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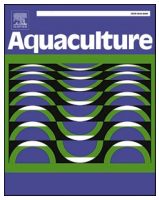

Deformities and their potential control in larviculture of the European eel *Anguilla anguilla*

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ABSTRACT

With the current assisted reproduction protocols, yolk-sac larvae of the European eel can be regularly produced. However, high mortality and deformity rates may exist during the first week after hatching with few larvae reaching the exogenous feeding stage. Recently, we suggested that larval survival could be impaired by pathogen pressure. Therefore, in this study, we tested the effects of antibiotics (rifampicin and ampicillin 50 mg L^{-1}) and egg surface disinfection treatment (povidone iodine 25 ppm) on hatching success, larval survival and the occurrence of deformities in wild and feminized eels. Differences in egg quality aspects existed between offspring of wild and feminized eels. Eggs produced by the wild females were more buoyant than the ones released by the feminized eels. Larvae of the wild females hatched at higher rates and survived longer than the ones produced by the feminized eels. Disinfection treatment negatively affected hatching success and larval survival but the application of antibiotics improved larval survival. While larvae survived until 5 days post-hatch (dph) in the untreated controls, larvae continued to survive up to 21 dph when treated with antibiotics. Despite the beneficial effects of antibiotics on larval survival, a wide diversity of deformities could be observed during early development of eels that have not been reported before. The deformity determination key for young eel larvae that is presented in this paper fills a gap in the knowledge about larval deformities during early ontogeny. The larvae showed pericardial oedemas, microcephaly, an enlarged-yolk sac, necrosis and notochord deformities. The treatment with antibiotics decreased the deformity rates although they remained high (75–85%). The causes behind each deformity need to be clarified in order to prevent them in future trials.

1. Introduction

The life cycle of the European eel has not been closed in captivity. Therefore, the aquaculture industry still depends on wild-caught juveniles that are grown to marketable size. The European eel population has declined over three decades from 1980 to 2011 and has been stable but low ever since (ICES, 2023). The species is listed on the IUCN Red List since 2008 (Pike et al., 2020). The need to be able to propagate eel under aquaculture conditions is eminent. With the current assisted reproduction protocols, several research groups have been able to produce yolk-sac larvae (Di Biase et al., 2017; Asturiano, 2020; Politis et al., 2021; Jéhannet et al., 2023a) but large variations in larval survival are often observed (Da Silva et al., 2018; Jéhannet et al., 2021). To overcome this bottleneck, hatchery practices have been extensively developed in European eels (Butts et al., 2014; Politis et al., 2018; Sørensen et al., 2014; Sørensen et al., 2015; Sørensen et al., 2016) but still, larval survival can vary from 0 to 93% during the first week of hatching (Da Silva et al., 2018). Even for the Japanese eel *A. japonica*, whose life cycle was closed more than one decade ago (Tanaka et al., 2003; Tanaka, 2015), low survival rates that occur a few days after hatching are frequently observed (Okamura et al., 2020).

To understand the reasons behind larval mortality in the European eel, we compared 1 dph larvae of batches that survived <3 days with 1 dph larvae of batches that survived for at least a week (Jéhannet et al., 2021). In this study, expression of genes related to inflammation was up-regulated in larvae that survived <3 dph when compared to the larvae

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that survived for at least a week. From this study, we suggested that larval survival was impaired by pathogenic pressure. Evidence in eels and other aquaculture fish species suggests that larval mortality is largely due to high bacterial densities in the rearing tanks (reviewed by Vadstein et al., 2013). To increase larval survival, antibiotics have been widely applied in aquaculture practices (reviewed by Limbu et al., 2021). In European eels, Sørensen et al. (2014) showed that hatching success and larval survival were significantly improved by the application of antibiotics treatments (rifampicin and ampicillin 50 mg L^{-1}). Egg disinfection treatments, like hydrogen peroxide (H_20_2), glutaraldehyde and povidone-iodine, have been employed to increase larval survival in fish (De Swaef et al., 2016; Yalsuyi et al., 2022). In contrast to other disinfectants like H_20_2, povidone-iodine reacts over a wide pH range and has a limited dependence on temperature (De Swaef et al., 2016). Disinfecting eggs with povidone-iodine (25 ppm) for 5 min has been shown to effectively kill bacteria in zebrafish (Chang et al., 2015; Chang et al., 2016). The disinfection timing may greatly impact hatching success and larval survival (reviewed by De Swaef et al., 2016). For instance, the morula stage was the most suitable stage when disinfecting with povidone-iodine in red seabream *Pagrus major* (Hirazawa et al., 1999). The role of the developmental stage in iodine disinfection tolerance was also demonstrated in red porgy *Pagrus pagrus* and white sea bream *Diplodus sargus sargus* (Katharios et al., 2007). This stresses that different developmental stages should be incorporated when assessing the efficiency of disinfectants (De Swaef et al., 2016).

Although antibiotics and egg disinfection treatments improve larval survival by preventing the proliferation of pathogens, they may also cause side effects such as reduced growth (Zhang et al., 2015; Limbu et al., 2018), body malformations (Toften and Jobling, 1996; Oliveira et al., 2013; Zhang et al., 2014; Escobar-Huerfano et al., 2020) and oxidative stress (Zhang et al., 2015; Limbu et al., 2018). Antibiotics can induce deformities including pericardial oedemas, a curved body axis and an abnormal yolk-sac and notochord (reviewed by Limbu et al., 2021). Similar to the negative effect of antibiotics on deformities, various studies have demonstrated the potency of disinfectants to induce malformations (reviewed by De Swaef et al., 2016). However, given the capacity of some bacteria (e.g., *Flavobacterium psychrophilum*, *Streptococcus agalactiae*, *Streptococcus iniae*) to induce body malformations (reviewed by Eissa et al., 2021), antibiotics and disinfectants could also have important positive impacts on the occurrence of deformities. To our knowledge, the effects of antibiotics and disinfectants on body malformations have not yet been reported in European eels.

