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Markers for egg quality in European eel derived from offspring of females subjected to different gonadotropic treatments

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ABSTRACT

Broodstock management techniques in general, and gonadotropic treatments for vitellogenesis induction in particular, can influence the maternal provisioning of different compounds to the eggs, thus affecting embryo and larval competence. Among these, the abundance of certain transcripts as well as lipid content and fatty acid composition have been used as egg quality markers alongside other egg morphological parameters, such as egg size and the occurrence of abnormal cleavages during the first stages of embryonic development. Here, we evaluated the use of different markers of egg quality from farmed-raised European eel, *Anguilla anguilla*, broodstock reared on the same formulated diet but subjected to different pituitary extract administration schemes: weekly injections of salmon pituitary extract (SPE) in a constant dose or weekly injections of carp pituitary extract (CPE) at a stepwise increasing dose. Egg batches were categorized in two quality groups (high and low) according to their survival to the first feeding larval stage. In agreement with previous studies, unfertilized eggs and 4-h post fertilization embryos (16-cell stage) from SPE treated females had higher *epcam* expression levels than CPE ones, which is a molecule involved in cell-cell adhesion. When comparing treatments, batches from the SPE treatment had a lower incidence of cleavage abnormalities which could originate from compromised cell adhesion. On the other hand, this difference was not observed when comparing high-quality batches (i.e. those surviving up to the first-feeding stage) and low-quality ones. From the five genes analysed: *epcam*, *dcbld1*, *plec*, *cenpf*, and *cenpk*, only the latter had a differential expression between high- and low-quality groups both in unfertilized eggs and 4 h-post-fertilization embryos. This gene, *cenpk*, is involved in kinetochore assemblage during cell division and appears to be promising as egg quality marker in European eel in combination with *epcam* and *dcbld1*. Common biomarkers, such as egg size, total protein and lipid content, were similar between groups. However, although fatty acid composition was similar between quality groups, DHA and EPA content in unfertilized eggs was affected by the gonadotropic treatment applied to the female broodstock, and higher for the CPE treated females than the SPE ones. This entails that the hormonal treatments applied for vitellogenesis induction can influence the amount of specific fatty acids being transferred to the eggs, even for female broodstock that were fed the same high-quality diet.

1. Introduction

The obtention of high-quality eggs, i.e. eggs that can be successfully fertilized and subsequently develop into normal embryos, is required for sustainable aquaculture practices (Bobe and Labbé, 2010). Poor egg quality can lead to low quality larvae and impaired larval survival, and negatively impact commercial hatcheries' production. Here, variation between offspring batches is often observed (Myers et al., 2020a, 2020b), which could be related to genetic differences or to environmental factors such as photoperiod, temperature, salinity or broodstock diets (Bobe and Labbé, 2010). Some of these environmental conditions are frequently manipulated in broodstock rearing to control reproduction (Mylonas et al., 2010).

In this context, egg quality can be influenced by application of

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assisted reproduction techniques, i.e. hormonal treatments that are used in some fish species to stimulate follicular development, vitellogenesis and maturation, while in others only to induce follicular maturation and ovulation (Bobe, 2015; Mylonas et al., 2010). Hormonal treatments are administered to species that otherwise would not reproduce in captivity such as long-migratory species (Migaud et al., 2013; Mylonas et al., 2010). For these, it is complicated to mimic in captivity the conditions that the fish experience during migration, and therefore they lack the proper environmental signals acting on the endocrine reproductive axis to achieve a normal reproductive performance (Mañanos et al., 2008; Mylonas et al., 2010). This is the case for eels (*Anguilla* sp.) which do not proceed through gametogenesis and spawning in captivity without repeated hormonal treatment and induced gamete development (Kagawa et al., 2005; Pedersen, 2003).

The European eel (*Anguilla anguilla*) start their migration from their continental habitats and towards their spawning area in the Sargasso Sea as silver eels (Schmidt, 1923; van Ginneken and Maes, 2005; Wright et al., 2022). At this point, they are still sexually immature and if migration is prevented, they remain blocked at this prepubertal stage (Dufour et al., 1983). This results from a deficient gonadotropic function in Anguillid eels as dopamine exerts an inhibitory control on the release of follicle stimulating (FSH) and luteinizing hormones (LH) at the brain-pituitary level (Vidal et al., 2004). Accordingly, sexual maturation and vitellogenesis in female eels can be induced by repeated administration of salmon (SPE), carp (CPE) pituitary extracts (Dufour et al., 1983) or recently by recombinant gonadotropic hormones (rGTH) (Jéhannet et al., 2023; Kazeto et al., 2019) to induce vitellogenesis, and maturation-inducing steroid (MIS)s, such as 17α,20β-dihydroxy-4-pregnen-3-one (DHP), to complete follicular maturation and induce ovulation (Kagawa, 2003; Rousseau et al., 2013). According to current production methods, females are treated weekly with GTH substitutes over approximately 12 to 16 weeks and one priming dose before administration of MIS (Jéhannet et al., 2023; Kazeto et al., 2019; Tomkiewicz et al., 2019).

These gonadotropic treatments may affect the maternal supply of molecules to the developing eggs, including egg lipid content and fatty acid composition (Rupia et al., 2014) and the expression of certain maternally derived mRNAs (Bonnet et al., 2007), Early embryonic development is directed exclusively by these maternal mRNA, as the embryonic genome is not yet transcriptionally active (Lee et al., 2014; Pelegri, 2003). Therefore, changes in the expression levels of these genes are expected to reflect embryonic competence and could be used as markers of egg quality (Brooks et al., 1997). The genes that have been linked to egg and embryo quality in fish carry out critical cellular functions during early embryonic development, such as cell adhesion (Sullivan et al., 2015).

The duration of the maternal control of embryonic development is species-specific and depends both on when the zygotic transcription begins and the persistence of maternal transcripts (Abrams and Mullins, 2009). In European eel, maternal to zygotic transition takes place around 10 h post fertilization (hpf), at a rearing temperature of 18 °C (Kottmann et al., 2020a). Cleavage takes place mainly before the zygotic to maternal transition where embryonic development is under maternal control and therefore it is a stage highly sensitive to depletion of maternally supplied mRNAs. One of the processes that these transcripts regulate is cell division. Here, disruption of cleavage was observed in relation to impaired karyokinesis and cytokinesis, i.e. the partitioning of the genetic material and the division of cytoplasm (Lubzens et al., 2017). In addition, several mutations associated with the cytoskeleton lead to defects in spindle and furrow formation, and negatively affected cell adhesion (Abrams and Mullins, 2009; Lubzens et al., 2017). Poor cell adhesion among blastomeres is one of several types of abnormalities that have been observed in early fish embryogenesis (Ajduk and Zernicka-Goetz, 2016; Avery et al., 2009). Batches with high percentages of abnormal cleavage patterns during early development may present higher embryonic mortality, lower hatch success or a higher incidence of larval deformities (Avery et al., 2009). Therefore, in some species the assessment of abnormal cleavage patterns can be used as an indicator of egg quality (Lahnsteiner et al., 2009).

