVA models included the larval age (0, 6, 12 dph) fixed effect, the random family effect, and the random age × family interaction.

Results

Survival

Eel larval survival on 12 dph was significantly ($p<0.01$) higher in the Slow (55 ± 8%) and Fast (51 ± 7%) salinity reductions, compared to the Control (27 ± 5%) and Drastic (24 ± 3%) treatments (Fig 3). A significant ($p<0.01$) effect of family cross was observed, explaining 59.6% of the total variance. No statistically significant treatment × family interaction was observed.

Biometrics

Larval body area on 12 dph was significantly ($p<0.001$) larger when salinity was decreased Slow (4.12 ± 0.1 mm²) and Fast (4.06 ± 0.1 mm²) compared to the Drastic (3.67 ± 0.1 mm²) and Control (3.54 ± 0.1 mm²) treatments (Fig 4A). At the same stage, significantly ($p<0.05$) more energy reserves (in terms of oil droplet area; Fig 4B) were retained by larvae subjected to the Slow reduction (0.015 ± 0.001 mm²) compared to full-strength seawater (Control; 0.012 ± 0.001 mm²). Eel larvae grew significantly ($p<0.001$) more when salinity was decreased Slow (0.185 ± 0.007 mm²/d) and Fast (0.181 ± 0.006 mm²/d), compared to the Drastic (0.148 ± 0.007 mm²/d) and Control (0.137 ± 0.010 mm²) treatments (Fig 4C). At the same time, no statistically significant difference in energy (oil droplet) reserve utilization (0.007 ± 0.0001 mm²/d) was observed among treatments (Fig 4D), which resulted in significantly ($p<0.001$) higher growth efficiency in eel larvae experiencing the Slow and Fast reduction, compared to the Drastic and Control treatments (Fig 4E). In contrast, the heart edema severity ratio (Fig 4F) was significantly ($p<0.01$) higher for larvae reared in all salinity reduced treatments (Slow, Fast and Drastic) compared to full-strength seawater (Control). Furthermore, the VCs for family were significant for all biometrical parameters, explaining 67.3% ($p<0.0001$), 21.1% ($p<0.05$), 57.5% ($p<0.001$), 77.6% ($p<0.0001$), 66.5% ($p<0.001$), and 69.1% ($p<0.001$) of the total variance observed in body area, oil droplet area, growth, oil droplet utilization, growth efficiency and heart edema severity ratio, respectively. No statistically significant treatment × family interactions were observed.

Gene expression

At mouth opening (6 dph), larvae reared in full strength seawater (Control) showed significantly ($p<0.05$) upregulated expression of *dio3* (deiodination), while at the first-feeding

stage (12 dph), they showed significantly ($p<0.05$) upregulated expression of *nkcc1a* (ion transport) compared to the salinity reduced treatments (Table 3). Moreover, the VC analysis revealed differing reaction norms relative to the environmental variable (salinity) investigated. As presented in Table 3, on 6 dph (mouth opening), the expression patterns of 13 genes, associated to immune response (*il1β*, *mhc2*, *tlr2*), deiodination (*dio1*, *dio2*, *dio3*), skeletogenesis (*admp*), stress (*crfr1*, *crfr2*), growth (*gh*, *igf1*), ion regulation (*nkcc2a*), and heart development (*npr1*) significantly ($p<0.05$) differed among families. At the same stage, 12 genes associated to stress/repair (*hsp90*), immune response (*mhc2*), neurogenesis (*neurod4*), deiodination (*dio2*), thyroid metabolism (*thαa*, *thαb*, *thβb*), energy metabolism (*atp6*), skeletogenesis (*bmp2b*, *bmp5*), growth (*igf2b*), and ion regulation (*nkcc2b*) were significantly ($p<0.05$) influenced by the family × treatment interaction. Similarly, on 12 dph (first feeding), the expression patterns of 14 genes associated to water transport (*aqp3*, *aqp*, *aqp1dup*), immune response (*il1β*, *mhc2*, *tlr2*), neurogenesis (*neurod4*), deiodination (*dio1*, *dio2*, *dio3*), ion regulation (*nkcc1a*, *nkcc2a*, *nkcc2b*), and heart development (*nppa*) significantly ($p<0.05$) differed among families. At the same stage, 5 genes associated to water transport (*aqp3*), immune response (*il1β*), thyroid metabolism (*thβb*), skeletogenesis (*bmp5*), and heart development (*nppb*) were significantly ($p<0.05$) influenced by the family × treatment interaction.

