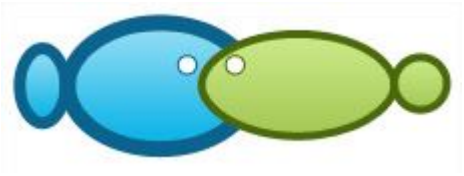

The influence of feeding reduction on the survival and growth of the larvae and postlarvae of freshwater prawn (*Macrobrachium rosenbergii*): an applied biofloc technology study

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Abstract. This study involves a research conducted in hatcheries concerning the impact of feed reduction on the survival, productivity and growth of the larvae and postlarvae (PL) of freshwater prawn (*Macrobrachium rosenbergii*). The experiment included cohorts with different daily feeding schedules, i.e., (i) *Artemia* twice + artificial feed thrice (control); (ii) *Artemia* twice + artificial feed twice; (iii) *Artemia* twice + artificial feed once; (iv) *Artemia* once + artificial feed thrice; (v) *Artemia* once + artificial feed twice; (vi) *Artemia* once + artificial feed once. Refined sugar was supplied as a carbon source to create biofloc from larval stage-4, and the C/N ratio was maintained at 17.5. A 250-L tank was used for larval rearing, stocking density was 60 ind L^{-1}, and salinity of 12 ‰. The results obtained imply that environmental factors, bacterial factors, and biofloc parameters were in a suitable range for PL to develop; the greatest PL15 length (10.03±0.51 mm) followed treatment (i), with significant differences ($p < 0.05$) observed for treatments (v) and (vi) but no significant differences ($p > 0.05$) observed for any of the remaining treatments. The highest survival rate and productivity were observed for PL15 under treatment (i) (56.8% and 27±1.05 ind L^{-1} respectively); although no significant differences ($p > 0.05$) were observed for treatment (ii), significant differences ($p < 0.05$) were observed for the remaining treatments. Towards the end of the experiment, the feeding regime was modified by removing one artificial feed instance; this did not negatively impact growth, survival, or productivity compared to the conventional feeding regime.

Key Words: *Artemia*, artificial feed, freshwater prawn larvae, biofloc.

Introduction. Freshwater prawn (*Macrobrachium rosenbergii*) is a heavily targeted species with a high value and an important role in world aquaculture. The global production of freshwater prawn reached 234,000 tons in 2018, with India, Thailand, Bangladesh, Indonesia, and Vietnam among the biggest contributors (FAO 2020). Economically, freshwater prawn has become the most important species for Vietnam's Mekong Delta. A common candidate for culturing in either fresh- or brackish-water ecosystems, target to 2025 of the productivity of freshwater prawn in the region is estimated to reach 50,000 tons, demanding the seeding of up to 2-3 billion postlarvae (PL) for commercial production (MARD 2020). However, freshwater prawn culture development has recently arrived at a bottleneck due to problems with PL quantity and quality.

To develop a proper biosecurity solution in the context of viable hatchery protocol, applying biofloc technology (BFT) is considered the most promising approach to ensuring PL quality. This technology is considered a friendly technique that can not only improve water quality but also provide floc particles as extra food for aquatic animals (Avnimelech & Kochba 2009; Ahmad et al 2017; Emerenciano et al 2017; Fischer et al 2020). Floc particles contain protein, lipids, minerals, vitamins, amino acids, fatty acids, as well as enzymes, stimulants, and probiotics, and most crustaceans, including freshwater prawn, can easily digest floc particles (Crab et al 2010; Emerenciano et al 2013).

There have recently been trials concerning applying BFT in freshwater prawn hatcheries (e.g., Pérez-Fuentes et al 2013; Miao et al 2017; Ballester et al 2017; Hai et al 2018; Nghi et al 2020; Truyen et al 2020). However, with the exception of the study by Vargas-Ceballos et al (2020), which considered direct intake of different food sources during the first zoea stages of *Macrobrachium tenellum*, few papers have proven the possibility of the larvae and postlarvae (PL) of freshwater prawn (*M. rosenbergii*) digesting floc particles directly (Vargas-Ceballos et al (2020), thus a study on the influence of feeding reduction on the survival and growth of the larvae and postlarvae of freshwater prawn applied biofloc techmology as an indirect study of biofloc taking up as food for larvae and PL via evaluating their performance was performed and evaluated.

Material and Method

Location and the period of study. This study was conducted on College of Aquaculture, Cantho University, Vietnam during September to October 2020.

Water resource. Tap water, the freshwater source, was mixed with brine (90 ‰ salinity) obtained from the Vinh Chau salt works (Soc Trang province) to produce a 12 ‰ saline solution. The saline solution was treated with chlorine at 50 g m^{-3}; then, chlorine residue was removed by strong and continuous aeration, and sodium bicarbonate ($NaHCO_3$) was added to balance and stabilize the alkalinity at 120 mg $CaCO_3$ L^{-1} throughout the culture (Tao & Phu 2015). Finally, the water was passed through a filter (5 µm mesh size) before being transferred into larval tanks.

