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Original Research

Growth Performance of Giant Freshwater Prawn (*Macrobrachium Rosenbergii*, De Man, 1879) in a Vertical Compartment Farming System with Different Biofilters and Varying Flow Rates

Wanna Thawinwan[1]*, Krishna R. Salin[1], Md. Lifat Rahi[2], Amara Yakupitiyage[1]

[1]Aquaculture and Aquatic Resources Management, School of Environment, Resources and Development, Asian Institute of Technology, Khlong Luang, Pathumthani 12120, Thailand
[2]Fisheries and Marine Resource Technology Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh

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Abstract

Heterogeneous individual growth and cannibalism are major impediments to farming giant freshwater prawn (GFP), *Macrobrachium rosenbergii*, and growing GFP individually in compartment farming units could eliminate both hindrances. This study conducted two pioneering trials to evaluate a newly designed indoor compartmental grow-out system for GFP. The first trial was designed to investigate the potential use of three commercial microbial supplements (two *Bacillus* spp. and one photosynthetic bacteria) for water treatment. The results of ammonium removal efficiency showed that the bacterial supplements with *Bacillus* spp. had the highest ammonia removal efficiency of 91.73% and 90.57%, respectively, compared to the commercial photosynthetic bacteria (86.69%). The second trial evaluated the effect of four water flow rates at 1, 2, 3, and 4 L/min on the growth performance of GFP juveniles. The mean weight gain of the prawns grown in the compartments subjected to the flow rates of 1 L/min and 2 L/min (11.57±0.2 g. and 11.46±0.5 g) was significantly higher than the prawns grown in compartments where the water flow rate was set at 3 L/min and 4 L/min (9.91±0.55 g and 9.65±0.45 g). These results suggest that administration of *Bacillus* strain in the rearing water and optimal flow velocity confer beneficial effects in vertical compartment culture of GFP.

Keywords: giant prawn, heterogeneous individual growth, ammonia removal, recirculating aquaculture system, eco-friendly aquaculture

*e-mail: wannathaw@gmail.com

Introduction

The giant freshwater prawn (GFP), *Macrobrachium rosenbergii*, is one of the most important crustaceans in Asian aquaculture. It is popular among farmers, especially in Thailand, due to its fast growth, attaining the marketable size of 60-80 g within 5-6 months from the post-larval stage, feasibility to culture under either mono or polyculture conditions, and command for a premium price in both domestic and international markets [1]. However, the aggressiveness and cannibalistic behavior of GFP males make it challenging to farm them at high stocking densities [2], and hence the recommended stocking density is approximately 4 prawns per square meter after the juvenile stage [1]. This low-density pond culture requires substantial land and water resources, fetches a relatively low yield per unit area of land, and causes environmental pollution from untreated effluent discharges. Additionally, because of the phenomenon of heterogeneous individual growth (HIG) pattern, three different morphotypes of male prawn are found, namely blue-clawed male (BC), orange-clawed male (OC), and small male (SM). Sometimes harvested prawns remain undersized by as much as 50% [3]. Thus, there is a need to develop innovative grow-out techniques for *M. rosenbergii* culture, and these issues might be resolved by growing prawns in individual compartments or growth chambers to allow the males to grow to maximum size without hierarchical social influence. It may also remove the effects of visual and somatic cues that might bring cannibalism and HIG in the male prawns, thereby increasing the survival rate.

Growing aquatic animals in individual compartments or growth chambers requires a well-designed water recirculation system with a suitable flow rate and an effective mechanism to provide enough dissolved oxygen and remove metabolic wastes. The elimination of ammonia in traditional recirculation systems is facilitated by biofilters that provide a large surface area for ammonia-oxidizing bacterial (AOB) species such as *Nitrosomonas* and *Nitrobacter* spp. to grow and convert ammonia into non-toxic nitrates. Heterotrophic and photosynthetic bacteria are also used to treat ammonia as these microbes convert ammonia into bacterial protein [4-5].

The objective of this trial was to evaluate three commercially available microbial products in Thailand to select the best performing and cost-effective commercial product to be used in a vertical compartment prawn culture system to control ammonia content in recirculating water. It is expected that the results presented here will help design a scaled-up vertical compartment recirculating aquaculture system for the indoor culture of giant freshwater prawns with an appropriate combination of probiotics and water flow rates.

