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Larval Rearing of Giant Freshwater Prawn (*Macrobrachium Rosenbergii*) Using Different Rearing Protocols

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Keywords: Giant freshwater prawn, *larvae* rearing, improved green-water, biofloc technology, open clear-water probiotic.

Abstract: This study aimed to assess the effectiveness of commonly adopted larval rearing protocols for giant freshwater prawn (*Macrobrachium rosenbergii*). The experiment was conducted using a completely randomized design (CRD) to compare the performance of four larval rearing systems: an improved green-water system (IGW), a biofloc technology system (BFT), an open clear-water system supplemented with probiotics (CWP), and a conventional open clear-water system (CW). Larvae and post-larvae reared in the BFT system showed the best performance among treatments, with a larval stage index of 10.64 ± 0.86 at 21 days after rearing and an average body length of 10.34 ± 0.71 mm at the post-larval stage (PL-15). In addition, the BFT treatment achieved the highest survival rate (83.0 ± 3.0%) and productivity (41.47 ± 1.27 individuals/m³). These results demonstrate the significant potential of biofloc technology for larval rearing of giant freshwater prawn, contributing to the production of high-quality post-larvae.

I. INTRODUCTION

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important aquaculture species in Vietnam, particularly in the Mekong Delta, and is characterized by its high economic value and significant role in the development of the national aquaculture sector [1]. In 2019, Vietnam produced 20,129 tons of giant freshwater prawns, accounting for approximately 7.35% of global production and ranking fourth worldwide, after China (139,609 tons), Bangladesh (52,197 tons), and Thailand (31,345 tons [2].

Despite its considerable potential, the giant freshwater prawn farming still faces several challenges. High initial investment costs, particularly for post-larvae and feed, reduce the farmers' economic efficiency. In

addition, inconsistent post-larval quality, low survival rates, and frequent disease outbreaks during the culture period remain common [1]. These limitations are closely associated with hatchery practices, especially the larval rearing stage, which plays a critical role in determining both survival and post-larvae quality.

Currently, several larval rearing protocols for the giant freshwater prawn are applied in practice, including biofloc technology [3], improved green-water systems, clear-water systems supplemented with probiotics, and open clear-water systems [4]. These protocols differ in water quality management, natural food availability, and microbial communities, thereby affecting larval growth, survival, and post-larvae quality. However, systematic and comprehensive comparative studies evaluating the effectiveness of these protocols remain limited. Therefore, studying the larval rearing of giant freshwater prawn under different protocols is necessary to assess their effectiveness and propose optimal solutions for juvenile production.

II. Research methodology

The present study was conducted between April and June 2025 at the giant freshwater prawn hatchery of the Department of Aquaculture, School of Agriculture and Aquaculture, Tra Vinh University.

Experimental Materials

Chemicals and reagents: pH test kits, ammonia/ammonium (NH_3/NH_4^+) and nitrite (NO_2^-) test kits, alkalinity test kits, chlorine, potassium permanganate ($KMnO_4$), EDTA, ET 800, formalin, mineral supplements (Mirano), shrimp flavor additive, etc.

Other materials: Granulated sugar, green water containing *Chlorella* algae, *Probiotic PC-01*, seawater with a salinity of 30‰.

Equipment: pH meter, thermometer, refractometer, and composite tanks.

Preparation of Hatchery, Tanks, and Equipment

The hatchery, tanks, and all experimental equipment were thoroughly cleaned and disinfected using chlorine at a concentration of 200 ppm before commencing the experiment.

Water Preparation and Treatment

Saline water was collected from Ba Đong Sea, and freshwater was obtained from the Long Binh River (Vinh Long Province, Vietnam). Both types of water were transported to storage tanks at the experimental site. Initially, the water was treated with potassium permanganate ($KMnO_4$) at a concentration of 2 ppm and aerated for 12 hours. Following aeration, the system was left undisturbed to allow the sediments to settle. The supernatant was then filtered into another tank using filter bags with a 5 µm mesh size and subsequently disinfected with chlorine at a concentration of 50 ppm. Continuous aeration was applied to remove residual chlorine completely. The water was finally filtered again through a finer filter bag (1 µm) before being transferred to a storage tank. The prepared saline and freshwater sources were then mixed to achieve a salinity of 12 ppt, which was used for larval rearing of giant freshwater prawn.