Larvae of freshwater prawn. Gravid females (i.e., carrying grey eggs in their abdomens) were bought in Can Tho; those considered healthy and of good quality with a bright color and no deformations were selected and reared in Cantho University's wet lab. The average size of the gravid females was from 40 to 50 g ind^{-1}. A 500-L tank was used as the hatching tank (12 ‰ salinity). Immediately following observation of stage 1 larvae, those with phototropism were collected and treated with formalin (200 ppm) to eliminate parasites on shrimp larvae before set-up.

Biofloc creation. Biofloc was created by adding a refined sugar solution (Nghi et al 2020) to larval tanks to maintain C/N at 17.5 (Truyen et al 2020). First, sugar was dissolved in the water at 60^{o}C at a 1:3 ratio (i.e., 1 sugar to 3 water by weight); then, it was well-mixed and incubated for 48 h before being added to larval tanks. Sugar began being added from larval stage 4 of freshwater prawn, and the amount of sugar added to the larval tanks followed Avnimelech (2015), which used Lansy PL (48% protein) as artificial feed.

Experimental design. There were six treatments that each followed a different daily feeding regime, each with three replicates featuring different randomized set-ups. Larvae were reared at a density of 60 ind L^{-1} in composite tanks (250-L) at 12 ‰ salinity.

- treatment 1: *Artemia* twice + artificial feed thrice (control; 2A3F);
- treatment 2: *Artemia* twice + artificial feed twice (2A2F);
- treatment 3: *Artemia* twice + artificial feed once (2A1F);
- treatment 4: *Artemia* once + artificial feed thrice (1A3F);
- treatment 5: *Artemia* once + artificial feed twice (1A2F);
- treatment 6: *Artemia* once + artificial feed once (1A1F).

Larval and post-larval zoo-technology. To observe larval behavior, water quality parameters were monitored periodically. Larval feeding followed the feeding regime (Table 1). Proper aeration ensured floc particles were maintained in suspension. There was no water exchange required by the BFT system; however, fresh seawater was added to compensate for evaporation loss.

Table 1

Type of food and feeding rate per feeding regime for freshwater prawn larvae (Nghi et al 2020)

Larval stages	*Foods*	*Feeding rate*	*Feeding times*
Stage 1*	No feeding (yolk-sac available)		
Stage 2-3	Newly hatched *Artemia*	1 *Artemia* mL^{-1} culture volume	As designed
Stage 4-5	Lansy PL	1 g m^{-3} feeding	As designed
	Newly hatched *Artemia*	3 *Artemia* mL^{-1} culture volume	
Stage 6-8	Lansy PL	1.5 g m^{-3} feeding	As designed
	Newly hatched *Artemia*	3 *Artemia* mL^{-1} culture volume	
Stage 9-PL15**	Lansy PL	2 g m^{-3} feeding	As designed
	Newly hatched *Artemia*	4 *Artemia* mL^{-1} culture volume	

* Stage 1 to postlarvae of freshwater prawn (Uno & Soo 1969); ** PL15 (postlarvae 15 days old).

Water parameters

Water quality. Temperature and pH were measured twice per day at 8 am and 2 pm using a thermometer and pH meter (HI-98127 Multi-Parameter Waterproof Meter, HANNA Instruments, Ltd.). Alkalinity, total ammonia nitrogen (TAN), and nitrite (NO^{-}_{2}) were determined every three days, with alkalinity measured by titration of acid, TAN measured using Indophenol Blue, and NO_2^- measured by the colorimetric method using 4500-NO^{-}_{2}B (APHA 2005).

Total bacteria and Vibrio levels. Sampling and determination of total bacteria and *Vibrio* were performed every 8 days for the water column and at the end of the experiment for the PL samples. The samples were diluted and spread over the sterilized Nutrient Agar, and 1.5% NaCl (NA) was added (Huys 2002). The samples were defined by the dilution method, in which the initial sample (i.e., concentration of 100%) was diluted in physiology solution (0.86%) into different concentrations (i.e., 10^{-1}, 10^{-2}, and 10^{-3}); then, a sample of 100 µL per concentration was spread over the sterilized TCBS (thiosulphate–citrate–bile salts–sucrose) agar for total bacteria and trypticase soy agar for *Vibrio* examination (Huys 2002). The formula below used to calculate either total bacterial count or *Vibrio* count:

$$\text{Bacterial count (CFU mL}^{-1}) = \text{Colony counted x dilution factor x 10}$$

Biofloc parameters. Floc volume (FV) was monitored at PL4, PL10, and PL15 stages by collecting a 1-L water sample and then inserting an Imhoff cone, which was left undisturbed for 30 min; settled floc volume was read and recorded as mL L^{-1} (Santhana et al 2018). Additionally, biofloc were also collected for particle size measurement, with 30 floc samples per tank observed and measured using a Nikon ECLIPSE Ti2 microscope with a DS-Qi2 camera (Nikon Corporation, Tokyo, Japan). Zooplankton composition was identified based on Shirota (1966) and Agrawal & Gopal (2013). Additionally, the approximate composition (i.e., protein, lipid, ash) of floc particles was analyzed at the end of the experiment (AOAC 2016).

