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Effect of biofloc volume on growth, survival, economics and proximate composition of *Macrobrachium rosenbergii* postlarvae cultured in brackish water biofloc system

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Abstract

Biofloc volume refers to the suspended solids in a biofloc system, consisting of bacteria, algae, protozoa, organic matter, and other microorganisms that serve as natural food for cultured fish and crustaceans. Furthermore, optimal biofloc volumes enhance water quality, provide live feed, and improve the overall health of cultured animals. Moreover, excessive biofloc can clog gills, degrade water quality, and reduce animal growth or cause mortality. However, the effects of different biofloc volumes on the production, nutritional quality, and economic viability of culturing giant river prawn, *Macrobrachium rosenbergii* remain underexplored. Therefore, a 4-weeks experiment was conducted to optimize the suitable biofloc volume of *M. rosenbergii* postlarvae (PLs). Growth, survival, proximate composition of *M. rosenbergii* and water quality, total bacteria and zooplankton community were compared among four biofloc (BF) volume groups of BF2 - 5, BF7 - 10, BF12 - 15 ml L^{-1} and BFZ/zero-solid removal biofloc system. Twelve 125 L polyethylene tanks with water volume of 100 L were used for this experiment. Each tank was stocked with 500 PLs (average initial weight 21.8 ± 2.36 mg). Each treatment was randomly assigned in triplicate. Temperature, nitrite-N and nitrate–N did not differ ($P > 0.05$) among four treatments. A lower dissolved oxygen concentration was remained ($P < 0.05$) in the BF-Z than three BF volume treatments. A lower *Vibrio* spp. density was found ($P < 0.05$) in the BF2 - 5 than BF12 - 15 and BF-Z treatments. Ciliates, rotifers and nematodes were significantly ($P < 0.05$) higher in the BF-Z than other biofloc volumes groups. PLs growth was similar ($P > 0.05$) among four BF volume groups. However, significantly ($P < 0.05$) a higher survival and economic return were obtained in the BF2 - 5 treatment when compared to those BF7 - 10, BF12 - 15 ml L^{-1} and BF-Z. In conclusion, our results show that the biofloc volume 2–5 ml L^{-1} is found suitable for *M. rosenbergii* PLs, ensuring higher survival and profit in nursery phase can be considered in management practices.

Highlights

- A lower *Vibrio* spp. density observed in the 2 - 5 ml L^{-1} floc volume level biofloc system
- The floc volume level 2 - 5 ml L^{-1} supported for more prawn postlarvae survival

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- The best FCR obtained in the 2 - 5 ml L^{-1}floc volume level biofloc system
- Simple cost analysis shows a floc volume levels 2 - 5 ml L^{-1} is economically viable than the floc volume levels of 7 - 10, 12 - 15 ml L^{-1} and zero-exchange biofloc system when culturing *Macrobrachium rosenbergii*

Keywords Giant river prawn, Floc volume, Prawn nursery, Solid removal, Zero-exchange system

Introduction

Giant river prawn, *Macrobrachium rosenbergii* is a delicious table food and high demand aquaculture commodity globally. This species is being cultured in the tropical and subtropical region, supporting employment opportunities, income generation, subsistence among prawn value chain actors in many Asian countries such as Bangladesh, Cambodia, India, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam [1–3]. This prawn naturally inhabits freshwater ecosystems, but berried animals migrate from freshwater to brackish water environment for spawning, where the larvae undergo 11 developmental stages to reach the postlarval stage (PLs). These PLs graze and forage in brackish water for several weeks and then begin to migrate towards freshwater for maturation [4, 5]. Thus, hatcheries usually use brackish water at salinities around 10–12 ‰ for commercial venture PLs production.

Two types of biofloc technology (BFT) systems *i.e.* zero-exchange and limited or minimal water have been well-documented for fishes and crustaceans. This aquaculture system is recognized as an environment friendly blue revolution technology, which augment productivity and survival as well as minimize food conversion ratio and maximize food utilization rate. This system maintains good water quality through ecological process by uptake of toxicant nitrogenous compound from aquaculture environments. A BFT system continuously proliferates microorganisms, which has been utilized by fishes, shrimps and prawns as a surplus natural live feed, providing nutrition and enhancing health status [6–8]. In aquaculture systems, higher level of slugs, and their total suspended solids (TSS) have the harmful effects and resulting the decreases of free oxygen and reduce light penetration, the latter of which interferes with the proliferation of algae, increasing COD and BOD level; thereby, these can be associated with poor water quality, stunted growth and survival and the reduced overall health of culture animal [9–13].

Recently, biofloc systems incorporating solids removal have been developed and introduced, contributing to the maintenance of TSS levels. Studies show that the low (100–300 mg L^{-1}) and medium (400–600 mg L^{-1}) TSSs based BFT system improves water quality conditions while reducing feed cost, augmenting production, growth and survival as well as ensuring overall animal health [14]. In contrast, the higher solids (600–100 mg L^{-1}) based BFT system is characterized by decreased shrimp productivity, growth and survival [15, 16]. This culture strategy has led to a higher degree of shrimp gill occlusion, which has resulted in reduced shrimp performance including mortality [15].

In BFT, floc volume is an empirical variable, gradually increasing from startup and typically accompanied by a steadily increasing trend in TSSs with increasing biofloc volume [17–20]. Relatively little attention has focused on the consequences of higher floc volume levels. In addition, floc volume level of 17 ml L^{-1} have been associated with mortality at the end of study as a result of gill clogging of *Farfantepenaeus duorarum* [20]. Furthermore, the higher biofloc volume consists of assemblages of higher heterotrophic microorganisms, which can reduced available oxygen concentrations as well as clogging gills; consequently, these authors have suggested 5–15 and 5–20 ml L^{-1} floc volumes for shrimp and tilapia fingerlings, respectively [6]. For these reasons, the optimal floc volume and their management for different aquaculture species are the considered to be most vital issues. However, there are currently no published reports available on the effects of different floc volume on water quality, total bacteria, *Lactobacillus* spp., *Vibrio* spp., zooplankton composition/abundance and biofloc proximate composition, or the subsequent effects of these biological and environmental variables on the performance of culture subjects in biofloc systems.

