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Determining the optimum temperature and salinity for larval culture, and describing a culture protocol for the conservation aquaculture for European smelt *Osmerus eperlanus* (L).

Running Title: Culture protocol for European smelt

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Summary

Populations of anadromous European smelt *Osmerus eperlanus* (L.) are declining across its geographical range in northern Europe, but no practical culture techniques exist to develop stock enhancement programmes for this species. In this study, a culture protocol is described to rear fish from fertilised eggs to mature adults in 2 years involving the use of 'green water', live feed and artificial diets. The sequence of embryonic development for eggs incubated at 10ºC/0 ppt was described and photographed. To determine the optimum conditions for larval culture, fertilised eggs were reared at a range of salinities (0 - 20 ppt) and temperatures (5 - 18ºC) until first feeding. Best hatching success (*ca.* 97%), size at hatch (*ca.* 0.8 mm) and survival to first feeding (*ca.* 96%) of larvae were achieved under combined conditions of low salinity (0 -10 ppt) and temperature (5 - 10ºC). No larvae survived a salinity of 20 ppt. The time taken from fertilisation to hatch (FtH) and hatching duration (HD) were temperature-dependent ranging from 42 days FtH and 10 days HD at 5ºC, to 10 days FtH and 2 days HD at 18ºC irrespective of salinity. The results indicate that conservation programmes could utilise existing salmonid hatchery facilities (i.e. freshwater, ≤ 10ºC water temperature) for stock enhancement. Since on-growing of smelt involves the logistical and technical problems

of live feed production, it is recommended that smelt enhancement programme utilise freshwater hatchery facilities to rear fish until hatching, and then stock out onto known spawning grounds in rivers allowing hatched larvae to drift into estuaries to complete the larval and juvenile phases. This approach would minimise the time spent in the hatchery post-hatching, eliminate the need for live food production, prevent the development of predator-naïve fish, and hence would mimic the natural life cycle of the species as closely as possible.

1 | INTRODUCTION

The European smelt, *Osmerus eperlanus*, is an anadromous species with a historic distribution in the coastal and estuarine Atlantic waters of western Europe ranging from the Garonne estuary in the south to the Baltic, Barents and White Seas in the north, including relict landlocked non-migratory, lacustrine populations occurring in Scandinavia, Baltic and White Sea/Barents Sea regions (Froese & Pauly, 2019). *O. eperlanus* is a small to medium-sized fish, typically ranging in size from 10 to 30 cm in anadromous populations (but can attain sizes of 45 cm; Froese & Pauly, 2019), that make seasonal migrations in early spring into the lower reaches of rivers to spawn (Lyle & Maitland, 1997). Smelt play an important role in food webs, both as a predator of zooplankton and as prey for larger piscivorous fish and birds in brackish and freshwater ecosystems (Nellbring, 1989; Sandlund et al., 2005; Žydelis, & Kontautas, 2008; Taal et al., 2014) and is also considered to be an indicator species, due to its sensitivity to pollution (Thomas, 1998).

Although currently listed in the 'Least Concern' category in the IUCN red list, European smelt has decreased considerably in some areas of its historic distribution such as the UK, where stocks of this once abundant species have declined. Smelt are now absent from approximately 33% of estuaries and rivers where historically once present in England and Wales (Maitland, 1999) and 80% in Scotland (Maitland & Lyle, 1996). Its decline is thought to be due to a variety of factors including pollution, overfishing, destruction of spawning grounds/nursery areas and the physical obstruction of spawning migrations by the erection of dams and weirs (Maitland, 2007). It is clear that targeted conservation efforts are needed to promote population recovery for this species. Recovery of declining fish populations entails removing the causative factors of decline and improving environmental conditions to allow natural recruitment, however, in some cases it is necessary to support habitat restoration activities with stock enhancement programmes. This has been a common activity in helping to restore declining salmonid populations for many years (Hendry et al., 2003; Molony et al.,

2005) and is an approach being adopted for other declining freshwater (Neufeld et al., 2011; Bartley et al., 2012) and anadromous fish species (Navarro et al., 2014; DiMaggio et al., 2015).

Conservation aquaculture involves the development of culture techniques to conserve or aid the recovery of threatened fish populations and is often used as part of a multifaceted approach involving habitat improvement and restoration, with the aim of conserving wild populations, their locally adapted gene pools, characteristic phenotypes and behaviours (Anders, 1998). Although laboratory culture of *O. eperlanus* larvae has been conducted (Ivanov and Volodin, 1981; Gorodilov & Melnikova, 2006), no larger-scale long-term artificial culture method has been described. Given its decline, the development of hatchery culture techniques for European smelt would contribute to restoration efforts for this species. A culture protocol has been established for the closely related rainbow smelt *Osmerus mordax* (Ayer et al., 2005; Fuda et al., 2007 Colburn et al., 2012) indicating that the early life stages of osmerids can be reared successfully in captivity and provides a culture technique that can be applied to *O. eperlanus*.

Survival and growth during the larval stages of fishes are determined by environmental conditions and it is well known that abiotic factors such as temperature, oxygen concentrations and salinity all influence larval performance in fishes (Blaxter, 1991). European smelt are euryhaline during the juvenile and adult life stages but the optimum temperature and salinity conditions for survival during the larval phase are not known. Therefore, the aims of this study are to (1) describe the development of European smelt under culture conditions, (2) examine the effect of temperature and salinity on the hatching success, size at hatching and survival to first feeding and (3) to describe a culture protocol for the species from egg fertilisation to adult maturity.

