Optimization of diet and culture environment for larvae and juvenile freshwater pearl mussels, *Hyriopsis (Limnoscapha) myersiana* Lea, 1856

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**Summary**

Culture of the freshwater pearl mussel, *Hyriopsis (Limnoscapha) myersiana*, was carried out in three consecutive steps: (1) culture of glochidia larvae in artificial media, (2) rearing the early juveniles (0–120 days old) in a nursery, and (3) rearing the juveniles (120–360 days old) in an earthen pond. The percentage survival of glochidia in standard tissue culture medium (M199) supplemented with common carp plasma was 95±2.5. All surviving larvae (100%) transformed to juveniles, the duration of transformation being 8 days. The early juveniles (0–60 days old) were fed with a mixture of four selected phytoplankton species (*Chlorella* sp., *Kirchneriella incurvata*, *Navicula* sp. and *Coccomyxa* sp.). The survival rate of juveniles was 8±0.2%. The average length of these juveniles increased from 0.13±0.01 mm to 1.41±0.16 mm and the average height from 0.16±0.01 mm to 0.98±0.09 mm. Subsequently, 60–120-day juveniles were fed with one of the same four phytoplankton species or a combination of the four. Feeding the juveniles with *K. incurvata* resulted in the highest survival rate (65±8.32%), with an average length of 3.46±0.04 mm and an average height of 1.94±0.04 mm. Finally, the 120–360-day juveniles were cultured in an earthen pond. There were progressive changes in average weight (0.0037±0.002 g to 11.24±5.02 g), length (3.48±0.39 mm to 54.08±6.21 mm), height (1.97±0.24 mm to 25.09±2.48 mm) and width (0.98±0.06 mm to 12.28±3.21 mm) from 120 to 360 days. The average growth rates per day of these parameters were 0.0497±0.01 g, 0.2414±0.15 mm, 0.0975±0.08 mm and 0.0493±0.03 mm, respectively. *H. (L.) myersiana* juveniles developed the complete structural composition of the adult by 160 days, and at 360 days, gametogenesis was complete.

**Key words**: Culture, rearing, glochidia, juveniles, adult, freshwater pearl mussel

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Introduction

*H. (L.) myersiana* is an endemic freshwater pearl mussel in Thailand. The nacreous mussel shell can be used for inlaying pearl furniture, ornaments, kitchen utensils and souvenirs. The mussels can also produce freshwater pearls and the meat is a source of protein for humans and animals. These mussels are suspension feeders and their filtration activities contribute to maintaining a clean environment and reducing pollution. *H. (L.) myersiana* is dioecious. The life cycle includes a unique parasitic larval stage known as the glochidium. Adults spawn from October to May and are able to do so 13–25 times a year (average 18.9 times), which is the number of times a female can release glochidia larvae in a year (Nagachinta and Meeguji, 1998). The peak of the spawning season is from December to January. Glochidia of *H. (L.) myersiana* are obligate ectoparasites on a specific fish host (Arayawatanaavij et al., 1992; Panha, 1992; Kovitvadhi et al., 2002, 2003). During this period, the glochidia larvae receive nourishment from their hosts through absorption. After the adult organs have developed, the glochidium bursts from the enclosing cyst and sinks to the bottom as an independent, free-living animal (Binhe, 1984).

The natural population of *H. (L.) myersiana* in Thailand has been drastically reduced to the extent that it is now nearly extinct. Thus, there is an urgent need to restore their numbers through aquaculture. Furthermore, culturing these freshwater mussels throughout their complete life cycle would lead to better understanding and contribute to the management and control of wild populations.

Freshwater mussel culture is divided into the three stages of the mussel’s life cycle (i.e., the parasitic glochidia larval stage, juveniles and adults, respectively). Glochidia larvae are obligatory ectoparasites on fish hosts until they become juveniles. Kovitvadhi et al. (2001b, 2002) succeeded in culturing glochidia of *H. (L.) myersiana* in artificial media with a survival rate of up to 93%, and 100% of these glochidia transformed into juveniles within 10 days. Juveniles of *H. (L.) myersiana* have been cultured using *Chlamydomonas* sp., *Monoraphidium* sp. and *Chlorella* sp. for food (Kovitvadhi et al., 2001b). These juveniles survived to 60 days, with the average length of 2.4 mm. However, information regarding the biology and culture of juvenile *H. (L.) myersiana* is still limited. The aims of this research were to identify an appropriate diet and a suitable artificial environment for the survival of juvenile *H. (L.) myersiana* and their development to adulthood.