Currently, BFT based systems have been shown viable to *M. rosenbergii*, where suitable carbon source, C-N ratio and salinity levels are examined [21–27]. More recently, the use of a maize starch carbon source has resulted in a higher survival rate than molasses and wheat bran, and in the molasses and wheat bran groups biofloc volume was determined to increase over time, probably presenting an obstacle for PL survival in zero-exchange BFT systems [24]. Maize starch addition to nitrogen ratios of 10–25 have been shown an increasing trend of biofloc volume in zero-exchange BFT condition at stocking density of 4 PLs L^{-1}, and this study suggests some water exchange beyond a month culture [21]. For this reason, Hosain et al. [23]

performed biofloc removal at the 19 th day in a maize starch based BFT system, which had maintained safe floc volume and higher biological solids including more total zooplanktons in 10 or 15‰ salinity biofloc culture environments, contributing to higher prawn PLs survival. *M. rosenbergii* growth and survival were better in a BFT than control, in which biofloc volume displayed an increasing trend, reaching < 35 ml L^{-1} within 90 days; however, the larger prawns (8.8 g) are probably able to tolerate this level of biofloc volume [26]. Moreover, the effects of floc volumes are still unknown to *M. rosenbergii* PLs performance produced in biofloc systems. Thus, the aim of this study was to compare the growth performance of *M. rosenbergii* PLs in the different floc volumes in the BFT system.

Materials and methods

Preparation of stock water, biofloc inoculum and biofloc enriched water

The stocked freshwater, seawater (28‰) and brackish water (6 and15‰) was prepared and kept in four fiberglass tanks (each tank 2.5 tonnes capacity); these were then treated with 5 ppm chlorine. Each stock water tank was then vigorously aerated with 8 air stones, installed near the edges of tank for several days to remove chlorine.

Three cylindrical polyethylene tanks (125 L water capacity) were used for the preparation of inoculums. These tanks were filled with 100 L dechlorinated brackish water (15‰). The inoculum materials included 2 kg pond soil, 1 g ammonium sulphate and 40 g maize starch as carbon source added to each inoculum tank [27]. These inoculum tanks were vigorously aerated with 4 air stones for 24 h. Ammonia–nitrogen was checked, confirming the absence of ammonia. Each inoculum was sieved using a 200 µm mesh benthos sieve box. These inoculums were then assigned to experimental biofloc stock water production tanks.

A total of 1000 individual *M. rosenbergii* juveniles (weight of 674.9 ± 74.87 mg) were acquired from International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia, Port Dickson, Negeri Sembilan, Malaysia. These prawn juveniles were distributed between two fiberglass tanks with water volume 900 L at freshwater conditions. In each tank, six air stones were installed at the edges of the tank. The salinity level was increased at 3‰ in each day by addition of dechlorinated sea water, while the desirable salinity level (15‰) was obtained at day- 5. On days 3 and 6, approximately 40% of water exchange was performed with 6‰ and 15‰ brackish water, respectively. At day 6, after water exchange, these each prawn culture tank was treated by the addition of 100 L biofloc inoculum (1% of tank volume). Prawn were fed shrimp pellet (40% protein) at the rate of 10% body weight twice a day. The daily maize starch was administered to the nitrogen ratio of 20. The biofloc volume (ml L^{-1}) was measured at 08:00 h every three days interval. In the second week, the bottom deposited solids were removed. After that, every three days, this process was performed in order to maintain a floc volume (12 ml L^{-1}) using equation i and ii (will be discussed in next section). Dechlorinated brackish (15‰) water was added to compensate the loss of floc removal. In day 21, this biofloc enriched water (12 ml L^{-1}) was transferred into experimental tanks of differing floc volumes; the procedure of three floc volume level adjustment will be discussed in next section.

Experimental animal source and biofloc volumes management

A total 8100 individual *M. rosenbergii* PLs were produced in 12‰ salinity at I-AQUAS prawn hatchery. A five days acclimation was performed in three 1-tonne tanks with water volume 900 L. These tanks were filled with 12‰ dechlorinated water. A total 2700 PLs (stocking density: 3 individuals L^{-1}) were stocked in each acclimation tank. In the second day, the salinity was increased to achieve the desired salinity of 15‰ by adding seawater. This salinity is reportedly preferable for *M. rosenbergii* PLs culture in BFT [23]. The PLs were fed the shrimp pellet feed (STAR Feedmills (M) Sdn. Bhd.) that contained 40% crude protein and 5% fat twice (09:00 and 18:00) daily to apparent satiation. Each tank was siphoned daily and approximately 40% of the water volume was exchanged.

This experiment was conducted with four treatments: floc volume 2–5 ml L^{-1} (BF2 - 5), floc volume 7–10 ml L^{-1} (BF7 - 10), floc volume 12–15 ml L^{-1} (BF12 - 15) and zero-exchange biofloc system (BF-Z). A total of twelve 125 L cylindrical polyethylene experimental tanks were randomly assigned in triplicate. Prior to this study, each tank water volume was 100 L, while nine tanks were filled with biofloc stock water at floc volume levels approximately 12 ml L^{-1}. In case of BF-Z treatment, three tanks were filled with 15‰ treated brackish water and 1% of biofloc inoculum. Each tank was aerated by 3 air stones, placed near the edge of the tank. Floc volume of each treatment tank was adjusted by using equations i & ii, and all experimental tanks were then left overnight. Then, a total of 500 PLs (average initial weight 21.8 ± 2.36 mg) were stocked (5 individuals L^{-1}) in each tank. The biofloc volume of all treatment tanks was measured daily

at 08:00 h using Imhoff cones. Biofloc removal was performed to maintain the designed volume levels. No floc/solid was removed from BF-Z treatment. To maintain the floc volumes, excessive bioflocs water of each tank was transferred into an outlet system, which was attached to an inlet of removable tank with a plankton net setting (100 µm mesh) to capture the flocs. These waters were then transferred back into the same tanks. Dechlorinated water was added to experimental tanks to compensate for water loss due to evaporation.

