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In Vitro Culture of Freshwater Pearl Mussel from Glochidia to Adult

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1. Introduction

The culture of freshwater pearl mussel is divided into three steps, i.e. glochidia, juveniles and adults. Juvenile has been successfully cultured in the laboratory by attaching glochidia to fish until they could transform into the early juvenile stage (Fukuhara et al., 1990; Panha, 1992; Buddensiek, 1995; Uthaiwan et al., 2003; Hanlon & Neves, 2006). Furthermore, sterilized artificial media could be utilized for the culture of glochidia (to bypass the parasitic stage); the progress of this technique can be followed in a succession of reports by Isom & Hudson (1982, 1984a,b), Keller & Zam (1990), Uthaiwan et al. (2001, 2002), Kovitvadhi et al. (2006, 2007, 2008, 2009), Areekijeree et al. (2006), Lima et al. (2006), Supannapong et al. (2008), Srakaew et al. (2010) and Chumnanpuen et al. (2011). Moreover, some species of freshwater mussel glochidia cultured in artificial media could develop to adulthood as well as inducing gonadal development to sexual maturity and the marsupia could develop, namely *Hyriopsis* (*Limnoscapha*) *myersiana* (Kovitvadhi et al., 2006, 2008), *Chamberlainia hainesiana* (Kovitvadhi et al., submitted) and *Hyriopsis* (*Hyriopsis*) *bialatus* (Kovitvadhi & Kovitvadhi., in preparation).

Therefore, in this chapter author will explain each step from preparation and culture of glochidia in artificial media as well as all techniques of rearing early juvenile through the adult. In addition, water qualities and food suitable for rearing from early juvenile until adult will be described including morphological development under light microscope and scanning electron microscope.

2. Culture of glochidia in artificial media

Glochidia were cultured in artificial media according to Kovitvadhi, (2000); Uthaiwan et al., (2002) and Kovitvadhi et al., (2006) until they transformed to 0-day-old juveniles. The details in each step of glochidia culture in artificial media were as follows:

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2.1 Composition of artificial media

The composition of artificial media for culture of glochidia is shown in Table 1. Artificial media were based on those improved by Keller & Zam (1990), which consisted of a modification from the formulae of Isom & Hudson (1982). The main differences concerned the composition of the commercial media M199. While the protein source was exclusively horse serum in Keller & Zam (1990) and common carp, *Cyprinus carprio* fish plasma was used as an alternative support to the medium for cultured glochidia of *H.* (*L.*) *myersiana* (Uthaiwan et al., 2002; Kovitvadhi et al., 2006, 2007, 2008, 2009), *Hyriopsis* (*Hyriopsis*) *bialatus* (Areekijeree et al., 2006; Supannapong et al., 2008; Srakaew et al., 2010; Chumnanpuen et al., 2011; Kovitvadhi & Kovitvadhi., preparation), *Chamberlainia hainesiana* (Kovitvadhi et al., submitted) and *Anodonta cygnea* (Lima et al., 2006). Glochidia could transform into juvenile in the media containing common carp fish plasma as protein source. These glochidia were completely transformed within 8-11 days with a survival rate up to 93% except 34% of *A. cygnea*. All surviving larvae transformed into the juvenile stage except *A. cygnea* <34%. For these reasons, composition of artificial medium (Table 1) was according to Uthaiwan et al. (2002), based on Isom & Hudson (1982) and Keller & Zam (1990), was proposed for culture of glochidia.

Composition of media	The ratio of artificial medium
M199	2
Common carp fish plasma	1
Antibiotics and antimycotic	0.5

Table 1. Composition of artificial medium for culture of glochidia

2.2 Preparation of glochidia media

2.2.1 M199 preparation

Dissolved one packet of M199 powder (Gibco, No. 6231100-035) in 1 liter volume of sterile distilled water and added 2 g of $NaHCO_3$. Thereafter, M199 was filtered through 0.45 and 0.20 μm filter paper, respectively and kept at 4 °C.

2.2.2 Fish plasma preparation

Common carp was anesthetized with 50 mg/l of quinaldine. Fish blood was collected from the caudal vein, in the tail area, using a syringe needle no. 18 which was coated with sodium heparin at 1000 unit/ml. The blood sample was placed into sterile plastic test tube and centrifuged at 1000 and 3000 rpm for 10 min each. Plasma portion (clear yellow in colour) was separated and placed into the new test tube and centrifuged at 3000 rpm for 10 min. Then, plasma was separated and filtered through 0.45 and 0.20 μm filter paper, respectively and kept at -10 to -20 °C.

2.2.3 Antibiotics and antimycotic preparation

The composition of antibiotics and antimycotic chemicals (Isom & Hudson, 1982) is shown in Table 2.