Furthermore, the abundance of some maternal mRNAs has been linked to embryonic survival and hatch success in European eel and are potential candidates for molecular markers of egg quality (Kottmann et al., 2021, Kottmann et al., 2020a; Rozenfeld et al., 2016). Here, the abundance of the products of genes related to cell adhesion, stability and structural support, and cell division have been linked to embryonic and larval survival in this and other species (Chapman et al., 2014; Kottmann et al., 2021; Żarski et al., 2017) and therefore could be used as molecular markers of egg quality. The epithelial cell adhesion molecule (EpCAM) is a glycoprotein involved in cell adhesion, proliferation, migration and differentiation involved in numerous processes throughout embryonic development (Trzpis et al., 2008; Trzpis et al., 2007). High expression of *epcam* has been observed during European eel embryonic development, which indicates that it is necessary for normal development (Kottmann et al., 2020a). The members of the Discoidin, CUB, and LCCL domain-containing protein receptor family play integral roles in development (Schmoker et al., 2020; Schmoker et al., 2019). Downregulation of *dcbld2* in zebrafish embryos leads to an impaired vascular development (Nie et al., 2013), while in European eel it was proposed that low *dcbld1* expression could result in incomplete cell adhesion leading to irreversible disc formation abnormalities (Kottmann et al., 2020a). Plectin is a member of the plakin protein family present in all vertebrates. It interacts with the intermediate filaments of the cytoskeleton, being essential to its organization and functionality (Wiche et al., 2015; Wiche and Winter, 2011). Centromere protein F and centromere protein K are constituents of the kinetochore, a protein assembly that facilitates chromosome segregation during mitosis (Perpelescu and Fukagawa, 2011) and are essential for cell viability (Okada et al., 2006). Both Plectin and centromere protein F transcript abundance in eggs was related to fertilization and hatching rates in *Dicentrarchus labrax* (Żarski et al., 2017). Similarly, higher larval survival was observed for eggs with high *cenpk* mRNA abundance in *Morone saxatilis* (Chapman et al., 2014). Due to their central role in early development, expression of these genes may be applied as egg quality markers and tools for identifying and resolving causes of poor-quality offspring.

Traditional biomarkers involve nutrients stored in the developing egg. Together with proteins, lipids are the main constituents of egg yolk (Reading et al., 2018). They are essential for embryonic development as they are structural components of cell membranes (Rainuzzo, 2020) and are used as source of metabolic energy both for embryos and larvae until first-feeding (Rainuzzo et al., 1997). Although nutritionally deficient or imbalanced broodstock diets are regarded as the main factor affecting egg lipid composition and thus their quality (Bobe and Labbé, 2010; Izquierdo et al., 2001), the egg quality can also vary among batches from females fed the same diet (Rainuzzo et al., 1997). Typically, both total lipid and fatty acid content have been used as egg quality indicators in fish (Rainuzzo et al., 1997). In particular, egg relative composition of the essential polyunsaturated fatty acids (PUFAs) C20:4n6 (ARA), C22:6n3 (DHA) and C20:5n3 (EPA) has been associated to egg viability, fertilization and hatch success, and larval survival in several marine species (Henrotte et al., 2010; Mazorra et al., 2003; Pickova et al., 1997; Rodríguez et al., 1998), including European eel (Kottmann et al., 2020b; Støttrup et al., 2016; Støttrup et al., 2013). In relation to this, egg size has also been proposed as an indicator of egg quality, as it may reflect egg yolk content (Rainuzzo, 2020). Here, eggs of bigger size and therefore a higher yolk content might result in higher larval survival during the pre-feeding stage (Lahnsteiner et al., 2009). In this context another possible factors to consider in relation to assisted reproduction is the scheme for the administration of pituitary extracts used for vitellogenesis induction, which can influence the process of accumulating lipids and the proportion of fatty acids in the oocytes (Rupia et al., 2014).

In the present contribution, we evaluated different egg quality indicators in batches with high and low survival of larvae originating from farm-raised females European eel subjected to two most common PE treatment schemes, i.e. constant dose for SPE and increasing dose for CPE (Ohta et al., 1997; Pérez et al., 2011; Tomkiewicz et al., 2019). The analysed egg quality parameters were: (a) egg size, (b) the occurrence of cleavage abnormalities, (c) egg lipid and protein content, (d) egg fatty-acid profile, and (e) the expression of specific mRNAs, which was monitored using quantitative real-time PCR (qPCR) in unfertilized eggs and in embryos at 4 h post-fertilization (hpf).

2. Material and methods

2.1. Ethics statement

All fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 86/609/EEC). Eel experimental protocols were approved by the Animal Experiments Inspectorate (AEI), Danish Ministry of Food, Agriculture and Fisheries (permit number: 2020-15-0201-00768). All eels were anesthetized before tagging, biopsies, and stripping and euthanized after the experiment. All efforts were made to minimize animal handling and stress.

2.2. Broodstock establishment

Female broodstock originated from a stock of wild-caught glass eels obtained as elvers from a commercial eel farm (Lyksad Fish Farm, Kolding, Denmark). These juveniles were reared in a freshwater recirculating aquaculture system (RAS) at the experimental facility EEL-HATCH, Technical University of Denmark, in Hirtshals at a temperature of ~22 ± 1 °C, salinity 6–8, and a photoperiod of 12 h light/12 h dark, and fed on a commercial eel diet (DAN-EX 2848, BioMar A/S, Denmark) for approximately 1 year. During the following two years, they were offered a customized diet (Gold-BEel) developed as part of present study. This diet that was designed based on the study by Kottmann et al. (2020b) focused on an increased level of ARA and optimization of the EPA/DHA ratio. The proximal composition is given in Supplementary Table A1. Fish were sorted by size periodically, which due to sexual size dimorphism led to female only broodstock (Holmgren and Wickström, 1993). For the experiment, females were randomly distributed in 1150 L tanks (~13 fish per tank) equipped with a closed recirculation system. One RAS unit was allocated for each hormonal treatment.

Male eels raised from glass eels on a commercial eel diet (DAN-EX 2848, BioMar A/S, Denmark), at a temperature of 23 ± 1 °C, salinity of 8 ± 1, and constant light, were obtained at a commercial eel farm (Royal Danish Fish A/S, Denmark) and transported to the EEL-HATCH facility. Upon arrival, males were distributed in three 450 L tanks (~20 fish per tank) connected to a RAS unit. Acclimatization took place over 14 days, during which salinity was gradually increased to 36 with Aquaforest Sea Salt (Brzesko, Poland). Subsequently, eels were anesthetized by submergence into an aqueous solution of benzocaine (ethyl p-aminobenzoate, Sigma-Aldrich, Germany) 20 mg/L and tagged with a passive integrated transponder.

From the beginning of the hormonal treatment and until spawning, female and male broodstocks were maintained under low intensity light (20 lx), and a 12 h light/12 h dark photoperiod. Salinity and temperature were kept constant at 35 ± 1 and 20 ± 2 °C, respectively. Eels were not fed during the experiment, mimicking the cease of feeding that characterize eels during spawning migration (van Ginneken and Maes, 2005).