Materials and Methods

All-male giant freshwater prawn (*M. rosenbergii*) juveniles were obtained from a commercial farm in Suphanburi Province, Thailand, and transported in oxygenated tanks to the Field Laboratory of Asian Institute of Technology, Thailand. The prawns were acclimatized for one week in a 500 L circular cement tank filled with freshwater at the ambient temperature of 27-30.5ºC under continuous aeration. Approximately 20% of the water from each acclimatization tank was exchanged daily. Prawns were fed *ad libitum* twice daily (8.00 h and 15.00 h) with 2 mm commercial feed pellets containing 32% crude protein (CP Company Ltd., Thailand). The uneaten feed and feces were removed daily by siphoning. The initial mean weight of the prawns was 5.76±0.25 g, and the initial length was 8.18±0.20 cm.

Vertical Compartment Prawn Culture System

The indoor vertical compartment culture facility was constructed using 60 custom-made HDPE boxes (40x25x20 cm), with each box as a growth chamber for stocking a single experimental animal. Five such HDPE boxes were vertically assembled using interlocking grips. Each 5-box unit with a mechanical filter, a biological filter, and a sump formed a small water recirculation system (Fig. 1) that can stock 5 prawns. There were 12 such compartmental water recirculation units (replicates) that were used to house a total of 60 prawns. Water from the sump tank was pumped to the top compartment using a submersible aquarium pump (Sonic Powerhead AP 2500) connected to a 12 mm (½ inch) PVC pipe. The flow rate was adjusted to the desired level using a flow sensor (YF-S 201 Louchen Zm: 1-30 L/min). Each growth chamber had a standpipe drain (10 cm in height) to maintain 10 L of water, while the excess water overflew to the unit below and ultimately to the mechanical filter (9 cm radius x 23 cm height filled with fiber mat) and a biofilter (45 x 32 x 20 cm) filled with 36 cm bio-balls that provided 400 cm^2 of surface area. Each compartment had a frontal flap that could be opened and closed, and it was used as both the observation window and feeding hole. The total water volume in one recirculation unit with five compartments was 77 L.

Trial 1: An Evaluation of Commercially Available Effective Microorganisms (EM)

There were three water treatments in this trial: use of (1) *Bacillus* species I; *B. subtilis*, *B. amyloliquefaciens*, *B. valismortis* (Baxtel Co. Ltd, Thailand), (2) *Bacillus* species II; *B. subtilis, B. megaterium*, *B. licheniformis* (MA Group, Thailand), and (3) Photosynthetic bacteria (PSB); *Rhodobacter sphaeroides*, *Rhodobacter capsulatus* (White Crane Co. Ltd., Thailand). The treatment with no microbes added to the biofilter

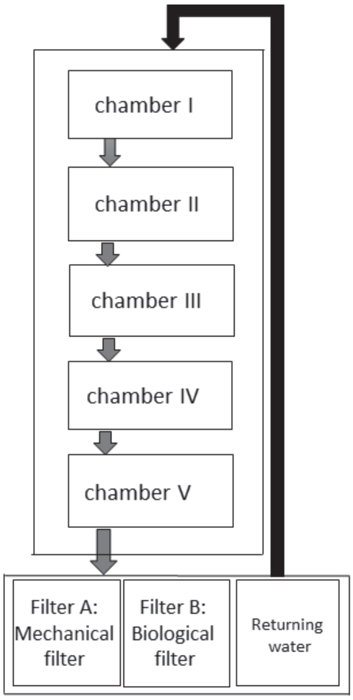

Fig. 1. One recirculation unit in the experimental vertical compartment prawn culture facility (12 such units assembled to form the total culture facility).

was the control. Three randomly selected experimental units were allocated to each treatment. The *Bacillus* and PSB solutions were added at rates of 0.02 ml L^{-1} and 0.1 ml L^{-1}, respectively, as recommended by the manufacturers. Ammonium chloride (NH_4CL) was added to each unit at 2 mg L^{-1} of TAN (total ammonia nitrogen) as the sole N source. Glucose was added to the two *Bacillus*-containing treatments as the carbon source to maintain a C:N ratio of 8. This trial was conducted without experimental animals.

Total ammonia nitrogen (TAN) concentration in the system was measured every 8 h using the indophenol method [6] for a total of 32 h until the TAN level was <0.5 mg L^{-1}. The pH was measured using a pH meter (YSI 310), and dissolved oxygen and temperature were measured using a DO meter (YSI 550A). Nitrite was measured by coupling diazotized sulfanilic acid-NED and nitrate by cadmium reduction methods [7]. The percentage of ammonia removal was calculated according to the following equation: Removal (%) = $\frac{(Ic-Fc)}{Ic}$ x 100 where *I*c is the initial concentration (mg L^{-1}) and *F*c is the final concentration (mg L^{-1}).

