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Effects of different types of artificial substrates on nursery production of freshwater prawn, *Macrobrachium rosenbergii* (de Man) in recirculatory system

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Abstract

An experiment was conducted for two months in a recirculatory system consisting 12 glass aquaria (size 41× 41 × 46 cm) to evaluate the effects of substrates on the growth and survival of *Macrobrachium rosenbergii* postlarvae (PL). Treatment T₁ having no substrate was considered as the control. Treatment T₂ contained pieces of hollow PVC pipe, treatment T₃ and T₄ were provided with high density polyethylene (HDPE) and black nylon netting, respectively. Each treatment had three replicates. PL-15 of *M. rosenbergii* (mean initial length 1.20 ± 0.02 cm; mean initial weight 27 ± 0.02 mg) were stocked at the rate of 75 PLs (1.25 PL L⁻¹) in each aquarium. At the beginning PLs were fed three times daily at the rate of 20% of their body weight which was reduced to 10% at the start of 2nd month. The ranges of water quality parameters recorded in different treatments were: temperature 26 - 29 °C; dissolved oxygen 6.2 - 8.1 mg L⁻¹; pH 6.8 - 8.1 and total ammonia 0.01 - 0.15 mg L⁻¹. The result of the study showed that there was no significant difference (P>0.05) in final length of PLs between treatments T₃ (HDPE netting) and T₄ (nylon netting) but these values were significantly higher than those in T₁ (control). There was no significant difference (P>0.05) in final weight and specific growth rates (SGR) of PLs in treatments T₃ and T₄ but these values were significantly higher than those in T₂ (PVC pipe) and T₁ (control). Final weights of PLs were 32.70%, 31.54% and 21.05% higher in treatments T₃, T₄ and T₂ respectively than T₁ (without substrate). The FCR values ranged between 1.85 and 1.88. There was no significant difference (P>0.05) between the survival of PLs which ranged between 80.33 and 83.00%. Result of the study indicated that growth of *M. rosenbergii* PLs improved in presence of artificial substrates but the artificial substrates did not improve survival. HDPE and nylon netting gave the best results compared to PVC pipes and control treatments in terms of growth and feed efficiency. Therefore, use of HDPE or nylon netting may be recommended as substrate for successful nursing of *M. rosenbergii* PLs. However, further studies using different substrates should be carried out in nursery ponds to ascertain the usefulness of these substrates for nursing of *M. rosenbergii* PLs.

Keywords: Nursery rearing, Freshwater prawn, Postlarvae, Artificial substrates

Introduction

The freshwater prawn (*Macrobrachium rosenbergii* de Man 1879) is indigenous to most Southeast Asian and South Pacific countries (New, 1982). Since its successful domestication the culture of freshwater prawn has gained great popularity worldwide (Ling, 1969), mostly in the tropics and subtropical regions with the limited production in temperate regions such as North America (D'Abramo *et al.*, 1989). There has been a very rapid global expansion of freshwater prawn farming since 1995; this is mainly because of the huge production in China, but also, in the last few years, because of a rapid take-off of farming in India and Bangladesh (New, 2005).

M. rosenbergii nursery system is an intermediate stage between larval rearing and grow-out, in which postlarvae (PL) are cultured at high densities from metamorphosis to juveniles. The use of nursery systems as an intermediary stage between hatcheries and grow-out ponds was initially suggested by Ling (1969) and later became especially important for locations where climatic and water restriction do not allow continuous culture (New, 1995; Tidwell *et al.*, 2005). Nursing of newly raised *M. rosenbergii* PL for 1-2 months period, prior to stocking in the grow-out pond, is an important step in freshwater prawn aquaculture in Bangladesh (Alam *et al.*, 1997), making it possible to obtain three yearly cycles and increase productivity (Zimmermann and Sampio 1998). The juveniles fetch higher price and the nursery farmers can make higher profit by nursing the PLs to juveniles. The prawn farmers are very keen to stock juveniles because of their higher survival rates compared to the PLs.

There are a number of potential benefits associated with the use of a nursery phase, including better control of stock inventory, greater uniformity of market size at harvest (Raánan *et al.*, 1984; Tidwell *et al.*, 2005), less predation, and the ability to extend the growing season (Malecha, 1986; Persyn and Aungst, 2001). Juvenile's survival rate is improved because larger animals are hardier (Alston, 1989). Juveniles are more resistant than PLs to predation (New and Singholka, 1985), cannibalism, and fluctuating environmental conditions in grow-out ponds (Fujimura and Okamoto, 1972).