2 | METHODS

2.1 | Broodstock acquisition and spawningeuropean

In March 2011, European smelt were caught at night using fyke nets at the Newton Stewart spawning grounds on the River Cree (Hutchinson & Mills, 1987) and held overnight at the nearby Torhouse Trout Farm, Wigtown (southwest Scotland). The day after capture, 7 mature females (Total Length [L_T] 26.0 ± 0.8 cm; 174.7 ± 15.8 g) and 12 mature males (24.2 ± 0.7 cm; 133.2 ± 9.9 g) were anaesthetised (2-Phenoxyethanol; 0.3 ml L^{-1}) and gametes obtained by massaging the ventral surface. The eggs were fertilised and their adhesiveness removed

(150 mg L^{-1} tannic acid solution) and disinfected (2000 µl L^{-1} [active ingredient] hydrogen peroxide) using protocols established for *O. mordax* (Ayer et al., 2005; Walker et al., 2010). In total 221 g of eggs were collected [mean egg weight 31.6 ± 5.4 g $female^{-1}$; 1.263 g eggs g^{-1} female]. Eggs from each female were mixed with milt from either 1 or 2 males to produce 17 families, the fertilised eggs were mixed together and distributed between eleven 2 L glass jars containing spring water (aerated by battery-operated air pump, packed in a PVC tray containing ice, and transported to Menai Bridge.

2.2 | Incubation of embryos

In the aquarium, eggs were added to 2 L round-bottomed Erlenmyer incubating flasks containing bottled spring water vigorously aerated to keep the eggs in suspension (Ayer et al., 2005). At 1 day post fertilisation (dpf), 30 eggs were removed and photographed to measure egg diameter (ImageJ v.1.44; NIH public domain software; Ferreira & Rasband, 2012). Each subsequent day, a further 30 eggs were removed at random and photographed in order to monitor embryonic development (see Figure 1 for photographs at different ages post-hatching). To eliminate the build up of deleterious nitrogenous compounds, 50% of the water in each incubation flask was changed daily.

2.3. | Determining optimum temperature and salinity for larval culture

At 7 dpf, 4000 fertile eggs were removed from the incubation flasks, with 50 eggs transferred by pipette to each of 80 polypropylene beakers containing 1 L bottled spring water mixed with synthetic sea salt (ZM Fish Food, UK) to salinities of 0, 5, 10, 15 and 20 ppt (16 beakers per salinity). Four beakers containing eggs from each salinity treatment were placed in each of 4 water baths (water circulated using a submersible water pump) either heated or cooled to maintain nominal temperatures of 5, 10, 15 and 18°C. To control for any effect of beaker position, beakers within each tray were moved at random every 2 days. Tray temperature was recorded daily and each beaker visually inspected for the initiation of hatching. The first 10 hatchlings in each beaker were removed and photographed on the day of hatching under a dissecting microscope to measure L_T (tip of the snout to the end of the tail). Water changes (50%) were carried out twice weekly with the salinity in each beaker rechecked using a hand held refractometer. The experiment was terminated when all un-hatched eggs within each temperature treatment appeared necrotic. Dead eggs were not removed from beakers during the experiment to keep egg density constant in each beaker and avoid potential injury to live larvae. All larvae from each beaker were counted to determine % hatching success. The

number of dead larvae in each beaker was recorded and used to determine % survival to first feed of the total number of hatchlings from each beaker. First feeding was defined as when the yolk sacs were largely diminished and mouthparts were agape. At the end of the experiment, live larvae were transferred back to stock tanks at the appropriate salinity for a subsequent, separate experiment (not reported here) and thus no measurements were taken at first feeding.

2.4 | Larviculture and ongrowing of juveniles to adulthood

Larviculture and ongrowing of *O. eperlanus* (10°C; 12 h Light:Dark cycle, 400 lx) were based on culture protocols developed for *O. mordax* (Ayer et al., 2005; Colburn et al., 2012). The feeding protocol is summarised in Figure 2. On hatching (Figure 3A), larvae were transferred to 25 L rectangular glass tanks (approximately 100 fish L^{-1}) containing bottled spring water (supplemental aeration provided by an air-driven sponge filter). To avoid 'walling' (where larvae are drawn to light or white objects), adhesive black plastic sheeting was applied to the tank exterior. At 2 days post-hatching (dph), live *Nannochloropsis occulata* ('green water') was added to each tank (2 x 10^5 cells ml^{-1}) to facilitate feeding and larvae were fed marine rotifers *Brachionus plicatilis* (10 rotifers ml^{-1}) (Figure 3B). In addition to rotifers, from 28 dph, *Artemia* sp. nauplii (ZM Fish Food, UK), enriched with ZM Artemia HUFA Enrichment for 22 hours, were added to each tank at a density of 5 *Artemia* ml^{-1} (Figure 3C). At 32 dph, feeding with rotifers and 'green water' supplementation was discontinued.