2.3. Hormonal treatment and gamete production

Vitellogenesis was induced by weekly intramuscular injections of either salmon (SPE) or carp (CPE) pituitary extracts using standardized procedures (Kottmann et al., 2020a). Extracts were prepared following Ohta et al. (1997, 1996) by grinding and diluting freeze-dried salmon (Argent Aquaculture LLC, Washington, USA) or carp (Ducamar, Vizcaya, Spain) pituitaries in NaCl 0.9 *g*/L. After centrifugation at 1275*g* for 20 min, supernatants were stored at −20 °C until use. One group of females ($n = 13$; mean initial body weight: 827 ± 281 g; mean initial body length: 69 ± 7 cm) received a weekly intermuscular injection of SPE in a constant dose of 20 mg/kg initial body weight, during approximately 15 ± 1 weeks. A second group of females (n = 13; mean initial body weight: 784 ± 201 g; mean initial body length: 69 ± 6 cm) received weekly injections of CPE at a stepwise increasing dose: 5 mg/kg for the first three weeks, 10 mg/kg from week 4–6, 15 mg/kg from week 7–9, and 20 mg/kg from week 10. These 2 PE schemes are considered the most optimal administration strategies for each extract type for European eel (da Silva et al., 2016; Jørgensen, 2020; Kottmann et al., 2020a). Body weight was registered weekly, and oocyte developmental stage was monitored through biopsies. At increasing body weight, an additional hormone injection was given as a primer according to Palstra et al. (2005) and da Silva et al. (2018). Final follicular maturation and ovulation was induced using the maturation inducing steroid, 17α,20β-dihydroxy-4-pregnen-3-one (DHP crystalline, Toronto Research Chemicals, Canada) at a dose of 2 mg/kg body weight (Kottmann et al., 2021). Females were strip-spawned 12–14 h after injection.

Induction of spermatogenesis was induced following standardized procedures (Kottmann et al., 2020a). Males (mean initial body weight: 120 ± 11 g) received weekly injections of human chorionic gonadotropin (Sigma-Aldrich, Germany) at 1.5 IU/g fish (Perez et al., 2000). Prior to fertilization, an additional injection was given and milt was collected by strip-spawning 12 h after administration of hormone (Koumpiadis et al., 2021). Milt samples from 4 to 5 males per female were pipetted into a P1 immobilizing, storage medium (Peñaranda et al., 2010) at a concentration of 1:99 (Ohta et al., 1996). The sperm density in the storage medium was standardized and used for fertilization within 4 h of collection (Butts et al., 2014; Sørensen et al., 2013).

2.4. Fertilization and embryonic incubation

The amount of eggs stripped from each female was weighed and fertilized separately by sperm dilutions from different groups of males. Gametes were swirled together and activated with artificial seawater, consisting of reverse osmosis water (Vertex Puratek 100 gpd RO/DI, Vertex Technologies Inc., CA, USA) salted up to 36 with Reef Salt (Aquaforest, Brzesko, Poland). After 5 min of gamete contact time, embryos were moved into 15 L of artificial seawater at 20 °C and incubated for an hour. Then, the buoyant egg layer was transferred to a second container with 15 L of artificial seawater. For each female, the weight of stripped eggs was recorded. The percentage of buoyant eggs (%) was determined 30 min after fertilization in a 25 mL volumetric column (Tomkiewicz, 2012).

At 2 hpf, buoyant eggs were moved to 60-L conical incubators supplied with conditioned and filtered seawater (flow through rate of ~350 mL/min) (Politis et al., 2018) at 18 °C (Politis et al., 2017) and a low light intensity of 10 lx (Politis et al., 2014). Gentle aeration was added after 4 hpf and sinking dead eggs were purged from the bottom valve of each incubator. Aeration was stopped at 52 hpf and hatching took place at 54 to 58 hpf.

2.5. Larval culture

Newly hatched larvae were transferred into 80-L tanks connected to a RAS system consisting of biofilter, trickle filter, UV (ProCristal UV-C 11 W, JBL GmbH & Co. Neuhofen, Germany), protein skimmer (Aqua-Medic 5000 single 6.0, Bissendorf, Germany), and reservoir for top-up. Larvae rearing aimed at a temperature of 20 °C and salinity of 36, and applied low-intensity illumination until reaching the first-feeding stage at 9 dph.

2.6. Sample and data collection

Samples of unfertilized eggs were collected immediately after stripping and stored at −20 °C for biochemical determinations or preserved in RNAlater® storage reagent (Sigma-Aldrich, Germany) and kept at −20 °C for gene expression analysis. A second sample (n ~ 100 eggs) was photographed at 4 hpf using an objective microscope (Eclipse 55i, Nikon Corporation, Japan) with a mounted camera (Digital Sight DS-Fi1, Nikon Corporation, Japan). Based on the images, cell cleavage patterns were categorized as normal (1a) or abnormal (1ab and 1b) following Sørensen et al. (2016b) (Fig. 1). Fertilization success was calculated as the percentage of fertilized eggs from the total number of eggs sampled (Sørensen et al., 2016b).

Embryonic survival was estimated at 4, 24, and 48 hpf from 3 × 15 mL water samples collected from each incubator. Survival at 4 hpf was used as the initial value for subsequent survival and hatch success estimations. A sample of ~30 embryos from each batch was haphazardly taken at 4 hpf for gene expression analysis and preserved as previously described. A sample of embryos (n ~ 100) was collected at 48 hpf and transferred into a 200-mL sterile tissue culture flasks (VWR, Denmark) containing culture water enriched with rifampicin and ampicillin (each 50 mg/L, Sigma-Aldrich, Germany) (Sørensen et al., 2014). The number of larvae and unhatched embryos was counted from each flask at 12 h post hatch (approximately 20 h after collecting the sample) for estimating hatching success as the percentage of hatched larvae from the initial number (Benini et al., 2022).

Larval survival was estimated at 10 dhp from 3 × 30 mL representative water samples collected from each 80-L tank as the percentage of surviving larvae from the total of hatched larvae. Batches from which larvae reached the first-feeding stage, around 10 dph (Sørensen et al., 2016b), were considered to contain eggs of good quality. Following this criterion, egg batches were categorized into two groups: high quality (*n* = 12, where 6 were originated from SPE treated females and 6 from CPE treated ones) and low quality (*n* = 14, originating from 7 SPE and 7 CPE treated females).

2.7. Biochemical composition of feeds and eggs

Analysis of proximate composition of feeds was performed by following the methodologies described by AOAC (2006). Dry matter after drying at 105 °C for 24 h; total ash by combustion (550 °C during 6 h) in a muffle furnace; crude protein (N × 6.25) by a flash combustion technique followed by a gas chromatographic separation and thermal conductivity detection with a Leco N Analyzer (Model FP-528, Leco Corporation, USA); Following an acid hydrolysis step, crude lipid was determined by dichloromethane extraction (40–60 °C) using a Soxtec™ 2055 Fat Extraction System (Foss, Denmark). Gross energy was measured in an adiabatic bomb calorimeter (Werke C2000 basic, IKA, Germany). For fatty acid analysis, lipids were first extracted according to the method of Folch et al. (1957). The fatty acid composition was determined by gas-chromatography analysis of methyl esters, according to the procedure of Lepage and Roy (1986).

2.8. Gene expression

Samples were homogenized and RNA was extracted using the NucleoSpin® RNA Kit (Macherey-Nagel) following the manufacturer's instructions. RNA integrity was assessed by agarose gel-electrophoresis, and RNA concentration and purity were determined by spectrophotometry using Nanodrop™ One (Thermo Scientific). All samples were normalized to a common concentration of 125 ng/μL with DNase/RNase free water and 1 μg of RNA from each sample was transcribed using the qScript XLT cDNA SuperMix (Quantabio) according to the manufacturer's instructions, including an additional gDNA wipe out step prior to transcription (Thermo Scientific DNase I, RNase-free). cDNA was stored at −20 °C until use.

