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First steps towards mass rearing of European smelt (*Osmerus eperlanus*, L.) using conventional hatchery equipment

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Abstract – Anadromous European smelt (*Osmerus eperlanus*) is a keystone species in lake and river ecosystems. In the past and present, stock declines in several of its habitats have been reported. The reasons for this are unclear. Experimental research on the early life stages could help to reveal the potential causes. For this purpose, knowledge on artificial propagation and rearing of early life stages is needed. Following from previous work, we show how to scale up and mass rear European smelt using conventional hatchery equipment and present a simplified protocol for first feeding. Smelt eggs, after egg adhesiveness is removed, can be incubated in standard hatchery equipment commonly used in aquaculture. Incubation in McDonald-type jars shows even improved results when settling of floating eggs is prevented. Next to avoiding egg loss this simultaneously reduces labor for daily care. First feeding of larval smelt can be achieved with decapsulated artemia cysts, eliminating the need for the labor-intensive green water production. Using the protocol presented, larvae of different stages can be produced in large quantities allowing further experimental studies.

Keywords: Delta smelt / Elbe river / first feeding / fish larvae / forage fish / Osmeriformes

1 Introduction

European smelt, *Osmerus eperlanus*, is a diadromous species inhabiting coastal and estuarine waters in western Europe. Landlocked populations exist in lakes of coastal areas. The current biomass situation of many smelt stocks is unclear as there is a limited amount of data on this species (Wilson and Veneranta 2019). For several stocks, a decline in biomass has been reported (Hutchinson and Mills, 1987; Keskinen et al., 2012; Arula et al., 2017; Sendek and Bogdanov, 2019; Keller et al., 2020). Next to high fishing pressure, smelt is sensitive to environmental disturbances like pollution, obstruction of rivers, physical barriers to migration and degradation of its spawning habitats (Hutchinson and Mills, 1987; Maitland and Lyle, 1990; Thiel et al., 1995; Sendek and Bogdanov, 2019). While former poor chemical water quality in European rivers has improved and smelt populations have recovered (Sepulveda et al., 1993; Thiel et al., 1995; Eick and Thiel, 2014), the lower reaches of major rivers are still heavily impacted by various anthropogenic activities. This may further contribute to the decline of smelt stocks and hamper their recovery.

The extent to which smelt is affected by these individual factors is unknown but experimental research can help disentangle the effects of the various stressors. Still, information on the husbandry of smelt is very limited. This is especially true for the artificial propagation and rearing of the early life stages (Lillelund, 1961; Keller et al., 2020; McCarthy et al., 2020) which is a prerequisite for conducting experimental research with this species. In a recent study, the general principles of artificial reproduction of smelt using a small-scale setup have been demonstrated (McCarthy et al., 2020). This study adopted previous research on rainbow smelt (*Osmerus mordax*, Mitchill) (Ayer et al., 2005; Walker et al., 2010). In the present study, we follow up on this by extending the present knowledge on artificial propagation of smelt using conventional hatchery equipment.

2 Materials and methods

2.1 Experimental design and artificial spawning

MacDonald-type jars (8 L volume) made of silica glass were used for egg incubation. For the experiment, four conventional jars were used as a control and four jars were provided with a technical modification. This modification consisted of a custom-made, beveled PVC-U fitting (2 cm height) sit atop each central delivery tube's ring to avoid eggs from settling (Fig. 1).