Trial 2: The Optimum Flow Rate and Growth Response of Prawns

*Bacillu*s supplement I from Trial 1 was selected to control ammonia in Trial 2, and it was added at a rate of 0.02 ml L^{-1} to the biofilters of all recirculation units. There were four flow rate treatments: 1, 2, 3, and 4 L min^{-1} for T1, T2, T3, and T4, respectively. Three sets of recirculation units were randomly allocated to each treatment, and the flow rate was adjusted in the inlet water of the top vertical compartment.

All-male giant freshwater prawn juveniles (5.76±0.25 g and 8.18±0.20 cm) were stocked individually in each compartment. Before stocking, the prawns were starved for 24 h. During the trial, the prawns were fed a commercial feed containing 32% crude protein at a rate of 3 % BW/day at 8.00 h and 15.00 h. Uneaten food and feces were siphoned out every morning, and lost water was replaced with treated water. The water temperature was kept between 27-30ºC. The pH of the culture water was kept between 7.5 and 8.5 by administration of sodium bicarbonate when pH dropped below 7.0. The photoperiod was constant at 12 h light: 12 h dark. The mechanical filter mats were cleaned every day. Dissolved oxygen (DO), pH and temperature were monitored at 8.00 am every day. Water samples were taken every three days to monitor the total ammonia nitrogen (TAN), nitrite, nitrate, alkalinity, and hardness. Total ammonia nitrogen (TAN), nitrite (NO_2-N), DO, pH, and temperature were measured as mentioned in section 2.2 above. Nitrite was measured by coupling diazotized sulfanilic acid-NED and nitrate by cadmium reduction methods [7]. The prawns were sampled every 15 days to measure length using a measuring scale and weight using a digital weighing balance (nearest 0.1 g, OHAUS Corporation, USA). The trial lasted 60 days when the prawns were counted, and each prawn was measured for length and weight. The following growth indices were calculated.

a. Average body weight (ABW)

b. Weight gain (g): Weight gain = Mean final weight – Mean initial weight

c. Specific growth rate (SGR %-day^{-1}) = $\frac{\ln W2 - \ln W1}{T2 - T1}$ × 100

Where, W1 = Initial weight (g) at time T1 (day); W2 = Final weight (g) at time T2 (day)

d. Average daily growth (ADG; g day^{-1}) = $\frac{Total\ weight\ gain}{Total\ days\ of\ culture}$

e. Survival rate = $\frac{Number\ of\ total\ surviving\ prawns}{Total\ number\ of\ prawns\ stocked}$ × 100

f. Feed Conversion Ratio (FCR) = $\frac{Total\ feed\ consumed(g)}{Total\ yield(g)}$

Statistical Analysis

Statistical analysis was performed using SPSS version 23.0. The mean values of responses were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at p<0.05 level.

Results and Discussion

The changes in ammonia concentration in water recirculating units are shown in Table 1 and Fig. 2.

Table 1. Total ammonia nitrogen (TAN) concentration in experimental units. Figures in brackets show % of the TAN removed relative to the initial concentration in Trial 1 (mean±SD, n = 3).

Treatment	0 hour mg L^{-1}	8 hour mg L^{-1} (%)	16 hour mg L^{-1} (%)	24 hour mg L^{-1} (%)	32 hour mg L^{-1} (%)
Bacillus spp. I	2.17	1.02	0.68	0.18	0.53
		(52.93±22.66)	(68.49±7.60)	(91.73±2.89)	(75.70±7.06)
Photosysthetic bacteria	2.15	1.61	1.10	0.29	0.35
		(25.21±9.31)	(48.98±20.37)	(86.69±1.89)	(83.52±5.05)
Bacillus spp. II	2.08	1.08	0.60	0.20	0.42
		(48.26±27.31)	(71.34±3.48)	(90.57±7.94)	(79.57±5.79)
Control	2.05	1.87	1.76	1.50	1.36
		(8.65±3.77)	(14.28±3.51)	(26.65±8.72)	(33.70±14.96)