At 60 dph, as well as *Artemia*, the larvae were offered 100-200 µm BernAqua (Switzerland) Caviar feed by hand five times daily and from 63 dph onwards feeding with *Artemia* was discontinued. At 114 dph, the larvae were offered a 50:50 mix of 100-200 µm and 200-300 µm Caviar feed at a ration of 3% bodyweight d^{-1}, and from 149 dph the smaller-sized diet was discontinued and the fish were fed by hand 5 times per day to excess. Between 130 and 140 dph, tank salinity was gradually increased at a rate of 3-4 ppt d^{-1} (by increasing the salinity of the replacement water each day) to 35 ppt.

To maintain water quality, the base of each glass tank was siphoned daily (6 mm hose internal diameter) to remove dead larvae, faeces and dead prey items, and 50% of the tank water was changed. To avoid injury to larvae or removal of live prey during water changes, the siphon hose (10 mm internal diameter) was placed inside a sieve (150 µm mesh base). After siphoning, each rearing tank was topped up with live *N. occulata* to maintain target cell density until 32 dph. Every 3 days, NH_3-N, NO_2-N, and NO_3-N were monitored using test

kits (API, USA) and pH (Mettler Toledo, USA) and salinity measured using hand-held monitors. Nitrogen levels in the tanks remained <0.01 mg L^{-1} for NH_3-N and NO_3-N, and <30 mg L^{-1} for NO_2-N. pH levels remained between 7.43 and 8.04 and salinity levels were at the target levels for that stage of culture.

At 149 dph the fry were transferred to 400 L glass fibre tanks supplied with natural seawater at a rate of 6 L min^{-1} in a recirculating system (water turnover *ca.* 10% d^{-1}). Fry were fed artificial feed by hand (5 times d^{-1}) increasing in size from 200-300 µm (Figure 3D) to 300-500 µm (both BernAqua Caviar) to 0.6-1.0 mm (NutraPlus 01; Skretting, Norway) until 213 dph when 24-hour clockwork belt feeders were used to deliver feed (Figure 2). At 307 dph, juvenile fish were fed a 50:50 mix of 0.6-1.0 and 1.0-1.7 mm (Skretting NutraPlus 02) feed until 423 dph when the smaller diet size was discontinued and the larger feed size offered until 443 dph when fish were fed on 1.0-1.5 mm pellets (Skretting Labrax) for the rest of the culture period (Figure 3E).

2.5 | Statistical analysis

All data are presented as mean values ± SEM. Data were tested for normality and homoscedasticity prior to analyses. Potential differences for L_T of hatchlings between temperature and salinity treatments were assessed using a general linear model (GLM) and where significant, multiple *post-hoc* comparisons were made using a Bonferroni test. Percentage data were arcsine transformed and examined using a GLM (% hatch) or a Kruskal-Wallis and Mann-Whitney U tests (% survival) respectively.

3 | RESULTS

3.1 | Embryonic development

Mean maximum egg diameter of fertilised elliptical eggs at 1 dpf (Figure 1A) was 1.54 ± 0.02 mm. Three random samples of 100 eggs taken from each of 4 incubating flasks at 1 dpf indicted that 99.1 ± 0.7% of the eggs were fertilised. As with *O. mordax* (Ayer et al., 2005), the ovulated eggs of *O. eperlanus* were highly adhesive. While the adhesive property of the eggs was eliminated following washing in tannic acid solution, a small number of eggs did adhere to the sides of the glass jars having initially made contact with the glass as the eggs were poured into the tannic acid solution. However, eggs that both retained and lost their adhesive property went on to hatch successfully.

The sequence of embryonic development, based on those eggs sampled daily from the incubating flasks, is shown in Figure 1 with different developmental stages identified based on Gorodilov & Melnikova (2006). At 10ºC/0 ppt, the Morula stage of development, with the presence of numerous blastomeres and the germ ring was observed at 1 dpf (Figure 1A), with the more advanced Blastula stage of development observed at 3 dpf (Figure 1B). Evidence of the early embryonic stage axis was observed at 4 dpf (Figure 1C) and further development, *i.e.* the formation of the cephalic region and the optic vesicles, was clearly distinguishable at 5 dpf (Figure 1D), and the embryonic fin fold was observed on 6 dpf (Figure 1E). Movement by smelt embryos was first recorded at 8 dpf when tail movement was observed in all embryos examined. During the late stages of development (13 dpf at 10ºC), embryos had increased in size so much so that they occupied much of the volume of the eggs (Figure 1F) and were entirely surrounded by the yolk sac, with the tail extending all the way to the head region. Hatching occurred in the stock incubating flasks (10ºC, 0 ppt) between 21 and 27 dpf.

3.2 | Determining optimum temperature and salinity for larval culture

In the temperature/salinity experiment, mean treatment temperatures are presented in Table 1. The salinity in each beaker did not vary from target values throughout the experiment. Time taken to hatch was dependent on water temperature. Embryos incubated at 5°C took 24 days longer to hatch than those incubated at 10°C, while there was only an 8 day difference in time to hatch between eggs incubated at 10 and 18°C (Table 1). In addition, hatching duration was also prolonged with decreasing water temperature irrespective of salinity (Table I).