In all treatments, 2 of 33 genes relating to water transport (*aqp1dup*) and skeletogenesis (*admp*) showed highest expression at larval hatch (0 dph) and significantly ($p<0.01$) decreased with increasing age (Fig 5). Moreover, a core group of genes, showing the same expression pattern, peaking on 6 dph (mouth opening) in all treatments, were linked to the molecular mechanisms of neurogenesis (*neurod4*, *ngn1*) and heart development (*nppb*, *nppa*). The other genes, significantly ($p<0.05$) but irregularly peaking among treatments on 6 dph, were relating to

skeletogenesis (*bmp2b*), thyroid metabolism (*thαb*, *thβb*), heart development (*npr2*), and immune system ontogeny or immune response (*il1β*, *tlr2*). Furthermore, the majority of genes, relating to almost all targeted molecular mechanisms (except neurogenesis and skeletogenesis), peaked on 12 dph. Here, 16 genes in the Control, 13 in the Slow, 17 in the Fast, and 14 in the Drastic treatments significantly ($p<0.05$) peaked at the first-feeding stage. On the other hand, the expression patterns of 8 genes in the Control, 12 genes in the Slow, 8 genes in the Fast, and 10 genes in the Drastic treatments were not significantly affected by larval age (Table 2). This revealed another core group of genes, showing a constant expression pattern over time in all treatments, which were linked to the molecular mechanisms of energy metabolism (*atp6*, *cox1*), deiodination (*dio1*, *dio3*), skeletogenesis (*bmp5*), and water transport (*aqp3*). The other genes, showing no significant expression pattern over time, were relating to skeletogenesis (*bmp2b*), deiodination (*dio2*), water transport (*aqp1*), ion transport (*nkcc1a*), stress response (*crfr2*), and immune system ontogeny or immune response (*il1β*, *mhc2*), with no regular pattern among treatments.

Discussion

European eels undertake a catadromous reproductive migration resulting in eel offspring naturally occurring in the ocean that is hyper-osmotic to teleost fishes. Here, plasma osmolality of the organism is lower than the environment (Lee et al., 2013). Therefore, eel offspring need to maintain osmotic balance through desalting processes to counteract osmotic water loss. In this regard, eel larvae are expected to be genetically pre-programmed and equipped with molecular mechanisms to thrive in oceanic conditions. However, larvae produced and reared in a hatchery,

benefit from an unnatural salinity reduction towards iso-osmotic conditions (Okamura et al., 2009; Ahn et al., 2015; Politis et al., 2018a).

Growth and survival

The results of the present study, show that the early and gradual salinity reduction led to an increased growth rate and resulted in larger larvae, irrespective of the change occurring Slow (over 24h) or Fast (within ~1h). At the same time, the salinity reduction influenced the expression of *dio3* (associated to growth and development), supporting the involvement of the deiodination mechanism and the thyroid endocrine system during early ontogeny of this species as well as its sensitivity to environmental parameters, confirming observations in other fish species (Orozco et al., 2002). Moreover, ion transport related expression of *nkcc1a* was stable throughout larval ontogeny in the salinity reduced treatments, but was upregulated in full strength seawater (Control), indicating an upregulation of the active Na^+, K^+, and Cl^- transport. As this mechanism requires energy, an increased transcellular ion transport translates into increased cellular energy consumption when eel larvae are reared in 36 psu. Moreover, generally associated to energy metabolism in teleost fishes (Bermejo-Nogales et al., 2015), the expression levels of ATP-synthase and cytochrome-c-oxidase were constant throughout the entire developmental period, suggesting that energy metabolism was stable and independent of salinity. As such, the decreased ion regulation and osmotic demands (i.e. lower *nkcc* expression) when eel larvae experienced an early salinity reduction, explain why they are able to use their energy reserves more efficiently than larvae reared in full strength salinity. Consequently, the more efficient energy utilization resulted not only in better growth but also improved survival, where

larvae experiencing the Slow and Fast salinity reduction, showed ~50% lower mortality. Similar survival rates have been observed in Japanese eel, where improved growth and survival were reported for larvae reared in ~50% reduced salinity (Okamura et al., 2009; Okamura et al., 2016). Reducing seawater salinity (with an osmolality of ~1050 mOsm kg^{-1} H_2O) to ~50%, facilitates an almost iso-osmotic environment for eel larvae, with a tissue osmolality of 360 - 540 mOsm kg^{-1} H_2O (Lee et al., 2013). This, probably decreases energy utilization due to lower osmoregulatory and metabolic expenses, enabling the survival of weaker larvae, which would not survive in a high salinity environment (Okamura et al., 2016). Interestingly though, in the current study, the survival of larvae experiencing a salinity reduction on 6 dph (Drastic), followed the same pattern as larvae experiencing no salinity reduction (Control), which indicates that the process of "saving" larvae possibly occurs earlier.