For qPCR, primers (Table 1) were designed using primer 3 software version 0.4.0 (http://frodo.wi.mit.edu/primer3/) based on cDNA sequences available in NCBI (https://www.ncbi.nlm.nih.gov/), for an amplification size ranging from 65 to 100 nucleotides and optimal Tm of ~60 °C and secondary structures ~0 (within and between each primer

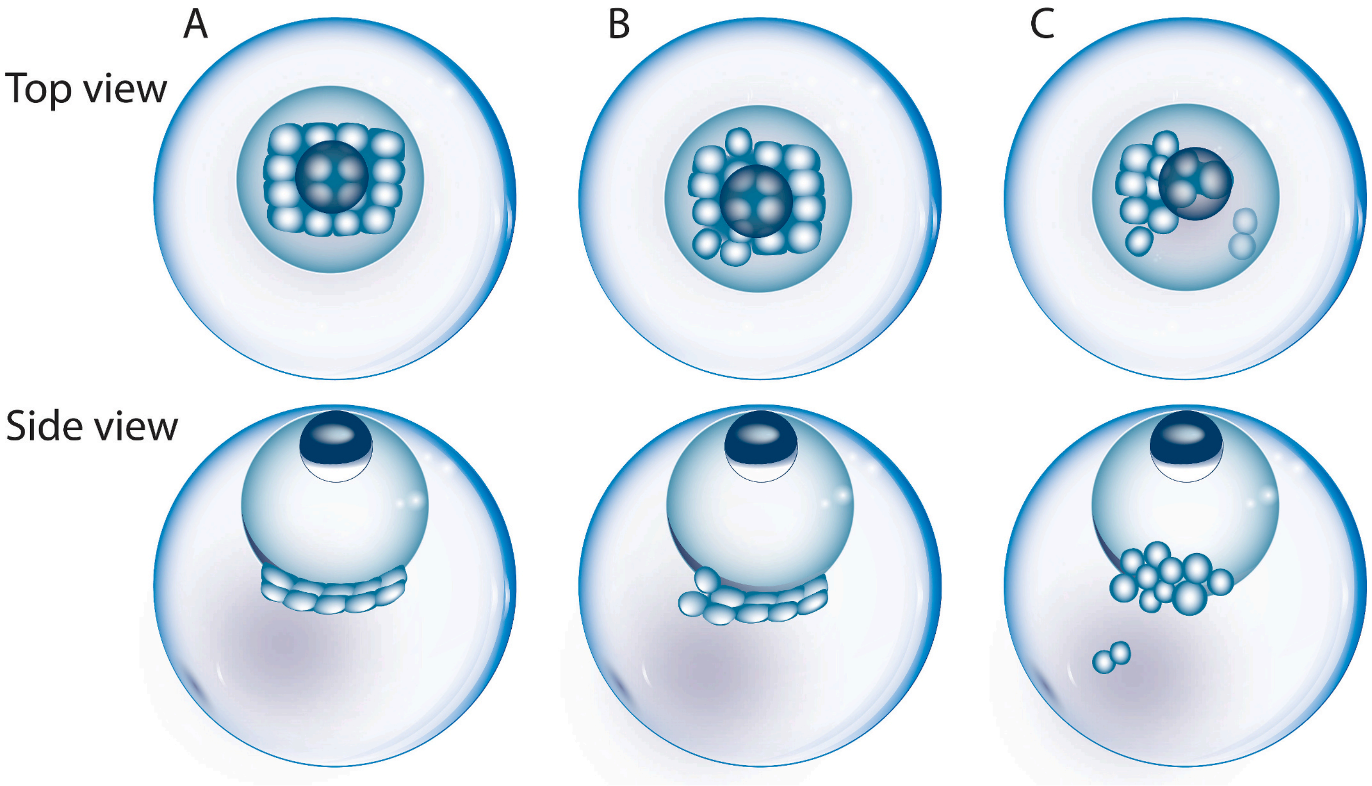

Fig. 1. Normal (A) and abnormal (B—C) cell cleavage patterns observed in European eel, *Anguilla anguilla*, embryos at 4 h post-fertilization.

Table 1
Sequences of European eel, *Anguilla anguilla* primers used for amplification of genes by qRT-PCR.

Full name	Abbreviation	Function	Accession number	Primer sequence (5′ 3′) (F: Forward; R: Reverse)
Cyclin A2	*ccna2*	Reference	XM_035424575.1	F: ATGGAGATAAAATGCAGGCCT R: AGCTTGCCTCTCAGAACAGA
Aurora kinase B	*aurkb*	Reference	XM_035432394.1	F: CCTCAAACACCCCAACATCC R: AGCTCCTCCATGTACGTTGC
Thyroid hormone receptor αa	*thαa*	Reference	KY082904.1	F: GCAGTTCAACCTGGACGACT R: CCTGGCACTTCTCGATCTTC
Insulin like growth factor receptor 1b	*igfr-1b*	Reference	XM_035396386.1	F: ATGGGAATCTTCAGCTCTTTAGA R: TCAAACTCCTCCTCCAAGCT
Epithelial cell adhesion molecule	*epcam*	Cell adhesion	XM_035401081.1	F: CTACTTGTGACGGAACCCCT R: AACTTAGTGCAGTCCAGGGG
Discoidin, CUB and LCCL domain containing 1	*dcbld1*	Cell adhesion	XM_035424145.1	F: ACCAGTCCACAGAGTTCACC R: CGTGTGCAGGTAGTCGTAGT
Plectin	*plec*	Structural protein	XM_035417968.1	F: TGGACACAACCTCATCTCCC R: CAAGTTCCTGACAACGTCCC
Centromere protein K	*cenpk*	Cell division	XM_035399751.1	F: CGAATGCTCTTACGGCTGAA R: ACAGCGAGCAAAACTTCAGG
Centromere protein F	*cenpf*	Cell division	XM_035410254.1	F: CAGAGCAAGCTAATGGACGC R: TCTTCACCTCCTCCTCCAGA

pairs). qPCR reactions were performed using 4 μL of cDNA as template, 5 μL of PowerTrack™ SYBR Green Master Mix (Applied Biosystems), and 0.25 μL of each primer in a final volume of 10 μl. Each sample was run in three technical replicates. After a 2 min incubation at 95 °C, the amplification was performed using the following cycle: 95 °C, 15 s; 60 °C, 1 min; 90 °C, 15 s, 40 times. Subsequently, amplification specificity was confirmed by performing a melting curve.

The relative quantity of target gene transcripts was normalized and measured using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The geometric mean of four reference genes (*ccna2*, *aurkb*, *thαa*, and *igfr-1b*) was used for normalization (Kottmann et al., 2021). The coefficient of variation (CV) of the technical replicates was calculated and checked to be <0.04.

2.9. Statistical analyses

Data were analysed through a series of two-way ANOVAs using R (version 4.1.2; R Core Team, 2023). For the gene expression and egg size variables, model assumptions were checked by testing residuals for normality (Shapiro-Wilk test) and homogeneity of variances (plot of residuals vs. fitted values). Models were fit using the nlme package (Pinheiro et al., 2024). All other variables were analysed with a generalized linear model (GLM) following a beta distribution (using "logit" as a link function) using the glmmTMB package (Brooks et al., 2017). Model diagnostics was performed using the package DHARMa for R (Hartig and Lohse, 2022). Alpha was set at 0.05 for testing main effects and interactions. Post-hoc analysis were performed using the emmeans package for R (Lenth et al., 2024). If a significant treatment × group interaction was detected, the model was decomposed into a series of reduced ANOVA models to determine the effect of treatment for each quality group. This was the case for C18:0, C20:0, and total lipids.