Larvae preparation

Larvae of the giant freshwater prawn used in this study were supplied by Phu Hung Company (Dong Thap Province, Vietnam), totaling 288,000 individuals. The larvae were tested for free from diseases and healthy.

Chlorella algae preparation

Chlorella algae were cultured in tanks fertilized with Nile tilapia (Oreochromis niloticus), which acted as a biofertilizer. Tilapia was reared in 0.5 m^3 composite tanks filled to a water depth of approximately 0.5 m. The initial salinity was set at 6‰ and gradually increased to 12‰ during the culture period. Tilapia, weighing between 20 and 50 g each, were stocked at a density of 20 fish per tank. The fish were fed daily with

commercial pellet feed at a rate of 5% of their total body weight. After about 7 days, the water in the tanks turned green, indicating the development of Chlorella algae. When the algal density reached approximately 5 × 10^6 cells/mL, the green-water culture was harvested and used for inoculating the larval rearing tanks.

Biofloc Preparation Biofloc was generated by using brown sugar as the carbon source. The sugar was dissolved in warm water at 60°C at a ratio of 1:3, thoroughly mixed, and allowed to ferment for 48 h before adding to the larval rearing tanks. The required amount of sugar solution to achieve a carbon-to-nitrogen (C/N) ratio of 17.5 [5].

Probiotic application

The probiotic used in this study was PC-01 Super Probiotic (Thuy Duy Thuc Trading, Service and Production Co., Ltd., Vietnam), which contains *Bacillus subtilis* (0.2 × 10^7 CFU/g), *Bacillus licheniformis* (1 × 10^7 CFU/g), *Bacillus polymyxa* (0.1 × 10^7 CFU/g), *Bacillus circulans* (0.15 × 10^7 CFU/g), and *Saccharomyces boulardii* (0.2 × 10^7 CFU/g). The bioproduct was administered daily at a dose of 0.4 g per tank (equivalent to 1 ppm).

Experimental Design

The one-factor experiment was designed in a Completely Randomized Design (CRD) to compare efficiency of four larval rearing methods. The present experiment included four treatments, as following:

IGW: Improved green-water system

BFT: Biofloc technology system

CWP: Open clear-water system with probiotic supplementation

CW: Open clear-water system

The treatments were replicated three times, each replication consisting of a 0.5 m^3 composite tank. Each tank contained 400 L of water at a salinity of 12 ppt and was stocked with 24,000 larvae, equivalent to 60,000 larvae per m^3. This experiment was conducted over a period of 30 days.

Experiment maintainance

Daily, the shrimp larvae were fed with proper type of feed (Table 1). Water quality parameters, including pH and temperature, were monitored twice daily at 08:00 and 14:00 using a pH meter. Alkalinity, total ammonia nitrogen (TAN), and nitrite (NO_2^-) concentrations were assessed every three days.

Table 1: Feed types and feeding regimes for different larval stages of giant freshwater prawn

Larval stage	Feed type	Feeding rate	Feeding frequency
Stage 1		No feeding	
Stage 2–4	Artemia nauplii (umbrella stage)	1–2 nauplii/mL	3 times/day (07:00, 11:00, 17:00)
Stage 5	Lansy Shrimp PL	1 g/m^3/feeding	3 times/day (09:00, 11:00, 14:00)
	Artemia nauplii (newly hatched)	3 nauplii/mL	2 times/day (07:00, 17:00)
Stage 6–8	Lansy Shrimp PL	1.5 g/m^3/feeding	3 times/day (09:00, 11:00, 14:00)

Stage 9 – Postlarvae	Artemia nauplii (newly hatched)	3 nauplii/mL	2 times/day (07:00, 17:00)
	Lansy Shrimp PL	2 g/m³/feeding	3 times/day (09:00, 11:00, 14:00)
	Artemia nauplii (newly hatched)	4 nauplii/mL	2 times/day (07:00, 17:00)

Note: PL: Post larvae

It should be noted that algal inoculation in the improved green-water system (IGW) was applied only once at the start of the rearing period. For the biofloc system (BFT), brown sugar was added daily as a carbon source to promote biofloc development. For each gram of artificial feed administered, 1.21 g of brown sugar was supplemented to maintain optimal biofloc formation. In both IGW and BFT treatments, no water exchange was conducted during the culture period; only evaporative losses were replenished.