Zootechnical parameters. They refer to growth of larvae and postlarvae. Metamorphosis of larvae was determined every 3 days. The length of larvae and PL was sampled and measured at larval stages 1, 5, and 11 and postlarval stages 1 and 15; 30 randomized samples per tank were measured, with length measured using a Nikon ECLIPSE Ti2 microscope with a DS-Qi2 camera (Nikon Corporation, Tokyo, Japan). Survival rates and productivity were defined at the end of the experiment by counting individual survivors.

Data analysis. Recorded data were used to estimate the mean and standard deviation in Microsoft Excel 2013. Differences between treatments were analyzed using one-factor ANOVA (Duncan test) when the dependent variable was normally distributed in each group at a significant difference level of 5% ($p < 0.05$). The SPSS 20.0 package was used for these calculations.

Results and Discussion

Culture conditions. Although the temperatures in the rearing tanks were rather stable, there were minor fluctuations between mornings and afternoons. Nonetheless, the average temperature varied in the range 28.17-29.86^{o}C. According to Hai et al (2017), the preferred temperature range for freshwater prawn in larviculture is 28-30^{o}C.

The pH level varied in the range 7.83-7.96; this variation was negligible. Furthermore, this indicated the optimum level for rearing freshwater prawn; according to Phuong et al (2003), the appropriate pH range is 7-8.5.

Table 2 displays that alkalinity during larval rearing was in the range 110.8-111.9 mg $CaCO_3$ L^{-1}; this is considered a suitable alkalinity condition. According to Tao & Phu (2015), appropriate alkalinity for rearing the larvae and PL of freshwater prawn is in the range 100-120 mg $CaCO_3$ L^{-1}.

This experiment's average TAN levels varied in the range 0.54-0.71 mg L^{-1}, with treatment 2A3F displaying the highest TAN (0.71±0.02 mg L^{-1}), differing significantly ($p < 0.05$) from treatment 1A1F but not differing significantly ($p > 0.05$) from the other treatments. It was concluded by Nghi et al (2020) as TAN recorded below 1.35 mg L^{-1} for freshwater prawn larvae in BFT contexts does not have any negative impact on the larvae, therefore TAN's recorded in current study are appropriate. Additionally, it was observed that TAN values were low when feeding rates were reduced. Moreover, sugar added as a carbon source degrades faster and hence promotes faster growth of heterotrophic bacteria, which, in turn, requires lower ammonia concentrations to maintain water quality and promote larvae and PL in terms of survival and growth rates.

Average NO^{-}_{2} varied in the range 0.41-0.78 mg L^{-1}; the lowest value was recorded for treatment 1A1F (0.41±0.21 mg L^{-1}), which differed significantly ($p < 0.05$) from all other treatments. No significant differences ($p > 0.05$) were observed between the other treatments (2A3F to 1A2F). According to Mallasen & Valenti (2006), survival, growth, and larval stage indices of freshwater prawn do not produce significant differences when NO^{-}_{2} remains below 2 mg L^{-1}. Thus, the parameters recorded by the current study (i.e., water temperature, pH, alkalinity, TAN, and NO^{-}_{2}) were appropriate for the cultivation of freshwater prawn larvae.

Table 2

Water parameters

Parameters		*Treatment*					
		2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
Temperature	am	28.21±0.99	28.23±0.96	28.27±0.99	28.17±0.97	28.19±0.96	28.24±0.97
	pm	29.86±1.12	29.78±1.15	29.81±1.14	29.75±0.16	29.80±1.14	29.84±1.11
pH	am	7.90±0.32	7.89±0.31	7.91±0.30	7.86±0.32	7.83±0.33	7.88±0.32
	pm	7.93±0.33	7.95±0.33	7.96±0.33	7.92±0.34	7.90±0.36	7.89±0.35
Alkalinity (mg $CaCO_3$ L^{-1})		111.9±0.3^{a}	111.8±0.4^{a}	110.8±1.2^{a}	110.9±0.5^{a}	111.2±0.4^{a}	111.8±0.7^{a}
TAN (mg L^{-1})		0.71±0.02^{b}	0.69±0.02^{b}	0.64±0.03^{b}	0.64±0.02^{b}	0.65±0.05^{b}	0.54±0.06^{a}
NO_2^{-} (mg L^{-1})		0.78±0.04^{b}	0.76±0.04^{b}	0.65±0.12^{b}	0.68±0.06^{b}	0.67±0.12^{b}	0.41±0.21^{a}

Values in the same row with different letters are significantly different ($p < 0.05$).

Total bacteria and Vibrio in water. The statistical analysis indicated that, between treatment regimes, there were no significant differences ($p > 0.05$) in the total bacteria levels in the culture medium after 8 days. The highest concentration of total bacteria was recorded for treatment 2A1F ($0.73x10^{4}$ CFU mL^{-1}) and the lowest concentration was recorded for treatment 1A1F ($0.27x10^{4}$ CFU mL^{-1}). On day 16, the total bacteria count was lowest for treatment 1A1F; however, this did not represent a significant difference ($p > 0.05$) from the other treatments. On day 24, the highest total bacteria count was observed for treatment 2A3F; again, however, this did not represent a significant difference from the other treatments. Near the experiment's end, treatment 1A3F demonstrated the highest total bacterial count, and the lowest count was recorded for

treatment 2A2F. Again, no significant differences ($p > 0.05$) between treatments were observed. Generally, the total bacterial count increased according to rearing time.

