Environmentally Friendly Diversification of Aquaculture.

Conservation aquaculture rearing techniques for the European smelt *Osmerus eperlanus*.

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Conservation aquaculture rearing techniques for the European smelt *Osmerus eperlanus*.

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Foreword

This culture manual is a deliverable of the SEAFARE project (project number 2009-1/123) under the subproject Environmentally Friendly Diversification of Aquaculture with the express purpose of promoting diversification in the aquaculture industry by providing a greater range of species and environmentally friendly production systems. This work was funded European Union Atlantic Area Transitional Programme (2007-2013). As part of this, the aim of the present study was to develop conservation aquaculture technologies that could be used in restoration projects for the threatened European smelt, *Osmerus eperlanus*.

For general advice on live food culture in the laboratory and the infrastructure to support this, including the culture of microalgae, marine rotifers and *Artemia*, Laing (1991) and Hoff & Snell (1987) are both excellent sources of information. We have not attempted to summarise these works here as we think it more pertinent and valuable to refer to them directly as required, due to their detailed and comprehensive nature.

The aim of this manual is to provide the end user with all the necessary information required for the successful culture of the European smelt *Osmerus eperlanus* for conservation aquaculture purposes. In addition, the information found within this manual will also be of benefit to those looking to culture this species commercially.
Chapter 1

The European smelt

Appearance

The European smelt *Osmerus eperlanus* (Fig. 1.1), also known as “the cucumber smelt” for their strong smell, or sparling in Scotland and Wales, is a small to medium sized fish typically ranging in size from 20 to 25 cm, but can attain lengths of up to 30 cm. European smelt are not to be confused with the superficially similar but smaller (15-20 cm) sand smelt *Atherina presbyter* (Fig. 1.2) which lack the distinctive fleshy adipose fin of *O. eperlanus* and the distinctive smell and possesses two dorsal fins.

![Fig 1.1 The European smelt, *Osmerus eperlanus* (from Maitland, 2007).](image1)

![Fig 1.2 The sand smelt, *Atherina presbyter* (FAO).](image2)
Distribution

The European smelt is an anadromous species primarily found on the Atlantic seaboard of northwest Europe from northern France and British Isles as far north as the White Sea in Russia (including the Baltic). However, its native distribution used to extend further south to Spain but these southerly Spanish and French populations are now thought to be extinct. Lacustrine populations do occur in Scandinavia, Poland and Russia. In the UK, the only recorded freshwater population of smelt in Britain was once found in Rostherne Mere (Cheshire, England), which became extinct in the 1920’s, probably as a result of eutrophication. Estuarine smelt populations once occurred all along the east coast of Britain from the Tweed to the Thames and on the west coast from the Solway to the Conwy.

Exploitation

Historically, smelt populations throughout the UK have supported small scale commercial fisheries. European smelt are fished for human consumption, animal feed and for bait (for pike Esox lucius). While O. eperlanus are of low commercial importance, with only small scale fisheries in Great Britain, Ireland and in the Baltic Sea, important fisheries once existed on the Thames estuary and the inland lakes of northern Poland. In the early 1980’s approximately 5 to 6 tonnes of smelt, equating to approximately 43 to 51,000 fish were caught annually during the spawning run on the River Cree in southwest Scotland. Although a fishery still exists on the Cree, catches are now so small that the fishery is barely viable, with no fish landed in some years. In 1978, commercial fishing for smelt on the River Conwy during their spawning run ended as a result of declining numbers of fish caught in the previous 5 to 6 year period.

Current status

Unfortunately the story of smelt in the UK is one of decline from one of healthy status and abundance before the Industrial revolution to its present status (see review by Maitland, 2003). Within the UK, 52 estuarine or tidal river systems (comprising of at least 26 populations) are known to have contained smelt at one time, however many of these are now extinct and currently there are 3 populations (Cree, Forth and Tay) remaining in Scotland, possibly 18 or more in England (Mersey, Ribble, Lune, Wyre, Solway, Tyne, Esk, Humber, Wash, Broads, Alde, Stour, Orwell, Blackwater, Crouch, Thames, Adur and Plymouth Sound) although we do not know for sure, and the Conwy and Dee populations in north Wales. The decline of the species is thought to not only be due to pollution, but also as a direct consequence of overfishing, the
destruction of spawning grounds and nursery areas, and the physical obstruction of spawning migrations by the erection of dams and weirs.

Ecology & life cycle

Like salmonids, smelt are thought to return to their natal river to spawn although the degree of fidelity may not be as strong as in Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*). Adult smelt run up rivers in small shoals to spawn in March-April in freshwater, just above the head of the tide on clean rivers (see Fig 1.3). Their eggs are small (ca. 1 mm in diameter). There is no excavation of a spawning nest (or redd), the eggs are released by the female into the water column and fertilised by the male who lies alongside her. Due to the adhesive outer coating, eggs rapidly attach onto any suitable substrate (clean gravel, stones, macrophytes (see Fig. 1.4)) and thus avoid being swept away. The length of embryonic development is short, usually 3-4 weeks in the wild, but will vary with temperature. On hatching, the larvae do not reside in freshwater, but are immediately swept downstream into the estuary where they complete the larval and juvenile stages, feeding on zooplankton. As *O. eperlanus* reach adulthood they are thought to feed in the estuary, local coastal waters and their natal river on small crustaceans and fishes. Maturing adults return to the outer part of the estuary during winter before undertaking their spawning migration in the spring. This spawning run usually takes place over 3-4 days, with communal spawning taking place at night.

![Spawning grounds at Newton Stewart on the River Cree, southwest Scotland. Photo courtesy of the Galloway Fisheries Trust.](image)
Smelt play an important role in food webs, both as a predator of zooplankton and as prey of large predatory fish. They are also considered to be indicator species, due to their sensitivity to pollution and therefore their presence or absence may be indicative of the overall health of an estuarine/river system.

**Smelt culture**

To date the culture of smelt from the family Osmeridae has been limited to the experimental laboratory culture of two smelt species under threat in North America. These are the rainbow smelt *Osmerus mordax* and the delta smelt *Hypomesus transpacificus*, found in the northeastern United States as well as Canada, and the Sacramento Delta respectively.

Historically, rainbow smelt have been subject to a large commercial and recreational fishery in the estuarine areas of the northwest Atlantic and northeast Pacific. While not only being important as food fish, rainbow smelt are also important prey species for piscivores including the Atlantic salmon and as such, live smelt (<10 cm) are prized baitfish, commanding high prices among the New England ice fishing community. Declines in the populations of this species over consecutive decades has prompted research into the culture of this species in order to satisfy increased consumer demand, but also for restoration and restocking programmes.
The delta smelt is a small osmerid that has been in a state of serious decline since the 1980’s as a result of environmental degradation. Since 2006 a refugial population has been maintained at the UC Davis Fish Conservation and Culture Lab with the aim of ensuring that firstly, there are fish available for restoration programmes should the species go extinct in the wild, and secondly to provide a source of fish for supplementation of wild stocks, should supplementation be deemed appropriate. Subsequently a culture programme was initiated to develop conservation aquaculture rearing techniques for all life stages of delta smelt.

Current bottlenecks hindering the culture of both species include difficulties in weaning larvae to accept commercially prepared diets as well as determining optimal environmental conditions during larviculture. Inappropriate environmental conditions are known to result in behaviours which lead to impaired feeding activity or physical malformations.

Summary

A restoration project for the European smelt is currently underway on the Rivers Cree and Fleet under the governance of the Galloway Fisheries Trust, although this has not included the development of culture techniques for the species. Such techniques have already been established for the closely related rainbow smelt therefore the knowledge gained from previous culture studies could be adapted for culturing European smelt. The aim of this project is to develop and record a protocol for rearing European smelt.
References


Thomas, M. 1998. Temporal changes in the movements and abundance of Thames estuary fish populations. In: A rehabilitated estuarine ecosystem: the environment - 11 -


www.baydeltaconservationplan.com
Chapter 2

Live Food Culture

Algal culture

Algae are at the base of the marine food chain, hence the use of marine microalgae in aquaculture is critical in achieving the correct nutritional requirements needed for optimal growth of larvae and juveniles maintained at high densities. In general, microalgal culture in aquaculture includes the culture of highly nutritious flagellate and diatom species, offered in unison. Most marine fish, molluscs and crustaceans are incapable of, or exhibit a limited ability to synthesise essential polyunsaturated fatty acids (PUFA) and cholesterol, yet these are essential components of their diet. However, both PUFAs and cholesterol are abundant in microalgae as they possess $\Delta 4$, 5 and 6 desaturase enzymes which facilitate the synthesis of essential PUFAs and many phytosterols.

In fish hatcheries, marine microalgae are used for the culture of zooplankton (rotifers, copepods and brine shrimp) which in turn serve as first foods for early stage fish larvae, as well as for maintaining the quality of the larval rearing medium. The ‘green water’ technique involving the suspension of algae such as the diatom *Nannochloropsis occulata* (see Fig 2.1) in larval rearing tanks during the first days of feeding is now considered a standard procedure for those rearing marine fish larvae. Our understanding of exactly how the presence of microalgae in fish larval culture systems benefits the larvae is not fully understood, yet there is evidence that its addition improves and stabilises water quality through oxygen production, while also stabilising pH. It has also been suggested that the addition of microalgae to culture systems facilitates the control of microbes, offers probiotic effects, stimulates immunity and provide a source of nutrition to the larvae. ‘Green water’ techniques have also been shown to have a pronounced effect on the onset of feeding, survival and growth of numerous species of larval fish. Commercially prepared algal concentrates can also be used as ‘green water’, but it is widely considered that the use of live microalgae is preferential.

*Fig 2.1.* Cells of the marine microalga *Nannochloropsis occulata* (2-4 µm in diameter).
Summary of microalgal culture

Detailed and comprehensive microalgal culture manuals which are available online have been produced by the FAO (Lavens & Sorgeloos, 1996; Helm et al. 2004) which should be directly referred to as excellent source references. Hoff & Snell (1987) have also produced an excellent plankton culture manual which gives additional information on the culture of both phytoplankton and zooplankton.

Figure 2.2. is a schematic diagram of the required inputs for successful microalgal culture, while Table 2.1 gives the tolerable and optimal environmental parameters for algal culture, although it should be noted that these parameters can be species-specific. The most important environmental parameters for regulating marine microalgal growth are nutrient quantity and quality, light, pH, turbulence, salinity and temperature.

![Figure 2.2: The various input required for the cultivation of microalgae. Adapted from Helm et al. 2004.](image-url)
**Fig 2.3** A generalised schematic showing the steps involved in the production of microalgae. From Helm et al. 2004.

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<th>Range</th>
<th>Optima</th>
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<td>16-27</td>
<td>18-24</td>
</tr>
<tr>
<td>Salinity (PSU)</td>
<td>12-40</td>
<td>20-24</td>
</tr>
<tr>
<td>Light intensity (lux)</td>
<td>1,000-10,000</td>
<td>2,500-5,000</td>
</tr>
<tr>
<td></td>
<td>(depends on volume and density)</td>
<td></td>
</tr>
<tr>
<td>Photoperiod (light:dark, hours)</td>
<td>16:8 (minimum)</td>
<td>24:0 (maximum)</td>
</tr>
<tr>
<td>pH</td>
<td>7.9</td>
<td>8.2-8.7</td>
</tr>
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A generalised method for microalgal culture involves a step by step approach to scale-up production starting with stock cultures of a small volume, gradually building up to larger production-scale volumes as shown in Figure 2.3 and PROTOCOL 1.