In the probiotic-supplemented clear-water system, the probiotic product PC-01 was applied at a daily dose of 1 ppm at 10:00 AM. Siphoning and partial water exchange were performed every three days, with lost water replenished using treated water. In the clear-water system, daily siphoning and water exchange were conducted, replacing 30–50% of the tank volume with fresh treated water each day

Data collection

The larval stage index (LSI) was evaluated starting from day 3, at three-day intervals, by randomly sampling 15 larvae from each tank. Larval length measurements were conducted at stages 5, 11, and the post-larval stage PL-15 (30 days after rearing), with 20 individuals randomly sampled per tank at each time point. Survival rates and productivity at the PL-15 stage were determined using the volumetric counting method.

Data Analysis

The collected data were analyzed to calculate mean values and standard deviations using Microsoft Excel 2010. Differences among treatments were evaluated using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS version 25.0, at a significance level of $p < 0.05$.

III. Results and Discussion

Water quality parameters

The results of the present study show that water quality parameters during the experimental period were within suitable ranges for larval rearing (Table 2). In particular, water pH in different treatments ranged from 7.7 to 7.9, which is in agreement with a previous study indicating that the optimal pH for giant freshwater prawn larval rearing is 7.4–8.0 [6]. Similarly, morning water temperatures in the experimental tanks ranged from 29.6°C to 29.7°C, while afternoon temperatures fluctuated between 30.5°C and 30.7°C. Tran [7] reported that temperature strongly affects molting and larval development, with an optimal range of 26–31°C and the best range being 28–30°C. Daily temperature fluctuations should not exceed 5°C, as excessively high or low temperatures can negatively affect larval activity and growth [8]. Consequently, the recorded water temperatures during the experimental period were suitable for post-larval growth.

In addition, alkalinity, total ammonia nitrogen (TAN), and NO_2^- concentration in the tank water ranged from 107–109 mg $CaCO_3$/L, 0.80–1.19 mg/L, and 0.07–1.31 mg/L, respectively. According to Chau [9], the optimal alkalinity for larvae and post-larvae of the giant freshwater prawn is 100–120 mg $CaCO_3$/L. TAN concentrations (0.80–1.19 mg/L) in the present study were lower than the recommended threshold of 2 mg/L for aquaculture ponds [10]. Previous research also showed that NO_2^- concentrations up to 2.95 mg/L did not negatively affect

larval development [11]. Overall, the water quality parameters during the experimental period, including pH, temperature, alkalinity, TAN, and NO_2^-, were suitable for the growth and development of giant freshwater prawn larvae and post-larvae.

Table 2: Fluctuation of water quality parameters in larval rearing tanks (mean±S.D.)

Parameter		IGW	BFT	CWP	CW
pH	7:00	7.8±0.30	7.8±0.28	7.9±0.25	7.9±0.27
	14:00	7.7±0.2	7.8±0.22	7.8±0.22	7.8±0.21
Temperature (°C)	7:00	29.7±0.47	29.7±0.46	29.7±0.50	29.6±0.44
	14:00	30.7±0.48	30.6±0.48	30.6±0.52	30.5±0.54
Alkalinity (mg $CaCO_3$/L)		109±7.67	110±5.94	107±8.95	109±9.95
TAN (NH_4^+/NH_3) mg/L		0.80±0.60	1.07±0.60	1.19±0.70	0.94±0.59
NO_2 (mg/L)		1.31±0.80	0.08±0.12	0.07±0.12	0.07±0.14

IGW: Improved green-water system; BFT: Biofloc technology system; CWP: Open clear-water system with probiotic supplementation; CW: Open clear-water system.

Larval stage index (LSI) of giant freshwater prawn larvae

The larval stage index (LSI) reflects the degree of metamorphosis and uniformity of giant freshwater prawn larvae in the rearing tanks. Larval development was monitored through successive molting and metamorphosis events. Larvae undergo 11 molting stages to reach the post-larval stage [4]. The duration of each molt stage depends on environmental conditions, nutrition, stocking density, and physiological status. New [11] reported that the rearing environment significantly influences the molting process of *Macrobrachium rosenbergii* larvae.