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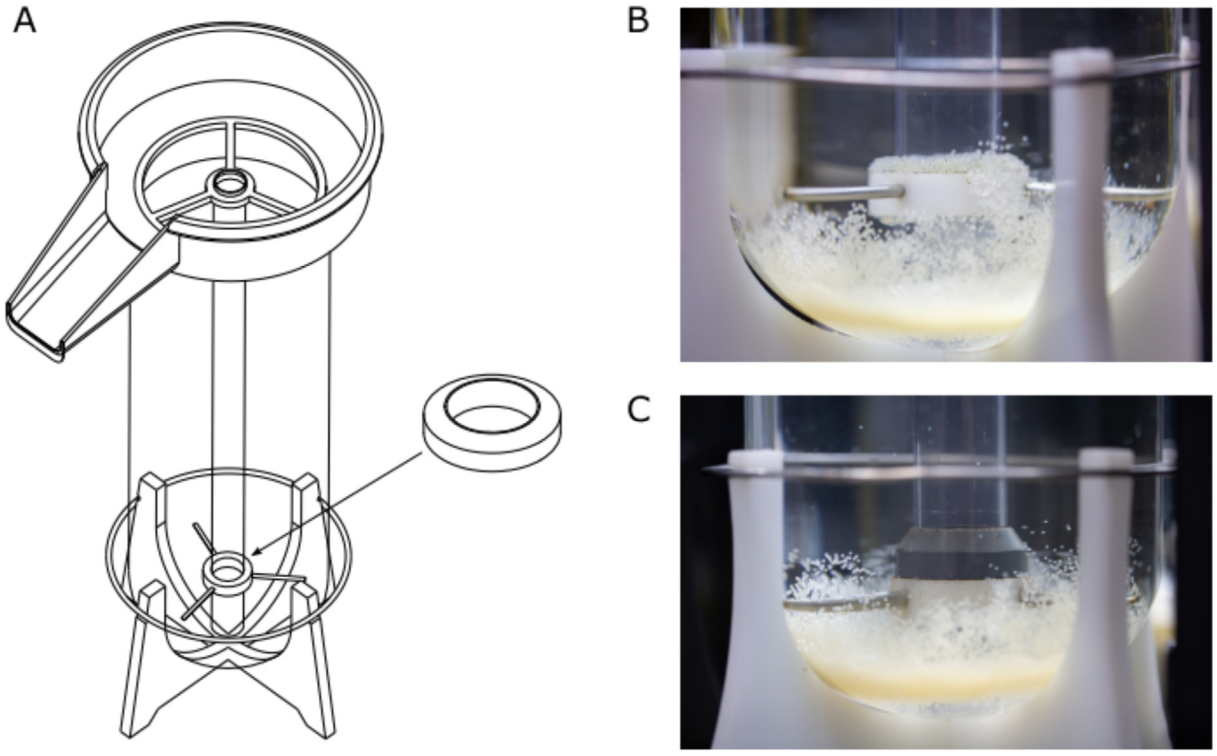

Fig. 1. Schematic drawing of a McDonald-type jar used for egg incubation and the beveled PVC-U fitting with the arrow indicating its position atop the central ring in modified jars (A). Pictures of jars loaded with fertilized smelt eggs in (B) control jars where eggs accumulate atop the ring and (C) modified jars without the accumulation.

Adult smelt were caught in February 2022 by a commercial fisherman using eel pots in the River Weser (53.060°N, 8.864 °E, Bremen, Germany) and transported alive to the Thünen Institute of Fisheries Ecology (Bremerhaven, Germany). Artificial spawning was conducted using standard techniques described in Appendix A. Individual egg mass varied between 0.32 and 0.64 mg egg^{-1}, with a mean (±SD) of 0.45 ± 0.07 g egg^{-1}, and was positively related to female body weight (Fig. A1 in Appendix A). A total mean of 22.3 ± 0.2 g eggs was incubated in each jar, totalling up to a mean (±SD) of 50,084±6,210 eggs per jar (Fig. A1 in Appendix A).

2.2 Egg incubation, animal husbandry, and sampling

Eggs were incubated with a constant flow of degassed well water at 1.7 mL min^{-1}, and mean (±SD) temperature of 12.1 (±0.1) °C, dissolved oxygen (DO) 10.6 (±0.2) mg mL^{-1}, and pH 8.19 (±0.02) (Multi 3510 DS, Xylem Analytics, Weilheim, Germany). Fertilisation success was determined after 24 h and reached 98.8%. Eggs were photographed daily between 1 and 10 days post fertilization (dpf) to document development (see Fig. 2A) (Leica M165FC, Leica, Wetzlar, Germany, and Nikon SMZ25, Nikon Europe, Amsterdam, Netherlands). Eggs were disinfected every three days at 3, 6, and 9 dpf (see initial disinfection in Appendix A). Egg survival was measured as the proportion of living eggs on 2, 4, 6, and 8 dpf. At these days, eggs that settled were removed and the amount counted per individual jar. Larvae started hatching at 10 dpf, and hatching success was measured by estimating abundances of larvae in the aquaria on 10, 11, 12, and 13 dpf. Mass hatching occurred at 12 dpf, and this day was set as 0 days post hatch (dph). Larvae hatched into rectangular 57.5L glass aquaria, shaded with black tarpaulin and a gray plastic board covering half of the surface. At 0 dph, five larvae were sampled from each aquarium for size-at-hatch measurements. Four glass aquaria were chosen as replicates for subsequently tracking growth, the onset of exogenous feeding, and larval mortality over the next 14 days. From these aquaria, larvae were sampled every other day. Larvae were euthanized by asphyxiation in carbon dioxide-enriched water, photographed (Leica M165FC, Leica, Wetzlar, Germany, and Nikon SMZ25, Nikon Europe, Amsterdam, Netherlands) and standard lengths were measured by analysing the images in FishSizer (v.3) (Rasmussen et al., 2022). Aquaria were cleaned daily, moribund and dead larvae siphoned out and counted. Larvae were kept at mean (±SD) temperature of 12.1 (±0.1) °C, 10.9 (±0.1) DO, and pH 8.13 (±0.05) at a density of about 525 larvae L^{-1}.

