

EVALUATING THE EFFICACY OF SUBSTRATE, INDIVIDUAL BOTTLE, AND BIOFLOC CULTURE METHODS ON THE GROWTH, FEED EFFICIENCY, AND SURVIVAL OF GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii* POST LARVAE

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Abstract: The Giant Freshwater Prawn (GFP) *Macrobrachium rosenbergii* holds significant commercial significance globally. Nevertheless, the optimal rearing system to enhance overall productivity for this species remains unclear. A 60-day rearing trial was conducted to examine the impact of different farming methods on growth performance, feed utilisation efficiency, survival rate, and cost-effectiveness of GFP. Three culture methods, with substrate (control, T1), individual bottle (T2), and biofloc technology (T3) were used with triplicate groups of GFP each. The results indicated that GFP in the T2 exhibited superior weight gain (185.56%) than T3 groups (91.03%) during the initial 30 days of the experiment. However, after 60 days of rearing, GFP in the T3 demonstrated significantly ($p < 0.05$) better growth performance (1188.16% of body weight gain) and Feed Conversion Ratio (FCR) (1.90) among all the culture methods due to increased nutrient availability and reduced cannibalism in the biofloc environment. In contrast, the T2-cultured GFP did not significantly improve survival and growth performance despite having a higher FCR and the highest operational cost. Therefore, T3 emerges as a more viable farming method that enhances the overall productivity of GFP.

Keywords: Giant freshwater prawn, farming technique, biofloc technology.

Introduction

The Giant Freshwater Prawn (GFP), *Macrobrachium rosenbergii* is commercially crucial worldwide. Its popularity is greatly due to its good taste and ability to be integrated with farms such as rice or fish production and its farming as an alternative to marine shrimp farming that has been greatly restricted by disease (Kim *et al.*, 2013). In Malaysia, the aquaculture production of GFP has reached 183 tonnes, valued at RM14.7 million in 2022 (DOF, 2022). Furthermore, GFP is indigenous to Southeast Asian countries, the farming of this species is commonly small-scale, and semi-intensive in Malaysia. This is largely due to the inherent nature of the species, including cannibalistic behaviour, longer larval development, and slower growth rate compared to marine prawns (New *et al.*, 2010).

In consideration of feeding, costs represent the major operational costs of a modern aquaculture farm. Any savings in feeding costs without affecting aquatic animals' growth, Feed Conversion Rate (FCR), survival rate, nutrient composition, and sensory attributes would greatly benefit the aquaculture industry (Kutty, 2005; Kim *et al.*, 2014). The Malaysian 11th plan emphasises that with continued uncertainties in the global economy, a greater resolve is needed to boost productivity and drive economic growth on the domestic front. Therefore, sustainable practices are vital for the continued growth of the aquaculture industry. In addition, optimising culture conditions by ensuring adequate space, appropriate habitat structures, and optimal stocking densities can significantly influence

social dynamics, enhancing growth and productivity in GFP (Tidwell & Coyle, 2008; Shivananda *et al.*, 2012). On the other hand, raising GFP in individual compartments within farming units could effectively enhance survival and growth in GFP by minimising cannibalism and preventing stunted growth (Thawinwan *et al.*, 2022). For instance, the individual culture method was proven to lower feed requirements since there is no feeding competition, though maintaining a consistent feeding rate is crucial to avoid overfeeding or underfeeding (Teoh, 2021, unpublished data).

The introduction of biofloc technology has become increasingly noticeable among farmers and growers in a culture of aquatic organisms (Abakari *et al.*, 2021). In particular, bioflocs are flocs of algae, bacteria, protozoans, and other kinds of particulate organic matter such as faeces and uneaten feed. Each floc is held together in a loose matrix of mucus secreted by bacteria, bound by filamentous microorganisms, or held by electrostatic attraction (Hargreaves, 2013). Notably, biofloc helps enhance feed utilisation efficiency and improves the survival rate and growth performance of aquatic cultured organisms (Pérez-Rostro *et al.*, 2014; Camarin *et al.*, 2023). This is attributable to the fact that the flocs are a supplemental food containing protein (25%-50% dry weight), vitamins, and minerals that can be grazed by the cultured organism amidst feedings of commercial pellets (Hargreaves, 2013). Additionally, the interaction between planktons and bacteria in biofloc helps in better removal of the harmful nitrogenous compounds in water (Becerril-Cortés *et al.*, 2017) as the nitrogen wastes are recycled and/or converted into microbial protein and ultimately improve water quality and health status of the cultured organisms (Emerenciano *et al.*, 2017).