Hatching success was significantly affected by salinity ($P < 0.001$) with lower hatching success rates observed at 15 and 20 ppt ($P < 0.001$) compared to salinities ≤10 ppt. Hatching success between 0 and 10 ppt was high (range; 82 - 96%) with a general reduction in hatching success at salinities >10 ppt with extremely poor hatching success (range; 0 - 17.3%) in embryos incubated at 20 ppt, irrespective of temperature treatment (Figure 4A). Temperature had no effect ($P > 0.05$) on hatching success, although this tended to be lower at 15 and 18°C. There was no interaction between temperature and salinity ($P > 0.05$) on the hatching success.

Size at hatch of surviving larvae ranged between 0.53 - 1.00 mm L_T (Figure 4B). Accurate measurements of L_T of larvae hatched at 20 ppt were not possible as those larvae that did hatch, died before they could be removed alive and subsequently decomposed preventing measurement. Size at hatch was significantly affected by salinity ($P < 0.001$) and temperature ($P < 0.001$) with a significant salinity*temperature interaction ($P < 0.001$) on L_T at hatch. The

multiple pairwise comparisons indicated that hatchlings from eggs incubated at 5°C and at 0 - 15 ppt were significantly larger ($P < 0.001$) than those incubated under the remaining temperature/salinity combinations, with all other treatments similar in size (all $P > 0.05$), except 20 ppt at 15 and 20°C that were significantly smaller ($P < 0.001$) (Figure 4B). The largest size at hatch was recorded in smelt incubated at 5°C/5 ppt where larvae ranged in size from 0.76 - 1.00 mm L_T with an average size of 0.85 ± 0.01 mm.

A Kruskal-Wallis test performed on the medians from the temperature and salinity combinations showed that there was a significant difference ($P < 0.001$) in survival of larvae to first feed between at least two of the treatments. Survival to first feed was significantly lower in larvae reared at 15 ppt (5 - 18°C) when compared to those reared at 0, 5 and 10 ppt (all $P < 0.001$) with a trend of increasing variability between treatments as salinity increased (Figure 4C). No larvae reared at 20 ppt survived to first feeding.

3.3 | Culture protocol for larviculture and ongrowing of juveniles to adulthood

The culture protocol summarised in Figure 2 was used to successfully rear European smelt from hatching (Figure 3A) to maturity (Figure 3F) with fish making the diet transitions from endogenous yolk resources to live feed (Figures 3B, C) and onto formulated feed during the fry (Figure 3D), juvenile (Figure 3E) and adult (Figure 3F) stages. Growth was rapid, especially after the transition to formulated feed at *ca.* 4 cm L_T (Figure 3D). The length-weight relationship is presented in Figure 5.

Fish were reared for 2 years under culture conditions and both male and female fish reached sexual maturity (Figure 3F). Although spawning did not occur in captivity, unfertilised eggs were observed on the floor of one of the rearing tanks. As observed in other studies (Colburn et al., 2012; DiMaggio et al., 2015), nodular growths on the lower jaw were observed in some juvenile and adult smelt (see female smelt in Figure 3F) as a result of collisions with the tank walls, although its occurrence was not quantified.

4 | DISCUSSION

The embryonic and larval development of European smelt has been described in detail (with accompanying line drawings) by Gorodilov & Melnikova (2006) and Melnikova & Gorodilov (2006) respectively, based on experimental rearing of early life stages in petri dishes with larvae hatching out of eggs attached to the dish. In the current study, embryonic development was monitored daily at 10°C/0 ppt and the 6 key stages of development defined

by Gorodilov & Melnikova (2006) were observed and photographed: fertilisation, cleavage, blastulation (Figure 1A-C), gastrulation (Figure 1D), somitogenesis (Figure 1E) and prehatching (Figure 1F). By removing the adhesive layer according to Walker et al. (2010), it was possible to upscale egg incubation to larger hatchery vessels (in this case, Erlenmeyer flasks) containing thousands of eggs that can facilitate the large-scale hatchery production of smelt eggs for on-growing and subsequent restocking into the wild. In the present study, 50% water changes were conducted daily due to logistical constraints with using bottled spring water. However, to minimise egg mortality, for future culture work where access to freshwater is not limited, we would recommend greater daily water replacement as seen in culture of *O. mordax* (90% replacement; Colburn et al., 2012) and *Alosa* spp. (200% replacement; DiMaggio et al., 2015).

It is well known that developmental rates of fish eggs and larvae are temperature-dependent (Blaxter, 1991) and, as expected, the time interval between fertilisation to hatching decreased with increasing temperature in this study (Table 1), as also observed by Gorodilov & Melnikova (2006) for *O. eperlanus* and Ayer et al. (2005) for *O. mordax*. Of the two abiotic factors examined in this study, salinity had the greater overall effect on larval performance (i.e. hatch success, size at hatch, survival to first feeding) than temperature across the ranges included in this study (5 - 18°C; 0 - 20 ppt). In the present study, the results of the temperature/salinity experiment indicated the best survival and growth of smelt larvae was achieved at both lower salinity (0 - 10 ppt) and temperature (5 - 10ºC) regimes.