Survival at the end of the nursery phase can vary substantially and may be related to the territorial and cannibalistic nature of prawn PLs when cultured at high densities. Nursery production may be enhanced by the addition of artificial substrates to increase the surface area upon which prawn graze and to use them as refuge. Several authors have suggested the use of artificial substrates to increase the amount of two dimensional spaces available to prawn and thereby yielding an increase in survival and production (Tidwell *et al.*, 1998; D'Abramo *et al.*, 2000). With the addition of artificial substrates, PLs utilize the three-dimensional volume of the tank, rather than only the walls and bottom.

Although considerable reports are available on the use of artificial substrates in prawn farming system, reports on improvement in the nursery rearing of freshwater prawn, *M. rosenbergii* PLs, using artificial substrates in recirculation tank (where, better environmental control is possible) is scanty (Tidwell *et al.*, 2005). Moreover, systematic studies for developing suitable substrates to produce more advance juveniles (>0.3g) of *M. rosenbergii* PLs have not been done. Therefore, investigation into potential benefits derived from using artificial substrates for prawn nursery is necessary. Thus, in the present study, an experiment was conducted in a recirculatory system to evaluate the effects of different artificial substrates on the growth, feed utilization and survival of *M. rosenbergii* PLs.

Materials and Methods

The experiment was conducted in a recirculatory system in the wet laboratory of the Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh for a period of 60 days during the month of June to August, 2006.

Experimental system

The experimental system consists of 20 rectangular glass aquaria (each of size 46× 41× 41 cm) containing about 65 L of water each. Altogether 12 aquaria were used for the study. All the aquaria were kept on a rack made of iron bar to facilitate better observation and accessibility. Each of the aquarium received water at the rate of 1.5 L/min. The outflow of water from the experimental aquaria drained through the standpipe. The opening of the standpipe is 1.5 cm inner and 2.0 cm outer diameter and covered by 1 mm mesh screen to prevent the escape of prawn PLs. Water was circulated through a common biological filter system under gravity before flowing into a sump tank so that all the replicate aquaria shared similar water. The biological filter drums contained net wrapped small plastic cans and small marble stones that promoted settling of wastes by increasing retention time and provided a substrate for attachment of nitrifying bacteria. At the mouth of the first filter tank a filtering device was placed to collect incoming solid wastes (uneaten food, faeces and other solid wastes) from the water. Water was pumped from the sump tank using a pump (Johnson pump, MDR-Series, Sweden) to the overhead tank for distribution into the experimental aquaria. Excess water from the overhead tank overflowed to the sump tank through a PVC pipe. A high degree of recirculation was maintained with minimal replacement of evaporation and splashing losses by fresh water make-up. An adequate level of oxygen in each aquarium was maintained through artificial aeration using an air pump (Hiblow Air Pump, SPP-100GJ-H, Techno Takatsuki Co. Ltd., Japan). Natural photoperiod of 12 h light and 12 h dark was maintained throughout the experimental period.

Source of postlarvae (PLs) and acclimatization

The PL-15 of *M. rosenbergii* collected from Freshwater Station, Bangladesh Fisheries Research Institute (BFRI), Mymensingh were brought to Bangladesh Agricultural University in oxygenated bags. The PLs were kept in the recirculatory systems for 7 days to acclimatize with the new environment and during this time they were fed a commercial prawn nursery feed (32% protein) from Saudi-Bangla Fish Feed Ltd., Bhaluka, Mymensingh, Bangladesh.

Experimental feed

A Saudi-Bangla nursery Golda feed containing 32% protein from Saudi-Bangla Fish Feed Ltd., Bhaluka, Mymensingh, Bangladesh were used for rearing the PLs in the recirculatory system (Table 1).

Table 1. Analyzed proximate composition of the nursery feed¹ used for rearing of *M. rosenbergii* PLs (% dry matter basis)

Components	Amount (%)
Dry matter	89.96
Crude protein	30.20
Crude lipid	11.60
Ash	22.68
Crude fibre	9.60
NFE ²	25.92

¹Saudi-Bangla Fish Feed Ltd., Bhaluka, Mymensingh, Bangladesh.

