CHAPTER 6 – THE PROPAGATION AND CULTURE
OF FRESHWATER MUSSELS

6.1 Introduction

In the previous chapters, it has been shown that mussels play important roles in rivers, and have potential uses in biomanipulation and drinking water clarification. If mussels are to be used in biomanipulations or industrially, very large numbers of mussels will be needed (in the millions). Such large numbers of mussels could not be sustainably harvested from existing populations in rivers or lakes in the UK. Therefore the only way to obtain such numbers is through the artificial propagation and culture of mussels.

Freshwater mussel culture is still in its early stages; relatively few studies have been done, and these have had variable success (Table 6.1). The earliest attempts to culture freshwater mussels took place at the beginning of the twentieth century; these early attempts hoped to provide a renewable source of mussel shells for the button industry (Coker et al., 1919-1920; Pritchard, 2001). More recent attempts have focused on rearing juveniles as part of conservation programmes for threatened species of mussel (e.g. Buddensiek, 1995; Jones and Neves, 2002; Araujo et al., 2003). Because of the high sensitivity of juvenile mussels to a number of contaminants, mussels have also been reared for use in toxicity testing (Warren et al., 1995). The most successful of these studies have reared several thousand juveniles (R. Neves, pers. comm.), suggesting that the large-scale culture of freshwater mussels is possible.

Most attempts to rear juveniles have focused on North American species (Table 6.1), with only two studies on European species, and both these have worked with Margaritifera spp. (Buddensiek, 1995; Araujo et al., 2003). This study represents the first attempt to rear four species of European unionid: A. anatina, A. cygnea, P. complanata and U. pictorum. The rearing of P. complanata is of particular importance because this species is currently listed as ‘near-threatened’ in the Red Data Book (IUCN, 2003). The culture of P. complanata juveniles may offer both the possibility of reintroducing this species to sites from which it has been lost, and the opportunity to study its juvenile habitat requirements in order to restore its natural habitat in rivers.
Table 6.1. Previous attempts at rearing juvenile freshwater mussels, showing the species which have been cultured, how long the juveniles were reared for, their survival and length, and the conditions under which they were cultured. *The survival and length of juveniles are given for the maximum juvenile age recorded in these studies; age is taken to mean time from excystment, and survival is measured as the proportion of juvenile mussels excysting from fish which survived through to this age. ‘n.d.’ = no data.

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Location</th>
<th>Age (days)</th>
<th>Survival*</th>
<th>Length (mm)*</th>
<th>No. produced</th>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coker et al., 1919-20</td>
<td>a number of different species</td>
<td>Fairport, Iowa</td>
<td>&gt; 730 (&gt; 2 years)</td>
<td>varying with species</td>
<td>91</td>
<td>several thousand</td>
<td>Various, including floating crates in a river and pond, bottom crates, a pen of galvanized netting and in concrete and earth ponds.</td>
</tr>
<tr>
<td>Hudson and Isom, 1984</td>
<td>Eukrohnia imbricata, Epiphragma triqueta</td>
<td>South Carolina</td>
<td>74</td>
<td>n.d.</td>
<td>3.1</td>
<td>n.d.</td>
<td>In 250ml beakers or 1.5 litre containers, using river water changed daily, with and without sift and fed a variety of algal species</td>
</tr>
<tr>
<td>Buddensiek, 1995</td>
<td>Margaritifera margaritifera</td>
<td>northern German rivers</td>
<td>1600</td>
<td>&lt;5%</td>
<td>3.6</td>
<td>9</td>
<td>Small cages placed in rivers, with gauze mesh to prevent juveniles escaping</td>
</tr>
<tr>
<td>Gatenby et al., 1996</td>
<td>Villona iris, Pygamonon grandis</td>
<td>Copper Creek, Virginia, Clayton Lake, Virginia</td>
<td>272/195</td>
<td>5/13%</td>
<td>4.5</td>
<td>n.d.</td>
<td>Initially in aerated 175ml glass culture dishes with fine sediment, fed Chlorella vulgaris, Ankistrodesmus falcatus and Chlamydomonas reinhardtii; later in aerated 4 litre tanks with sediment and internal circulation, with the same food</td>
</tr>
<tr>
<td>Gatenby et al., 1997</td>
<td>Villona iris</td>
<td>Clinch River, Virginia</td>
<td>140</td>
<td>30%</td>
<td>1.7</td>
<td>n.d.</td>
<td>Aerated glass dishes with fine river sediment, fed Sestodinella plathabeldae, Bracteacoccus grandis and Phaeodactylum tricornutum</td>
</tr>
<tr>
<td>O’Beirn et al., 1998</td>
<td>Villona iris, Lampsilis fasciola</td>
<td>Upper Tennessee River System</td>
<td>273</td>
<td>23%</td>
<td>5.7</td>
<td>n.d.</td>
<td>In recirculating system (approx. 400 litres capacity), with juveniles in petri dishes with fine sediment</td>
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<tr>
<td>Starkey et al., 2000</td>
<td>Ectobacula umbrella</td>
<td>Sheepdred Branch Creek, South Carolina</td>
<td>117</td>
<td>7%</td>
<td>18.9</td>
<td>7</td>
<td>A partitioned aquaculture system containing several algal species including Chlorella, Ankistrodesmus, Coelastrum and Scenedesmus</td>
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<tr>
<td>Henley et al., 2001</td>
<td>Lampsis fasciola</td>
<td>Tennessee</td>
<td>112</td>
<td>29%</td>
<td>1.62</td>
<td>n.d.</td>
<td>A mini-recirculating unit (volume eight litres), fed Scenedesmus</td>
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<tr>
<td>Eads and Layzer, 2002</td>
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<td>Tennessee</td>
<td>578 (19 months)</td>
<td>48%</td>
<td>17.4</td>
<td>n.d.</td>
<td>An indoor recirculating system, fed with Bracteacoccus grandis and Ankistrodesmus sp., with and without sediment</td>
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<td>Cystoseirina stegaria</td>
<td>Clinch River, Tennessee</td>
<td>28</td>
<td>37%</td>
<td>0.31</td>
<td>7.92</td>
<td>Non-recirculating tank with sand or fine sediment and fed Scenedesmus quadricauda</td>
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<td>Aragno et al., 2003</td>
<td>Margaritifera auricularia</td>
<td>Canal Imperial, Zaragoza, Spain</td>
<td>35</td>
<td>11%</td>
<td>0.325</td>
<td>13</td>
<td>In recirculating tanks containing well water and green water, sediment, soil and vegetation from the Ebro River; juveniles were suspended in containers with gauze-mesh bottoms with fine sediment</td>
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</tbody>
</table>

6.1.1 Freshwater mussel reproduction and juvenile mussel ecology

Freshwater mussels have an unusual life cycle: their larvae, called glochidia, are parasites of fish, attaching to their fins, scales or gills (Kat, 1984). They remain encysted on the fish for a number of days or weeks, during which time the glochidia metamorphose into juvenile mussels. After excystment from the fish, juveniles fall onto the sediment where they start to grow. In this way freshwater mussels are dispersed away from the parent mussels.