In this context, the aim of this study was to test the effects of antibiotics (rifampicin and ampicillin 50 mg L^{-1}) and disinfection (povidone-iodine, 25 ppm) treatment, alone or in combination at 2 and 10 h post-fertilization (hpf), on (i) hatching success, (ii) larval deformities, and (iii) larval survival, in both wild and feminized eels.

2. Materials & methods

2.1. Animals ethics

Experimental protocols complied with the current laws of The Netherlands and were approved by the CCD (Central Committee for Animal Experiments) project numbers AVD401002017817 and AVD40100202215, and by the DEC (Animal Experiments Committee) and IvD (Authority for Animal Welfare), experiment numbers 2017.D-0007.005, 2017.D-0007.006 and 2022.D-0005.001.

2.2. Broodstock and housing

2.2.1. Wild eels

Wild male (100–200 g) and female (300–900 g) silver eels were caught in the Harinxma canal (The Netherlands) with fyke nets during the migratory season (Oct-Nov), transported to the CARUS experimental facilities (Wageningen University & Research, The Netherlands) where they were not fed and used for experimentation over the following year. Then, eels were slightly anesthetized, PIT-tagged (Trovan, DorsetID, Aalten, The Netherlands) and housed in 373-L tanks with artificial seawater at 36 ppt and 16 °C under dark conditions.

2.2.2. Feminized eels

Young elvers (10 g) and male eels were obtained from Palingkwekerij Koolen B.V (Bergeijk, The Netherlands) and transferred to the CARUS facilities. The young elvers were feminized according to the procedure published by Chai et al. (2010) using 2 mm pellets (Alltech-Coppens, Helmond, the Netherlands) that were coated with estradiol (Sigma Aldrich, Saint Louis, Missouri, USA) over a 6 month period. Subsequently, they were fed with a custom-made diet (Jéhannet et al., 2023a) until satiation using automatic feeding belts. The males and feminized eels (250–400 g) were then slightly anesthetized, PIT-tagged (Trovan, DorsetID, Aalten, The Netherlands) and housed under the same conditions as the wild ones.

2.3. Egg collection

Both wild and feminized eels were artificially matured as previously described (Jéhannet et al., 2021, 2023b). Briefly, females were matured by weekly injections with carp pituitary extracts at a dose of 20 mg kg^{-1} to induce oocyte growth (Fontaine et al., 1964). Ovulation was induced when oocytes were predominantly in stage 4 (Palstra et al., 2005) by injection with the maturation-inducing hormone (17α,20β-dihydroxy-4-pregnen-3-one, DHP, or progesterone, P) at a dose of 2 mg kg^{-1} (Ohta et al., 1996). Injected females were joined with 3 to 6 spermiating males in a separate tank and water temperature was increased from 18 up to 20 °C. When eggs were released, females were slightly anesthetized and stripped by applying gentle pressure on the abdomen to collect eggs into dry bowls. Males were matured with one single 1000 IU hCG injection to induce spermiation (Kahn et al., 1987) and another 250 IU hCG injection 24 h before stripping the sperm to optimize motility. Just before fertilization, males were slightly anesthetized to collect the sperm into artificial eel plasma (Peñaranda et al., 2010). The stripped eggs and diluted sperm were gently mixed together. Artificial seawater (36 ppt salinity, 18 °C) was added to the mixed gametes for activation. After 5 min of gamete activation, ~10 g of eggs were placed in a 100 mL cylinder filled with artificial seawater to determine the percentage of floating eggs one hour later. The remaining eggs were placed in 3 L-beakers filled with artificial seawater. After 1 hpf, the floating egg layer was collected, rinsed gently over a sieve to remove waste. The floating eggs were then transferred into a new 5 L-beaker filled with fresh artificial seawater (36 ppt, 18 °C), kept in the dark and gently aerated by a glass pipette until the start of the experiment.

2.4. Experimental set-up

A schematic overview of the experimental set-up is shown in Fig. 1. At 2 hpf, floating eggs were collected from the 5-L beaker with a sieve to weigh 63 g of eggs. Weighted eggs were equally distributed over twenty-one 1800 mL beakers to obtain 3 g (~ 2600 eggs) per beaker. Eggs were placed in (i) three beakers containing artificial seawater as control (**C**) and (ii) three beakers containing artificial seawater (Tropic Marine, Wartenberg, Germany) supplemented with antibiotics that consisted of ampicillin and rifampicin at a dose of 50 mg L^{-1} each (**A2**). From the weighted eggs, 18 g was disinfected with povidone-iodine at 25 ppm for 5 mins, rinsed three times and divided over (i) three beakers containing artificial seawater (**D2**) and (ii) three beakers containing artificial seawater supplemented with the previously described antibiotics (**AD2**). Remaining eggs were placed in nine beakers containing artificial seawater until 10 hpf. At 10 hpf, antibiotics were added to three beakers (**A10**). Eggs from the remaining six beakers were pooled, disinfected, rinsed three times, and divided over (i) three beakers containing artificial seawater (**D10**) and (ii) three beakers containing artificial seawater