3. Results

3.1. Reproductive success and quality group assignment

Female information and reproductive success for each treatment and quality group is presented in Table 2 (individual data can be found in Supplementary Table A2). To this end, larval survival at 10 dph (first-feeding stage) was used as an ultimate measure of egg quality. Here, batches from the low-quality group did not survive to 10 dph whereas those from the high-quality group had a mean survival of 16.1 ± 12.5%. Female weight was similar among the four groups ($p = 0.280$) and treatments ($p = 0.359$), and therefore it was excluded as a covariate in the subsequent analyses. Likewise, fertilization success was similar for the quality groups ($p = 0.450$) and between SPE and CPE treated females ($p = 0.373$). However, and in agreement with the criteria used for quality assignment, embryonic survival was on average 1.8 times higher in the high-quality than in the low-quality group at 24 hpf ($p = 0.017$), and this difference increased through development, being 4.4 times higher at 48 hpf ($p = 0.001$). In both cases, embryonic survival was similar between treatments ($p = 0.052$ and $p = 0.732$, respectively). Similarly, although hatch success was not influenced by the hormonal treatment ($p = 0.516$), it was 5.8 times higher for the high-quality batches ($p = 0.001$). Variability was high within all groups.

3.2. Embryonic morphological traits

Egg diameter was not influenced by the hormonal treatment ($p = 0.171$) and was similar for both egg quality groups ($p = 0.924$) (Fig. 2A;

Table 2
Summary of female data and reproductive success for PE treatments and quality groups. Survival at 4 hpf was used as the initial value for estimating survival at 24 and 48 hpf, and hatch success. Larval survival was estimated as the percentage of surviving larvae from the total of hatched larvae.

Treatment	Egg quality	Number of females	Weight (g)	Length (cm)	Eggs (g)	Fertilization success (%) 4hpf	Survival at 24 hpf (%)	Survival at 48 hpf (%)	Hatch success (%)	Survival at 10 dph (%)
SPE	High	6	950.00 ± 312.15	72.67 ± 7.67	456.80 ± 212.78	32.90 ± 23.02	57.90 ± 22.24	20.51 ± 10.35	16.26 ± 10.29	19.62 ± 10.42
CPE	High	6	752.67 ± 134.98	70.08 ± 4.84	308.67 ± 120.40	42.92 ± 29.70	40.65 ± 15.79	16.55 ± 15.41	12.99 ± 16.21	12.53 ± 14.34
SPE	Low	7	722.57 ± 227.77	66.57 ± 6.67	297.71 ± 116.83	27.12 ± 28.99	26.16 ± 25.09	5.18 ± 12.20	3.94 ± 9.96	0.00 ± 0.00
CPE	Low	7	726.86 ± 256.24	69.07 ± 6.90	327.14 ± 188.59	31.12 ± 31.19	27.29 ± 12.50	3.18 ± 3.28	1.06 ± 1.24	0.00 ± 0.00

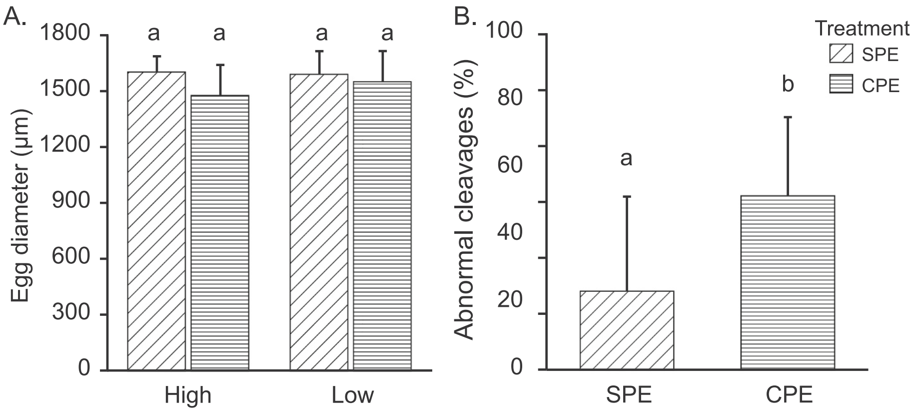

Fig. 2. Egg diameter (A) and percentage of abnormal cleavages (B) of 4 h post-fertilization embryos of European eel, *Anguilla anguilla*, from high and low egg quality batches originating from females subjected to SPE or CPE hormonal treatments. Data were analysed through a series of two-way ANOVAs. Alpha was set at 0.05. Different letters indicate statistically significant differences among groups.

Supplementary Table A2). The percentage of abnormal cleavages at 4 hpf, on the other hand, was 2 times higher for the CPE than for the SPE treatment ($p = 0.030$), with similar values for the quality groups ($p = 0.728$) (Fig. 2B; Supplementary Table A2).

3.3. Biochemical composition of eggs

Total protein content of unfertilized eggs was similar for both hormonal treatments and for both quality groups ($p = 0.785$, $p = 0.868$, respectively) (Fig. 3A; Supplementary Table A2). A significant hormonal treatment x group interaction was found for the unfertilized eggs' total lipid content ($p = 0.003$). Therefore, the effect of the hormonal treatment on this variable was evaluated for each group. No significant effect was found between treatments for the low-quality group ($p = 0.210$). However, lipid content was significantly influenced by hormonal treatment in the high-quality group ($p = 0.004$), being 1.1 times higher for the batches from SPE treated females than CPE ones (Fig. 3B; Supplementary Table A2).

Egg relative fatty acid composition according to groups is shown in Table 3. The sum of SFA was similar for the two quality groups and for both treatments. Thus, no differences were found among groups or treatments for C14:0, C15:0, and C16:0. However, an effect for the treatment x group interaction was found for C18:0 and C20:0. In both cases, the relative levels were higher for the high-quality group than the low-quality within the CPE treatment ($p = 0.008$, $p = 0.046$, respectively) but similar within the SPE treatment batches. No significant differences were found for the relative levels of C16:1 and C20:1 neither for the quality groups nor the hormonal treatments. However, there was a treatment effect for C18:1n9 levels as well as for the sum of MUFA, with higher levels for the CPE treatment ($p = 0.025$, $p = 0.047$, respectively). Similarly, the sum of PUFA, sum of n-3 PUFA, n-3/n-6 ratio, as well as the relative levels of EPA and DHA were significantly higher for the batches from CPE treated females compared to those from SPE treated females ($p = 0.013$, $p = 0.001$, $p = 0.006$, $p = 0.018$, $p = 0.001$, respectively), irrespective of quality group. The levels of the other n-3 PUFA and n-6 PUFA did not differ among treatments and quality group, neither did the EPA/ARA and DHA/EPA ratios differ.

3.4. Gene expression

Gene expression levels in terms of relative transcript abundance of *plec*, *cenpf* and *dcbld1* (Fig. 4A; Supplementary Table A4) were similar for the two quality groups ($p = 0.332$, $p = 0.690$, $p = 0.737$, respectively) and in batches from both hormonal treatments ($p = 0.775$, $p = 0.726$, $p = 0.208$, respectively). No significant differences were found between the expression of these genes in unfertilized eggs and in embryos at 4 hpf ($p = 0.422$, $p = 0.107$, $p = 0.099$, respectively).