Juvenile mussels are rarely found in the field (Isely, 1911; Coker et al., 1919-20; Neves and Widlak, 1987; pers. obs.), and consequently little is known about their ecology. The lack of juveniles seen in the field may result from their occupying different habitats from adult mussels, as found by Isely (1911) and Neves and Widlak (1987). Other possible reasons for the difficulty in locating juveniles are that recruitment is sporadic and only occurs in particularly ‘good’ years (Kat, 1982); that the distribution of juveniles is highly clumped (Coker et al., 1919-20; Neves and Widlak, 1987); and that juveniles suffer very high mortality, due to their falling into unfavourable habitat and being predated by
organisms such as flatworms and fish (Coker et al., 1919-1920; Young and Williams, 1984; Neves and Widlak, 1987).

When young juveniles excyst from the fish, they do not yet have the adult form, and lack siphons, labial palps, outer gills and sex glands (Coker et al., 1919-20). They burrow below the sediment surface, are more mobile than adult mussels, and may have a byssal thread which they use to attach to pebbles (Isely, 1911; Coker et al., 1919-20; Yeager et al., 1994). These differences in the morphology and behaviour of juvenile and adult mussels suggest that the mode of life of juveniles is very different from that of adults.

6.1.2 Propagation and culture of mussels in the laboratory

In order to artificially propagate freshwater mussels, fish may either be artificially infected with glochidia, or in vitro transformation of glochidia may be used (Lefevre and Curtis, 1912; Isom and Hudson, 1982). Transformation of glochidia on fish may take between six days and several weeks depending on mussel species. Most mussel species are host-fish specific, so that transformation will only be completed successfully if the host fish species are known (Coker et al., 1919-1920). Infecting fish is the most reliable way of producing juveniles, and has been used in most previous attempts to rear them (e.g. Coker et al., 1919-1920; Buddensiek, 1995; Gatenby et al., 1996 and 1997; Eads and Layzer, 2002; Araujo et al., 2003). In vitro transformation has also been successful with a number of mussel species (e.g. Isom and Hudson, 1982; Keller and Zam, 1990; Dimock, 2000); however, some species fail to complete metamorphosis when propagated in this way (R. Neves, pers. comm.).

Following propagation of juvenile mussels from glochidia, the juveniles must be reared, and this has proved to be the most difficult stage in the production of freshwater mussels. The success of previous rearing attempts has been highly variable (Table 6.1). This may have been caused by differences between species: Coker et al. (1919-1920) note that while some species were easy to rear under all conditions tested (e.g. the Lake Peppin Mucket, Lampsilis siliquoidea), others could only be reared successfully under certain conditions, and no success at all was had with some species. A variety of apparatus has been used to rear the juveniles (Table 6.1), and some groups have succeeded in rearing mussels through to a reproductive age (J. Layzer, pers. comm.). However, most studies have reared juveniles for less than a year, and few juveniles have reached more than 10mm in length (Table 6.1).
The variable success in these attempts can be attributed to our lack of knowledge of the habitat requirements of juvenile freshwater mussels (as described above). Most culture attempts have been forced to rely largely on trial and error to achieve suitable rearing conditions for mussels. The main factors believed to be important for the successful rearing of juvenile mussels are sediment, flow and food (Hudson and Isom, 1984; Gatenby et al., 1997; O’Beirn et al., 1998; Henley et al., 2001). These factors are described in more detail below.

6.1.3 Requirements to rear juveniles in the laboratory

Sediment

The importance of sediment to juvenile mussel survival is much debated: suggestions include that it may offer food (in the form of organic matter or microorganisms) (Yeager et al., 1994); it may provide stability and help juveniles to orientate themselves properly for feeding (O’Beirn et al., 1998); and it may aid digestion by helping to grind open algal cells (Gatenby et al., 1996).

Hudson and Isom (1984) found that the addition of silt enhanced survival and growth of juveniles, and the need of juveniles for sediment has been shown in subsequent studies (Gatenby et al., 1996 & 1997; O’Beirn et al., 1998). The depth of sediment and sediment type may also be important (Hudson and Isom, 1984; Warren et al., 1995; Jones and Neves, 2002). The depth of sediment required is unknown: Hudson and Isom (1984) report no difference in growth between juveniles provided with a minimal amount of silt (provided as a cloudy silt suspension) and those provided with a 1cm layer of silt. However Yeager et al. (1994) report that Villosa iris juveniles preferred to bury in the sediment so that they were not exposed to the overlying water. The type of sediment can also affect juvenile survival: Jones and Neves (2002) found higher survival of Cyprogenia stegaria in fine sediment (<105μm) than in sand (300-500μm). Lastly, sediment may have contaminants attached to particles which can cause juvenile mortality (shown by Warren et al., 1995, in the field) so the “pollution history” of sediment is also important.

Flow

The flow requirements of juveniles may vary by species: O’Beirn et al. (1998) and Henley et al. (2001) found that a flow-through system gave good survival and growth of juveniles of Villosa iris, Elliptio complanata, and Lamspsilis fasciola, while Hudson and Isom (1984), Gatenby et al. (1996 & 1997), Dimock (2000) and Jones and Neves (2002)
all used a static system, again with good survival and growth of juveniles of their respective species: *Anodonta imbecilis*, *Villosa iris* and *Pyganodon grandis*, *Pyganodon cataracta*, and *Cyprogenia stegaria*. The variation in flow requirements is likely to reflect differences in the habitats of adult mussels; however it is possible that the habitat requirements of juveniles may differ from those of adults, as discussed earlier.

**Food**

The diet of adult freshwater mussels is poorly known. It has generally been assumed that adult mussels feed primarily on phytoplankton, in the same way as marine bivalves (Ziuganov *et al.*, 1994; Gosling, 2003). However Nichols and Garling (2000) showed that freshwater mussels gained most of their carbon from bacterial sources. Most culture studies to date have fed juvenile mussels a suspension of algae (Hudson and Isom, 1984; Yeager *et al.*, 1994; Gatenby *et al.*, 1996 & 1997, O’Beirn *et al.*, 1998; Dimock, 2000; Henley *et al.*, 2001; Jones and Neves, 2002). Gatenby *et al.* (1996 & 1997) looked at a variety of foods for juveniles, including various algal combinations, bacteria, and sediment. They found that sediment alone supported *V. iris* juveniles, but the addition of algae increased their survival and growth; there was no enhancement of growth when bacteria were added to the diet. However a different study by Yeager *et al.* (1994) found that three to five-day-old *V. iris* juveniles contained flagellated bacteria in their guts, with smaller quantities of algal cells. Therefore it is likely that juveniles gain their nutrition from both small algal cells and bacteria.