Estimation of excess biofloc volume in culture tank (BFV*ex*) = (W*v* × BFV*ct*) − (Wv × BFV*prs*) (i). Where, W*v* = experimental water volume (L), BFV*ct* = Biofloc volume present in culture tank (ml L^{-1}), BFV*prs* = Preselected biofloc volume (ml L^{-1}).

Estimation of water volume for biofloc removal in culture tank = (W*v* × BFV*ex*)/(W*v* × BFV*ct*) (ii).

Giant river prawn feeding and carbon source management

The giant river prawn PLs were fed with the same shrimp pellet used during acclimatization, with a feeding rate 40% of PL's biomass [23, 28]. A set total 4.5 g feed was supplemented twice (9:00 and 18:00 h) per day during this study. Maize starch carbon source (24) at a rate of C-N ratios 20 was used for this study [21]. The amount of daily carbon source was estimated according to De Schryver et al. [29]. Maize starch was then prepared as described by Romano [30] with some modification. It was mixed with dechlorinated brackish (15‰) water (1:10) and left overnight in 12 beakers for different biofloc groups. After that it was applied to each of the floc treatments and to the zero water-exchange tank at 10:00 h. Sodium bicarbonate ($NaHCO_3$) was also administered to each treatment tank to maintain pH and alkalinity with recommended dose [31, 32].

Water quality parameters

Temperature, dissolved oxygen and pH were measured twice a week 11:00 h with replication using a multi parameter (YSI Model 556, YSI Incorporated, Yellow Springs, Ohio, USA). Total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) contents were determined with API® commercial test kits (API® Aquarium Pharmaceuticals, North America) on a weekly basis. Total suspended solids (TSS) and total volatile suspended solid (VSS) were determined at day 9, 18 and 27. Water samples (50 ml) were collected from each tank at around 16: 00 h. These were then filtered under vacuum pressure through pre-dried and pre weighed GF/C filter paper. TSS and VSS were analyzed according to ESS Method (340.2) [33]. Filter papers, wet and dried samples were weighed to 0.01 mg using a Mettler AC 100 balance. The biofloc volume (ml L^{-1}) was measured using the Imhoff cones daily at 08:00 h according to Avnimelech [34]. Floc volume data were presented on a weekly basis.

Biofloc total bacteria, Lactobacillus and Vibrio spp., and zooplankton

Biofloc microorganism communities *i.e.* total heterotrophic bacteria (TB), *Lactobacillus* spp., *Vibrio* spp., ciliates, rotifers and nematodes were determined at the end of the experimental day. Total bacteria estimation was performed using tryptic soya agar (TSA; Difco, Detroit, MI, USA) under the dilution factors of 10^5, 10^6, 10^7 and 10^8. The *Lactobacillus* spp. were grown using *Lactobacillus* MRS agar medium (HiMedia, India) under the dilution factor of 10^0, 10^1, 10^2 and 10^3. For the enumeration of *Vibrio* spp. number, thiosulphate citrate bile salt sucrose (TCBS) (Difco Laboratories ®, Detroit, MI, USA) was used with dilution factors of 10^0, 10^1, 10^2 and 10^3. After preparing the agar plates, these were incubated at 37 °C for 24 h for later colony forming unit (CFU) counting. The data of total bacteria, *Vibrio* spp. and *Lactobacillus* spp. are presented as CFU ml^{-1}.

For zooplankton enumeration, a 50 ml water sample from each biofloc tank was collected and preserved in 4% buffered formalin for further analysis [23]. The ciliates, rotifers and nematodes were identified to the generic level with the aid of a light microscope [35–38]. In order to measure the abundance of zooplankton, 1-ml concentrated water sample was transferred to a Sedgwick-Rafter counting cell. A total 300 fields of SR-cells were counted for each sample with triplicates for each biofloc volume group. The abundance of ciliates, rotifers and nematodes were expressed and presented as individuals L^{-1}.

Giant river prawn growth performance and economic analysis

Fortnightly samples of 30 PLs were randomly collected from each tank to measure their body weights. After 28 days of rearing, all PLs were counted to determine survival, while 30 PLs from each experimental tank were also measured for their body weight and the growth performance was determined. Survival, final weight, weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) were estimated using following equations [39]:

$$\text{Weight gain} = [\text{final weight} - \text{initial weight}]$$

$$\text{Specific growth rate (SGR, \%}day^{-1}) = [(\ln Wf - \ln Wi)]/t \times 100,$$

where Wf = final weight, Wi = initial weight, and t = time in days

$$\text{Survival rate (\%)} = [\text{final number of prawns / initial number of prawns}] \times 100$$

$$\text{Feed conversion ratio as FCR} = \text{total diet fed (mg)/total wet weight gain (mg).}$$

A simple economic analysis was done to estimate the benefit–cost ratio (BCR) of giant prawn PLs produced within the three experimental biofloc volume groups and the zero-exchange BFT system. The following formula was used to estimate the profitability of *M. rosenbergii* PLs raised this study:

$$R = P_{bi}B_i - \left(P_{xj}X_j + TFC\right)$$

where, R= net return, P_{bi}= unit price of ith products (RM individual^{-1}), B_i= quantity of ith products sold (total number), Pxj= unit price of jth inputs, Xj= quantity of jth inputs, i= 1, 2, 3,... n, TFC= fixed costs (23).