Culture of glochidia

Fifty male and fifty female adult freshwater mussels, *H. (L.) myersiana*, were collected from the Mae Klong River, Kanchanaburi Province, Thailand. These individuals had an average weight of 120.95±50.7 g, width of 3.8±0.5 cm, length of 13.73±2.05 cm and height of 6.09±2.02 cm. The culture method for *H. (L.) myersiana* larvae was modified from that of Kovitvadhi et al. (2001a, 2002): 5,000–6,000 glochidia per replicate (three replicates) were placed in a culture dish (90×15 mm) containing 10 ml of artificial medium composed of Medium199 (Gibco, No.6231100-035), fish plasma (common carp, *Cyprinus carpio*) and antibiotics/antimycotic (100 µg/ml carbenicillin, 100 µg/ml gentamycin sulfate, 100 µg/ml rifampin, and 5 µg/ml amphotericin B) in a ratio of 2:1:0.5, respectively. The culture dishes were placed in a low temperature incubator at 25°C with 5% CO₂. The culture medium was removed and replaced with fresh medium on day 4. Finally, 4 ml of sterilized distilled water was added to the culture dish on day 7 to stimulate the transformation of glochidia into juveniles.

Selecting phytoplankton food species for juveniles

Phytoplankton were collected from the gastrointestinal tract of 15 mature mussels from the Mae Klong River, Kanchanaburi Province, and transferred into 1 l of f/2 media (Guillard and Ryther, 1962) and cultured under fluorescent light of 10,000 lux intensity for 18 h/day with a continuous supply of 3% CO₂. The cultured phytoplankton were sub-cultured and purified every 10 days using a streak plating technique, as described by Hoshaw and Rosowski (1973). Single colonies of these phytoplankton were then identified and mass-cultured to be used for juvenile feeding.

Phytoplankton selection was based on three criteria: (1) the ability of mussels to pass each specific type of plankton through the gastrointestinal tract, (2) digestibility of plankton, and (3) ability to mass-culture that phytoplankton species. In testing ingestion and digestibility of each phytoplankton species with juveniles, 1×10^3 cells of each species of phytoplankton were dispersed in 50 ml dechlorinated water and used to feed 50–100 early juveniles in a 100 ml beaker; three replicates per phytoplankton species were set up. After 90 min, 30 juveniles were randomly collected from each replicate to observe plankton ingestion and digestibility under the light microscope. Ingestion was determined by the presence of phytoplankton in the gastrointestinal tract the phytoplankton being clearly visible through the transparent shells of the juveniles.
Digestibility of phytoplankton was determined from the structural and color changes of the phytoplankton in the digestive gland, as well as from the feces.

From the ten different types of phytoplankton species (Ankistrodesmus gracilis, Chlamydomonas sp., Chlorella sp.1, Chlorella sp.2, Kirchneriella incurvata, Monoraphidium irregularare, Navicula sp., Scenedesmus sp., Stichococcus sp. and Coccomyxa sp.) found in the gastrointestinal tract, four species of phytoplankton (Chlorella sp.2, K. incurvata, Navicula sp. and Coccomyxa sp.) were selected as potential food sources for juveniles based on their sizes, shapes, the ability of cilia around the foot, the mantle and gill to transport them into the gastrointestinal tract, and digestibility.

