Aquaculture 606 (2025) 742569

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

# European eel larvae are sensitive to vibration and light

Fan Yang [a,1], Elisa Benini [a,b,1], Sune R. Sørensen [a], Christoffer Moesgaard Albertsen [a], Johanna Kottmann [a,c], Ian A.E. Butts [d], Jonna Tomkiewicz [a], Sebastian N. Politis [a,*]

[a] National Institute of Aquatic Resources, Technical University of Denmark, Denmark
[b] Department of Veterinary Medical Sciences, University of Bologna, Italy
[c] AquaGen, Puerto Varas, Chile
[d] School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, AL, USA

ARTICLE INFO

*Keywords:*
Fish larvae
Early ontogeny
Hatchery techniques
Escape probability
Escape behavior

ABSTRACT

European eel (*Anguilla anguilla*) earliest life stages haven't been encountered in nature, thus, studying behavior of hatchery-reared larvae is crucial for understanding their ecology and developing rearing protocols. Utilizing computer-assisted video tracking we investigated the behavior of hatchery-reared eel larvae from hatching to the first-feeding stage at 12 days post hatch (dph). Trials focused on escape responses linked to mechanical (vibration) and visual (light) stimuli, repeated at 60 s intervals and selected to mirror stimuli encountered in hatchery settings. Variables included distance moved in single-frame intervals, distance moved in 120 ms (3 frames), initial escape speed, total distance moved in 5 s, mean and maximum speed for the entire response. Escape probabilities were estimated by the hidden Markov model. Results revealed that eel larvae's responses to stimuli were stage-specific, corresponding to the development of sensory systems, where the lowest and highest escape probabilities were observed at hatch and 12 dph, respectively. Larvae demonstrated fast reactions to mechanical stimuli (vibration), but a stronger sensitivity to sudden changes in light intensity, with average maximum escape response speeds up to 40 cm/s. Moderate but consistent escape responses to repeated vibrations, suggest a potential inherent ability to detect and respond to mechanical stimuli, while a decreasing escape response trend with repeated light exposures might indicate a degree of habituation potential to visual stimuli. In conclusion, we highlight the importance of tailoring rearing protocols for eel larvae, avoiding excessive disturbances, especially during highly sensitive stages, to reduce unnecessary stress for improved welfare in hatchery settings.

## 1. Introduction

Fish face several critical developmental events, fundamental to their survival and transition to adulthood, especially during their early life stages, including embryogenesis, hatching, and during larval ontogeny, characterized by early development of essential morphological, physiological and behavioral traits (Yúfera and Darias, 2007; Benini et al., 2022a). Generally, early life history is marked by high mortality and linked to development of essential features for swimming, surrounding sensing, predator avoidance and feeding (Voesenek et al., 2018). In fact, when facing challenging situations, fish larvae exhibit specific behaviors, including characteristic swimming patterns, vertical migrations, and/or escape responses (Houde, 2008). During the larval stage, many species also show high vulnerability, being particularly sensitive to external drivers and environmental factors (Koenker et al., 2018).

In this regard, the early life stages of fish are often considered the most challenging phases in aquaculture (Hamre et al., 2013; Ribeiro et al., 2022). The vulnerability of fish larvae during these early developmental stages poses significant obstacles for successful farming (Føre et al., 2018). Rearing fish larvae is a complex process that requires careful attention to their delicate needs and inherent behaviors. Factors such as nutrition and water quality are crucial, and the success of hatchery protocols heavily depend on understanding and accommodating the intricate behavioral complexities of these early life stages (Rønnestad et al., 2013). Unlike adults with established swimming behaviors, larvae are constantly evolving and adapting behaviors that are critical for their survival. From the specific ways they search for food to the nuanced escape responses triggered by perceived threats, larval

* Corresponding author.
*E-mail address:* snpo@aqua.dtu.dk (S.N. Politis).
[1] Shared 1st authorship.

https://doi.org/10.1016/j.aquaculture.2025.742569
Received 1 February 2025; Received in revised form 31 March 2025; Accepted 8 April 2025
Available online 11 April 2025

behaviors require careful consideration when designing rearing environments (Hamre et al., 2013; Ribeiro et al., 2022). Failure to account for these complex needs and preferences can result in stress, stunted growth, deformities, and ultimately, high mortality rates. Especially from a hatchery technology perspective, understanding how larvae respond to various external stimuli, including changes in light, turbulent flows, and rearing system dynamics or handling techniques, is instrumental in designing any effective rearing protocol. These aspects, including insights into behavioral responses to different stimuli, can lead to optimized husbandry practices that improve survival and overall well-being, thereby contributing to the development of effective aquaculture techniques (Conte, 2004; Fisheries, F.A.O, 2022).

An effective approach to gather knowledge on the matter involves observation of the targeted species within corresponding native habitats, in order to gain insights regarding natural behavior dynamics and responses to environmental stimuli (Rønnestad et al., 2013; Martorell-Barceló et al., 2023). By studying how fish larvae interact with their surroundings and other organisms can lead to a comprehensive understanding of their unique requirements for optimal growth and development (Ribeiro et al., 2022). In this regard, replicating possible stimuli found in natural habitats, and applying it into controlled experiments and hatchery environments, is a challenging yet essential step in developing aquaculture practices. Unfortunately, acquiring comprehensive information on larval behavior is not always feasible, particularly in the case of European eel, *Anguilla anguilla*. As far as we know, eel larvae hatch in the Sargasso Sea and are then passively drifting with ocean currents for several months before reaching European waters (Arai et al., 2020). Thus, in combination with the elusive and migratory nature of adult European eel, only few detailed field observations have been made regarding their early life stages (Miller et al., 2019; Righton et al., 2016; Wright et al., 2022). In fact, no eggs or pre-feeding larvae have ever been discovered in nature. As such, behavioral aspects of European eel early life stages can only be deducted from hatchery-reared offspring, which is now possible due to progressive development of controlled propagation techniques (Tomkiewicz et al., 2019).

Analyzing eel larval behavior in the hatchery is challenging due to their small sizes and transparent bodies. For instance, European eel larvae emerge from their eggs with underdeveloped features, including a prominent yolk sac and oil droplet (Sørensen et al., 2016a). When raised at ~18–20 °C, eye pigmentation only becomes visible around 10–12 days post-hatch (dph), together with the development of the feeding apparatus, characterized by the emergence of teeth, shortly before yolk depletion (Sørensen et al., 2016a; Politis et al., 2017). In this regard, some experimental studies have explored rearing conditions for European eel larvae during the above mentioned stages, including biotic (Sørensen et al., 2014; Benini et al., 2022b; Bandara et al., 2023; Politis et al., 2023) and abiotic factors, such as temperature, salinity, and light (Politis et al., 2017; Politis et al., 2014; Butts et al., 2016; Syropoulou et al., 2022). However, despite those important insights, survival rate of eel larvae during early life stages remains variable (Benini et al., 2023).

Therefore, it is hypothesized that disturbances during hatchery practices, including monitoring and handling procedures, could significantly impact the physiological and behavioral responses of fish larvae, potentially leading to impaired growth and development. The intricate interplay between stress and the behaviors of developing larvae highlights the importance of understanding and mitigating stress factors to ensure successful early life culture of European eel. To address this issue, we utilized computer-assisted video tracking technology to explore behavioral patterns of eel larvae from hatch to the first-feeding stage (12 dph). The focus was on identifying and characterizing the larvae's escape responses to mechanical and visual stimuli, triggered by vibration and sudden shifts in light intensity, respectively. These stimuli were intentionally selected to mirror conditions typically encountered in current hatchery settings.

## 2. Materials and methods

### 2.1. Ethical statement

All fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 86/609/EEC). Broodstock assisted reproduction protocols were approved by the Animal Experiments Inspectorate (AEI), Danish Ministry of Food, Agriculture and Fisheries (permit numbers: 2015-15-0201-00696). Briefly, broodstock eels were anesthetized by short submergence in an aqueous ethyl p-aminobenzoate (benzocaine) before tagging, biobsies and stripping and euthanized subsequently by longer exposure.