Starting from 5 dph, fish were fed decapsulated artemia eggs and nauplii at 50 nauplii larva^{-1} day^{-1} (for details see Appendix A). Artemia were added to the aquaria between 8:00 and 19:00 at hourly intervals (Reef Doser EVO 4, AB Aqua Medic GmbH, Bissendorf, Germany). Light regime was set as 12:12 L:D (8:00-20:00). Starting from 6 dph, the proportion of larvae consuming feed was visually determined by inspecting 10 larvae per tank and day for the presence of artemia in the digestive track three hours after the onset of feeding. Rearing trials were terminated after 14 days.

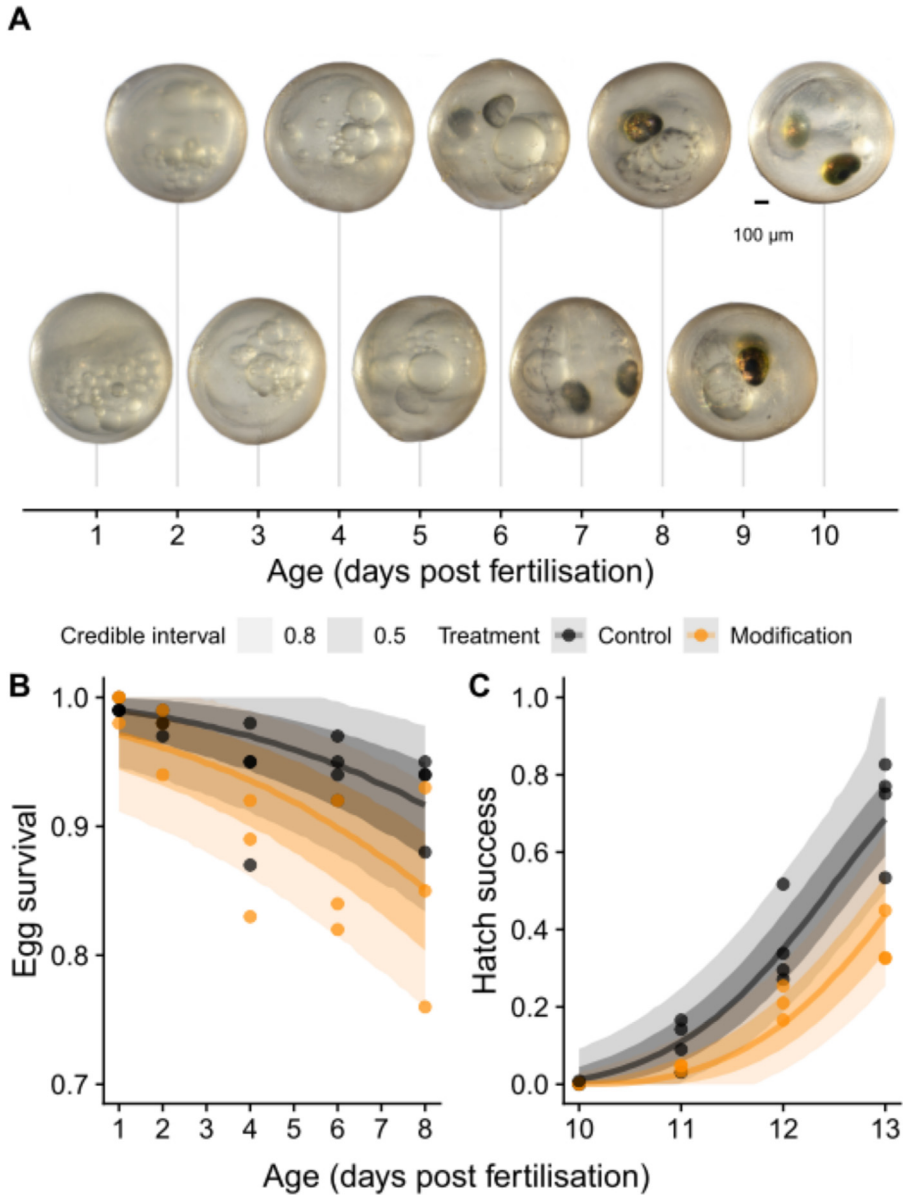

Fig. 2. Development of smelt eggs incubated at ~12°C over a period of 10 days (A). Hatch started at 10 days post fertilisation (dpf) and peaked at 12 dpf. Panel B shows the proportion of living eggs during the incubation between 1 and 8 dpf. From the model's posterior distributions, the medians are shown as the black (control, $n = 4$) and orange (modified jars, $n = 3$) regression line and the shaded areas show the 0.5 and 0.8 quantile-based credible intervals. Panel C shows the relative hatching success in control (black, $n = 4$) and modified jars (orange, $n = 3$). From the model's posterior distributions, the medians are shown as the regression line and the shaded areas show the 0.5 and 0.8 quantile-based credible intervals.

2.3 Statistical analyses