Proximate analysis

The biofloc samples as well as 30 prawn PLs from final samples were obtained from each tank, these were dried in an oven at 55 °C until constant weight and preserved at − 20 °C for further analyses. The bioflocs and prawn PLs whole body proximate composition analyses were performed in triplicate following standardized methods [40]. Dry matter was estimated at 105 °C until constant weight and then the samples were used to measure the ash content at 600 °C for 5 h. The Foss Tecator Lipid Analyzer (Foss Tecator, Soxtec™ 8000) was used to estimate the lipid content by petroleum ether extraction. Crude protein percentage was determined using a protein analyzer (Foss 2400 Kjeltec Analyzer Unit) following a 60 min acid digestion.

Statistical analysis

Statistical analysis was performed using SPSS version 25 for Windows. The homogeneity of the variances was determined using Levene`s test for analysis of the statistical data. Differences among treatments were determined by using one-way analysis of variance (ANOVA). Tukey's tests were used for post hoc comparison of mean between different groups. Additionally, Games-Howell post hoc test was done for the data of floc volume, total suspended solids and volatile suspended solids to compare the mean values of the different groups. All the data were compared and presented in the text, figures and tables as mean ± standard error and significant difference at α 5% (P< 0.05).

Results

Water quality parameters and biofloc volume

Temperature and pH were not significantly different (P> 0.05) among four different treatments (Table 1 and Fig. 1a & b). There was significantly (P< 0.05) lower DO in the BF-Z than those of BF2 - 5 and BF7 - 10 treatments (Table 1 and Fig. 1c). Although dissolved oxygen was similar at week 1 and 2 (P> 0.05), this significantly decreased by week 3 and 4 in the BF-Z treatment (Fig. 1c). Ammonia was significantly (P< 0.05) higher in BF2 - 5 treatment. However, ammonia was similar among BF7 - 10, BF12 - 15 and BF-Z treatments (Table 1 and Fig. 1d). Nitrite-N and nitrate–N concentrations were similar (P> 0.05) in the four treatments at each week and in final mean values (Table 1 and Fig. 1e & f). A higher TSS level (P< 0.05) was detected in the BF-Z treatment than in BF7 - 10 or BF2 - 5 (Table 1 and Fig. 2a). A lower VSS was remained (P< 0.05) in the BF2 - 5 than those of BF7 - 10, BF2 - 5 and BF-Z treatment (Table 1 and Fig. 2b). Floc volume was significantly higher in the BF-Z than the other treatments (Table 1 and Fig. 2c). Significantly lower biofloc volume was remained in BF2 - 5 than those of BF7 - 10, BF12 - 15 and BF-Z treatments (Table 1); floc volume was steadily increased in every week in the BF-Z treatment (Fig. 2c).

Table 1 Physicochemical features of the water at different floc volume levels biofloc systems and zero-exchange biofloc system when culturing *Macrobrachium rosenbergii* post larvae for 28 days

Variables	BF2 - 5	BF7 - 10	BF12 - 15	BF-Z
Temperature (°C)	27.60 ± 0.04^{a}	27.56 ± 0.06^{a}	27.58 ± 0.06^{a}	27.72 ± 0.06^{a}
pH	7.71 ± 0.03^{a}	7.76 ± 0.04^{a}	7.79 ± 0.05^{a}	7.65 ± 0.03^{a}
DO (mg L^{-1})	5.46 ± 0.04^{b}	5.48 ± 0.07^{b}	5.36 ± 0.04^{ab}	5.10 ± 0.10^{a}
Ammonia-N (mg L^{-1})	0.52 ± 0.08^{b}	0.31 ± 0.03^{a}	0.27 ± 0.03^{a}	0.35 ± 0.03^{ab}
Nitrite-N (mg L^{-1})	0.33 ± 0.06^{a}	0.20 ± 0.06^{a}	0.33 ± 0.06^{a}	0.27 ± 0.05^{a}
Nitrate–N (mg L^{-1})	50.0 ± 10.0^{a}	60.0 ± 10.44^{a}	61.25 ± 9.79^{a}	63.33 ± 8.73^{a}
TSS (mg L^{-1})	294.44 ± 9.24^{a}	423.40 ± 18.93^{b}	472.14 ± 13.26^{bc}	603.37 ± 82.01^{c}
VSS (mg L^{-1})	180.07 ± 1 5.10^{a}	235.51 ± 9.07^{b}	241.11 ± 9.52^{b}	288.07 ± 41.24^{b}
Floc volume (ml L^{-1})	3.83 ± 0.32^{a}	8.75 ± 0.32^{b}	13.16 ± 0.34^{c}	30.41 ± 8.29^{bc}

Similar superscript letters in the same row indicate the lack of significant difference (P> 0.05); different letters (P< 0.05) reflect significant differences