### 2.2. Broodstock management, gametes production and larval rearing

Female silver eels were obtained from lake Vandet, Denmark, while male eels were obtained from a Danish commercial eel farm. At a DTU Aqua facility male and female eels were kept separately during reproductive development at 20 °C and 36 psu (Tropic Marin® Sea Salt, Dr. Biener Aquarientechnik, Germany). Prior to experiments, fish were anesthetized (ethyl p-aminobenzoate, 20 mL saturated solution $L^{-1}$; Sigma–Aldrich Chemie, Steinheim, Germany) and individually tagged with a passive integrated transponder (PIT tag) for individual identification. No feed was provided, as sexually maturing eels cease feeding (Pankhurst and Sorensen, 1984). To induce development of gametes, females received weekly injections of salmon pituitary extract and males weekly injections of human chorionic gonadotropin (Sigma-Aldrich, Denmark A/S) following previously described protocols (Kottmann et al., 2020).

Females used for experiments ($n = 4$) had a mean (± S.E.) standard length and initial body weight of 40 ± 3 cm and 135 ± 25 g, respectively. Gametes were harvested by strip spawning and fertilization performed as described in (Butts et al., 2014). After fertilization, buoyant eggs were incubated in natural seawater, which was adjusted from 32 to 36 psu using reef salt at 20 ± 0.5 °C (Politis et al., 2018; Sørensen et al., 2016b). Eggs were reared in 60 L black conical incubators with aeration, while water flow was kept at a rate of 350 ± 50 mL $min^{-1}$ and initial egg density aiming at 100 g per incubator (Sørensen et al., 2016a). Non-buoyant eggs were purged daily. The newly hatched larvae were reared at low light conditions (Politis et al., 2014), 36 psu salinity and temperature of 20 ± 0.5 °C (Sørensen et al., 2016a). The larval rearing tanks were connected to a recirculation system (RAS) consisting of a 300 L reservoir, 0.8 μm cartridge filter (Atlas Filtri Ltd., Padua, Italy), 36 W UV (ProCristal UV-C 36 W, JBL GmbH & Co. KG, Neuhofen, Germany), 150 L coarse gravel biofilter, and trickle filter (0.5 $m^3$ BioBlok 200, Expo-Net, Hjørring, Denmark). Temperature was regulated by 2 × 500w Titanium Heaters (T500 titanium heater, Aqua Medic GmbH, Bissendorf, Germany) connected to a temperature control unit (Aqua Medic T-computer, Aqua Medic GmbH, Bissendorf, Germany).

### 2.3. Experimental set-up

Larval behavior was recorded at 0, 3, 6, 9, and 12 dph, with these time points chosen for their developmental significance in European eel. The development at each time point selected in this study is presented in Fig. 1. In brief, newly hatched larvae (0 dph) are still underdeveloped, characterized by a prominent yolk sac and a large oil droplet, measuring approximately 3.7 mm in length (Sørensen et al., 2016a). During the first few days, larvae primarily focus on length growth while developing optic capsules, a visible hindbrain, and a pericardium. By 3 dph, the heart and brain become clearly visible, and larvae reach an estimated length of 5.3 mm (Sørensen et al., 2016a). At 6 dph, the oral opening appears as a small channel behind the eyes, marking the initial formation of the mouth and eye structures (Politis et al., 2021). By 9 dph, eye pigmentation begins, and both upper and lower jaws start to develop.


**Fig. 1.** Morphological development of European eel (*Anguilla anguilla*) larvae from hatch to the first-feeding stage at 20 °C.

Simultaneously, the tail elongates, the abdomen extends into the yolk sac, and caudal pigmentation emerges. At this stage, larvae reach a length of approximately 6.6 mm, and the head angle shifts, causing the mouth to point forward (Sørensen et al., 2016a).

Larvae reach the first feeding stage around 10–12 dph at 18–20 °C (Sørensen et al., 2016a; Politis et al., 2017). During this period, they are observed ingesting food, confirming their transition to active feeding (Benini et al., 2023).

At each targeted age, 3 replicate groups of 5 randomly selected eel larvae were transferred to a specifically designed aquarium (3.7 × 5.1 × 0.5 cm). The experiments were conducted with eel larvae from 4 different batches, each representing a distinct parental crossing. These larvae were placed in a separated room maintained in darkness for video acquisitions. To capture high-quality videos in complete darkness, an infrared (IR) sensitive camera (GigE, monochrome ½" CCD, 5.3 × 5.3 μm pixel size, Basler acA1300-60gmNIR C-mount) with a suitable lens and an 850 nm IR pass filter was used. The camera was mounted on a tripod held ~4 cm in front of the aquarium and connected to a laptop for remote video control, minimizing disturbances to the larvae. The larvae were filmed in visual darkness, exposed only to an IR light source (46 × 66 cm, Noldus Information Technology, Wageningen, Netherlands) positioned behind the aquarium. Videos were recorded at 25 frames per s (fps) with a resolution of 1280 × 1080 $px^2$. A conceptual overview of the experiment is presented in Fig. 2.

### 2.4. Experimental protocols

For eel larvae at each age (0, 3, 6, 9, 12 dph), 3 replicated trials were conducted with randomly selected groups of eel larvae (5 larvae per group) from four batches, examining both escape responses and undisturbed control behaviors with or without external stimuli. Larvae were allowed to acclimate in the experimental aquarium for 300 s. An overview of the behavioral trials for each batch at different ages is presented in Fig. 2E.

#### 2.4.1. Undisturbed "control" trials

Undisturbed "control" behavior, defined as the activity of eel larvae in rearing conditions without external disturbances, was recorded for 3

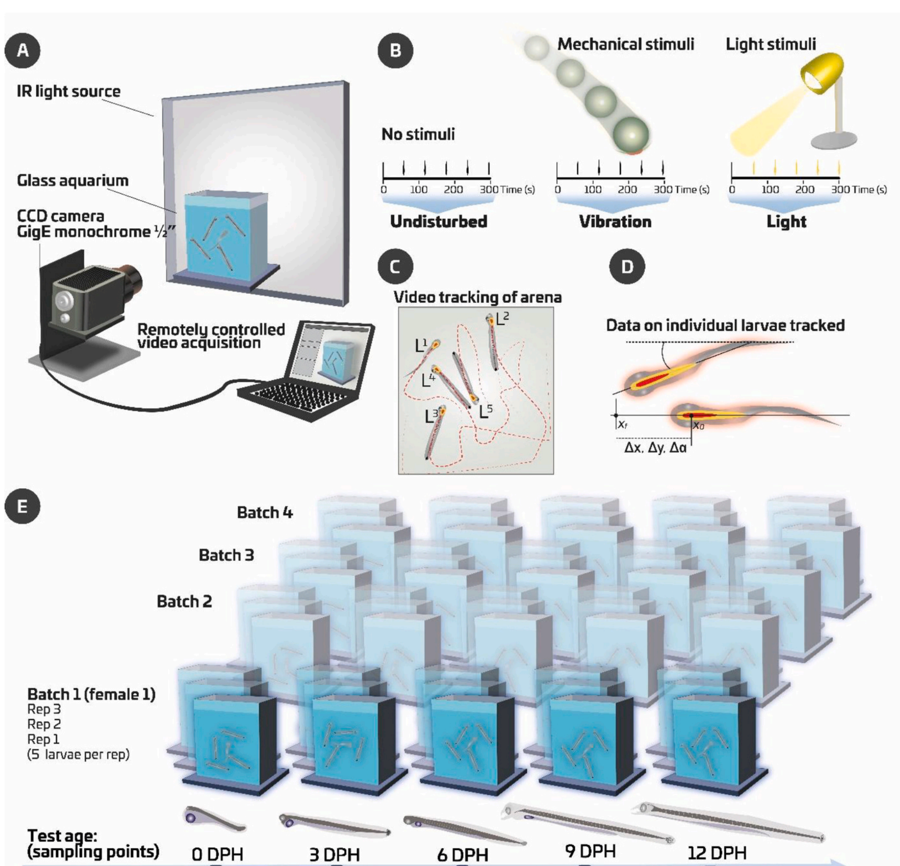


**Fig. 2.** Conceptual overview. A) Experimental set-up for remotely controlled video acquisition of European eel (*Anguilla anguilla*) larvae reared in an aquarium placed in front of an infrared (IR) light source. B) Larvae were exposed to no external stimuli (undisturbed control), repeated vibration stimuli, and repeated light stimuli. C) Behavioral responses of eel larvae were analyzed using the Noldus tracking software EthoVision. D) To accurately detect the transparent larvae, dynamic subtraction was used to compensate for temporal changes in the background, and a detection reference image was generated by averaging multiple images, while the X and Y coordinates of each larva's center of mass were tracked, to minimize tracking errors. E) Trials were conducted at different ages in days post hatch (DPH), while five larvae were used for each age ($n$ = 5), batch ($n$ = 4) and replicate ($n$ = 3).

replicated groups of 5 larvae, from each batch, and at each age. The undisturbed behavior of each larval group was filmed for 300 s following the acclimatization period in the aquarium.