Compound	Concentration (μg / ml)
Antibiotics	
Carbenicillin	100
Gentamicin sulfate	100
Rifampin	100
Antimycotic	
Amphotericin B	5

Table 2. Combination of antibiotics and antimycotic for culture of glochidia.

2.3 Glochidia media preparation

Medium199 (see Section 2.2.1), fish plasma (see Section 2.2.2) and antibiotics/antimycotic (see Section 2.2.3) were mixed in the ratio 2:1:0.5, respectively (Table 1). The artificial media were divided into sterile plastic test tubes and kept at -10 to -20 °C for stocking culture media.

2.4 Glochidia preparation

Adult freshwater mussel were collected from the natural habitat. They were sexually identified by microscopic observation of sperms and eggs in fluid sucked from the gonads by use of a sterile syringe. Fifteen female and fifteen male adult mussels were cultured together in a cylinder net cage (diameter 50 cm × height 50 cm) in an earthen pond for the production of mature glochidia. They were allowed to feed freely on natural food. After 1-2 weeks, all females were observed marsupial colour by tongs to open the shell slightly, which marsupial colour indicates the development of larval stage. In the immature stage, glochidia was yellow in colour, while partially brown colour was at the beginning of maturity. Only completely brown marsupia of gravid mussel was selected in order to examine the strong and suitable glochidia for culturing in artificial media. Thereafter, the outer shell of gravid mussel with completely brown marsupia was washed with tap water and then sterile tap water. The glochidia were sucked by using a sterilized 1 ml syringe and discharged into depression or well slide with sterilized distilled water. Then, the glochidia were observed under a light microscope (×400). If their shells periodically closed, they were sucked according to abovementioned. Later, they were cleaned to eradicate tissue residues, mucus and glochidia shell fragments by spraying sterilized distilled water onto them. Complete cleaning and stronger glochidia were used for culture. Glochidia from gravid mussels should be cultured in artificial media within 5 h after harvesting.

2.5 Glochidia culture

Approximately 5000–6000 glochidia were transferred into a culture dish (90×15 mm) containing 10 ml of artificial medium (Table 1). The culture dishes were placed in a low-temperature incubator at 25 °C with 5% CO_2. The culture medium was removed and replaced with fresh medium in the middle of cultured period. Finally, 4 ml of sterilized

distilled water was added to the culture dish to stimulate the transformation when the mantle (Fig. 1A) was observed before 1 day of transformation from glochidia into juveniles. Juvenile transformation was observed under a light microscope (×400) for the movement of juvenile foot (Fig. 1B) and also juvenile movement as an indicator of the glochidia transformation success into juvenile stage.

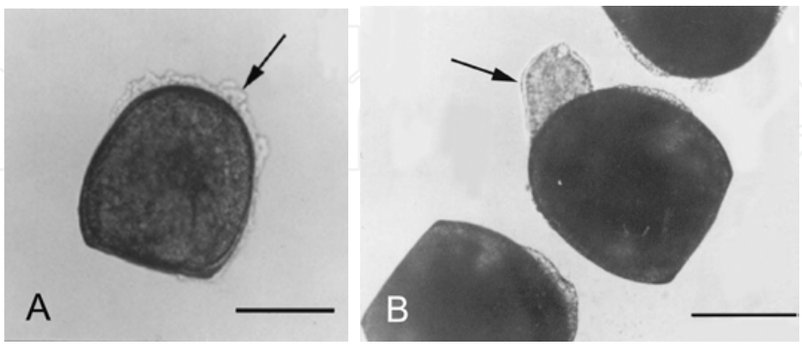


Fig. 1. Light microscope of glochidial development to early juvenile. A; Glochidial development with the mantle edge bordering shell outside (arrow). B; Early juvenile with a foot (arrow). Bar = 100 μm (Uthaiwan et.al., 2001).

