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A laboratory-scale recirculating aquaculture system for juveniles of freshwater pearl mussel *Hyriopsis* (*Limnoscapha*) *myersiana* (Lea, 1856)

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Abstract

Growth and survival rates of juvenile freshwater pearl mussels *Hyriopsis* (*Limnoscapha*) *myersiana* (Lea, 1856) were compared at 0–120 days when reared in two closed recirculating aquacultural systems. System I was composed of a glass aquarium with a filter cabinet (combination of pebbles, ground freshwater mussel shells and nylon fiber), a UV tube, a resting cabinet, and a plastic culture unit. The system II was composed of 5 cabinets: a particulate filter cabinet, a macrophyte (*Limnophila heterophylla*) filter cabinet, a biological filter cabinet, a water resting cabinet and plastic culture units. Water flowed through the juvenile culture units at 20 ml/min in both systems. In each system juveniles were stocked at day 0 with sand at <120 μm and were fed twice a day on a 1:1 mixture of *Chlorella* sp. and *Kirchneriella incurvata*. Over the 120 days, average growth rate per day and rate of survival were higher in system II. Free carbon dioxide, total ammonia nitrogen, nitrate, phosphate and silica of second system were significantly lower in system II. The relationship between shell length (L) and age of the freshwater pearl mussels cultured in system II was $L=0.6164-0.0809\ \mathrm{Day}+0.0032\ \mathrm{Day}^2-1\times10^{-5}\ \mathrm{Day}^3$, $R^2=0.983$.

Keywords: Freshwater mussel; Juvenile; Growth; Survival; Recirculating system

1. Introduction

Hyriopsis (*Limnoscapha*) *myersiana* (Lea, 1856) is a freshwater pearl mussel endemic to Thailand. At present it has a decreasing population, so culture is considered to be important in order to provide material for nacre inlays for furniture, nuclei for the cultured pearl industry and data to assist conservation measures. Freshwater pearl mussel culture can be divided into the three stages of the life cycle: the parasitic glochidia larval stage, juveniles and adults. Only a small amount of data on freshwater juvenile biology and culture is available; juvenile survival from culture in artificial media has been assessed in only three groups of research experiments under laboratory conditions (Hudson and Isom, 1984; Uthaiwan et al., 2001; Kovitvadhi et al., 2006). Only Kovitvadhi et al. (2006) succeeded in culturing glochidia of *H.* (*L.*) *myersiana* to adulthood. However, early juveniles (0–60 days) cultured with a mixture of four phytoplankton species (*Chlorella* sp., *Kirchneriella incurvata*, *Navicula* sp. and *Coccomyxa* sp.) had a survival rate of only 8±0.2%. The cause of low survival rate is due to a higher total ammonia nitrogen of the water quality under laboratory conditions than in the mussels' natural habitat equal to 0.42; ammonia nitrogen must be removed from the culture water, as it is toxin. Glochidia and juvenile mussels are more sensitive to some chemicals such as copper, ammonia and chlorine when compared to commonly tested aquatic organisms (Wang et al., 2007).

Many species can be successfully grown and have high survival rates in recirculating (closed) aquaculture systems (RAS)

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due to the high-quality culture water. Therefore, RAS have been used extensively for rearing and maintaining adult and juvenile marine bivalves in captivity (Epifanio et al., 1974; Spotte, 1979; MacMillan et al., 1994), and have also been used for some juvenile and adult freshwater mussels (Coker et al., 1921; Gatenby et al., 1996; Dunn and Layzer, 1997; O'Beirn et al., 1998; Henley et al., 2001; Kovitvadhi et al., 2006). Therefore, the objective of the present study was to increase survival rate and growth of the freshwater pearl mussel at the juvenile stage (0-60 days) by a comparison of two culture systems: system I, used by Kovitvadhi et al. (2006) and system II, using a biological filter (macrophytes and bioball) in the filter system. The water quality in these two systems was compared in order to ascertain the suitable water quality for culturing this freshwater pearl mussel.

2. Materials and methods

2.1. System design and components

Growth and survival rates of juvenile freshwater pearl mussel, *Hyriopsis* (*Limnoscapha*) *myersiana* (Lea, 1856), were compared in two closed recirculating systems. System I (Fig. 1A) was the system adopted by Kovitvadhi et al. (2006) for culturing juveniles of 0–60 days. This system consisted of a particulate filter cabinet (Length × Width × Height × Water level = 50 × 46 × 35 × 30 cm) was divided into two equal section, resting cabinet (50 × 26 × 35 × 30 cm) and plastic culture unit (20 × 11 × 8 × 7 cm). Water from the culture unit flowed into the first section of the filter cabinet, which was composed of three layers: a nylon filter layer, a gravel layer and a layer of ground freshwater mussel shells, then flowed to the second section. Filtered water flowed through a UV tube and collected in the resting cabinet, whence it flowed into the culture unit at 20 ml per minute. The inside of the culture unit was divided into two sections. The first