No significant differences were detected for *cenpk* (Fig. 4B; Supplementary Table A4) between treatments ($p = 0.727$) and developmental stages ($p = 0.182$). However, this gene showed a higher expression in the low-quality group compared to the high-quality batches ($p = 0.047$).

There was a significant treatment x developmental stage interaction

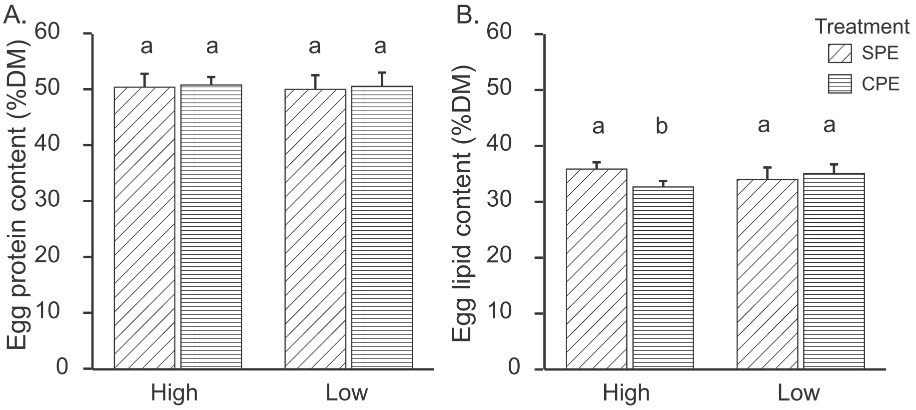

Fig. 3. Egg total protein content (A) and total lipid content (B) of European eel, *Anguilla anguilla*, unfertilized eggs from high- and low-quality batches, originating from females subjected to SPE or CPE hormonal treatments. Data were analysed through a series of two-way ANOVAs. Alpha was set at 0.05. For total lipids, a significant treatment × group interaction was detected, and the model was decomposed into a series of reduced ANOVA. Different letters indicate statistically significant differences among groups; for total lipids different letters indicate statistically significant differences between treatment within each quality group.

Table 3
Relative fatty acid composition (% of total fatty acids) of total lipids extracted of unfertilized eggs of European eel females according to high- and low-quality groups and hormonal treatments.

Fatty acid (FA; % Total FA)	SPE		CPE	
	High	Low	High	Low
C14:0	1.81 ± 0.34	1.86 ± 0.53	1.49 ± 0.20	1.58 ± 0.30
C15:0	0.19 ± 0.02	0.19 ± 0.03	0.17 ± 0.01	0.19 ± 0.02
C16:0	16.88 ± 0.67	16.90 ± 0.59	16.83 ± 0.60	16.76 ± 0.43
C16:1	4.21 ± 0.69	4.24 ± 0.93	3.85 ± 0.62	3.96 ± 0.53
C18:0	3.50 ± 0.20	3.510 ± 0.32	3.80 ± 0.14 ^{A}	3.41 ± 0.07 ^{B}
C18:1n9	28.78 ± 0.87 ^{a}	28.30 ± 1.36 ^{a}	26.90 ± 1.04 ^{b}	27.17 ± 2.04 ^{b}
C18:2n6	5.90 ± 0.48	5.67 ± 0.61	6.22 ± 0.13	6.11 ± 0.78
C18:3n3	5.21 ± 0.90	4.86 ± 0.75	5.43 ± 0.28	5.16 ± 0.72
C18:3n6	1.07 ± 0.08	0.99 ± 0.11	1.11 ± 0.15	1.15 ± 0.18
C18:4n3	0.15 ± 0.04	0.20 ± 0.12	0.23 ± 0.04	0.24 ± 0.12
C20:0	0.15 ± 0.04	0.16 ± 0.03	0.19 ± 0.01 ^{A}	0.15 ± 0.03 ^{B}
C20:1	2.52 ± 0.67	2.85 ± 1.01	1.86 ± 0.46	2.29 ± 0.94
C20:4n3	0.37 ± 0.08	0.33 ± 0.09	0.41 ± 0.03	0.38 ± 0.05
C20:4n6 (ARA)	5.59 ± 0.34	5.55 ± 0.73	5.96 ± 0.31	5.68 ± 0.79
C20:5n3 (EPA)	2.91 ± 0.24 ^{a}	3.42 ± 0.87 ^{a}	3.57 ± 0.05 ^{b}	3.45 ± 0.33 ^{b}
C22:5n3	2.13 ± 0.26	2.11 ± 0.32	2.29 ± 0.15	2.25 ± 0.10
C22:6n3 (DHA)	10.13 ± 0.74 ^{a}	10.80 ± 0.88 ^{a}	11.67 ± 0.75 ^{b}	11.58 ± 0.81 ^{b}
Σ SFA	22.54 ± 0.86	22.62 ± 0.95	22.47 ± 0.74	22.09 ± 0.67
Σ MUFA	35.51 ± 1.65 ^{a}	35.38 ± 2.54 ^{a}	32.60 ± 1.65 ^{b}	33.42 ± 3.36 ^{b}
Σ PUFA	33.47 ± 2.26 ^{a}	33.92 ± 2.52 ^{a}	36.89 ± 0.81 ^{b}	36.01 ± 2.87 ^{b}
Σ n-3	20.91 ± 1.58 ^{a}	21.72 ± 1.39 ^{a}	23.60 ± 0.40 ^{b}	23.06 ± 1.54 ^{b}
Σ n-6	12.56 ± 0.80	12.21 ± 1.23	13.30 ± 0.41	12.94 ± 1.61
n-3/n-6	1.67 ± 0.08 ^{a}	1.79 ± 0.09 ^{a}	1.78 ± 0.03 ^{b}	1.80 ± 0.17 ^{b}
EPA/ARA	0.52 ± 0.07	0.64 ± 0.25	0.60 ± 0.04	0.61 ± 0.08
DHA/EPA	3.49 ± 0.36	3.33 ± 0.82	3.27 ± 0.24	3.38 ± 0.31

Note: Data were analysed through a series of two-way ANOVAs. Alpha was set at 0.05. If a significant treatment × group interaction was detected, the model was decomposed into a series of reduced ANOVA models to determine differences in quality groups for each treatment. This was the case for C18:0, C20:0. Different superscript letters indicate statistically significant differences among groups; for C18:0 and C20:0 different letters indicate statistically significant differences between quality groups within each treatment.

for *epcam* (Fig. 4C; Supplementary Table A4) expression ($p = 0.003$). The relative expression levels of this gene were similar for the quality groups ($p = 0.460$) and hormonal treatments ($p = 0.061$) in unfertilized eggs. On the other hand, higher *epcam* expression was observed in 4 hpf embryos for the SPE treated batches compared to the CPE treatment ($p < 0.001$).

4. Discussion

In this study, we evaluated the use of different markers of egg quality from farmed-raised female European eel broodstock subjected to different PE administration schemes. It is the first time for that the two most common SPE and CPE treatments schemes constant and increasing doses, respectively, have been compared, and associated with an analysis of potential biomarkers. The integration and evaluation of molecular, biochemical, and morphological biomarkers provides a first framework for selection and utilization of these tools in future work enhancing embryonic and larval survival.