3. Selection of phytoplankton food species for juveniles

Phytoplankton has proven to be a vital source of nutrient for several species of freshwater mussel juveniles (Hudson & Isom, 1984; Gatenby et al., 1996; Gatenby et al., 1997; ÓBeirn et al., 1998; Uthaiwan et al., 2001). Moreover, Kovitvadhi et al. (2000) reported that phytoplankton contributed to 99% of the gastrointestinal tract content of the adult freshwater pearl mussel, *H. (L.) myersiana* in natural habitat. Consequently, phytoplankton from the gastrointestinal tract of adult were cultured and selected for juvenile feeding. Collecting mussels of different sizes from natural habitat, which phytoplankton existed in the gastrointestinal tract then it was cutting and sucking to culture in sterilized water with f/2 media (Guillard & Ryther, 1962). Then, they were cultured under light not less than 10,000 Lux for 12 h as well as in mixed about 3% carbon dioxide. From there, phytoplankton to be cultured were in the process of sub-culture and purified every 2-10 days by streak plating technique (Hoshaw & Rosowski, 1973). Streak plates were placed under light until the single colonies of phytoplankton appeared which might last for 10-30 days. Thereafter, those phytoplankton were kept in slant tube. Whenever phytoplankton were required for feeding juveniles, those phytoplankton were multiplied in 1 liter bottle by using the same separated formulae. Kovitvadhi et al. (2006) as abovementioned found ten species of phytoplankton; *Ankistrodesmus gracilis*, *Chlamydomonas* sp., *Chlorella* sp.1, *Chlorella* sp.2, *Kirchneriella incurvata*, *Monoraphidium* sp., *Navicula* sp., *Scenedesmus* sp., *Stichococcus* sp. and *Coccomyxa* sp. Thereafter, all phytoplankton were selected for suitable cultured juvenile which should be considered based on 6 criteria according to Areekijseree et al. (2006); Kovitvadhi et al. (2006); Supannapong et al., (2008): (1) size (2) capability of filter into gastrointestinal tract by observing under microscope within 30 min and 1.30 h after giving phytoplankton (3) the movement of cilia at gill, mantle and foot by observing under microscope (4) color changes in gastrointestinal tract which should be from green to yellow or brown and shape from normal to debris (5) carbohydrate and protein contents of

phytoplankton (6) efficiency of digesting carbohydrate and protein of phytoplankton by using crude enzyme extracts from juveniles (*in vitro* digestibility). The major phytoplankton were suitable for culturing juvenile of freshwater mussel, namely *Chlorella* sp. 2 and *K. incurvata* (Table 3).

Phytoplankton	Size[1] (µm)	Filtration time (h)[1]		Cilia activity[1]		Phytoplankton change[1]	Carbohydrate[2] (%)	Protein[2] (%)	Digestibility[2]	
		0.30	1.30	Gill	Foot				Carbohydrate	Protein
Ankistrodesmus gracilis	3×20	-	-	-	-	-	43.50	13.67	122.22	9.93
Chlamydomonas sp.	8×9	-	-	-	-	-	25.67	20.00	158.74	11.50
Chlorella sp.1	3	+	+	-	+	+	45.80	22.50	130.74	11.25
Chlorella sp.2	4	+	+	-	+	+	37.32	25.45	231.77	15.95
Kirchneriella incurvata	4	+	+	+	+	+	21.64	12.27	248.82	16.67
Monoraphidium sp.	12×20	-	+	-	-	+	26.29	17.50	155.08	11.23
Navicula sp.	4×16	+	+	+	+	+	24.2	8.31	-	-
Scenedesmus sp.	16×20	-	+	-	+	-	37.47	16.00	158.73	12.67
Stichococcum sp.	3-4	+	+	-	-	-	82.31	16.33	131.95	11.46
Coccomyxa sp.	3×5	+	+	+	+	+	21.89	25.83	168.47	9.50

Table 3. Characteristic of phytoplankton isolation from gastrointestinal tract of adult freshwater mussel in natural habitat and *in vitro* digestibility for carbohydrate (µg maltose mg plankton^{-1}) and protein (µg DL-alanine equivalent mg plankton^{-1}) of ten algal species at seven days old, using crude enzyme extracts from 30-day-old juveniles. [1] Kovitvadhi et al. (2006), [2] Supannapong et al. (2008).

4. Juvenile culture

After glochidia transformed to juvenile in artificial medium, water of recirculating aquaculture system was added in the ratio of medium to water equaled 3:1, 1:1, 1:3 for 30 min, added *K. incurvata and Chlorella* sp. in ratio 1:1 at density of 1×10^5 cells per ml in ratio medium to water equaled 1:3. From there, cultured juvenile was transferred to culture units. Culture of juvenile stage could be divided into 3 stages, namely first stage beginning from 0 days old juvenile until two shell mussel completely closed. Kovitvadhi et al. (2008) cultured juvenile 0-120 days old of freshwater mussel, *H.* (*L.*) *myersiana* by recirculating aquaculture system (Fig. 2). This system comprised of three filter cabinets made of 6 mm thick acrylic (particulate filter cabinet, macrophytes filter cabinet and biological filter cabinet), one water resting cabinet (Fig. 2E) and nine plastic culture units (Fig. 2F). The size of particulate filter cabinet (Length×Width×Height×Water level = 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 (Fig. 2B). Water flowed through this filter and via the second to the macrophytes filter cabinet. The size of macrophytes filter cabinet (Length×Width×Height×Water level = 80×40×51×42 cm) 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 (Fig. 2C). 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 water surface equal to 5320 lux; 24 h) (Fig. 2G). The water then flowed into the biological filter cabinet (60×34×51×42 cm) filled with bioball to full capacity (Fig. 2D) 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 (<120 μm) at 0.27 g/cm^2. The inside of the culture unit was divided into two section, as described previously, but of different sized (section 1-66.1×14×15×7 cm; section 2-17.9×14×15×7 cm). The first section also consisted of five acrylic sheets jutting from the walls on alternate sides. Juveniles were fed *Chlorella* sp. and *K. incurvata*. Each species of algae 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 to an algae 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.