#### 2.4.2. Escape trials

For the escape trials, the larval groups (from each batch and age) were recorded for 300 s, while exposed to 5 repeated vibrations (applied at 60 s intervals) and then another 300 s, while exposed to 5 repeated light exposures (applied at 60 s intervals).

To induce vibrations, a rubber ball ($D = 3.2$ cm) was dropped from a height of 20 cm above the table where the aquarium was placed. The flashlight stimuli were manually applied using a daylight lamp (Falcon Eyes ML-40 W, 5400–5600 K, 2100 lm, Benèl BV Hoogeveen, Netherlands) positioned 30 cm from the aquarium at an intensity of 840 lx.

In total, including the acclimatization period, the undisturbed "control" trial and the escape trials, the larvae were kept in the experimental aquarium for a maximum of 1200 s = 20 min. Water applied to the test arena was natural seawater adjusted to 36 PSU, using Red Sea salt (Red Sea International, Eilat, Israel) filtered through 0.2 μm (Minisart® hydrophilic syringe filters Sartorius Stedium Biotech GmbH, Germany) prior to use to ensure water quality. After each trial (20 min) new water was applied.

### 2.5. Behavioral measurements

Behavioral responses of eel larvae in the control and escape trials (vibrations and light stimuli) were analyzed using the tracking software EthoVision XT v17.5 (Noldus et al., 2001). To accurately detect the transparent larvae, dynamic subtraction was used to compensate for temporal changes in the background, and a detection reference image was generated by averaging multiple images. To minimize tracking errors, only the *X* and *Y* coordinates of each larva's center of mass were tracked. The percentage of "subject-not-found" errors was 9.63 ± 11.21 % across all tracked videos, indicating good tracking quality.

Based on the obtained tracks, larval behaviors were quantified in two aspects: escape probabilities modelled by the hidden Markov chain (described in detail in Section 2.6) and behavioral response variables that were defined as follows:

(1) Distance moved in single-frame intervals ($D_{esc\_40ms}$), representing displacement from the first contraction of larvae
(2) Distance moved in 3 video frames ($D_{esc\_120ms}$), indicating the contribution of the fast-start portion of the response
(3) Initial escape speed ($U_{init}$), which is the swimming speed calculated during the initial 120 ms of the escape response (also referred to as the burst speed)
(4) Total distance moved in 5 s ($D_{esc\_05s}$), indicating the distance moved from initiating the response to where escape speeds decline markedly
(5) Mean speed for the entire response ($U_{mean}$)
(6) Maximum speed for the entire response ($U_{max}$).

### 2.6. Statistical analyses

To estimate the escape probabilities of eel larvae to no-stimuli (control), vibration, and light, distances moved were modelled by hidden Markov models (HMM) (Zucchini and MacDonald, 2009). Note that the behavioral responses of larvae during the 300 s of either no-stimuli (control), vibration, or light stimuli were treated as independent processes in the model. The HMM consisted of two parts: (1) a discrete Markov chain to model the unknown behavioral states, classified as "escape" or "resting"; (2) a log-normal distribution for the observed distance moved, left-censored at 0.458 cm/s. The parameters of the log-normal distribution depended on the estimated behavioral state of larvae.

Behavioral states to different stimuli were obtained through the transition matrix in the Markov chain. The transition matrix in the HMM was parametrized by $\Gamma_i = exp(Q_i)$, where $Q_i$ was a matrix of transition intensities, with off-diagonal elements being positive and row-sums equal to zero. Each transition intensity was modelled by a log-linear function of potential influencing factors: *Larval Age* (days-post-hatch, dph), *Stimulus Type* (vibration, light, or none), *nStimuli* (the number of same-typed stimuli), *Trial Time*, and *Time Since Stimuli*. The log-normal distributions of distance moved were estimated using the maximum likelihood for each larva. The likelihood function and smoothing state probabilities were calculated by the forward-backward algorithm, while the most likely state sequence for each larva was obtained using the Viterbi algorithm (Zucchini and MacDonald, 2009; Viterbi, 1967). Finally, the most likely state sequences were used for post-hoc analysis of movement during escape events.

To explore the influence of potential factors on the probability of different behavioral states, we modelled the state transition probabilities as functions of potential influencing factors (Langrock et al., 2012; McClintock and Michelot, 2018). Non-linear effects of *Trial Time* and *Time Since Stimuli* were modelled using splines, while *nStimuli* was studied with a third-order polynomial. *Larval Age* and *Stimulus Type* were treated as categorical variables with separate coefficients for each level. Model selection was performed using the Akaike Information Criterion (AIC) to identify the combination of covariates that best explained the behavioral state transitions (Akaike, 1974; Byrnes et al., 2021). The final model for both, vibration and light stimuli followed the *formula* $\sim$ *Age* + *Trial Time* + *Stimulus Type* + *Stimulus Type* × *nStimuli* + *Stimulus Type* × *Time Since Stimuli*, where the interactive effects among *Stimulus Type*, *nStimuli,* and *Time Since Stimuli* were considered.

Additionally, linear mixed models were employed to test the effects of *Age* and *nStimuli* on the escape behavior of larvae. *Larval Batch*, fish identity (*fishID*), and trial number (*Trial*) were included as random factors to account for repeated measurements. For escape trials, six response variables ($D_{esc\_40ms}$, $D_{esc\_120ms}$, $D_{esc\_05s}$, $U_{init}$, $U_{mean}$, and $U_{max}$) were modelled, while only $D_{esc\_05s}$, $U_{mean}$, and $U_{max}$ were considered for control trials. The Bayesian Information Criterion (BIC) was applied to balance model fit and complexity. The selected models were: $response.variables \sim Age + nStimuli + (1|Batch) + (1|fishID) + (1|Trial)$ for escape trials, and $response.variables \sim Age + (1|Batch) + (1|fishID) + (1|Trial)$ for control trials. Herein, the factor "*Age*" was used to investigate how larvae react to stimuli in different developmental stages, while "*nStimuli*" was used to evaluate responses to repeated use of the same stimuli. The normality and homoscedasticity of the model residuals were checked using Q-Q plots and residuals vs. fitted values plots. All response variables were log-transformed to comply with model assumptions. Differences between groups were interpreted by examining the overlap of confidence intervals (CI) for the predicted means. Significant differences were identified if the fraction of CI overlap between two groups was <0.5 (Cumming et al., 2007).

The function "*lmer*" (https://cran.R-project.org/web/packages/lme4/index.html) was used for model specification, while the function "*DHARMa*" (https://CRAN.R-project.org/package=DHARMa) was used to check model assumptions with residual diagnostics. All statistical analyses were done in R v4.3.0 (http://www.R-project.org/).

## 3. Results

### 3.1. Estimated escape probabilities

Four covariates (i.e., *Larval Age*, *nStimuli*, *Trial Time*, and *Time Since Stimuli*) significantly affected the estimated escape probabilities during the periods with vibration, light, or no stimuli. As shown in Fig. 3A, there is generally no or very low probability (0.07 ± 0.01) of escape-like responses without external stimuli, with significantly lowest (0.03 ± 0.01) escape probabilities of undisturbed larvae at 0 dph and highest (0.12 ± 0.01) at 12 dph (Fig. 3D).