The findings of the present study indicated differences in LSI values among treatments during the experimental period (Table 3). On day 3 after treatment, LSI values did not differ significantly among treatments ($p > 0.05$). By day 6, the BFT treatment exhibited the highest LSI (5.49 ± 0.50), while the CW treatment had the lowest. Although differences in LSI between the BFT and IGW treatments were not statistically significant ($p > 0.05$), LSI in the BFT treatment was significantly higher than in the CWP and CW treatments ($p < 0.05$).

On day 9, the BFT and CW treatments had the highest LSI values (6.47 ± 0.50 and 6.47 ± 0.73, respectively). These values did not differ significantly from the CWP treatment but were significantly higher than the IGW treatment (Table 3).

By days 12 and 15, LSI values were similar across all treatments ($p > 0.05$). However, on day 18, the BFT treatment again had the highest LSI (10.60 ± 0.72), significantly higher than CWP, but not significantly different from IGW and CW. At the final sampling day (day 21), LSI in the BFT treatment remained the highest (10.64 ± 0.86), significantly higher than in all other treatments ($p < 0.05$).

Tran [13] reported that larvae and post-larvae of *Macrobrachium rosenbergii* can utilize substrates in biofloc as a nutrient source. The biochemical substrates of biofloc include protein, lipids, carbohydrates, fiber, amino acids, fatty acids, cholesterol, antioxidants, and several bioactive compounds such as organic acids, polyhydroxyacetate, and polyhydroxybutyrate [14–17]. In addition, Nguyen [11] demonstrated that the LSI after 27 days of larval rearing of giant freshwater prawn fed commercial feed five times per day was 10.6. In the

present study, the LSI in the BFT treatment on day 21 reached 10.64, indicating superior larval development under the biofloc rearing system.

Table 3: Larval stage index (LSI) of giant freshwater prawn larvae (mean±S.D.)

Sampling day	IGW	BFT	CWP	CW
LSI – day 3	4.82±0.49^{a}	4.91±0.60^{a}	4.69±0.60^{a}	4.80±0.46^{a}
LSI – day 6	5.42±0.50^{bc}	5.49±0.50^{c}	5.27±0.45^{ab}	5.11±0.32^{a}
LSI – day 9	6.11±0.49^{a}	6.47±0.50^{b}	6.33±0.52^{ab}	6.47±0.73^{b}
LSI – day 12	7.09±0.67^{a}	7.22±0.74^{a}	7.09±0.85^{a}	6.91±0.85^{a}
LSI – day 15	8.11±0.75^{a}	8.36±0.61^{a}	8.29±0.87^{a}	8.33±0.80^{a}
LSI – day 18	10.31±0.67^{ab}	10.60±0.72^{b}	10.04±0.90^{a}	10.40±0.75^{b}
LSI – day 21	10.02±0.72^{a}	10.64±0.86^{b}	10.16±0.85^{a}	10.13±0.723^{a}

IGW: Improved green-water system; BFT: Biofloc technology system; CWP: Open clear-water system with probiotic supplementation; CW: Open clear-water system; In the same row, means with different letters are significantly different at the $p < 0.05$ level according to Duncan's multiple range test.

Growth in body length (mm) of giant freshwater prawn larvae and post-larvae

Body lengths of giant freshwater prawn at the larval and post-larval stages among treatments are presented in Table 4**.** In particular, body length at larval stage 1 was the same among treatments (1.93 ± 0.19 mm). At stage 5, the body length parameter showed slight differences among treatments, ranging from 3.42 ± 0.38 to 3.50 ± 0.43 mm. A similar trend was also observed at larval stage 11 (Table 4). Consequently, differences in this parameter at larval stages 5 and 11 were not statistically significant ($p > 0.05$).

At the post-larval stage PL-15, the BFT treatment exhibited the highest body length (10.34 ± 0.71 mm), which was significantly greater than that in the other treatments ($p < 0.05$). According to Xu [18], biofloc functions as a nutritious natural feed in aquaculture systems, with high protease and amylase activities that facilitate the breakdown of proteins, carbohydrates, and other nutrients into simpler forms for efficient digestion and absorption. Yu [19] reported that prawns reared with biofloc show improved growth performance and higher survival rates, contributing to increased production efficiency.