There are a number of anadromous teleost species in temperate latitudes where early life stages are exposed to oligohaline/mesohaline conditions, *e.g.* members of the *Alosa* genus (shads, river herring and alewife) and *Osmerus* genus (smelts) (Froese & Pauly, 2019). These species often spawn in tidal freshwater where eggs (*Alosa* spp.) and larvae (*Alosa* and *Osmerus* spp.) passively drift downstream into oligohaline/mesohaline conditions in estuaries and tidal reaches of rivers (Ayer et al., 2005; Navarro et al., 2014). Research on *Alosa* spp. indicates that low salinity conditions are most suitable although the optimal conditions for hatching success, survival and growth vary between species (Limburg & Ross, 1995; Bardonnet & Jatteau, 2008; Navarro et al., 2014; DiMaggio et al., 2016). Similarly in osmerid smelts hatching success is highest at salinities and temperatures between 0 - 10 ppt and at ≤10°C (*O. eperlanus*, this study; *O. mordax*, Ayer et al. 2005; see also Nellbring, 1989). This study showed that embryonic and larval mortality increased with increasing salinity, a relationship also observed for *O. mordax* (Ayer et al., 2005; Fuda et al., 2007) and *Alosa* spp. (Bardonnet & Jatteau, 2008; Navarro et al., 2014).

The fact that best survival and growth of European smelt larvae from fertilisation through hatching to first feeding larvae includes rearing in freshwater and at low water temperature (≤ 10ºC) will facilitate the use of existing salmonid hatcheries in smelt conservation aquaculture programmes. Since mortality rates during the egg and larval phase are extremely high, stock enhancement programmes usually culture fish beyond this stage and release fish into the wild as juveniles (Hendry et al., 2003; Molony et al., 2005) although culture for too long in artificial conditions can reduce survival in the wild by producing predator-naïve fish (Molony et al., 2005). On-growing of smelt would include the logistical and technical problems of live feed production, however, it may be preferable in enhancement programmes to rear fish until hatching and then stock out onto known spawning rivers, and allow hatched larvae to drift down into estuaries to complete the larval and juvenile phases. This approach would minimise the time spent in the hatchery post-hatching, eliminate the need for live food production, prevent the development of predator-naïve fish and would mimic the natural life cycle of the species as closely as possible.

In summary, this study has shown that the techniques developed to rear the early life stages of rainbow smelt in captivity (Ayer et al., 2005; Fuda et al., 2007; Colburn et al., 2012; Walker et al., 2010) can be successfully applied to rear European smelt from fertilisation to maturity in a 2 year culture cycle. The same culture methodology, with minor modifications, has also recently been applied to rear other anadromous species of conservation concern such as alewife (*Alosa pseudoharengus*) and river herring (*A. aestivalis*) (DiMaggio et al., 2015). Thus, a conservation aquaculture protocol has been established that has the potential to be applied to a number of anadromous [*e.g.* clupeids such as allis (*A. alosa*) and twaite shad (*Al. fallax*)] and freshwater [*e.g.* coregonids such as vendace (*Coregonus albula*), the endemic powan (*C. clupeoides*) and European whitefish (*aka* schelly or gwyniad; *C. lavaretus*] species that are declining in abundance in the UK and are also of conservation concern in other areas of Europe. The egg rearing protocol described in this study is one tool that could be used for reintroducing fish into former rivers or boosting numbers in extant populations by providing high numbers of hatched larvae that could be used for out-stocking without the need for on-growing. However, the continued ongrowing to maturity achieved in the current study does allow for the possibility of broodstock to be reared/retained in captivity, although further research to stimulate volitional spawning, or to induce spawning using hormones (Abraham, 2007), may be necessary.

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DATA AVAILABILITY STATEMENT

Raw data can be requested from the corresponding author.

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Table 1. The effect of water temperature (T°C; Mean ± SEM) on the time to first hatch (dpf, days post fertilisation) and hatching duration (days) on embryos of European smelt *Osmerus eperlanus* (L.).

Recorded T°C	dpf to first hatch	Hatching duration
5.1 ± 0.10	42	10
10.9 ± 0.01	18	7
14.9 ± 0.08	13	6
18.3 ± 0.20	10	2

FIGURE 1 Development of laboratory-incubated European smelt *Osmerus eperlanus* (L.) embryos at ~10°C. (A) 1 dpf (days post-fertilisation). (B) 3 dpf. (C) 4 dpf. (D) 5 dpf. (E) 6 dpf. (F) 13 dpf. BL, blastomeres; YO, part of the yolk sac not covered with blastoderm; GR, germ ring; B, blastula; EA, embryonic axis; BLA, blastoderm; MI, micropile; OV, optic vesicle; YS, yolk sac; FF, fin fold; Y, yolk; OG, oil globule; AV, auditory vesicle; M, myomeres; CR, caudal region. Scale bars = 0.5 mm.

FIGURE 2 Summary outline of the feeding protocol for larviculture and ongrowing of European smelt *Osmerus eperlanus* (L.).

FIGURE 3 European smelt *Osmerus eperlanus* (L.) at various stages of culture from hatched larva to mature adult. (a) hatched larva at 1 dph (day post-hatching; 0.80 cm L_T), (b) larvae at 8 dph (0.86 cm L_T; rotifers in gut), (c) larvae at 28 dph (1.6 cm L_T; *Artemia* in gut), (d) fry at 180 dph (4.3 cm L_F; granulated diet in gut), (e) juvenile at 450 dph (*ca.* 7.0 cm L_F), (f) mature adult male (upper; 16.0 cm L_F) and female (lower; 18.3 cm L_F). L_T = Total Length, L_F = Fork Length

FIGURE 4 The effects of temperature (5, 10, 15 & 18°C) and salinity (0, 5, 10, 15 & 20 ppt) on (a) % hatching success, (b) size (Total Length, L_t, mm) at hatching and (c) % survival to first feeding of European smelt *Osmerus eperlanus* (L.) larvae. Data are presented as Mean ± SEM and data points are slightly offset for clarity.