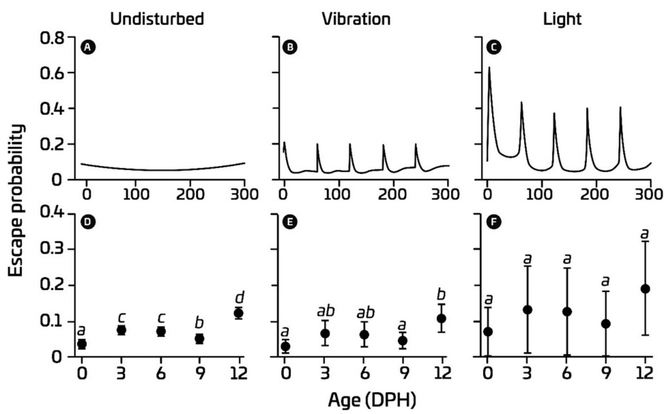


**Fig. 3.** Escape probabilities of European eel (*Anguilla anguilla*) larvae as functions of trial time and age in days post hatch (dph) when exposed to (A, D) no external stimuli, (B, E) repeated vibration stimuli, and (C, F) repeated light stimuli. Error bars represent 95 % confidence intervals. Different lowercase letters indicate statistically significant differences between groups ($p < 0.05$).

In contrast, eel larvae showed consistent moderate escape probabilities (0.20 ± 0.005) when exposed to repeated vibration stimuli (Fig. 3B), whereas the escape probabilities were relatively uniform from hatch until 9 dph and reached highest values at 12 dph (Fig. 3E). On the other hand, eel larvae showed increased escape probabilities (0.45 ± 0.01) when exposed to repeated light stimuli (Fig. 3C). In this case, no significant effects of "*Age*" were observed on the escape probabilities of eel larvae (Fig. 3F).

### 3.2. Influencing factors for escape variables

Larval behavior results in response to control and escape trials are summarized in Table 1, presenting the effects of "*Age*" and "*nStimuli*" on escape variables (i.e., $D_{esc\_40ms}$, $D_{esc\_120ms}$, $D_{esc\_05s}$, $U_{init}$, $U_{mean}$, and $U_{max}$). When no stressor was applied (control trials), *Age* had significant ($p < 0.001$) influence on the variables measured in 5 s (i.e., $D_{esc\_05s}$, $U_{mean}$ and $U_{max}$). As shown in Fig. 4A5, $U_{mean}$ was highest for eel larvae at 6 dph, while $D_{esc\_05s}$ and $U_{max}$ reached peak values at 3 dph (Fig. 4A3 and A6, respectively).

When larvae were exposed to vibration, $D_{esc\_40ms}$, $D_{esc\_120ms}$ and $U_{init}$ peaked at 3 dph (Fig. 4B1, B2 and B4), while highest $U_{max}$ was observed at 6 dph (Fig. 4B6). When larvae were exposed to light, the three escape variables measured in 5 s (i.e., $D_{esc\_05s}$, $U_{mean}$, and $U_{max}$) were significantly affected by *Age*, with larvae at 0 dph displaying lowest sensitivity (Fig. 4C3, C5 and C6).

Furthermore, effects of stimuli times when exposed to vibration and light stimuli are visualized in Fig. 5A(1–6) and B(1–6), respectively. Repeated vibration stimuli imposed consistent escape responses with no significant difference across stimuli times on most escape variables, except for $D_{esc\_120ms}$, which showed a peak value at 3 dph (Fig. 5A2). In contrast, when larvae were exposed to light stimuli, generally highest values were registered at 1st exposure and demonstrated a decreasing trend with increasing stimuli times (Fig. 5B1–6), indicating a degree of habituation to repeated light stimuli.

## 4. Discussion

### 4.1. Escape speeds and setup optimizations

Understanding the behavioral responses of fish larvae is essential for optimizing hatchery practices (Rønnestad et al., 2013; Martins et al., 2012). From a kinematic perspective, fish escape responses typically consist of three stages (Weihs, 1973; Domenici and Blake, 1997; Eaton et al., 2001). Stage 1, a preparatory rapid body bend with minimal movement of the center of mass; stage 2, a propulsive stroke where the fish accelerates away; and stage 3, where the fish either continues swimming or begins gliding (Weihs, 1973). Most research has focused on stages 1 and 2, although stage 1 alone can occur as a single-bend response (Domenici and Blake, 1997). During stage 1, the body assumes either a 'C' shape (C-start) or an 'S' shape (S-start). For adult fish, the response latency (stage 1) and escape duration (stages 1 and 2) typically range between 5–150 ms and 30–200 ms, respectively (Domenici and Blake, 1997; Roche et al., 2023). However, limited examples exist about these behavioral stages in fish larvae. For instance, Batty (1989) measured the latency of C-start responses of herring (*Clupea harengus*) larvae to flash-light stimuli, which was around 40 ms and 120 ms in dark and light conditions, respectively. On the other hand, (Skajaa and Browman, 2007) found mean escape latency of cod (*Gadus morhua*) larvae in response to pipette suction to be around 10 ms, which is considerably faster compared to herring larvae.

Interestingly, even though the average escape speeds (U*mean*) in our study have generally been low, the max speeds of the startle response (U*max*) were higher than previously reported for other species, including cod larvae (0–28 dph), winter flounder (*Pleuronectes americanus*) (1–50 dph), herring, capelin (*Mallotus villosus*), and shanny (*Lipophrys pholis*) (Williams et al., 1996). In these species, at comparable developmental stages, the max escape speeds reached ~15 cm/s in cod, ~11 cm/s in flounder, ~17 cm/s in herring, ~14 cm/s in capelin, and ~22 cm/s in shanny. In contrast, the max escape speed (U*max*) of European eel larvae in this study reached values above 40 cm/s, demonstrating a markedly faster response potential. This could be an indication that, besides relying on tactics such as hiding or using cryptic coloration,

**Table 1**
Effects of *Age* and *nStimuli* on distance moved in the first-frame intervals ($D_{esc\_40ms}$), distance moved in 120 ms ($D_{esc\_120ms}$), total distance moved in 5 s ($D_{esc\_05s}$), initial escape speed ($U_{init}$), mean speed during the entire escape ($U_{mean}$), and maximum speed ($U_{max}$) during the behavioral response of European eel (*Anguilla anguilla*) larvae under vibration, light or no stimuli. Note that only effects of *Age* were considered for the escape variables during the undisturbed period. d.f.: degrees of freedom, sum sq.: sum of squares. * $p < 0.05$.; ** $p < 0.01$.; *** $p < 0.001$.

| Variable | Factors | d.f. | Undisturbed | | | Vibration | | | Light | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sum sq. | F | *p*-value | Sum sq. | F | *p*-value | Sum sq. | F | *p*-value |
| $D_{esc\_40ms}$ | *Age* | 4 | 9.11 | 1.36 | $p > 0.05$ | 54.21 | 5.72 | *** | 12.69 | 1.18 | $p > 0.05$ |
| | *nStimuli* | 4 | \ | | | 15.28 | 1.61 | $p > 0.05$ | 18.83 | 1.75 | $p > 0.05$ |
| $D_{esc\_120ms}$ | *Age* | 4 | 9.51 | 1.58 | $p > 0.05$ | 60.23 | 6.48 | *** | 8.80 | 0.84 | $p > 0.05$ |
| | *nStimuli* | 4 | \ | | | 20.81 | 2.34 | * | 25.97 | 2.48 | * |
| $D_{esc\_05s}$ | *Age* | 4 | 27.84 | 7.24 | *** | 12.54 | 2.00 | $p > 0.05$ | 15.32 | 2.42 | * |
| | *nStimuli* | 4 | \ | | | 4.77 | 0.76 | $p > 0.05$ | 140.70 | 22.19 | *** |
| $U_{init}$ | *Age* | 4 | 11.77 | 1.90 | $p > 0.05$ | 57.51 | 6.66 | *** | 10.31 | 0.97 | $p > 0.05$ |
| | *nStimuli* | 4 | \ | | | 15.53 | 1.80 | $p > 0.05$ | 22.88 | 2.16 | * |
| $U_{mean}$ | *Age* | 4 | 21.49 | 5.78 | *** | 2.61 | 0.59 | $p > 0.05$ | 20.16 | 3.28 | * |
| | *nStimuli* | 4 | \ | | | 2.09 | 0.47 | $p > 0.05$ | 83.21 | 13.53 | *** |
| $U_{max}$ | *Age* | 4 | 50.85 | 5.84 | *** | 45.81 | 3.45 | * | 24.31 | 2.03 | * |
| | *nStimuli* | 4 | \ | | | 7.46 | 0.56 | $p > 0.05$ | 196.45 | 16.39 | *** |