Table 3

Total bacterial count in water culture (10^4 CFU mL^{-1})

Day	Treatment					
	2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
8	0.63 ± 0.15^a	0.70 ± 0.36^a	0.73 ± 0.21^a	0.37 ± 0.31^a	0.43 ± 0.15^a	0.27 ± 0.21^a
16	1.83 ± 0.50^a	1.53 ± 0.47^a	2.93 ± 1.62^a	2.33 ± 2.22^a	1.70 ± 0.52^a	0.63 ± 0.06^a
24	3.20 ± 0.46^a	2.37 ± 0.42^a	2.93 ± 0.46^a	2.10 ± 0.79^a	2.53 ± 0.91^a	2.03 ± 1.46^a
32	3.97 ± 0.21^a	3.20 ± 1.10^a	4.07 ± 0.75^a	4.53 ± 0.66^a	4.37 ± 0.15^a	4.30 ± 0.95^a

Values in the same row with different letters are significantly different ($p < 0.05$).

Table 4 displays the *Vibrio* concentration for different treatments, which varied between 0.37×10^2 and 4.80×10^2 CFU mL^{-1}. On day 8, *Vibrio* density was highest for treatment 2A1F, and on day 16 it was highest for treatment 2A2F with no significant difference ($p > 0.05$) compared to the other treatments. On day 24 day (towards the end of the experiment), the lowest *Vibrio* density was observed for treatment 1A1F; again, this was not significantly different ($p > 0.05$) from the other treatments. *Vibrio* levels recorded were lower than those observed by Nghi et al (2020). The use of sugar as a carbon source which is similar to molasses might have improved water quality and reduced the *Vibrio* concentration (Huang et al 2017).

These results indicate that rearing freshwater prawn larvae using sugar as a carbon source facilitated enhanced total count bacteria and simultenously inhibited the invasion of *Vibrio* as competition for nutrients and energy (Fuller 1989). It has been stated that *Vibrio* can only harm the larvae of freshwater prawn at densities of 10^5-10^7 CFU mL^{-1} (Hoa et al 2004). By increasing the total bacterial count, *Vibrio* levels were decreased. Therefore, no negative impact on larvae or PL was recorded.

Table 4

Vibrio concentration in water culture (10^2 CFU mL^{-1})

Day	Treatment					
	2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
8	0.47 ± 0.06^a	0.37 ± 0.21^a	0.50 ± 0.30^a	0.40 ± 0.26^a	0.37 ± 0.21^a	0.37 ± 0.15^a
16	1.47 ± 0.21^a	1.70 ± 1.04^a	1.53 ± 0.49^a	1.47 ± 0.58^a	1.10 ± 0.87^a	1.27 ± 0.51^a
24	2.47 ± 0.40^a	3.00 ± 1.18^a	2.57 ± 0.38^a	2.83 ± 0.55^a	2.57 ± 1.56^a	2.27 ± 0.42^a
32	3.57 ± 0.51^a	4.80 ± 1.45^a	4.23 ± 0.86^a	3.83 ± 0.55^a	3.90 ± 1.14^a	3.27 ± 0.42^a

Values in the same row with different letters are significantly different ($p < 0.05$).

Total bacteria and Vibrio in freshwater prawn. The total bacteria count in the PL freshwater prawn varied between 2.8×10^5 and 6.6×10^5 CFU g^{-1} and the fluctuation was not significant different among treatments. That is, although the concentration was highest for treatment 1A3F, this did not represent a significant difference ($p > 0.05$) compared to the other treatments. Meanwhile, concentration was lowest for treatment 1A1F, but again, this did not represent a significant difference ($p > 0.05$) compared to the other treatments. These counts are significantly lower than the counts obtained by Truyen et al (2020). This could be explained by the higher TAN values recorded by that study in comparison to the current study.

Vibrio levels in PL freshwater prawn varied between 0.2×10^2 and 0.6×10^2 (CFU g^{-1}). Again, no significant differences ($p > 0.05$) were recorded between feeding regimes (Table 5). However, when applied BFT with different carbon sources, *Vibrio* levels can reach $15.8 \pm 1.05 \times 10^3$ CFU mL^{-1} without harming to the postlarvae rearing of freshwater prawn (Hai et al 2018).

Table 5

Total bacteria count (10^5 CFU g^{-1}) and *Vibrio* (10^2 CFU g^{-1}) in postlarvae of freshwater prawn

Parameter	Treatment					
	2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
Bacteria	5.2±2.6[a]	4.7±2.4[a]	4.5±2.7[a]	6.6±1.4[a]	4.8±3.0[a]	2.8±2.4[a]
Vibrio	0.2±0.1[a]	0.4±0.3[a]	0.6±0.3[a]	0.5±0.3[a]	0.4±0.2[a]	0.5±0.2[a]

Values in the same row with different letters are significantly different ($p < 0.05$).