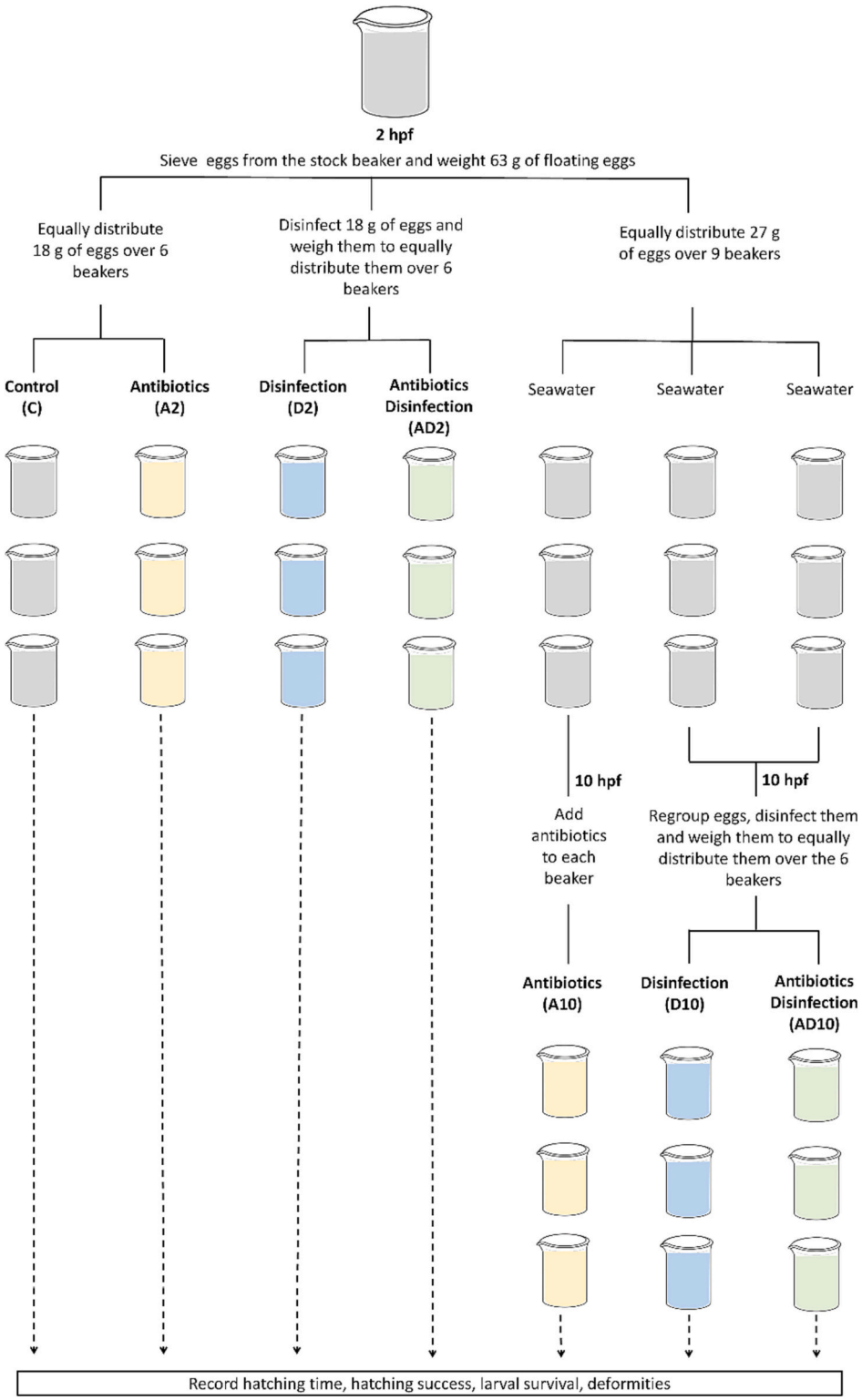


Fig. 1. Overview of the experimental set-up to test the effects of antibiotics and disinfection treatment, alone or in combination, at 2 and 10 h post-fertilization (hpf).

supplemented with antibiotics (**AD10**). Eggs and larvae were reared in artificial seawater (18 °C, 36 ppt) under continuous darkness and gently aerated through a glass pipette throughout the entire trial. Every 24 h, dead eggs and larvae were removed by siphoning and half of the water was refreshed with the previously described artificial seawater (C, D2, D10) or artificial seawater supplemented with antibiotics (A2, A10, AD2, AD10). Dissolved oxygen levels remained 9.0–9.4 $mg.l^{-1}$ during the period of egg and larval rearing.

2.5. Data collection

2.5.1. Hatching success

Hatching success was determined for the larval batches of wild females. Hatching time was monitored every 12 h. Once hatched, larvae were counted in each experimental unit. When few larvae hatched ($\leq$50), larvae were directly counted in the beaker to assess hatching success. When many hatched larvae were observed per beaker ($>$ 50),

larvae were gently transferred to a 3.1 L rectangular container (18 × 27 cm) in which six squares of 9 × 9 cm were drawn at the bottom. Larvae were then counted in two squares, averaged, and then multiplied by the number of total squares to get an estimation of the total number of larvae. After getting an estimation of the total number of larvae, the larvae were placed back in their original beaker. Hatching success was calculated as the total number of counted larvae divided by the number of eggs incubated at 2 hpf (x 100%).

2.5.2. Larval survival

Larval survival was determined for the larval batches of wild females. For estimating larval survival, larvae were daily counted. Larval numbers were estimated in similar fashion as the number of newly-hatched larvae (see 2.5.1). Larval survival was calculated as the number of larvae surviving at the start of each day as a percentage of the initial number, from 0 to 21 dph. In addition, larval longevity (i.e., the number of days that larvae survived) was daily recorded for each batch until the complete demise of all larvae.