Relating the occurrence of cleavage abnormalities to molecular markers has been used to identify quality parameters for European eel reproductive success (Kottmann et al., 2020a), based on the cleavage patterns described by Sørensen et al. (2016a) for this species. The occurrence of abnormal cleavages (cells that are not regularly or symmetrically cleaved) or cells displaying weak or no cell adhesion have been linked to a reduced embryonic survival and therefore related to poor egg quality in several species (Avery et al., 2009). Each species displays specific cell cleavage patterns and abnormalities that can be used as indicators of egg quality. Also, fertilized eggs with incomplete cleavages have been observed in eggs with an underdeveloped perivitelline space resulting from an incomplete cortical reaction (Valdebenito et al., 2015). Therefore, these eggs could have a smaller size after fertilization than good quality ones. In the present study, egg diameter was similar for all groups. However, embryos from SPE treated females showed a lower frequency of abnormal development than embryos from the CPE treatment, in all quality groups. These results support previous studies comparing constant SPE and CPE doses (Kottmann et al., 2020a). The occurrence of abnormal development relates to attenuated cell adhesion, which has been previously linked to a low relative transcript abundance of *epcam* and *dcbld1* genes (Kottmann et al., 2020a). Here, maternal supply of mRNA to the developing egg can be affected by the conditions experienced by the mother (Adrian-Kalchhauser et al., 2020; Brooks et al., 1997). This is particularly relevant in the context of aquaculture practices, as induced maturation and ovulation by light regime manipulation or hormonal treatment (Bonnet

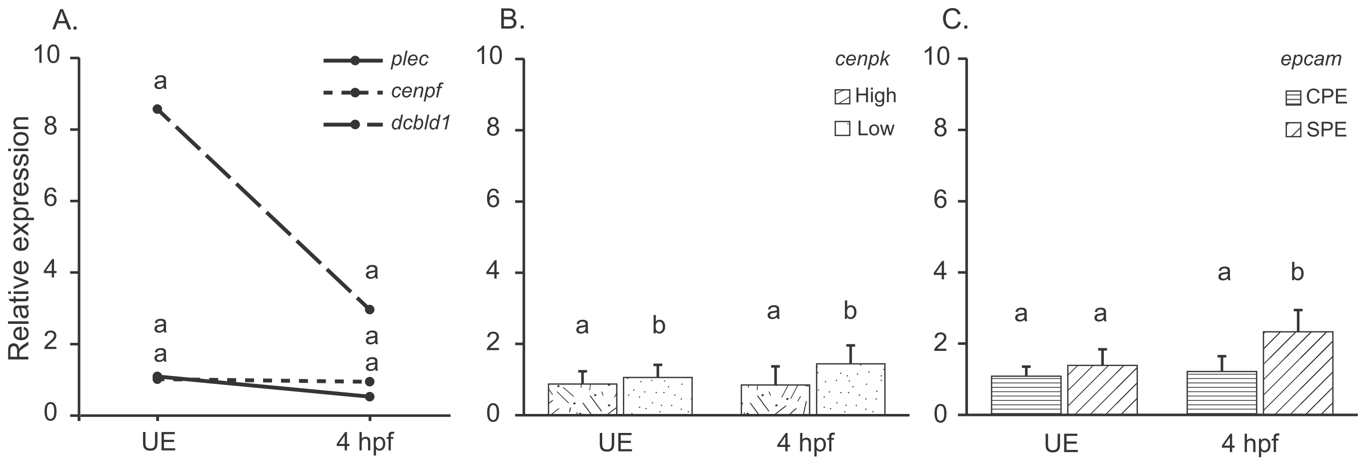

Fig. 4. mRNA transcript abundance in European eel, *Anguilla anguilla* unfertilized eggs and embryos at 4 h post-fertilization for (A) *plec*, *cenpf*, and *dcbld1* (B) *cenpk*, and (C) *epcam*. Different letters represent significant differences between treatments/groups within each stage ($p < 0.05$). Data were analysed through a series of two-way ANOVAs. Alpha was set at 0.05. Different letters indicate statistically significant differences among groups.

et al., 2007) as well as the induction of vitellogenesis by hormonal administration (Kottmann et al., 2020a) may result in changes in the abundance of mRNA transcripts that are transferred to the developing eggs and can affect their developmental potential.

Previous studies have shown that *epcam* mRNA is detectable in fertilized eggs and 2-cell stage embryos (Trzpis et al., 2008; Trzpis et al., 2007), and distributes uniformly in all blastomeres from 4 to 16-cell stages (Slanchev et al., 2009). This gene is involved in cell-cell adhesion, and plays an essential role during epiboly by promoting the integrity of the enveloping layer (Slanchev et al., 2009). In the present study, no initial differences in *epcam* transcript levels were found between treatments, i.e., in unfertilized eggs. However, lower *epcam* mRNA levels were observed in 4 hpf embryos (16-cell stage) for batches from CPE treated females compared to SPE ones, in agreement with previous studies (Kottmann et al., 2020a). The function of *dcbld1* on the other hand remains largely unknown (Schmoker et al., 2019), but the gene is considered to be involved in fundamental cellular processes during development, such as cell adhesion (Schmoker et al., 2020; Schmoker et al., 2019). Although previous studies have found a higher *dcbld1* transcript levels in 2 hpf embryos from females treated with a constant SPE dose compared to females administered with a similar CPE scheme (Kottmann et al., 2020a), our results show no difference in *dcbld1* in initial transcript abundance levels between hormonal treatments. This does not appear to be sufficient to compensate for low *epcam* transcript levels as observed in embryos from CPE treated females, leading to a compromised cell adhesion and coinciding with a higher frequency of abnormal cleavages.

Low levels of *epcam* and *dcbld1* mRNA transcripts have previously been associated with a higher incidence of cleavage abnormalities leading to embryonic mortality (Kottmann et al., 2020a). However, the present study showed no significant differences in *epcam* or *dcbld1* mRNA levels between quality groups in unfertilized eggs. Similarly, a study in Atlantic cod found that *dcbld1* expression was not significantly correlated to egg quality, yet showing a great variability among batches (Rise et al., 2014). As batches with lowest and highest egg quality showed a 50-fold difference, the authors suggested that although *dcbld1* may not be used as a single egg quality marker, it may potentially be analysed in combination with other genes as a suite of biomarkers. In this regard, based on the present and previous (Kottmann et al., 2020a) studies on eel, it appears that *epcam* and *dcbld1* transcripts are linked to cell cleavage and adhesion involved in normal versus abnormal development patterns. Therefore, these genes appear promising as indicators of egg quality for European eel. However, further studies are needed to fully elucidate the roles of these genes and to which extent different cleavage abnormalities will affect the progress of embryonic development in individual eggs considering differences among cleavage abnormalities.

Among other genes, the cytolinker protein plectin anchors the intermediate filaments to different cytoskeletal junctional complexes and organelles, and interacts with the actomyosin machinery and microtubules (Wiche, 2021; Wiche et al., 2015; Wiche and Winter, 2011). These maternally derived transcripts that control cell division during early development regulate spindle and furrow formation and promote cellular cohesiveness delivering adhesive molecules to the cell surface (Lubzens et al., 2017). Differential mRNA abundance of genes coding for cytoskeleton proteins was observed in batches of high and low egg quality in rainbow trout originating from females subjected to photoperiod manipulation or hormonal ovulation induction (Bonnet et al., 2007). Moreover, a higher *plec* transcript level was found to be linked to high egg quality in sea bass, suggesting it could be used as a molecular marker of egg quality (Żarski et al., 2017). However, in the present study no differences were found in *plec* expression for batches in high- and low-quality groups, independently of the hormonal treatment applied.