**Culture of H. (L.) myersiana juveniles**

The culture of juvenile *H. (L.) myersiana* was undertaken in five stages, based on the passage of time: 0–60, 60–120, 120–180, 180–270 and 270–360 days. Culture conditions were as follows:

**Culture of 0–60-day juveniles**

About 5,000–6,000 newly transformed juveniles per replicate (three replicates) were transferred to a plastic culture unit (20×11×8 cm) containing 20 g of sand (<120 mm grain size) about 3 mm deep. This sand was collected from the natural habitat of *H. (L.) myersiana*, passed through a 120-mm mesh, washed several times with dechlorinated water and oven dried at 180°C for 24 h. Each culture unit was maintained in a glass aquarium, the filter cabinet (dimensions, 50×46×35 cm). A combination of 5 kg of pebbles, 2 kg of ground freshwater mussel shells and 240 g of nylon fiber (~46×35×2 cm in size and ~30 g per piece) was used as filter material. The water was then passed through a UV tube into a resting cabinet (40 l capacity), but no resting cabinet or UV tube. The flow rate of water recirculation was kept at 20 ml/min. The sand and 25% of the water in the system was replaced every other day. All mussels from each replicate were measured both for length and height and rinsed every 10 days. The survival rate was also recorded. The 60–120-day juveniles were fed with one of the four species of phytoplankton (see above) or a combination of these species at a ratio of 1:1:1:1. All the feeds were given at the concentration of 1×10^5 cells/ml.

**Culture of 60–120-day juveniles**

Sixty juveniles (60–120 days old) were placed in a recirculating water system similar to that used for the previous stage, involving 20 g of coarser sand (<250 mm in size), a smaller filtration unit (40 l capacity), but no resting cabinet or UV tube. The flow rate of water recirculation was kept at 20 ml/min. The sand and 25% of the water in the system was replaced every other day. All mussels from each replicate were measured both for length and height and rinsed every 10 days. The survival rate was also recorded. The 60–120-day juveniles were fed with one of the four species of phytoplankton (see above) or a combination of these species at a ratio of 1:1:1:1. All the feeds were given at the concentration of 1×10^5 cells/ml.

**Culture of 120–180-day juveniles**

Juveniles 120 days old of approximately the same size were selected to rear in an earthen pond. Thirty-five juveniles were transferred to each of three culture units (each 20×12×72 cm). The culture units had all four vertical sides lined with nylon net (0.42 mm mesh size) and each had a plastic lid with holes to cover the top. The lower part of the culture unit consisted of a section 2 cm in height, which fitted snugly into the culture unit, from which it could be removed (Fig. 2A). This lower part contained 400 g of sand (<425 mm in size). The juveniles were placed directly on the sand. The culture unit was then hung in the earthen pond, located at the Department of Aquaculture, Faculty of Fisheries, Kasetsart University. The pond was about 2 m deep with a total surface area of 0.8 ha and filled with rain water. The base of the culture unit was adjusted to a position approximately 50 cm below the water surface. The juveniles fed by filtering phytoplankton from the water in the earthen pond. All mussels from each culture unit were weighed, measured for length, height and width, and rinsed every 10 days. The survival rate was recorded.

**Culture of 180–270-day juveniles**

Thirty-five 180-day-old juveniles per unit were placed in culture units similar to those described above, but with nylon net lining with a mesh size of 2.0 mm. Three culture units were used. These juveniles
were exposed to the same conditions and feeding regime as those 120–180 days old. All mussels from each culture unit were weighed, measured (length, height and width) and rinsed every 10 days. The survival rate was recorded.

Culture of 270–360-day juveniles

Thirty-six 270-day juveniles per pocket net were transferred to three pocket nets (Fig. 2B). Each net was equally divided into six compartments using string and six juveniles were introduced through the opening at the top of each compartment. The nets were hung 50 cm below the water surface of the earthen pond. All mussels were weighed, measured (length, height and width) and rinsed every 10 days until they reached 360 days. Their survival rate was recorded.

Water analysis

Water samples from laboratory culture and from the earthen pond were collected and analyzed for water temperature, pH, dissolved oxygen (azide modification), total alkalinity (phenolphthalein methyl orange indicator), free carbon dioxide (titrimetric), total hardness (EDTA titrimetric), total ammonia nitrogen (direct nesslerization), calcium (EDTA titrimetric), orthophosphate (ascorbic acid method) and silicate (molybdosilicate method). The analysis was carried out weekly for laboratory water samples and every other week for water in the earthen pond.