2.5.3. Deformities

Deformities were investigated on a total of 150 larvae, collected at 100 hpf from egg batches originating from five wild females (2A9B, FBDO, 6F73, FEEF, F92C). From each beaker, ~10 larvae were randomly sampled and digitally imaged using a Moticam camera (Motic, Hong Kong) connected to a light microscope. Larvae showing one or more developmental abnormalities were classified as deformed.

2.6. Statistical analysis

The percentage of floating eggs, hatching success and larval longevity were compared between wild and feminized eels using the non-parametric Wilcoxon signed-rank test. Hatching time, hatching percentage, larval longevity and the number of larvae were compared between treatments and timepoints by using the following linear mixed model:

$$ln(y_{ijk}) = \mu + \mathrm{TR}_i + \mathrm{T}_j + \mathrm{TR}_i \times \mathrm{T}_j + \mathrm{A}_k + e_{ijk}$$

where y_{ijk} is the variable to be explained (hatching time, hatching percentage, larval longevity, the number of larvae and cumulative survival) from treatment i (control, antibiotic, disinfection and antibiotic-disinfection) at time j (2 and 10 hpf) observed for female eel k (k = 1–10). In this model, μ is the overall mean, TR_i is the fixed effect of the i^{th} treatment, T_j is the fixed effect of the j^{th} time, $\mathrm{TR}_i \times \mathrm{T}_j$ is the fixed interaction effect for the i^{th} treatment and j^{th} time, A_k is the random effect of the k^{th} eel and e_{ijk} is the residual. Separate general linear models were run at 1, 2, 3, 4 and 5 dph to assess the effects of each treatment on the number of larvae. For comparing the percentage of normal larvae between treatments and timepoints, slight adjustments were made to the previously described linear mixed model. In this model, y_{ijk} is the percentage of larvae showing normal development from treatment i (control and antibiotics) at time j (2 and 10 hpf) observed in female eel k (k = 1–5). All data were analysed using R (version 3.2.4; R foundation for statistical computing, Vienna, Austria). Differences were considered significant when $P < 0.05$.

3. Results

3.1. Reproduction parameters of feminized and wild eels

Wild females required 11.9 ± 4.2 weekly CPE injections to mature, feminized eels 12.5 ± 2.4 weekly injections, no significant difference. Wild females released eggs 11.9 ± 1.0 h after MIS injection, feminized eels 11.8 ± 0.6 h, again no significant difference. An overview of the reproduction parameters of feminized and wild eels is shown in Table 1. The percentage of floating eggs was significantly lower in feminized eels (average: 32 ± 23%, range: 5–80%) than in wild females (average: 65 ± 24%, range: 22–96%) ($P = 0.007$). While all ten wild females gave eggs that developed into larvae, only three egg batches out of the twelve from feminized eels developed into larvae. Hatching rates of egg batches from the feminized eels were extremely low (average: 0.4 ± 1.3%, range: 0–4.6%). Hatching rates of egg batches from wild females (average: 6.1 ± 6.3%, range: 0.1–17.6%) were significantly higher ($P = 0.0003$). As determined for the controls, larvae that developed from eggs of the feminized eels died earlier (average: 1 ± 1 dph, range: 0–4 dph) than larvae from the wild eels (average: 3 ± 2 dph, range: 0–5 dph) ($P = 0.003$).

Table 1
Reproduction parameters of feminized ($N = 12$) and wild ($N = 10$) females. **Tag**, PIT-tag code of the female. **Origin**, feminized or wild. **Floating eggs**, percentage of floating eggs 1 h after fertilization. **Hatching percentage**, percentage of larvae that hatched (in the controls). **Longevity,** number of days that the larvae survived after hatching (dph) (in the controls). Eels are ordered according to the longevity of their larvae.

Tag	Origin	Floating eggs (%)	Hatching percentage (%)	Longevity (dph)
D3B2	Feminized	20	4.6	4
DE99	Feminized	80	0.3	3
CF73	Feminized	5	0.2	2
5407	Feminized	30	0	0
4566	Feminized	30	0	0
C468	Feminized	20	0	0
32E4	Feminized	30	0	0
2CFF	Feminized	15	0	0
D065	Feminized	10	0	0
4992	Feminized	60	0	0
D86E	Feminized	20	0	0
BA7B	Feminized	60	0	0
6F73	Wild	55	3.5	5
32CC	Wild	22	17.6	4
F92C	Wild	45	12.6	4
FEEF	Wild	80	13.5	4
40E1	Wild	96	5.1	4
FBD0	Wild	85	4.8	4
CFC9	Wild	80	2.5	3
2A9B	Wild	44	0.4	1
D732	Wild	83	0.4	1
5051	Wild	55	0.1	0

3.2. Antibiotics and disinfection treatments

3.2.1. Hatching time and percentage

Hatching time was between 48 and 100 hpf and not affected by treatment nor time of the treatment. The hatching percentage was significantly affected by treatment ($P < 0.001$). Hatching rates were similar between egg batches of the antibiotic treatments (A2, A10) and those of the control treatment (Fig. 2). Hatching rates were significantly lower in the egg batches of the disinfection treatments (D2, D10) when compared to egg batches of the controls ($P < 0.02$) and of the antibiotic treatments (A2, A10) ($P < 0.001$) (Fig. 2). Hatching rates tended to be lower for the egg batches that were disinfected and treated with antibiotics (AD2, AD10), but this was not statistically different from the control ($P = 0.11$) because of the high variation (Fig. 2). It is important to note that numerous eggs died following the disinfection treatment. While 2.4 ± 0.7 g (13 ± 4%) of the eggs died following the disinfection treatment at 2 hpf, 8.6 ± 3.9 g (37 ± 14%) of eggs died after disinfection at 10 hpf. From 2 to 10 hpf, the weight of the eggs increased from the initial 18 g to 23.2 ± 4.3 g (29 ± 24% increase).