²Nitrogen free extract calculated as 100- % (protein + lipid + ash + crude fiber)

Experimental design and feeding rate

The experiment was conducted in completely randomized design. Three artificial substrates were assigned to three different treatments. Each treatment had three replicates. Treatment T₁ received no substrate and considered as the control. Treatment T₂ contained 2 pieces of hollow PVC pipe (15 cm long, 2 cm internal diameter) placed on the bottom of each aquarium. The PVC pipes increased the surface area and hollow areas provided more space for hiding of PLs. Treatment T₃ was provided with HDPE (High Density Polyethylene) black netting (1.2 cm mesh) oriented vertically to increase available surface area in the aquarium by about 40% and attached with the aquarium wall by binder clip (Shihmark® Binder clip, SQ-155, China). Black nylon netting (1.0 cm mesh) was used as substrate in treatment T₄. Piece of net material was installed vertically across the aquarium and tied with binder clips. Each net material installed in an aquarium had an area of about 1620 cm² to increase available surface area by about 40 %.

PLs were hand-counted and stocked at the rate of 75 PL (1.25 PL L⁻¹) in each aquarium. The mean initial length and weight of PLs were 1.20 ± 0.02 cm and 27 ± 0.02 mg, respectively. PLs were fed at a rate of 20% of their body weight at the beginning and feeding rate was gradually reduced to 10% at the start of 2nd month. The total amount of feed for a day was divided in to three equal proportions and delivered at 900, 1300 and 1700 h daily. Uneaten feed, feces, and exuviae were removed by siphoning from tank bottoms immediately prior to each morning and afternoon feeding. A record of supplied feed was kept for determining the feed conversion ratio (FCR) and protein efficiency ratio (PER).

Fortnightly sampling of about 25% of the total PLs was done to observe the growth of PLs and to adjust the feeding rate. The weight of PLs during each sampling was measured by a digital electronic balance (OHAUS, Model CT1200-S, Princeton, NJ, USA). Total length of *M. rosenbergii* PL/juvenile at the start and end of the experiment was measured from the tip of the rostrum to the tip of the telson by a centimeter scale. The sampled PLs were handled very carefully as the species is very susceptible to handling stress.

Water quality parameters viz. water temperature, dissolved oxygen, pH and total ammonia were monitored weekly throughout the experimental period. Water temperature and pH were measured by a Hach pH meter (model Sension 1, Germany) and dissolved oxygen by Hach DO meter (model Sension 6, Germany) and total ammonia by a Hach kit (model DR 2010) following the standard method. At the end of the experiment all the juveniles from each aquarium were counted and batch weighed.

Calculations and statistical analysis

Weight gain (g), specific growth rate (SGR % day⁻¹), food conversion ratio (FCR), protein efficiency ratio (PER) and survival (%) of the PLs were calculated according to Castell and Tiews (1980). Food conversion ratio and protein efficiency ratio were considered as apparent as no correction was made for uneaten food left (if any). The proximate composition of the feed was analyzed according to AOAC (2000). Crude protein was determined by the Kjeldahl method (total N × 6.25) with a Kjeldahl System (1007 Digestion System, 1002 Distilling unit, and Titration unit, Tecator, Hoganas, Sweden) using boric acid to trap released ammonia. Lipid was determined by Soxhlet method using diethyl ether as the organic solvent. Crude fibre contents in diet samples were determined using a Fibretech system (model M1017 Hot Extractor, Tecator, Hoganas, Sweden). The nitrogen-free extract was calculated by difference. Data on weight gain, SGR, FCR, PER, and survival were analyzed using one-way ANOVA (MSTAT-C package program). Significant variation between the means was evaluated by Duncan's new multiple range test (Duncan, 1955). A significance level of P<0.05 was used.

Results and Discussion

Water quality parameters such as temperature, pH, dissolved oxygen and total ammonia were measured weekly during the experimental period are shown in Table 2. The ranges were: temperature 26-29 °C; dissolved oxygen 6.2-8.1 mg L⁻¹; pH 6.8-8.1 and total ammonia 0.01-0.15 mg L⁻¹. All the water quality parameters were well within the acceptable range for PL nursing.