Phytoplankton communities

During the culture of the 120–360-day juveniles, phytoplankton in the earthen pond was collected from a depth of 50 cm using a 10 l Van Dorn sampler container every month, with two replicates. The samples were filtered through a 40-mm mesh size net, preserved with 1% acidic Lugol’s solution, and counted under an inverted microscope. Species identification were based on Prescott (1951), Desikachary (1959) and Wongrat (1998, 1999). All samples were examined in triplicate.

Results

Culture of glochidia

The adult gravid freshwater mussels collected from the Mae Klong River and cultured in the earthen pond successfully produced glochidia during October–May, similar to those in their natural habitats. The average survival of glochidia in the artificial medium was 95±2.5%. All surviving glochidia transformed to juveniles within 8 days.

Selecting phytoplankton food species for juveniles

Ten species of phytoplankton (Ankistrodesmus gracilis, Chlamydomonas sp., Chlorella sp.1, Chlorella sp.2, K. incurvata, M. irregulare, Navicula sp., Scenedesmus sp., Stichococcus sp. and Coccomyxa sp.) were found in the gastrointestinal tracts of 15 mature H. (L.) myersiana and all grew well in the f/2 media.
Fig. 3. Phytoplankton species for culture of *H. (L.) myersiana* juveniles. (A) *Chlorella* sp. 2; (B) *Kirchneriella incurvata*; (C) *Navicula* sp.; (D) *Coccomyxa* sp. Scale bar = 20 µm.

Table 1. Average growth rate and survival rate of 0–60-day juveniles of *H. (L.) myersiana*. Juveniles were fed with a mixture of four phytoplankton species (*Chlorella* sp. 2, *Coccomyxa* sp., *Kirchneriella incurvata* and *Navicula* sp.) at a ratio of 1:1:1:1; 60–120-day juveniles were fed with four separate species of phytoplankton and a mixture of these species at the ratio of 1:1:1:1; 120–360-day juveniles were reared in the earthen pond.

<table>
<thead>
<tr>
<th>Mussel age (days)</th>
<th>Food type</th>
<th>Average growth rate (±SD)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weight (g/day)</td>
<td>Length (mm/day)</td>
</tr>
<tr>
<td>0–60</td>
<td>Mixture of four phytoplankton</td>
<td>—</td>
<td>0.021±0.01</td>
</tr>
<tr>
<td>60–120</td>
<td><em>Chlorella</em> sp. 2</td>
<td>—</td>
<td>0.0207±0.004</td>
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<tr>
<td></td>
<td><em>Coccomyxa</em> sp.</td>
<td>—</td>
<td>0.0078±0.004*</td>
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<tr>
<td></td>
<td><em>Kirchneriella incurvata</em></td>
<td>—</td>
<td>0.0364±0.002</td>
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<tr>
<td></td>
<td><em>Navicula</em> sp.</td>
<td>—</td>
<td>0.0173±0.004**</td>
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<tr>
<td></td>
<td>Mixture of four phytoplankton</td>
<td>—</td>
<td>0.0350±0.0002</td>
</tr>
<tr>
<td>120–360</td>
<td>Food in the natural habitat</td>
<td>0.0497±0.01</td>
<td>0.2414±0.15</td>
</tr>
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</table>

Remark: Groups with the same superscript letter are not significantly different (*P* >0.05).
*Juveniles fed with *Coccomyxa* sp. only survived to 90 days.
**Juveniles fed with *Navicula* sp. only survived to 100 days.