### 6.1.4 Aims

This study aims to rear juveniles of four British freshwater mussel species, *A. anatina*, *A. cygnea*, *P. complanata* and *U. pictorum*, for the first time. No data are available on the habitat requirements of juveniles of any of these species; therefore the guiding principles in these rearing attempts have been to follow the techniques used in previous culture attempts with other species (Table 6.1), and to provide a wide variety of conditions for juveniles in the hope that at least some of these will support juvenile survival and growth.
6.2 Methods

6.2.1 Production of juveniles

Gravid female mussels of the species *A. anatina*, *P. complanata* and *U. pictorum* were taken from the R. Great Ouse at Smithey Fen (grid reference: TL448720), and gravid females of *A. cygnea* were taken from the Cambridge University Botanic Gardens pond (grid reference: TL453571) (Figure 6.1). Mussels were collected during their gravid periods, which were January to March for the *Anodonta* spp.; April to June for *Unio pictorum*; and September to April for *Pseudanodonta complanata* (Aldridge, 1999; McIvor, 1999).

The 3-spined stickleback *Gasterosteus aculeatus* is known to be a suitable host-fish for the glochidia of these mussel species (Berrie and Boize, 1985; Aldridge, 1997). 3-spined stickleback were caught from local streams using hand-nets. Fish were taken from streams where freshwater mussels were not present, to ensure that fish had no previous exposure to mussel glochidia. Only fish between 5cm and 8cm in length were kept for use in experiments. The fish were held for several days prior to infection with glochidia to make sure they were healthy, and to adapt them to living in tanks and eating the dried bloodworms which formed their staple diet during the infection.

Mussels and fish were kept in a constant temperature room. The temperature was kept close to river temperature for the time of year, and a 12 hour light/dark cycle was maintained throughout the experiments. Cambridge tap water was used in all tanks; it was dechlorinated by aeration for 24 hours prior to use, and contained 285mg/l calcium carbonate (Cambridge Water Company, 2004).

Fish were infected with *A. anatina*, *A. cygnea* and *P. complanata* glochidia on 10 January 2002, and with *U. pictorum* glochidia on 31 May and 1 June 2002. For *A. anatina*, *A. cygnea* and *U. pictorum*, the mussels and fish were placed together in tanks, and the temperature was raised by approximately 2°C. This caused the mussels to release glochidia naturally on mucus threads, and the fish became infected after one or two days (Figure 6.2).

For *P. complanata*, glochidia were removed from the gills of gravid mussels by making a small slit in the ventral margin of the gill and injecting water into the anterior part of the gill. The water coming out of the slit in the gill contained suspended glochidia, and these were collected in a beaker. Glochidial viability was confirmed by adding saturated salt solution to a sub-sample of these glochidia: >95% of glochidia should close their valves.
Figure 6.1. Collection sites for gravid mussels and release sites for juveniles. **Left:** the River Great Ouse at Smithey Fen (where *A. anatina, P. complanata* and *U. pictorum* were collected). **Right:** the Cambridge University Botanic Gardens pond (where *A. cygnea* was collected).

Figure 6.2. *U. pictorum* releasing glochidia on mucus threads into the water column. **Inset:** infected 3-spined stickleback, with glochidia visible on fins and scales.
(Weaver et al., 1991; Watters and O’Dee, 1998). Fish were then placed in a suspension of the glochidia for a few seconds until they became infected. The desired infection rate was approximately 15 to 20 glochidia per fish. This procedure was carried out under Home Office project licence no. PPL 80/1444.

The infected fish were placed in aquaria with aeration and daily feeds, and were held there until all juveniles had excysted. The bottoms of all aquaria were siphoned daily to pick up all particulate matter from the floor of the tanks. Additionally all aquaria were given an 80% water change once a week. The siphonate from the floor of the tanks was passed through a fine mesh to strain out the juvenile mussels; the mesh size used was 300μm for A. anatina, A. cygnea and P. complanata, and 150μm for U. pictorum. A black mesh made juveniles easier to see. Under a dissecting microscope, all glochidia/juveniles were picked off the mesh individually (using a syringe tip and scalpel blade to pick them up) and placed in a dish of water. Live juveniles kept their valves shut, except when using their foot to move around the dish; the shells of dead juveniles gaped open.

6.2.2 Rearing the juveniles

6.2.2.1 A. anatina, A. cygnea and P. complanata (January 2002)

Between 10 and 30 juveniles were placed either in pyramidal mesh bags (mesh size: 140μm; side length: 6cm) or in plastic trays (3.7 by 5.7 by 2.1cm) in the following six treatments (shown in Figures 6.3a-d and 6.4):

1) a 25 litre flow-through tank (Figures 6.3a and 6.4a). Flow was approximately three litres/minute. Juveniles were placed in mesh bags.
2) as in 1), except a thin layer of sediment was placed on the bottom of the tank.
3) as in 1), except that juvenile mussels were placed in plastic trays.
4) a 5 litre tank with a recirculating aerator (Figures 6.3b and 6.4b), with juveniles in trays.
5) a 5 litre tank with aeration only (Figure 6.3c), with juveniles in trays.
6) in mesh bags in the river/pond where the parent mussels had been taken from (Figures 6.1 and 6.3d). Juveniles were placed in mesh bags, and three mesh bags were placed in each wire cage (Figure 6.3d). Wire cages were attached to the bank with string and anchored in the sediment with 15cm long metal pegs. The mesh bags were hung on threads within the cages to keep them above the sediment surface.
The numbers of juveniles placed into each bag or tray and the number of replicate bags and trays placed in each treatment are shown in Table 6.2. Some sieved sediment (particle size < 300μm) was also placed into each bag or tray; approximately 1mm$^3$ of sediment was placed in the mesh bags, and sediment was placed in trays to a depth of approximately 2mm. This sediment had been taken from the river at Smithey Fen (where the parent mussels had come from), and was heated to 80°C to kill any organisms that might prey on the juvenile mussels before use. The mesh bags were labelled with combinations of coloured beads and the trays were labelled with a waterproof marker pen.

![Diagram](image.png)

**Figure 6.3.** Tanks, containers and cages into which juvenile mussels were placed. For the *Anodonta* spp. and *P. complanata*, the apparatus shown in a) – d) was used. For the later rearing attempts with *U. pictorum* juveniles, a), c) and e) were used.

- a) a 25 litre flow-through tank: water is pumped from a large tank into a smaller tank suspended in the large tank. The water flows back into the large tank through small holes in the base of the small tank. Juveniles were placed in the small tank either in plastic trays, pyramidal mesh bags or larger glass dishes as shown; further details are given in the text.
- b) a 5 litre recirculating tank, driven by the aerator, with juveniles in trays.
- c) a 5 litre aerated tank, with juveniles in trays.
- d) juveniles placed in mesh bags in cages in the river/pond from which the parent mussels had been taken (R. Great Ouse at Smithey Fen for *A. anatina* and *P. complanata*, the Botanic Gardens pond for *A. cygnea*).
- e) a 15 litre stream-flow tank, with water pumped from a sump, flowing down by gravity and returning to the sump, and juveniles kept in trays.
Figure 6.4. Tanks where juvenile mussels were placed. (a) 25-litre flow through tank. (b) five litre recirculating tank driven by the aerator.