Although some nutrition studies on GFP have been conducted, the data surrounding the rearing system for optimising the overall productivity of GFP lacks clarity at best. Hence, this study aimed to investigate the impacts of different rearing methods, including biofloc technology, on the feed utilisation efficiency,

survival rate, and growth performance of GFP larvae and to define the optimal culture condition for better productivity of GFP nurseries.

Materials and Methods

Three different rearing methods were adopted in the present study: Treatment 1 (T1), conventional method (control); Treatment 2 (T2), individual bottle; and Treatment 3 (T3), biofloc. The individual bottle-rearing method was assessed in a preliminary experiment and reported to be promising in terms of survival and FCR compared to the conventional method (Teoh, 2021, unpublished data). As such, biofloc technology was applied and compared to other methods to determine the efficacy of the three tested methods in rearing GFP.

Rearing System Set Up and Rearing Experiment

Nine units of 500 L fibreglass tanks were randomly assigned to the experimental rearing methods, with three replicate tanks per method. Each tank was filled with 300 L of filtered water and covered with nets to prevent or avoid invasion. Two handfuls of coral bones were added into each tank to provide calcium and minerals for the GFP to improve their shell growth (Chowdhury *et al.*, 1993; Ballester *et al.*, 2017). At the same time, a 3-in-1 top filter system was provided to each of the T1 and T2 tanks for continuous aeration and water circulation throughout the rearing trial.

On the other hand, molasses (55.5 g) was added into the T3 tanks one week before prawn distribution to promote the development of the biofloc (De Schryver *et al.*, 2008). After that, the same amount of molasses was added to all the T3 tanks every three days to maintain the carbon-to-nitrogen ratio of 15:1 (Wei *et al.*, 2016). Note that each of the T3 tanks was equipped with two air stones for aeration and keeping the solids suspended in the water column. For each of the T2 tanks, 20 units of 1.5 L plastic bottles, with their bottoms removed and covered with nylon mesh, were hung, and submerged within the water column. Each bottle housed one prawn,

with 20 prawns stocked individually per tank. Polyvinyl Chloride (PVC) pipes (80 mm (L) × 25 mm (D)) were added into all the T1 and T3 tanks as a substrate to provide shelter for smaller size prawns. In contrast, no substrate was needed for T2 tanks, whereby each prawn was individually stocked into a bottle.

The prawn rearing was carried out in the Aquaculture Facilities (AQF) at Universiti Tunku Abdul Rahman (UTAR), Kampar, Perak (4°20'25.7"N 101°08'07.6"E). The GFP post larvae (PL₁₄) were obtained from Pusat Perternakan Udang Galah in Kampung Acheh. Upon arrival at the AQF, the Post Larvae (PL) were acclimatised in a stocking tank and fed with commercial prawn crumble (Star Feedmills (M) Sdn. Bhd.) for one week. After one week of conditioning, 180 healthy and similar sizes of PL with an average weight of 0.13 g and average length of 2.68 cm were selected, weighed, and distributed randomly into the nine experimental culture tanks.

Prawns were fed with commercial prawn feed (Star Feedmills (M) Sdn. Bhd.) twice a day at 09:00 and 17:00 until apparent satiation for 60 days and feed consumption was recorded daily. Water temperature (°C), pH, and dissolved oxygen (DO, mg/L) were measured daily using a portable pH metre (HI8424, HANNA instruments, USA) and portable DO metre (ECDO1101, EUTECH, Singapore). Total ammonia nitrogen (TAN), nitrite, and nitrate

were measured using a Freshwater Master Test Kit (API, USA) every three days. The data were validated using a colourimeter (DR/890, HACH, USA) once a week or when the concentration exceeded the detection limit of the test kit. Total Settleable Solid (TSS) was measured using an Imhoff cone (Avnimelech, 2015) every 10 days. Water change was performed when water parameters exceeded the acceptable range (Baloi *et al.*, 2013) and water was topped off for evaporation.