Deformities

It has been reported that rearing eel larvae at reduced salinity can cause deformities, such as pericardial edema (Okamoto et al., 2009). More specifically, the rate of deformed larvae, including kyphosis and scoliosis, was higher in salinity reduced rearing conditions, but the most of severely deformed larvae did not survive in full strength seawater, implying that the advantage of salinity reduction for rearing eel larvae outweighs the risk of deformities (Okamura et al., 2016). Additionally, the authors of the same manuscript stated that it is not salinity alone to be seen as the cause of deformities in eel larvae, but other factors influencing deformities such as temperature, water flow or nutrition should also be taken into consideration. The results of the present study are in agreement with those findings, as larvae with an increased pericardial edema

severity ratio were observed in the salinity reduced treatments, irrespective of whether the reduction occurred Slow, Fast or Drastic. Thus, it is arguable that reducing salinity is a tradeoff process, which improves survival and growth but also induces an edematous state of the larval heart. Interestingly, the expression of *nppa* and *nppb* peaked at 6 dph, demonstrating that key processes of cardiovascular development occur at this larval stage. However, the increased pericardial edema severity ratio observed in this study cannot be linked to a general malfunction or failure of this molecular mechanism in response to salinity reduction, as the expression patterns were not driven by the treatment effect. Nevertheless, the timing of salinity reduction and the trade-of between survival and deformities as well as the implications on feeding capacity, is of key importance for further development of eel hatchery techniques and thus needs to be further explored.

Timing and functionality of molecular mechanisms

The expression patterns of 33 genes were investigated to elucidate timing and functionality of targeted molecular mechanisms, driving and/or documenting health and development throughout early eel ontogeny (Fig 5). Four different patterns were identified, were gene expression either significantly peaked at hatch (0 dph), mouth opening (6 dph), and first-feeding (12 dph) or was unaffected by larval age/stage. Irrespective of treatment, two genes relating to water transport (*aqp1dup*) and skeletogenesis (*admp*) showed highest expression at larval hatch and decreased with increasing age. The same expression pattern was previously observed in European eel for genes relating to water transport (*aqp1dup*), thyroid metabolism (*thba*), growth (*igf2a*) and immune system ontogeny (*c3*, *igm*) (Politis et al., 2017, 2018a, 2018c; Miest et al.,

2019), revealing that those genes might be of primary importance during earlier development or were potentially transferred maternally to offspring (Kottmann et al., 2020). Moreover, a group of genes linked to neurogenesis (*neurod4*, *ngn1*) and heart development (*nppb*, *nppa*) peaked on 6 dph and in most cases decreased to basal levels again beyond that, demonstrating that key processes of neuronal differentiation and cardiovascular development occur at this stage, irrespective of environmental drivers and largely coincide with the genetically pre-programmed timing of primary organogenesis. Furthermore, the majority of genes, relating to almost all targeted molecular mechanisms, peaked at first-feeding corresponding to a timing of organogenesis refinement and/or specific functional tissue development, in order to ensure optimal transition from endogenous to exogenous feeding (Sørensen et al., 2016; Butts et al., 2016; Politis et al., 2018b). Finally, another group of genes, appearing unaffected by larval age, with a consistent expression pattern over time in all treatments, were linked to energy metabolism (*atp6*, *cox1*), thyroid metabolism/deiodination (*dio1*, *dio3*), skeletogenesis (*bmp5*) and water transport (*aqp3*). The function of those genes and their associated mechanisms probably are of basal importance for development, metabolism, and homeostasis throughout eel larval ontogeny from hatch until first-feeding.

Genotype and environment interaction

The VC analysis of this study showed that the majority of gene expression patterns were driven by "family". In this regard, a "good genes" hypothesis, could translate into genetically preprogrammed "better" or more sensitive molecular mechanisms, endowed by the parents, to control early ontogenetic processes. However, for each genotype, phenotypic trait, and

environmental factor, a different reaction norm can exist, resulting in interrelationships between genetic and environmental factors (Pfennig et al., 2010; Kelly et al., 2012; Jarquin et al., 2014). Typical genetic × environment interactions have been observed in several fish species and reviewed by Oomen and Hutchings. (2015). In the current study, the VC analysis revealed differing reaction norms, regarding larval eel phenotypic traits, of each genotype (family) to the environmental variable (salinity) investigated. As such, these results reveal a phenotypic plasticity to salinity, as each genotype can produce different phenotypes (in terms of gene expression) when exposed to different environments (salinity treatments). This translates into a variable sensitivity of each batch to salinity, which should be taken into consideration in future larval culture of this species, as uncontrolled variability in extrinsic parameters might unnecessarily challenge the condition and development of some eel offspring and thus negatively impact larval survival and production efficiency.