3.2.2. Larval longevity

A significant time by treatment interaction ($P < 0.02$) was found for larval longevity (Fig. 3). Larvae survived significantly longer in the antibiotics (A2: $P = 0.006$; A10: $P = 0.02$) than in the control treatment (Fig. 3). While larvae survived on average 3 dph in the controls, they

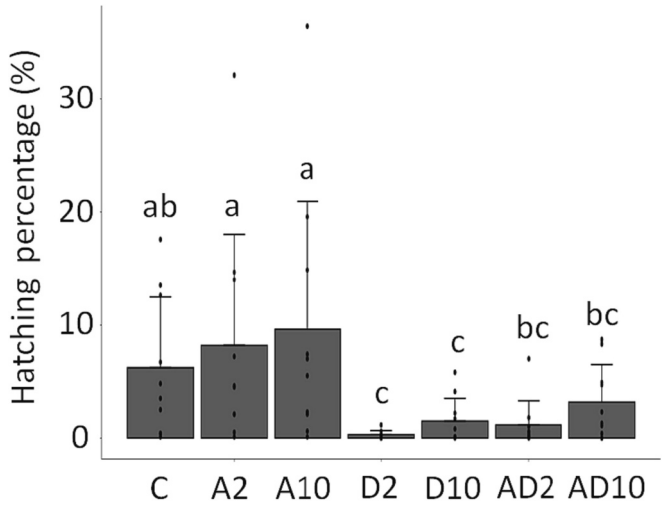


Fig. 2. Hatching percentages when egg batches were treated with antibiotics and/or disinfection, at 2 hpf and at 10 hpf. Hatching percentages were similar for both the antibiotic treatments (A2, A10) and the control (C) but significantly lower for the disinfection treatment (D2, D10) Bars with no overlap in letters indicate significant difference ($P < 0.05$). Data are shown as bar plots with averages ± standard deviation and individual datapoints as circles. Data are based on larvae originating from $N = 10$ wild eels.

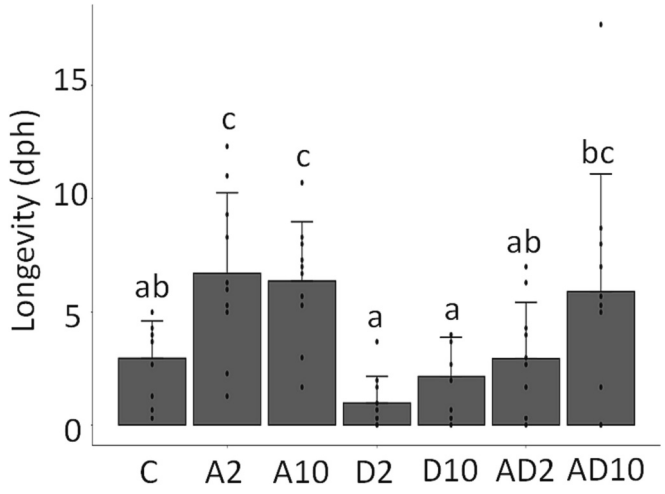


Fig. 3. Larval longevity when egg batches were treated with antibiotics and/or disinfection, at 2 hpf and at 10 hpf. Larvae survived longer in the antibiotic treatments (A2, A10) than in the control (C). Disinfection did not affect larval longevity when compared to the control. Bars with no overlap in letters indicate significant difference ($P < 0.05$). Data are displayed as bar plots with averages ± standard deviation and individual datapoints as circles. Data are based on larvae originating from $N = 10$ wild eels.

survived on average 7 dph and 6 dph in the antibiotics at 2 hpf and 10 hpf, respectively. No difference in larval longevity was detected between the antibiotic treatments at 2 hpf and 10 hpf. Disinfection, applied at both 2 hpf and 10 hpf, or in combination with antibiotics at 2 hpf, did not affect larval longevity when compared to the control. The combined treatment of disinfection and antibiotic at 10 hpf tended to increase larval longevity when compared to the same treatment at 2 hpf ($P = 0.05$) and the control ($P = 0.06$).

3.2.3. Larval survival

From 1 to 5 dph, the number of larvae decreased for each of the treatments (Fig. 4). From 6 dph onwards, larvae were only alive in the antibiotic treatments (A2, A10, AD2, AD10) (Fig. 4). Based on these results, general linear models were run at 1, 2, 3, 4 and 5 dph to assess the effects of treatments on the number of larvae. At each of these time points, treatment significantly affected the number of larvae ($P < 0.01$). From 1 to 3 dph, the number of larvae was significantly lower in the disinfection treatments (D2, D10, AD2, AD10) when compared to the antibiotic treatments (A2, A10). Over the same period, the number of larvae was similar between the antibiotic treatments (A2, A10) and the controls (C). From 4 to 5 dph, the number of larvae was significantly higher for the antibiotic treatments (A2, A10) when compared to any other treatment (D2, D10, AD2, AD10) and the controls.

Since a significant effect of treatment on hatching percentage was observed (Fig. 2), data were normalized as cumulative survival percentages per treatment over the experimental period (Fig. 4). As shown for the number of larvae, the cumulative larval survival decreased over time and the application of antibiotic treatments increased larval survival (Fig. 4).