Sample Collection and Calculation

20 days into the rearing trial, the prawns from each tank were collectively weighed to determine their body weight and to monitor the feed utilisation efficiency, survival rate, and growth performance. After the end of the rearing trial, all prawns were individually weighed, and their total length was measured. Meanwhile, prawn growth performance was analysed in regard to weight gain and Specific Growth Rate (SGR). In addition, feed utilisation efficiency was determined by FCR. The survival rate (%) was analysed by calculating the mortality rate of prawns. The operational cost for the respective culture setup included nets, a water test kit, and feed costs. There were additional operational costs for biofloc and individual bottle methods whereby molasses were added as a carbon source while 1.5 L plastic bottles and nylon nets were used in the latter, respectively. However,

$$\text{Weight gain (\%)} = \frac{[\text{Final mean weight} - \text{Initial mean weight}] \text{ (g)}}{\text{Initial mean weight (g)}} \times 100$$

$$\text{SGR (\%/day)} = \frac{[\ln(\text{final mean weight}) - \ln(\text{initial mean weight})] \text{ (g)}}{\text{Days of culturing trial}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Final prawn number}}{\text{Initial prawn number}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry feed (g)}}{\text{Wet weight gained (g)}} \times 100$$

$$\text{Operational cost (RM)} = \text{Apparatus and material costs (RM)} + \text{Feed cost (RM)}$$

electricity and water utilities were not included in the cost. All the parameters were determined using the formula below:

Statistical Analysis

All data were presented in mean \pm Standard Error (SE) and subjected to the Shapiro-Wilk test for normality and a one-way Analysis of Variance (ANOVA) using the statistical program IBM SPSS version 16.0 (SPSS Inc., IL, USA) to determine if significant differences occurred among culture methods. Simultaneously, differences among means were determined by Duncan's multiple range test and considered to be significant at the level of 0.05.

Results

Growth Performance and Survival Rate

After 30 days of rearing, weight gain and SGR of GFP cultured in T2 were the highest, which were significantly higher ($p < 0.05$) than that of GFP in T3 yet not significantly ($p > 0.05$) different than those in T1 (Table 1). However, percentage weight gain was the highest in T3 groups (1188.16%) after 60 days of rearing, significantly higher than those reared with

the other two methods (352.69%-395.49%). Likewise, GFP reared with T3 recorded the highest SGR (4.17%.day⁻¹) among all the experimental prawns while no significant difference was observed between T1 and T2. On the other hand, GFP in T1 achieved the highest survival rate (70.83%), followed by those in T2 (67.50%), and T3 groups recorded the lowest survival rate (50.42%) throughout the 60 days of rearing. However, no significant difference was observed among the rearing methods.

FCR and Production Cost

GFP in T3 revealed the lowest FCR with a value of 1.90, followed by T1, 2.29, and T2-cultured GFP was observed to have the highest FCR, 2.40, after the 60-day rearing period (Table 1). However, no significant difference was observed among the rearing treatments. On the other hand, a significant difference was noted in total operational cost among the experimental groups. In particular, T1 groups reported the lowest operational cost (RM35.00), followed by T3 (RM36.13), and T2 was observed to require the highest operational cost (RM60.13), which was caused by the higher system establishment cost.

Table 1: Growth performance, survival rate, FCR, and input cost of GFP cultured with different rearing methods¹

Parameters	Rearing Methods ²		
	T1	T2	T3
After 30 days			
Weight gain (%)	148.29 \pm 8.73 ^{ab}	185.56 \pm 28.11 ^b	91.03 \pm 26.20 ^a
SGR ³	3.03 \pm 0.12 ^{ab}	3.47 \pm 0.32 ^b	2.09 \pm 0.83 ^a
After 60 days			
Weight gain (%)	352.69 \pm 48.93 ^a	395.49 \pm 17.76 ^a	1188.16 \pm 405.51 ^b
SGR	2.50 \pm 0.19 ^a	2.67 \pm 0.06 ^a	4.17 \pm 0.77 ^b
Survival rate (%)	70.83 \pm 0.83	67.50 \pm 1.44	50.42 \pm 21.11
FCR ⁴	2.29 \pm 0.14	2.40 \pm 0.13	1.90 \pm 0.38
Operational cost ⁵ (RM)	35.00 \pm 0.01 ^a	60.13 \pm 0.00 ^c	36.13 \pm 0.06 ^b