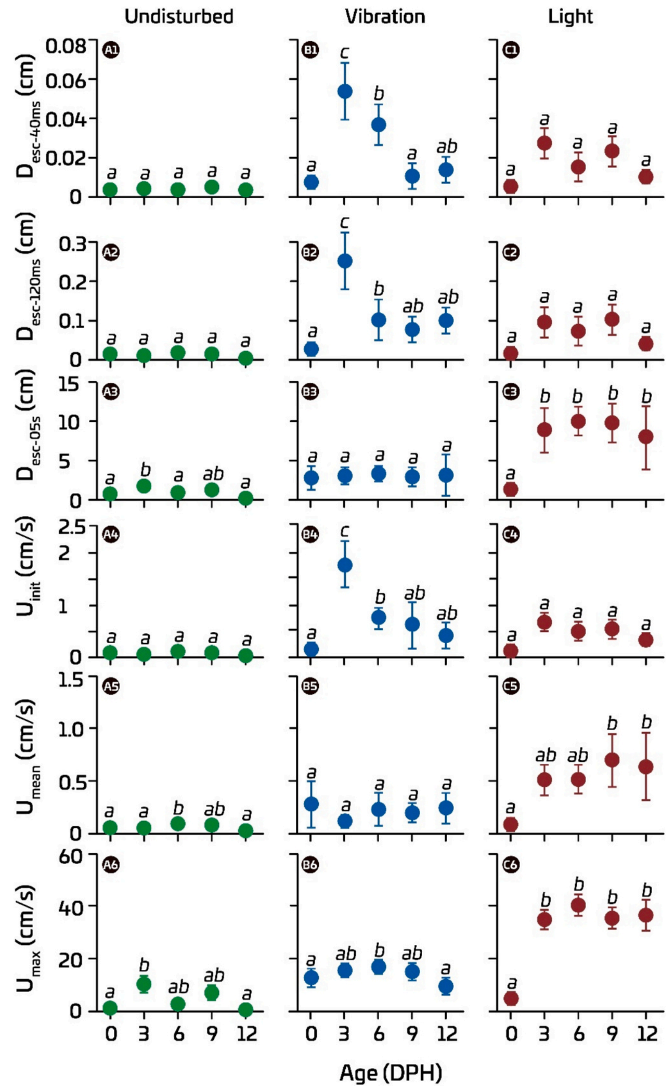


**Fig. 4.** Effects of age in days post hatch (dph) on behavioral responses of European eel (*Anguilla anguilla*) larvae during an (A) undisturbed period, or when exposed to repeated (B) vibration and (C) light stimuli. Behavioral responses of larvae were indicated by (1) distance moved in the first-frame intervals (Desc_40ms), (2) distance moved in 120 ms (Desc_120ms), (3) total distance moved in 5 s (Desc_05s), (4) initial escape speed (Uinit), (5) mean speed during the entire escape (Umean), and (6) maximum speed (Umax). Plots display raw data in mean ± s.e.m., while letters indicate significant differences among treatments.

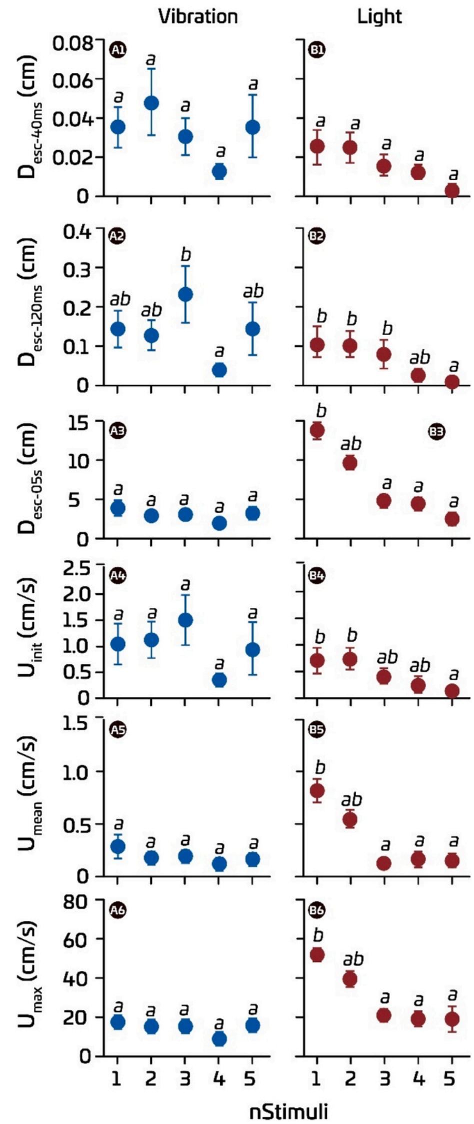


**Fig. 5.** Effects of *nStimuli* (stimuli times) on the behavioral responses of European eel (*Anguilla anguilla*) larvae when exposed to (A) vibration and (B) light stimuli. Behavioral responses of larvae were indicated by (1) distance moved in the first-frame intervals ($D_{esc\_40ms}$), (2) distance moved in 120 ms ($D_{esc\_120ms}$), (3) total distance moved in 5 s ($D_{esc\_05s}$), (4) initial escape speed ($U_{init}$), (5) mean speed during the entire escape ($U_{mean}$), and (6) maximum speed ($U_{max}$). Plots display raw data in mean ± s.e.m., while letters indicate significant differences among treatments.

eel larvae have developed a strategy, counting on speed to evade predators, which could be an adaptation to their natural environment. At this point it is important to mention, that this extremely high speed of eel larvae, combined with the low frame rate (25 Hz) and the minimum time interval (40 ms) of the recording system, limited the extent to which their escape responses could be accurately quantified. The large time intervals between frames also increased the difficulties in maintaining the identity of each larva during the early response stages. In addition, suboptimal lighting conditions and low filming resolution, made it difficult to obtain precise tracking data for the snout and tail, which was difficult enough already due to the larvae's transparent bodies. As such, tracking was limited to the center of mass, preventing the calculation of common escape performance variables such as turning radius, turning rates, and turning angles, resulting in the loss of valuable kinematic information regarding how larvae react to external stimuli.

Based on these considerations, to accurately capture different stages of an escape response, future studies on fish larvae should target a frame rate that can cover a minimum of five frames during stage 1, which usually lasts for 5–20 ms (Roche et al., 2023; Gingins et al., 2017). Moreover, future research should utilize cameras with higher frame rates/resolutions and regarding eel larvae, employ fully transparent tank designs or if possible, studying larval behaviors during actual rearing conditions, which would be even more valuable to provide accurate insights from a hatchery perspective. Nevertheless, the present study described the early-life behavior of European eel larvae exposed to repeated vibration and light stimuli, with findings contributing to the development of more effective hatchery protocols for improving survival, growth, and overall health during these critical early stages.

### 4.2. Developmental stage sensitivity

Analysis of video tracking data revealed stage-specific variations in escape behavior ability and probability, where specifically just after hatch (at 0 dph), eel larvae were least responsive to external stimuli, potentially due to their still underdeveloped sensory systems. From that point onwards, eel larvae progress their ontogenetic development and organogenesis (Sørensen et al., 2016a), where their sensory system should be genetically programmed to develop a physiological basis that enhances sensitivity to external stimuli, which is a critical adaptation for predator detection in natural settings. Similar developmental patterns have been observed in zebrafish (*Danio rerio*) larvae at a comparable age (Colwill and Creton, 2011; Creton, 2009). In fact, zebrafish larvae start swimming around 4 dph, when they are visually capable of following moving objects. By 5 dph, they develop color vision, allowing more vigorous escape responses to stimuli, while at 6–7 dph, they can distinguish colors such as green and red, which influences their circadian rhythm (Colwill and Creton, 2011). Moreover, studies have shown that escape responses of walleye pollock (*Theragra chalcogramma*) larvae to predators (Sugisaki et al., 2001) and Atlantic cod larvae to sudden water movement (Skajaa and Browman, 2007) increase with progressive development. As such, our results agree with these findings, as eel larvae exhibited increased sensitivity with advancing developmental stages.