Microalgae are cultured under sterile conditions in filtered seawater, enriched with culture nutrients. In order to reduce the risk of contamination, Helm et al. (2004) give a detailed description of suitable sterile transfer techniques which should be adhered to. The sterilisation of the culture media may be achieved through either pasteurisation, by chemical means or by autoclaving. Nutrients added to the culture media are a blend of macro and micro nutrients, such as Guillards F/2 and Walse’s medium which are suitable for the culture of most microalgal species and which are common place at aquaculture facilities, while other commercial enrichment products such as Cell-hi are also readily available. When culturing diatoms a source of silicates must also be added to the culture media as this is used in the formation of the external test. The addition of silicates compensates for any natural deficiencies in the seawater, allowing the culture of such species at high densities which would otherwise not be possible. In order to attain optimal growth, diatoms are typically cultured in diluted seawater at 20 to 25 PSU (practical salinity units= parts per thousand) and flagellates at 30 PSU.

In summary, monospecific stock cultures (or master cultures (≤ 250 ml in volume)) in a sterile culture medium, maintained under light and climate control (low temperature: 4-12 °C) are used to inoculate starter cultures. Stock cultures are neither aerated nor supplemented with CO₂. The purpose of such cultures is to maintain a culture of the pure species in good condition, without promoting rapid growth, examples of which can be seen in Figure 2.4. It is important to note that both stock and starter cultures should be handled and maintained using sterile procedures set out by Helm et al. (2004) in order to reduce the risk of contamination.

Starter cultures (250 ml to 4 litres in volume) are used to inoculate larger culture volumes. Unlike stock cultures, growth is rapid in starter cultures and they are maintained at higher temperatures and light intensities, at a pH of between 7.5 to 8.2 and if deemed necessary can also be supplemented with carbon dioxide enriched air (2% CO₂). Once growth of the starter culture is sufficient (usually 3 to 5 days for diatoms; 7 to 14 days for flagellates) the starter culture is used to inoculate an intermediate-scale culture (4 to 20 L in volume) maintained under similar conditions to the starter culture (see Figure 2.4).
Fig. 2.4 *Nannochloropsis occulata* stock, starter and intermediate-scale cultures (right to left). Note the lack of aeration in the stock cultures.

Fig. 2.5 Large-scale production of *Nannochloropsis occulata* in clear polythene bags, suspended vertically from a metal frame.
Intermediate-scale algal cultures are generally maintained either as a batch or semi-continuous cultures and at this stage may used as a larval food source or for inoculating large-scale cultures (≥ 50 litres in volume). Where space is sufficient, large-scale microalgal cultures can be maintained in large, clear polythene bags, sealed at one end, suspended from a frame or supported within a mesh frame (see Figure 2.5), or alternatively maintained in open topped tanks although the risk of contamination is greater in open culture.

Protocol 1

Semi continuous production of *Nannochloropsis occulata* cultures as ‘green water’ in larval fish rearing systems:

1) Approximately 4-6 weeks prior to green water being required, acquire a *Nannochloropsis occulata* algal disk and store in a cool, dark place such as a refrigerator. This will come in the form of a gelatine filled petri dish.

2) Take 2 x 250 ml and 2 x 1,000ml conical pyrex flasks (see Figure 2.6). Fill these with 0.2 µm UV filtered seawater and add Cell-hi WP (similar to Walne’s medium) liquid nutrient. Add 1 ml of liquid nutrient per litre of filtered seawater. Place cotton wool in the neck of each flask to produce a bung and tightly cover the mouth and neck of each flask in aluminium foil. Take 2 x rubber bungs with glass tube (for later use in the 1,000 ml flasks) and wrap tightly in aluminium foil. Place these items in an autoclave (heat sterilisation) and allow to cool. In the absence of an autoclave, a pressure cooker can be used. Failing that, the procedure should be undertaken in as clean an environment as possible.

![Figure 2.6 Conical flasks used for starting *N. occulata* cultures.](image-url)
3) In a laminar flow hood, remove a small volume (~ 20 ml) of now sterilised water from one of the conical flasks using a sterile pipette. Remove the lid of the algal disk and fill the dish with the sterile water, replacing the lid afterwards. Allow to stand for 24 h at 20 °C in moderate illumination. This will cause the algal cells to detach from the gelatine.

4) Once 24 h has elapsed and in a laminar hood, use a sterile Q-tip or syringe to remove the algal cells (see Figure 2.7), dividing them approximately equally among each of the four flasks (N.B. Do not add the gelatine culture medium to the flasks). In the absence of a laminar flow hood, undertake this procedure in a clean, dust free area.

5) Sterilise the inner and outer necks of each flask using the naked flame from either a Bunsen burner/blow torch/camping stove (see Figure 2.8). Remove the aluminium foil from the remaining items.

Fig 2.7 Sterile removal of *N. occulata* cells from an algal disk.

Fig 2.8 Sterilising the neck of an algal culture flask in a laminar flow cupboard using a blow torch prior to the transfer of algae.
6) **250 ml stock cultures** - Place cotton wool in the neck of the flask to create a bung, label the flask as appropriate (e.g. species and culture date) and maintain at 20 °C under constant illumination, gently swirling the flask twice daily.

1,000 ml **starter cultures** - Place a rubber bung with glass rod in the neck of the flask. Maintain at 20 °C under constant illumination and aerate vigorously. Once the culture is dark green in colour, the contents of the flask can be transferred either to an intermediate-scale (4 L) culture or to a large-scale culture.

7) **Large-scale culture** - 10 days prior to smelt egg collection set up 2 x 20 litre large-scale bags using standard clear polythene layflat tubing (10” x 500 gauge x 170 m long).

8) Cut the layflat tubing (see polybags.co.uk) to the appropriate size and seal one end using a heat sealer. Tie a knot in the sealed end. Rinse the bag in seawater in order to remove any unwanted debris. Add 5 litres of seawater, suspend the part full bag from the frame, tying the open end off at top with an adjustable cable tie. Make an incision near top of bag with scissors or blade, through which nutrients and water can be added. Add more seawater (to ~75% full). Puncture a small hole near the bottom of the bag using a pipette tip (see Figure 2.9). Insert the air supply hose into the hole. Add nutrients (1 ml of nutrients per litre of culture water), add the contents of the starter or intermediate culture, fill the bag to the top and aerate vigorously at 20 °C under constant illumination. Harvest as needed, topping up each time with filtered seawater and the appropriate volume of nutrients. After 4 weeks the bag should be stripped down and a new large-scale culture started prior to this if needed.

![Fig 2.9 Puncturing a small hole in the side of an algal culture bag with a pipette tip. An air line is then placed in the hole and pushed through until it reaches the centre of the bag.](image-url)
Neubaur Improved Bright-Line haemocytometers comprise of a grid of 25 intermediate squares, each containing 16 smaller squares, all set within one large square on each side of the counting chamber. The depth of the counting chamber is 0.100 mm, while the surface area of one of the smallest squares is 0.0025 mm$^2$.

1) Clean the haemocytometer and its glass cover slip with tissue paper.

2) Gently press the cover glass onto the slide until the Newton diffraction ring appears (Figure 2.10).

Fig 2.10 Newton diffraction ring.

3) Remove a small (~ 25 ml) subsample of the algal culture and place in a glass beaker.

4) To fix the subsample in order to facilitate the counting of the cells, add either 5 drops of lugols iodine or 4% formalin. Dilute the subsample if necessary.

5) Using a Pasteur pipette (1 ml), add the algal suspension to both sides of the counting chamber, by adding a smooth flow of the sample at the edge of the cover slip. N.B. Avoid adding air bubbles.
6) Under a compound microscope (x 40 objective) count the number of algal cells in 80 small squares, by counting the number of cells in 5 of the intermediate squares (each containing 16 small squares) in a diagonal from top left to bottom right (Figure 2.11). **N.B Include in your counts those cells that touch the upper and left hand borders, but exclude those which touch the lower and right hand borders of each small square (Figure 2.12).**

**Fig. 2.11** Haemocytometer counting grid (a) and counting directions (b).

**Fig 2.12** Represents a small square of a haemocytometer, and shows highlighted red or blue borders indicating whether algal cells that lie on those borders should be included or excluded in the count.
7) Repeat this step on the opposite side of the chamber.

8) **Calculate the number of algal cells per µl of sample using the equation:**

\[
\text{number of cells. µl}^{-1} = \frac{(n_1 + n_2)}{2} \times 250
\]

where:
- \( n_1 \) = mean number of algal cells in an intermediate square (left hand side of chamber)
- \( n_2 \) = mean number of algal cells in an intermediate square (right hand side of chamber)

Multiple this number by 1000 to get the number of algal cells per ml.

9) **To order to determine the volume of algal culture to harvest as green water for each individual larval rearing tank the following equation can be used:**

\[
\text{volume of culture to harvest} = \frac{(\text{cell concentration required (ml}^{-1}) \times \text{tank volume (ml)})}{\text{observed cell concentration (ml}^{-1})}
\]
Rotifer culture

What are rotifers and why are they important?

The phylum Rotifera consists of approximately 2,000 holoplanktonic species, 90% of which inhabit freshwater environments, where they may account for as much as 50% of the zooplankton population (Hoff & Snell, 1987). Rotifers range in size (length) from 100 to 2,000 µm and are either herbivores, bacterivores or predators. In marine environments rotifers are largely found in estuaries, consisting of predominantly herbivorous species less than 400 µm in length, consuming phytoplankton 3 to 17 µm in diameter. The larval stages of a number of marine fish species are spent in estuaries where rotifers, due to their size, slow swimming behaviour and digestibility are predated upon by larval fishes. As such they are frequently used in aquaculture, with the marine rotifer Brachionus plicatilis (Figure 2.12) being the most commonly cultured rotifer species. Marine rotifers play a key role as a first food in the intensive culture of most marine fish larvae and to date, no adequate substitute has been found.

![Diagram of Brachionus plicatilis](www.fao.org)

**Fig. 2.12** Female (left) and male (right) *Brachionus plicatilis*. From www.fao.org.
Prey size is of the upmost importance in prey selection by larval fishes, with the width of the prey being critical for successful ingestion. At first feeding, most fish larvae consume prey between 50 and 100 µm in width (Hoff & Snell, 1987). Some fish species lack fully functioning stomach and gastric glands at first feeding, which impedes absorption due to the lack of specific enzymes. However, the enzymes which facilitate digestion are abundant in live zooplankton such as rotifers. Subsequently, aside from being a source of nutrients for larval fish, live zooplankton also contribute to larval growth by indirectly facilitating digestion through the ingestion of these enzymes.

Life cycle

Rotifers exhibit a cyclically parthenogenetic life cycle (Figure 2.14), comprising of both asexual and sexual phases. The majority of the life cycle is spent in the asexual phase, yet under certain environmental conditions such as stressors (i.e. lack of food) both reproductive phases may occur simultaneously.

During asexual reproduction female rotifers produce up to 7 genetically identical diploid daughters. At a temperature of 25 °C the life-span of female rotifers is approximately 6 to 8 days and 2 days for males. Under the correct environmental conditions female rotifers become sexually mature in 18 hours, with eggs hatching after 12 hours. Fecundity of a single female rotifer over its entire life is between 20 to 25 daughters (under optimal environmental conditions).