¹Results are presented as mean \pm SE of triplicate groups of prawn (n = 3). Values in the same row with different superscripts in the same row indicate significant differences in Duncan's multiple range test at $p < 0.05$. ²Experimental culture method nomenclature: T1 = conventional method (control); T2 = individual bottle; T3 = biofloc technology. ³SGR = Specific Growth Rate, ⁴FCR = Feed Conversion Ratio, ⁵Operational costs include the expenses for feed (calculated as total feed given (g) multiplied by feed price in RM/g) and culture setup apparatus and materials, excluding electricity and water utilities

Water Quality Parameters

Throughout the experiment, no significant differences were noted in the water temperature, pH, and DO among all the culture tanks, with the average water temperature of tanks ranging from 26.8°C to 27.3°C. The average water pH ranged from 7.01 to 7.07 while the average DO was 6.43 ppm (Table 2). On the other hand, TAN in the T1 tank was significantly higher compared to T3, yet not significantly different than T2. However, water in the T3 tank contained the highest nitrite

(0.07 ppm) and nitrate (17.54 ppm), significantly higher than those in the T2 and T1 tanks. TSS fluctuated in the T3 tank (Figure 1) with an average reading of 20.89 mL.L⁻¹, which was the significantly highest among all the culture tanks. Conversely, the average TSS value was recorded as 0.20 ppm for the other two rearing methods (Table 2). Water change was conducted on day 40 when TSS spiked 70.00 mL.L⁻¹, dropping to 5.33 mL.L⁻¹ by day 50.

Table 2: Water quality of experimental tanks throughout the 60 days rearing trial¹

Water Quality Parameters	Rearing Methods ²		
	T1	T2	T3
Temperature (°C)	27.1 ± 0.24	27.3 ± 0.19	26.8 ± 0.17
pH	7.07 ± 0.05	7.00 ± 0.04	7.01 ± 0.06
DO ³ (ppm)	6.45 ± 0.17	6.42 ± 0.16	6.43 ± 0.13
Total ammonia nitrogen (ppm)	0.12 ± 0.04 ^b	0.11 ± 0.04 ^{ab}	0.01 ± 0.00 ^a
Nitrite (ppm)	0.01 ± 0.00 ^a	0.00 ± 0.00 ^a	0.07 ± 0.01 ^b
Nitrate (ppm)	1.88 ± 0.12 ^a	1.47 ± 0.11 ^a	17.54 ± 2.80 ^b
TSS ⁴ (mL.L ⁻¹)	0.20 ± 0.05 ^a	0.20 ± 0.05 ^a	20.89 ± 10.08 ^b

¹Results are presented as mean ± SE of triplicate groups of prawn (n = 3). Values in the same row with different superscripts in the same row indicate significant differences in Duncan’s multiple range test at *p* < 0.05, ²Experimental culture method nomenclature: T1 = conventional method (control); T2 = individual bottle; T3 = biofloc technology, ³DO = Dissolved Oxygen, ⁴TSS = Total Settleable Solids

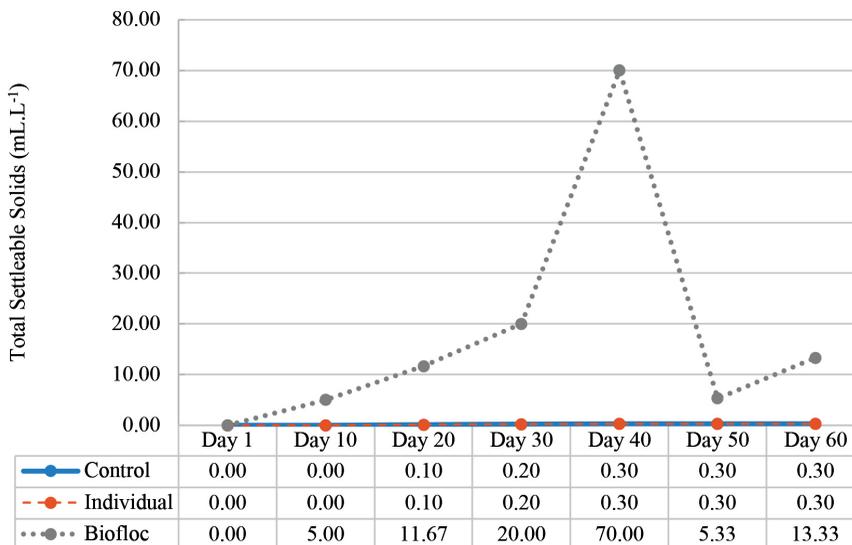


Figure 1: The average readings of total settleable solids in the water of the experimental systems throughout the 60 days of the rearing trial (n = 3)