Second stage, thirty-five juveniles (120 days old) were transferred to culture units (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. 3). This lower part contained 400 g of sand (<425 μm in size). The juveniles were placed directly on the sand. The culture unit was then hung in the earthen pond; 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 the culture unit were rinsed every 10 days.

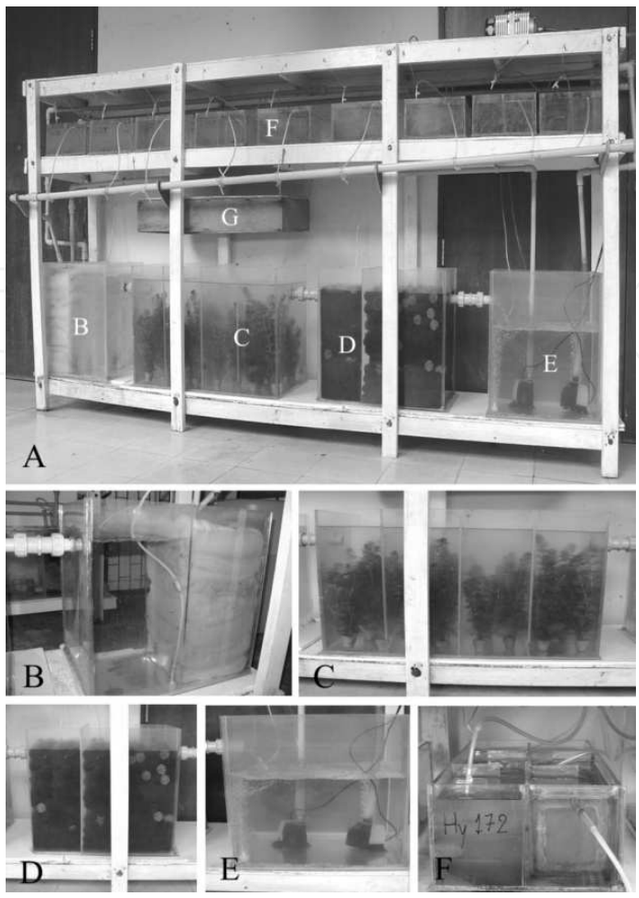


Fig. 2. Photographs of the recirculating used to rear freshwater pearl mussel juveniles. A; Recirculating aquaculture system, B; Particulate filter cabinet, C; Macroplants filter cabinet, D; Biological filter cabinet, E; Water resting cabinet, F; Plastic culture unit, G; Fluorescent box.

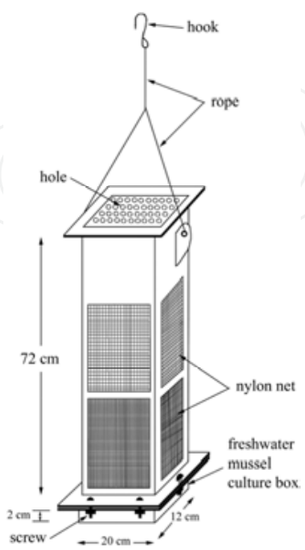


Fig. 3. Rearing container of juveniles (Kovitvadhi et al., 2006).

Third stage, juveniles (180 days old) were transferred to culture into natural habitat or the earthen pond by a cylinder net cage until adult.

5. Adult culture

At present, adult of some species freshwater pearl mussels had been successfully cultured in an earthen pond and natural habitat. They had high survival and could produce glochidia stage such as *H.* (*L.*) *myersiana* (Uthaiwan et al., 2002; Kovitvadhi et al., 2006, 2007, 2008, 2009), *H.* (*H.*) *bialatus* (Areekijeree et al., 2006; Supannapong et al., 2008; Srakaew et al., 2010; Chumnanpuen et al., 2011; Kovitvadhi & Kovitvadhi., preparation), *C. hainesiana* (Kovitvadhi et al., submitted). Fifteen female and fifteen male adult mussels, were cultured together in a cylinder net cage (diameter 50 cm × height 50 cm). Then, it was hung under the raft (Fig. 4) at 1.5-2 m deep from water surface which phytoplankton were plenty at this level. The cage was shaken every week for protecting biofouling attachment which could mass mortality.