Four species, namely, *Chlorella* sp.2, *K. incurvata*, *Navicula* sp., and *Coccomyxa* sp. (Fig. 3) were selected as the preferred feed due to their size, shape, speed of movement into the gastrointestinal tract, and digestibility, as observed from the feeding behavior and the feces of the juveniles. During the feeding period, it was observed that phytoplankton cells needed to reach the gastrointestinal tract of the juvenile within 90 min, since any further delay caused nutritional deficiency and the juveniles became weak and finally died. As for the size and form of the phytoplankton, it was found that the small, round or oval-shaped cells could enter the juvenile mouth more readily than large and long ones (Fig. 3). *Chlorella* sp.2 is round (4 mm in diameter), *K. incurvata* oval (5×6 mm), *Navicula* sp. elliptical (4×26 mm) and *Coccomyxa* sp. has a short bar shape (2×4 mm). The digestibility of the food was assessed by the changes in form of each species of phytoplankton after passing through the gastrointestinal tract to the anus. In addition, the feces were inspected for phytoplankton remains. The remains of *K. incurvata* were found to be severely transformed from the original shape and form, indicating that it had the best digestibility, followed by those of *Chlorella* sp.2, *Navicula* sp. and *Coccomyxa* sp.

**Culture of H. (L.) myersiana juveniles**

**Culture of 0–60-day juveniles**

Having been fed with the mixture of four phytoplankton species for 60 days, the length and height of juveniles had changed from 0.13±0.01 mm to 1.41±0.16 mm and 0.16±0.01 mm to 0.98±0.09 mm, respectively (Fig. 4A, 4B). The survival rate of these juveniles over the 60-day period was 8±0.2% (Table 1).

**Culture of 60–120-day juveniles**

Culture of 60–120-day juveniles until 120 days with individual species of phytoplankton and the mixture of four phytoplankton species, respectively, showed that mussels fed exclusively with *K. incurvata* reached the highest average body length (3.46±0.04 mm) and height (1.94±0.04 mm). These results are not significantly different (*P* >0.05) from the growth
Fig. 4. Development of *H. (L.) myersiana* juveniles. (A,B) Average shell length and height of 0–120-day juveniles fed with different phytoplankton: ○ *Chlorella* sp.2, × *Coccomyxa* sp. ▲ *Kirchneriella incurvata*, ■ *Navicula* sp., ● mixture of four phytoplankton; (C–F) Average weight and shell size (length, height and width) of 120–360-day juveniles reared in the earthen pond (total n = 300, 180, 105, 105 and 108 individuals of 0–60, 60–120, 120–180, 180–270 and 270–360-day-olds, respectively).

measurements of mussels fed with the mixture of four phytoplankton species, but those fed with *Chlorella* sp.2 had significantly (*P* < 0.05) lower growth rates (length 2.61±0.22 mm, height 1.53±0.08 mm) (Fig. 4A, 4B). Moreover, juveniles fed only with *Navicula* sp. or *Coccomyxa* sp. lived only until 100 days and 90 days, respectively. The survival rates from 60–120 days for juveniles fed with *K. incurvata*, *Chlorella* sp.2 and the mixture of four phytoplankton species were 65±8.32, 61±9.43 and 27±17.63%, respectively (Table 1).

**Culture of 120–360-day juveniles**

Culture of juveniles in the earthen pond from 120–360 days resulted in changes of average weight from 0.0037±0.002 to 11.24±5.02 g, length from 3.48±0.39 to 54.08±6.21 mm, height from 1.97±0.24 to 25.09±2.48 mm, and width from 0.98±0.06 to 12.28±3.21 mm (Fig. 4C–F). The average growth rate (weight, length, height and width) was 0.0497±0.01 g/day, 0.2414±0.15 mm/day, 0.0975±0.08 mm/day and 0.0493±0.03 mm/day, respectively (Table 1). The survival rate from 120–360 days approached 100%. 
The morphological development of *H. (L.) myersiana* juveniles in culture (0–360 days old) is shown in Fig. 5. Anterior shell growth was clearly seen in the first 30 days of juvenile development, while posterior shell growth followed afterwards. The rim of the shell became spread out to form an anterior and a posterior wing. The posterior wing was more prominent and became more upwards-pointing as the juveniles progressed in their development. The juveniles assumed the mature form at the age of 160 days. The shells of 0–40-day-old juveniles were thin and transparent as seen under light microscope. The inner organs (i.e., stomach, intestine, gills, heart, foot, mantle, and cilia at the gills, mantle and foot) were clearly observed through the shell at this period. The shell, however, became thicker during the developmental process and covered all the inner organs. Male and female reproductive organs were inspected when they reached 360 days and mature gametes were found in both.
Water analysis

The range of physicochemical properties of the water in the culture system and the earthen pond during the 360 days of culturing *H. (L.) myersiana* juveniles did not differ greatly from those of the natural river water where mussels are found. The results are shown in Table 2.