All statistical analyses were conducted within the R statistical and graphical environment (v. 4.1.1) (R Development Core Team, 2021) and are described in Appendix A.

3 Results

3.1 Egg survival and hatching success

Across treatments, smelt egg survival declined by about 2% per incubation day (–0.02 [–0.03, −0.01], average marginal effect with 0.95 credible intervals) (see Fig. 2B). Yet, on the last sampling day before hatch (i.e. 8 dpf), the proportion of living eggs in control and modified hatching jars was still 0.91 [0.84, 1.00] and 0.85 [0.73, 0.76], respectively (values provided as medians and 0.80 lower and upper highest posterior density intervals (HPD) [HPD_{low}, HPD_{high}]). Although egg survival was higher in the control, there was only weak evidence for better survival in control hatching jars (i.e. credible intervals did intersect with zero in post-hoc contrasts, −0.59 [–0.99, 0.13]). The Bayesian R^2 was 0.58 [0.24, 0.69] for the egg survival model (contrasts and Bayesian R^2 as median and 0.95 [HPD_{low}, HPD_{high}]).

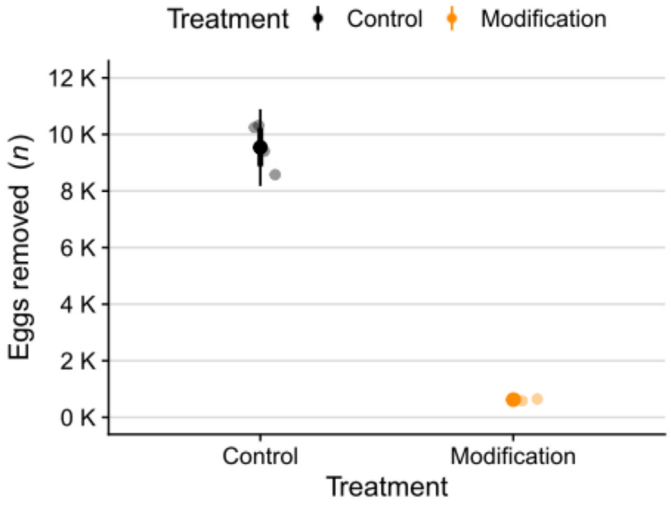

Fig. 3. Number of accumulated eggs collected every other day from control (black) and modified (orange) jars during 2–8 days post fertilisation (dpf). Raw data for each jar are shown as semi-transparent point symbols, while point intervals show medians and 0.5 and 0.8 quantile-based credible intervals (thick and thin lines, respectively) from the model's posterior distribution.