In the laboratory treatments (1 – 5), all tanks were kept well-oxygenated using airstones. Juveniles were fed every 3 to 4 days with a suspension of the marine alga *Nannochloropsis* (supplied in concentrated form by Reed Mariculture Inc., San Jose, California), to an initial concentration of approximately 500,000 cells/ml, which is equivalent to a chlorophyll *a* concentration of approximately 20µg/l. The mean cell size of *Nannochloropsis* was 2µm. Although *Nannochloropsis* is a marine alga, very little salt remains once the concentrate has been diluted (R. Reed, pers. comm.). Tanks were cleaned and had a complete water change approximately once every month.

For *A. anatina* and *A. cygnea* held indoors, juveniles were monitored after nine and approximately 40 days; juveniles placed outdoors were monitored after nine, 40 and 120 days. *P. complanata* juveniles were monitored after approximately 20 and 80 - 100 days (both indoor and outdoor treatments).

After nine or 20 days (depending on species), one mesh bag or tray from each of the indoor treatments was checked to monitor the survival and growth of juveniles; for outdoor treatments, three mesh bags were examined at each time for *A. anatina* and *A. cygnea*. Juveniles were found by wet-sieving the sediment from the mesh bag or tray through a 300µm steel sieve. The particles retained in the sieve were then washed onto a piece of black mesh with a similar mesh size, and the juvenile mussels were picked off individually using a syringe tip and scalpel blade under a dissecting microscope. All live juveniles and dead juvenile shells were counted, and live juveniles were measured using an eyepiece graticule under the dissecting microscope.
After approximately 40 or 80 - 100 days (Anodonta spp. and P. complanata respectively), all remaining containers were checked (except some outdoor ones). The juveniles were counted and measured in the same way as above, and then juveniles were pooled and placed in larger glass dishes (10cm in diameter and 5cm deep) with more sediment (1cm depth). These dishes were placed in the 25 litre flow through tanks used in treatments 1, 2 and 3. The juveniles in these glass dishes were monitored at approximately 120, 180, 250 and 390 days post-excystment.

### 6.2.2.2 U. pictorum (June 2002)

Based on the results from the earlier study using the Anodonta spp. and P. complanata, juveniles of U. pictorum were placed in some new treatments, including a new tank set-up and some new variations of the treatments used previously. Mesh bags were not used with U. pictorum juveniles, and no juveniles were placed outdoors.

As the juveniles excysted from the fish, they were divided into groups of ten, and these were placed in plastic trays with approximately 2mm sediment. The sediment was sieved to only include particles less than 200μm in diameter, and was heated to 80°C prior to use. The trays were divided between a number of different experiments as described below:

### Table 6.2. The number of juveniles placed into each treatment, separated by species. Dates vary according to the date juveniles excysted from fish. The first number given is the number of juveniles placed in that treatment on that date; these juveniles were divided evenly between the number of replicate mesh bags/trays shown in parentheses. (FT tank = flow-through tank.)

<table>
<thead>
<tr>
<th>Date</th>
<th>A. anatina</th>
<th>A. cygnea</th>
</tr>
</thead>
<tbody>
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<td>25/2/02</td>
<td>3 (1)</td>
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</tr>
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<table>
<thead>
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</table>

*On some days it was impossible to pick out all the juveniles individually, so juveniles were placed in larger glass dishes in a different 25L FT tank.
A. different tank set-ups: a 25 litre flow-through tank (12 trays; Figures 6.3a and 6.4a); a 15 litre stream-flow tank (seven trays; Figure 6.3e) and a five litre aerated tank (four trays; Figure 6.3c). The number of juveniles placed in each tank on each day are shown in Table 6.2. Flow through the 25 litre tank and the 15 litre tank was approximately three litres/minute.

B. with different depths of sediment in the trays: the sediment was 1, 2, 4 or 8mm deep (one tray per depth), placed in the 25 litre flow-through tank.

C. in different-depth trays: three normal trays (height 21mm) and three shallower trays (height 7mm), all with 2mm depth of sediment, placed in the 25 litre flow-through tank.

D. with different levels of disturbance: juveniles which had been checked once after nine days were checked again after 40 days, and their survival and growth after 40 days was compared to that of juveniles that had been left undisturbed until their 40-day check. Four disturbed trays were compared with six undisturbed trays.

All tanks were aerated and juveniles were fed *Nannochloropsis* concentrate as described above. Tanks were cleaned and given a 100% water change approximately once a month. Juveniles from the different tank set-ups (experiment A above) were checked after nine days (one tray from each treatment) and approximately 40 days for survival and growth. Juveniles from trays with different sediment depths, and different-sized trays (B and C) were checked after 40 days only. After 40 days, all juveniles were pooled into larger glass dishes (10cm in diameter, 5cm deep with 1cm depth of sediment) and placed in the 25 litre flow-through tanks. They were then checked at 66, 102, 176 and 274 days post-excystment.

### 6.3 Results

#### 6.3.1 Production of juveniles

Between 252 and 854 juveniles of the different mussel species were produced by infecting fish (Figure 6.5 and Table 6.3). The infection rate of fish varied between eight and 34 glochidia per fish, and fish survival was highest at lower temperatures (Table 6.3). The period of glochidial encystment varied with species: *U. pictorum* remained on the fish for the shortest time (24 days), while *P. complanata* remained on the fish for longest (53 days; Table 6.3). Between 58% and 89% of the glochidia that had attached to fish excysted successfully as live juveniles (Table 6.3).
Figure 6.5. The time that glochidia of different mussel species spent encysted on fish.

<table>
<thead>
<tr>
<th>date of infection</th>
<th>10/1/02</th>
<th>10/1/02</th>
<th>10/1/02</th>
<th>31/5/2002 to 1/6/2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>mussel species</td>
<td>A. anatina</td>
<td>A. cygnea</td>
<td>P. complanata</td>
<td>U. pictorum</td>
</tr>
<tr>
<td>number of fish</td>
<td>29</td>
<td>30</td>
<td>22</td>
<td>141</td>
</tr>
<tr>
<td>(Gasterosteus aculeatus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fish survival</td>
<td>86%</td>
<td>93%</td>
<td>91%</td>
<td>67%</td>
</tr>
<tr>
<td>approximate number of glochidia per fish</td>
<td>26</td>
<td>34</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>temperature range (°C)</td>
<td>11</td>
<td>11</td>
<td>11 to 15</td>
<td>15</td>
</tr>
<tr>
<td>median day of excystment</td>
<td>30</td>
<td>34</td>
<td>53</td>
<td>24</td>
</tr>
<tr>
<td>days between which 80% of juveniles excysted from fish</td>
<td>28 to 34</td>
<td>31 to 36</td>
<td>48 to 59</td>
<td>21 to 26</td>
</tr>
<tr>
<td>proportion of encysted glochidia which successfully metamorphosed into juveniles</td>
<td>80%</td>
<td>85%</td>
<td>58%</td>
<td>89%</td>
</tr>
<tr>
<td>number of live juveniles produced</td>
<td>599</td>
<td>854</td>
<td>252</td>
<td>703</td>
</tr>
</tbody>
</table>

Table 6.3. Fish infection data, showing the number of juveniles produced.

