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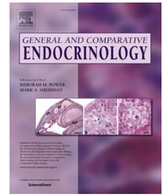

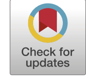

Recent insights into egg quality and larval vitality of the European eel *Anguilla anguilla*

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ABSTRACT

To date, the eel industry still depends on wild-caught juveniles that are grown to marketable size. There is an urgent need to close the eel life cycle in captivity to make aquaculture independent of the natural population. With this artificial reproduction protocol, yolk-sac larvae can be produced but egg quality may be impaired. Low survival rates and high deformity rates are frequently observed during the first week after hatching. Over the past four years, we have conducted studies with the aim to optimize the artificial reproduction protocol, thereby focussing on increasing egg and larval quality. Weekly carp or salmon pituitary extract (PE) treatment was successfully replaced with recombinant gonadotropins (rGTHs) to mature female eels and produce larvae. 17α,20β-dihydroxy-4-pregnen-3-one (DHP) was replaced with upstream precursor progesterone (P) to induce the endogenous production of DHP by the female eel. DHP and P were found equally potent in inducing oocyte maturation and ovulation. The effects of antibiotics on larval survival and the occurrence of deformities were investigated. Antibiotic treatment increased survival and decreased the occurrence of deformities indicating bacterial infection as an important cause. A deformity determination key for young eel larvae has been developed that provides a framework of reference for larval deformities which will be instrumental with gaining insights on the reasons behind each larval deformity. These improvements of the artificial reproduction protocol and hatchery practices will contribute to the production of robust eel larvae that survive, grow and metamorphose into juveniles that will later be able to reproduce in captivity.

1. Introduction

1.1. Eel propagation

Since the European eel cannot be propagated in captivity yet, the eel industry still depends on wild-caught glass eels that are grown to marketable size. There is an urgent need to close the life cycle of this species in captivity to make aquaculture independent of the natural population. In captivity, females are weekly injected with carp or salmon pituitary extract (PE) to induce oocyte growth. When the oocytes are becoming mature, the females are injected with an extra PE booster injection, followed by 17α,20β-dihydroxy-4-pregnen-3-one (DHP) injection to induce oocyte maturation and ovulation. With this hormonal treatment, larvae batches are now regularly produced by several European groups (Mordenti et al., 2013; Asturiano et al., 2016; Jéhannet et al., 2021; Benini et al., 2022) but egg quality is insufficient as exemplified by low hatching rates and larval survival (Da Silva et al., 2018). Further advances in increasing the reproductive success are expected to come from the improvements of artificial reproduction protocols and hatchery practices with the ultimate goal of producing high-quality eggs and larvae that are able to survive, feed and grow.

1.2. How to increase egg quality?

Egg quality has been defined by several authors as the ability of an egg to be fertilized and subsequently develop into normal offspring (Brooks et al., 1997; Bobe and Labbé, 2010). In European eel, poor egg quality is a limiting factor for mass production of juveniles. Good-quality eggs have been defined in eels as those with high fertilization rates, normal cell division patterns, high hatching success and larval survival (Furuita et al., 2003; Da Silva et al., 2018; Kottmann et al., 2020b). A wide variety of factors like the broodstock diet, the environmental

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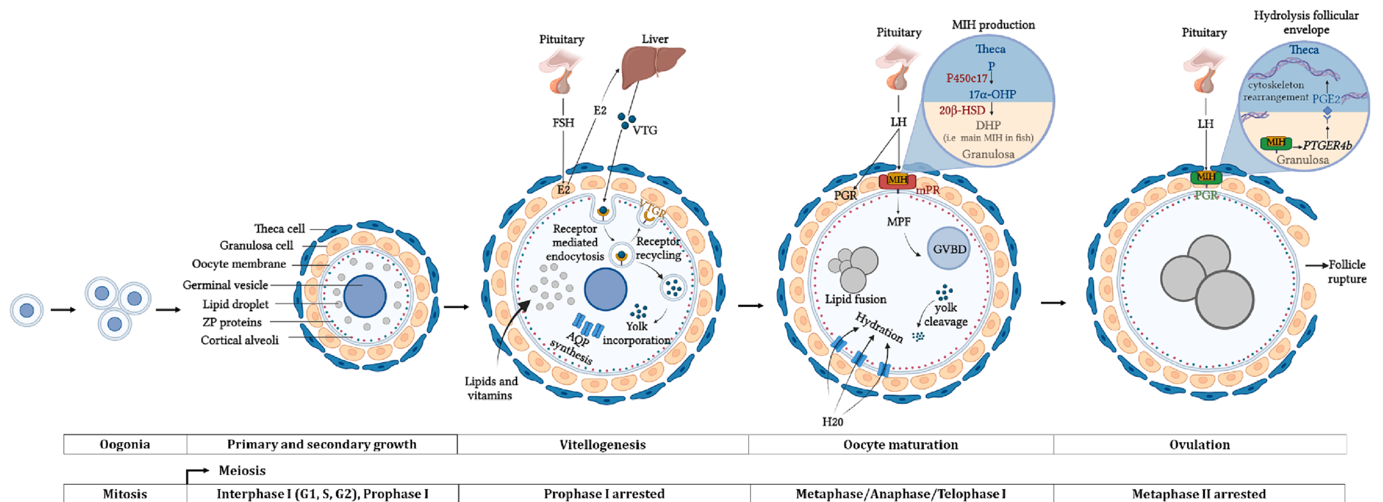