Embryos started hatching at 10 dpf and completed hatching after 13 dpf (see Fig. 2C). The overall hatching success was 0.69 [0.47, 1.00] and 0.44 [0.20, 0.65] in control and modified egg jars, respectively (median and 0.80 [HPD_{low}, HPD_{high}]). The post-hoc contrasts revealed strong evidence for higher hatching success in the control jars (i.e. credible intervals not intersecting with zero, −0.95 [–1.42, −0.46]). The hatching success model's Bayesian R^2 was 0.90 [0.80, 0.92] (contrasts and Bayesian R^2 as median and 0.95 [HPD_{low}, HPD_{high}]).

The total amount of eggs removed during incubation, was about 20 times higher in control (20% of originally loaded eggs; $n = 9{,}629$ [7,079; 12,910]) than in the modified jars (1% of originally loaded eggs; $n = 624$ [464; 842], medians and 0.8 [HPD_{low}; HPD_{high}] reported) (Fig. 3). Consequently, there was strong evidence (–2.73 [–2.90, −2.55]) for the technical modification keeping the egg count high and minimizing the amount of work (Bayesian R^2 of 0.99 [0.94, 0.99]; contrasts and Bayesian R^2 as median and 0.95 [HPD_{low}, HPD_{high}]).

3.2 Larval length, feeding, and mortality

Smelt larvae hatched at a size (in mm) of 5.59 [4.66, 6.58] (median and 0.80 [HPD_{low}, HPD_{high}]), and continued to grow about 0.15 mm d^{-1} until 14 dph, when the trial was terminated (see Fig. 4A). We found strong evidence that age had a positive effect on larval length (post-hoc contrast 0.15 [0.14, 0.17]; Bayesian R^2 of 0.62 [0.56, 0.66] (median and 0.95 [HPD_{low}, HPD_{high}]). Yolk reserves were depleted by 6 dph, while oil globules were still present at 14 dph (see Fig. 4B).

Prey was added from 5 dph, and when larvae were examined at 6 dph a proportion of 0.40 [0.00, 0.65] (median and 0.8 [HPD_{low}, HPD_{high}]) already had prey in the gut (Fig. 4C). There was some weak evidence that larval age and the proportion of larvae with ingested artemia cysts and/or nauplii was positively correlated (post-hoc contrast 0.15 [–0.06, 0.39]; Bayesian R^2 of 0.37 [0.00, 0.62] (both as median and 0.95 [HPD_{low}, HPD_{high}]). Still, the proportion of larvae with prey items increased at a rate of about 4% per day (0.04 [0.02, 0.05], average marginal effect with 0.95 credible intervals). At 14 dph, about three quarters of all examined larvae had prey items in the gut (0.74 [0.00, 1.00], median and 0.8 [HPD_{low}, HPD_{high}]).

Larval mortality decreased at a rate of about 15 individuals per day (–14.89 [–20.16, −10.56], average marginal effect with 0.95 credible intervals) over the course of the experiment. Most unfit larvae were removed within the first week, and the post-hoc contrast revealed strong evidence for age playing an important role (–0.13 [–0.17, −0.10]; Bayesian R^2 of 0.44 [0.20, 0.69] (both as median and 0.95 [HPD_{low}, HPD_{high}])). Overall, the number of dead larvae decreased from 211 [77, 392] at 2 dph to 43 [14, 80] at 14 dph (median and 0.8 [HPD_{low}, HPD_{high}]).

4 Discussion

Control over artificial propagation, early rearing and first feeding are the most critical steps in introducing new fish species into an aquaculture environment. Different jar types are used for egg incubation (Billard et al., 1995; Jensen et al., 2008; Harper et al., 2010). Zug-type or funnel-shaped jars are tapered at the bottom and are connected to the water supply from below. In McDonald-type jars, water is transported via a delivery tube to the concave bottom of the jar and creates an upwelling effect. A previous study on striped bass (*Morone saxatilis*) showed no difference in percent survival between the two jar types with a non-significant tendency to higher survival in McDonald-type jars (Harper et al., 2010). In burbot (*Lota lota*), embryo survival was higher in Zug-type jars (Jensen et al., 2008). While McDonald-type jars can be setup easily, the delivery tube has to aligned perpendicularly. For this purpose, spacers are added at its lower end. In previous incubation trials (data not shown), we observed eggs to accumulate on the ring where spacers are attached. Even with regular disinfection as described in previous studies (Ayer et al., 2005; McCarthy et al., 2020), fungal growth was rapid in some jars. In the present study, the addition of the beveled fitting has helped prevent the accumulation of settling eggs during incubation. We intervened every second day and removed accumulating eggs in all jars, resulting in more eggs being removed from the control group. Even if the effect of this intervention was not identical between control and modified jars, it was required in order to avoid compromising successful incubation, especially in the control. The modification is thus recommended as it helps eliminating the problem of fungal infections and decreases the amount of work required for maintenance and cleaning.