Table 2. Ranges of water quality parameters observed during the experimental period

Parameters	Ranges
Water temperature (°C)	26.0-29.0
Dissolved oxygen (mg L ⁻¹)	6.2-8.1
pH	6.8-8.1
Total ammonia (mg L ⁻¹)	0.01-0.15

There was significant (P<0.05) effects of substrates on final length of juveniles. However, there was no significant difference (P>0.05) in final length of juveniles between treatment T₃ (HDPE netting) and T₄ (nylon netting) and these values were significantly higher than those in T₁ (control). Again there was no significant difference (P>0.05) between the final length of T₁ and T₂ (Table 3).

Table 3. Growth performance in terms of length of *M. rosenbergii* PLs

Parameters	Treatments			
	T ₁	T ₂	T ₃	T ₄
Initial length (cm)	1.20 ± 0.02	1.20 ± 0.02	1.20 ± 0.02	1.20 ± 0.02
Final length (cm)	4.21 ^{b1} ± 0.27	4.50 ^{ab} ± 0.33	4.89 ^a ± 0.14	4.86 ^a ± 0.13
Gain in length (cm)	3.01 ^a ± 0.27	3.23 ^a ± 0.42	3.69 ^a ± 0.14	3.66 ^a ± 0.13
% Length gain	251.16 ^b ± 22.86	274.99 ^{ab} ± 27.63	307.77 ^a ± 11.31	305.28 ^a ± 11.10

¹Values are mean ± standard deviations and values in the same row having same superscripts are not significantly different (P>0.05)

The result of this study showed that there was significant (P<0.05) substrate effects on growth performance of prawn. The growth of PLs reared at treatment T₃ (HDPE netting) and T₄ (nylon netting) were significantly (P<0.05) higher than those reared at T₁ (control) and T₂ (PVC pipes). There was no

significant ($P>0.05$) difference between the final weight gain of PLs in treatments T_3 and T_4 . Final weight was 32.70%, 31.54% and 21.05% higher in treatments T_3 (HDPE), T_4 (nylon netting) and T_2 (PVC pipes) respectively than T_1 (without substrate). Mean final weight of PLs in treatment T_2 was significantly lower than T_3 and T_4 but the weight was significantly higher than that of T_1 (control). The SGR of PLs in different treatments ranged from 4.72 to 5.19 (Table 4). However, there was no significant difference ($P>0.05$) between the SGR of PLs in treatment T_3 and T_4 but these value were significantly higher than those in treatment T_2 and T_1 . Although the SGR values in treatment T_2 was significantly lower than that of T_3 and T_4 , this value was significantly higher than that of T_1 (control).

Table 4. Growth and feed utilization of *M. rosenbergii* PLs under different experimental treatments

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Initial weight (mg)	$27^a \pm 0.02$	$27^a \pm 0.02$	$27^a \pm 0.02$	$27^a \pm 0.02$
Final weight (mg)	$460.67^c \pm 9.50$	$557.67^b \pm 17.95$	$611.33^a \pm 19.14$	$606.00^a \pm 11.36$
Weight gain (mg)	$433.67^c \pm 9.50$	$530.67^b \pm 17.95$	$584.33^a \pm 19.14$	$579.00^a \pm 11.36$
% weight gain	$1605.67^c \pm 35.02$	$1965.00^b \pm 66.14$	$2163.67^a \pm 71.07$	$2144.33^a \pm 41.65$
SGR(%day ⁻¹) ¹	$4.72^c \pm 0.04$	$5.04^b \pm 0.05$	$5.19^a \pm 0.05$	$5.18^a \pm 0.03$
FCR ²	$1.88^a \pm 0.01$	$1.87^{ab} \pm 0.02$	$1.85^c \pm 0.01$	$1.86^{bc} \pm 0.02$
PER ³	$1.83^a \pm 0.21$	$1.98^a \pm 0.02$	$2.01^a \pm 0.02$	$1.98^a \pm 0.01$
Survival (%) ⁴	$80.33^a \pm 3.51$	$80.33^a \pm 5.13$	$83.00^a \pm 2.65$	$83.00^a \pm 2.65$

¹ Values are mean \pm standard deviations and values in the same row having same superscripts are not significantly different ($P>0.05$).

¹ Specific growth rate (SGR% day⁻¹) = 100 (ln final body weight – ln initial body weight) / total number of experimental days.