Fig. 1. Oocyte development in fish, with emphasis on oocytes containing lipid droplets that eels have. For inducing vitellogenesis, follicle-stimulating hormone (FSH) stimulates the production of ovarian estrogen (E2) that in turn promotes the production of vitellogenin (VTG) that binds to its receptor (VTGR) on the oocyte membrane. VTG is then incorporated into small egg yolk proteins. Lipids and vitamins are deposited within the ooplasm. Aquaporins (AQP) are synthesized during the vitellogenic growth phase. For inducing oocyte maturation, luteinizing hormone (LH) induces the production of maturation-inducing hormone (MIH) as follows: progesterone (P), which is formed in the theca cells, is converted into 17α-hydroxyprogesterone (17-OHP) that diffuses into the granulosa cells to be converted into 17α,20β-dihydroxy-4-pregnen-3-one (DHP, i.e. the main MIH in fish). Two enzymes, 17α-hydroxylase cytochrome P450 (P450c17) and 20β-hydroxysteroid dehydrogenase (20 β-HSD), are responsible for the conversion of P into DHP. Following its production, MIH binds to its membrane progestin receptors (mPR) to activate the formation of the maturation-promoting factor (MPF) that promotes germinal vesicle breakdown (GVBD). In addition, LH prepares the oocyte for ovulation by stimulating the synthesis of the nuclear progestin receptor (PGR). Lipid droplets fuse together to form a few lipid globules and the oocyte hydrates. For hydration to occur, the AQPs are translocated into the oocyte membrane where they can mediate water transport. Water is driven within the oocyte by an increased osmotic pressure that results from the cleavage of the yolk into free amino acids. Prior to ovulation, MIH binds to its PGR that is anchored in the granulosa cells to act as a critical transcription factor of the prostaglandin receptor (PTGER4b). Following the binding of prostaglandin (PGE2) to its receptor, the follicle cells undergo cytoskeleton rearrangement that leads to follicle rupture and the release of the oocyte from its surrounding follicles. Following ovulation, the oocyte is ready to be fertilized. The schematic overview was created with Biorender and inspired by Nagahama and Yamashita, 2008, Cerdà 2009, Lubzens et al., 2010 and Hara et al., 2016.

conditions, the hormonal stimulation of gonad development and the age of the fish are thought to influence egg quality (for review see Brooks et al., 1997; Bobe and Labbé 2010). In this mini-review, we present our recent findings on the importance of the used broodstock and the hormonal therapy to induce oocyte development in dictating egg quality in European eels.

1.2.1. Broodstock conditioning

At our facilities, experiments have been performed with wild and feminized eels. For inducing the feminization, juveniles are fed with commercial pellets coated with E2 for two months. Besides shortening the generation time to just two years, feminized eels seem to be more sensitive to hormonal stimulation later in life (Ijiri et al., 1998; Chai et al., 2010; Palstra et al., 2023). Feminized eels and wild eels performed equally well when it came to the percentage of females reaching sexual maturation, which increased up to 97 % in 2022 in our facilities. However, feminized eels released their eggs later after the final DHP injection than wild females (Jéhannet et al., 2023a). In eels, it is known that females that spawn rapidly following the DHP injection, produce better-quality offspring than females that release their eggs relatively late (Ohta et al., 1997; Palstra and van den Thillart, 2009). This is in line with our recent study showing that feminized eels had lower hatching rates and that their larvae died earlier than the larvae produced by wild eels (Jéhannet et al., 2024). Our study suggest that egg quality is lower in feminized eels when compared to wild females which is consistent with the results obtained in Japanese eels (Ijiri et al., 1998). The superior quality of eggs of wild eels over eggs of farmed eels is believed to be largely influenced by the broodstock diet (Støttrup et al., 2015; Kottman et al., 2020a). Since a large amount of lipid is incorporated in the ovaries during oocyte development in European eel (Palstra et al., 2006), the broodstock diet highly impacts the egg quality (Heinsbroek et al., 2013; Støttrup et al., 2015). Thus, in order to improve egg quality and larval vitality, studies should be performed using wild females. When juveniles can be produced and are subsequently used for closing the cycle, procedures for feminization, broodstock diets and naturally induced silvering require renewed research attention.