Phytoplankton communities

Of the phytoplankton species found in the earthen pond during the culture of 120–360-day juveniles, 21 taxa were identified, including filament, colony and unicellular types. Three phytoplankton phyla are predominant, namely, Chlorophyta (92%), Chromophyta (6%) and Cyanophyta (0.8%). The most abundant were classified as belonging to four genera, namely, *Phacus* (39%), *Euglena* (17%), *Euastrum* (16%) and *Pediastrum* (9%).

Discussion

Culture of glochidia

Keller and Zam (1990) succeeded in culturing glochidia of the freshwater mussel, *Utterbackia imbecillis*, with a high transformation percentage (81.8%) using a complex artificial medium described by Isom and Hudson (1982), with added fish plasma. An alternative, more simple medium (M199) with horse serum supported a lower transformation percentage (65.4%) (Keller and Zam, 1990). Subsequently, Kovitvadhi et al. (2002) cultured glochidia of *H. (L.) myersiana* in M199 with fish plasma (common carp, *Cyprinus carpio*). The survival rate of 150–200 glochidia/3.5 ml of culture medium was 93±3.0%, of which 100% transformed to juveniles within 10 days at about 23°C. With the present study, we have made an improvement by culturing a large population of 5,000—6,000 glochidia in 30 ml of artificial medium, giving a higher survival rate (95±2.5%) and an earlier transformation (8 days) at 25°C. A further modification to previous culture methods is the replacement of the culture medium with fresh medium at days 3 and 6, thus allowing the glochidia to have greater access to food. The higher culture temperature, a continuous supply of 5% CO₂ and the stimulation provided by sterilized distilled water, may also have shortened the transformation period by 2 days.

Phytoplankton selection for culturing juveniles

Phytoplankton has proven to be a vital source of nutrient for several species of freshwater mussel juveniles (Hudson and Isom, 1984; Gatenby et al., 1996; Gatenby et al., 1997; ÓBeirn et al., 1998; Tankersley and Butz, 2000; Henley et al., 2001; Kovitvadhi et al., 2001b). Similarly, Kovitvadhi et al. (2000) reported that phytoplankton contributed to 99% of the gastrointestinal tract content of the adult freshwater mussel, *H. (L.) myersiana*. This finding is consistent with gut content analyses from other bivalve species (Gale and Lowe, 1971; Huca et al., 1983; Paterson, 1986; Parker et al., 1998). Consequently, phytoplankton from the gastrointestinal tract of adult *H. (L.) myersiana* were cultured and selected for juvenile feeding. Since the size and shape of phytoplankton also determine the ease of their passage into the gastrointestinal tract (Gatenby et al., 1996; Gatenby et al., 1997; ÓBeirn et al., 1998; Henley et al., 2001; Kovitvadhi et al., 2001b), the morphological features of phytoplankton were taken into account. Selection of suitable phytoplankton food species was based on size, morphology, digestibility, and appropriate coordination of cilia around the foot, mantle and gill to move each type of phytoplankton. Kovitvadhi et al. (2001a) found that the gills of the early juvenile *H. (L.) myersiana* (<13 days old) were not fully developed for filtering food. In the present study, it was observed that early juveniles (0–40 days) have to use cilia around the foot, the mantle and gill to move phytoplankton into the mantle cavity and ultimately to the mouth. We describe this type of food intake at the juvenile stage as “pedal-mantle-gill-feeding”, rather than “pedal-feeding” as used by other research groups (Reid et al., 1992; Yeager et al., 1994; Gatenby et al., 1997). This type of feeding behavior is also different from “suspension-feeding” at the more mature stage of mussel development where an incurrent siphon, gill and labial palp are involved in selective intake of food.