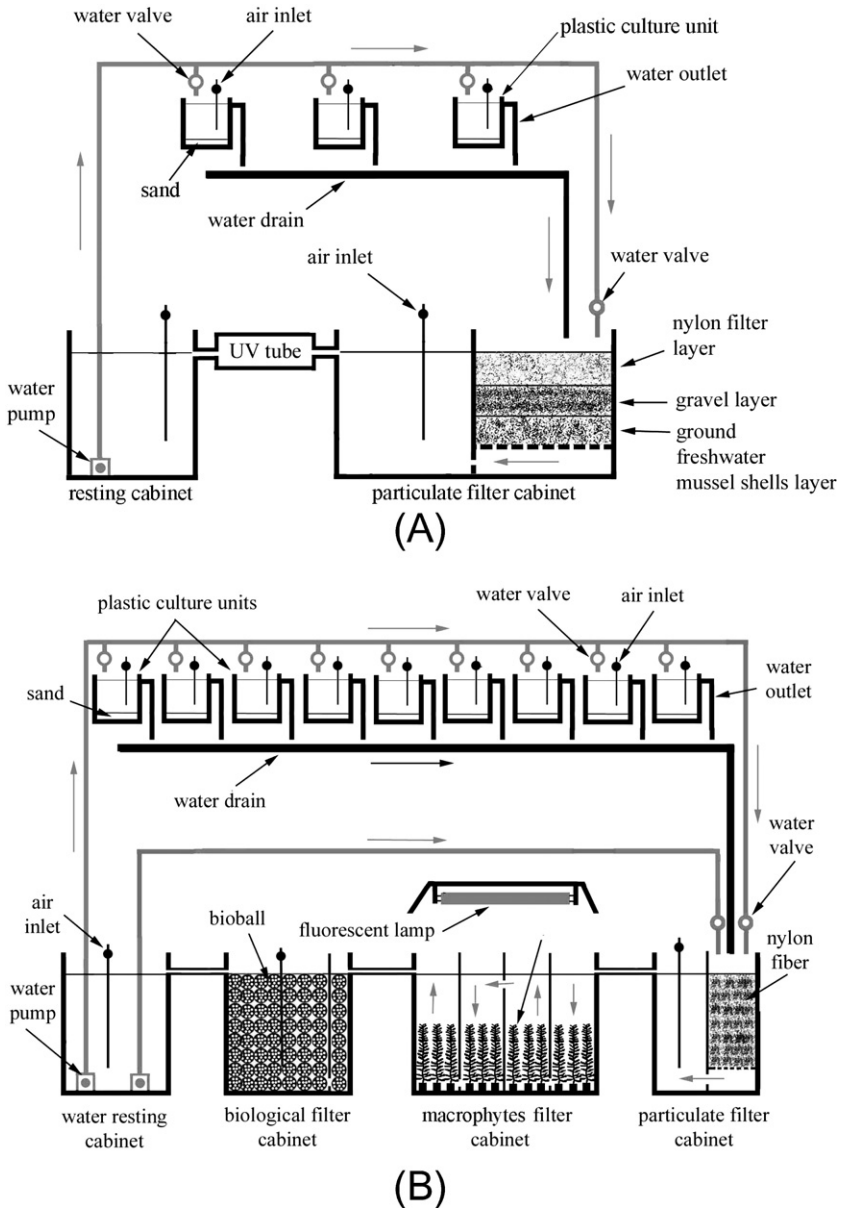

Fig. 1. Schematic diagram of the recirculating systems I (A) and II (B) used to rear freshwater pearl mussel juveniles (0–120 days).

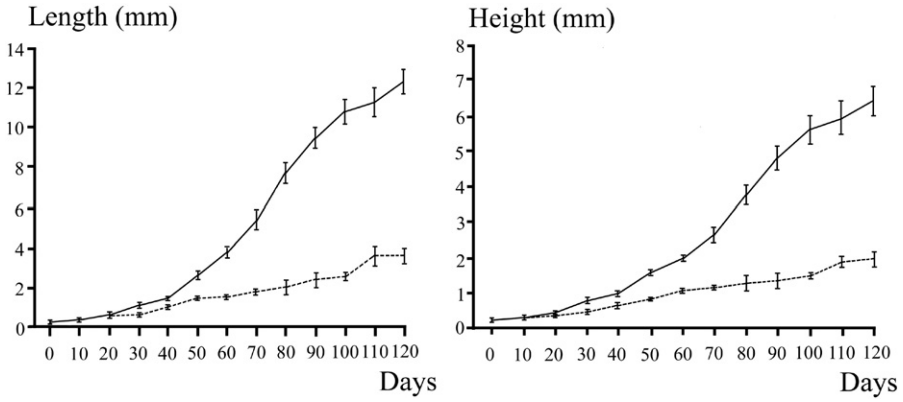

Fig. 2. Development of *H.* (*L.*) *myersiana* juveniles. Average shell length (±SD) and height of 0–120 day-old juveniles cultured in systems I (————) and II (——).