1.2.2. Hormonal stimulation of oocyte development

1.2.2.1. Oocyte development. It is essential to understand the mechanisms of oocyte development since good egg quality depends on the correct progression through each developmental phase (Brooks et al., 1997). The mechanisms of oocyte development, with special emphasis on eels (Fig. 1), were investigated to identify potential treatments to improve the current artificial reproduction protocols. In fish, oocyte development is controlled by a cascade of hormones along the brain-pituitary–gonadal (BPG) axis (reviewed by Nagahama, 1997; Swanson et al., 2003; Zohar et al., 2010; Levavi-Sivan et al., 2010). When the oocyte enters vitellogenesis (reviewed by Planas & Swanson 2008; Lubzens et al., 2010; Hara et al., 2016), it grows considerably by accumulating egg yolk proteins that are stored as nutrients for the development of embryos and yolk-sac larvae (Sire et al., 1994; Montserrat et al., 2004; Hara et al., 2016). As previously reported for other fish species (Planas & Swanson 2008; Lubzens et al., 2010), we provided evidence that specifically FSH induces vitellogenic growth in European eels (Jéhannet et al., 2023b).

After the vitellogenic growth period, the oocyte enters maturation to resume meiosis. Oocyte maturation is characterized by several events such as hydration, lipid fusion and germinal vesicle breakdown (GVBD). In eels, like in other marine species producing buoyant eggs, oocyte hydration is very pronounced (Cerdà et al., 2007) and water contributes up to 95 % of the final volume of the egg (Seoka et al., 2003). Water intake is driven by the cleavage of the yolk that was stored during vitellogenesis into free amino acids (Greeley et al., 1986). While

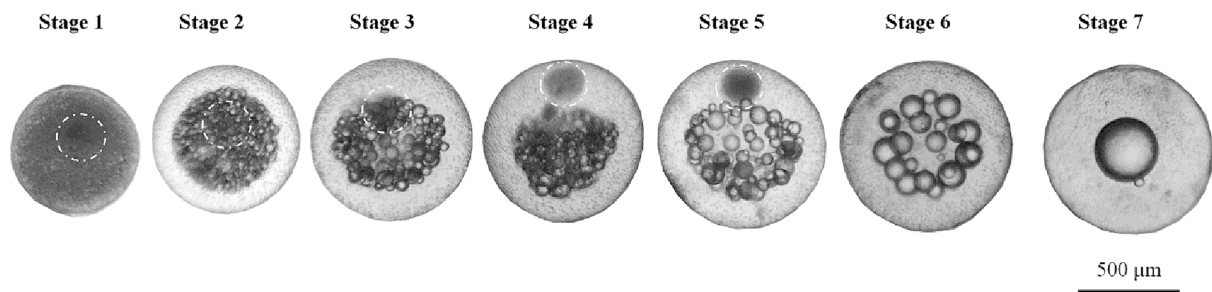

Fig. 2. Oocyte maturation in European eels on basis of the staging described by Palstra et al. (2005). Oocytes were fixed in Serra's solution to stain the germinal vesicle (encircled in white). During oocyte maturation, the germinal vesicle migrates from the center of the oocyte towards the periphery (stages 1–5) where the nuclear envelope disintegrates (stages 6–7). In addition, the oocyte clarifies and increases in size due to the fusion of the lipid droplets and hydration response, respectively.

hydrating, the lipid droplets fuse together in just a few lipid globules at the moment of fertilization (Palstra et al., 2005). For inducing GVBD, LH stimulates maturation-inducing hormone (MIH) production (Nagahama & Yamashita, 2008). For Japanese eel, it was found that oocytes were able to produce MIH when treated with a high dose of PE mimicking the LH surge (method developed by Dr. Kagawa and described by Ijiri et al., 2023). For European eel, the production of MIH by the ovarian follicles after treatment with PE has not been shown. Following MIH production, MIH binds to the membrane progestin receptors (mPRs) on the oocyte surface to induce GVBD (Ikeuchi et al., 2002; Nagahama & Yamashita, 2008). In European eel, we showed that mPR expression remained stable in our *in vitro* experimentations of maturating oocytes (Jéhannet et al., 2023b) which may imply that the mPRs were already present on the oocyte surface during oocyte maturation. Alike the LH effect on oocyte maturation, the action of LH on ovulation is indirect since it appears to be mediated by the MIH (Takahashi et al., 2019). Based on the work of Hagiwara et al. (2014) in medaka, MIH binds to the nuclear progestin receptor (PGR) to act as a transcriptional factor of the prostaglandin receptor (PTGER4b) during ovulation. Our recent findings in European eel are consistent with this mechanism as we found that MIH treatment (1) downregulated both progestin receptors (*pgr1* and *pgr2*), and (2) increased *ptger4b* expression in maturing oocyte of eels *in vitro* (Jéhannet et al., 2023a). Surprisingly, the follicle-stimulating hormone receptor (FSHr) appeared to play a role during ovulation in European eels since its transcript increased in a similar fashion as *ptger4b* in maturing oocytes of eels (Jéhannet et al., 2023a). For ovulation to occur, it is known that the follicle cells undergo cytoskeleton rearrangement to allow follicle rupture and the release of the oocyte from its surrounding follicles (Takahashi et al., 2019). This is in line with our transcriptomic analysis in which we showed that numerous transcripts related to apoptosis, cytoskeleton organization, and extracellular matrix remodeling changed between the time of spawning induction and egg release (Jéhannet et al., 2023a).