Culture of 0–360-day juveniles

The high survival rates (65±8.32%) and large shell size of juveniles fed with *K. incurvata* during the 60–120-day period suggests that this is the most promising phytoplankton species for use as feed for the culture of *H. (L.) myersiana*. Although the mixture of four phytoplankton species gave good results for the first 60 days of culture, the survival rate with this mixture over the 60–120-day period was markedly lower (27.5±17.63%). An attempt to use *K. incurvata* alone as feed, from day 0 to day 120, should be made to increase the survival rate after 60 days. From our observations, the first 30 days are the most sensitive of juvenile life, so that the low survival rate during this stage was expected. A similar observation was made by Hudson and Isom (1984) who observed juveniles of *U. imbecillis* in their natural habitat.
Attempts to culture juveniles of U. imbecillis using lake water with different species of phytoplankton were made by Hudson and Isom (1984), while Kovitvadhi et al. (2001b) raised H. (L.) myersiana juveniles using dechlorinated tap water supplemented with Chlamydomonas sp., Monoraphidium sp. and Chlorella sp. as food. However, these groups were only able to raise the juveniles for 74 days and 60 days, respectively, reaching maximum lengths of 5.1 mm and 2.4 mm, respectively. The 0–60-day H. (L.) myersiana juveniles in this study grew to the maximum length of 3.46 mm and could be reared to maturity with little or no mortality. It should be noted here that the juveniles used in the experiments of Hudson and Isom (1984), Kovitvadhi et al. (2001b), and in this study, were developed from glochidia which had been cultured in artificial media, while Gatenby et al. (1997) cultured Villosa iris juveniles derived from parasitic glochidia on host fish. They could rear them to 60 days, to maximum lengths of 450 µm, and the survival rate at that stage was 66.5%.

Transferring 120-day juveniles to the earthen pond until they reached 360 days enabled us to observe and record in detail the development of these mussels. Although the overall weight and size of juveniles increased throughout the culture period, their sizes (length, height, width) changed most markedly during the 120–180-day period, while the weight increase was most prominent during the 280–360-day period, when it reached an average of 0.0633 g/day (Fig. 4C and Table 1).

Adding sand to the culture unit for 0–270-day juveniles to a depth of 3–5 mm appears to improve survival rates. The sand may help grind the food in the gastrointestinal tract. The same effect was reported by Isom and Hudson (1982, 1984), for U. imbecillis, and Buddensiek (1995) for Margaritifera margaritifera.

**Water analysis**

Water quality of the juvenile culture medium at two different stages (0–60 days and 60–120 days) of the freshwater mussel, H. (L.) myersiana, was comparable to that in their natural habitat (Kovitvadhi et al., 1998), except for the values of free carbon dioxide, hardness and calcium. The high values of water hardness and calcium found in the water of the 0–60-day juvenile culture could result from the higher level of free carbon dioxide due to the recirculation of CO₂ under closed culture conditions depressing the pH, so that calcium from ground mussel shell in the filtration unit could readily dissolve and raise the calcium value and hence water hardness. Water samples from the earthen pond during the culture of 120–360-day juveniles were found to have similar qualities to that of the mussels’ natural habitat. However, the values of free CO₂, hardness and silicate were higher.

**Phytoplankton communities**

Gale and Lowe (1971), Huca et al. (1983), Binhe (1984), Paterson (1986) and Kovitvadhi et al. (2000) found more phytoplankton than zooplankton in the gastrointestinal tract of adult freshwater mussels. They demonstrated that most phytoplankton were those of green algae, of unicellular or small colony types, long filament algae being rarely found. Kovitvadhi et al. (2000) reported that the relative abundance of phytoplankton species within the gastrointestinal tract of adult H. (L.) myersiana from the Mae Klong River, Kanchanaburi Province, corresponded to their prevalence in the environment. According to the present study, the main phytoplankton species in the earthen pond related closely to the species in the gastrointestinal tract contents of adult H. (L.) myersiana. Consequently, the 120–360-day juveniles reared in the earthen pond were able to grow vigorously and achieve survival rates approaching 100%.

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