Total bacteria and Vibrio in biofloc. The total bacteria levels observed in biofloc ranged between $1.7x10^6$ and $2.1x10^6$ CFU g^{-1} (Table 6). The highest total bacteria levels were observed for the biofloc of treatment 2A3F; this did not represent a significant difference ($p > 0.05$) compared to the other treatments. Meanwhile, the lowest total bacteria levels were observed for the biofloc of treatment 2A1F.

Table 6

Total bacteria count (10^6 CFU g^{-1}) and vibrio (10^4 CFU g^{-1}) in biofloc

Parameter	Treatment					
	2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
Total bacteria	2.1±0.6[a]	1.9±0.2[a]	1.7±0.2[a]	1.8±0.4[a]	2.0±0.3[a]	1.8±0.4[a]
Vibrio	0.5±0.1[a]	0.4±0.3[a]	0.5±0.3[a]	0.8±0.2[a]	0.6±0.3[a]	0.5±0.4[a]

Values in the same row with different letters are significantly different ($p < 0.05$).

Table 6 shows that the *Vibrio* in biofloc levels varied between $0.4x10^4$ CFU g^{-1} and $0.8x10^4$ CFU g^{-1}. The levels were highest for treatment 1A3F and lowest for treatment 2A2F; however, no significant differences ($p > 0.05$) were observed among treatments. *Vibrio* count is most commonly related to pathogenic bacteria; that is, higher bacteria and *Vibrio* counts increase the likelihood of a shrimp disease outbreak. However, the total bacteria and *Vibrio* concentrations recorded by the current study did not demonstrate any negative effect on the survival or growth of larvae or PL freshwater prawn.

Biofloc parameters

Floc volume. Accumulation of floc volume was recorded over time and demonstrated significant differences ($p < 0.05$) between treatments (Table 7). For PL stage 5, the highest floc volume was recorded for treatment 2A3F (0.71 ml L^{-1}); this was not significantly different ($p > 0.05$) from treatment 1A3F, but it was significantly different ($p < 0.05$) from the other treatments. In PL stage 10, floc volume was lowest for treatment 1A1F; this was not significantly different ($p > 0.05$) from treatment 2A1F, but it was significantly different ($p < 0.05$) from the other treatment regimes. Towards the end of the experiment, accumulated floc volume was highest for treatment 2A3F (2.53 mL L^{-1}); this was not significantly different ($p > 0.05$) from treatment 2A2F or 1A3F, but it was significantly different ($p < 0.05$) from the other treatment regimes. Floc volume varies according to the type of carbon source used in the culture medium. In a study by Hai et al (2019), floc volumes recorded in the context of rearing freshwater prawn in hatcheries ranged between 0.63 and 2.33 mL L^{-1} when rice flour was used as carbon source; these floc volumes did complicate larval rearing. This indicates that the floc volumes recorded in the current study were appropriate for the larvae or PL of freshwater prawn to survive and develop.

Table 7

Floc volume and dimensions

Items		Treatment					
		2A3F	2A2F	2A1F	1A3F	1A2F	1A1F
Floc volume	PL5	0.71±0.04^{c}	0.50±0.05^{b}	0.36±0.05^{a}	0.66±0.02^{c}	0.45±0.09^{ab}	0.35±0.08^{a}
	PL10	1.47±0.14^{c}	1.25±0.07^{b}	0.87±0.08^{a}	1.46±0.10^{c}	1.19±.003^{b}	0.92±0.06^{a}
	PL15	2.53±0.11^{c}	2.41±0.02^{c}	1.33±0.09^{a}	2.51±0.04^{c}	1.99±0.07^{b}	1.23±0.07^{a}
Length (mm)	PL5	0.59±0.08^{a}	0.57±0.05^{a}	0.60±0.01^{a}	0.85±0.06^{b}	0.64±0.03^{a}	0.61±0.07^{a}
	PL10	0.98±0.04^{d}	0.76±0.01^{b}	0.75±0.04^{b}	0.85±0.03^{c}	0.70±0.03^{a}	0.67±0.01^{a}
	PL15	0.92±0.08^{c}	0.89±0.06^{bc}	0.84±0.03^{ab}	1.01±0.03^{d}	0.82±0.03^{ab}	0.78±0.01^{a}
Width (mm)	PL5	0.28±0.04^{a}	0.29±0.03^{a}	0.33±0.01^{a}	0.46±0.06^{b}	0.37±0.07^{a}	0.29±0.05^{a}
	PL10	0.57±0.03^{b}	0.38±0.03^{a}	0.38±0.01^{a}	0.50±0.01^{ab}	0.44±0.18^{ab}	0.35±0.03^{a}
	PL15	0.55±0.03^{b}	0.53±0.01^{b}	0.49±0.02^{a}	0.59±0.03^{c}	0.48±0.02^{a}	0.48±0.01^{a}

Values in the same row with different letters are significantly different ($p < 0.05$).