3.3. Deformities

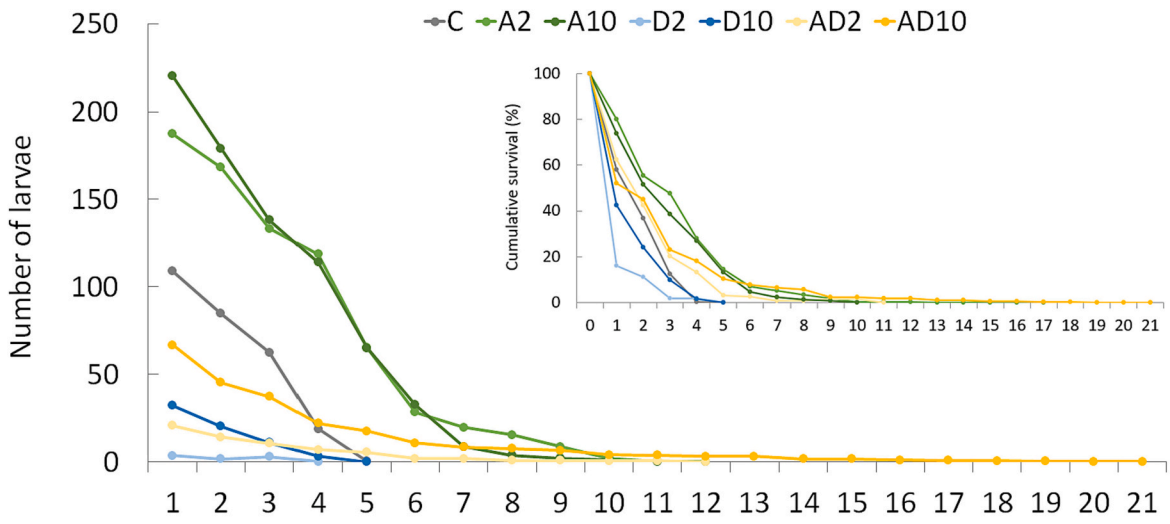
The observed developmental abnormalities as shown in Fig. 5 were separated into nine types:

- Notochord deformities that were classified to pre-anal (**1**) and post-anal (**2**), according to the affected body area. The anterior/posterior part of the body axis was curved dorso-ventrally and/or the notochord presented local defects.
- General axial deformity (**3**): the general body axis was curved on the sagittal plane to various degrees.
- Pericardial oedemas (**4**) of various severity degrees.
- Misplaced and/or elongated lipid droplet (**5**).
- Microcephaly (**6**): abnormal reduction of the head.
- Enlarged and/or fragmented yolk-sac (**7**).
- Abnormalities of the primordial marginal finfold (**8**): mostly in the form of wrinkled or necrotic fin tip.
- Necrosis (**9**): this abnormality included opacifications of the tissues in any parts of the larvae (head, trunk, tail).

Percentages of normally developed larvae were compared between antibiotic (A2, A10) treatments at 100 hpf vs. the controls. Antibiotic treatment significantly affected the percentage of normally developed larvae ($P < 0.01$). The percentage of larvae without any type of deformity was higher in the antibiotic treatments (A2, A10) when compared to the controls (A2: average 15.0 ± 16.3%, range: 0–42.9%, A10: average: 25.0 ± 8.4%, range: 14.3–33.3%; $P = 0.009$). For all five egg batches, all larvae in the control group showed at least one type of deformity (i.e., 0% normally developed larvae).

4. Discussion

High larval mortality can jeopardize closing the life cycle of the European eel. Although eel larvae can be produced by several groups, relatively few larvae reach the exogenous feeding stage. High bacterial densities in the rearing tanks are considered to affect eel larviculture (Sørensen et al., 2014; Jéhannet et al., 2021). Antibiotic treatment (rifampicin and ampicillin 50 mg L^{-1}) has been shown to improve larval survival in European eels (Sørensen et al., 2014). Besides antibiotics, egg disinfection treatments have been commonly employed to increase larval survival in fish (reviewed by De Swaef et al., 2016). Although antibiotics and disinfection treatments have the potency to increase larval survival, these hatchery practices may induce body malformations (Toften and Jobling, 1996; Oliveira et al., 2013; Zhang et al., 2014; De Swaef et al., 2016; Escobar-Huerfano et al., 2020; Limbu et al., 2021). In our study we have investigated the effects of antibiotics and disinfection treatments on the occurrence of deformities in European eels. The findings suggest that antibiotic treatment, but not disinfection, leads to lower mortality and deformity rates.



dph	C	A2	A10	D2	D10	AD2	AD10
1	ab	a	a	b	b	b	b
2	ab	a	a	b	b	b	b
3	ab	a	a	b	b	b	b
4	a	b	b	a	a	a	a
5	a	b	b	a	a	a	a

Fig. 4. Number of larvae when eggs were treated with antibiotics and/or by disinfection at 2 hpf and 10 hpf. The number of larvae decreased over the experimental period, but antibiotic treatment increased larval survival. The insert shows a similar graph but with the data expressed as cumulative survival percentages. In the table, the number of larvae is significantly different when there is no overlap in letters ($P < 0.05$). Data are based on larvae originating from $N = 10$ wild eels.