When rotifers become stressed due to adverse environmental conditions they alter their sexual phase, producing haploid-identical eggs, with only half the genetic material. The resulting offspring are small, fast swimming, non feeding and short-lived males, their only purpose to fertilise more haploid eggs. These fertilised eggs, or cysts are approximately 110 µm in diameter and can be differentiated from asexual eggs by their reddish colour. Rotifer cysts may remain dormant for several years (store for >1 year in a freezer, or for 1 year at room temperature), yet when maintained at 25 °C under a moderate lighting regime they will hatch within 24 to 48 hours. Rather than being used as food, the emerging female rotifers are used to inoculate cultures as they are capable of asexual reproduction after approximately 18 hours therefore high culture densities can be reached within days.
Fig 2.14 The rotifer life cycle showing both asexual and sexual phases (adapted from King and Snell, 1977).
There are a number of benefits to using rotifer cysts to inoculate cultures:

1) Eliminates the need to maintain stock cultures, saving time and money.
2) Reduces the risk of contamination with ciliates and pathogenic bacteria.
3) Cysts can be treated with antibiotics, resulting in bacteria-free cultures.
4) The time required for upscaling is reduced by using large numbers of cysts as inocula.

**Culture requirements**

The marine rotifer *Brachionus plicatilis* is found in a variety of marine environments, from low salinity estuarine water to highly saline brine lakes. Therefore, its physiology has evolved sufficiently to tolerate a wide range of environmental conditions. When maintaining rotifers in an aquaculture setting it is important to promote high rates of asexual reproduction, suppressing sexual reproduction. This is achieved by the provision of optimal environmental conditions within the culture media (see Table 2.1).

**Table 2.2** A generalised set of conditions for culturing rotifers (following Hoff & Snell, 1987).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
<th>Optima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (^{\circ}{\text{C}})</td>
<td>20-30</td>
<td>25</td>
</tr>
<tr>
<td>Salinity (psu)</td>
<td>1-97</td>
<td>10-20</td>
</tr>
<tr>
<td>Light intensity (lux)</td>
<td>2000-5000</td>
<td></td>
</tr>
<tr>
<td>Photoperiod (light:dark, hours)</td>
<td>16:8 (minimum)</td>
<td>24:0 (maximum)</td>
</tr>
<tr>
<td>pH</td>
<td>6.5-8.0</td>
<td>7.3-7.8</td>
</tr>
</tbody>
</table>

While marine rotifers are euryhaline, salinity has a significant effect on reproduction which only takes place at salinities less than 35 PSU. Rapid salinity changes of 10 PSU or more are likely to kill *B. plicatilis*, however mortality can be greatly reduced through gradual acclimation. If possible, rotifers should be cultured at a similar salinity to that in the larval rearing tanks. This reduces the osmotic stress on the rotifers at the point of transfer to the larval rearing tanks, reducing rotifer mortality and promoting normal swimming behaviour. It is thought that when rotifers are
rapidly transferred to a different salinity, their swimming behaviour changes, making them less appealing prey items to larval fishes.

While filtration is not required if NH$_3$ concentrations are kept below 1 mg.l$^{-1}$, moderate to low aeration should be provided, even though they can tolerate dissolved oxygen levels as low as 2 mg.l$^{-1}$. Moderate aeration keeps the rotifers and their food in suspension, while strong aeration may physically damage the rotifer population.

When culturing rotifers it is also important to check for the presence of ciliates in the culture as these compete with the rotifers for food, although they may help remove detritus and bacteria from the culture. Their presence is usually a sign of sub-optimal conditions within the culture. Once contaminated, ciliates can be eliminated or their numbers reduced by rinsing the culture water through a plankton sieve with a mesh size of <50 µm.

Diet

Rotifers have a high metabolic rate hence in the absence of a suitable food source, they may lose between 18-26 % of their body weight per day when maintained at 25 °C. It is therefore of the upmost importance to feed rotifers regularly (twice daily, morning and evening) as even a 24 hr lapse in feeding causes the nutritional quality and calorific content of rotifers to rapidly deteriorate.

Marine microalgae are the optimum diet for rotifers as very high culture densities can be achieved under the right conditions. *Brachionus plicatilis* will accept a variety of live microalgal species, but *Nannochloropsis*, *Isochrysis* and *Tetraselmis* are considered to be the best nutritionally. Under optimal conditions, a single rotifer may consume up to 200 *Nannochloropsis* cells per minute, while a concentration of 2 million cells.ml$^{-1}$ is sufficient to maintain maximum ingestion rates. The disadvantages of requiring high algal concentrations are the associated cost, time and space needed for large-scale production, although the feeding of rotifers on live algae will yield higher rotifer reproduction rates than other diets. Therefore in most instances, live algae are only used to start rotifer cultures or for enrichment shortly prior to harvesting.

Historically, rotifer cultures have been fed baker’s yeast as its particles are of an appropriate size (5-7 µm in diameter), it is high in protein (45-52 %) and is cheap to buy. Approximately 1 g.million$^{-1}$ rotifers should be fed on a daily basis although this may vary with the strain of rotifer and culture conditions. Instant baker’s yeast, marine yeast (*Candida*) and caked yeast (*Rhodotorula*) may also be used. However, yeast is hard to digest and nutritionally deficient, therefore it should not be fed as a monodiet, although it can be enriched through the addition of vitamins (B12 (1.4
µg.ml⁻¹, A (0.1 µg.ml⁻¹), D (0.5 µg.ml⁻¹), E (0.4 µg.ml⁻¹)) as well as essential fatty acids (EFA) (n-3). Alternatively, yeast can be fed to rotifers alongside a microalgal species such as *Nannochloropsis* at a ratio of 1:1.

More recently, spray-dried algal diets have been developed for the commercial production of rotifers. These provide much of the nutritional value of live cells although the cost of such diets is high.

It is also possible to preserve microalgae for up to a year at -20 °C as a concentrated algal paste yet again these can be expensive. Marine rotifers fed on concentrated *Nannochloropsis* pastes have a reproductive rate of more than 80 % of that of those animals reared on live *Nannochloropsis*. Frozen algal pastes have the advantage of being able to be stored with minimal loss of nutritional value. The fatty acid composition of rotifers fed frozen *Nannochloropsis* versus live *Nannochloropsis* are similar and therefore do not require further enrichment. Their use also eliminates the time and space needed for producing large volumes of live microalgae.

Rotifers can also be fed formulated diets which have been formulated as a complete replacement for live microalgae, giving high incorporation of vitamins and EFA. Such diets are generally of a reasonable price compared to spray-dried and frozen algal pastes.

To conclude, a number of different diets are readily available for feeding and enriching rotifers. The choice of which diet or enrichment product to use is at the discretion of the user who may wish to take in to account factors such as cost, suitability for a given requirement and ease of use.

**Enrichment**

It is beneficial to enrich rotifers shortly prior to feeding to larval fish. Some live microalgae such as *Nannochloropsis occulata* and *Isochrysis galbana* are high in essential fatty acid eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3 respectively and can be successfully used as an enrichment boost. Rotifers maintained in these algae at concentrations of approximately 5 x 10⁶ cells.ml⁻¹ incorporate the EFA within hours. However, as previously mentioned, the culture of live microalgae can be costly both in terms of time spent maintaining the cultures and in space. Consequently, rotifers are usually enriched using commercially prepared algal pastes before being placed in larval rearing tanks containing ‘green water’.
PROTOCOL 2 (a)
Continuous culture of marine rotifers *Brachionus plicatilis*:

1) 300 ml of live marine rotifers *Brachionus plicatilis* (L strain) were purchased from an aquarium supplier.

2) Rotifer culture vessels consisted of cylindro-conical culture vessels ((see Figure 2.15) 90 litre volume, 70 litre working volume). The culture media consisted of dilute synthetic seawater (made up using synthetic sea salt mixed with mains tap water) at 20 PSU and 23 °C.

3) Add 20 g synthetic sea salt.\(\text{l}^{-1}\). Aerate vigorously and allow to reach the target temperature. Add \(\text{Na}_2\text{S}_2\text{O}_3\) (sodium thiopsulphate) at a concentration of 0.3 g.litre\(^{-1}\) of tap water to remove chlorine present in the tap water. Aerate for 12 hours. N.B. Estimates of local (by post code) chlorine concentrations in your tapwater can be found online from your local water authority.

4) Add concentrated *Nannochloropsis occulata* at a concentration of between 0.2-1 ml for every 10 litres of culture water. This should be enough to turn the culture a light green colour. N.B. Dark green culture water (i.e. dense algal concentration) elevates the pH and inhibits growth and reproduction. It is best to do this twice daily (morning and evening) to ensure that there is always ample food available. If the culture water remains cloudy prior to the second feed, do not administer a second feed. Note: Concentrated *Nannochloropsis* can be stored in a fridge for short term use (3 months) or for long term use (2 years) in the freezer (-20 °C).

5) Initially add 5 litres of the 20 PSU water to the rotifer culture vessel, and apply moderate aeration. Inoculate the culture media with concentrated *Nannochloropsis* followed by the live rotifer starter culture.

6) Continually add 1-2 litre(s) of 20 PSU water daily. N.B. When upscaling the culture daily it is advisable to vigorously mix the required volume of concentrated *Nannochloropsis* into the 1-2 litres of 20 PSU water that is being added to the tank. A flocculation trap (see Figure 2.15) can be added to the rotifer culture vessel (Figure 2.16 (a) and Appendix 2) at this stage. This consists of a 20 cm x 10 cm strip of polyester filter wool matting suspended
from a length of fishing line. The flocculation trap should be cleaned/replaced regularly (twice weekly).

Fig 2.15 A flocculation trap suspended from a pvc pipe by a piece of fishing line above a rotifer culture.

7) Two cultures were initially set up, a third a week later and a fourth the following week (Figure 2.16 (b)). More cultures can be established as required to ensure that cultures do not need to be depleted of rotifers on a daily basis. The use of a staggered culture approach reduces the number of ‘old’ cultures and also safeguards against crashes.
Fig 2.16 (a) Internal view of a 70 litre bin used to culture rotifers. (b) A number of rotifer culture bins adapted from a system previously used for rearing crustacean larvae.

PROTOCOL 2 (b)
Continuous culture of marine rotifers *Brachionus plicatilis* starting with cysts:

1) Resting marine rotifer cysts can be purchased from an aquarium or aquaculture supplier.

2) Place approximately 25 ml of culture water (see Protocol 2 (a).2.) in a small, clear container such as a petri disc or glass beaker.

3) Add the cysts to the culture water and cover the rotifer culture vessel. Maintain the culture at approximately 25 °C, and illuminate continuously under moderate light (1000 to 4000 lux). At this temperature, the cysts should hatch within 24 to 48 h. To check for hatching, remove a small (1 ml) sample of culture water and view it under a 8x magnifying glass or under a low powered microscope.

Culture maintenance

Rotifer cultures should be cleaned every 3-6 weeks. Transfer 10-20 % of the old water to the new culture vessel. Initial survival after transfer is not high if 100 % of the culture water is replaced. Filter the rotifers from the old vessel using a zooplankton sieve (Figure 2.17) and discard the water. While in the plankton sieve, wash with clean seawater to remove ciliates. Detritus settled on the bottom of the tank can be removed daily, by opening the ball valve at the base of the culture vessel. This will help maintain a healthy (ciliate free) culture.

To harvest the rotifers, pour or siphon the required volume of culture water (see formula for determining volume to be harvested) through a zooplankton sieve with a mesh size of 53 µm (see Figure 2.17) and rinse in freshwater. Then place the washed rotifers back into a container with 20 PSU water and apply moderate aeration.

N.B. Cultures should not be harvested until densities reach 50 rotifers.ml⁻¹. These densities may be reached after approximately 10 days.