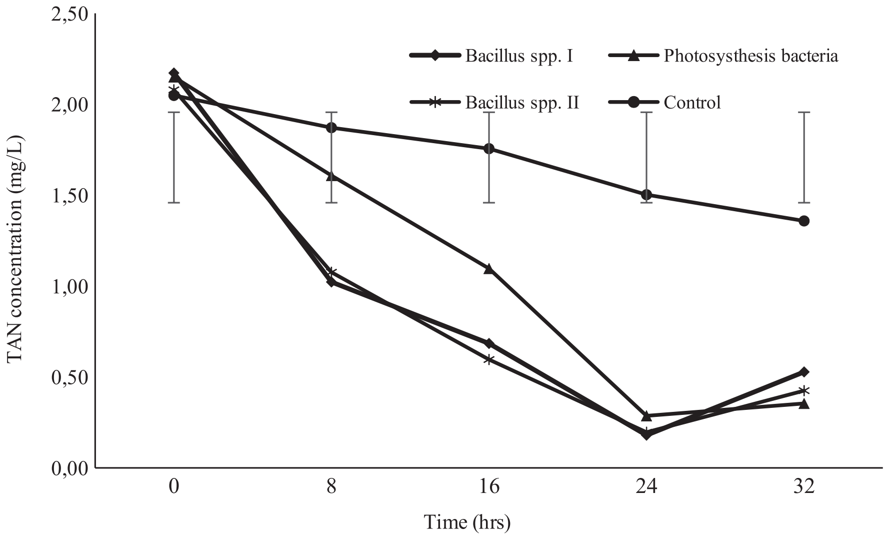

Fig. 2. Mean TAN concentration (mg L^{-1}) of different experimental units of the vertical compartment system during Trial 1 (vertical lines show ±SD).

Commercial microbial products containing *Bacillus* spp. reduced 90.57% and 91.73% of initial TAN concentration within 24 h, while PSB-containing product was also as effective as those containing heterotrophic bacteria. There was no significant difference in ammonia removal rate between the experimental units containing Bacillus I and II. Nitrite concentrations in heterotrophic and photosynthetic bacteria treatments were minimal (<0.2 mg L^{-1}), increasing up to 1 mg L^{-1} in control (Fig. 3). Water quality variables were stable and within the acceptable range for freshwater prawns for the entire 60 days of the experimental period (Table 2).

The mean initial weight of the prawns stocked in flow rate treatments of 1, 2, 3, and 4 L min^{-1} were 5.79±0.08, 5.86±0.34, 5.65±0.24, and 5.76±0.34 g. The growth pattern of *M. rosenbergii* in all the flow rate treatments was linear (Fig. 4). The treatments significantly ($p<0.05$) affected both the final weight and weight gain of the experimental prawns (Table 3). The mean final weight of freshwater prawns in the flow rate 1 and 2 L min^{-1} was significantly higher than that in the flow rate 3 and 4 L min^{-1}. This was also reflected in the total weight gain and average daily gain (ADG) of the experimental prawns (Table 3). Relatively slower growth rates at the higher flow rate were observed during the second half of the experiment (Fig. 5). No significant effect of flow rate on the specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) of the experimental prawns were observed ($p>0.05$). The water flow rate affected the size distribution of the prawns (Fig. 6), and a more uniform size distribution was observed with two lower water flow rates, i.e., 1 and 2 L min^{-1}.

This study indicated that the three commercially available microbial products in Thailand were effective for controlling ammonia levels when used in a vertical compartment recirculating aquaculture system. In comparing the different bacteria tested in