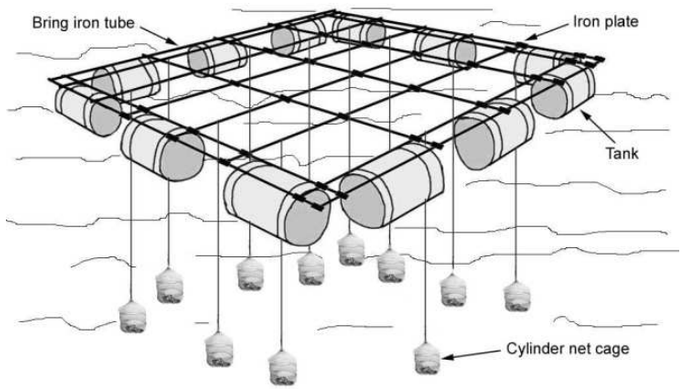


Fig. 4. Raft for adult freshwater pearl mussel culture. (Kovitvadhi, 2008).

6. Water analysis

Prior to culturing mussel, water quality in habitat, was studied in water improvement suitable to growth and survival which conformed to Kovitvadhi et al. (2006, 2008) that juvenile cultured in the laboratory and the earthen pond nearby natural habitat (Kovitvadhi et al., 1998) (Table 4). Water quality parameters should be analyzed for freshwater pearl mussel culture: water temperature, pH, tubidity, conductivity, dissolved oxygen, total alkalinity, free carbon dioxide, total hardness, total ammonia nitrogen, nitrite, nitrate, phosphorus, silica and calcium. In this connection, juvenile stage had been more sensitive to environmental changes than another stages, particularly water qualities suitable and rather stable; water temperature, pH, free carbon dioxide, dissolved oxygen, nitrate and phosphorus and decreasing values; total alkalinity, total hardness, total ammonia nitrogen, silica, and calcium except nitrite that had increasing value (Fig. 5). When averaged water quality value was calculated to relationship with averaged survival value and shell length with equation: $Y=b_0+b_1X+b_2X^2+b_3X^3$ where Y is the survival or shell length, X is age (days), and b_0, b_1, b_2 and b_3 are parameters. It was found that survival of 0-120-day-old juveniles would have direct relationship with pH, total alkalinity, total hardness, silica and calcium with highly significant difference ($P<0.01$) and with reverse relationship to free carbon dioxide and nitrite (Table 5) (Kovitvadhi et al., 2008).

Water quality	Culturing of juvenile in laboratory[1]		Culture of mussel in the earthen pond[1]	Culture of mussel[1]	Mussel Habitat[2] (min. – max.)
	0 - 60 day old (mean±SD)	60 - 120 day old (mean±SD)	120 -360 days old (mean±SD)	0 -360 days old (mean±SD)	
Water temp. (°C)	25±0.74	28±0.54	26.5±1.1	24.5-28.5	23.8-31.6
pH	7.03±0.02	7.51±0.04	7.65±0.46	6.85-8.08	6.92-8.14
Dissolved oxygen ($ppmO_2$)	8.1±0.07	7.5±0.04	5.0±0.6	4.2-8.2	2.5-9.0
Total alkalinity ($ppmCaCO_3$)	52±1.41	52.75±0.35	83.7±20.3	50-114	62.5-115.0
Free carbondioxide ($ppmCO_2$)	10±0.04	4.25±0.35	3.95±2.7	0-10.2	0-6.0
Total hardness ($ppmCaCO_3$)	154±2.83	123±9.90	196.8±12.6	121-222	90-133
Ammonia nitrogen ($ppmNH_3$-N)	0.42±0.02	0.28±0.01	0.44±0.19	0.20-0.82	0.22-0.88
Calcium ($ppmCaCO_3$)	139±9.9	89±4.24	101.1±5.1	86-142	65-105
Phosphorus (ppmP)	0.12±0.06	0.19±0.07	0.17±0.1	0.01-0.45	0.08-0.88
Silica ($ppmSiO_2$)	4.85±0.6	4.05±0.5	5.75±1.6	3-8	0.2-5.5

[1]Kovitvadhi et al. (2006), [2]Kovitvadhi et al. (1998).

Table 4. Water quality during culturing of 0-360 day-old juveniles and the adult mussel habitat of *H.* (*L.*) *myersiana* in the Mae Klong River, Kanchanaburi Province.

Parameter	Survival	Shell length	Shell height
Water temperature	-0.093^{ns}	0.075^{ns}	0.107^{ns}
pH	0.716**	-0.597*	-0.590*
Dissolved oxygen	-0.118^{ns}	-0.055^{ns}	-0.035^{ns}
Total alkalinity	0.841**	-0.849**	-0.827**
Free carbon dioxide	-0.634*	0.481^{ns}	0.476^{ns}
Total hardness	0.769**	-0.764**	-0.751**
Total ammonia nitrogen	-0.152^{ns}	-0.051^{ns}	-0.061^{ns}
Nitrite	-0.716**	0.709**	0.688**
Nitrate	0.203^{ns}	-0.200^{ns}	-0.218^{ns}
Phosphorus	0.003^{ns}	-0.085^{ns}	-0.091^{ns}
Silica	0.914**	-0.913**	-0.091**
Calcium	0.817**	-0.751**	-0.761**

Table 5. Coefficient of correlation between average survival rate and water quality; average growth rate and water quality of juvenile *H.* (*L.*) *myersiana* cultured in recirculating aquaculture system every 10 days. (Kovitvadhi et al., 2008) (* = $P<0.05$, ** = $P<0.01$, *ns* = not significant difference, $P>0.05$).