Fig 2.17 Zooplankton sieves used for harvesting rotifers and Artemia. Note the difference in mesh size (53 and 125 µm zooplankton sieves).
Estimating rotifer density

1) Remove 100-200 ml of culture from each, well-mixed, culture vessel.

2) With a one ml pipette, stir the sample well and remove 1 ml of the sample and add this to a Sedgewick rafter counting cell (Figure 2.18 (a)).

3) In order to fix the sample, add 2 drops of Lugol’s iodine.

4) Place the counting cell under a compound microscope (x 40 objective) and count the number of rotifers in the entire sample (see Figure 2.18). Repeat this 3 times in total with separate 1 ml samples and calculate the average of the 3 counts. Multiply this number by the number of ml in the culture to get an estimate of the total number of rotifers in the culture vessel.

5) Observe a separate 1 ml sample of the live rotifers for swimming speed and for the presence of male rotifers within the culture. Males (see Figure 2.12) are small, clear, fast swimming and have a black dot towards their posterior. Also check for ciliates (flat and oblong in shape, 60 x 30 µm in size). Ciliates (see Figure 2.19) swim faster than rotifers, and in a twisting motion. The presence of either is usually an indicator of forthcoming problems in rotifer production, possibly due to sub-optimal environmental parameters.

6) Record the number of female rotifers with single and multiple eggs. A large number of females with either single or no eggs indicates that production is slow. This may be caused by a paucity of food, sub optimal water quality and/or the presence of contaminants. High numbers of females bearing multiple eggs show that production is high, usually indicating that the culture environment is optimal.
Fig 2.18 (a) A Sedgewick Rafter counting cell. (b) A Sedgewick Rafter counting cell positioned under a compound microscope (x 40 objective).

Fig 2.19 A ciliate (~70 µm in length) viewed under a compound microscope.
The volume rotifer culture to be harvested daily can be calculated using the following formula:

\[
\text{Volume of rotifer culture to harvest} = \frac{\text{Rotifer concentration required (ml}^{-1}) \times \text{Tank volume (ml)}}{\text{observed rotifer concentration (ml}^{-1})}
\]

This number is then divided by 1,000 to determine the volume in litres to be harvested from the culture vessel.

\textbf{N.B.} Background counts of rotifers already present in the larval rearing tanks should be made daily in order to determine the quantity of rotifers to add to maintain the required concentration of rotifers in the larval tanks. This can be done by taking 3 x 1 ml replicate samples from selected tanks and determining the average concentration of rotifers already present in the larval rearing tanks.

To harvest, pour the required volume of culture water through a 53 µm zooplankton sieve and rinse with clean seawater and place in a container of a known volume. Top up the container with clean water of the correct salinity, and distribute between your larval rearing tanks. For your records, record the volume added to each larval rearing tank per feed.

A rotifer culture trouble shooting guide has been produced by Hoff & Snell (1987) and is worth consulting if problems arise during culture.
**Artemia culture**

**What are *Artemia* and why are they important?**

The brine shrimp *Artemia* (Figure 2.20) are members of the phylum Arthropoda, class Crustacea. *Artemia* have been used as a live food in both marine and freshwater aquaculture for fish and invertebrate culture since the 1920's and are used in virtually all (> 85 %) commercial hatcheries, hence the harvesting of *Artemia* cysts is big business, with prices fluctuating according to supply and demand. The provision of live *Artemia* to marine fish larvae ensures a high quality, complete protein diet (~60 % protein) which yields higher survival, faster growth rates and fuller colour developments than other diets.

*Artemia* are euryhaline organisms able to tolerate salinities as high as 340 PSU, with a circumglobal distribution in waters 6 to 35 °C. While they may thrive in coastal waters and open sea, their distribution is largely limited to extreme environments such as saline lakes and coastal salt works with a salinity >70 PSU. This is because *Artemia* are vulnerable to predation, with the threat of predation being greatly reduced under such inhospitable conditions where predators are scarce. *Artemia* are even able to tolerate high ammonia concentrations up to 90 mg.l⁻¹.

*Fig 2.20*  *Artemia* nauplii viewed under a dissecting microscope.
Life cycle

The *Artemia* life cycle is shown in Figure 2.21 below. Under the right environmental cues *Artemia* hatch from encysted embryos that are metabolically inactive, called cysts. If kept dry, cysts may remain dormant for several years, only resuming development once re-hydrated in seawater. When maintained at 25 °C the cysts burst after 15-20 hr, initiating the umbrella stage of development where the embryo is positioned underneath the shell, while still enveloped within a hatching membrane. The first larval stage (Instar I) is a non-feeding stage of development, relying on nutrition from yolk reserves. At this stage the nauplii range in length from 400 to 500 µm. Approximately 12 hr post hatch, the nauplii molt into the second larval stage (Instar II) at which point they are able to filter feed on particles suspended in the water column between 1 to 40 µm in diameter, including microalgae, bacteria and detritus. After a total of 15 molts, some 8 days after hatching, *Artemia* reach adulthood. Adult *Artemia* from populations with both males and females attain a maximum length of 8 mm, while those in polyploid parthenogenetic populations may reach a maximum of 20 mm in length. Male *Artemia* can be easily differentiated from females by the presence of muscular graspers (modified antennae) on the head region, while females have a brood pouch (see Figure 2.22). Adult *Artemia* are short-lived, with a life span of up to 4 months under optimal environmental conditions during which time females produce up to 300 nauplii or cysts every four days. Under high salinity, eutrophication caused by large O₂ fluctuations during night and day and food shortages, causes adult *Artemia* to produce dormant cysts (oviparous reproduction). These cysts float on the highly saline water and are blown ashore by winds where they accumulate and dry. Once re-hydrated, development will commence.

Fig 2.21 The *Artemia* life cycle.
**Culture requirements**

While *Artemia* are able to tolerate a wide range of environmental parameters, growth is optimal under specific conditions (see Table 2.3) which may differ from strain to strain. Such information should be available from the supplier and is usually given on the tin. In general, most strains prefer constant, rather than fluctuating temperatures, although adult *Artemia* can tolerate brief exposure to temperatures between -18 to 40 °C, while dry, dormant cysts are able to withstand temperatures from -273 to 60 °C without detriment. When storing cysts for long periods of time they should be kept in a cool, dark place with low humidity such as a refrigerator.

*Artemia* are excellent iono/osmoregulators, with the ability to maintain salt concentration in their tissues within 9 PSU, irrespective of the salinity of their surrounding environment. They are also able to tolerate brief periods (up to 5 hr) in freshwater before dying.

When culturing *Artemia*, dissolved oxygen concentrations should be in excess of 2 mg.l\(^{-1}\) achievable through aeration of the culture media. Interestingly, when oxygen concentrations are optimal, growth and live nauplii production is rapid, but the switch to consuming bacteria and detritus under sub-optimal conditions, leads to a switch in reproductive strategy (oviparity) where dormant cysts are produced.

Fig 2.22 Male and female *Artemia salina*. 
Table 2.3 A generalised set of conditions for maintaining and hatching *Artemia* (adapted from Hoff & Snell, 1987; Lavens & Sorgeloos, 1996).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
<th>Optima*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>-18 to 40</td>
<td>25 to 28</td>
</tr>
<tr>
<td>Salinity (PSU)</td>
<td>15 to 180</td>
<td>15 to 35</td>
</tr>
<tr>
<td>Light intensity (lux)</td>
<td>(light is required for hatching)</td>
<td>2000</td>
</tr>
<tr>
<td>pH</td>
<td>5 to 10</td>
<td>8 to 9</td>
</tr>
</tbody>
</table>

*Dependent on strain, consult supplier for strain-specific optima.

_Artemia as food_

*Artemia* are non-selective filter feeders and as such have been cultured using a variety of live and inert foods ranging in particle size from <50 to 60 µm. Newly hatched *Artemia* nauplii (0.6 mm in length) have a high fat content (13 to 32 %) of the dry weight, with fat levels decreasing as development takes place [metanauplius stage (2.5 mm in length)=16.5 %, pre-adult stage= 7 %], while the protein content ranges from 42.5 % in newly hatched nauplii to 62.8 % in adult *Artemia* (Hoff & Snell, 1987). This information is critical in determining at what developmental stage the *Artemia* should be harvested, as young fish larvae require a diet that is high in fat, while older juvenile fish require more protein. Like rotifers, the nutritional value of *Artemia* can be enhanced by feeding them with live microalgae or by supplementation using commercially formulated enrichment products.

_Decapsulation_

Decapsulation is the removal of the hard, external shell of the *Artemia* cyst or chorion through chemical means. This is beneficial for a number of reasons; the outer shells are indigestible, and if ingested can cause obstruction, leading to death, the decapsulation process effectively disinfects the cysts, leaving them free from bacteria and pathogens, higher hatching success rates are achieved, the emerging nauplii have a higher nutritional value as energy expenditure is reduced when hatching, while also delivering a 100 % edible particle, regardless of hatching success.

*Artemia* cysts should be re-hydrated in freshwater prior to decapsulation at a concentration of 1 g cysts per 30 ml water (or <100 g.l⁻¹) at 25 °C for 60 to 90
minutes (hydration will take longer at lower temperatures). The re-hydration process should not exceed 120 minutes as this will result in a reduced hatch. Ideally, cysts should be re-hydrated in a conical flask, with good aeration in order to keep the cysts in suspension. Once re-hydrated, the cysts can be drained and rinsed on a 100 to 125 µm sieve. Ideally the decapsulation process should then be initiated immediately, but if refrigerated at 4 °C, the cysts can be stored for several hours.

Detailed decapsulation protocols can be found in Lavens & Sorgeloos, (1996) as well as Hoff & Snell (1987). It is also possible to purchase decapulated *Artemia* cysts but these will have a much shorter shelf life than unaltered cysts. *Artemia* cysts were not decapsulated for use in the present study as this was not done by Ayer et al. (2005) whose methodology for the culture of the rainbow smelt *Osmerus mordax* was being followed.

It should also be noted that noxious gases are released when the agents used to decapsulate the cysts are mixed and therefore appropriate risk assessments should be made in advance, and the correct PPE (personal protective equipment), such as breathing apparatus worn, or the work should be undertaken in a fume hood, outdoors or in an area with good ventilation. This is particularly important for female workers, as the gases can have deleterious effects on ovulation.

**Hatching *Artemia* cysts**

Optimal environmental conditions for hatching *Artemia* cysts can be found in Table 2.3. *Artemia* should be hatched in containers that allow good water circulation such as round bottomed flasks using natural seawater, or using synthetic seawater made from commercially formulated synthetic sea salts. The culture media should also be vigorously aerated to ensure that cysts are suspended and that the media is saturated with oxygen.

Most cyst producers recommend hatching cysts at a specific density in order to ensure optimal hatching rates, although a density of 5.5 g cysts.L⁻¹ yields consistently high hatch rates. Depending on the quality, size and weight of cysts, approximately 200,000 to 300,000 nauplii hatch from one gram of cysts. Hatching success can vary depending on the strain and the environmental conditions under which the cysts are hatched.
In order to determine the total number of *Artemia* are required to achieve specific stocking densities the following formula can be used:

\[
\text{Number of } \text{Artemia required} = \text{Target density } \text{Artemia.}\text{l}^{-1} \times \text{Total volume of culture vessels (l)}
\]

In order to determine the weight of cysts required to be hatched the following formula can be used:

\[
\text{Dry weight of cysts to hatch} = \text{Number of cysts.}\text{g}^{-1} \times \text{Hatching success(0.9)}
\]

(N.B. The approximate number of cysts.g\(^{-1}\) and the approximate hatching success should be available from the supplier or given on the product packaging).