Furthermore, the marked escape behaviors of European eel larvae observed in response to vibration and light at different stages, could potentially be physiologically connected to an early development of Mauthner (M-) cells, which are a bilateral pair of reticulospinal neurons critical for initiating a rapid escape response, which allows fish to evade possible predators (Machnik et al., 2018). They are involved in escape responses that can include a powerful bending of the body and tail, propelling the fish away from the threat in a quick and efficient manner. M-cells receive surrounding information from multi-sensory modalities, including eyes and the lateral line. As soon as fish detect a sudden stimulus, such as a flash of light or a sound wave, they fire off an action potential that travels down the spinal cord and activates muscles on one side of the body, causing the fish to bend sharply in the opposite direction, away from the stimulus (Eaton and Emberley, 1991; Medan and Preuss, 2014). As such, the mechanical and visual stimuli tested in the present study could have triggered an early version of M-cells to start escape responses even in such early life stages of European eel. Given the lack of research on eel, future studies would greatly benefit from investigating the ontogeny of structures such as M-cells in this species.

### 4.3. Sensitivity to stimulus types

When focusing only on the earliest response, in the present study quantified in 40 ms ($D_{esc\_40ms}$) and 120 ms ($D_{esc\_120ms}$) (Fig. 4B1, B2, B4), escape responses were quite strong when eel larvae were exposed to vibration, especially on 3 dph. However, when focusing on the longer-term effects ($D_{esc\_05s}$), measured within 5 s (Fig. 4C3, C5, and C6), escape responses were strongest when eel larvae were exposed to visual stimuli (light), which translated into generally higher escape probabilities (Fig. 3C). Similar escape responses have also been observed in herring larvae, where escape latency to a visual stimulus was found to be longer than latency to a mechano-acoustic stimulus (Batty, 1989; Domenici and Hale, 2019). This is further supported by experiments on goldfish (*Carassius auratis*), where (Mirjany and Faber, 2011) similarly described how visual stimuli can trigger the function of M-cells through a longer pathway than mechano-acoustic stimuli. As such, it seems likely that eel larvae also react faster to mechanical stimuli, such as vibration, but are generally more sensitive to sudden changes in light intensity, despite a longer reaction time during these early life stages.

At this point it is also valuable to mention that compared to other fish species, eel larvae display a delayed development of their visual system, as eye pigmentation and potentially functional vision only emerge after 10–12 dph at 18–20 °C (Sørensen et al., 2016a; Politis et al., 2017). Nonetheless, light conditions can still influence eel early life stages, as demonstrated by (Politis et al., 2014), where intensity and spectral composition affected development and survival, providing evidence that limited light detection can be possible even without functional eyes. However, at the first-feeding stage, eel larvae must have functional vision to successfully initiate feeding (Butts et al., 2016). Thus, the stage-specific sensitivity to external stimuli observed in this study appears to be closely linked to the genetically programmed development and functionality of their sensor systems.

### 4.4. Effects of stimulus times

Consistent escape performance has been observed in sea bass (*Dicentrarchus labrax*), where fast-start escape responses were assessed at both short (30-min intervals repeated five times) and medium (30-day intervals repeated twice) time scales (Marras et al., 2011). Similarly, when repeated stimuli were imposed at 15 min intervals on the coral reef fish (*Amblyglyphidodon curacao*), maximum escape performances were repeatable through time, while considerable variations in the response magnitude were found both among and within individual fish (Jornod and Roche, 2015). However, in the present study on European eel larvae, repeated vibration stimuli imposed consistent escape responses with no significant difference across stimuli times on most escape variables (Fig. 5A1–6), potentially demonstrating an inherent ability to detect and respond to this type of stimuli. In contrast, when larvae were exposed to light stimuli, generally highest values were registered at 1st exposure and demonstrated a decreasing trend with increasing stimuli times (Fig. 5B1–6). From one side, this decrease may result from the short time intervals (60 s) between stimuli, which might not allow sufficient recovery time, but at the same time it could be indicating a degree of habituation potential to repeated light stimuli. A recent study investigated the ability of larval zebrafish to learn through repeated exposure to the same stimuli, as demonstrated by startle reflex habituation (SRH), a basic form of non-associative learning (Beppi et al., 2021). In SRH, motor responses to sudden or unexpected stimuli diminish over time as the stimuli are recognized as non-threatening. This learning process involves both peripheral sensory adaptations, in which sensory cells become less responsive, and central habituation, where the motor reaction is reduced despite continued sensory processing (Roberts et al., 2016). However, the duration of habituation varies depending on the frequency and duration of the stimuli, ranging from minutes to days. Therefore, the reduction in escape responses to visual stimulation observed in eel larvae could potentially result from acquiring knowledge about the specific repeated stimulus. This habituation mechanism enables the larvae to recognize the visual stimuli as non-threatening, thereby conserving energy by avoiding unnecessary startle responses.

## 5. Conclusions

To summarize, the findings of this study provide crucial insights into the early-life behavioral responses of European eel larvae to environmental stimuli, emphasizing the need for refined rearing protocols in hatchery settings. The observed stage-specific sensitivity to mechanical and light stimuli highlights the importance of handling larvae with extreme care, minimizing water disturbances and sudden changes in light intensity. Such precautions are essential to prevent unnecessary escape responses, which lead to increased energy expenditure from the limited nutrients in the yolk sac, nutrients that would otherwise support growth and development.

The study also revealed that larvae exhibit a faster reaction to mechanical stimuli (vibration) but a stronger sensitivity to sudden changes in light intensity, despite a longer reaction time. The moderate yet consistent escape responses to repeated vibrations suggest an inherent ability to detect and react to mechanical disturbances, while the decreasing escape response trend with increasing light stimuli may indicate a degree of habituation to visual stimuli.

Overall, these findings underscore the necessity of controlled and stable environmental conditions to reduce stress and optimize larval development. By tailoring rearing protocols to avoid excessive mechanical disturbances and sudden light shifts, especially during highly sensitive early life stages, hatcheries can improve survival rates, promote better growth, and enhance overall larval welfare.

## CRediT authorship contribution statement

**Fan Yang:** Software, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Elisa Benini:** Formal analysis, Writing – original draft, Writing – review & editing. **Sune R. Sørensen:** Investigation, Visualization, Writing – review & editing. **Christoffer Moesgaard Albertsen:** Software, Formal analysis, Writing – review & editing. **Johanna Kottmann:** Investigation, Writing – review & editing. **Ian A.E. Butts:** Funding acquisition, Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Jonna Tomkiewicz:** Funding acquisition, Writing – review & editing, Project administration. **Sebastian N. Politis:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition.

## 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 article.

## Acknowledgements

This study was part of the projects "Improve Technology and Scale-up Production of Offspring for European Eel Aquaculture" (ITS-EEL) and Eel Hatchery Technology for a Sustainable Aquaculture (EEL-HATCH) supported financially by the Innovation Fund Denmark (www.innovationsfonden.dk) under the grant agreements 7076-00125B and 5184-00093B as well as by a grant from "Elforsyningen Nordvendsyssel Fund" (ENV-"Fonden"), administered by Nord Energi, Hjørring, Denmark (www.nordenergi.dk). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We thank Christian Graver, Peter Lauesen and Maria Krüger Johnsen for broodstock management and provision of larvae for the experiment.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19 (6), 716–723. https://doi.org/10.1109/TAC.1974.1100705.

Arai, T., Taha, H., Mohd-Riduan, M.N., Mokti, S.S.A., 2020. Molecular and morphological evidence for the identity of the giant mottled eel, *Anguilla marmorata* in Southeast Asia. Trop. Ecol. 61 (3), 429–436. https://doi.org/10.1007/s42965-020-00096-4.