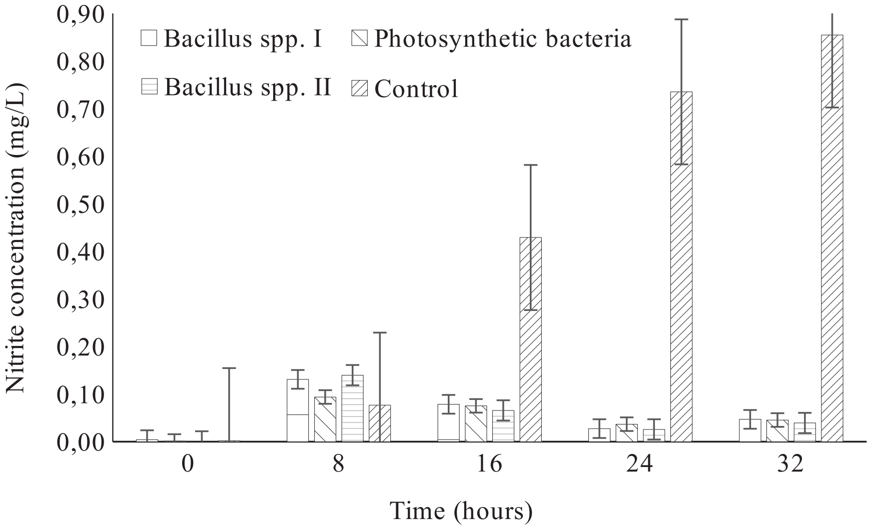

Fig. 3. Mean nitrite concentration (mg L^{-1}) of different experimental units of the vertical compartment system during Trial 1 (vertical lines show ±SD).

Table 2. Average water quality parameters (means±SD) during the experimental period of Trial 2.

Flow rate (L/min)	Temperature (ºC)	DO (mg/l)	pH	Total Alkalinity (mg/L)	TAN (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Hardness (mg/L)
1	29.72±0.6	5.22±0.5	7.56±0.3	106.41±8.9	0.30±0.1	0.15±0.1	38.33±18.1	115.00±11.5
2	29.72±0.7	5.24±0.4	7.52±0.2	107.04±10.3	0.27±0.1	0.16±0.1	36.85±18.8	113.67±10.2
3	29.59±0.7	5.45±0.5	7.62±0.2	112.70±9.6	0.26±0.1	0.15±0.1	36.85±18.8	116.33±9.2
4	29.63±0.7	5.50±0.4	7.63±0.2	113.33±9.4	0.23±0.1	0.16±0.1	34.07±18.5	113.74±11.5

this trial, it was found that the *Bacillus* group I and II gave the highest TAN reduction. While in control (without bacteria), ammoniacal nitrogen concentration developed and remained above the lethal level. The levels of ammoniacal nitrogen of all bacteria biofilters were brought to optimum levels for aquatic animals within 24 h. While no significant difference was observed with regard to the ammonia removal ability of biofilters with either *Bacillus* group I or II, both combinations also presented an excellent ammonia

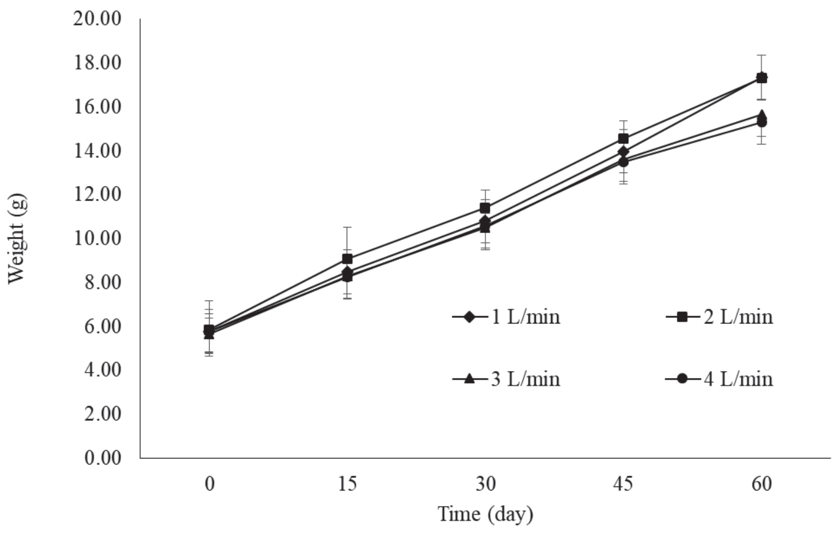

Fig. 4. Growth pattern of experimental prawns during Trial 2 (vertical lines show ±SD).

Table 3. The initial and final weight, weight gain, average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) of prawn (mean±SD, n = 3) in different flow rate treatments of Trial 2.