The following protocol below details how *Artemia* cysts were hatched in the present study. Other hatching methodologies are described in detail in Hoff & Snell (1987) as well as Lavens & Sorgeloos (1996). N.B. It is advisable to handle and culture *Artemia* cysts away from microalgal and rotifer cultures as the cysts are easily air borne and can contaminate other cultures.

**PROTOCOL 3**

**Batch culture of brine shrimp *Artemia* spp:**

1) Purchase premium grade *Artemia* cysts and refrigerate prior to use.

2) Fill a 2 L glass, round-bottomed flasks (Figure 2.23.a.) with UV filtered seawater (35 PSU). N.B. Alternatively, synthetic sea salts can be added to dechlorinated tap water with similar results.

3) Place the flasks in a warm location (constant temperature of ~28 °C). Add vigorous aeration and the required quantity of cysts. Place cotton wool in the neck of the flask to prevent cysts from being lost and also to reduce the risk of contamination. Leave to hatch for 24 hours under constant illumination.

4) After 24 hours, stop the air flow and allow the cysts to float to the surface of each flask. Place a light source beneath the flask as newly hatched nauplii are attracted to light and will congregate at the bottom of the flask.
5) Siphon the nauplii into a plankton sieve (120 µm mesh), and rinse with filtered seawater.

6) Add the nauplii to a clean 2L glass, round bottomed flask (Figure 2.23.b.), fill with 35 PSU filtered seawater and apply heat and aeration as before. Add an *Artemia* enrichment product according to the manufacturer's instructions. The time taken for the *Artemia* to be fully enriched will vary between products.

7) Once enriched, stop the aeration and allow any remaining cysts to float to the surface and the nauplii to sink as before.

8) Once again, siphon the nauplii into a plankton sieve (120 µm mesh), and rinse with filtered seawater.

9) Add the nauplii to a container of a known volume (~ 2 L) and fill with filtered seawater (35 PSU).

10) Place this container on a bed of crushed ice within a polystyrene box. This ensures that the nauplii maintain a high nutritional profile throughout the day. Record the volume fed out to each larval rearing tank.

**Fig 2.23** (a) Aerated and illuminated, round bottomed *Artemia* hatching flasks (b) enrichment flasks sat on a light box.
Procedure for the determination of *Artemia* culture concentration using a Sedgewick Rafter counting cell.

Mix well the concentrated and now harvested *Artemia* culture and take a 10 ml sample.

1) Using a Pasteur pipette, add 1 ml of *Artemia* culture to a Sedgewick rafter counting cell.

2) In order to fix the sample, add 5 drops of Lugol’s iodine.

3) Under a compound microscope (x 40 objective) count the number of *Artemia* nauplii in your 1 ml sample. Repeat steps 1 to 3 a further two times and calculate the average number of nauplii among the three samples with a separate 1 ml sample for each count.

4) To determine the volume of *Artemia* culture to harvest as food for each individual larval rearing tank the following equation can be used:

\[
\text{volume of culture to harvest} = \frac{(\text{Artemia concentration required (ml}^{-1}) \times \text{tank volume (ml)})}{\text{observed Artemia concentration (ml}^{-1})}
\]

Once this figure is determined, divide it by the number of times per day that you intend to feed the *Artemia* to the tank (i.e. 4-5 times daily, minimum) to determine the volume to use for each meal.
References


Chapter 3  
Broodstock  
Site selection

In Wales European smelt (Figure 3.1) are found in the Rivers Dee and Conwy in the north. In the Conwy River, smelt are known to spawn in March/April in Llanrwst, approximately 13 miles from the river mouth, yet the spawning grounds on the Dee are unknown. Both smelt populations in Wales have been poorly studied, there is limited legislation in place to conserve them and they are thought to be small compared to others within the UK. Therefore, it was considered to be unwise to remove fish from these two rivers for spawning. A large smelt population exists in the river Thames although spawning runs are not closely monitored. Also, Thames smelt are not thought exhibit site fidelity when spawning although they are believed to spawn within a large area between Battersea Park and Wandsworth in March/April. Of the three Scottish smelt populations, the River Cree population has been particularly well studied and staff from the Galloway Fisheries Trust (GFT) are able to model the timing of the spawning run within a few days. The main spawning ground, known as Rag Run in Newton Stewart is approximately 100m either side of the A75 road bridge hence access to this site is good. Also GFT staff are involved in broodstock collection for restoration work consequently, *O. eperlanus* broodstock were collected from the River Cree, southwest Scotland.

![Fig 3.1](image.jpg) An adult European smelt from the River Cree, Scotland. Photo courtesy of the Galloway Fisheries Trust.
Timing of spawning

*Osmerus eperlanus* are known to spawn once river temperatures reach between 4 and 9 °C, with rising temperature being the main cue that triggers spawning. River Cree smelt make their spawning run in large shoals when water temperatures reach 5 °C, when tides are at their highest and there is a full or new moon. Water flows in the river also need to be low. Historical anecdotes state that the spawning run of smelt moving up the Conwy River was once visible as a wave of fish heading up river. The timing of spawning can be predicted by the build up of predators such as otters, herons and other piscivorous birds following it upstream. Spawning on the Cree lasts for approximately 1 week, yet spawning duration and timing may be affected by high water flow as smelt are known to be poor swimmers under conditions of elevated water flow. Storms, bright moonlight and human interference are also known to alter spawning runs.

Collection

When collecting fish as broodstock, it is advisable to collect them early on in the spawning run. From previous studies of smelt on the River Cree it was found that sex ratios change dramatically over time, with many females leaving the spawning grounds after spawning, resulting in an abundance of males. Prior to this there is an approximately equal ratio of males: females.
During the spawning run, smelt can be collected at night using long-handled dip nets with a 38 mm stretched mesh. This technique allows large numbers of fish to be collected over the short time period in which they spend within the spawning grounds. Alternatively, smelt can also be collected by setting Fyke traps or by angling during their spawning migration upstream. Anecdotal evidence suggests that adult smelt are sensitive to handling, resulting in scale loss, but we found no evidence of scale loss when handling adults.

Adult fish can then be transported back to the hatchery/laboratory in cool boxes. If the fish are to be transported over short distances, supplementary aeration is not necessary due to the cold water temperatures. As previously stated, smelt spawn in darkness. If the fish are close to spawning, such activity can be temporarily prevented by leaving the lid off the cool box, allowing light penetration.

### Protocol 4

**Gamete collection, fertilisation, removal of adhesiveness and disinfection:**

1) **Gamete collection**

Anaesthetise adult smelt in river water with the addition of either MS-222 (Tricaine Methane Sulphonate) at a concentration of 100 mg.l⁻¹, or 2-Phenoxethanol (2-PE; Phenoxythol) at 0.1 to 0.5 ml.l⁻¹. **N.B. If undertaken for scientific purposes rather than management or husbandry, such a procedure would be subject to legislation under the Animals (Scientific Procedures) Act 1986, and as such, training and licensing must be sought prior to undertaking this procedure.**

**Males**

- Anaesthetise the male fish.
- Weigh the male if required.
- Blot the ventral area of the fish dry with absorbent paper towel.
- Express the milt by applying gentle pressure to the ventral area by abdominal massage and collect the milt using a polypropylene transfer pipette.

- Transfer the milt to a 1.5 ml microcentrifuge tube and store on ice (~5 °C). Milt from 2 or 3 males can be stored in each tube.

- Use the milt within 30 minutes.

**Females**

- When sedated, weigh females prior to egg collection.

- Gently blot dry the area surrounding the genital area and anal fin using absorbent paper towel.

- Express the eggs by applying gentle pressure to the belly of the fish, slowly moving the region where pressure applied from the belly forwards to the tail and collect the eggs in a tared polystyrene weigh boat placed on a balance. Record the mass of the eggs. N.B. eggs do not stick to plastic weigh boats.

- Pool the eggs from several females, or keep them separately by family as required.

- Female fecundity is ~226 eggs.g$^{-1}$ body mass. For an average sized European smelt from the River Cree (175 g) this equates to a fecundity of ca. 39,500 eggs.

2) **Fertilisation**

- Activate the milt by adding to chilled (5 °C) river water. Activation can be verified by checking milt motility under a compound microscope. Milt can be pooled and activated in one batch or be kept in separate batches as required.

- Fertilise the eggs by adding 2-3 drops of milt (300-500 µl) and 25 ml freshwater to activate milt/fertilise the eggs in the weighing boat. **Tip:** The
eggs can also be fertilised by simply squirting milt from the male directly onto the activated eggs and then adding water.

- Swirl the gametes in a weigh boat for 3 minutes (Figure 3.4) before pouring into a glass jar containing 2 litres of freshwater to wash the eggs.

- Fertilisation success can vary between batches, but should be in the region of 60 to 99 %. Viable embryos appear translucent under a dissecting microscope, whilst non-viable eggs are opaque.

![Fig 3.2 Collection of milt from a male O. eperlanus by abdominal massage.](image1)

![Fig 3.3 Collection of eggs from a female O. eperlanus by abdominal massage.](image2)
Fig 3.4  Manual fertilisation of *O. eperlanus* eggs in a weighing boat.

Fig 3.5  Swirling technique used to remove the eggs adhesiveness and disinfect smelt eggs.
3) **Removal of adhesiveness and disinfection**

- Remove most of the water from the 2 L glass jar and transfer the fertilised eggs into a second glass jar containing a 150 mg.l\(^{-1}\) tannic acid solution. **Tip:** The eggs are extremely sticky at this stage and may stick to the glass when undertaking this procedure. To prevent this, slowly pour the eggs into the glass jar containing the tannic acid, while also gently swirling the tannic acid.

- Swirl the eggs for 10 minutes (Figure 3.5), keeping the eggs in suspension throughout. This process removes the adhesive outer layer from the eggs.

- Decant off the tannic acid solution and rinse the eggs twice with 1 litre of chilled water (5 °C).

- Eggs from multiple spawns can be pooled and incubated for 15 minutes prior to disinfection.

- Following de-adhesion, disinfect the eggs by adding 2000 µl.l\(^{-1}\) (active ingredient) hydrogen peroxide solution to the glass jar. Swirl the eggs every 30 seconds for 15 minutes, then rinse with 1 litre of freshwater (N.B. use chilled (4 °C) spring/mineral water, not river water to avoid contaminating the eggs).

**Note:** Low concentrations of calcium hypochlorite (25-75 mg.l\(^{-1}\)) or polyvinylpyrrolidone (25 mg.l\(^{-1}\)) can also be used to disinfect eggs without deleterious effects, however they are not as effective in preventing bacterial growth.

**Egg transport**

Eggs can then be incubated *in situ* or transported elsewhere for incubation. To transport the eggs, they should be kept in the glass jars in which they have been disinfected. Each jar can then be aerated during transport using a battery operated air pump or a similar device (Figure 3.6). In order to keep the eggs at a stable temperature (~5 °C) during transport, pack the jars on a bed of ice within a cool box. At 1 dpf eggs will measure ~1.5 mm in diameter.
Fig 3.6 Cooled glass jars containing smelt eggs shortly prior to transport. Each jar is aerated using a battery operated air pump.
References


Chapter 4

Egg Incubation

The egg stage

The egg and larval stages of fishes are the most sensitive of the teleost life cycle, where sub-lethal variations in environmental parameters may affect egg and larval physiology, impacting later growth and survival. By understanding the preferred environmental parameters of fish eggs and larvae, hatchery production can be optimised. Therefore, the methodology used to incubate marine fish eggs is critical in determining the success or failure of a batch of eggs. Fish eggs are more tolerant of mechanical shock and chemical changes than newly hatched larvae, as the protective shell surrounding the egg acts as an osmotic membrane, preventing rapid chemical changes within the egg.