Eggs of the feminized broodstock eels showed significantly lower hatching rates than eggs from the wild females. Moreover, larvae produced by the feminized eels died earlier than their wild counterparts. Overall, this study demonstrates that wild females produced better-quality eggs than feminized eels. The superior quality of eggs produced by the wild females over the ones produced by the cultured broodstock is not restricted to eels but also involves other fish species like Atlantic cod *Gadus morhua* (Salze et al., 2005) and lumpfish *Cyclopterus lumpus* (Pountney et al., 2022). It is commonly believed that the superior quality of wild vs. cultured broodstock is influenced by the diet (Brooks et al., 1997) and particularly its lipid content. Since a large amount of fat is incorporated into the oocytes of European eels (Palstra, 2006), the broodstock diet is strongly believed to affect egg quality in this species (Heinsbroek et al., 2013; Støttrup et al., 2015). The lower reproductive success with feminized eels suggests that the quantity and/or quality of the broodstock diet needs further improvements.

When eggs are overgrown by bacteria, egg development is hampered, and subsequently hatching rates and larval health are affected (reviewed by Olafsen, 2001). Disinfection with iodophors has shown to decrease the bacterial load in numerous fish species (Katharios et al., 2007; Overton et al., 2010; Stuart et al., 2010). Still, we observed that disinfecting eggs with povidone-iodine negatively affected hatching success and larval survival in European eels. The observed negative effect of povidone-iodine on hatching and larval survival might be explained by the introduction of pathogens after disinfecting. Although egg disinfection is commonly employed for microbial control in intensive larviculture (De Swaef et al., 2016), this treatment disturbs the balance between microbial communities, allowing exponential growth of pathogenic bacteria (reviewed by Vadstein et al., 2013). Another possible hypothesis for the reduced hatching success and larval survival is that eggs were too sensitive for the disinfection treatment at the used dose at 2 and 10 hpf.

We have shown in this study that the use of antibiotics increased larval survival during the yolk-sac larval stage in European eels. Our results are consistent with the study of Sørensen et al. (2014) who showed that ampicillin and rifampicin improved larval longevity in European eels. In that study, the authors showed that ampicillin and rifampicin acted bactericidal as there was a decrease in cell density from 4.1×10^6 to 6.2×10^3 in 24 h. Our results and those from this previous study (Sørensen et al., 2014; Jéhannet et al., 2021) strongly suggest that reducing bacterial egg coverage is essential to increase larval survival in eels. Although antibiotics increased larval longevity, we observed only a low percentage of larvae without any type of deformities. High deformity rates of 50 up to 80% have been reported at the first-feeding stage in both European (Politis et al., 2018; Politis et al., 2021) and Japanese eel larvae (Okamura et al., 2007; Kurokawa et al., 2008; Okamoto et al., 2009). An overview of the deformities observed during early ontogeny is shown in Fig. 5. Although larvae with pericardial-oedemas, axial curvature and microcephaly have been commonly observed in eels (European eel: Politis et al., 2018; Politis et al., 2021; Japanese eel: Kurokawa et al., 2008; Okamoto et al., 2009; Okamura et al., 2016), also other types of abnormalities (e.g. necrosis, enlarged yolk-sac, elongated lipid droplet) were observed in this study. In addition, the classification that we propose clearly makes a distinction between notochord and gross axial deformities, which have been previously mingled together (Kurokawa et al., 2008). In contrast to previous studies (Okamura et al., 2007; Kurokawa et al., 2008), larvae with abnormal jaw development were not observed here, probably because these 2 dph larvae were too young for developing this deformity. In European eels, the mouth only starts developing lower and upper jaws around 8 dph at 18 °C (Jéhannet et al., 2021). Fig. 5 represents a deformity determination key for young eel larvae providing a framework of reference for assessing larval deformities during early ontogeny.

The precise mechanisms causing deformities remains largely unknown in fish. Pathogens, inappropriate rearing conditions, pollutants, spawning induction therapies and the broodstock diet may cause