Flow rate L/min	Initial weight (g)	Final weight (g)	Total weight gain (g)	ADG (g/day)	SGR (%/day)	FCR	SR (%)
1	5.79±0.08 a	17.36±0.25 a	11.57±0.24 a	0.19±0.00 a	1.83±0.03 a	1.59±0.15 a	100.00±0.00 a
2	5.86±0.34 a	17.27±0.45 a	11.46±0.58 a	0.19±0.01 a	1.81±0.10 a	1.49±0.32 a	86.67±11.55 a
3	5.65±0.24 a	15.64±0.66 b	9.91±0.55 b	0.17±0.01 b	1.70±0.23 a	1.72±0.06 a	86.67±11.55 a
4	5.76±0.34 a	15.34±0.49 b	9.65±0.45 b	0.16±0.01 b	1.66±0.07 a	1.78±0.11 a	93.33±11.55 a

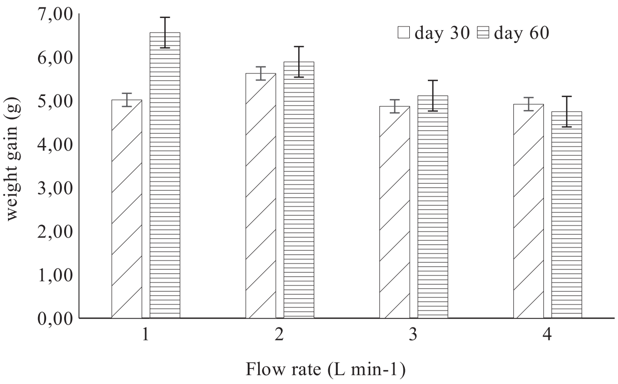

Fig. 5. Effect of flow rates on weight gain of experimental prawns during the first and second 30-days of Trial 2 (vertical lines show ±SD).

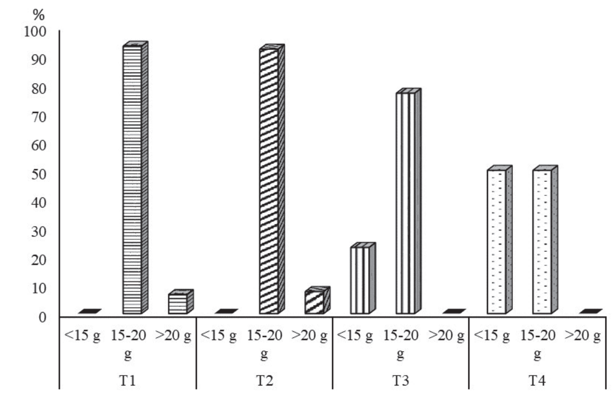

Fig. 6. Final size distribution of the experimental prawn (*M. rosenbergii*) in Trial 2.

removal profile reaching a TAN removal percentage of 91.73% and 90.57%, respectively. Previous studies on ammonia nitrogen removal in synthetic wastewater using seaweed and *Bacillus* spp. had found that aerobic conditions were the best, giving an ammonia removal rate of 94% [8]. Studies on different strains of *Bacillus* sp., a heterotrophic-nitrifying and aerobic-denitrifying bacterium, have shown that *Bacillus* sp. isolated from aquaculture ponds exhibited an enhanced removal efficiency for ammonia, nitrite, and nitrate [9]. The findings of the present trial concur *with other studies that assessed the capacity of removing ammonia* by the genus *Bacillus.* The efficiency of *Bacillus* in modulating water quality is greatly dependent on factors such as mode of application, dissolved oxygen, pH, temperature etc. Providing optimum water quality conditions to achieve the desired efficiency of *Bacillus* in controlling water quality need to be considered [10]. Increased temperature (30ºC) and higher DO concentration have been identified as advantageous for stimulating nitrite oxidizing bacteria (NOB) activity [11].

The results from our study found the efficacy of photosynthetic bacteria (PSB) to be lower than that of *Bacillus* sp. A wild strain of photosynthetic bacteria used to treat wastewater containing high NH_4^+-N could remove up to 83.2% of NH_4^+-N [12]. However, another study had shown efficient ammonium removal of up to 99.67% by a novel photosynthetic bacteria strain of *Rhodopseudomonas* isolated from natural landscape water with a high ability to remove ammonium [13]. Studies on grass carp culture water also revealed that supplementation with photosynthetic bacteria could significantly reduce the levels of ammonia nitrogen and nitrite nitrogen [14].

In experiment II of the present study, varying water flow rates were tested to help design the optimal flow rate for vertical farming of freshwater prawns. The results showed that the highest weight gain and average daily gain of the prawns were obtained from rearing in flow rates of 1 and 2 L min^{-1}. Relatively higher flow rates of 3 and 4 L min^{-1} might have stressed the juvenile freshwater prawns resulting in a reduction of weight gain, average daily gain, and specific growth rate. However, no statistically significant effects were detected on the specific growth, FCR, and survival rate of the prawns. Water flow rate is an important parameter for the success of a recirculating system. Too slow flow rate leads to an increase in suspended solids, while too fast recirculation flow rates lead to less contact time for water in biofilter that can adversely affect the nitrification rate and nitrogen removal efficiency of biofilter [15]. Systematic studies on the effect of flow rates on freshwater prawn growth in recirculating aquaculture systems are scarce. A previous study on the effect of flow rate for juvenile seahorse nursing indicated that water flow rate could significantly affect the growth and survival of seahorse juveniles when cultured in captive conditions [16].