FIGURE 5 Length-weight relationship for European smelt *Osmerus eperlanus* (L.) reared under *ad-libitum* feeding at 10°C

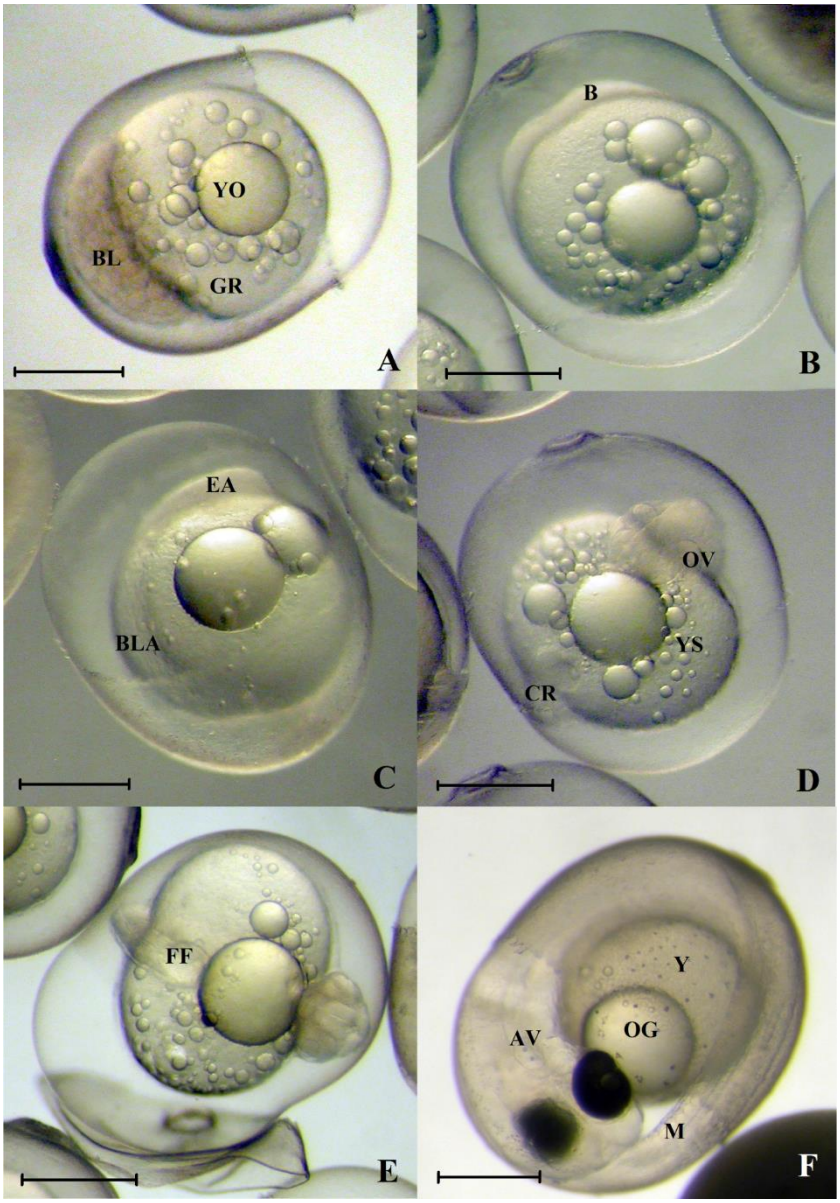