Biotic and abiotic interactions

Part of the observed variability in reaction norms can possibly be attributed to the relationship between biotic and abiotic interactions occurring within a RAS system. Salinity fluctuations can influence the microbial community composition (Navada et al., 2019) and impact the nitrification process (Kinyage et al., 2019). As such, the abiotic change in salinity applied in the current study, could have directly and indirectly, impacted the RAS biofilter, system water, and eel larval microbiome composition as well as their interactions. Consequently, this could partly explain the observed differential reaction norms regarding physiological responses and the interlinked gene expression of the eel larvae from different family crosses.

From a RAS functionality point of view, there is no advantage of small compared to large steps of salinity modification, as long as there is adequate time allowing for appropriate adjustment (Navada et al., 2019). However, the salinity modifications suggested in eel larviculture do not allow for long acclimatization periods, challenging the stability of each RAS unit. In Japanese eel aquaculture, where the eel life cycle has been closed, larviculture is based on flow-through systems, which is economically and ecologically not the most resource efficient and sustainable procedure regarding water management. Interestingly though, intentional "priming" of water systems, by preceding exposure to salinity changes, leading to alterations in microbial community compositions, can result in "trained" RAS systems, able to accommodate improved nitrification during future salinity challenges (Navada et al., 2020). This could be an interesting microbial management strategy and tool for addressing issues in eel larviculture. Nonetheless, the current study has explored the possibility of applying a "Drastic" salinity reduction, where larvae are directly moved from seawater to ~50% reduced salinity. If suited timing is identified, applying this technique can circumvent technical problems and improve efficiency as it requires only 2 RAS at the desired salinity levels.

Conclusion and future perspectives

To summarize, the current study elucidated European eel larval development and performance under different salinity regimes. We conclude that an early and progressive salinity reduction (Fast or Slow), reduces ion-regulatory demands and thus eel larvae are able to use their energy reserves more efficiently, leading to increased growth and improved survival. Here, a genetic (parental) programming of molecular mechanisms and intrinsic (batch-specific)

sensitivity to extrinsic (environmental) drivers need to be considered in future eel farming, especially when breeding programs become a reality. However, challenges such as the edematous state of the larval heart and potential negative implications need to be addressed in future studies. On the other hand, we show that biotechnical difficulties for introducing salinity reductions in European eel hatcheries, can be circumvented by applying "Drastic" changes, directly moving larvae from full strength seawater to iso-osmotic conditions. As such, the current study revealed the applicability of an ecologically viable and economically efficient salinity reduction protocol for eel larviculture, requiring only two stable RAS units, "primed" at the desired salinity levels. However, the technically most efficient and biologically suited timing for applying this "Drastic" salinity reduction needs to be addressed in future studies.

Author's contribution

JT, SP and SS provided funding and designed the study. SS constructed systems and rearing tanks. JT established the assisted broodstock protocols providing eggs for the experiment. SP, EB and ES conducted the experiment and collected samples. SP, EB and JM conducted gene expression analysis. IB and SP ran statistics and analyzed data. SP and SS made illustrations. FB and SP designed primers. SP wrote original manuscript draft. All authors contributed to data interpretation, manuscript revision and approved the submitted version.

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Figure Captions

Figure 1. European eel (*Anguilla anguilla*) larvae were reared throughout the endogenous feeding stage (from 0 to 12 days post hatch (dph)) in 8 L acrylic Kreisel tanks (n = 3 for each family and each treatment). The Kreisel tanks of each treatment were connected to a separate Recirculating Aquaculture System (RAS) unit, where flow rates of conditioned filtered seawater were kept at ~500 ml min^{-1}. Each RAS unit facilitated a sump reservoir of ~1 m^3, from where water entered a 1 m^3 biofilter, followed by a similar sized trickle filter and then re-entered the bottom reservoir. Here, a protein skimmer was included for removal of waste protein. A UV lamp was also included to treat the newly filtered water.