1.2.2.2. Hormonal therapy to induce vitellogenic growth. Currently, female European eels are sexually matured by injecting carp or salmon PE as this hormonal mixture contains FSH and LH (Minegishi et al., 2012). Besides FSH and LH, PE may also include other hormones like growth hormone (GH), prolactin (PRL), somatolactin (SL) and thyroid-stimulating hormone (TSH) that regulate other physiological processes. For better control of artificial reproduction protocols, recombinant gonadotropins (rGTHs) have been developed for numerous fish species (Molés et al., 2020). In contrast to PE, the developed eel rGTHs are eel specific and highly stable. Kazeto et al. (2008) produced rGTHs with Drosophila S2 cells for Japanese eel that showed bioactivity *in vitro*, but not *in vivo*. rGTHs produced in the ovary cells of Chinese hamsters (CHO) were able to induce spermiation in male European eel (Peñaranda et al., 2018). Very recently, we tested the effects of CHO-cell produced eel rGTHs on oocyte development in European eels (Jéhannet et al., 2023b). In this study, females were weekly injected with (i) CPE as control, (ii) rFSH followed by CPE, and (iii) rFSH followed by rLH. For all treatment groups, larvae were produced, and for the first time with rGTHs treatment alone. However, the rGTHs protocol still needs optimization for the production of good-quality eggs. This is supported by the fact that females injected with rFSH had very high gonadosomatic index (GSI) values that have never been reported before. While the GSI values of the females injected with CPE are within the range (50–60) of what has been previously reported in eels (Palstra et al., 2005), eels injected with rFSH had GSI values of 75 up to 80. The reasons behind the high GSI values remain unknown: does rFSH synchronize the oocyte development better and is the asynchronous development that is often observed with the routine PE protocol a flaw of PE treatment? Or, does oocyte maturation need to be induced earlier by injecting PE or rLH sooner and/or in higher doses? The second statement is supported by the fact that a spontaneously matured female eel in a Finnish aquarium showed asynchronous development and had an estimated GSI value of 47 (Palstra et al., 2020). Besides inducing oocyte maturation sooner, rGTHs probably need to be injected at longer intervals than weekly due to their higher stability. Also very recently, Kazeto et al. (2023) reported on fully matured Japanese eels induced by CHO-produced rGTHs.

1.2.2.3. Hormonal therapy to induce oocyte maturation and ovulation. When oocytes are maturing, ovarian biopsies are usually taken to monitor oocyte development (Palstra et al., 2005; Jéhannet et al., 2017; Da Silva et al., 2018). Based on the seven-stage scale developed for European eels (Palstra et al., 2005), an additional dose of PE is given when most oocytes are in stage 3 (i.e. transparent oocytes with migrating germinal vesicle) (Fig. 2). One day later, another biopsy is taken to check the progression of oocyte maturation. When most oocytes are around stage 4 (i.e. transparent oocytes with germinal vesicle at the periphery) (Fig. 2), females are injected with DHP in the ovarium. Females injected with DHP need to ovulate rapidly to release good quality eggs. Eels must spawn within 18 h after DHP injection as fertility and hatching rates decrease drastically after this period (Ohta et al., 1997; Kim et al., 2007; Palstra and van den Thillart, 2009). To increase egg quality, spawning induction therapies have focused on factors that act higher in the BPG axis to induce the endogenous production of DHP (Mylonas and Zohar, 2007). Progesterone (P), an upstream precursor of DHP in the steroidogenic pathway, induces the last steps of oocyte development in numerous fish species (Adachi et al., 2003; Upadhyana and Haider 1986; Jalabert, 1976; Haider & Inbaraj, 1989). It is commonly believed that P acts *indirectly* on oocyte maturation and ovulation by being converted into DHP. Based on the two-type cell model developed in salmonids (Nagahama, 1997), P, which is formed in the theca cells, is converted into 17α-hydroxyprogesterone that diffuses into the granulosa cells and is converted into DHP. However, we cannot rule-out that P *directly* induces oocyte maturation and ovulation since low doses of P were able to effectively induce GVBD *in vitro* in Japanese