section (18×11×8×7 cm) was to put sand and juveniles. This sand was collected from the natural habitat of *H.* (*L.*) *myersiana*, passed through a 120-μm mesh, washed several times with tap water and oven dried at 180° C for 24 h. Then the water from the first section flowed through the screen (120-mm mesh) to the second section (2×11×8×7 cm) which there was no sand and juveniles for trapping juveniles and at the end of the second section was an outlet for overflow water to collect into the particulate filter cabinet via a trough. The water circulation was turned off for 1 h during feeding. System II (Fig. 1B) comprised three filter cabinets made of 6 mm thick acrylic (particulate filter cabinet, macrophytes filter cabinet and biological filter cabinet), one water resting cabinet and nine plastic culture units. The particulate filter cabinet (46×35×51×42 cm) was divided into two equal parts, of which the first part was filled with a 30 cm thick nylon filter. Water flowed through this filter and via the second part to the macrophytes filter cabinet (80×40×51×42 cm) which was divided into four equal units. Each unit contained 57 ambulia plants, *Limnophila heterophylla* (Raxb.) Bentham; these, 228 plants in total, were introduced when they were 6 cm in height and had an average weight of 2.69±0.13 g. The plants were removed and replaced when their tips reached the water surface. The upper parts of the cabinets were equipped with three fluorescent lamps (each 20 W) 25 cm above the water surface (light intensity at the water surface, 5320 lux; 24 h). The water then flowed into the biological filter cabinet (60×34×51×42 cm) filled with BioBall to full capacity, and then to the resting cabinet (46×41×51×42 cm). In the resting cabinet there were two water pumps: the first returned water to the particulate filter cabinet at the rate of 1 l per minute continuously and the second pumped water at 20 ml per minute to nine plastic culture units (each 84×14×15×7 cm). This pump was stopped for 1 h after phytoplankton was introduced into the culture unit. The bottom of the culture unit was filled with sand at 0.27 g/cm^2. The preparation of sand was the same as for system I. The inside of the culture unit was divided into two sections, as described previously, but of different sizes (section 1−66.1×14×15×7 cm; section 2−17.9×14×15×7 cm). The first section in this experiment also consisted of five acrylic sheets jutting from the walls on alternate sides.

2.2. Preparation of juvenile mussels

Fully grown adult male and female freshwater pearl mussels, *H.* (*L.*) *myersiana*, were collected in September 2005, at Mae Klong River (13°57'30" N; 99°45'00" E), Kanchanaburi Province, and held in an earthen pond (≈8000 m^2) on Department of Aquaculture, Faculty of Fishery, Kasetsart University, culture in November 2005. Mature glochidia were sucked from gravid females to culture in artificial medium according to Kovitvadhi et al. (2006). The 0-day-old juveniles that developed were released into the two culture systems at a density of 4 juveniles per sq cm (790 and 3700 juveniles per unit, in total 2370 and 33,300 juveniles per system, respectively).

2.3. Food and feeding

Juveniles were fed *Chlorella* sp. and *Kirchneriella incurvata* over the course of the experiment. These two species were purified from the digestive tract of freshwater pearl mussels from the Mae Klong River by the streak plate technique

Table 1
Average growth rate and survival rate of 0–120-day-old juveniles of *H.* (*L.*) *myersiana* cultured in systems I and II

Mussel age (days)	Growth rate (average±SD)						Survival (%)			
	Length (mm/day)			Height (mm/day)						
	System I	System II	Sig.	System I	System II	Sig.	Days	System I	System II	Sig.
0–10	0.02±0.003	0.02±0.002	*ns*	0.01±0.002a	0.01±0.002b	*ns*	10	90.57±3.25	98.54±2.29	*ns*
10–20	0.01±0.006a	0.02±0.006b	*	0.0003±0.004a	0.01±0.005b	**	20	87.42±0.27a	96.44±1.24b	*
20–30	0.01±0.007a	0.05±0.007b	**	0.01±0.005a	0.03±0.007b	**	30	77.01±0.13a	95.67±1.09b	*
30–40	0.03±0.015a	0.04±0.009b	**	0.019±0.006a	0.021±0.009b	*	40	62.42±2.04a	94.53±2.14b	**
40–50	0.04±0.009a	0.18±0.026b	**	0.02±0.006a	0.06±0.014b	**	50	39.72±3.44a	92.40±3.15b	**
50–60	0.01±0.007a	0.11±0.031b	**	0.02±0.004a	0.04±0.012b	**	60	29.55±3.56a	91.54±3.24b	**
60–70	0.02±0.009a	0.16±0.052b	**	0.01±0.007a	0.06±0.026b	**	70	25.82±2.42a	90.22±2.54b	**
70–80	0.02±0.040a	0.24±0.071b	**	0.01±0.023a	0.11±0.037b	**	80	22.42±2.61a	89.24±2.13b	**
80–90	0.04±0.031a	0.17±0.053b	**	0.01±0.016a	0.10±0.041b	**	90	19.75±3.25a	88.56±3.21b	**
90–100	0.01±0.058a	0.13±0.068b	**	0.01±0.029a	0.08±0.054b	**	100	17.84±2.44a	85.39±2.14b	**
100–110	0.04±0.084a	0.10±0.055b	**	0.03±0.059a	0.04±0.022b	**	110	15.62±1.85a	84.61±1.22b	**
110–120	0.0001±0.006a	0.10±0.068b	**	0.01±0.032a	0.05±0.057b	**	120	12.45±2.14a	82.74±1.47b	**
0–120	0.03±0.003a	0.10±0.006b	*	0.01±0.002a	0.05±0.003b	**				

Different letters at each age within each system denote significantly different values ($P<0.05$).
*=$P<0.05$, **=$P<0.01$, *ns*=not significant difference, $P>0.05$.