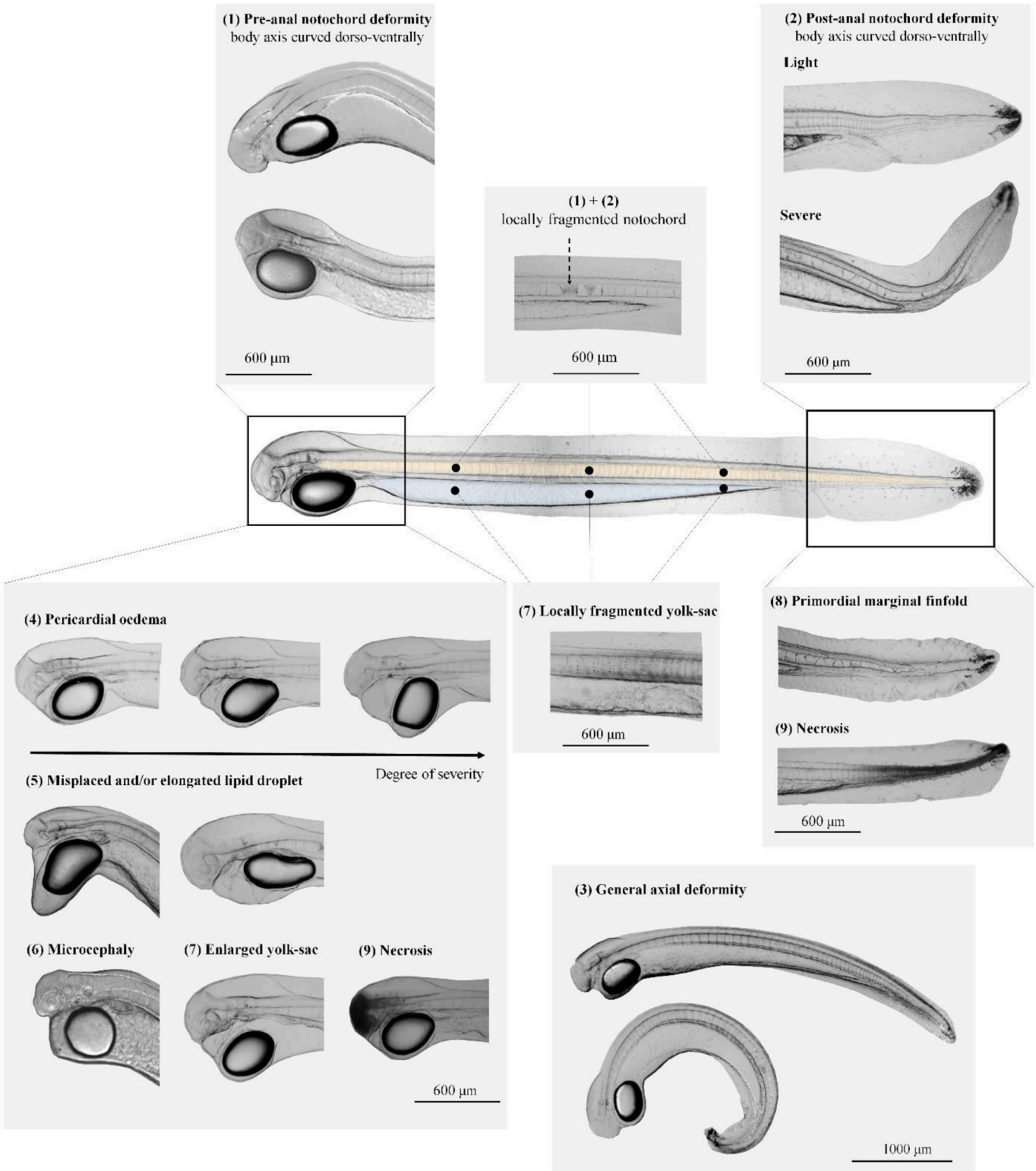


Fig. 5. Deformity determination key (DDK) of the observed abnormalities at 100 h post-fertilization (hpf) in European eel larvae. In the middle, a normal larva is shown. Deformed larvae had notochord deformities that were classified to (**1**) pre-anal and (**2**) post-anal, according to the affected body area. Besides the anterior/posterior part of the body axis being curved dorso-ventrally, the notochord could present localized defects (dashed arrow). Deformed larvae also had (**3**) general axial deformity; (**4**) pericardial oedemas; (**5**) misplaced and/or elongated lipid droplet; (**6**) microcephaly; (**7**) enlarged and/or fragmented yolk-sac, (**8**) abnormalities and (**9**) necrosis. It is important to note that larvae presented more than one deformity type simultaneously.

deformities (reviewed by Boglione et al., 2013; Eissa et al., 2021). In European eels, temperature (Okamura et al., 2007; Kurokawa et al., 2008), salinity (Okamoto et al., 2009; Okamura et al., 2016; Politis et al., 2018) and water current velocity (Kuroki et al., 2016) have been found to affect the occurrence of deformities. We report here, for the first time, that antibiotics decreased the occurrence of deformities during early ontogeny in European eels. While all larvae reared in the control group showed at least one type of deformity, 15–25% of the larvae in the antibiotics treatment groups did not show any type of deformity. Some bacteria like *S. agalactiae*, which have been reported to induce ulcerative or inflammatory lesions in the tegument and muscle of fish, may cause skeletal abnormalities in Nile tilapia *Oreochromis niloticus* (Pasnik et al., 2007). Similarly, in zebrafish, pro-inflammatory signals within the spinal cord indicated inflammation-induced skeletal deformities (Van Gennip et al., 2018). Here, we hypothesize that larvae treated with antibiotics developed normally due to the lack of impact of pathogenic bacteria. This hypothesis is in line with our recent transcriptomic study (Jéhannet et al., 2021). From our study, it remains unknown, specifically, what type of deformities was reduced when applying the antibiotics. The deformities that were observed, especially the notochord ones 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 can reduce swimming and feeding ability and thus impede exogenous feeding that should occur around 15 dph. Therefore, it is important to understand the reasons behind these deformities, to control them and produce healthy larvae that are able to feed, grow and survive. Current procedures for eel propagation must be critically reviewed and to give one example of future investigation, the routine procedure of selecting floating eggs may cause hypoxia in the floating egg layer which on its turn may cause skeletal malformation centrum defects such as reported for in red sea bream *Pagrus major* (Sawada et al., 2006).

5. Conclusions

In the present study, we demonstrated that wild European eels produced eggs of superior quality over eggs of feminized European eels. In addition, we showed that the application of antibiotics increased larval survival. Moreover, antibiotics decreased the occurrence of deformities although the deformity rates remained high. These results demonstrate that antibiotics can benefit early eel larvae in terms of lower mortality and improved development. However, for the longer term, the use of antibiotics for eel larviculture is not a sustainable solution. Alternative solutions, aiming at increasing larval health, should therefore be developed in European eels.

Author statement

Jéhannet, P: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft, Visualization. **Havinga, M**: Methodology, Investigation. **Koumoundouros, G**: Formal analysis, Writing – Review & Editing. **Swinkels, W**: Conceptualization, Funding acquisition. **Heinsbroek, L.T.N**: Conceptualization, Methodology, Investigation. **Palstra, A.P.**: Conceptualization, Methodology, Investigation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The co-authors W.S. and L.T.N.H. are employees of a company. The other authors have no competing interests.

Data availability

Data will be made available on request.

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