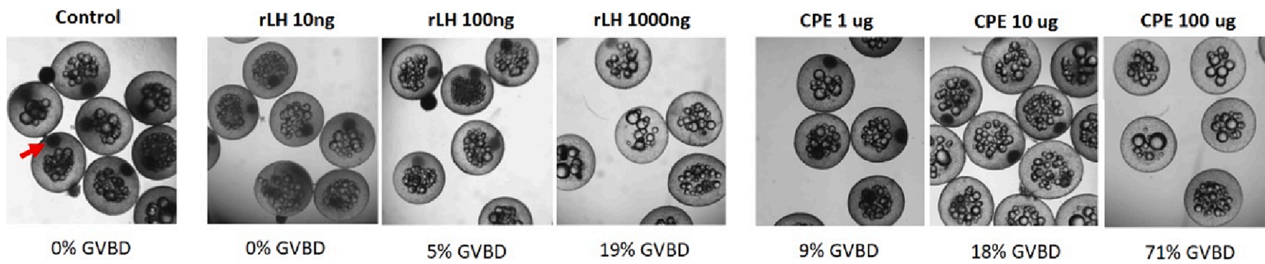

Fig. 3. The percentage of oocytes that underwent GVBD when treated with various dosages of rLH (10, 100, 1000 ng mL^{-1}) and CPE (10, 100, 1,000 µg mL^{-1}) *in vitro* after 18 h of incubation at 20 °C. Oocytes were fixed in Serra's solution to stain the germinal vesicle (GV; red arrow). Oocytes incubated without hormones have a visible GV. GVBD was induced in ~ 20 % of the oocytes at a dose of 1,000 ng rLH and at a dose of 10 µg CPE. GVBD was induced in ~ 70 % of oocytes at a dose of 100 µg CPE.

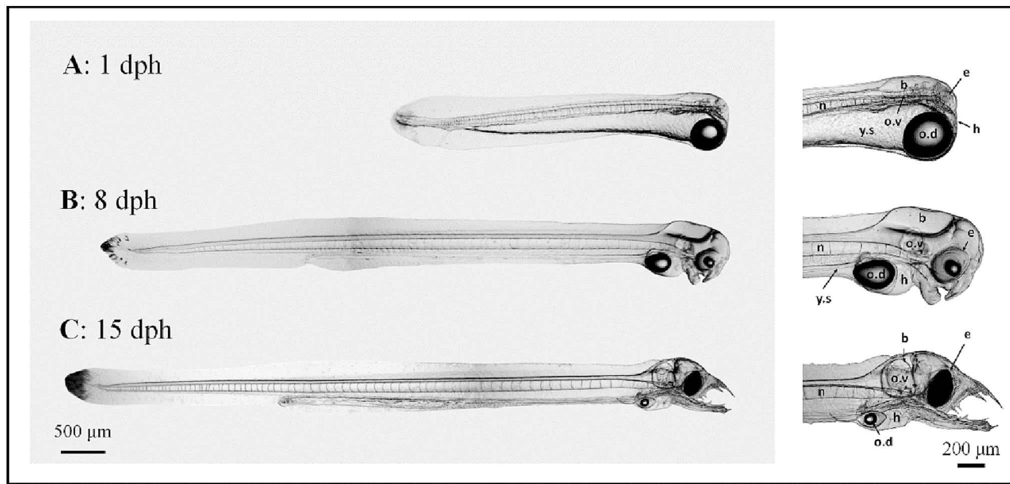

Fig. 4. Early ontogeny of the European eel at 18 °C. **A**: 1 dph larva with a prominent oil globule and yolk-sac; **B**: 8 dph larva with pigmented eye and distinguishable upper and lower jaws; **C:** 15 dph larva with the terminal mouth and needle-like teeth. Insets to Fig. 4A-C zoom in on the head with **e**: eye; **b**: hindbrain; **o.d**: oil droplet; **o.v**: otic vesicle; **y.s**: yolk-sac; **n**: notochord and **h**: heart.

eels (Adachi et al., 2003). Recently, we showed that both P and DHP work equally well in European eels (Jehannet et al., 2023a). This conclusion was supported by our observations that the effective dose to induce oocyte maturation and ovulation was similar for both steroids in our *in vitro* and *in vivo* experimentation. Therefore, it is possible that P acts either *directly* as MIH or *indirectly* by being converted into DHP. As DHP induced GVBD at lower effective dose than P, the latter is still more probable. Since we could not induce the release of better-quality eggs with P, future studies should investigate the effects of factors that act even more upstream in the BPG axis to induce endogenous MIH production. For instance, the use of rLH as spawning induction therapy holds promise since this hormone was able to induce GVBD at 20 °C in preliminary *in vitro* follow-up studies (Fig. 3).

1.3. How to increase larval quality?

Larvae belonging to the superorder Elopomorpha (eels and their relatives) are unique with their distinctive ontogeny. The leptocephalus larvae of eels are transparent, hatch in a precocious stage, have a long larval period (~1–3 years; Arai et al., 2000; Bonhommeau et al., 2009; Tesch, 2003) and can grow to large size (>80 mm; Miller et al., 2015). Since eel larvae have unique characteristics, research efforts aimed to comprehend their early ontogeny (Mazurais et al., 2013; Ahn et al., 2015; Bouillart et al., 2015; Sørensen et al., 2016). The description of early larval development, as reviewed here, provides a framework for the assessment of offspring quality. With this in mind, the effects of extrinsic factors (e.g. temperature, light, salinity) that critically influence early ontogeny, have been assessed in order to increase larval survival. However, despite standardized hatchery practices (Sørensen et al., 2014; Sørensen et al., 2016; Politis et al., 2014; Politis et al., 2017; Politis et al., 2018; Politis et al., 2021), larval survival remains low, especially during the first week of hatching.