(Hoshaw and Rosowski, 1973) on solid mixed f/2 medium (Guillard and Ryther, 1962), and placed under fluorescent lamps at 10,000 lux intensity for 18 h/day. Then the algae were separated to culture in liquid f/2 medium in pointed test tubes of 250 ml volume for 7 days under fluorescent lamps for 18 h/day and 3% carbon dioxide mixed with air (24 h). They were then increased to 1 l volume by culturing in pointed test tubes under the same conditions of light and air for

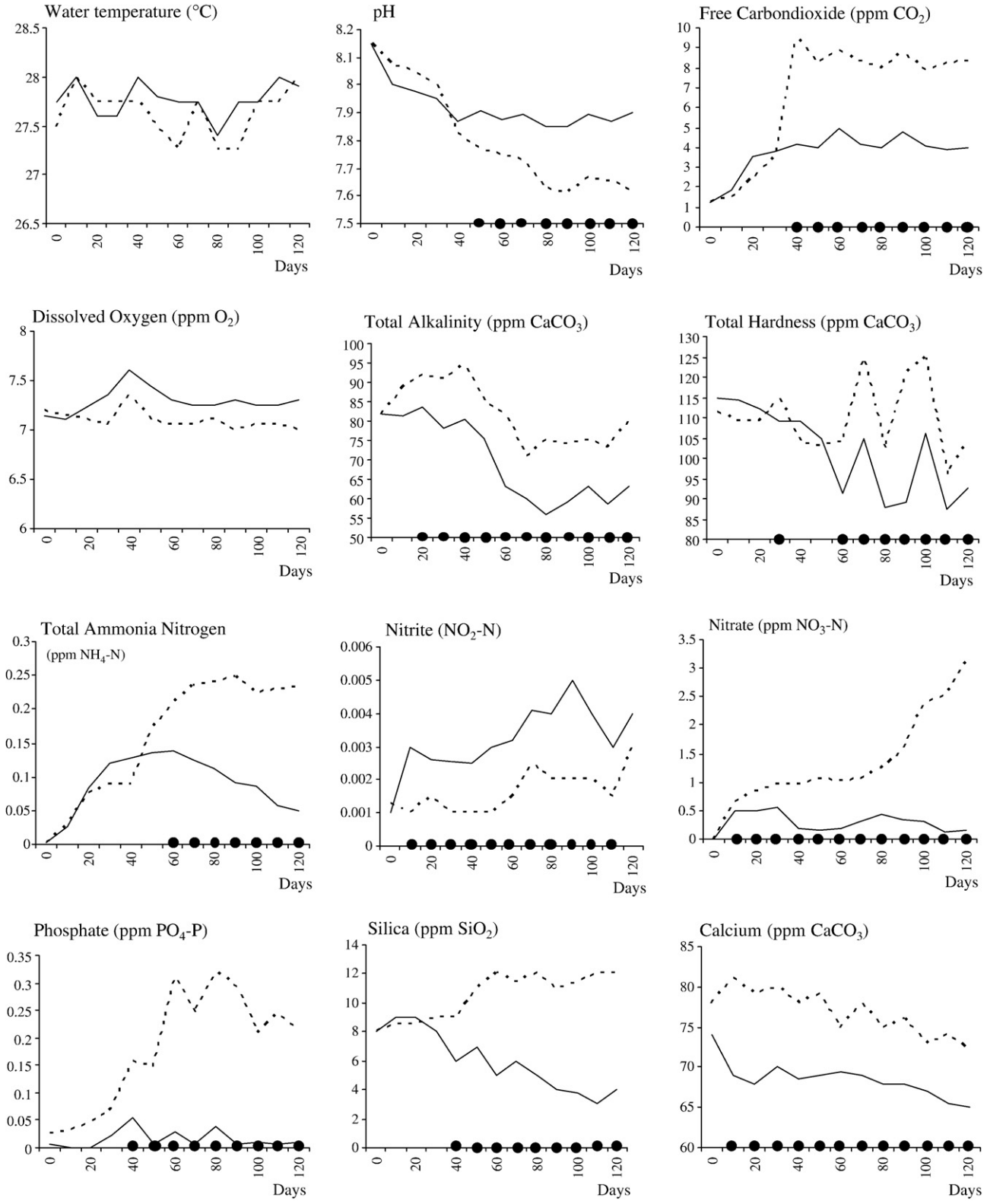

Fig. 3. Water quality during culture for 0–120 days of *H.* (*L.*) *myersiana* in system I (————) and system II (——). Dots on the 'Days' axes indicate that water quality in systems I and II was significantly different ($P<0.05$).

Table 2
Coefficient of correlation between average survival rate and water quality; average growth rate and water quality of juvenile *H.* (*L.*) *myersiana* cultured in systems I and II every 10 days

Parameter	Survival		Shell length		Shell height	
	System I	System II	System I	System II	System I	System II
Water temperature	0.237^{ns}	−0.093^{ns}	0.030^{ns}	0.075^{ns}	−0.012^{ns}	0.107^{ns}
pH	0.983**	0.716**	−0.881**	−0.597*	−0.905**	−0.590*
Dissolved oxygen	0.568*	−0.118^{ns}	−0.559*	−0.055^{ns}	−0.579*	−0.035^{ns}
Total alkalinity	0.745**	0.841**	−0.685**	−0.849**	−0.726**	−0.827**
Free carbon dioxide	−0.874**	−0.634*	0.706**	0.481^{ns}	0.729**	0.476^{ns}
Total hardness	−0.001^{ns}	0.769**	−0.144^{ns}	−0.764**	−0.104^{ns}	−0.751**
Total ammonia nitrogen	−0.982**	−0.152^{ns}	0.849**	−0.051^{ns}	0.885**	−0.061^{ns}
Nitrite	−0.664**	−0.716**	0.676**	0.709**	0.722**	0.688**
Nitrate	−0.771**	0.203^{ns}	0.928**	−0.200^{ns}	0.911**	−0.218^{ns}
Phosphate	−0.903**	0.003^{ns}	0.711**	−0.085^{ns}	0.762**	−0.091^{ns}
Silica	−0.968**	0.914**	0.849**	−0.913**	0.888**	−0.091**
Calcium	0.797**	0.817**	−0.862**	−0.751**	−0.882**	−0.761**

(*=$P<0.05$, **=$P<0.01$, *ns*=not significant difference, $P>0.05$).