Most fish larvae, marine species in particular, rely on live prey before they can be gradually weaned and adapted to dry feed. In the field, smelt larvae start feeding at a body length of 7–9 mm (Arula et al., 2017 and references therein) and depend on the presence of *Eurytemora* spec. and their copepodite stages (Lillelund, 1961; Sepulveda et al., 1993; Thiel, 2001). The cultivation of copepods can be tedious and time consuming and appropriate protocols must be established. Natural plankton may serve as an alternative, however,

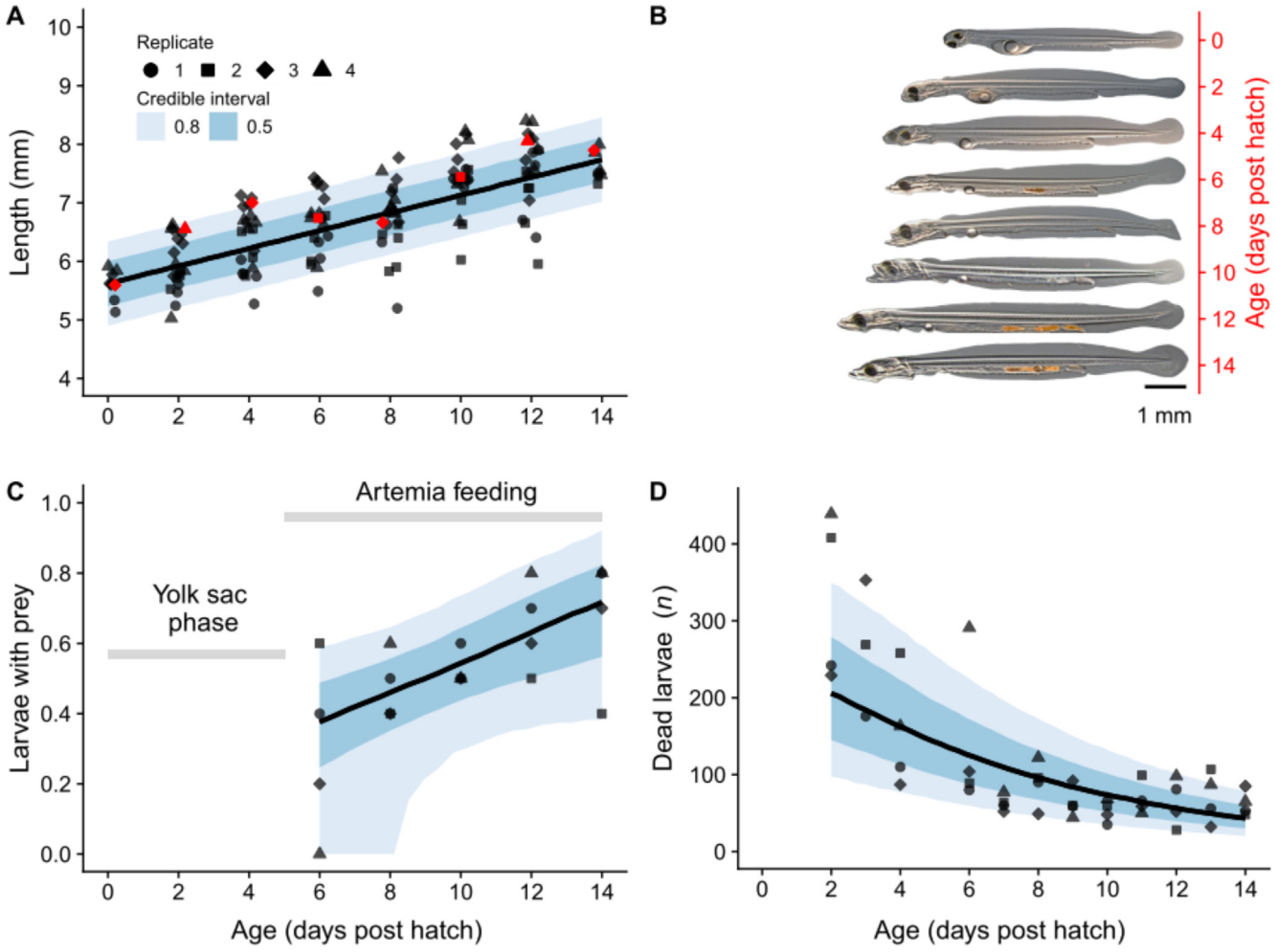

Fig. 4. Length-weight relationship of European smelt (*Osmerus eperlanus*) larvae (0–14 days post hatch (dph), $n = 157$). In panel A, the overall relationship of larvae is shown. Red data points indicate individuals shown in panel B that depicts larval development between 0 and 14 dph. Panel C shows the transition from endo- to exogenous feeding and the relative number of larvae with prey items in the gut across time. Panel D shows the relationship of age and the number of dead larvae removed per aquarium. In panels A, C and D the medians, from the model's posterior distributions, are shown as the regression line and the shaded areas show the 0.5 and 0.8 quantile-based credible intervals.

previous attempts using natural plankton have not been successful (Lillelund 1961). Green water and rotifers are often used to rear marine fish larvae. This approach proved successful for smelt as shown by McCarthy et al. (2020). In pre-trials (data not shown), we found smelt feeding on freshly hatched and screened artemia starting at 14 dph. Yet, feeding on unhatched artemia