6.3.2 Survival and growth of juvenile mussels

6.3.2.1 A. anatina

The mean length of newly excysted juveniles was 339 µm (S.D. = 10.8, n = 22), and the juveniles had not grown during their time encysted on the fish (mean length of glochidia = 344 µm, S.D. = 17.9, n = 22; Mann-Whitney U test comparing glochidia and juvenile lengths: W = 558, p > 0.05).
Juveniles were pale brown in colour (Figure 6.6). Gill bars were just visible through the juvenile shell, and the paired adductor mussels could be seen at either end of the dorsal margin of the shell (i.e. the hinge) (Figure 6.6). In older juveniles, new shell growth was white, and the early juvenile shell continued to be visible amidst the new shell growth for over a year (Figure 6.6 and 6.7). At nine and 40 days, juvenile shells were round, but by 120 days post-excystment, juveniles had the appearance of young adult mussels (Figures 6.6 and 6.7). The digestive gland and gut were visible at 40 days; by 120 days, mussels had a fully formed siphon (Figure 6.7). Young juveniles (0 to 40 days post-excystment) rapidly burrowed below the surface of the sediment in which they were placed, and could not be seen once buried. 120-day old juveniles burrowed until they were positioned just below the surface of the sediment, with their siphons visible from above.

During the first nine days after excystment, there was high mortality in all treatments except in the five litre aerated tank (Figure 6.8). Between nine and 40 days, mortality was again high in all treatments except among juveniles held in trays in the 25 litre flow-through tank. By 120 days, most juveniles in this treatment had also died, but those juveniles which had been transferred after 40 days to larger glass dishes with deeper sediment had high survival (almost 100%). This cohort continued to show high survival until they were 250 days old (Figure 6.9). Following this (during the winter) there was higher mortality. For juveniles held in the most successful treatments (i.e. in trays in a 25 litre flow-through tank up to 40 days, and then transferred to larger glass dishes with more sediment in the same tank), survival was approximately 51% up to 250 days, and 21% at 398 days (where survival is measured as the proportion of juveniles excysting from fish that survived to this age). No juveniles survived in the outdoor mesh bags to 120 days.

By nine days after excystment, *A. anatina* juveniles from indoor treatments had reached a mean length of 476µm (S.D. = 43.8µm, n = 29; Figures 6.6 and 6.8). There was no difference between juvenile lengths in the different indoor treatments (ANOVA, $F_{(4,28)} = 0.74$, $p > 0.5$), but juveniles in indoor treatments had grown significantly more than those juveniles placed in the river (mean length of river juveniles: 363µm, S.D. = 7.56µm, n = 7; $t$-test comparing the lengths of indoor versus outdoor juveniles: $t = 14.4$, d.f. = 38, $p < 0.001$) (Figure 6.8).
Figure 6.6. *Anodonta* species juveniles; there was no discernable difference between *A. anatina* and *A. cygnea* juveniles, so the two species are presented together here. (a) – (b) newly excysted juveniles with foot, lengths approximate 330μm; (b) pictures of a juvenile taken at 5 second intervals, showing movement across the dish; (c) – (f) 9-day old juveniles, showing variable shell growth, lengths 340 to 540μm; (d) preferred orientation of juveniles for moving, (e) new shell has grown inside of glochidial hooks (length of juvenile is 630μm); (f) some juveniles were covered in black spots, but seemed otherwise healthy (juvenile length is 510μm).
Figure 6.7. Older *Anodonta* spp. juveniles: (a) – (d) 40-day old juveniles, showing early juvenile shell (e.j.s.), new shell growth (n.s.g.), digestive gland (d.g.), gut (g.), gill bars (g.b.), and foot (f.) used to move across dish; (a) and (b) 1110μm in length; (c) and (d) length 930μm; (e) and (f) 120-day old juveniles, showing gut, foot, and siphon (s.); (e) lengths 3.10 – 6.32mm; (f) moving across dish, length 4.62mm; (g) 398-day old *A. anatina* juveniles, lengths 13.4mm and 9.12mm; (h) 382-day old *A. cygnea*, length 12.4mm.
Figure 6.8. Survival and growth of *A. anatina* (a & b), *A. cygnea* (c & d) and *P. complanata* (e & f) juveniles in different tanks and containers up to 120 days after excystment. Note the logarithmic scale on the y-axis of (b) and (d). In (b) and (d), error bars have been omitted for juveniles at 9 and 40 days, as they are impossible to distinguish (see text for a statistical comparison of treatments). The dotted line shows the survival and growth of juveniles pooled from all other containers after 40 days. The survival and growth of these pooled juveniles is continued in Figures 6.9 and 6.10. Key to legend: FT = flow-through.
Figure 6.9. Survival of the different juvenile mussel species with age. The data shown here are from the treatments with the highest juvenile survival.

After 40 days, the largest juveniles had doubled in length, and these juveniles were found in the mesh bags in the 25 litre flow-through tank (treatment 1). Again, all juveniles held in indoor treatments were significantly larger than juveniles placed in the river, and additionally, juveniles held in mesh bags in the 25 litre flow-through tank with mud (treatment 2) were larger than those in trays in the 25 litre flow-through tank with mud (treatment 3) (ANOVA, $F_{(4,36)} = 6.8$, $p < 0.001$; significant differences between treatments were identified using Tukey’s pairwise comparisons, $p < 0.05$).

Juveniles held in trays in the 25 litre flow-through tanks had poor growth between 40 and 120 days, but those which had been transferred to larger glass dishes with deeper sediment continued to grow until the autumn, when the temperature was lowered in line with outdoor temperatures and growth decreased (Figure 6.10). Juveniles reached a maximum length of 14.1mm by 398 days post-excystment, representing a 43-fold increase in length (mean length = 11.2mm, S.D. = 1.7mm, n = 10).
Figure 6.10. The growth of juveniles of the different mussel species in the most successful treatments (i.e. in trays in the 25 litre flow through tank up to 40 days, and then in glass dishes with deeper sediment in the same tank). The temperature at which juveniles were held is also shown.