5 days and then culturing for increased volume in plastic tanks (100 l) in the open air with f/2 medium and continuous aeration for 5 days.

Each species of alga was collected from the 100 l by being pumped through 0.3 μm ceramic filters and then separated from the water by centrifuging at 8000 ×*g*. The sediments of the two algal species were mixed at a ratio of 1:1 wet weight and kept in a freezer. When required, the mixture was brought to room temperature then sucked by Pasteur pipette into the all plastic culture unit in both system to an algal density of 1×10^5 cells per ml. Algae were supplied twice a day (06.00 h and 18.00 h), and the frozen stock was usually used within 7 days of collection.

2.4. Water analysis

The water used in culturing juveniles was free from chlorine. In both systems the following water quality parameters were analyzed every 10 days: water temperature (Hg thermometer), pH (pH meter), dissolved oxygen (azide modification), total alkalinity (phenolphthalein methyl orange indicator), free carbon dioxide (titration), total hardness (EDTA titration), total ammonia nitrogen (phenate method), calcium (EDTA titration), nitrite (colorimetry), nitrate (cadmium reduction), orthophosphate (ascorbic acid method) and silica (molybdosilicate method) (APHA, AWWA, WPCF, 1998).

2.5. Statistical analysis

The mussels were sampled by isolate juvenile form sand with screen (120-μm mesh) every 10 days for growth during the experiment was $n=50$ from each culture unit. Growth of juveniles was assessed by recording increments of shell size (shell length and shell height). Juveniles were measured using a light microscope with a calibrated ocular micrometer to the nearest 0.01 mm. Growth rate were calculated as average growth rate in mm per day=(average shell length or average shell height at the end of every 10 days-average shell length or average shell height at before 10 days/total growth period in days (10 days).

Survival was calculated using the average number of living juveniles at the beginning of the experiment and at the end of every 10 days.

Group comparison (*t*-test) was used to compare values between two systems for growth rate, survival and water quality in different every 10 days.

The coefficient of correlation (r) of linear regression was used in relationship of water quality and survival rates or shell size (length and height) which was calculated by using averages of water quality characteristics with averages of survival rates and average shell size (shell length and shell height) throughout experiment.

The relationship between the shell size and age was expressed by the equation:

$$Y = b_0 + b_1X + b_2X^2 + b_3X^3$$

where Y is the shell size (shell length or shell height in mm), X is age (days), and b_0, b_1, b_2 and b_3 are parameters. The all group comparison and regressions analysis was used the statistical program SPSS (SPSS Inc,).

3. Results

3.1. Growth and survival of cultured juveniles

Growth and survival rate of 0–10-day-old juveniles of freshwater pearl mussels did not differ between systems I and II; however, for 10–120-day-old juveniles there were significant ($P<0.05$) and highly significantly ($P<0.01$) differences between the two recirculating

Table 3
Summary of system composition and other in system I and II

Item	System I	System II
Water volume (l)/each unit		
Culture unit	1.54	8.23
Number of culture unit/system	3	9
Particulate filter cabinet	69.0	67.62
UV tube	1.0	–
Macrophytes filter cabinet	–	134.4
Biological filter cabinet	–	85.68
Water resting cabinet	39.0	79.21
Total water volumes	113.62	440.98
Total mussel/system	2,370	33,300
Mussel/l	20.86	75.51
Survival rate (average±SD)	12.45±2.14	82.74±1.47
Growth rate of shell length of 0–120 day juveniles (mm/day±SD)	0.03±0.003	0.1±0.006
Water quality (average±SD)		
Water temperature (°C)	27.6±0.19	27.8±0.17
pH	7.81±0.05	7.92±0.02
Free carbon dioxide (ppm CO_2)	6.52±0.6	3.73±0.2
Dissolved oxygen (ppm O_2)	7.10±0.06	7.29±0.1
Total alkalinity (ppm $CaCO_3$)	81.79±1.1	69.5±1.2
Total hardness (ppm $CaCO_3$)	109.85±2.1	101.85±2.1
Total ammonia nitrogen (ppm NH_4-N)	0.159±0.01	0.089±0.01
Nitrite (ppm NO_2-N)	0.0016±0.001	0.0032±0.001
Nitrate (ppm NO_3-N)	1.331±0.14	0.289±0.19
Phosphate (ppm PO_4-P)	0.178±0.015	0.015±0.004
Silica (ppm SiO_2)	10.46±0.7	5.98±0.1
Calcium (ppm $CaCO_3$)	76.8±1.68	68.5±0.88