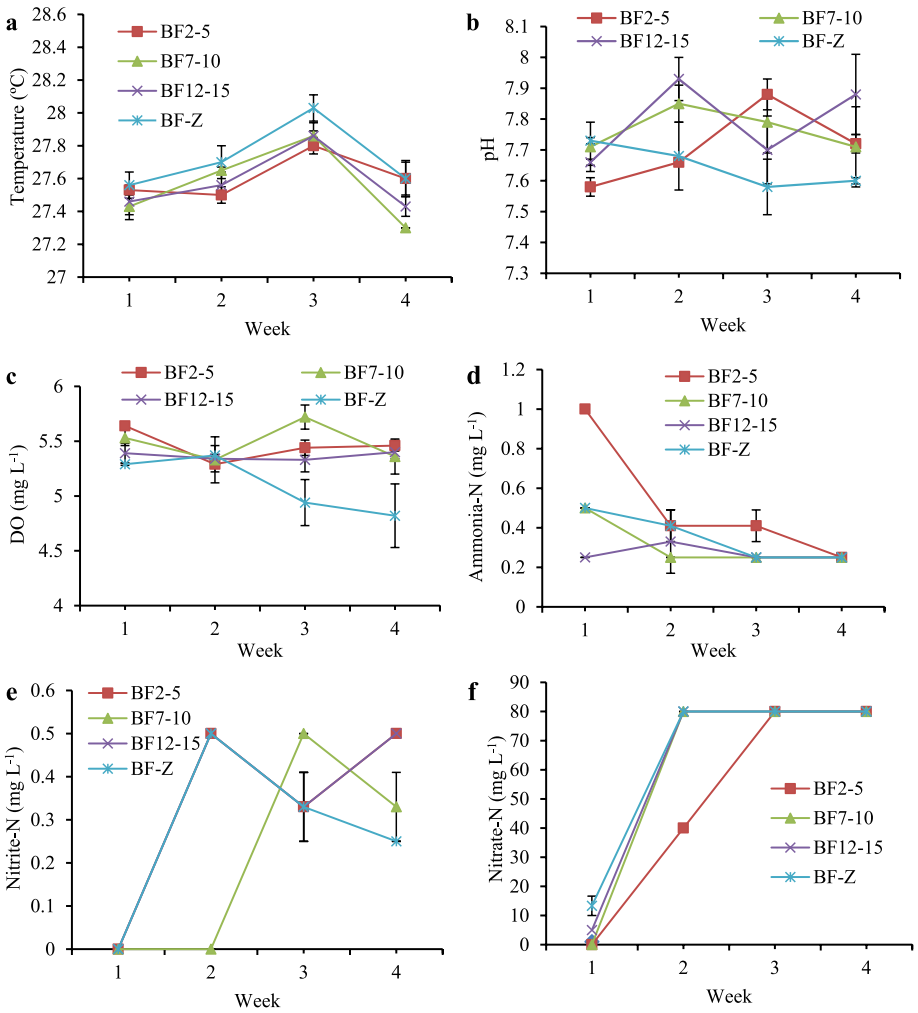

Fig. 1 Weekly mean (± SE) temperature (°C) (**a**), pH (**b**), dissolved oxygen (DO) (mg L^{-1}) (**c**), ammonia–nitrogen (mg L^{-1}) (**d**), nitrite-nitrogen (mg L^{-1}) (**e**), and nitrate-nitrogen (mg L^{-1}) (**f**) in different floc volume levels in solids removal and zero-exchange biofloc systems for *Macrobrachium rosenbergii* post larvae

In this study, it was apparent that four different biofloc volume treatments were capable of eliminating the nitrogenous toxicants. The dissolved oxygen concentration was decreased in week 3 and 4 as related to steadily increasing biofloc volume and its solids in in the BF-Z treatment. The lower biofloc volume treatment BF2 - 5 was recorded, indicating that a minimum level of solids ensured improved water quality parameters in the *M. rosenbergii* postlarvae culture tank.

Total bacteria, Lactobacillus, Vibrio and zooplankton abundances

A total 16 genera of zooplankton were identified, of which, six genera belonged to ciliates, nine to rotifers and one to nematode (Table 2). Ciliates, rotifers and nematode abundance were significantly ($P < 0.05$) higher in the BF-Z than other treatments, while those were similar ($P > 0.05$) among the BF2 - 5, BF7 - 10 and BF12 - 15 treatments (Table 3). Total bacteria counting was similar ($P > 0.05$) among the treatments (Table 3). *Lactobacillus* spp. counting was significantly lower ($P > 0.05$) in the BF-Z than BF12 - 15, while this was similar among the BF2 - 5, BF7 - 10, and BF12 - 15 treatments (Table 3). *Vibrio* spp. abundance was similar ($P > 0.05$) between BF2 - 5 and BF7 - 10, these were significantly lower ($P < 0.05$) than those in the BF12 - 15 and BF-Z treatments (Table 3).

This study showed that the increasing biofloc volume treatments did not increase total bacterial count. While, zero-solids removal treatments BF-Z significantly increased the ciliate, rotifer and nematode abundances as compared with results with the three solids removal

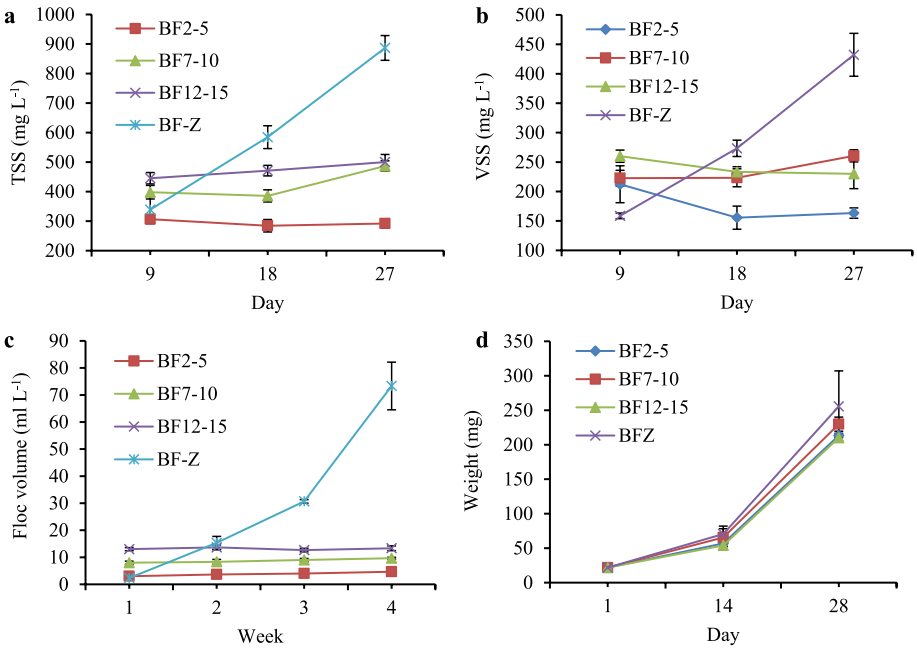

Fig. 2 Weekly mean (± SE) total suspended solid (TSS) (mg L^{-1}) (**a**), total volatile suspended solids (VSS) (mg L^{-1}) (**b**), biofloc volume (ml L^{-1}) (**c**) and prawn weight (mg) (**d**) in different floc volume levels in solids removal and zero-exchange biofloc systems for *Macrobrachium rosenbergii* post larvae