Moreover, Chau [19] also found that the average body lengths of giant freshwater prawn larvae at stages 5, 11, and post-larval stage PL-15 were 2.81 mm, 6.69 mm, and 9.06 mm, respectively. The findings of the present study show a similar trend to previous studies. Furthermore, larval and post-larval growth in the current experiment exhibited better performances than previously reported values.

Table 4: Growth in Body Length (mm) of Giant Freshwater Prawn Larvae (mean±S.D.)

Stage	IGW	BFT	CWP	CW
Stage 1	1.93±0.19^{a}	1.93±0.19^{a}	1.93±0.19^{a}	1.93±0.19^{a}
Stage 5	3.42±0.38^{a}	3.47±0.33^{a}	3.50±0.43^{a}	3.48±0.43^{a}
Stage 11	7.66±0.47^{a}	7.76±0.53^{a}	7.63±0.49^{a}	7.62±0.51^{a}

PL-15	9.77±0.73[a]	10.34±0.71[b]	9.91±0.52[a]	9.92±0.79[a]

IGW: Improved green-water system; BFT: Biofloc technology system; CWP: Open clear-water system with probiotic supplementation; CW: Open clear-water system; PL: Post-larvae; In the same row, means with different letters are significantly different at the $p < 0.05$ level according to Duncan's multiple range test.

Survival rate (%) and productivity (individuals/m³) of giant freshwater prawn after 30 days (PL-15)

The survival rate (%) and productivity (individuals/m³) of giant freshwater prawns across the different treatments over the 30-day rearing period are presented in Table 5. Survival rates among treatments ranged from 68.0% to 83.0%. The BFT treatment achieved the highest survival rate (83.0 ± 3.0%), which was significantly higher than in the IGW treatment ($p < 0.05$), but not significantly different from CWP and CW ($p > 0.05$). Productivity of post-larvae was highest in BFT (41.47 ± 1.28 individuals/m³) and lowest in IGW (33.98 ± 3.75 individuals/m³), showing a significant difference compared with IGW ($p < 0.05$), but no significant difference compared with CWP and CW ($p > 0.05$).

According to Tran [21], survival rates of 30-day reared giant freshwater prawns using biofloc technology ranged from 30.7% to 40.9%, and PL-15 post-larvae productivity varied from 18.41 to 24.56 individuals/m³. Zemor [22] reported that suspended biofloc particles in water reduce cannibalism among prawns, thereby increasing productivity. Zhou [23] stated that biofloc can improve growth performance and immune efficiency of giant freshwater prawns by regulating bacterial communities in water and the gut, increasing actinomycete populations, and activating the prawn immune system.

The results of the present study indicate that the survival rate and PL-15 productivity observed were higher than those reported in previous studies. Overall, the biofloc technology system (BFT) **achieved superior results** in all measured parameters, including larval stage index (LSI), body length, survival rate, and productivity, compared with the other rearing systems.

Table 5: Survival rate and productivity of giant freshwater prawns after 30 days (mean±S.D.)

Parameter	IGW	BFT	CWP	CW
Survival rate (%)	68.0±7.0[a]	83.0±3.0[b]	72.3±10.0[ab]	79.3±8.0[ab]
Productivity (individuals/m³)	33.98±3.75[a]	41.47±1.28[b]	36.04±4.84[ab]	39.58±3.96[ab]

IGW: Improved green-water system; BFT: Biofloc technology system; CWP: Open clear-water system with probiotic supplementation; CW: Open clear-water system; In the same row, means with different letters are significantly different at the $p < 0.05$ level according to Duncan's multiple range test.

IV. Conclusion and Recommendations

The environmental parameters, including pH, temperature, alkalinity, TAN, and NO_2 concentration, were within suitable ranges for the growth and development of larvae and post-larvae of *Macrobrachium rosenbergii*. The BFT treatment (using biofloc technology) showed the best performance in comparison with the other treatments, with the highest values for larval stage index (LSI; 10.64 ± 0.86), body length growth (10.34 ± 0.71 mm), survival rate (83.0 ± 3.0%), and productivity (41.47 ± 1.27 individuals/m³). The application of biofloc technology for rearing giant freshwater prawn larvae should be promoted and scaled up in practical aquaculture.

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