Floc dimension. The largest average length of biofloc particles during PL stage 5 was recorded for treatment 1A3F, differing significantly ($p < 0.05$) from the other treatment regimes (Table 7). During PL stage 10, the largest length was recorded for treatment 2A3F, differing significantly ($p < 0.05$) from the other treatment regimes. During PL stage 15, the smallest floc particles were recorded for treatment 1A1F; these particle sizes were not significantly different ($p > 0.05$) from those of treatment 2A1F and 1A2F but were significantly different ($p < 0.05$) from those of the other treatment regimes. The widths of floc particles during PL stage 15 were larger than the widths recorded during PL stages 5 and 10, being largest for treatment 1A3F (0.59±0.03 mm), representing a significant difference ($p < 0.05$) from the other treatment regimes. Floc dimension varies according to, for example, bacteria density, organic matter, and faeces, which are functions of environmental parameters that correspond to culture protocols. In the current study, floc particle dimensions seemed to increase towards the end of the experiment. Floc particles have been observed to contribute to water quality as well as provide supplementary feed particles in prawn farming contexts (Avnimelech 2015).

Floc composition. Similar set-up and maintenance were applied for all treatments except feeding regimes in freshwater prawn rearing. Therefore, floc composition did not demonstrate significant differences between treatments, with observations under microscope detecting Cyanobacteria, Bacillariophyta, Euglenophyta, Dinophyta, Protozoa, and Rotifera (Figure 1); these results are similar to those obtained by Avnimelech (2015).

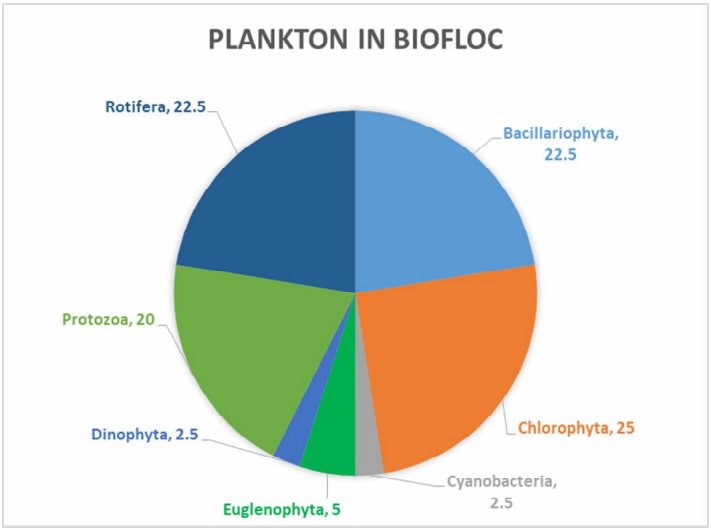

Figure 1. Plankton composition (%) in biofloc.

Algae were found belonging to the relative dominant groups (Figure 1), especially *Chlorella variegatus* (Chlorophyta), *Chlamydomonas cingulata* (green algae), *Navicula* (Bacillariophyta), *Nitzschia* (Bacillariophyta), *Euplotes* (Protozoa), and *Euchlanis dilatata* (Rotifera). The plankton composition breakdown recorded by the current study conformed to the findings of Phuong (2016) in the context of a grow-out pond of whiteleg shrimp. The presence of Euglenophyta could imply that the culture medium was rich with organic matter and presented as organically polluted water (Trang et al 2018); however, at the level of approximately 5%, this group did not negatively impact larvae or PL survival or growth.

Proximate composition of biofloc. Relative levels of protein, lipid, and ash did not differ significantly ($p > 0.05$) between treatment regimes; protein content varied in the range 28.4-29.0%. The proximate composition of floc depends on various factors, including culture density, aeration intensity, oxygen concentration, carbon source type and availability, temperature, salinity, and pH, as well as the type of microbial community that has developed (Avnimelech 2007) and possibly the food provided. However, in this case, different feeding regimes did not change the proximate composition of biofloc as expected. Using different types of carbon sources could lead to differences in the protein content of floc particles (Day 2018).

Protein in biofloc was highest proportion in treatment 1A2F, while it was smallest in treatment 1A3F, however, the difference among treatments was not statistically significant ($p > 0.05$). Lipid content did not differ substantially between treatments, varying in the range 2.8-3.3%; although the content was highest for treatment 1A2F and lowest for treatment 2A3F, there were no significant differences ($p > 0.05$) between treatment regimes (Table 8). In the study of Emerenciano et al (2011), when the carbon source applied was a mixture of molasses (90%) and rice-bran (10%), protein content was 30.4% and lipid content was 0.47%. Although the current study only used sugar, and lipid content was higher for all treatment regimes, proximate composition relies on other factors in addition to carbon source (Avnimelech 2007). A similar consideration was made when interpreting the differences in ash content levels recorded between treatment regimes.

Table 8

Proximate composition of biofloc

Items	*Treatment*					
	2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
Protein (%)	28.7±0.1[a]	28.8±0.1[a]	28.7±0.2[a]	28.4±0.4[a]	29.0±0.5[a]	28.9±0.8[a]
Lipid (%)	2.8±0.3[a]	3.0±0.6[a]	2.8±0.5[a]	3.1±0.1[a]	3.3±0.1[a]	3.0±0.2[a]
Ash (%)	29.7±0.2[a]	29.8±0.2[a]	29.7±0.3[a]	29.4±0.3[a]	29.9±0.4[a]	29.8±0.7[a]

Values in the same row with different letters are significantly different ($p < 0.05$).