Under optimum conditions egg survival during incubation and subsequent hatching success will be high, ensuring a maximum return in terms of the number of larvae hatched for the effort invested. Also, large larval hatchlings will be produced which is particularly important if incubating eggs for restocking or translocation purposes. If eggs are to be incubated in a hatchery prior to the translocation of larvae to rivers, the size of the larvae will be critical to the fate of the fish following release. Larval size is considered to be a good indicator of larval vigour, with larger larvae being less susceptible to predation and damage, better at capturing prey, less sensitive to starvation, and they should also have a greater swimming ability, enabling them to be retained in favourable nursery areas.

Egg fertilisation

After 1-2 dpf the egg fertilisation levels can be assessed by removing the eggs from the rearing vessel using a small (3 ml) pipette, placing them in a petri dish containing water from the rearing vessel. Fertilised eggs can easily be differentiated from unfertilised eggs at this stage by the presence or absence of the embryo (see Figure 4.1). The eggs can then be returned to the incubation vessel.
Protocol 5 (below) proved successful for incubating the eggs of *O. eperlanus* in a laboratory setting. However, the same techniques apply to those wanting culture this species on a commercial scale. Information gathered on the incubation requirements of *O. eperlanus* embryos is presented in Table 4.1.

**Table 4.1** A generalised set of conditions for incubating *Osmerus eperlanus* eggs.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
<th>Optima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>5-20</td>
<td>5-10</td>
</tr>
<tr>
<td>Salinity (PSU)</td>
<td>0-15</td>
<td>0-10</td>
</tr>
<tr>
<td>Photoperiod (light:dark, hours)</td>
<td>12:12</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.5-8.5</td>
<td>6.0-8.0</td>
</tr>
<tr>
<td>Dissolved O₂</td>
<td>&gt;20 % saturation</td>
<td></td>
</tr>
</tbody>
</table>

An experiment was run to determine the optimal environmental conditions (temperature and salinity) for incubating *O. eperlanus* embryos. Eggs were incubated in 1 L polypropylene beakers placed in heated or chilled water baths (Figure 4.2). Our study showed that the time taken to hatch was dependent on water temperature (see Table 4.2), with hatching duration reduced with increasing water temperature.
Salinity had a significant effect on hatching success (see Figure 4.3 (A)), which was poor (0 to 17 %) in eggs incubated at 20 PSU, irrespective of the temperatures investigated. Hatching success at 0 to 10 PSU ranged between 82 and 96 %.

Salinity and temperature were found to affect the length of hatchlings (see Figure 4.3 (B)), with the hatchlings from those eggs incubated at 5 °C and at 5, 10 and 15 PSU being significantly larger than those incubated under the other temperature and salinity combinations investigated. The size of smelt larvae at hatch ranged between 0.53 and 1.00 mm total length (TL). The greatest average TL of larvae was 0.85 mm in those embryos incubated at 5 °C and 5 PSU, ranging in size between 0.76 and 1.00 mm.

The survival of smelt larvae to first feed (see Figure 4.3 (C)) was significantly higher in larvae reared at 0 to 10 PSU than those at 15 PSU, while no larvae reared at 20 PSU survived to first feed. From these findings we were able to conclude that the optimum temperature and salinity combinations for incubating and rearing *O. eperlanus* eggs and larvae are 5 to 10 °C at 0, 5 or 10 PSU.

![Fig 4.2 A racking system used to incubate eggs under different temperature and salinity combinations.](image)
Fig 4.3 (A) The combined effect of temperature and salinity on the hatching success of *Osmerus eperlanus* embryos. (B) The combined effect of temperature and salinity on the mean size (total length) of *Osmerus eperlanus* larvae on hatching. (C) The combined effect of temperature and salinity on the survival of *Osmerus eperlanus* larvae to first feed. N.B. Data for all temperatures are slightly offset for clarity.
Table 4.2. The effect of water temperature on the time to first hatch and hatching duration on embryos of the European smelt *Osmerus eperlanus*.

<table>
<thead>
<tr>
<th>Target temperature (°C)</th>
<th>Mean recorded temperature (°C)</th>
<th>Days to first hatch (dpf)</th>
<th>Hatching duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5.1 ± 0.1</td>
<td>42</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10.9 ± 0.006</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>15</td>
<td>14.9 ± 0.08</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>18.3 ± 0.2</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

dpf, days post fertilisation

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Protocol 5

The incubation of *Osmerus eperlanus* embryos.

1) In a temperature-controlled room (~10 °C), transfer the smelt eggs to 3 litre round bottom flasks filled with 2 litres of water. In the absence of a temperature-controlled room, incubation flasks could be placed in a water bath and cooled using an aquarium chiller, or incubated outdoors, or in an outbuilding. The eggs should be incubated in water with salinity no greater than 10 PSU. This can be made up using de-chlorinated spring with the addition of synthetic sea salt to the desired salinity, or alternatively the eggs can successfully be incubated in freshwater.

2) When making up synthetic seawater, add 1 g of synthetic sea salt for every litre of water in order to achieve the desired salinity. Aerate vigorously until all the salt is dissolved.

3) The chlorine present within the tap water is highly toxic to most aquatic organisms and therefore it must be removed by chemical means. Dechlorinate the synthetic seawater using sodium thiosulphate (see chapter 2 for suggested concentration) or use bottled spring/mineral water although this can be expensive.

4) Place the container in the temperature controlled room and leave until the temperature matches that of the room.
5) Vigorously aerate the water in the incubation flask enough to keep the eggs in suspension. This can be done by attaching a glass tube to a compressed air supply, placing one end of the glass tube ~1 to 2 cm off the bottom of the flask (see Figure 4.4). Tilting the flask to one side at an angle of approximately 30° will improve water circulation within the flask (see Figure 4.4). Alternatively, a specialised fish egg incubator such as a McDonald hatching jar (Figure 4.5).

6) Place a rubber bung or cotton wool in the neck of the flask. This will reduce the risk of contamination.

7) Replace 90% of the water daily in order to eliminate the build up of nitrogenous compounds within the incubating flask. \( \text{NH}_4 \), \( \text{NO}_2 \) and \( \text{NO}_3 \) are all toxic to varying degrees and their concentrations should be maintained at or close to zero.

8) Remove a sub sample of approximately 30 eggs daily until hatch basis to monitor their development (see Figure 4.6). If possible, remove any opaque, dead eggs although this may not be practical if large numbers of eggs are incubated.

![Fig 4.4 Glass round bottomed flasks used to incubate smelt eggs.](image-url)
Fig 4.5 McDonald hatching jars used for incubating *Osmerus mordax* eggs at the University of New Hampshire. Note that high densities of eggs can be reared if good water quality is maintained. Photo courtesy of UNH staff.

Hatching success may vary between batches depending on egg and sperm quality, but under the conditions given above, hatching success should range between 80 - 100 %. Time to hatch is dependent on water temperature, but if incubated under optimal temperature regimes, this will take between 18 and 42 days. Figure 4.6 shows the embryonic development of *O. eperlanus* incubated at 10 °C. Hatching duration will also vary between 7 and 10 days accordingly. Water temperature can be manipulated by placing the incubation vessels in baths (Figure 4.2), with water temperature being maintained using aquarium grade chillers or heaters. Once hatched, the larvae gain nutrition from an external yolk sac which becomes diminished after approximately 8 days post hatching (dph) depending on temperature at which point the larvae are capable of hunting and consuming live prey items.
Fig 4.6 Development of laboratory incubated *O. eperlanus* embryos at ~10 °C. (A) 1 dpf. (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. Hatching took place at 18 dpf.
References


Chapter 5

Larval rearing

The larval stage is defined as the point from hatching to metamorphosis, the point at which the fish has developed the external morphological features and the same internal organs as adults of the species. Larval rearing is the most critical and time consuming phase of fish culture, with a fine line between success and failure.

Larval rearing tanks (experimental)

On hatching, *O. eperlanus* larvae (between 5 and 9 mm in total length) should be transferred to either glass or polypropylene tanks (25 l) at a stocking density of >100 l⁻¹. As mentioned in Chapter 4, once hatched, the larvae gain nutrition from an external yolk sac which becomes diminished after approximately 8 days post hatching (at ~ 10 °C) at which point the larvae are capable of hunting and consuming live feeds.

In order to avoid “wallowing”, where larvae are drawn to light or white objects, the sides of the larval rearing vessel should be dark in colour. This can be achieved by simply using a dark coloured polypropylene tank or by painting the exterior of a glass tank. Alternatively, sticky-backed coloured plastic sheeting can be applied to the exterior of glass tanks (see Figure 5.1a).

The water in the larval rearing tank (Figure 5.1b) should be lightly aerated not only to oxygenate the water, but in order to help keep the larvae and prey species in suspension. To do this, an air supply (air pipe) can be positioned in the centre of a tank floor and set to release approximately two air bubbles per second. The tank may not contain a filter, in which case the tank water is changed (20 to 50 %) daily, alternatively, an air-driven sponge filter can be added at one end of the tank, requiring 20 % daily water changes. In the second instance the filter sponge should be rinsed twice weekly using waste tank water in order to remove large organic waste particles. Using tap water containing chlorine and other chemicals to do this will harm the nitrifying bacteria on the sponge, reducing the capacity of the filter. The addition of such filters will help reduce the build up of harmful nitrogenous compounds.
Fig 5.1. (a) Blacked out 25 L glass larval rearing tanks in a temperature-controlled room. (b) Schematic drawing of a tank set up used for rearing larval *O. eperlanus*.

Smelt larvae can be reared under continuous illumination, or on a 12h light: 12h dark photoperiod at a light intensity of approximately 400 lux. To achieve this, ceiling-mounted fluorescent strip lights can be positioned above the larval rearing tanks.
Care should be taken to not place the lights too close to the surface of the water as heat emitted by the lights may heat the larval rearing tank water. Alternatively, LED lights can be used as these emit very little heat. While LED lights are expensive to purchase, they are cheaper to run than other lighting units.

In small systems such as the one described here, the larval rearing tank water temperature can be maintained by placing the tanks in a heated/chilled water bath depending on the ambient water temperature. Alternatively, the tanks can be maintained in a temperature-controlled room at approximately 10 °C. A generalised set of environmental conditions for rearing *O. eperlanus* larvae is given in Table 5.1.

<table>
<thead>
<tr>
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</thead>
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</tr>
<tr>
<td>Salinity (PSU)</td>
<td>0-15</td>
<td>0-10</td>
</tr>
<tr>
<td>Light intensity (lux)</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Photoperiod (light:dark, hours)</td>
<td>12:12 (minimum)</td>
<td>24:0 (maximum)</td>
</tr>
<tr>
<td>pH</td>
<td>5.5-8.5</td>
<td>7.3-7.8</td>
</tr>
</tbody>
</table>

### Larval development and husbandry

At the point of hatching (Figure 5.2a.), larvae are extremely delicate and great care should be taken to ensure that the larvae are handled in a delicate manner, whilst preventing the larvae from being exposed to air at any point. This can be done by carefully drawing up the larvae into a wide bored pipette (smallest internal diameter of 6 mm), or by collecting them in a small container such as a beaker or weigh boat. First feeding (with rotifers) is at 8 dph (Figure 5.2b.) by which point the yolk sac is largely exhausted with only the oil globule remaining while by 58 dph (Figure 5.2c.) the oil globule is absent. By 180 dph (Figure 5.2d.)
Fig 5.2 Development of laboratory reared \textit{O. eperlanus} larvae at \~{}10 °C. (a) 1 dph, 0.8 cm TL. (b) 8 dph, 0.86 cm TL. (c) 58 dph, 1.6 cm TL. (d) 180 dph, 4.3 cm TL.