Table 2 Taxonomic composition of zooplankton communities at different floc volume levels biofloc systems and zero-exchange biofloc system when culturing *Macrobrachium rosenbergii* post larvae for 28 days

Group	Genus	BF2 - 5	BF7 - 10	BF12 - 15	BF-Z
Ciliata	*Euplotes*	√	√	√	√
	Aspidisca			√	√
	Coleps	√	√	√	√
	Paramecium	√	√	√	√
	Vorticella		√	√	√
	Acineta	√	√	√	√
Rotifera	*Brachionus*	√	√	√	√
	Euchlanis	√	√	√	√
	Lecane	√	√	√	√
	Colurella	√	√	√	√
	Lepadella	√	√	√	√
	Gastropus	√	√	√	√
	Habrotrocha	√	√	√	√
Nematoda	*Philodina*	√	√	√	√
	Rotaria	√	√	√	√
	Rhabditis	√	√	√	√

treatments. On the other hand, the lower *Vibrio* spp. and higher *Lactobacillus* spp. density in BF2 - 5 than the BF-Z treatment likely contributed to a relatively more healthy culture environment during this study.

Giant river prawn growth performance and an economic analysis

Growth performance of *M. rosenbergii* postlarvae and an economic analysis over the time period are shown in Table 4 and Fig. 2d. The mean final weight, weight gain and specific growth rate of PLs were not significantly different ($P > 0.05$) among four treatments. Food conversion ratios were similar ($P > 0.05$) among the BF2 - 5, BF7 - 10 and BF12 - 15 treatments, these were significantly improved ($P < 0.05$) as compared with the BF-Z treatment. PL survival was significantly ($P < 0.05$) higher in the BF2 - 5 than other treatments. The gross return, net return and BCR were significantly higher ($P < 0.05$) in BF2 - 5 compared to BF7 - 10 and BF12 - 15.

This study exhibited that different level of biofloc volume as well as zero-solids removal biofloc system did not impact on *M. rosenbergii* growth. However, the higher survival was recorded in the BF2 - 5, which led to higher economic return.

Proximate composition of M. rosenbergii postlarvae and biofloc

Prawn whole-body dry matter, protein, lipid, ash and carbohydrate contents were not significantly different ($P > 0.05$) among the different treatments (Table 5). The biofloc dry matter, protein and lipid contents were also not significantly different ($P > 0.05$) among the different

Table 3 Total bacteria, *Lactobacillus, Vibrio* and zooplankton abundances at different floc volume levels biofloc systems and zero-exchange biofloc system when culturing *Macrobrachium rosenbergii* post larvae

Variables	BF2 - 5	BF7 - 10	BF12 - 15	BF-Z
Total bacteria (CFU $\times 10^{10}$ ml^{-1})	4.73 ± 0.60^{a}	7.60 ± 0.98^{a}	6.43 ± 1.39^{a}	4.96 ± 0.76^{a}
Lactobacillus spp. (CFU $\times 10^{3}$ ml^{-1})	15.03 ± 0.73^{ab}	13.86 ± 3.04^{ab}	17.96 ± 2.16^{b}	8.96 ± 0.44^{a}
Vibrio spp. (CFU $\times 10^{3}$ ml^{-1})	4.13 ± 0.40^{a}	4.66 ± 0.97^{a}	199.33 ± 20.73^{b}	245.33 ± 19.37^{b}
Ciliates (ind. $\times 10^{5}$ L^{-1})	2.25 ± 0.62^{a}	1.80 ± 0.40^{a}	2.71 ± 0.23^{a}	16.50 ± 0.72^{b}
Rotifers (ind. $\times 10^{4}$ L^{-1})	3.8 ± 1.01^{a}	8.83 ± 1.20^{a}	7.83 ± 1.69^{a}	21.33 ± 3.82^{b}
Nematodes (ind. $\times 10^{4}$ L^{-1})	7.50 ± 0.57^{a}	7.16 ± 0.44^{a}	9.33 ± 0.60^{a}	77.33 ± 5.23^{b}

Superscript similar letter in the same row did not differ significantly ($P > 0.05$), and different letters indicate significant differences ($P < 0.05$)

Table 4 Growth performance and an economic analysis of culturing *Macrobrachium rosenbergii* post larvae in different floc volume levels biofloc systems and zero-exchange biofloc system after 28 days culture

Variables	BF2 - 5	BF7 - 10	BF12 - 15	BF-Z
Final weight (mg)	213.36 ± 3.15^{a}	229.83 ± 10.23^{a}	210.06 ± 4.13^{a}	255.53 ± 51.55^{a}
Weight gain	193.26 ± 3.15^{a}	209.73 ± 10.23^{a}	189.96 ± 4.13^{a}	235.43 ± 51.55^{a}
SGR (%day^{-1})	8.14 ± 0.05^{a}	8.40 ± 0.15^{a}	8.10 ± 0.06^{a}	8.63 ± 0.77^{a}
FCR	1.52 ± 0.02^{a}	1.60 ± 0.01^{a}	2.07 ± 0.06^{a}	8.67 ± 2.70^{b}
Survival (%)	82.33 ± 1.76^{d}	72.33 ± 2.72^{c}	62.0 ± 2.08^{b}	14.0 ± 1.15^{a}
[a]Economic analysis				
[b]Gross cost	36.56 ± 0.0	36.56 ± 0.0	36.56 ± 0.0	36.56 ± 0.0
[b]Gross return	123.90 ± 2.49^{d}	108.07 ± 4.10^{c}	92.90 ± 2.98^{b}	20.80 ± 1.47^{a}
[b]Net return	87.33 ± 2.49^{d}	72.13 ± 4.10^{c}	56.33 ± 2.98^{b}	- 15.76 ± 1.47^{a}
[b]BCR	2.38 ± 0.06^{d}	1.97 ± 0.11^{c}	1.54 ± 0.08^{b}	- 0.43 ± 0.04^{a}