1.3.1. Larval development

An overview of the early ontogeny of the European eel is shown in Fig. 4. European eel larvae hatch around 48–60 h post fertilization (hpf) at 18–20 °C (Pedersen, 2004; Sørensen et al., 2016). New hatchlings hang vertically in the water column due to their prominent oil globule (Sørensen et al., 2016). Like in other teleost species (Zambonino-Infante et al., 2008), eel larvae have poorly differentiated digestive systems when hatching occurs (Mazurais et al., 2013). In European eels, the digestive tract, observed close to the oil droplet at 1 day post-hatch (dph), develops into a straight tube with a tight lumen at 6 dph at 20 °C (Mazurais et al., 2013). Although not visible by light microscopy in Fig. 4, scanning electron microscope observations of eel larvae showed that the mouth appears as a slit, just after hatching (Ahn et al., 2015). This underdeveloped mouth allows larvae to drink as early as hatching (Ahn et al., 2015), to compensate for water loss in the hyperosmotic environment. The mouth develops into a hole-like shape at 2 dph (Ahn et al., 2015), upper and lower jaws are developed at 8 dph (Sørensen et al., 2016), and can move when the musculoskeletal anatomy has sufficiently developed at 12 dph (Bouillart et al., 2015). Other organs that are essential for digestive functions (liver, pancreas, gallbladder), were observed histologically at 12 dph at 20 °C (Mazurais et al., 2013). Enzymes, related to appetite and digestion, increase during early ontogeny to reach optimal values prior to or at the onset of

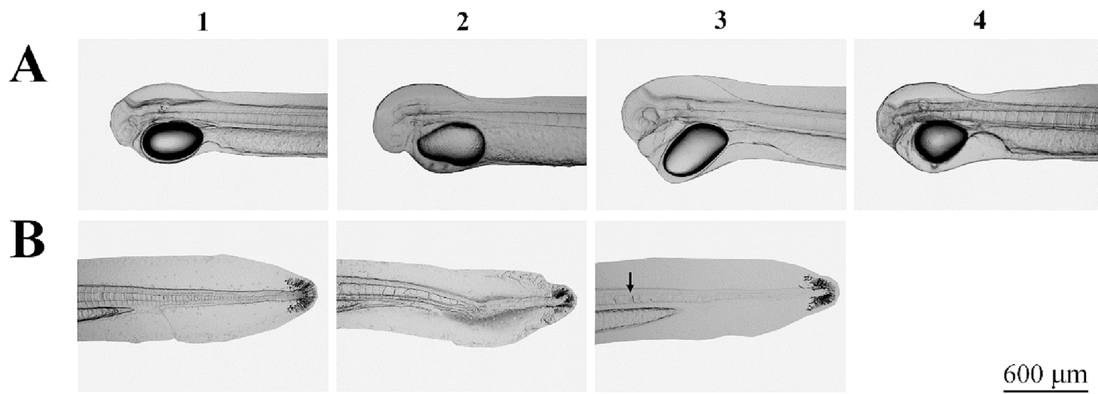

Fig. 5. Some examples of deformities observed during early ontogeny in European eel larvae at 2 dph. Around the head region (**A**), larvae showed (**1**) normal development; (**2**): an abnormal lipid droplet; (**3**) pericardial oedema and (**4**) an abnormal yolk-sac. At the posterior tip of the tail (**B**), larvae showed (**1**) normal development; (**2**) post-notochord deformities and abnormalities of the primordial marginal finfold and (**3**) a locally fragmented notochord (black arrow). It is important to note that most deformed larvae showed multiple deformities.

exogenous feeding (Mazurais et al., 2013; Politis et al., 2018; Parmeggiani et al., 2020). At 15 dph, larvae should start exogenous feeding since their protruding teeth are formed and their yolk reserves are almost depleted. The function of these peculiar protruding teeth remains questionable but it has been suggested that these large teeth are used to pierce and grasp soft material like the mucous houses of larvaceans (Mochioka, 2003). Few studies reported on captive eel larvae ingesting food (Butts et al., 2016; Politis et al., 2018; Benini et al., 2022; Benini et al., 2023) since high mortality is often experienced during early ontogeny. Periods of high mortality occur, especially during the first week of hatching. Only 20–30 % of the larvae in the better batches are still alive at 7 dph (Sørensen et al., 2014; Miest et al., 2019). Despite intense research efforts to understand larval mortality, it remains unclear what goes wrong during early larval development in eels. For answering this question, we recently studied the transcriptomics of 1 dph larvae of batches that survived less than 3 dph (non-viable larvae) with batches that survived for at least a week (viable larvae; Jéhannet et al., 2021). Importantly, we found that non-viable larvae were sensitive to pathogens since numerous genes related to inflammation were up-regulated when comparing non-viable with viable larvae.