6.3.2.2 A. cygnea

The mean length of newly excysted juveniles was 326μm (S.D. = 24.1, n = 12), and they had not grown during their time encysted on the fish (mean length of glochidia = 342μm, S.D. = 11.1, n = 21; Mann-Whitney U test comparing glochidia and juvenile lengths: \( W = 403, p > 0.05 \)). Juveniles had pale brown shells (Figure 6.6), and were identical in appearance and burrowing behaviour to A. anatina juveniles at all ages; Figures 6.7g and 6.7h show the two species at a similar age (380-400 days post-excystment).

A. cygnea juveniles suffered high mortality in all treatments indoors and outdoors at nine and 40 days post-excystment, with the exception of juveniles placed in trays in the 25 litre flow-through tank, which showed very low initial mortality (<15% at 40 days) (Figure 6.8). As with the A. anatina juveniles, there was then high mortality in these trays (98%), but those juveniles which had been transferred to deeper sediment in larger glass dishes had 47% survival at 179 days (Figure 6.9). After 179 days, mortality increased, and by 382 days there were 19% surviving (Figure 6.9). No juveniles survived in the outdoor mesh bags to 120 days.

Nine days after excystment, juveniles held indoors had reached a mean length of 441μm (S.D. = 38.0μm, n = 43); the juveniles placed outdoors were smaller than juveniles
in the 25 litre flow-through tank in mesh bags and in trays (mean length of outdoor juveniles = 373\(\mu\)m, S.D. = 101\(\mu\)m, n = 3; ANOVA, \(F_{(5,40)} = 3.62, p < 0.01\); significant differences between treatments were identified using Tukey’s pairwise comparisons, \(p < 0.05\)) (Figure 6.8). After 40 days, the juveniles grown in trays in the 25 litre flow-through tank were significantly larger than those in all other treatments (with the exception of those in the 5 litre recirculated tanks, where only two juveniles survived) (ANOVA, \(F_{(4,40)} = 10.36, p < 0.001\); significant differences between treatments were identified using Tukey’s pairwise comparisons, \(p < 0.05\); Figure 6.8).

By 120 days, the juveniles held in trays in the 25 litre flow-through tank had shown little growth (reaching 800\(\mu\)m in length), but those juveniles that had been transferred to larger glass dishes grew to 4.5mm in the same time period. These juveniles continued to grow, reaching 11.6mm after 382 days (S.D. = 1.92mm, n = 7), with the largest being 13.7mm in length (Figures 6.8 and 6.10).

6.3.2.3  *P. complanata*

The mean length of newly excysted juveniles was 350\(\mu\)m (S.D. = 24.7, n = 25), and they had not grown during their time encysted on the fish (mean length of glochidia = 361\(\mu\)m, S.D. = 14.5, n = 20; Mann-Whitney U test comparing glochidia and juvenile lengths: \(W = 504, p > 0.05\)). Juveniles had white shells, and were more rounded in appearance, making them distinguishable from *Anodonta* spp. juveniles (Figure 6.11). One newly excysted juvenile had byssus-like threads extending from the mid-ventral margin, slightly anterior to the glochidial hook (Figure 6.11a and b).

Approximately 20 days post-excystment, survival was less than 50% in all treatments except the five litre recirculating tank (Figure 6.8). By 100 days, there were only three surviving juveniles (two from the cages outdoors, and one from the trays in the 25 litre flow-through tank). These juveniles were not refound, and presumably died.

After approximately 20 days, there were some differences in growth between *P. complanata* juveniles in different tanks and containers (ANOVA: \(F_{(5,49)} = 5.13, p = 0.001\); Figure 6.8); juveniles grown in mesh bags in the 25 litre flow-through tank were larger than those in the same tank with extra sediment, those in the aerated tank and those in the river (significant differences between treatments were identified using Tukey’s pairwise comparisons, \(p < 0.05\)). After 80-100 days, the two juveniles that survived in the outdoor cages were both 540\(\mu\)m in length, and the single survivor from a tray in the 25 litre flow-through tank had reached 740\(\mu\)m.
Figure 6.11. Juvenile *P. complanata*: (a) newly excysted juveniles with white shells and 351μm in length; one of these juveniles has byssus-like threads, which are shown more clearly in (b); (c) and (d) 20-day old juveniles, showing shell growth (original glochidial shell-shape is clearly visible), movement of the foot and gill bars (visible in (d)); the juvenile in (c) is 540μm in length, and the juvenile in (d) is 495μm long; (e) and (f) a 100-day old juvenile showing shell growth and movements of the foot; the juvenile is 740μm long. Gills bars are just visible in (e).
6.3.2.4 *U. pictorum*

*U. pictorum* juveniles were smaller than those of the other species (mean length 213μm, S.D. = 11.4, n = 23), and had white rounded shells (Figure 6.12). As with the other species, juveniles did not grow during their time on fish (mean length of glochidia = 208μm, S.D. = 8.97, n = 26; Mann-Whitney U test comparing glochidia and juvenile lengths: $W = 584, p > 0.05$).

After nine days, survival was high in all tanks (Figure 6.13); after approximately 40 days, survival had decreased and was highly variable between trays, but there was no significant difference between survival in the different tanks (Kruskal-Wallis test: $H = 3.98$, d.f. = 2, $p = 0.14$; Figure 6.13). Mortality remained high thereafter, with juveniles all placed in larger glass dishes in the 25 litre flow-through tank, and by 274 days post-excytment there were only eight surviving individuals out of the original 700 (1.6% survival; Figure 6.9).

After nine days, juveniles from the 25 litre flow-through tank were slightly larger than juveniles from other tanks; by 40 days, these juveniles from the 25 litre tank were significantly larger than those in the 15 litre stream-flow tank (ANOVA on tray means of juvenile lengths: $F_{(2,16)} = 6.61, p < 0.01$; significant differences between treatments were identified using Tukey’s pairwise comparisons, $p < 0.05$; Figure 6.14). Juveniles continued to grow slowly after 40 days when they were pooled into larger glass containers, reaching a maximum length of 900μm after 274 days, with a mean length of 778μm (S.D. = 108μm, n = 8; Figures 6.12 and 6.15). Figure 6.10 compares the growth of *U. pictorum* juveniles with the growth of the other mussel species.