² Feed conversion ratio (FCR) = total dry feed fed (g) / total live weight gain (g)

³ Protein conversion ratio (PER) = live weight gain (g) / dry protein fed (g)

⁴ Survival (%) = number of juvenile harvested / number of postlarvae stocked \times 100

There was no significant difference ($P>0.05$) between FCR values in treatment T_1 and T_2 and these values were significantly higher than that in T_3 . Again there was no significant difference between the FCR values in treatment T_3 and T_4 . Similarly, there was no significant difference between the PER values which ranged between 1.83 and 2.01. There was no significant difference between the survivals of PLs in different treatments which ranged from 80.33 to 83.00% (Table 4).

Survival at the end of the nursery phase can vary substantially and may be related to the territorial and cannibalistic nature of *M. rosenbergii* PLs when cultured at high densities. Nursery production may be enhanced by the addition of artificial substrates to increase the surface area upon which prawns graze and to serve as refuge. The present study investigated the effects of different artificial substrates on the growth and survival of *M. rosenbergii* PLs in a recirculation system.

In the present study, the water temperature ranged between 26 and 29 °C. Ang *et al.* (1992) concluded that, temperature above 35 °C or below 14 °C are generally lethal, 18-22 °C markedly stunts growth and 29-31 °C is optimal for *M. rosenbergii* reared in cages. Adult prawns are tolerant of wide range of water temperature (18-34 °C) but temperatures ranging from 27 to 32 °C are believed to be optimal (Tidwell *et al.*, 2005). Hossain and Islam (2006) reported a suitable temperature of 27-29 °C in a recirculatory system for rearing of *M. rosenbergii* PLs. Indulkar *et al.* (1994) reported that water temperature ranging from 25 °C to 27 °C was suitable for the growth of postlarvae of *M. rosenbergii*. The water temperature recorded in the present experimental system is more or less agreed with the above findings.

Dissolved oxygen (DO) concentrations less than 3 mg/l are stressful and lower concentrations can be lethal. Therefore, levels of dissolved oxygen concentration in the water should not be below 3 mg L⁻¹ (D'Abamo *et al.*, 1995). Hossain and Islam (2006) reported dissolved oxygen levels between 6.5 and 8.4 mg L⁻¹ in a recirculatory system for *M. rosenbergii* PLs rearing which is similar to those in the present study (6.2 to 8.4 mg L⁻¹). The better oxygen levels in the present study could be due to the continuous aeration of the experimental water using an air pump.

Although freshwater prawns have been raised in ponds with a pH range of 6.0 to 10.0 with no apparent adverse effect on survival, a pH range between 6.5 and 9.5 is recommended (D'Abramo *et al.* 1995). Boyd and Zimmermann (2000) showed that the ideal environment for nursing of prawn postlarvae should have pH values of 7.0-8.5. In the present study, pH range varied from 6.8 to 8.1. Hossain and Islam (2006) reported that pH range from 6.8 to 8.3 was suitable for PLs rearing in a recirculatory system.

Levels of un-ionized ammonia, exceeding 0.1 mg L^{-1} , can be very detrimental in fish ponds. Concentration of un-ionized ammonia as low as 0.26 mg L^{-1} at a pH of 6.83 have been reported to kill 50% of the prawn population within 144 hours (Armstrong *et al.*, 1978). Strauss *et al.* (1991) reported that prawn juveniles should not be exposed to $\text{NH}_3\text{-N}$ concentration higher than 1 mg L^{-1} or 2 mg L^{-1} at pH values of 9 and 8.5, respectively. Hossain and Islam (2006) reported a concentration of 0.01-0.25 mg L^{-1} total ammonia in a recirculatory system for rearing of *M. rosenbergii* PLs. Total ammonia in the present study ranged between 0.01 and 0.15 mg/l which might be considered safe for *M. rosenbergii* PLs.

In the present study, freshwater prawn PLs reared in treatment T_3 (HDPE netting) and T_4 (nylon netting) aquaria exhibited significantly higher ($P<0.05$) final length than T_1 (control) i.e. without substrate. Gwak (2003) reported that the provision of shelter significantly ($P<0.05$) increased the mean body length of black sea bass (*Centropristis striata* L.) and it was 5.7% higher than unsheltered fish.