Lipid content did not differ substantially between treatments, varying in the range 2.8-3.3%; although the content was highest for treatment 1A2F and lowest for treatment 2A3F, there were no significant differences ($p > 0.05$) between treatment regimes. In Emerenciano et al (2011), when the carbon source applied was a mixture of molasses (90%) and rice-bran (10%), protein content was 30.4% and lipid content was 0.47%. Although the current study only used sugar, and lipid content was higher for all treatment regimes, proximate composition relies on other factors in addition to carbon source (Avnimelech 2007). A similar consideration was made when interpreting the differences in ash content levels recorded between treatment regimes.

Larval stage index of freshwater prawn larvae. The larval stage index (LSI) indicates the homogenous growth and size of larvae throughout the experiment in terms of the metamorphosis of freshwater prawn. According to Phuong et al (2003), freshwater prawn larvae go through 11 metamorphosis cycles (i.e., molting) to reach PL, with each molting cycle depending on the culture medium, nutrition, rearing density, and their

physiological situation. Table 9 presents the LSI for each treatment, indicating differentiation and significant differences ($p < 0.05$) between day 3 and day 21. The highest LSI was recorded for treatment 2A3F (10.53±0.21), which differed significantly ($p < 0.05$) from treatment 1A2F and 1A1F but did not differ significantly ($p > 0.05$) from the other treatment regimes. A study by Hai et al (2019) observed different LSIs in the range 10.9-11.2 when rearing freshwater prawn using an applied BFT featuring the addition of carbon during different larval stages. Interestingly, that study also observed freshwater prawn larvae and PL taking in floc particles as an extra food source throughout the experiment.

Table 9

Larval stage index

Day	Treatment					
	2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
3	$3.63±0.06^{c}$	$3.40±0.10^{b}$	$3.37±0.06^{ab}$	$3.43±0.06^{b}$	$3.23±0.06^{a}$	$3.30±0.10^{ab}$
6	$5.93±0.15^{b}$	$5.47±0.40^{a}$	$5.67±0.15^{ab}$	$5.67±0.12^{ab}$	$5.53±0.06^{a}$	$5.30±0.17^{a}$
9	$7.13±0.45^{d}$	$6.77±0.15^{cd}$	$6.33±0.21^{bc}$	$6.60±0.10^{c}$	$6.10±0.26^{ab}$	$5.80±0.20^{a}$
12	$8.30±0.20^{c}$	$7.57±0.25^{b}$	$7.47±0.15^{b}$	$7.27±0.12^{b}$	$6.73±0.15^{a}$	$6.90±0.00^{a}$
15	$9.30±0.80^{b}$	$8.83±0.45^{ab}$	$8.20±0.36^{a}$	$8.80±0.26^{ab}$	$8.73±0.15^{ab}$	$8.40±0.46^{a}$
18	$10.00±0.26^{d}$	$9.30±0.40^{bcd}$	$8.97±0.75^{abc}$	$9.83±0.47^{cd}$	$8.70±0.36^{ab}$	$8.17±0.65^{a}$
21	$10.53±0.21^{b}$	$10.30±0.36^{b}$	$10.00±0.46^{b}$	$9.97±0.21^{b}$	$9.20±0.30^{a}$	$8.73±0.23^{a}$

Values in the same row with different letters are significantly different ($p < 0.05$).

Length of larvae and postlarvae. Length varied, with some significant differences ($p < 0.05$), between treatments. At stage 1, the average larvae length was 2.07 mm. At stage 5, the larvae with the longest length were observed for treatment 2A3F (4.19±0.11 mm); this did not represent a significant difference ($p > 0.05$) with treatment 1A3F but did represent a significant difference ($p < 0.05$) compared to the other treatment regimes. At stage 11, the longest length was again for treatment 2A3F (8.01±0.17 mm), which did not differ significantly ($p > 0.05$) from treatments 2A2F or 2A1F but did differ significantly ($p < 0.05$) from the other treatments. At PL stages 1 and 15, the longest lengths were observed for treatment 2A3F, which differed significantly ($p < 0.05$) from treatments 1A2F and 1A1F but did not differ significantly ($p > 0.05$) from the other treatments (Table 10). In a study by Nghi et al (2020), the lengths of larvae in stage 5 and PL in stages 1 and 15 were 3.5±0.2 mm, 8.6±0.1 mm, and 11.7±0.3 mm respectively when sugar was used as a carbon source in the applied BFT; although the corresponding lengths for this current study were similar but with significant difference ($p < 0.05$) given stunted growth was recorded in those treatment regimes featuring reduced feeding (i.e., treatments 1A3F, 1A2F, and 1A1F).