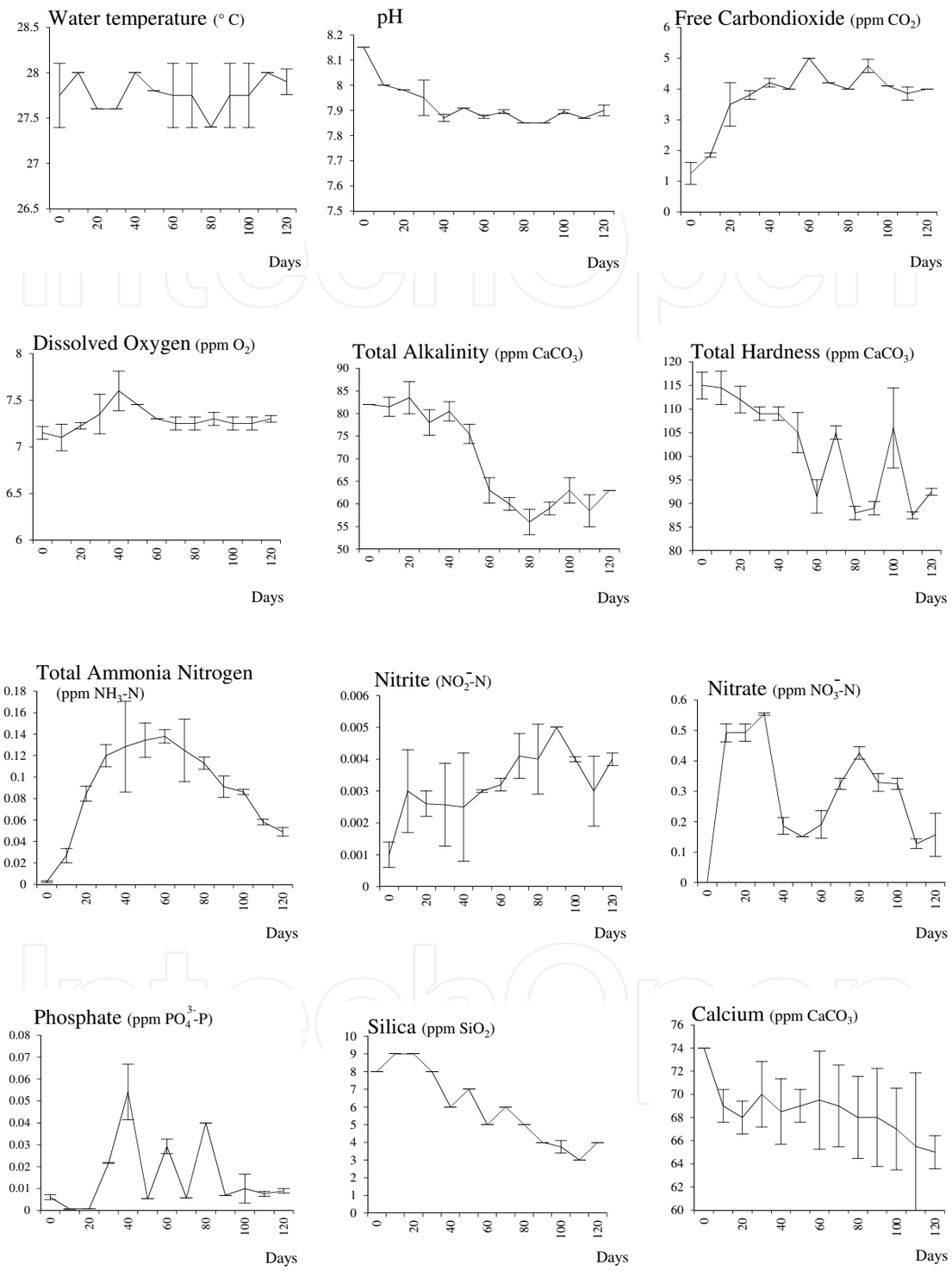


Fig. 5. Water quality during culture for 0-120 days of *Hyriopsis* (*Limnoscapha*) *myersiana* juveniles in recirculating aquaculture system (Kovitvadhi et al., 2008).