1.3.2. Hatchery practices

Since larval survival was impaired by pathogen pressure (Jéhannet et al., 2021), we tested the effects of antibiotics (Jéhannet, 2024). As previously reported (Sørensen et al., 2014), we showed that antibiotics increased larval survival (Jéhannet et al., 2022). It has been suggested that detrimental fish-microbe interaction is the main cause of larval mortality in numerous aquaculture species (Vadstein et al., 2018). Although larval survival of eel is increased by the application of antibiotics (Sørensen et al., 2014, Jéhannet et al., 2024), still less than 10 % of the larvae reached the exogenous feeding stage (Jéhannet et al., 2024). Other factors than bacterial infections may therefore be responsible for larval mortality. Larval survival is influenced by the rearing conditions such as light (Politis et al., 2014), temperature (Politis et al., 2018; Okamura et al., 2007) and salinity (Okamura et al., 2009; Okamoto et al., 2009; Okamura et al., 2016; Politis et al., 2018; Politis et al., 2021). Among the studied extrinsic factors, salinity is the one that received the most attention in both European eel (Politis et al., 2018; Politis et al., 2021) and Japanese eel (Okamura et al., 2009; Okamoto et al., 2009; Okamura et al., 2016). Reducing the salinity improves larval survival in eels (Okamura et al., 2016; Politis et al., 2018; Politis et al., 2021; Syropoulou et al., 2022). Since the energy costs of osmoregulation are high (Varsamos et al., 2005), salinity reduction might lower the costs of maintaining homeostasis and ultimately increase larval growth and survival (Politis et al., 2018; Politis et al., 2021; Okamura et al., 2009). In Japanese eels, larvae reared in 50 % seawater

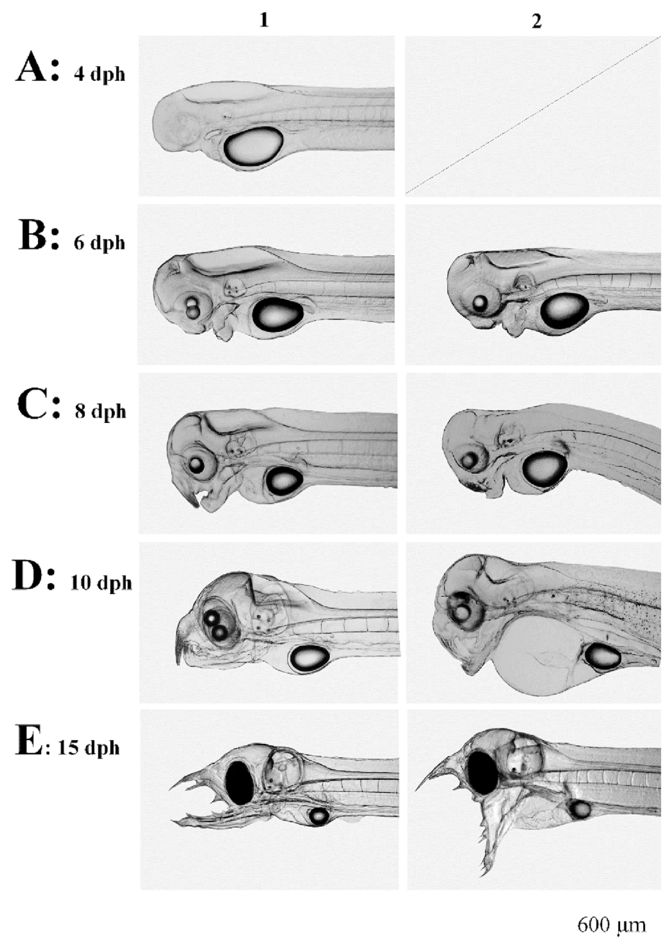

Fig. 6. Larvae showing (**1**) normal and (**2**) abnormal jaw development at (**A**): 4; (**B**): 6; (**C**): 8; (**D**): 10 and (**E**): 15 days post-hatch (dph). Jaw deformities were not observed at 4 dph. When the upper and lower jaws started to develop at 6 dph, larvae started to show jaw abnormalities. At 6 dph, the jaw abnormality is subtle but the upper and lower jaws were not aligned. At 8 and 10 dph, the lower jaws were longer than the upper ones. At 15 dph, the larva showed a severely depressed lower jaw that will directly impede its feeding ability.