The base of each rearing tank should also be siphoned daily in order to remove any faeces, dead larvae, or dead/decaying prey items using a pvc or silicone hose with an internal diameter of \~{}6 mm. When undertaking water changes, place a larger hose (\~{}10 mm internal diameter) inside a zooplankton sieve with a 150 µm mesh base (Figure 5.3). Once the siphon is started the sieve will prevent both fish larvae and prey species from being removed. In order to reduce any deleterious effect of rapid changes in water parameters or potential injury to the larvae as a result of turbulence, the clean top up water of the desired salinity should be siphoned into the tank. Top-up water should be the same temperature and salinity as the larval rearing tank water. To do this, prepare the water 24 h in advance and leave it in the temperature-controlled room until at ambient temperature. After the siphoning process, top up the larval rearing tank (see Figure 5.5) with live \textit{N. occulata} in order to maintain the target cell density.
**Fig 5.3** System used for siphoning water from larval rearing tanks.

**Fig 5.4** System used to top up larval rearing tanks with clean water.
Unlike the closely related rainbow smelt, *O. eperlanus* do not appear to tolerate salinities of 20 PSU or above soon after hatching, yet will thrive at a salinity of between 0 and 15 PSU. Between 130 and 140 dph gradually increase the salinity in larval rearing tanks (from 5 to 15 PSU) to 35 PSU. This can be done by increasing the salinity of the top-up water used in daily water changes. The following week, move the fish (using a hand net) to large (400 L) tanks on either a flow-through, semi-enclosed or recirculation system using natural seawater at 35 PSU. The flow rate in each tank should be approximately 6 l.min⁻¹.

By 180 dph (Figure 5.2d.) the smelt will be fully formed, resembling smaller versions of the adult fish. Once this stage is reached they can be considered and treated as juvenile fish (see Chapter 6).

**Husbandry routines for rearing *O. eperlanus* larvae**

**Daily:**
- Record water temperature.
- Carry out water change (see above).
- Siphon tank floor to remove waste.
- Record and remove dead larvae (if present).
- Feed a minimum of 5 x daily.

**Every 3 days:**
- Record NH₄, NO₂, NO₃, pH and salinity. These parameters can be measured using standard liquid test kit or test strips available from aquarium or aquaculture suppliers, although salinity should be measured using a refractometer (salinometer), again available from aquarium or aquaculture suppliers. Increase the percentage volume of water changed if NH₄ or NO₂ concentrations are >0.01 and 0.50 mg.l⁻¹ respectively, or if NO₃ concentrations are >30 mg.l⁻¹.

**Larval nutrition**

Newly hatched marine fish larvae lack the ability to effectively utilise commercially prepared diets, thought to be as a result of the low affinity of proteolytic enzymes in the digestive tracts of immature fishes for the proteins that are present in these diets,
effectively making the proteins indigestible. It is thought that prepared diets also lack certain enzymes, hormones or their regulators, or growth factors that are present in live feeds. Live feeds also contain thyroid hormones which play an important role in metamorphosis and growth.

Larval fish consume more food per unit body weight (50-300 %) than sub-adult fish (2-10 %), therefore, it is imperative that larval fish are fed frequently throughout the day (5 times per day, minimum).

**Feeding regime**

Throughout the entire culture process, it is essential that smelt larvae and juveniles receive a regular supply of appropriately-sized and nutritious food that promotes growth and healthy fish. Malnutrition will result in poor growth, aggressive behaviour towards tank mates and may ultimately lead to death. In the first instance most marine fish larvae should be fed on live feeds such as rotifers (see Figure 5.5a.), copepods and *Artemia* (see Figure 5.5b.) before being weaned to accept formulated diets by co-feeding and gradually reducing the availability of live prey species.

Optimal growth rates for larval smelt are achieved using commercial ‘crumb’ diets with a protein content of 50 %. The most common method of dispensing dry feed in aquaculture is by hand. While this is labour intensive, it can be advantageous as it allows the fish to be inspected regularly, especially if they feed on the surface. However, a variety of automatic feeders are widely available which continually deliver food to larval and grow-out tanks, meaning that optimal growth rates can be achieved. In our experience we found this to be the best method for feeding juvenile smelt but can only be used with diets above a certain size (≥600 µm) as smaller particles may remain adhered to the feeder as a result of static and/or the humidity of an aquarium.

To calculate the weight (g) of food to be fed each day the following equation can be used:

\[
\text{Average weight of fish (g)} \times \text{Number of fish} \times \text{\% body weight ration} \div 100
\]

- From 2 days post-hatching (dph) larvae should be offered marine rotifers *Brachionus plicatilis* that have been enriched for >8 h with either live, or concentrated *N. occulata*, or a commercial product (see chapter 2) A density of 10.ml\(^{-1}\) should be maintained. This can be done through daily enumeration
and supplementation in the rearing tank. In addition, supplement the tank with ‘green water’ (see Chapter 2) at a density of 200,000 cells.ml\(^{-1}\).

- **At 28 dph** enriched *Artemia* nauplii (Instar 1) should be added to the larval rearing vessel at a density of 5.ml\(^{-1}\) as well as enriched rotifers.

- **At 32 dph** discontinue feeding with rotifers and ‘green water’ supplementation.

- **At 60 dph** (Figure 5.2c.) the larvae should be offered a commercially prepared, granulated diet (100-200 µm) in addition to *Artemia*. Such diets are available from manufacturers such as Skretting and BERNAQUA. In our experience, fine, powdered diets stick to belt feeders therefore it should be fed by hand. **N.B.** These diets are powder-like, and tend to float on the surface of the rearing tank. To make it sink, first swirl the food in a water-filled container before adding to the larval rearing tank. Add close to the tank inflow pipe so that the food is dispersed throughout the water tank.

- **At 63 dph** discontinue feeding with *Artemia*.

- **At 114 dph** offer the larvae a 50: 50 mix of 100-200 µm & 200-300 µm diets. Feed at 3 % body weight (BW) per day.

- **149 dph**, discontinue feeding 100-200 µm diet and feed exclusively on 200-300 µm diet. Feed a minimum of 5 times daily by hand.
Large scale rearing of smelt larvae

Many of the principles given above for rearing smelt larvae under experimental conditions can be up scaled for larger larval rearing systems that are likely to be used in a commercial hatchery. The following draws on our own experience of rearing *O. eperlanus* under experimental conditions while incorporating published protocols developed for rearing larval rainbow smelt larvae on a large scale.

Larval smelt can successfully be reared in moderate/large scale (3,500 L) recirculation systems, in glass fibre or polyethylene tanks up to 700 litres in volume. Once again, the optimal environmental conditions for doing so are given in Table 5.1 above. Larval stocking densities should be in the region of between 10 and 100 fish/l, with a flow rate of between 1 and 2 l.min⁻¹. The larval rearing tank can be designed so that the water level in each tank can be adjusted using an external stand pipe, with water leaving each tank from a central standpipe. Outflow holes in the standpipe should be covered with a 500 µm mesh screen in order to prevent larvae from escaping. As standard, each tank should include an air diffuser, while standard recirculation filtration equipment such as biological, mechanical and chemical filtration equipment (for more details see Chapter 6, Nursery & grow-out)
should be incorporated. In large systems without filtration, between 25 and 50 % of
the tank water should be replaced daily. In such circumstances the importance of
good husbandry practices is increased.

When working at large volumes it is expensive to make up low salinity seawater
therefore the use of freshwater may be a cheap alternative to low salinity seawater
yet this is at the discretion of the user. However, if natural seawater is available then
low salinity seawater can be made up by adding this to dechlorinated tap water. The
salinity of the available estuarine or seawater will determine what volume will need to
be added to the tap water to achieve the target salinity.

From 2 dph, the same feeding regime as given above for experimental smelt larval
rearing systems should be followed. However, when working with larval rearing
tanks of a large volume, the provision of live microalgae as ‘green water’ is time
consuming, costly, labour intensive and requires a great deal of space, therefore it
can be beneficial to replace live microalgae with a concentrated *Nannochloropsis* sp.
(such as Instant Algae, Reed Mariculture) by adding 0.5 ml of concentrated algae to
each larval rearing tank (see manufacturers guidelines). Such products are readily
available from aquarium and aquaculture suppliers. Once again, the choice is at the
discretion of the user.
References


Chapter 6
Nursery & grow-out

Rearing systems
Juvenile and adult rearing tanks should consist of one or more large (400-700 l) glass fibre (see Figure 6.1) tanks plumbed into a flow-through, partial reuse (PRAS) or recirculating aquaculture system (RAS). Water circulation is optimal in round tanks, although square or rectangular tanks can also be used. In traditional flow-through aquaculture systems, water is passed through the culture tanks once and is then discharged. PRAS involves partial treatment of the waste water, allowing larger volumes of the water to be reused, while RAS maximise water re-use through simple waste water treatment technology. Detailed information on the design and benefits of all three systems is widely available elsewhere and therefore such details are not given here (see Lekang, 2007).

Fig 6.1 Three 400 litre glass fibre tanks attached to a partial reuse or recirculating aquaculture system (see Figure 6.2) used for rearing European smelt larvae and juveniles. Note the 24h belt feeders (black boxes with green lids) used to deliver the commercial diets to the tanks.
In this instance, a PRAS was used (Figure 6.2). Such systems may include mechanical filtration (i.e. vortex (or swirl)/filter socks/brushes) for removing particulate matter, a biological filtration (i.e. bio tower) for removing NH$_4$ and NO$_2$, chemical filtration such as a protein skimmer (foam fractionater) for removing organic compounds (including proteins and amino acids) before they break down into nitrogenous compounds as well as ultra violet (UV) filters which kill bacteria, viruses and micro-organisms. In the PRAS system used for smelt, seawater from an external reservoir entered the sump tank of the PRAS at a rate of 2 l.min$^{-1}$.

**Fig 6.2** A standard seawater filtration unit used for a partial reuse or recirculating aquaculture system.

Figures 6.1 and 6.3 show the tanks used to rear *O. eperlanus*. Water can be circulated around the tank using an inflow pipe positioned close to the surface of the water. Water should enter each rearing tank at a rate of approximately 6 l.min$^{-1}$,
while waste water should leave the tank via a surface skimmer. A mesh screen (2 mm diameter) is fitted around the surface skimmer in order to prevent fish from escaping. Smelt require highly oxygenated water therefore an air stone should be added to each rearing tank in order to provide supplementary aeration.

**Fig 6.3** The interior of a 400 litre glass fibre tank used to rear and maintain *O. eperlanus*.

**Juvenile and adult husbandry**

The husbandry requirements for juvenile and adult smelt (Figure 6.4) are similar to those of the husbandry of the latter larval stages. Table 5.1 (Chapter 5) gives a generalised set of tolerable and optimal environmental conditions for rearing juvenile smelt.
Fig 6.4  (a) 407 dph juvenile *O. eperlanus* (~7 cm FL). (b) 513 dph *O. eperlanus* (~13 cm FL) in a 400 L rearing tank.
Husbandry routines for rearing *O. eperlanus* juveniles and adults

Daily:

- Record water temperature.
- Siphon tank floor (Figure 6.5).
- Record and remove any dead juveniles (if present).
- Ensure that automatic feeding devices are clean and topped up.
- Clean mesh screen around surface skimmer.