Bandara, K.A., Benini, E., Politis, S.N., Conceição, L.E., Santos, A., Sørensen, S.R., Tomkiewicz, J., Vadstein, O., 2023. Exploring bacterial community composition and immune gene expression of European eel larvae (*Anguilla anguilla*) in relation to first-feeding diets. PLoS One 18 (7), e0288734. https://doi.org/10.1371/journal.pone.0288734.

Batty, R.S., 1989. Escape responses of herring larvae to visual stimuli. J. Mar. Biol. Assoc. U. K. 69 (3), 647–654. https://doi.org/10.1017/S0025315400031039.

Benini, E., Politis, S.N., Nielsen, A., Sørensen, S.R., Tomkiewicz, J., Engrola, S., 2022a. Type of hormonal treatment administered to induce vitellogenesis in European eel influences biochemical composition of eggs and yolk-sac larvae. Fish Physiol. Biochem. 48 (1), 185–200. https://doi.org/10.1007/s10695-021-01042-4.

Benini, E., Engrola, S., Politis, S.N., Sørensen, S.R., Nielsen, A., Conceição, L.E.C., Santos, A., Tomkiewicz, J., 2022b. Transition from endogenous to exogenous feeding in hatchery-cultured European eel larvae. Aquac. Rep. 24, 101159. https://doi.org/10.1016/j.aqrep.2022.101159.

Benini, E., Bandara, K.A., Politis, S.N., Engrola, S., Nielsen, A., Conceição, L.E.C., Santos, A., Sørensen, S.R., Tomkiewicz, J., 2023. Exploring first-feeding diets for European eel larval culture: insights at morphological, nutritional, and molecular levels. PLoS One 18 (4), e0283680. https://doi.org/10.1371/journal.pone.0283680.

Beppi, C., Beringer, G., Straumann, D., Bögli, S.Y., 2021. Light-stimulus intensity modulates startle reflex habituation in larval zebrafish. Sci. Rep. 11 (1), 22410. https://doi.org/10.1038/s41598-021-00535-9.

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

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

Byrnes, E.E., Daly, R., Leos-Barajas, V., Langrock, R., Gleiss, A.C., 2021. Evaluating the constraints governing activity patterns of a coastal marine top predator. Mar. Biol. 168 (1), 11. https://doi.org/10.1007/s00227-020-03803-w.

Colwill, R.M., Creton, R., 2011. Imaging escape and avoidance behavior in zebrafish larvae. Rev. Neurosci. 22 (1), 63–73. https://doi.org/10.1515/rns.2011.008.

Conte, F.S., 2004. Stress and the welfare of cultured fish. Appl. Anim. Behav. Sci. 86 (3–4), 205–223. https://doi.org/10.1016/j.applanim.2004.02.003.

Creton, R., 2009. Automated analysis of behavior in zebrafish larvae. Behav. Brain Res. 203 (1), 127–136. https://doi.org/10.1016/j.bbr.2009.04.030.

Cumming, G., Fidler, F., Vaux, D.L., 2007. Error bars in experimental biology. J. Cell Biol. 177 (1), 7–11. https://doi.org/10.1083/jcb.200611141.

Domenici, P., Blake, R.W., 1997. The kinematics and performance of fish fast-start swimming. J. Exp. Biol. 200 (8), 1165–1178. https://doi.org/10.1242/jeb.200.8.1165.

Domenici, P., Hale, M.E., 2019. Escape responses of fish: a review of the diversity in motor control, kinematics and behaviour. J. Exp. Biol. 222 (18), jeb166009. https://doi.org/10.1242/jeb.166009.

Eaton, R.C., Emberley, D.S., 1991. How stimulus direction determines the trajectory of the Mauthner-Inthated escape response in a teleost fish. J. Exp. Biol. 161 (1), 469–487. https://doi.org/10.1242/jeb.161.1.469.

Eaton, R.C., Lee, R.K.K., Foreman, M.B., 2001. The Mauthner cell and other identified neurons of the brainstem escape network of fish. Prog. Neurobiol. 63 (4), 467–485. https://doi.org/10.1016/S0301-0082(00)00047-2.

Fisheries, F.A.O, 2022. The State of World Fisheries and Aquaculture. Towards Blue Transformation. https://policycommons.net/artifacts/8945635/the-state-of-world-fisheries-and-aquaculture/9788497/.

Føre, M., Frank, K., Norton, T., Svendsen, E., Alfredsen, J.A., Dempster, T., Eguiraun, H., Watson, W., Stahl, A., Sunde, L.M., Schellewald, C., 2018. Precision fish farming: a new framework to improve production in aquaculture. Biosyst. Eng. 173, 176–193. https://doi.org/10.1016/j.biosystemseng.2017.10.014.

Gingins, S., Roche, D.G., Bshary, R., 2017. Mutualistic cleaner fish maintains high escape performance despite privileged relationship with predators. Proc. R. Soc. B Biol. Sci. 284 (1853), 20162469. https://doi.org/10.1098/rspb.2016.2469.

Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L.E.C., Izquierdo, M., 2013. Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. Rev. Aquac. 5 (s1), S26–S58. https://doi.org/10.1111/j.1753-5131.2012.01086.x.

Houde, E.D., 2008. Emerging from Hjort's shadow. J. Northwest Atl. Fish. Sci. 41. https://doi.org/10.2960/J.v41.m634.

Jornod, M., Roche, D.G., 2015. Inter- vs intra-individual variation and temporal repeatability of escape responses in the coral reef fish *Amblyglyphidodon curacao*. Biol. Open 4 (11), 1395–1399. https://doi.org/10.1242/bio.013508.

Koenker, B.L., Copeman, L.A., Laurel, B.J., 2018. Impacts of temperature and food availability on the condition of larval Arctic cod (*Boreogadus saida*) and walleye pollock (*Gadus chalcogrammus*). ICES J. Mar. Sci. 75 (7), 2370–2385. https://doi.org/10.1093/icesjms/fsy052.

Kottmann, J.S., Jørgensen, M.G.P., Bertolini, F., Loh, A., Tomkiewicz, J., 2020. Differential impacts of carp and salmon pituitary extracts on induced oogenesis, egg quality, molecular ontogeny and embryonic developmental competence in European eel. PLoS One 15 (7), e0235617. https://doi.org/10.1371/journal.pone.0235617.

Langrock, R., King, R., Matthiopoulos, J., Thomas, L., Fortin, D., Morales, J.M., 2012. Flexible and practical modeling of animal telemetry data: hidden Markov models and extensions. Ecology 93 (11), 2336–2342. https://doi.org/10.1890/11-2241.1.

Machnik, P., Schirmer, E., Glück, L., Schuster, S., 2018. Recordings in an integrating central neuron provide a quick way for identifying appropriate anaesthetic use in fish. Sci. Rep. 8 (1), 17541. https://doi.org/10.1038/s41598-018-36130-8.

Marras, S., Killen, S.S., Claireaux, G., Domenici, P., McKenzie, D.J., 2011. Behavioural and kinematic components of the fast-start escape response in fish: individual variation and temporal repeatability. J. Exp. Biol. 214 (18), 3102–3110. https://doi.org/10.1242/jeb.056648.

Martins, D.A., Rocha, F., Martínez-Rodríguez, G., Bell, G., Morais, S., Castanheira, F., Bandarra, N., Coutinho, J., Yúfera, M., Conceição, L.E.C., 2012. Teleost fish larvae adapt to dietary arachidonic acid supply through modulation of the expression of lipid metabolism and stress response genes. Br. J. Nutr. 108 (5), 864–874. https://doi.org/10.1017/S0007114511006143.

Martorell-Barceló, M., Signaroli, M., Barcelo-Serra, M., Lana, A., Aspillaga, E., Grau, A., Arlinghaus, R., Alós, J., 2023. Chronotypes-personality behavioural syndromes in wild marine fish. Sci. Rep. 13 (1), 20281. https://doi.org/10.1038/s41598-023-45579-1.

McClintock, B.T., Michelot, T., 2018. momentuHMM: R package for generalized hidden Markov models of animal movement. Methods Ecol. Evol. 9 (6), 1518–1530. https://doi.org/10.1111/2041-210X.12995.

Medan, V., Preuss, T., 2014. The Mauthner-cell circuit of fish as a model system for startle plasticity. J. Physiol. Paris 108 (2), 129–140. https://doi.org/10.1016/j.jphysparis.2014.07.006.