The depth of sediment into which juveniles were placed had no effect on juvenile survival or growth after 40 days (survival: $\chi^2 = 3.3$, d.f. = 3, $p > 0.25$; growth: ANOVA: $F_{(3,33)} = 0.53, p > 0.5$). Likewise tray depth had no effect on survival or growth of 40-day old juveniles (survival: analysis of deviance using the F-test (Crawley, 2002): $F = 0.21$, d.f. = 4, $p > 0.5$; growth: ANOVA with individual trays nested within treatment (tray depth): $F_{(1,27)} = 2.20, p > 0.2$). Survival and growth of juveniles was also similar in disturbed and undisturbed trays; i.e. there was no difference in survival or growth of juveniles that had been disturbed by removing them from the sediment after nine days, and those which were looked at for the first time after 40 days (survival: analysis of deviance using the F-test: $F = 0.64$, d.f. = 12, $p > 0.4$; growth: ANOVA with individual trays nested within treatment (disturbed or undisturbed): $F_{(1,57)} = 0.15, p > 0.5$).
Figure 6.12. Juvenile *U. pictorum*: (a) newly excysted juveniles, 215 μm in length; (b) newly excysted juveniles showing movements of the foot; (c) 40-day old juveniles, shell lengths between 290 and 330 μm, showing shell growth (original glochidial shell-shape is clearly visible) and movement across the dish with the foot; (d) 100-day old juvenile, shell length 600 μm, showing shell growth and movement with the foot; (e) a 274-day old juvenile, 650 μm in length.
Figure 6.13. Survival of *U. pictorum* juveniles in different tanks. Only one tray in each tank was checked at nine days. The points showing survival at 40 days have been staggered slightly so that the error bars can be seen. Standard deviations are calculated from ten trays (25 litre flow through tank), five trays (15 litre stream flow tank) and three trays (5 litre tank).

Figure 6.14. Growth of *U. pictorum* juveniles in different tanks up to 40 days post-excytment. The data points have been staggered slightly so that error bars are visible.
6.4 Discussion

Four species of freshwater mussel have been successfully reared for the first time. Two of these species, *A. anatina* and *A. cygnea*, were reared for over a year, with relatively high survival (20%) and growth (reaching >10mm length) after approximately 380 days. The two remaining species, *U. pictorum* and *P. complanata*, were also reared for long periods (274 days and 100 days respectively), although these species showed lower survival and growth. The survival of the *Anodonta* species is higher than in most previous culture attempts (Figure 6.16), and only three previous studies have reared juveniles for longer (Table 6.1). Their growth also compares favourably with the growth of other species of juvenile mussels reared in previous studies (Figure 6.17).

6.4.1 The conditions required to rear juveniles

The apparatus and conditions used in this study to rear juveniles were relatively similar to those used in previous studies (Table 6.1). Comparing the survival and growth of juveniles in flowing and static tanks, juveniles of all species faired better in the flow-through tanks. Most other recent attempts have also preferred to use recirculating tanks.
Figure 6.16. A comparison of the survival of juveniles in this study (open symbols) with the survival of juveniles of other species in previous studies (closed symbols, with each point representing a different study). The data from which these points have been plotted is given in Table 6.1. A log-scale has been used for “age” in order to include the previous studies in which juveniles survived for a number of years; consequently survival of juveniles from this study is shown from 9 days post-excystment.

Figure 6.17. Comparing the maximum lengths of juvenile mussels from this study (open symbols) with those of other species from previous studies (closed symbols; Table 6.1 summarizes the growth data from these previous studies).
(Table 6.1), although some species have done well in static tanks (e.g. *Anodonta imbecillis* and *Epioblasma triquetra*, cultured by Hudson and Isom, 1984).

Juveniles of both *Anodonta* spp. had higher survival in trays than in mesh bags. The trays contained a deeper layer of sediment than the mesh bags and this may have benefited the juveniles (although sediment was also placed in the mesh bags, much of it washed out through the mesh); alternatively the mesh bags may have limited the circulation of water over the juveniles, reducing the supply of oxygen and food particles. The low survival in mesh bags may also explain the low survival outdoors; it was not possible to place juveniles in trays outdoors because the juveniles would have been lost.

The algal species *Nannochloropsis* has not been fed to freshwater mussels in previous studies (presumably because it is a marine alga), but may have aided survival because of its high levels of essential fatty acids compared to other algal species (37% dry weight; Reed, 2004); essential fatty acids have been shown to enhance the growth of cultured oysters (Gosling, 2003). Although the sediment was heated to 80ºC to kill predators such as flatworms (Zimmerman and Neves, 2003), some bacteria will have survived; if juveniles feed on bacteria, this may also have facilitated high survival and growth (although Gatenby *et al.*, 1996, found no increase in growth for *V. iris* and *P. grandis* fed bacteria alongside algae). Additional factors which may have contributed to the success of these rearing attempts include the use of sediment from the parent mussel sites (which must have supported the growth of juvenile mussels in the past), and the use of calcium-rich water, with calcium levels being kept high by changing the water in the juvenile tanks on a regular basis.

### 6.4.2 Differences in survival and growth between species

There are many possible reasons for the lower survival and growth of *U. pictorum* and *P. complanata* in this study. Juveniles of these two species may have different habitat requirements from the *Anodonta* species, and the conditions provided here may have catered better for the needs of the *Anodonta* species. Alternatively, *P. complanata* and *U. pictorum* juveniles may have naturally lower survival and slower growth than the *Anodonta* spp., in line with other factors such as differences in breeding time, numbers of glochidia produced and infection rates of fish.

The lower survival of *P. complanata* may have been caused by unsuitable rearing conditions. *P. complanata* juveniles had similar growth both indoors and outdoors, even though temperatures were lower outdoors so that outdoor juveniles would have been
expected to grow more slowly. This implies that some basic requirement was missing for
the juveniles grown indoors. *P. complanata* is the only species used in this study which is
not generalist in its use of habitat; the other three species are widely distributed throughout
both small and large rivers and lakes (Kerney, 1999). *P. complanata* is found mostly in
larger rivers in the UK (McIvor, 1999; Müller, 1999), and is generally rare, with a few
dense populations in the lower tidal freshwater stretches of rivers (Müller, 1999). It may be
necessary to provide tanks with higher flows to rear *P. complanata*, to simulate the flow
conditions seen in these larger rivers. Further work is needed to test different conditions to
rear this species indoors, including different flow conditions and different foods.

*U. pictorum* excysted from fish later in the season, so only had three to four months
to grow before temperatures started to decrease in September/October. *Anodonta* juveniles
which excysted in February had seven to eight months to grow before temperatures
decreased in September/October. Therefore the growth of *Anodonta* species in the first year
is expected to be greater than that of *U. pictorum*. This is supported by Negus (1966), who
measured the mass of different aged mussels (where age was estimated from the number of
growth rings on the shell), and found that *A. anatina* in their second year weighed 1.2g,
while the same age *U. pictorum* weighed only 0.33g. If *U. pictorum* overwinters as small
juveniles, it may experience higher mortality than the larger *Anodonta* spp. juveniles:
Buddensiek (1995) found that only the larger *M. margaritifera* juveniles survived their first
winter. *U. pictorum* may make up for this lower survival by producing more glochidia
(Bauer, 1994).

With the *U. pictorum* juveniles, some additional hypotheses were tested, with
regard to the role of sediment depth, tray depth and disturbance of juveniles. None of these
had any effect on juvenile growth and survival. However this may be because sample sizes
were too small, or because some other factor was more limiting, so that the effects of the
factors tested were not apparent. Again further work is required to test whether the low
survival and growth of *U. pictorum* reflects naturally lower growth and survival, or whether
the conditions provided to rear *U. pictorum* were sub-optimal.