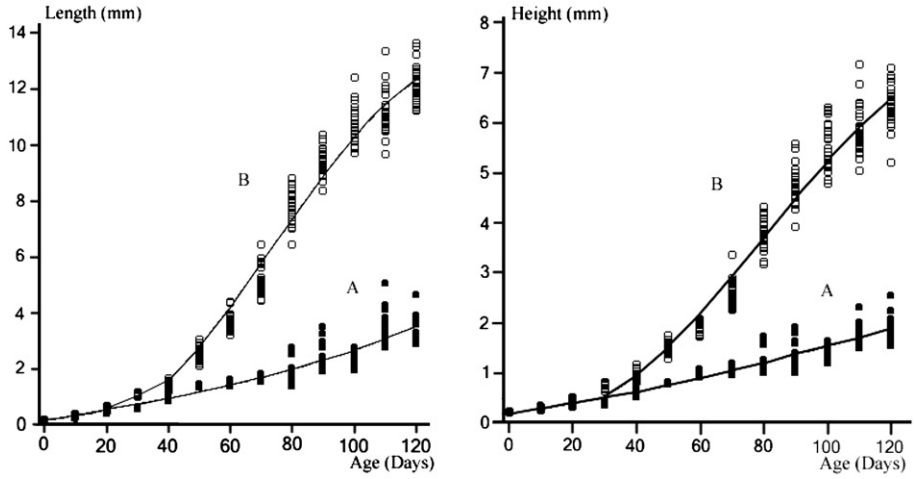

Fig. 4. Relationship curves between age and shell size (length and height) in systems I (A) and II (B).

systems. Shell length changed from 0.19±0.01 mm to 3.45±0.40 mm in system I and 12.17±0.68 mm in system II, and shell height changed from 0.22±0.01 mm to 1.89±0.23 mm in system I and 6.33±0.41 mm in system II (Fig. 2). Shell length and height of juveniles cultured in each system were significantly different ($P<0.05$) from 30 to 120 days. Average growth rates calculated from shell lengths and shell heights were 0.03±0.003 mm/day and 0.01±0.002 mm/day for system I, and 0.10±0.006 mm/day and 0.05±0.003 mm/day for system II, respectively (Table 1). Survival rates in culture were calculated up to 120 days. Juveniles in system II had a higher survival rate (82.74±1.47%) than in system I (12.45±2.14%) (Table 1), with differences between the two systems being significant ($P<0.05$) and highly significantly ($P<0.01$) from 20 to 30 days and 40 days onward, respectively.

3.2. Water quality

Water temperature and dissolved oxygen did not significantly ($P>0.05$) differ between the two systems, with ranges of 27.4–28° C and 7.1–7.6 ppm O_2. Total ammonia nitrogen, free carbon dioxide, nitrate, phosphate and silica of system I increased and were significantly ($P<0.05$) greater than in system II. Total alkalinity and total hardness of water in system I were also higher than in system II. Free carbon dioxide, pH, total ammonia nitrogen, phosphate, and silica were not significantly different between the two systems at an early culture stage (0–30 days) but there were significant differences ($P<0.05$) at the end of the experiment (Fig. 3). Highly significant positive correlations with survival indicated that pH, total alkalinity, and calcium were the most consistently important water quality factors in each system. These were followed by free carbon dioxide and nitrite, which had significant negative correlations with survival in each system. Whereas total ammonia nitrogen, nitrate, phosphate and silica showed significant negative correlations with survival in system I, none of these variables was correlated with survival in system II except silica. Correlations between water quality and growth (shell length and shell height) in both systems were similar to those between water quality and culture system in shell growth. Growth was significantly negatively correlated with pH, total alkalinity, and calcium in both systems (Table 2). As a result of the water quality in system II suitable to cultured juveniles, they had higher survival rate and growth development than in system I. Summary of system composition, stocking density, survival rate, growth rate and water quality in system I and II shown in Table 3.

3.3. Macrophytes

In system II the average initial weight was 2.69±0.13 g/plant. When the plant tips touched the water surface, the ambulia plants were replaced; this occurred 7 times, with an average of 15.14±0.64 days per cycle and an average weight increase of 12.07±0.02 g/plant (about a 4.5 fold increase).

3.4. Length-at-age and height-at-age curves

Growth was greater in system II than in system I, as a result of a more rapid increase in growth rate with increasing age (Fig. 4). The age and size relationships of freshwater pearl mussel for both culture systems are summarized in Table 4.

3.5. Morphological development of H. (L.) myersiana

The morphological development of *H.* (*L.*) *myersiana* juveniles in culture (0–120 days) was shown in Fig. 5. During days 0–30 the anterior shell use elongates instead of grew more than the posterior shell. At 40 days, the two sides were similar in size. Subsequently, the posterior part of the shell use elongates instead of grew more and expands in width. The incurrent and excurrent siphons were seen clearly from 50 days onward. The shell of 0–40-day-old juveniles was thin and transparent as seen under the light microscope. The inner organs (i.e. stomach, intestine, gills, heart, foot, mantle and cilia of the gills) were clearly seen through the shell at this period. When the

Table 4
Relationships of age with shell length and age with shell height in *H.* (*L.*) *myersiana* juveniles (0–120 days) in systems I and II

Regression	b_0	b_1	b_2	b_3	R^2
Age and length (n=390)					
System I	0.1474	0.0212	-5×10^{-5}	9.3×10^{-7}	0.926
System II	0.6164	−0.0809	0.0032	-1×10^{-5}	0.983
Age and height (n=390)					
System I	0.1960	0.0083	6.5×10^{-5}	-1×10^{-7}	0.935
System II	0.3954	−0.0313	0.0014	-6×10^{-6}	0.978

Regression equation: Shell size=b_0+b_1 Day+b_2 Day^2+b_3 Day^3.
R^2=coefficient of determination.