Miller, M.J., Westerberg, H., Sparholt, H., Wysujack, K., Sørensen, S.R., Marohn, L., Jacobsen, M.W., Freese, M., Ayala, D.J., Pohlmann, J.-D., Svendsen, J.C., Watanabe, S., Andersen, L., Møller, P.R., Tsukamoto, K., Munk, P., Hanel, R., 2019. Spawning by the European eel across 2000 km of the Sargasso Sea. Biol. Lett. 15 (4), 20180835. https://doi.org/10.1098/rsbl.2018.0835.

Mirjany, M., Faber, D.S., 2011. Characteristics of the anterior lateral line nerve input to the Mauthner cell. J. Exp. Biol. 214 (20), 3368–3377. https://doi.org/10.1242/jeb.056226.

Noldus, L., Spink, A., Tegelenbosch, R., 2001. EthoVision: a versatile video tracking system for automation of behavioral experiments. Behav. Res. Methods Instrum. Comput. 33, 398–414. https://doi.org/10.3758/BF03195394.

Pankhurst, N.W., Sorensen, P.W., 1984. Degeneration of the alimentary tract in sexually maturing European *Anguilla anguilla* (L.) and American eels *Anguilla rostrata* (LeSueur). Can. J. Zool. 62 (6), 1143–1149. https://doi.org/10.1139/z84-165.

Politis, S.N., Butts, I.A.E., Tomkiewicz, J., 2014. Light impacts embryonic and early larval development of the European eel, *Anguilla anguilla*. J. Exp. Mar. Biol. Ecol. 461, 407–415. https://doi.org/10.1016/j.jembe.2014.09.014.

Politis, S.N., Mazurais, D., Servili, A., Zambonino-Infante, J.-L., Miest, J.J., Sørensen, S. R., Tomkiewicz, J., Butts, I.A.E., 2017. Temperature effects on gene expression and morphological development of European eel, *Anguilla anguilla* larvae. PLoS One 12 (8), e0182726. https://doi.org/10.1371/journal.pone.0182726.

Politis, S.N., Sørensen, S.R., Mazurais, D., Servili, A., Zambonino-Infante, J.-L., Miest, J. J., Clemmesen, C., Tomkiewicz, J., Butts, I.A.E., 2018. Molecular ontogeny of first-feeding European eel larvae. Front. Physiol. https://doi.org/10.3389/fphys.2018.01477.

Politis, S.N., Syropoulou, E., Benini, E., Bertolini, F., Sørensen, S.R., Miest, J.J., Butts, I.A. E., Tomkiewicz, J., 2021. Performance thresholds of hatchery produced European eel larvae reared at different salinity regimes. Aquaculture 539, 736651. https://doi.org/10.1016/j.aquaculture.2021.736651.

Politis, S.N., Benini, E., Miest, J.J., Engrola, S., Sørensen, S.R., Syropoulou, E., Butts, I.A. E., Tomkiewicz, J., 2023. First assessment of prebiotics, probiotics, and synbiotics affecting survival, growth, and gene expression of European eel (*Anguilla anguilla*) larvae. Aquac. Res. 2023, 1–13. https://doi.org/10.1155/2023/1260967.

Ribeiro, L., Hubert, F.N., Rodrigues, V., Rojas-Garcia, C., Dinis, M.T., 2022. Understanding fish larvae's feeding biology to improve aquaculture feeding protocols. Oceans 3 (1). https://doi.org/10.3390/oceans3010009. Article 1.

Righton, D., Westerberg, H., Feunteun, E., Økland, F., Gargan, P., Amilhat, E., Metcalfe, J., Lobon-Cervia, J., Sjöberg, N., Simon, J., Acou, A., Vedor, M., Walker, A., Trancart, T., Brämick, U., Aarestrup, K., 2016. Empirical observations of the spawning migration of European eels: the long and dangerous road to the Sargasso Sea. Sci. Adv. 2 (10), e1501694. https://doi.org/10.1126/sciadv.1501694.

Roberts, A.C., Pearce, K.C., Choe, R.C., Alzagatiti, J.B., Yeung, A.K., Bill, B.R., Glanzman, D.L., 2016. Long-term habituation of the C-start escape response in zebrafish larvae. Neurobiol. Learn. Mem. 134, 360–368. https://doi.org/10.1016/j.nlm.2016.08.014.

Roche, D.G., Tytell, E.D., Domenici, P., 2023. Kinematics and behaviour in fish escape responses: guidelines for conducting, analysing, and reporting experiments. J. Exp. Biol. 226 (14), jeb245686. https://doi.org/10.1242/jeb.245686.

Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., Boglione, C., 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. Rev. Aquac. 5 (s1). https://doi.org/10.1111/raq.12010.

Skajaa, K., Browman, H.I., 2007. The escape response of food-deprived cod larvae (*Gadus morhua* L.). J. Exp. Mar. Biol. Ecol. 353 (2), 135–144. https://doi.org/10.1016/j.jembe.2007.01.014.

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

Sørensen, S.R., Tomkiewicz, J., Munk, P., Butts, I.A.E., Nielsen, A., Lauesen, P., Graver, C., 2016a. Ontogeny and growth of early life stages of captive-bred European eel. Aquaculture 456, 50–61. https://doi.org/10.1016/j.aquaculture.2016.01.015.

Sørensen, S.R., Butts, I.A.E., Munk, P., Tomkiewicz, J., 2016b. Effects of salinity and sea salt type on egg activation, fertilization, buoyancy and early embryology of European eel, *Anguilla anguilla*. Zygote 24 (1), 121–138. https://doi.org/10.1017/S0967199414000811.

Sugisaki, H., Bailey, K., Brodeur, R., 2001. Development of the escape response in larval walleye pollock (*Theragra chalcogramma*). Mar. Biol. 139 (1), 19–24. https://doi.org/10.1007/s002270100563.

Syropoulou, E., Benini, E., Sørensen, S.R., Butts, I.A.E., Tomkiewicz, J., Politis, S.N., 2022. Early and abrupt salinity reduction impacts European eel larval culture. Fish Physiol. Biochem. 48 (2), 355–366. https://doi.org/10.1007/s10695-022-01056-6.

Tomkiewicz, J., Politis, S.N., Sørensen, S.R., Butts, I.A., Kottmann, J.S., 2019. European eel–an integrated approach to establish eel hatchery technology in Denmark. In: Eels Biol Monit Manag Cult Exploit Proc First Int Eel Sci Symp, pp. 340–374.

Viterbi, A., 1967. Error bounds for convolutional codes and an asymptotically optimum decoding algorithm. IEEE Trans. Inf. Theory 13 (2), 260–269. IEEE Transactions on Information Theory. https://doi.org/10.1109/TIT.1967.1054010.

Voesenek, C.J., Muijres, F.T., van Leeuwen, J.L., 2018. Biomechanics of swimming in developing larval fish. J. Exp. Biol. 221 (1), jeb149583. https://doi.org/10.1242/jeb.149583.

Weihs, D., 1973. Hydromechanics of fish schooling. Nature 241 (5387), 5387. https://doi.org/10.1038/241290a0.

Williams, P.J., Brown, J.A., Gotceitas, V., Pepin, P., 1996. Developmental changes in escape response performance of five species of marine larval fish. Can. J. Fish. Aquat. Sci. 53 (6), 1246–1253.

Wright, R.M., Piper, A.T., Aarestrup, K., Azevedo, J.M.N., Cowan, G., Don, A., Gollock, M., Rodriguez Ramallo, S., Velterop, R., Walker, A., Westerberg, H., Righton, D., 2022. First direct evidence of adult European eels migrating to their breeding place in the Sargasso Sea. Sci. Rep. 12 (1), 15362. https://doi.org/10.1038/s41598-022-19248-8.

Yúfera, M., Darias, M.J., 2007. The onset of exogenous feeding in marine fish larvae. Aquaculture 268 (1–4), 53–63. https://doi.org/10.1016/j.aquaculture.2007.04.050.

Zucchini, W., MacDonald, I.L., 2009. Hidden Markov Models for Time Series: An Introduction Using R. Chapman and Hall/CRC. https://doi.org/10.1201/9781420010893.