In the present study, growth of *M. rosenbergii* PL was significantly influenced by the use of substrates. The final weight of PLs in treatment T_3 and T_4 was significantly higher than T_2 (PVC pipes) and T_1 (control) although there was no significant difference between the final weight of PLs in T_3 and T_4 . Final weight was 32.70%, 31.54% and 21.05% higher in treatments T_3 (HDPE), T_4 (nylon netting) and T_2 (PVC), respectively than those in T_1 (without substrate). Cohen *et al.* (1983) reported that total production and average weight of *M. rosenbergii* increased 14% and 13%, respectively, when artificial substrates were added to ponds in Israel. Similar improved growth and productions were also reported elsewhere (Tidwell *et al.* 1998; Ráanan *et al.* 1984). Tidwell *et al.* (1999) found no significant interaction between the additions of substrates and increased stocking density, although substrate did increase the total production by 18%, the average harvest size by 13% and marketable production by 25%. Tidwell *et al.* (2002) reported that orientation of substrates (horizontal or vertical) had no significant ($P>0.05$) impact on prawn production or population structure. However, total yield (kg/ha) was significantly greater ($P<0.05$) in ponds with 100% increase in surface area (2,653 kg/ha) than in control ponds (2,140 kg/ha). Moss and Moss (2004) stated that there was highly significant ($P<0.05$) substrate effects on final weight in nursery rearing of white shrimp (*Litopenaeus vannamei*). The final weight values obtained in the present study are similar to that reported by Tidwell *et al.* (1998; 1999) and Moss and Moss (2004) but slightly higher than that of Cohen *et al.* (1983). This probably resulted from an increased periphyton/biofilms growing on the substrates which acted as food and use of substrate as refuge by the *M. rosenbergii* PLs.

The SGR of prawn PLs in the present study ranged between 4.72 and 5.19%. The SGR values were significantly higher ($P<0.05$) in treatments T_3 and T_4 which were provided with net materials. Hossain and Islam (2006) reported SGR values between 2.93 and 3.32% for *M. rosenbergii* PLs in a recirculatory system for 60 days rearing which are lower than those observed in the present study. Gwak (2003) found significantly higher ($P<0.05$) specific growth rates in substrate provided treatments than with no refuge treatments.

In the present study, the FCR values ranged between 1.85 and 1.88. These values were similar (1.73-1.93) to that reported by Hossain and Islam (2006) when prawn PLs fed formulated diets in a recirculatory system. Posadas *et al.* (2003), Gwak (2003) and Tidwell *et al.* (2002) showed that substrates had no statistically significant impact ($P<0.05$) on feed conversion ratios. On the other hand, Tidwell *et al.* (2000) reported that FCR decreased from 2.8 in control ponds with no added substrate to 2.4 in ponds with substrate. Tidwell *et al.* (1999) also reported that added substrate improved feed conversion efficiencies. This is probably due to the increases in periphyton production and the resulting increases in natural food availability associated with the increased amount and complexity of benthic substrate (Tidwell *et al.*, 1999).

In the present study, survival ranged between 80.33% and 83.0% and there was no significant ($P>0.05$) variation in survival of PLs among the treatments. Posadas *et al.* (2003) and Tidwell *et al.* (2000) reported that addition of increasing amounts of substrate had no significant ($P<0.05$) impact on survival of freshwater prawn. Moss and Moss (2004) also stated that there was no density, substrate, or interaction effect on survival of pacific white shrimp which ranged between 89.1 and 93.2%. The survival obtained in the present study were slightly lower than those of Moss and Moss (2004) and Tidwell *et al.* (2000) but higher than those of Hossain and Islam (2006) who reported a survival range of 62 to 76% for *M. rosenbergii* PLs in a recirculatory system without substrates.

Results of the present study indicated that growth of *M. rosenbergii* PL improved in the presence of artificial substrates but the artificial substrates did not improve the survival. High density polyethylene (HDPE) and nylon netting gave the best result compared to PVC pipes and control treatment in terms of growth and feed efficiency. Therefore, use of HDPE or nylon netting may be recommended as substrates for successful nursing of *M. rosenbergii* PL. However, further studies using different substrates should be carried out in nursery ponds to ascertain the usefulness of the substrates for nursing of *M. rosenbergii* PLs.

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