Weekly:

- Record NH₄, NO₂, NO₃, pH and salinity. Increase seawater inflow rate if NH₄ or NO₂ concentrations are >0.01 and >0.50 mg.l⁻¹ respectively, or if NO₃ concentrations are >30 mg.l⁻¹.
- Clean skimmate (foam/waste water) from protein skimmer collection cup.

Twice weekly:

- Remove and spray clean mechanical filter bags under a freshwater hose. These are cylindrical polypropylene bags through which all tank water is passed in order to filter out solid waste. If the bags continually clog, replace them (if not, replace them every 6 weeks).

Monthly:

- Clean the filter baskets located in the circulation pumps.

6 months-annually:

- Remove and replace UV lamps.
Feeding regime

As for smelt larvae, the highest growth rates for juvenile and adult smelt are achieved using diets with a protein content of 50% or above. In the present study, juvenile European smelt were initially fed a commercially-formulated trout diet, whilst during the latter stages a commercially-formulated sea bass diet was introduced. From this point on, the juvenile fish should be fed using automated feeders such as belt feeders (see Figures 6.1 and 6.5) while in this instance, smelt were also fed by hand once a day. Due to the small size of the diets, the feed can stick to belt feeders, especially in a humid environment such as an aquarium. In order to prevent this, it is advisable to place the food on the feeder in single heaped line (see Figure 6.5), rather than spreading it out across the belt. The automatic feed should be situated above, or to one side of the tank, ideally positioned close to the seawater inflow pipe so that once the food is dispensed from the feeder it is quickly dispersed throughout the tank. If optimal growth rates are required it is advantageous to feed the fish using a belt feeder (or similar automated feeder) as soon as possible.
Fig 6.6 Length-Weight relationship for laboratory-reared juvenile *O. eperlanus* maintained under ambient seawater temperatures (9-15 °C).

It is far easier to visually estimate the length of a fish than it is its weight. Using the data presented in Figure 6.6 it is possible to accurately estimate the weight of smelt of a given size. This information can then be used to calculate the daily feeding ration to be offered.

- At 187 dph, juvenile smelt should be fed a formulated diet 300-500 µm in size.

- At 208 dph, start weaning the fish onto a diet with a larger crumb size by feeding a 50: 50 mix of 300-500 µm and 0.6-1.0 mm diet [for example Skretting Nutra Plus 01 (57 % protein)].

- At 213 dph, discontinue feeding the 300-500 µm diet and feed exclusively on a diet 0.6-1.0 mm in size. At this stage the diet should be large enough not to stick to a belt feeder.
- At 307 dph, wean the fish onto a larger crumb by feeding a 50: 50 mix of 0.6-1.0 mm and 1.0-1.7 mm diet [for example Skretting Nutra Plus 02 (53 % protein)].

- Discontinue feeding the 0.6-1.0 mm crumb at 423 dph.

- At 443 dph, the fish can be weaned onto small (1.0-1.5 mm) pellets using the same weaning methods as above [for example Skretting labrax 2 (54 % protein)].

**Fig 6.6** An internal view of a mechanical belt feeder used for feeding smelt larvae, juveniles and adults. Note how the feeder is positioned above the tank inflow pipe. Note heaps of food.

**Grading**

The regular grading (or sorting by size) of fish helps to prevent small individuals from being outcompeted, ensuring a more uniform size range within the culture tanks. A uniformity in the size of fish is beneficial in many ways. Firstly, it will help prevent
cannibalism which has been found to be a problem in cultured post-metamorphic rainbow smelt juveniles. For a cannibalistic act to take place, the smaller fish will need to be less than half the size (length) of the larger fish therefore, grading should be undertaken frequently to ensure a uniform size of fish in a given tank. However, cannibalism has not been recorded in the culture of *O. eperlanus* to date. Secondly, grading promotes growth by preventing competition between small fish and their larger and more dominant kin. Furthermore, if fish in a tank are approximately equal in size then it is easier to accurately determine the total weight of the fish, and therefore it is easier to determine the amount of food to be fed to them in terms of percentage body weight. In addition, this removes the need to mix food of different sizes.

In small experimental systems, such as the 400 L tanks used here, grading can be undertaken with the use of a hand net, simply sorting fish of different sizes by eye. However, this may stress the fish therefore the process should be undertaken quickly, yet as delicately and efficiently as possible in order to reduce excessive stress. An appropriately-sized hand net with a soft mesh should be used in order to avoid physically damaging the fish as scale loss or damage to the mucus membrane surrounding the fish can lead to secondary infection. In large commercial scale systems where fish are maintained in tanks >2000 L, seine nets may used to corale fish into an area of the tank where they can then be easily caught in a hand net and passed through a commercial grader.

**Anaesthesia**

From time to time it may be necessary in experimental research to anaesthetise or sedate fish in order to undertake certain scientific procedures, or to measure the average fish size for husbandry purposes to determine the correct quantity of food to be fed. In such circumstances, anaesthesia or sedation can be used to prevent pain, prevent or reduce stress, or calm a fish which might otherwise struggle and injure itself.

Anaesthesia (general anaesthesia) is a reversible, generalised loss of sensory perception accompanied by a sleep-like state induced through drugs or physical means. Sedation is defined as a calming effect and is considered to be a preliminary state of anaesthesia which involves some loss of sensory perception. Several behavioural stages and planes that occur during anaesthesia in fish are given in Table 5.1. (from Betts & Dolan, 2011).

In fish, a state of anaesthesia is typically induced chemically by inhalation/immersion in an anaesthetic solution which is taken up across the fish’s gills. The anaesthetic dose is calculated according to the volume of water in which the fish is to be held.
during the procedure. The quantity of anaesthetic required may vary according to the fish and its condition as well as other factors such as species, size, lipid content, sex, spawning phase, health and condition, developmental stage as well as water temperature. Therefore, if no anaesthetic data is available, trial runs are recommended when using a novel anaesthetic or data for related species/taxa can be used.

2-phenoxethanol (2-PE; Phenoxythol) is an oily liquid that is moderately water soluble and therefore requires vigorous mixing with water prior to its use. This can be done simply by stirring the anaesthetic in water. It also has bactericidal and fungicidal properties which make it a useful anaesthetic to use for surgical procedures. Adult *O. eperlanus* can be successfully anaesthetised using 2-phenoxethanol at a concentration of 0.1-0.5 ml.l$^{-1}$ (as determined for use on carp). However, our experience showed that the use of this anaesthetic was occasionally found to be lethal to larval and juvenile European smelt.

MS-222 (Tricaine methanesulphonate; 3-aminobenzoic acid ethyl ester) is a white powdery anaesthetic that readily dissolves in water. However, when added to freshwater in particular it has a tendency to reduce pH and therefore the treatment water should be buffered with Sodium bicarbonate (NaHCO$_3$) at a concentration of 200 mg.l$^{-1}$. Studies suggest that salmonids should be anaesthetised with MS-222 at a concentration of 50-100 mg.l$^{-1}$. Our own observations showed that a concentration of 75 mg.l$^{-1}$ was sufficient to anaesthetise juvenile *O. eperlanus*. This was our preferred anaesthetic for juvenile *O. eperlanus*. For more information on the use of MS-222 see Topic Popovic et al. (2012).

**N.B.** Smelt should be anaesthetised with extreme caution. Our observations showed that juvenile *O. eperlanus* are extremely susceptible from what we believe to be confinement stress. Therefore, we recommend returning fish to their rearing tanks as soon as possible after they have recovered from anaesthesia.

Clove oil is a natural anaesthetic that is widely used in fisheries management, hatcheries and research facilities yet its improper use can affect the viability and physiology of the fish, leading to death. To date, we have not used clove oil to anaesthetise *O. eperlanus*. For more information on the use of clove oil as an anaesthetic see Javahery et al. (2012).
**Table 5.1** Classification of the behavioural changes that occur in fishes during anaesthesia (from Betts & Dolan, 2011).

<table>
<thead>
<tr>
<th>Level of anaesthesia</th>
<th>Behavioural responses</th>
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<tr>
<td>**Stage</td>
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<td>0</td>
<td>Normal</td>
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**N.B.** In order to anaesthetise fish a certain degree of training is advisable and may be a legal requirement in the country where the work is undertaken. The purpose of the work may determine whether licensing is required. In the UK, if the work is to be undertaken for science then this will fall under the Animals (Scientific Procedures) Act 1986 therefore a license will be required, but the controls of the act do not extend to procedures undertaken for recognised culture or animal husbandry practice. **It is the reader’s responsibility to ensure that their work is conducted within the legal framework for their country.** For a full methodology of how to anaesthetise fish see Ross & Ross (2008). Some of the equipment required to anaesthetise fish is shown in Figures 6.7 and 6.8.

Following anaesthesia, fish are carefully transferred to vigorously-aerated anaesthetic-free water (see Figure 6.9), which allows the anaesthetic to be cleared from the fish by the gills. If deemed necessary, the recovery can be assisted by passing a stream of aerated water across the gills, by gently pumping water into the fishes mouth using a soft plastic pipette, or by flexible outlet hose from a small submersible pump such as a powerhead. Before returning the fish to its tank it should be allowed to recover fully, maintaining normal swimming behaviour. This prevents the fish being attacked from others if swimming abnormally on its return. However, in our experience confinement (and stress) can lead to mortality so we try to handle/anaesthetise our fish as little as possible.
Fig 6.7 Equipment used to anaesthetise, weigh and measure smelt.

Fig 6.8 A juvenile smelt in an anaesthetic (MS-222) batch. Note the small water volume and also that no supplemental aeration is used.
Fig 6.9 A juvenile smelt in a recovery bath following anaesthesia. Note the increased water volume and aeration provided by a battery powered air pump.

Breeding in captivity

To date, we have not attempted to spawn our laboratory-reared European smelt although we aim to hold these fish through to maturity at which point we will attempt to hand-strip them. While information is sparse, we understand from a 2011 article in Hatchery International (July-August) that researchers at IMARES (Institute for Marine Resources and Ecosystem Studies) in the Netherlands were recently able to spawn wild caught smelt from Ijsselmeer Lake in the laboratory by manipulating light, water temperature and water flow in their tanks.
References


Appendix

Appendix 1. Schematic drawing of algal culture vessels. N.B. Not to scale.

Microalgal starter cultures are used to inoculate progressively larger culture volumes. The components required for upscaling microalgal cultures are given above along with standard microalgal culture set ups for culture vessels of increasing volume.
Appendix 2. Schematic drawing of a rotifer culture vessel.

Rotifers can be successfully cultured in a variety of different culture vessels ranging in volume from 2 litre soft drink bottles to large open topped tanks in excess of 1,000 litres in volume depending on your requirements. Appendix 2 above shows a 70 litre (working volume) culture vessel used to culture rotifers for rearing smelt larvae. Aeration is provided by an externally located air compressor attached to an aquarium grade silicone air line with an attached air stone. For ease of maintenance and in order to keep the culture clean, a drainage tap was fitted to the base of the culture vessel, while a flocculation trap was suspended in the culture water. The flocculation trap should be cleaned regularly (twice weekly). Waste material will collect in the bottom of the culture vessel, forming a dark green or brown coloured sludge. In order to reduce the risk of the build up of ciliates that benefit form the presence of this waste material, the drainage tap should be briefly opened (for 1 to 2 seconds) on a daily basis in order to flush the sludge from the culture.
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