Another study on the effect of flow velocity on growth, stress, and immune response of turbot, *S. maximus,* in recirculating aquaculture systems found that excessively high velocities elicit some stress for turbot, inducing an immune response in the skin, which is sensitive to such environmental changes [17]. A Nile tilapia recirculating aquaculture system tested with different water flow rates revealed that the ammonia removal efficiency reduced substantially with increasing flow rates from 75% at 2.0-3.0 L min^{-1} to 9% at 8.0-10.0 L min^{-1} [18].

There was no significant difference in freshwater prawn survival rates, ranging from 86.7 to 100%, among the four groups evaluated in the present study, indicating that flow rates did not affect prawn survival in the individual grow-out compartments. The mean survival rate and SGR of prawn, *M. rosenbergii,* at a water flow rate of 1.6 L min^{-1} in an aquaponics system were higher than those maintained at 0.6, 2.6, and 3.6 L min^{-1} [19]. The present study showed that different flow rates had no significant effect on feed conversion ratios (FCR) of freshwater prawns in compartment culture. However, relatively higher FCRs and lower SGRs were observed at the faster flow rates of 3 and 4 L min^{-1}, indicating that slower water flow rates are favorable for better growth performance of prawns in a vertical compartment farming system.

The survival rates, FCRs (ranging from 1.49 to 1.78), and SGRs (1.83-1.66) obtained for prawns in the present study were better than some previous reports of prawn growth performance under various farming systems. All-male, mixed-sex, and all-female prawns tested in a commercial pond culture system at a low stocking density of 2.5 juveniles m^{-2} resulted in survival rates of 89%, 85%, and 80%, respectively [20]. In another study, the survival of mixed-sex prawns in ponds ranged from 62 to 72% [21]. In a prawn polyculture system in rice fields stocked at 4 prawns m^{-2}, survival rates of 64%, 49%, and 54% were obtained for all-male, mixed-sex, and all-female stocks, respectively, while the FCR values ranged from 2.74-2.95 [22, 33]. The mixed-sex prawn survival rates ranged from 36.9 to 55.3% in the cages set in freshwater lakes, while the FCR ranged from 2.1 to 3 [23]. The lowest FCR for juvenile prawns grown in an intensive tank culture system was 3.7 at the stocking density of 5 prawns m^{-2}, yielding an average daily growth (ADG) of 0.17 g and an SGR of 1.66 [24]. However, the highest ADG of prawns (0.19 g day^{-1}) obtained in the present study for a rearing period of 60 days in the compartment farming system was lower than the reported ADGs from pond culture of 0.5, 0.4, and 0.37 g day^{-1} for all-male, mixed, and all-female prawns for 150 days of culture [20]. Longer culture durations in a compartment farming system might result in comparable results with pond farming conditions.

Conclusion

This study provided preliminary data on the indoor compartmental culture of giant freshwater prawn (GFP), which will be valuable in developing compact prawn culture systems in locations unsuitable for conventional prawn farming. The results showed that juvenile GFP could be cultured in individual compartments to minimize the influence of heterogeneous individual growth and cannibalism. Although most of the growth performance parameters were better during the culture period of 60 days in the present study, the average daily growth rate was not as high as that of male *M. rosenbergii* cultured in ponds for longer durations of up to 5 months. Hence, further long-term studies similar to typical pond culture durations (5-6 months after nursery stage) are warranted to test potential improvements and refine the technology for improved growth and profitability of culturing male GFP in compartmental recirculating aquaculture systems (RAS). This study also demonstrated that it is possible to use commercially available probiotic products to reduce ammonia in compartmental RAS. A significant reduction of ammoniacal nitrogen was found after treating water with *Bacillus* spp. and photosynthetic bacteria, and either product can be used to reduce NH_3-N to a non-toxic level. Relatively slower flow rates (1-2 L min^{-1}) can achieve the best growth performance of prawns in a vertical compartment farming system.

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