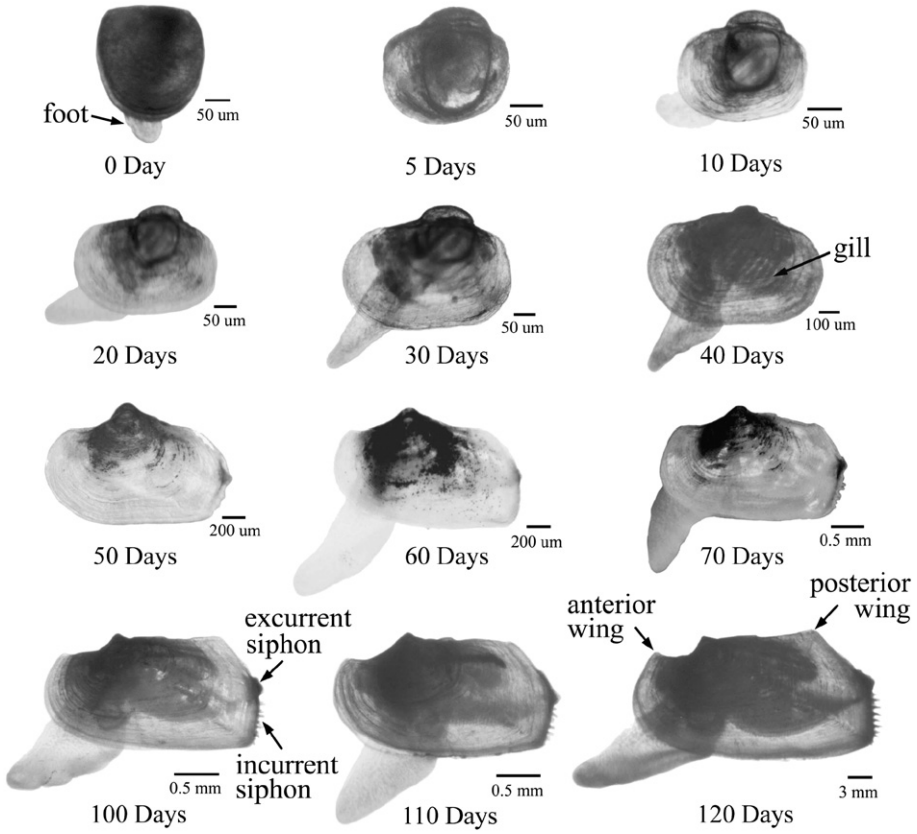

Fig. 5. Morphological development of 0–120-day-old juveniles of *H.* (*L.*) *myersiana*.

juvenile was older than 40 days, the shell thickens until the internal organs could hardly be seen. The anterior and posterior wings began to show at 100 days and could be seen clearly at 120 days.

4. Discussion

Generally, the larval stage of aquatic animals is more sensitive to environmental conditions than the adult stage and this is true for the freshwater pearl mussel in its juvenile stage (Gosling, 2003). This may be because the juvenile shell is not able to close completely against unfavorable environmental conditions or predators, since the foot is of relatively larger size than in the adult. In addition, 0–50-day-old juveniles have not yet completely developed the organs necessary for ingesting food, namely the incurrent and excurrent siphon and gills with fingerlike paired lobes (Hudson and Isom, 1984; Uthaiwan et al., 2001; Kovitvadhi et al., 2006). Juveniles began to close their shells completely at 40 days, and their organs were fully developed after>50 days, in the study by Kovitvadhi et al. (2006). Therefore, the culture system and quality of water used to rear the juvenile stage must provide suitable conditions for food ingestion and digestion.

Several culture systems have been assessed, but mostly a recirculating system has been used. Water used for rearing may come from a natural water resource or from a dechlorinated supply. Phytoplankton has been a vital source of nutrients in the culture of early juvenile *H.* (*L.*) *myersiana,* (Kovitvadhi et al., 2006; Uthaiwan et al., 2001, and the present study), and other freshwater mussel species (Hudson and Isom, 1984; Gatenby et al., 1996, 1997; O'Beirn et al., 1998; Tankersley and Butz, 2000; Henley et al., 2001; Lima et al., 2006). Phytoplankton should have the size and shape to easily pass into the gastrointestinal tract so that the juveniles can digest them (Gatenby et al., 1996, 1997; O'Beirn et al., 1998; Henley et al., 2001; Uthaiwan et al., 2001; Kovitvadhi et al., 2006), and should also be appropriate for the co-ordination of cilia around the foot, mantle and gill to move the phytoplankton into the mouth of the juveniles (Kovitvadhi et al., 2006). For the present study, culturing systems and phytoplankton for juveniles were developed from Kovitvadhi et al. (2006). It was found that growth and survival rates of the early juvenile *H.* (*L.*) *myersiana* in system II were higher than in system I. At 60 days, survival was 91.54±3.24% in system II and 29.55±3.56% in system I, and at 120 days 82.74±1.47% and 12.45±2.14%, respectively. These survival rates are higher than in Kovitvadhi et al. (2006), in which a system similar to system I but with different algal species achieved 8±0.2% survival at 60 days and 65±8.32% at 120 days. In fact, the present results suggest that the significantly higher ($P<0.05$) survival rate in system II may be due to one or both of the following reasons: (1) a specific diet more appropriate for effective digestion; (2) a better chemical water quality as a result of the recirculating system. In this study, the two algae were mixed in the ratio 1:1 (by wet weight) as diet through out the experiment. The relevant diet differences in both actual comparative experiments, *Chlorella* sp. and *Kirchneriella incurvata* were prove to be greater efficiency