The centromere proteins (CENPs) are also essential for cell division. They are part of the mitotic/meiotic kinetochore which assembles ensuring correct chromosome attachment to the microtubules and movement towards the poles (Cheeseman et al., 2008; Perpelescu and Fukagawa, 2011). Expression of genes encoding for different CENPs has also been related to egg quality. The ovarian expression of *cenpk* was downregulated in female striped bass that produced low quality eggs (Chapman et al., 2014). Similarly, the *cenpf* transcript level was significantly lower in low quality eggs of sea bass (Żarski et al., 2017). In the present study, although *cenpf* transcript levels were similar for all groups and treatments, we found that *cenpk* was differentially expressed between egg quality groups. In contrast to results obtained for striped bass, as we found that *cenpk* transcript levels were higher in unfertilized eggs of low quality. This differential transcript level continued to be observed in embryos 4 h post fertilization. *Cenpk* might therefore also be useful as a biomarker for egg quality in European eels, although further studies are needed to better understand the transcript profiles of *cenp* genes in this species during early development and how they functionally relate to egg quality. Considering the often high variability, larger scale evaluation of biomarkers is also recommended to ascertain significance.

The second aspect of the present study concerns biochemical markers. Here maternal supply of fatty acids in eggs have a direct impact on embryo and early larval viability (Rainuzzo, 2020). In particular, normal embryonic development depends on the content of specific PUFAs from the n-3 and n-6 series, termed essential fatty acids (Tocher, 2010). In the present study, broodstock females were fed an enhanced diet formulated based on previous studies by Støttrup et al. (2016, 2013) and further developed by Kottmann et al. (2020b), which contains similar EPA and DHA levels as the former but higher ARA, and consequently a lower EPA/ARA ratio. Higher dietary ARA intake results in an accumulation of this fatty acid in the ovaries and is positively correlated with egg ARA content in European eel (Støttrup et al., 2016). Accordingly, the increase in broodstock dietary levels of ARA was reflected in an overall higher ARA level in the unfertilized eggs in this study compared to the values previously reported for farm-raised broodstock in this species (Kottmann et al., 2020b).

Previous studies in European eels indicate that egg batches from farmed-reared broodstock with higher ARA content had a better performance in terms of fertilization success and larval survival (Kottmann et al., 2020b). In the present contribution, unfertilized eggs from low and high quality batches had similar ARA content, similar to the results obtained for wild-caught European eel broodstock (da Silva et al., 2018). ARA is the main precursor of eicosanoids which are cyclic compounds involved in ovulation, spawning, and osmoregulation, among other processes (Di Costanzo et al., 2019). However, EPA competitively interferes with ARA as substrate for the eicosanoid-producing enzymes, and it is converted to less biologically active compounds (Sargent et al., 2003). For this reason, the EPA/ARA ratio in eggs may affect the embryonic development and subsequent larval survival (Bell et al., 1997). In Japanese eel, increasing levels of EPA compared to ARA were associated to low quality in eggs with a lower fertilization rate (Furuita et al., 2007; Furuita et al., 2006). Furthermore, low fertilization and hatching rates, and larval survival were obtained for eggs containing high EPA/ARA ratios in Eurasian perch (Henrotte et al., 2010). However, in this study, unfertilized eggs of low and high quality displayed similar EPA/ARA ratios, as well as individual EPA and ARA levels, which supports that quality differences were not related to dietary constraints of these fatty acids or their ratios.

Alongside ARA and EPA, the importance of adequate DHA levels in eggs for embryonic development and larval survival has been highlighted for several species, including Japanese eel (Furuita et al., 2007), Atlantic bluefin tuna (Morais et al., 2011), Atlantic halibut (Evans et al., 1996) and Eurasian perch (Henrotte et al., 2010). DHA, which is the desired main fatty acid in the phospholipids of cell membranes, as it tends to increase their fluidity, is highly required for larval neural function and therefore, larval competence is dependent on egg DHA content (Bell et al., 1997). Moreover, as DHA competes with other n-3 and n-6 PUFA for the enzymes involved in chain elongation and further desaturation reactions, the DHA/EPA ratio can also impact egg quality, as was observed for cod where DHA/EPA ratio was positively correlated to hatch success (Pickova et al., 1997; Røjbek et al., 2014). However, in the case of European eel, DHA level and DHA/EPA ratio were similar for low and high quality eggs both in wild-caught (da Silva et al., 2018) and farmed broodstock (present study).

On the other hand, in this study the levels of EPA and DHA in unfertilized eggs differed between hormonal treatments irrespectively of quality groups and was higher for batches from CPE treated females, while ARA levels were similar for both treatments. Previous studies in wild-caught European eel found no influence of treatment, using weekly administration of a constant SPE or CPE dose, on EPA, ARA, and DHA levels in embryos and larvae, while their relative levels increased during embryonic and yolk sac larval development (Benini et al., 2022). In this regard, probably due to the high lipid utilization during embryonic development, the small initial differences found in this contribution between treatments were not observed in later larval stages.

The amount and composition of lipids accumulated in the egg during its formation is another factor that can affect egg quality, as lipids play key roles for embryos, such as energy storage and cell membrane structure (Bobe and Labbé, 2010; Reading et al., 2018). High-quality unfertilized eggs from batches of females from the SPE treatment contained a higher amount of total lipids. However, this difference was not observed in the low-quality group. Interestingly, fertilized embryos from females treated with a constant dose of CPE had a higher amount of lipids than SPE ones (Benini et al., 2022). This suggests that not only the hormone source but also the administration scheme can affect the amount of nutrients that are deposited in the oocytes during vitellogenesis.

5. Conclusions

Based on the results obtained for *cenpk* expression, this gene could potentially be used as an egg quality indicator in European eel in combinations with those previously described for the species. Defining and testing such molecular biomarkers is necessary for understanding the causes for high mortality in early stages of eel development. As such, these promising first results can be used as basis for further large scale studies.

In general, batches from females subjected to the SPE treatment had a lower percentage of cleavage abnormalities than those from CPE treated females. Accordingly, a higher *epcam* transcript level was observed in unfertilized eggs and 4 hpf embryos from those batches. Further studies evaluating individual embryonic development and larval performance are needed to assess to which extent different types of abnormal cleavages lead to a reduced survival or to the incidence of larval deformities. Based on the results obtained, *cenpk* transcript levels appear useful as an egg quality indicator in this species, possibly in combination with those previously described for the European eel, including *epcam* and *dcbld1* transcript abundances as egg quality markers. These biomarkers may prove useful for future improvement of new and existing methodologies inducing vitellogenenesis.

Overall, the customized broodstock diet appears to be adequate in terms of essential fatty acid provisioning to the developing eggs. Fatty acid composition in unfertilized eggs was in general similar for batches, surviving or not up to the first-feeding larval stage, although the PE administration scheme applied influenced the maternal provisioning of DHA and EPA. This suggests that even in female broodstock fed the same high-quality diet, the hormonal treatments applied for vitellogenesis induction can influence the amount of specific fatty acids being transferred to the eggs.

CRediT authorship contribution statement

Daniela Eliana Sganga: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Francesca Bertolini:** Writing – review & editing, Methodology. **Luis E.C. Conceição:** Writing – review & editing, Resources, Methodology. **Paraskevas Koumpiadis:** Writing – review & editing, Investigation. **Jonna Tomkiewicz:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741512.

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