cysts resulted in significant mortality. Lillelund (1961) reported, that smelt larvae survived between 9 and 14 days without feed at temperatures of 17.7 °C and 8.8 °C, respectively. We presumed that larvae were willing to feed on these small particles, but were not able to digest ingested cysts. We thus used decapsulated artemia providing unhatched decapsulated cysts next to freshly hatched nauplii from 5 dph onwards. Feeding was observed at 5 dph which is considerably earlier compared to previous studies (McCarthy et al., 2020). The presented findings can assist in further experimentally investigating cause-and-effect relationships between performance of smelt early life stages and changing environmental factors, and contribute to starting a conservation aquaculture program of smelt.

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Appendix: A

A.1 Artificial spawning

Only live and vital smelt were used (Lillelund, 1961). Fish were euthanized by percussive stunning. Two to three females were used per jar and eggs were fertilized with the

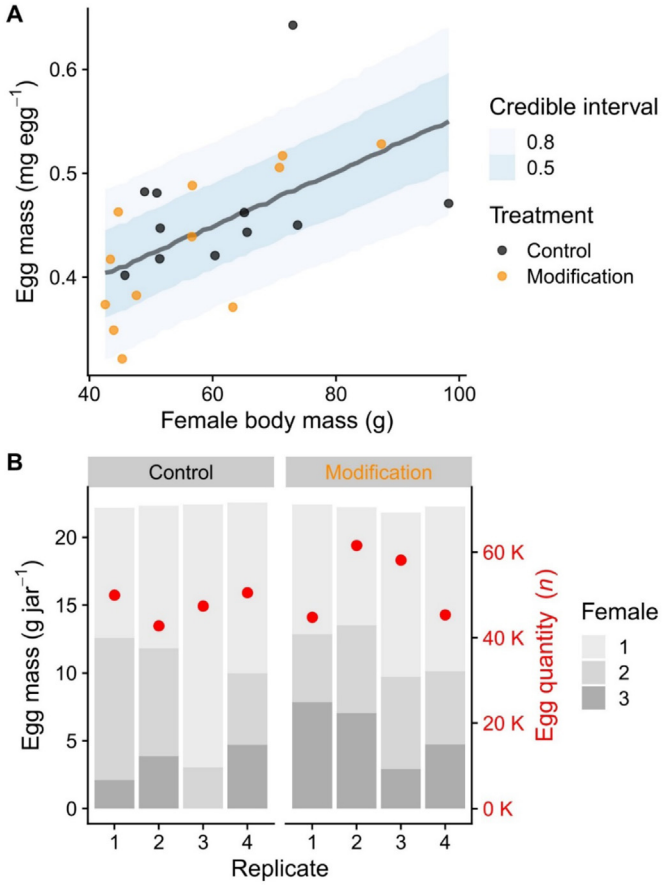

Fig. A1. Relationship of body mass and individual egg weight of female smelt ($n = 23$) used in the trial (A). From the model's posterior distribution, the median, combined for both treatments, is shown as the black regression line and the blue areas show the 0.5 and 0.8 quantile-based credible intervals. The model explained 33 [4, 53]% of the observed variability (Bayesian R^2, median and 95% lower and upper quantile intervals reported). Panel B shows the number of strip-spawned female smelt, egg mass and the correspondent number of eggs per jar.