Figure 2: A) European eel (*Anguilla anguilla*) larvae reared in 4 different salinity treatments. In the Control, salinity was kept constant at 36 psu. Moreover, starting on day 3, salinity was successively reduced to 18 psu, in steps of 4 psu per day either within 1 h (Fast) or 24 h (Slow). In the Drastic treatment, the salinity reduction from 36 to 18 psu was applied on 6 dph, with the change occurring within 1 h. B) Sampling points on day0 (hatch), day6 (mouth opening) and day12 (first feeding) post hatch.

Figure 3. Survival (%) of European eel (*Anguilla anguilla*) larvae reared over 12 days post hatch (dph) under four different salinity scenarios (Control, Fast, Slow and Drastic). Values represent means of 3 family crosses. The main model variables were salinity treatment (fixed effect), family (random effect), and the salinity treatment × family interaction (random effect). Small letters represent significant differences among treatments and asterisks represent significant

variance components (VC). The VCs were generated using the Restricted Maximum Likelihood estimation method and expressed as a percentage. Alpha was set to 0.05.

Figure 4. Body area (A), oil droplet area (B), growth/day (C), oil droplet utilization/day (D), growth efficiency (E), and heart edema severity ratio (F) of European eel (*Anguilla anguilla*) larvae reared over 12 days post hatch (dph) under four different salinity scenarios (Control, Fast, Slow and Drastic). Values represent means of 3 family crosses. The main model variables were salinity treatment (fixed effect), family (random effect), and the salinity treatment × family interaction (random effect). Small letters represent significant differences among treatments and asterisks represent significant variance components (VC). The VCs were generated using the Restricted Maximum Likelihood estimation method and expressed as a percentage. Alpha was set to 0.05.

Figure 5. Expression patterns of genes linked to key molecular mechanisms (ion regulation, water transport, thyroid metabolism, deiodination, heart development, stress, stress/repair, immune response, growth, skeletogenesis, energy metabolism, and neurogenesis) throughout early larval ontogeny of European eel, *Anguilla anguilla*. Larvae were reared over 12 days post hatch (dph) under four different salinity scenarios (Control, Fast, Slow and Drastic). Values represent means of 3 family crosses. Genes were grouped into three expression patterns, either significantly peaking at hatch (0 dph), at mouth opening (6 dph) or first-feeding (12 dph), elucidating timing and functionality of the associated mechanisms. Alpha was set to 0.05.

Table 1. European eel, *Anguilla anguilla* primers used for amplification of genes by qRT-PCR and designed based on sequences available on Genbank databases. The table lists function, gene name and abbreviation as well as sequences for forward and reverse primers.