(~18 ppt) had significantly higher body depth (1.58 ± 0.47 mm *vs.* 1.32 ± 0.35 mm) and survival rates (18.2 % *vs.* 8.2 %) than larvae reared in 100 % seawater (~36 ppt) (Okamura et al., 2009). Similarly, a gradual

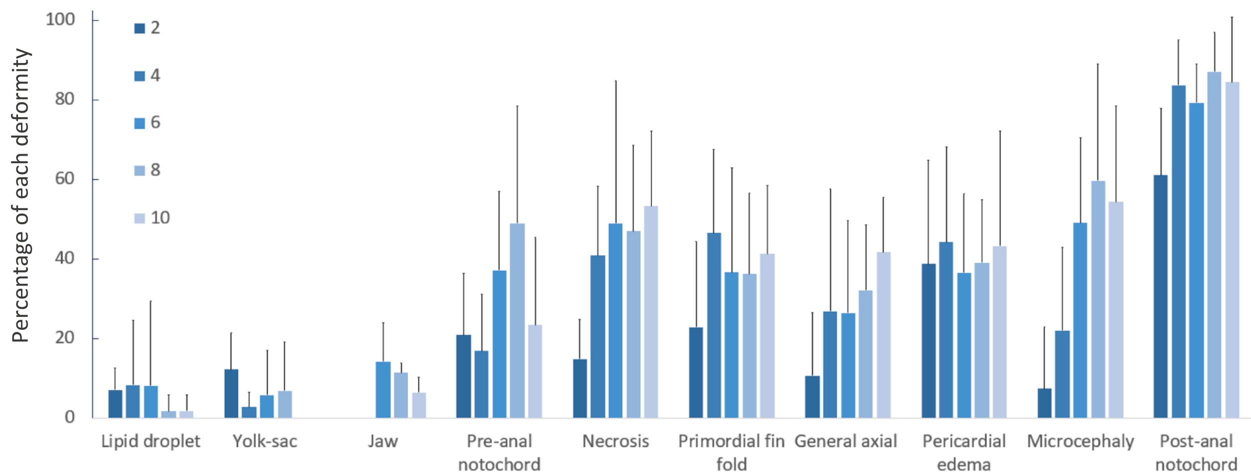

Fig. 7. Percentage of each deformity observed at 2, 4, 6, 8 and 10 days-post hatch (dph) during early ontogeny of the European eel. Data are displayed as bar plots with averages ± standard deviation. Data is coming from 2 (N = 125), 4 (N = 198), 6 (N = 148), 8 (N = 108) and 10 (N = 86) dph larvae that were reared in antibiotics.

salinity reduction towards isosmotic conditions (36 to 18 ppt) initiated at 3 dph, enhanced larval survival and growth in European eels (Politis et al., 2021).

1.3.3. *Decreasing the deformity rates*

Besides increasing larval survival, the application of antibiotics reduced the occurrence of deformities although the deformity rates remained high (75–85 %) during early ontogeny (Jéhannet et al., 2024). Similar deformity rates have been previously reported in both European eel (Politis et al., 2018; Politis et al., 2021) and Japanese eel (Okamoto et al., 2009; Okamura et al., 2007). We developed a deformity determination key for young eel larvae that provides a framework of reference for assessing larval deformities during early ontogeny (Jéhannet et al., 2024). Among others, larvae collected at 2 dph showed pericardial oedema, locally fragmented and/or enlarged-yolk sac, misplaced and/or elongated lipid droplet, and notochord deformities (Fig. 5). With the development of the upper and lower jaws at 6 dph, larvae started to show jaw deformities (Fig. 6). When considering the percentages of occurrence of each deformity during early ontogeny, the post-anal notochord deformity was the most dominant abnormality observed in 2, 4, 6, 8 and 10 dph larvae (Fig. 7). The deformities that were observed are frequently observed in other fish species and are considered as lethal when the degree of severity is high (Boglione et al., 2013). Even minor deformities, especially the notochord ones, can reduce swimming and feeding ability. With this in mind, the percentage of the most common deformities should be drastically reduced before starting feeding trials. The reasons behind the most common larval deformities remain largely unknown in European eels. More efforts are needed to understand the reasons behind larval deformities in order to prevent them in future trials and ultimately produce larvae that are able to survive, grow and feed.

2. Conclusion

For improving egg and larval quality, studies should be restricted, for now, to wild females. Once larvae can survive, feed, grow and metamorphose into juveniles that can subsequently be used for closing the life cycle, procedures for feminization, broodstock diets and naturally induced silvering will require renewed research attention. Besides the origin of the broodstock, it is important to take into consideration the hormonal therapy that is used to induce gonad development, for increasing the release of better-quality eggs. The use of rGTHs looks promising but the protocol still needs optimization. Since we could not induce the release of better quality eggs with P, factors that act higher in the BPG axis (e.g. rLH) should be investigated for their induction capacity of endogenous MIH production. With the application of antibiotics, we could increase larval survival. Besides lowering mortality, antibiotics treatment decreased the occurrence of larval deformities but it still remained high (75–85 %). Future studies should focus on understanding the reasons behind larval deformities in order to prevent them in future trials.

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CRediT authorship contribution statement

Pauline Jéhannet: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Leon T.N. Heinsbroek:** Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **William Swinkels:** Resources, Funding acquisition, Conceptualization. **Arjan P. Palstra:** Writing – review & editing, Supervision, Methodology, Investigation, 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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