milt of three males. Smelt were of mean (±SD) total lengths (TL) of 20.5±1.2 cm and 19.3±1.2 cm, respectively. Eggs and milt were strip-spawned by pressing the fish's ventral surfaces, and some eggs were sampled into pre-weighed 1.5 mL Eppendorf vials for subsequent weighing and counting. Eggs were then fertilized using the dry fertilization method. Subsequently, the eggs' adhesiveness was removed (1.2 g L^{-1} tannic acid solution for 10 min) and the eggs were disinfected (2 mL L^{-1} hydrogen peroxide, 35%, for 15 min, both chemicals Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), according to methods described in McCarthy et al. (2020).

Animal husbandry and all experimental procedures were conducted in accordance with European directive 2010/63/EU on the protection of animals used for scientific purposes.

A.2 Female body mass vs. egg weight and setup of jars

A.3 Decapsulation of artemia cysts

Artemia cysts were soaked in dechlorinated tab water for 1 h, screened and added to a detergent solution containing 5% chlorine (active compound: 2.8 g NaOCl 100g^{-1}). Cysts were shaken for 3–4 min until the color changed to orange, screened, and rinsed with tap water. Then, the sieve was placed in a mild acetic acid (5%) and gently shaken for 1 min. After a final rinse, decapsulated cysts were incubated in 25 ppt seawater at ~28 °C in vigorously aerated balloon tanks. Following 24 h of incubation, artemia were screened (100 μm). Subsamples of harvested artemia were sedated with EtOH (78%) and counted to determine and adjust densities.

A.4 Statistical analyses

Bayesian (generalized) linear mixed regression models were used for analysing the data in package 'brms' (v.2.16.3) (Bürkner et al., 2021) and, as some of the data were distributed between 0 and 1, in package 'ordbetareg' (v.0.2.1) (Kubinec 2022). Multiple candidate models were fit for the different response variables at the egg and larval stages, and all candidate models were run with three chains, 4000 iterations, a warm-up phase of 1000, and default, non-informative priors. Model selection was based on leave-one-out cross-validation techniques supplied by the 'loo' package (v.2.5.1) (Vehtari et al., 2022). In all models testing the effects of the modified technical component the categorical variable 'treatment' was added as random-intercept variable. In models assessing the effect of development, age was added as categorical variable (in days post fertilization (dpf) for eggs or days post hatch (dph) for larvae). Replicate jars (or aquaria during larval stage) were included as random effects. The selected models (with specified family functions) were: (1) individual egg mass ~ female body weight (gaussian), (2) egg survival ~ age + treatment + (1|replicate) (ordbetareg), (3) hatch success ~ age + treatment + (1|replicate) (ordbetareg), (4) removed eggs ~ treatment + (1|replicate) (lognormal with link = identity, and link_sigma = log), (5) larval length ~ age + (1|replicate) (gaussian), (6) prey consumption ~ age + (1+age |replicate) (ordbetareg), and (7) larval mortality ~ age + (1|replicate) (gamma_hurdle). Model validations were done by examining MCMC diagnostics with package 'bayesplot' (v.1.9.0) (Gabry et al.. 2022), and inspecting residuals using package 'DHARMa' (v.0.4.5) (Hartig and Lohse, 2022). Post-hoc comparisons were made using the 'emtrends' and 'emmeans' functions in package 'emmeans' (Lenth et al., 2022). The Bayesian R^2, a data-based estimate of the proportion of variance explained for new data, was calculated using function 'bayes_R2' for each selected model (Gelman et al., 2019). Coefficients from models using the 'ordbetareg' specification were turned into more meaningful marginal effect estimates with package 'marginaleffects' (v.0.6.0) (Arel-Bundock, 2022). Predictions

from the best fitting models were compiled with package 'tidybayes' (v.3.0.2) (Kay and Mastny, 2022), and visualized with the packages 'ggplot2' (v.3.3.5) (Wickham et al., 2021) and 'cowplot' (v.1.1.1) (Wilke, 2020).

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