Function	Gene	Abbreviation	5' forward 3'	5' reverse 3'	Accession Nr
Reference	Atrial natriuretic peptide receptor 3	*npr3*	AACCCTCCACGTGTAC[illegible]G	TGACCAGAATTGCTCCCTCTT	XM_035380325
	Elongation factor 1	*ef1*	CTGAAGCCTGGTA[illegible]GTGGT	CATGGTGCATTTCCACAGAC	EU_407824
Stress/repair	Heat shock protein 70	*hsp70*	TCAACCCAGA[illegible]AGCAGTG	GCAGCAGATCCTGAACATTG	AZBK_01685255
	Heat shock protein 90	*hsp90*	ACCATTGCCA[illegible]CAGGAAC	ACTGCTCATCGTCATTGTGC	AZBK_01838994
Growth	Insulin-like growth factor 1	*Igf 1*	TTCC[illegible]TAGC[illegible]GGGCTTTG	AGCACCAGAGAGAGGGTGTG	EU_018410
	Insulin-like growth factor 2b	*Igf 2b*	A[illegible]AGC[illegible]TGGGACAGCTTCA	CGCAGCTGTGTACGTGAAAT	AZBK_01622663
	Growth hormone	*gh*	TGAACAAGGGCATCAATGAA	CGGAGCTTTCTCACATCCTC	AZBK_01601863
Stress	Corticotropin-releasing factor receptor 1	*crfr1*	GCATGAAGAGGATGAAGGCG	ATAGATGGGATCGGCACCTG	XM_035400367
	Corticotropin-releasing factor receptor 2	*crfr2*	CAGGAGGAGGAAGATGGCTG	CTGGAACCTGATCACCACCT	XM_035431405
Skeletogenesis	Anti-dorsalizing morphogenetic protein	*admp*	TCTGTGAAGAGGACCAGCATG	CTGGATGGCAGACGAGGG	XM_035413656
	Bone morphogenetic protein 2b	*bmp2b*	AGCAAGCTGGACGAGAAGAA	CGTATGATTGGCACTGCGTT	XM_035406657
	Bone morphogenetic protein 5	*bmp-5*	CGCAATAATCCAGTCCTGCC	GCACAAGGGAGGAGACCAAA	XM_035399631
Energy	Cytochrome-C-Oxidase	*cox-1*	CTACTCCTCTCCCTGCCAGT	CTTCTGGGTGGCCGAAGAAT	NC_006531
	ATP synthase F0 subunit 6	*atp6*	GGCCTGCTCCCATACACATT	GACTGGTGTTCCTTCTGGCA	NC_006531
Ion transport	Na+K+2Cl- Cotransporter 1α	*nkcc1a*	CCAAGGCTCAGATCTTCCTG	TTTCCGAATGGTAACCGAAG	AJ_486858
	Na+K+2Cl- Cotransporter 2α	*nkcc2a*	ACGTGGTTGGGTTTTCAGAG	GTGAGATCCCCAAAAGCAAA	AJ_564602
	Na+K+2Cl- Cotransporter 2b	*nkcc2b*	AGCCAAAGTGGTGGATGTTC	TGTCAGCCTCTCCAGTTCCT	AJ_564603
Heart development	Atrial natriuretic peptide A	*nppa*	CCTGAAGGCACACGACTACT	ACCACACCAGACGACCTTTT	XM_035383076
	Atrial natriuretic peptide B	*nppb*	ACAGCGACAAATGGACCAAC	TTCTCTTGAGGTTGCTCGCT	XM_035383163
	Atrial natriuretic peptide receptor 1	*npr1*	ACCTCCATCAGCACAGGATC	GCATGTACACCTCCCTCAGT	XM_035427656
	Atrial natriuretic peptide receptor 2	*npr2*	AAACCCGATGCGTTCTTTGG	CGAGTGTAGGTAATGGGCGA	XM_035391652
Neurogenesis	Neuronal Differentiation 4	*neurod4*	TTCCTGTCCTCGCACCAGTA	AAGGAGTCGAAGGCCATGTC	MT_531400
	Neurogenin 1	*ngn1*	CAGGATGCACAACCTCAATG	TGCAATTCGGATTGTCTCTG	MT_531401

Thyroid metabolism	Thyroid Hormone Receptor aA	*thαa*	GCAGTTCAACCTGGACGACT	CCTGGCACTTCTCGATCTTC	KY_082904
	Thyroid Hormone Receptor aB	*thαb*	GAAGCCTTCAGCGAGTTCAC	ACAGCCTTTCAGGAGGATGA	KY_082905
	Thyroid Hormone Receptor bB	*thβb*	GAAGACTGAGCCCTGAGGTG	AGGTAATGCAGCGGTAATGG	KY_082907
	Deiodinase 1	*dio1*	AGCTTTGCCAGAACGACTGT	TTCCAGAACTCTTCGCACCT	Eeel2-c186
	Deiodinase 2	*dio2*	GAAGAGGAGGATCGCCTACC	GCACTCTACCTCCGTCCAAA	g12347
	Deiodinase 3	*dio3*	TACGGGGCGTATTTTGAGAG	GCTATAACCCTCCGGACCTC	Eeel-c22164
Immune response	Major histocompatibility complex, II	*mhc2*	TCAAATTGACCTGGCTGAGAG	TTTCCATTAGCCAGCTCCTC	AF_134926
	Interleukin 1β	*il1β*	ATTGGCTGGACTTGTGTTCC	CATGTGCATTAAAGCTGACCTG	AZBK_01652159
	Toll like receptor 2	*tlr2*	TGGTTCTGGCTGTAATCGAG	CGAAATGAAGGCATGGTAGG	AZBK_01853964
Water transport	Aquaporin 1 duplicate	*aqp1dup*	GAATTCCTGGCAACCTTTCA	CAAGATGACCCAGACCCACT	AJ_564421
	Aquaporin 3	*aqp3*	GCTCTCATGCCTTCTTCCTC	AAGGTCACAGTGGGGTTCAG	AJ_319533
	Aquaporin e	*aqpe*	TGGGCAGCTCACAGTAACAG	AATCACCTGGTCCACAAAGC	AJ_784153

Table 2. Overview of statistical analysis regarding the effect of European eel, *Anguilla anguilla* larval age on expression patterns of genes relating to different key molecular mechanisms at each treatment. Larvae were reared over 12 days post hatch (dph) under four different salinity scenarios (Control, Fast, Slow and Drastic). Genes were grouped into 4 expression patterns, either significantly peaking on 0 dph (↘), 6 dph (↑), and 12 dph (↗), or showing no significant changes over time (↔) to elucidate timing and functionality of the associated mechanisms. Small letters represent significant differences among treatments. Alpha was set to 0.05.