Superscript similar letter in the same row show the lack of significant differences ($P > 0.05$), different letters within the row indicate significant differences ($P < 0.05$)

[a] This economic analysis did not include laboratory equipment, provided by I-AQUAS or Department of Aquaculture, Universiti Putra Malaysia. Calculation was done based on 500 PLs in each tank

[b] The values are mean ± SE (RM 500 PLs^{-1}, 1 USD = 4.24 RM)

Table 5 Proximate composition of *Macrobrachium rosenbergii* post larvae and bioflocs obtained in different floc volume levels biofloc systems and zero-exchange biofloc system after 28 days culture

Variables	BF2 - 5	BF7 - 10	BF12 - 15	BF-Z
M. rosenbergii				
Dry matter (% WW)	25.02 ± 0.15^{a}	25.11 ± 0.08^{a}	24.94 ± 0.11^{a}	25.03 ± 0.18^{a}
Protein (% DW)	64.33 ± 0.21^{a}	64.12 ± 0.07^{a}	64.42 ± 0.56^{a}	65.53 ± 0.23^{a}
Lipid (% DW)	4.18 ± 0.08^{a}	4.20 ± 0.07^{a}	4.18 ± 0.10^{a}	4.40 ± 0.23^{a}
Ash (% DW)	18.05 ± 0.14^{a}	18.50 ± 0.50^{a}	18.75 ± 0.83^{a}	17.14 ± 0.18^{a}
*CHO (% DW)	13.42 ± 0.19^{a}	13.16 ± 0.54^{a}	13.63 ± 1.29^{a}	12.91 ± 0.48^{a}
Bioflocs				
Dry matter (% WW)	9.29 ± 0.41^{a}	9.22 ± 0.30^{a}	9.56 ± 0.27^{a}	10.88 ± 0.50^{a}
Protein (% DW)	29.43 ± 0.39^{a}	29.89 ± 0.04^{a}	31.26 ± 0.31^{a}	31.29 ± 0.73^{a}
Lipid (% DW)	2.12 ± 0.18^{a}	2.15 ± 0.07^{a}	2.16 ± 0.11^{a}	2.27 ± 0.12^{b}
Ash (% DW)	28.54 ± 0.45^{a}	29.60 ± 0.28^{a}	35.62 ± 1.55^{b}	35.58 ± 1.10^{b}
*CHO (% DW)	39.89 ± 0.58^{b}	38.34 ± 0.28^{b}	30.94 ± 1.69^{a}	30.84 ± 0.50^{a}

* Carbohydrate (CHO) % = 100 − (% protein + % lipid + % ash). Similar superscript letters in the same row indicate the absence of statistically significant differences ($P > 0.05$); different letters indicate the presence of significant differences ($P < 0.05$)

treatments (Table 5). However, biofloccs ash contents were signifyingly higher ($P < 0.05$) in BF12 - 15 and BF-Z compared to BF2 - 5 and BF7 - 10. The carbohydrate level was significantly higher ($P < 0.05$) in BF2 - 5 and BF7 - 10 compared in BF12 - 15 and BF-Z (Table 5). This study showed that biofloc volume did not impact prawn whole-body dry matter, protein, lipid, ash and carbohydrate contents. While carbohydrate levels were relatively underutilized in BF2 - 5 and BF7 - 10 treatments as compared with those of BF12 - 15 and BF-Z.

Discussion

Biofloc technology systems have relied on the addition of carbon sources (sugar or starches) with optimum carbon to nitrogen ratios, which proliferates heterotrophic microorganisms, also resulting with an aggregation as 'bioflocs'[41]. The study showed ammonia concentration was higher at the BF2 - 5 in week 1 than the other treatments, but these did not exceed levels considered to be safe [4, 42, 43]. After that, ammonia decreased at each week in the BF2 - 5, which was similar to the other treatments. Overall, the removal of ammonia and nitrite occurred, while an accumulation of nitrate was found in this study. This finding along with similar level of total bacteria counting in all groups in BFT, likely indicates the presence of nitrifying bacteria converting the ammonia to nitrite and then to nitrate [44]. This viable establishment of heterotrophic bacteria in this study was likely due to the addition of biofloc-enriched water or an inoculum in the three the floc volumes treatments, or in the BF-Z treatment, respectively. Thus, treatments used in this study maintained safe levels of ammonia and nitrite for giant river prawn culture [4, 43]. Nitrate levels in the current study do not threaten toxicity to *M. rosenbergii* PLs since they were within established safe levels for PLs [45].

Measured pH and dissolved oxygen during the present study remained within safe limits as recommended by New [4] and Pérz-Fuentes et al. [25]. The addition of sodium bicarbonate ($NaHCO_3$) has maintained the suitable pH level in four different biofloc groups [31, 32]. A lower dissolved oxygen concentration in BF-Z group is likely due to more microorganism assemblages particularly zooplankton, probably reflecting their biological oxygen demand. This study showed that ciliate, rotifer and nematode abundances were significantly higher in the BF-Z group than in the different floc volume/solids removal groups. This indicates that zooplankton assemblages require substrate/solids. Likewise, the solids removal BFT have reduced the rotifers and nematodes abundances when white leg shrimp cultured [12]. Despite the presence of higher zooplankton in BF-Z groups, a lower prawn survival was observed. In this case, the increasing trend of floc volume with TSSs levels over the period likely caused prawn PL mortalities in the BF-Z treatment, as previously reported for fish [41] and crustaceans [14, 15, 46]. In contrast, the current study has maintained three levels of floc volume at 2–5, 7–10 and 12–15 ml L^{-1}; which do not exceed suitable TSS levels recommended by recommended by Avnimelech [47] and Samocha et al. [48]. Thus, the higher survivals obtained in the BF2 - 5 and BF7 - 10 as compared with the BF12 - 15 or BF-Z treatments, suggest the potentially most suitable floc volume (2–10 mg L^{-1}) for *M. rosenbergii* nursery phase. This range of floc volumes has been recommended as safe levels for white leg shrimp culture in the BFT based systems [47]. On the other hand, these two groups TSS levels were within the range of suitable levels [14, 15, 49], allowing adequate assemblages of heterotrophic organisms as substrate and contributing to the available live-feed based nutrition for giant freshwater prawn post larvae.