Discussion

Growth Performance and Survival Rate

After one month into the rearing period, GFP in biofloc and individual bottle tanks demonstrated the lowest and highest growth performance, respectively. However, GFP in biofloc tanks recorded the highest percentage of weight gain and SGR after 60 days of rearing. This is in line with a previous study (Emerenciano *et al.*, 2017), where the consumption of biofloc enhanced the growth performance of shrimp. On the contrary, GFP in the control group has the lowest growth performance due to competition. Indeed, GFP, with a faster growth rate, becomes dominant, causing smaller prawns to grow even slower (FAO, 2006).

As such, competition between the GFP for food and space was the major factor for the inferior growth performance in the control system. In addition, mortality was higher in the biofloc group due to the excessive level of molasses, which was beyond the desired range of settleable solid concentration of 10 to 15 mL.L⁻¹ for shrimp (Hargreaves, 2013). With the smaller number of prawns in the tank due to higher mortality, the remaining GFP in the biofloc tank was able to grow larger than those in control and individual bottle tanks. According to Suneetha *et al.* (2018), the survival rate of GFP in biofloc systems should be higher since the heterotrophic bacteria can reduce the spread of pathogens. Likewise, Hussain *et al.* (2015) reported that the survival rate of green tiger shrimp (*Penaeus semisulcatus*) was better in the biofloc system compared to the conventional culture method. Hence, it can be concluded that GFP can perform well in a suitable range of biofloc density.

FCR and Operational Cost

In the present study, biofloc technology rearing ameliorated the FCR of GFP despite no significant improvement being noted. Hence, biofloc technology has been suggested to be able to improve the FCR of cultured aquatic organisms in the sense that the production of

protein and lipid-rich microorganisms provide extra nutrients for the cultured organisms (Kim *et al.*, 2014; Avnimelech, 2015; Emerenciano *et al.*, 2017). In the present study, the feed was in crumble form, which is more suitable for PL feeding. The commercial feed contained approximately 40% crude protein, which met the nutrient requirement of the early stage of GFP (Emerenciano *et al.*, 2012). Considering the better FCR and lower operational cost driven by the biofloc system, it is thus suggested that the biofloc farming system can act as a viable and cost-effective rearing method for improving the FCR of the GFP and the overall productivity of the prawn aquaculture. On the other hand, individual bottle rearing required a 66% higher operational cost than the other two methods. Considering the extremely high operational cost yet the unexceptional survival rate of GFP, it appears that the individual bottle culture method is not an economical approach for improving GFP's survival rate and growth performance in commercial-scale farming.

Water Quality Parameters

Throughout the 60 days of rearing, water temperature, pH level, DO, ammonia, and nitrite levels were in the acceptable range for GFP rearing, regardless of the culture techniques. Particularly, the lowest ammonia, together with the highest nitrite and nitrate levels in the water of the biofloc tank, evidenced the efficacy of the existing biofloc system in which the ammonia was taken up by the heterotrophic nitrifying bacteria and converted to nitrite and ultimately nitrate (Nandlal & Pickering, 2006). Furthermore, the TSS level fluctuated in the biofloc tank. It has been reported that the optimal biofloc density for shrimp culture ranges between 10 to 15 mL.L⁻¹ (Hargreaves, 2013). Therefore, the dramatic increase of TSS day 30 is considered one of the main causes leading to the low survival rate in the biofloc group as excessive flocs density clogging the gills or circulatory system of the prawn (Kavitha *et al.*,

2017). Correspondingly, excess sludge should be removed occasionally to maintain the optimal level of flocs density.

Conclusions

In conclusion, individual bottle rearing is not recommended for commercial farming since the operational cost is relatively higher compared to the conventional culture and biofloc methods. However, it did not result in a profound improvement in GFP's growth and survival rate. On the other hand, biofloc rearing is proven to be a more viable method for improving GFP's FCR and growth performance. Furthermore, the present study suggested that the water quality should be monitored daily and the biofloc density should be maintained at the desired range for the optimal performance of GFP.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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