Function	Gene ID	Control					Slow					Fast					Drastic				
		Age	0	6	12		Age	0	6	12		Age	0	6	12		Age	0	6	12	
Stress/Repair	*hsp70*	***	a	b	c	↗	*	a	ab	b	↗	*	a	a	b	↗	**	a	a	b	↗
	hsp90	*	ab	a	b	↗	**	a	a	b	↗	**	b	a	c	↗	*	ab	a	b	↗
Water transport	*aqp3*	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔
	aqpe	*	a	ab	b	↗	ns	.	.	.	↔	**	a	a	b	↗	**	a	a	b	↗
	aqp1	**	b	a	a	↘	**	b	a	a	↘	**	b	a	a	↘	**	b	a	a	↘
Immune response	*il1β*	*	a	ab	b	↑	*	a	ab	b	↗	*	a	ab	b	↗	ns	.	.	.	↔
	mhc2	ns	.	.	.	↔	ns	.	.	.	↔	*	a	ab	b	↗	ns	.	.	.	↔
	tlr2	***	a	b	c	↗	**	a	b	c	↗	**	a	b	c	↗	**	a	b	b	↗
Neurogenesis	*neurod 4*	**	a	b	a	↑	**	a	b	a	↑	**	a	c	b	↑	**	a	b	a	↑
	ngn1	***	a	c	b	↑	**	a	c	b	↑	**	a	b	a	↑	**	a	b	a	↑
Deiodination	*dio1*	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔
	dio2	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔	*	a	ab	b	↗
	dio3	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔

Thyroid metabolism	*thαb*	***	a	b	c	↗	**	a	b	b	↑	**	a	b	c	↗	**	a	b	c	↗
	thαa	**	a	a	b	↗	*	a	ab	b	↗	*	a	ab	b	↗	**	a	a	b	↗
	thβb	*	a	b	b	↑	*	a	ab	b	↗	**	a	b	b	↑	**	a	b	b	↑
Energy	*atp6*	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔
	cox1	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔
Skeletogenesis	*admp*	***	b	a	a	↘	** *	b	a	a	↘	** *	b	a	a	↘	** *	b	a	a	↘
	bmp2b	*	a	b	ab	↑	ns	.	.	.	↔	*	a	b	ab	↑	ns	.	.	.	↔
	bmp5	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔
Stress	*crfr1*	***	a	b	c	↗	**	a	b	c	↗	**	a	a	b	↗	**	a	b	c	↗
	crfr2	*	a	ab	b	↗	ns	.	.	.	↔	**	a	b	b	↗	*	a	ab	b	↗
Growth	*gh*	**	a	a	b	↗	**	a	a	b	↗	**	a	a	b	↗	**	a	a	b	↗
	igf1	**	a	a	b	↗	** *	a	b	c	↗	**	a	a	b	↗	** *	a	b	c	↗
	igf2b	***	a	b	c	↗	** *	a	b	c	↗	**	a	a	b	↗	**	a	a	b	↗
Ion regulation	*nkcc1a*	*	a	a	b	↗	ns	.	.	.	↔	ns	.	.	.	↔	ns	.	.	.	↔
	nkcc2a	***	a	b	c	↗	** *	a	b	c	↗	**	a	a	b	↗	** *	a	b	c	↗
	nkcc2b	***	a	b	c	↗	** *	a	b	c	↗	** *	a	b	c	↗	** *	a	b	c	↗
Heart development	*nppa*	***	a	c	b	↑	** *	a	b	a	↑	**	a	b	ab	↑	*	a	b	ab	↑
	nppb	*	a	b	b	↑	*	a	b	ab	↑	*	a	b	ab	↑	*	a	b	ab	↑
	npr1	***	a	a	b	↗	*	a	ab	b	↗	**	a	a	b	↗	**	a	a	b	↗

npr2	***	a	b	c	↗	**	a	b	b	↑	*	a	a b	b	↗	*	a	b	b	↑

p < 0.05	*	↘	Decrease from day0
p < 0.01	**	↑	Increase on day6
p < 0.001	***	↗	Increase on day12
		↔	Constant

Table 3. Variance component (%) analysis on the expression patterns of genes linked to key molecular mechanisms of European eel, *Anguilla anguilla* larvae at the mouth opening stage on 6 days post hatch (dph) and the first feeding stage on 12 dph. Larvae were reared under four different salinity scenarios (Control, Fast, Slow and Drastic). Values represent means of 3 family crosses. The main model variables were salinity treatment (fixed effect), family (random effect), and the salinity treatment × family interaction (random effect). Alpha was set to 0.05.