FIGURE 1

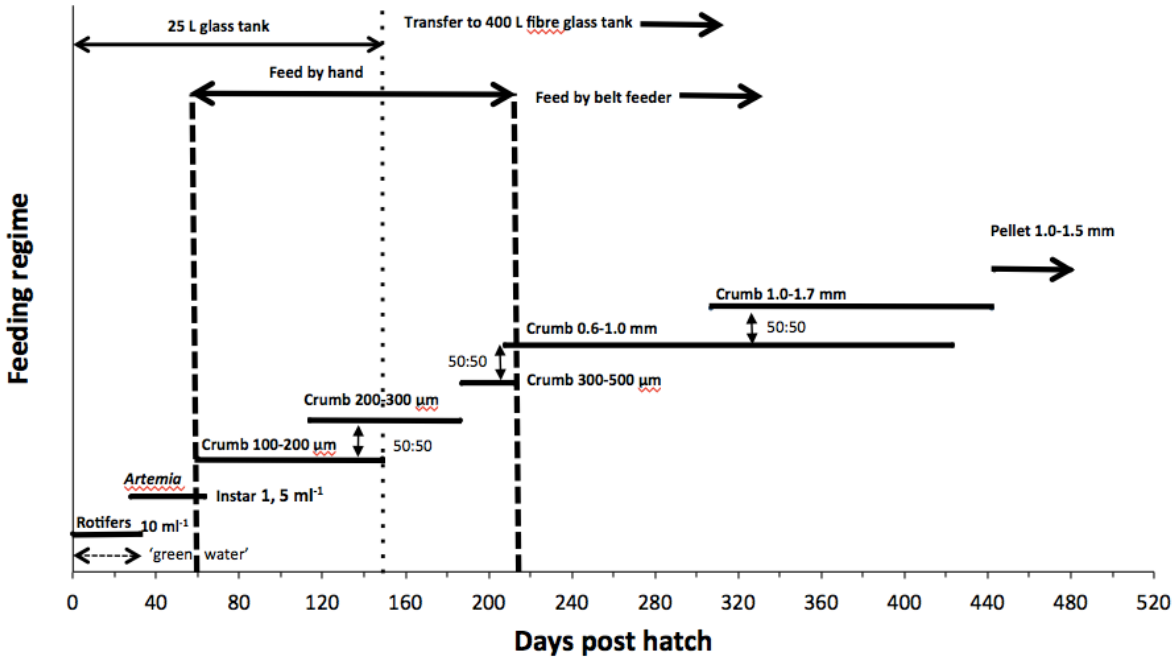


FIGURE 2

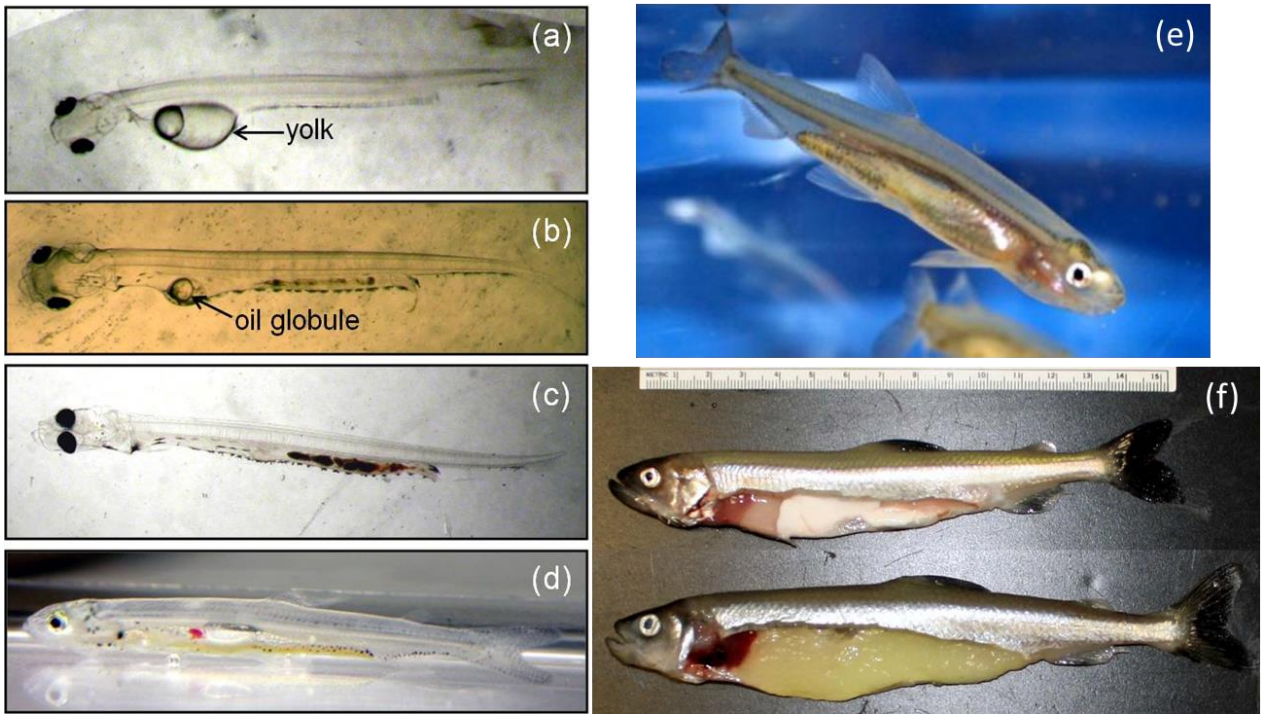
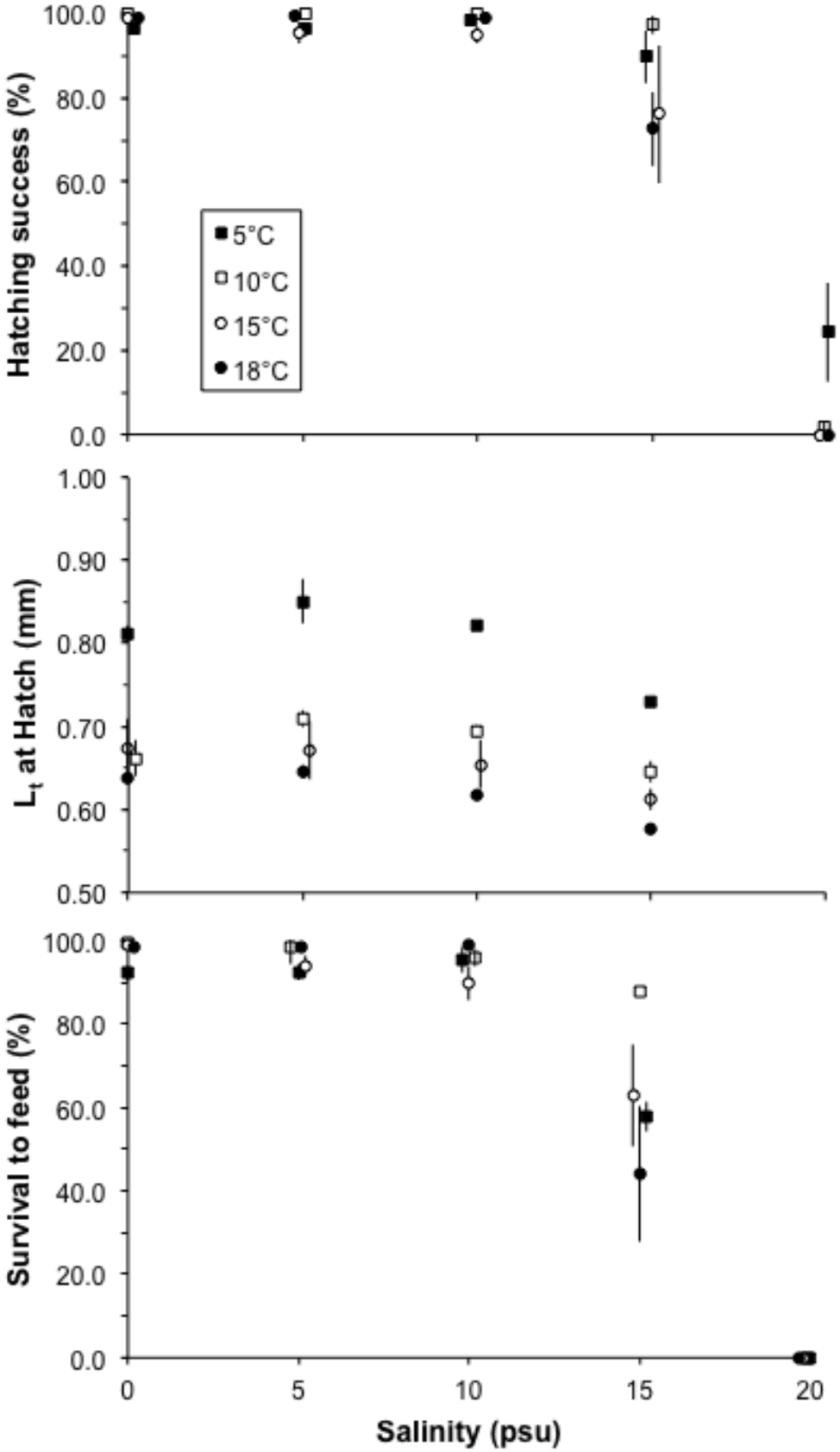