Vibrio spp. bacteria are viewed an extreme nuisance in *M. rosenbergii* hatcheries [50, 51], but they are common in brackish to marine water environments or aquaculture systems [52, 53]. In contrast, the presence of *Lactobacillus* spp. is considered as beneficial to fish, shrimp/prawn intestinal health and generally to water quality in aquaculture systems [54–57]. In this study, *Lactobacillus* spp. in the three biofloc volume level treatments were significantly more abundant than in the zero-exchange biofloc system. Typically, *Lactobacillus* can be ingested by *M. rosenbergii*, which has improved the humoral and hepatopancreatic immunity, although prawn growth was not augmented substantially in a biofloc system [22]. The current study shows that increasing populations of *Lactobacillus* spp. in the three biofloc volumes groups was accompanied by a decrease in *Vibrio* spp. Similarly, the remarkable removal of pathogenic bacteria including *Vibrio*'s by administration of the *Lactobacillus* (JK- 8 and JK- 11) spp. have been reported by Ma et al. [58]. Thus, our microbial observations confirm that water quality was improved in the three biofloc volume treatments, compared with the BF-Z treatment, apparently as a consequence of the higher abundance of *Lactobicillus* spp.

This study shows similar growth under culture conditions characterized by different levels floc volume treatments and zero-exchange BFT. This result is similar to those reported by Schveitzer et al. [15], who observed similar growth among different TSS levels (200, 400–600 and 800–1000 mg L^{-1}) BFT groups during *L. vannamei* grow-out. In this study, the better FCR values were in the BF2 - 5, BF7 - 10 and BF12 - 15 treatments (1.52, 1.60 and 2.07, respectively) compared to that of 8.63 in BF-Z. The FCR values of BF2 - 5, BF7 - 10 and BF12 - 15 treatments were lower than that (2.25) reported by Ballester

et al. [59], during *M. rosenbergii* culture in a biofloc system. Furthermore, significantly higher survival and better FCR obtained in lower (100–300 mg L^{-1}) and medium (400–600 mg L^{-1}) TSS levels BFT compared to high TSS level (600–1000 mg L^{-1}) BFT, during white leg shrimp culture using biofloc systems [14, 15]. Therefore, it is suggested that low floc volume in BFT system for prawn and shrimp could enhance survival by reducing FCR, thereby increasing sustainability.

This study indicates that *M. rosenbergii* whole-body protein and lipid were not altered by either differing biofloc volumes or zero-exchange BFT. This is due to similar level of protein and lipid in supplemented diet and or in biofloc in the current prawn juvenile production system. Typically, *M. rosenbergii* proximate composition (dry matter, protein and lipid) have improved in biofloc culture systems owing to the consumption of biofloc organisms including highly nutritious assemblages zooplankton [25]. The prawn PLs protein levels in current study is consistent with zooplankton (adult *Artemia*, *Tubifex* worms and *Moina*) fed *M. rosenbergii* PLs [60]. However, the prawn lipid content was lower, in contrast with results reported by Indulkar and Belsare [60] and Pérez-Fuentes et al. [25]. This may be due to the juvenile prawns expending more energy for growing rather than storing lipids as energy. In summary, this study clearly revealed that different biofloc volume levels can provide nutrition supplement to giant river prawn postlarvae, thereby elevating their nutritional profiles, while biofloc volume maintained within 2–10 ml L^{-1} has the potential to enhance sustainability and viable economic return.

Conclusions

This study demonstrates that lower biofloc volume treatment (2–5 ml L^{-1}) obtains a higher survival (82.33%) and a preferable food conversion ratio (1.52). This lower biofloc volume group ensures good water quality condition including a suitable total suspended solids level (294.44 mg L- 1). The proliferation of total bacteria and *Lactobacillus* spp. were similar between lower biofloc volume and zero-solids removal group, while lower *Vibrio* spp. abundance was detected in the lower biofloc volume BFT system. Thus, periodical solids removal is recommended to maintain acceptable floc volume and TSS for *M. rosenbergii* postlarvae rearing in biofloc system, which can augment prawn performance, production and profitability. This culture technique could therefore be considered as an important management strategy in the commercial prawn nursery operations. This study did not explore the bacterial diversity, virulent *Vibrio*'s or probiotic *Bacillus*, *Lactobacillus* or other probiotic strains of bacteria, which may have been present in the four different biofloc volume treatments. Therefore, further research should be conducted to explore detailed elements of the diversity of bacterial populations, and its potential roles in pathogenicity, probiotic activity as well as immune gene expression of prawn culture in nursery phase and grow-out production. Additionally, the profile of amino acids and fatty acids of prawn and biofloc should be determined besides those bacterial insights for zero-solids and solid removal biofloc systems.

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Authors' contributions
Md. Eilious Hosain, S. M. Nurul Amin, Murni Karim, Aziz Arshad, Mohd Salleh Kamarudin participated in conceptualization, methodology, resources and validation. Md. Eilious Hosain, S. M. Nurul Amin prepared original draft. S. M. Nurul Amin, Murni Karim, Aziz Arshad, Mohd Salleh Kamarudin, Shamarina Shohaimi, Md. Niamul Naser and Christopher L. Brown participated in review, editing and critical insights.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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