when there were used individually in juveniles after 60 days old (Kovitvadhi et al., 2006). Areekijseree et al. (2006) compared the protein, carbohydrate and lipid digestibility of four different phytoplanktons (*Chlorella* sp., *K. incurvata*, *Navicula* sp. and *Coccomyxa* sp.) by using crude enzyme extracts from 15 days old juvenile *Hyriopsis* (*Hyriopsis*) *bialatus*. It was indicated that a combination of *K. incurvata* and *Chlorella* sp. is a suitable food formula for juvenile culture. This aspect seems to suggest that the diet composition is very important for supporting the survival of the juvenile mussels from 60 to 120 days old, whereas the water quality is more crucial at 0–60 days old. In system II, sand was placed in the culturing container since it could be a source of food such as organic matter or microorganisms (Vogel, 1981; Mann and Lazier, 1991). Juveniles can develop when sand is present, and survival rates are higher than without sand, because they can burrow into the sand as they do in nature, and this helps to prevent the attachment to the shell of feces and pseudofeces with many protozoa and later flatworms and eventual death of the juveniles (Hudson and Isom, 1984; Gatenby et al., 1996; O'Beirn et al., 1998; Kovitvadhi et al., 2006).

The difference between the two systems was the filter cabinet. System I used nylon fiber, gravel and ground freshwater mussel shells as filter materials within the same cabinet but system II used nylon fiber, macrophytes (*Limnophila heterophylla*) and BioBall and these were separated from each other. In addition, the cabinets in system II were larger. These differences resulted in different water quality. Quantities of free carbon dioxide, phosphate, total ammonia nitrogen and nitrate in system II were significantly lower than in system I from days 40, 40, 60 and 10 of rearing, respectively, because the macrophytes could tolerate the environment, and had good growth and filamentous leaves that resulted in more surface area touching the water and absorbing more substance efficiently. The introduction of macrophytes in recirculating systems for sea mussel culture is common (Neori et al., 2000) but for freshwater juvenile culture no report has been found. The pH of system II was between 7.85 and 8.15 which was close to the values measured where growth of blue mussel, *Mytilus edulis*, was slow at pH 6.7–7.1 and better at 7.4–7.6, although the growth increments were not significantly different from those at normal pH 8.1 (Berge et al., 2006). Redding et al. (1997) found that the presence of the emergent plant *Rorippa nasturtium-aquaticum*, the free-floating plant *Azolla filiculoides* or the submerged plant *Elodea nuttalli* significantly reduced total ammonia nitrogen, nitrate and phosphate in comparison with systems lacking macrophytes. Ammonia is the main nitrogenous waste produced by aquatic organisms via metabolism and through the decomposition of organic wastes such as uneaten food and feces. (Goudreau et al., 1993). Sand, a nylon filter layer and BioBall stick provide a place for nitrifying bacteria that will convert toxic ammonia and nitrites into non-toxic nitrates (Al-Hafedh et al., 2003). Layzer et al. (1999) reported that total ammonia nitrogen should be lower than 0.25 ppm to be safe to freshwater unionids. For marine bivalves, MacMillan et al. (1994) reported that the highest level of nitrite and nitrate should not exceed 0.01 ppm and 19.16 ppm, respectively. In this study the total ammonia nitrogen, nitrate and nitrite of system I was higher than system II throughout the experiment but still lower than those two limits. In addition, the nylon fiber layer trapped particles from the water. Calcium is a major component of freshwater pearl mussel shells. Furthermore, silica, sodium, magnesium and iron are essential for growth (Binhe, 1984). Therefore, system II may have had less silica and calcium than system I as a result of their use for shell growth. The coefficient of correlation (r) between average water quality and average survival rate in system II indicated that pH, total alkalinity, total hardness, silica and calcium were important factors in juvenile survival, with a highly significant ($P<0.01$) positive correlation, while for free carbon dioxide and nitrite there was a significant negative correlation ($P<0.05$). Water temperature, dissolved oxygen, total ammonia nitrogen, nitrate and phosphate were not significantly correlated with survival rate ($P>0.05$) since those values were uniformly controlled at very low level by filter cabinets. This is in line with the report of Buddensiek (1995) who compared the coefficient of correlation between water quality and survival rate and growth of the freshwater pearl mussel, *Margaritifera margaritifera* culture in four rivers; that study found that there was a different correlation, with water temperature being an important factor in development of this mussel in all water resources and there was a highly significant difference ($P<0.01$) between rivers. Hence, the present study suggests that the values of water quality parameters has mostly highly significant relationship on growth rate, survival rate or development of juveniles, whereas a gradual decrease in free carbon dioxide and nitrites is beneficial. With regard to dissolved oxygen, total ammonia nitrogen, nitrate, phosphate and temperature, it can be concluded that constant maintenance at the experimental values is very important and probably determinant for correct development.

Therefore, when macrophytes and a biological filter are used in a system developed to a large scale for culture of the freshwater juvenile phase at an industrial level, according to the water recirculating system II, it is important to control free carbon dioxide and total ammonia nitrogen. Finally, from the present study it is possible to propose a very profitable aquaculture system to maintain and control a population of *H.* (*L.*) *myersiana* under excellent conditions from the glochidia in the laboratory to the adults in the natural pond. Additionally, from this research status it should be possible to extend this aquaculture system with few modifications to other freshwater bivalve species.

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