6.4.3 The levels of replication in these experiments

It should be noted that there are many possible levels of replication in these
experiments, from the different juveniles held in each mesh bag or tray, to the different
bags and trays within each tank, to the different tanks. Where possible in these
experiments, replication was at the level of different containers (trays/mesh bags). However
there has been little replication at the level of tanks, because it was not known which tanks would support juvenile survival and growth, and therefore it was considered more important to place juveniles in many different tanks rather than in many tank replicates. This has resulted in some pseudoreplication (as described by Hurllbert, 1984). This is a common problem for experiments in aquaculture (Smart et al., 1997 and 2001; Riley and Edwards, 1998), and many previous freshwater mussel culture experiments have suffered from the same low levels of replication (e.g. O’Beirn et al., 1998; Jones and Neves, 2002). In future studies, replication at the level of tanks or recirculating systems should be attempted where possible.

6.4.4 The feasibility of rearing juvenile mussels as biofilters

These first attempts at rearing British freshwater mussels suggest that it would be possible to rear large numbers of the *Anodonta* species for use as biofilters. The culture techniques used here supported high survival and rapid growth of juveniles, at a low cost and with a minimum of equipment. However in order to produce larger numbers of juveniles, a large amount of space will be required for the mussel and fish tanks, and labour costs may be high as some parts of the propagation are labour-intensive (particularly when juveniles are excysting from fish).

Although the experiments conducted here were in a temperature controlled room, this may not be necessary, and keeping juveniles at room temperature might encourage faster growth. However the infection of fish with mussel glochidia would still need to take place in a constant temperature room, as there was a high incidence of disease in fish at higher temperatures (pers. obs.).

An important question is how large the mussels need to be in order to be of use as biofilters. The filtration rate of mussels increases disproportionately with length (between $L^{1.44}$ and $L^{2.62}$ as discussed in Chapter 3). Therefore larger mussels are best for use as biofilters, as they filter the most water per unit size. However it may take several years for a mussel to reach a large size. A suitable size might be reached at 3 – 4 years of age (approximately 40mm in length; Aldridge, 1999). An additional problem is that adult mussels may be difficult to maintain for long periods in laboratory conditions (Gatenby et al., 1999; pers. obs.).

Realistically in order to produce the massive numbers of mussels required, culture efforts need to focus on rearing juveniles through the most vulnerable developmental stage, after which they could be placed in defined “grow-out” areas of rivers and lakes, where
they can continue to grow under natural conditions. Once mussels have reached the desired size in these “grow-out” sites, they could then be harvested and moved to the waterbodies needing to be filtered, e.g. eutrophic waterbodies or water treatment works.

This is the approach that Jones and Neves (2002) have taken towards the production of juvenile mussels: they cultured juveniles for only one to two weeks before replacing them in the river. This is clearly a cost-effective approach; however problems arise from a lack of knowledge as to exactly which parts of mussel life cycles are most vulnerable (i.e. where the bottleneck is which limits population numbers). It has generally been assumed that the early juvenile stage represents the most vulnerable part of the life cycle (Coker et al., 1919-1920; Neves and Widlak, 1987; Buddensiek, 1995); in addition the process of attaching to fish may represent a limiting stage (Neves and Widlak, 1987; Buddensiek, 1995). Predation and mortality of adult mussels seems to be generally low: in the UK, only rats are known to predate freshwater mussels (D. Aldridge, pers. comm.). If early juveniles suffer the highest mortality, the important question then becomes at what age survival increases (i.e. at what age mussels could be placed in a “grow-out” site and have the lowest mortality).

For both *A. anatina* and *A. cygnea*, mortality was initially high, before levelling out at approximately 50% when juveniles reached 120 days post-excystment (Figure 6.8). This suggests that the first four months of life are the most vulnerable life history stage. This may be explained by these young juveniles having a different mode of life to older juvenile and adult mussels. The morphology of juvenile mussels at nine and 40 days post-excystment was very different from that of adult mussels (Figures 6.6 and 6.7), and juvenile mussels buried to a depth below the sediment surface. By 120 days post-excystment, juveniles had the form of small adults (Figure 6.7), and were visible in the sediment with their siphons just below the level of the sediment-water interface, suggesting that 120-day old juveniles had assumed the adult mode of feeding.

Therefore 120-day old juveniles may have passed through the early vulnerable stage, and 120 days post excystment could be a suitable age to place juveniles back in a river or lake. Further experiments are needed where juveniles of different ages are put in rivers and lakes and their survival and growth monitored. Such experiments are difficult to carry out because of the high mobility of juvenile mussels, and therefore the high loss rates that may be expected (in addition to loss rates due to mortality). Recent advances in marking mussels using stains which are incorporated into the mussel shells will make such studies possible in the future (Eads and Layzer, 2002).
6.4.5 **Rearing juveniles in the conservation of freshwater mussels**

This study represents the first attempt to rear the rare species, *P. complanata*. Young juveniles of this species have not been found in the field, so the juvenile *P. complanata* reared here offer the first opportunity to study the morphology and habitat requirements of juveniles of this species.

A byssus-like structure was observed on one newly-excysted *P. complanata* juvenile (Figure 6.11). Isely (1911) and Coker *et al.* (1919-1920) found that several unionid species had byssal threads in their early juvenile life. Smith (2000) studied the byssus of *Elliptio complanata* and *Lampsilis radiata*: he found that the byssus originated from the visceral mass of the foot, and was present as a single strand. It was not possible to see where the byssal threads originated from in the *P. complanata* juvenile, and many threads were visible (Figure 6.11). Isely (1911) also observed a branched byssus on some specimens. Coker *et al.* (1919-1920) reported that *Megalonaias nervosa* (then called *Quadrula heros*) had a byssus-like secretion “a very few days after leaving the fish” as seen in the *P. complanata* juvenile here. Therefore it seems likely that the structure seen here was indeed a byssus. No other *P. complanata* were observed with byssus threads, suggesting that byssal threads may only form under certain circumstances (e.g. high disturbance). As *P. complanata* is a large river species, the possession of a byssus may prevent juveniles being washed downstream during high flows.

The oldest *P. complanata* juveniles in this study survived to 100 days post-excystment, with lower growth than that seen in the *Anodonta* species. The lower survival and growth of this species suggests that *P. complanata* may have more specific habitat requirements than the other species used in this study. Further attempts to culture this species are needed in order to refine the rearing conditions and to make a detailed study of these habitat requirements. If juvenile habitat differs from that of adult mussels, and if juveniles represent the most vulnerable life history stage, then the study of juvenile habitat requirements is vital for this species’ conservation. Loss of juvenile habitat may have contributed to the decline of this species; only through an understanding of habitat requirements can we hope to restore mussel habitat for the species’ continued survival. In addition, rearing juvenile mussels may offer the possibility of reintroducing *P. complanata* to sites from which it has been lost.