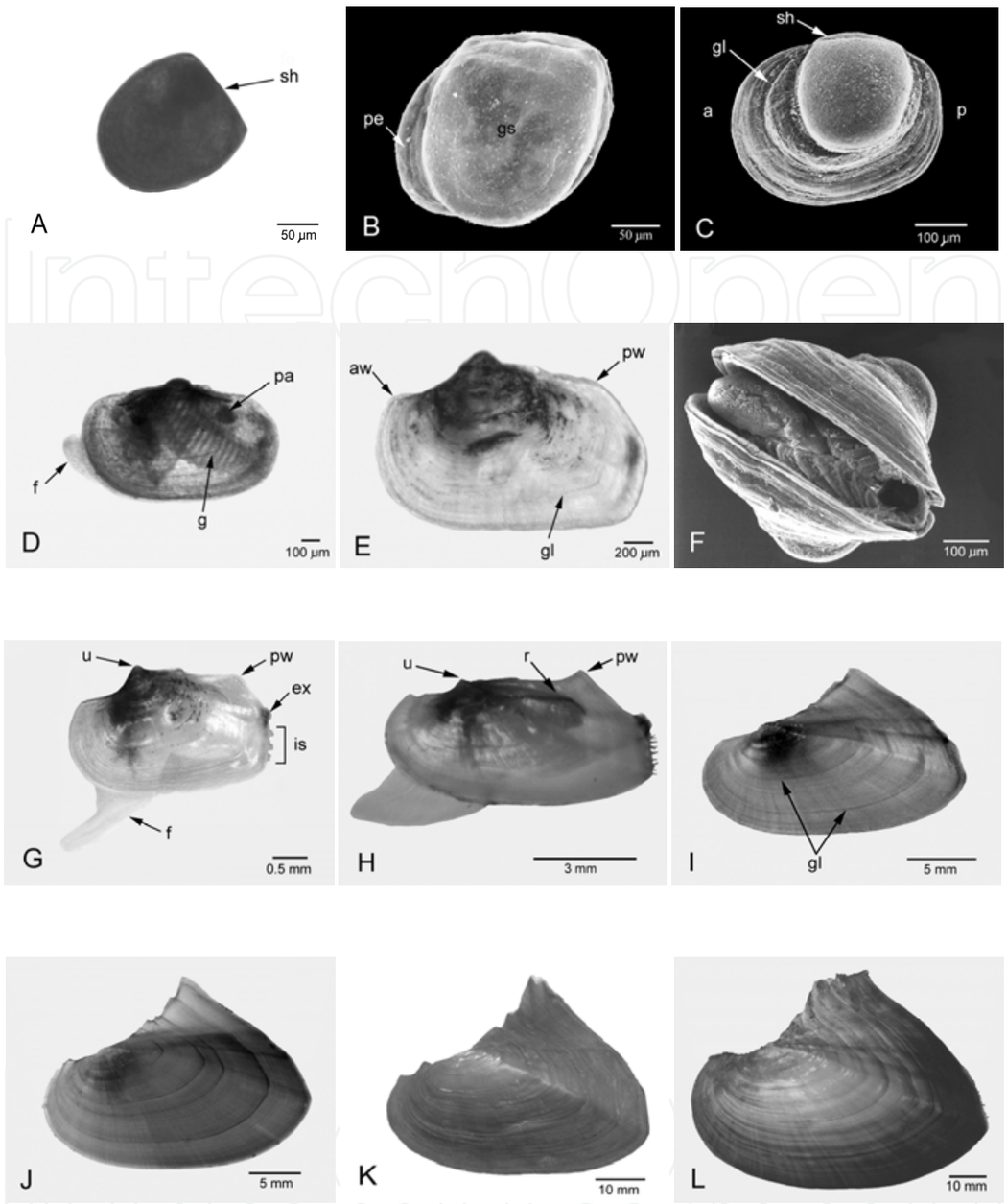
7. Phytoplankton communities

Phytoplankton was found in the gastrointestinal tract, which it was a source of nutrient for several species of freshwater mussel (Hudson and Isom, 1984; Gatenby et al., 1996; Gatenby et al., 1997; ÓBeirn et al., 1998; Kovitvadhi et al., 2000, 2001). This finding is consistent with gut content analyses from other bivalve species (Gale & Lowe, 1971; Huca et al., 1983; Paterson, 1986; Parker et al., 1998). From abovementioned data, it is confirmed that freshwater pearl mussels will filter phytoplankton as the main food. Therefore, culture of freshwater pearl mussels from juvenile to adult, it is necessary to have available phytoplankton species used as food for juvenile and in suitable amount throughout culturing period both in the laboratory and natural resource since freshwater mussel has to filter phytoplankton all the time. Thus, density of phytoplankton used for feeding juvenile or adult is not necessary in surplus but the food must be available throughout the culturing period which will result in increasing survival and growth.