FIGURE 3



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479 FIGURE 4

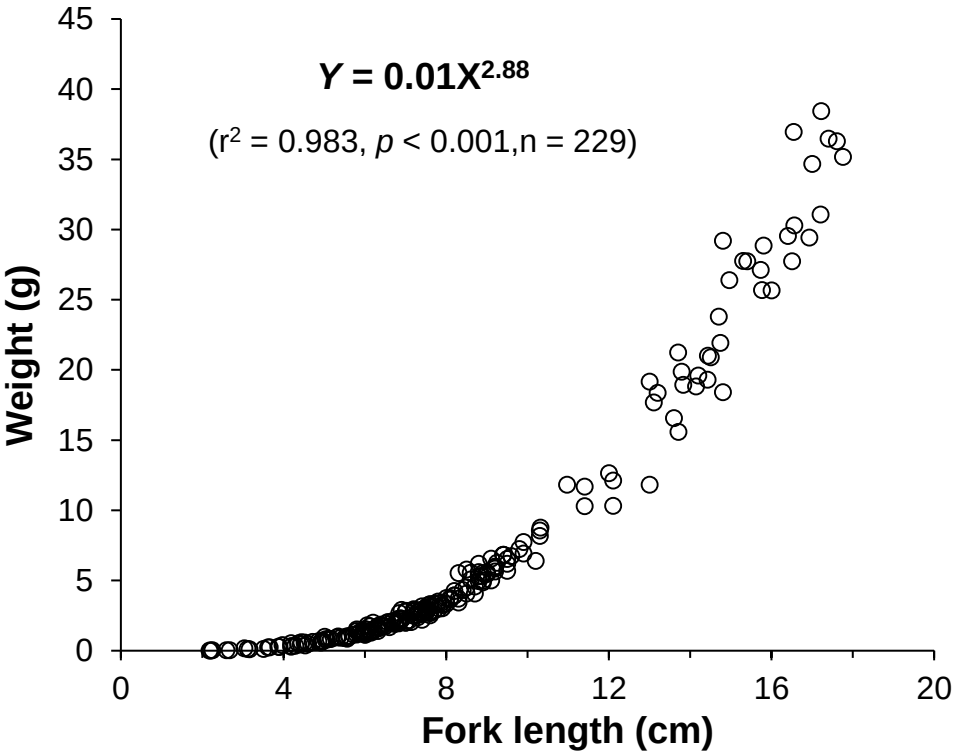
Y = 0.01X2.88
(r2 = 0.983, p < 0.001,n = 229)
Weight (g)
Fork length (cm)
0
5
10
15
20
25
30
35
40
45
0
4
8
12
16
20

FIGURE 5