Gene Function	Abbreviation	Mouth opening (day 6) Variance Component Estimate (%)						First feeding (day 12) Variance Component Estimate (%)					
		Fam		Fam x Treat		Error		Fam		Fam x Treat		Error	
Stress/Repair	*hsp70*	7,7		0,2		92,1		3,2		35,0		61,8	
	hsp90	0,0		57,2	*	42,8		27,7		8,8		63,5	
Water transport	*aqp3*	13,6		16,0		70,4		53,7	*	38,5	***	7,7	
	aqpe	0,0		0,0		100,0		58,9	*	10,1		31,0	
	aqp1	0,0		37,1		62,9		71,2	**	9,7		19,1	
Immune response	*il1β*	76,6	***	0,0		23,4		65,8	*	20,0	*	14,3	
	mhc2	96,6	***	1,9	*	1,5		36,8	*	0,0		63,2	
	tlr2	79,5	***	2,0		18,5		27,5	*	0,0		72,5	
Neurogenesis	*neurod4*	0,0		73,0	**	27,0		27,3	*	0,0		72,7	
	ngn1	12,2		36,2		51,5		9,3		0,0		90,7	
Deiodination	*dio1*	76,5	***	0,0		23,5		73,8	***	0,0		26,2	
	dio2	71,9	**	14,0	*	14,1		81,3	***	5,4		13,3	
	dio3	48,4	**	0,0		51,6		78,8	**	5,7		15,5	
Thyroid metabolism	*thαb*	0,0		85,6	***	14,4		0,0		0,0		100,0	
	thαa	7,1		79,2	***	13,7		0,0		46,0		54,0	
	thβb	0,0		61,0	**	39,0		32,7		37,2	*	30,1	
Energy	*atp6*	0,0		46,8	*	53,2		0,0		21,3		78,7	
	cox1	0,0		36,9		63,1		16,8		7,0		76,2	
Skeletogenesis	*admp*	64,7	**	8,9		26,4		0,0		35,9		64,1	
	bmp2b	24,7		38,9	*	36,4		0,0		21,7		78,3	
	bmp5	42,0		33,9	*	24,1		24,0		52,6	**	23,4	
Stress	*crfr1*	34,3	*	0,0		65,7		0,0		0,0		100,0	
	crfr2	32,3	*	0,0		67,7		0,0		26,6		73,4	
Growth	*gh*	81,7	**	0,0		18,3		23,3		18,4		58,3	
	igf1	52,0	*	7,4		40,6		12,6		0,0		87,4	
	igf2b	13,8		66,2	**	20,0		19,9		4,2		75,9	
Ion regulation	*nkcc1a*	33,2		29,8		37,1		35,2	**	0,0		64,8	
	nkcc2a	50,2	*	17,0		32,8		51,9	*	0,5		47,6	
	nkcc2b	14,1		53,6	*	32,3		47,9	**	0,0		52,1	
Heart development	*nppa*	0,0		38,2		61,8		77,8	*	1,1		21,0	
	nppb	21,6		5,7		72,7		3,3		57,4	*	39,3	
	npr1	50,6	*	9,2		40,2		21,8		17,9		60,3	
	npr2	25,3		0,0		74,7		13,2		0,0		86,8	

<0.05	*
<0.01	**
<0.001	***

Credit Author Statement

JT, SP and SS provided funding and designed the study. SS constructed systems and rearing tanks. JT established the assisted broodstock protocols providing eggs for the experiment. SP, EB and ES conducted the experiment and collected samples. SP, EB and JM conducted gene expression analysis. IB and SP ran statistics and analyzed data. SP and SS made illustrations. FB and SP designed primers. SP wrote original manuscript draft. All authors contributed to data interpretation, manuscript revision and approved the submitted version.

Declaration of interests

X The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Reducing salinity towards iso-osmotic conditions benefits eel larval survival
- Genetically programmed developmental timing of key molecular mechanisms
- Gene expression patterns were driven by genotype × environment interactions
- Applicability of efficient salinity reduction protocols for eel larviculture