8. Morphological development of freshwater mussel

Since the glochidia freshwater pearl mussel of *H.* (*L.*) *myersiana* was cultured in the artificial media that could develop to the adult (Kovitvadhi et al., 2006). Therefore, Kovitvadhi et al. (2007) could study for the morphological development of the juvenile through the adult *H.* (*L.*) *myersiana*. The mussels were collected in sequential developmental stages between 0 and 360 days old. Morphological development was observed by light microscope and SEM. SEM observations were prepared in fixative solution containing 10% neutral buffered formalin for 24 h and stored in 5% neutral buffered formalin for further process. The samples for SEM were thoroughly washed under running water for 30 min and then dehydrated in a graded series of ethanol and dried to critical point. Thereafter, they were mounted on SEM specimen stubs with conductive silver paint and coated with gold and observed with a Jeol Model JSM-5410LV scanning electron microscope operated at 25 KV. All samples before fixation, they were anesthetized in 2% chloral hydrate to observe the internal regions.

The morphological development of *H*. (*L.*) *myersiana* juveniles in culture (0-360 days old) is shown in Fig. 6. The early juvenile of *H*. (*L.*) *myersiana* at 0 days old after transformation has semi-oval, equivalve shells with an equilateral valve, presenting the same size and shape as the glochidium (Fig. 6A). Anterior shell growth was clearly seen in the first day of juvenile development (Fig. 6B), while posterior shell growth followed afterwards (Fig. 6C). The shells of 0-40-day-old juveniles were thin and transparent as seen under light microscope (Fig. 6D). 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 in this period (Fig. 6E). The shell, however, became thicker during the developmental process and covered all the inner organs (Figs. 6E-L). The first anterior and posterior wings appear in 50-day-old juveniles (Fig. 6E), with the posterior wing becoming dominant relative to the anterior from the 140-day-old stage (Fig. 6H). The mantle lobes of 0-50-day-old juveniles are joined dorsally and are free ventrally (Fig. 6F). The incurrent siphon and excurrent siphon appear after 50 days (Figs. 6G-6L). The complete adult morphology is apparent from 160 days old (Fig. 6I). Males and females reproductive organs are sexually mature about 270-360 days old mussel which depend on environment (Kovitvadhi et al., 2006; Srakaew et al., 2010) (Figs. 6K-6L).



A; Light microscopy of early juvenile (after transformation, 0-day-old) shell, note shell hinge (sh). B; SEM micrograph of 1-day-old juvenile, appearance in anterior region of new soft periostracum (pe), note glochidium shell (gs). C; Juvenile 10 days old, anterior (a) region appears before and grows more than the posterior (p), note growth line (gl). D; Light microscopy of development of shell, 40-day-old juvenile, note foot (f), gill (g), posterior adductor muscle (pa). E; Light microscopy of development of shell, 50-day-old juvenile, note anterior wing (aw); posterior wing (pw). F; SEM of ventral side of 50-day-old juvenile. G; Light microscopy of external morphology of 90-day-old juvenile, note excurrent siphon (es), incurrent siphon (is), umbo (u). H; Light microscopy of external morphology of 140-day-old juvenile, note rectum (r). I-L; External morphology of shell, 160, 180, 270 and 360 days old, respectively.

Fig. 6. Morphological development of 0–360-day juveniles of *Hyriopsis* (*Limnoscapha*) *myersiana*. (Kovitvadhi et al., 2006; 2007).

9. Summary

Culture of freshwater pearl mussel is divided into the three consecutive steps: (1) culture of glochidia larvae in artificial media, (2) rearing juveniles and (3) rearing adult. The results of several studies indicate that glochidia in some species of freshwater mussel could be cultured in artificial media containing mixtures of M199, common carp plasma, antibiotics and antimycotic, and could have fully developed adult and gametogenesis was complete. The important factors in juvenile culture included culturing systems, water quality, substrate (sand) and food. The laboratory-scale recirculating aquaculture system, which water quality change was rather stable and sand could attached materials for food such as organic matter or microorganisms. Furthermore, the juveniles can burrow into the sand as they do in nature, and this helps them to prevent the attachment to the shell of feces and pseudofeces with many protozoa and later flatworms and eventual death of the juveniles. However, the size of sand should appropriate for each size of the juvenile. Phytoplankton was a vital source of nutrients, which has suitable size and shape to move into the mouth of the juveniles so that juveniles can digest them. Prior to transfer to outdoor, mussel organs have to fully developed for ingesting food, particularly gills, the incurrent and excurrent siphon, and their shells must close completely. Moreover, the water quality and food were also important factors to growth and survival.

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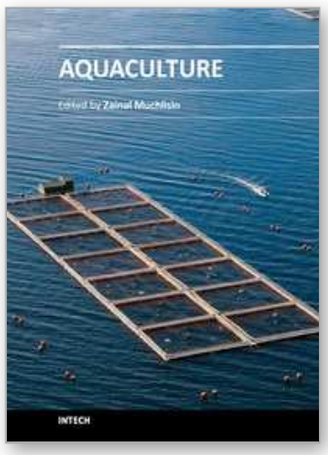

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