Table 10

Length of larvae and postlarvae of freshwater prawn

Stage	Treatment					
	2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
1	$2.07±0.01^{a}$	$2.07±0.01^{a}$	$2.07±0.01^{a}$	$2.07±0.01^{a}$	$2.07±0.01^{a}$	$2.07±0.01^{a}$
5	$4.19±0.11^{c}$	$3.99±0.06^{ab}$	$4.02±0.07^{b}$	$4.04±0.10^{bc}$	$3.84±0.13^{a}$	$3.90±0.05^{ab}$
11	$8.01±0.17^{c}$	$7.91±0.11^{c}$	$7.75±0.09^{bc}$	$7.63±0.22^{b}$	$7.59±0.11^{b}$	$7.33±0.12^{a}$
PL1	$8.22±0.02^{c}$	$8.16±0.03^{c}$	$8.10±0.06^{c}$	$8.18±0.08^{c}$	$7.91±0.13^{b}$	$7.70±0.13^{a}$
PL15	$10.03±0.51^{b}$	$9.82±0.23^{b}$	$9.70±0.09^{b}$	$9.67±0.19^{b}$	$9.13±0.26^{a}$	$9.09±0.12^{a}$

Values in the same row with different letters are significantly different ($p < 0.05$).

Survival and productivity. Survival at PL stage 15 varied in the range 12.7-56.8% (Table 11); although there was no significant difference ($p > 0.05$) between treatments 2A3F and 2A2F, these two demonstrated significant differences ($p < 0.05$) compared to the remaining treatment regimes. According to Nghi et al (2020) and Truyen et al

(2020), PL stage 15 freshwater prawn could reach over 50% survival if C:N is maintained at 17.5 and sugar is added as a carbon source; although such survival rates were observed for treatments 2A3F and 2A2F, other treatments (with reduced feeding regimes) demonstrated lower survival rates.

The productivity of PL stage 15 varied in the range 6-27 ind L^{-1} between treatments, with treatment 2A3F demonstrating the highest productivity, which did not differ significantly ($p > 0.05$) from treatment 2A2F but did differ significantly ($p < 0.05$) from the remained treatment regimes. Reducing feeding clearly considerably impacted survival and productivity (i.e., treatments 2A1F, 1A3F, 1A2F, and 1A1F); an exception was treatment 2A2F, which featured a 30% reduction in artificial feeding but survival and productivity were the same to the control (i.e. 2A3F). Notably, biofloc as food for whiteleg shrimps has been well-documented, with Khatoon et al (2016) observing that, at the grow-out phase, whiteleg shrimp reached higher survival and growth rates (compared to commercial feed only) when they were fed up to 50-75% biofloc.

Table 11

Survival and productivity at PL stage 15

Items	*Treatment*					
	2A3F	*2A2F*	*2A1F*	*1A3F*	*1A2F*	*1A1F*
Survival (%)	56.8 ± 2.19^{c}	54.8 ± 16.90^{c}	27.2 ± 12.30^{ab}	30.8 ± 1.30^{b}	17.1 ± 3.18^{ab}	12.7 ± 0.72^{a}
Productivity (con L^{-1})	27 ± 1^{c}	26 ± 8^{c}	13 ± 6^{ab}	14 ± 1^{b}	8 ± 2^{ab}	6 ± 1^{a}

Values in the same row with different letters are significantly different ($p < 0.05$).

Conclusions and Recommendations. Rearing freshwater prawn larvae and PL using applied BFT displayed advantages in terms of water quality and prawn performance. Even when the feeding regime was reduced by up to 30% artificial feed (i.e., treatment 2A2F), neither survival nor productivity was significantly different ($p > 0.05$) compared to the control. Therefore, such feeding regimes have been proven undoubtedly economical and efficient protocols for rearing freshwater prawn. This can have benefits for not only the environment but also for the capacity to produce high-quality PL in the hatchery context.

However, a larger-scale study verifying the application of the reduced feeding regime for freshwater prawn is needed before applying to production scale.

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References

Agrawal A., Gopal K., 2013 Analysis of phytoplankton and zooplankton: qualitative and quantitative. In: Biomonitoring of water and waste water. Gopal A. A. (ed), Springer India, pp. 33-40.

Ahmad I., Rani A. M. B., Verma A. K., Maqsood M., 2017 Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. Aquaculture International 25(3):1215-1226.

American Public Health Association (APHA), 2005 Standard methods of examination of water and wastewater. 21st edition. Washington, D.C, 2671 pp.

AOAC, 2016 Official methods of analysis of AOAC International. 20th edition. Association of Official Analysis Chemists International, 3172 pp.

Avnimelech Y. 2007 Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264:140-147.

Avnimelech Y., 2015 Biofloc technology - a practical guide book. 3rd edition. The World Aquaculture Society, Baton Rouge, Louisiana, United States, 182 pp.

Avnimelech Y., Kochba M., 2009 Evaluation uptake and excretion by tilapia in biofloc tanks, using ^{15}N tracing. Aquaculture 287:163-168.
Ballester E. L. C., Marzarotto S. A., de Castro C. S., Frozza A., Pastore I., Abreu P. C., 2017 Productive performance of juvenile freshwater prawns *Macrobrachium rosenbergii* in biofloc system. Aquaculture Research 48(9):4748-4755.
Crab R., Chielens B., Wille M., Bossier P., Verstraete W., 2010 The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquaculture Research 41(4):559-567.
Day P. V., 2018 [Rearing freshwater prawn (*Macrobrachium rosenbergii*) larvae applied biofloc technology]. MSc thesis in Aquaculture, Cantho University. [in Vietnamese]
Emerenciano M., Ballester E. L. C., Cavalli R. O., Wasielesky W., 2011 Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: growth performance, floc composition and salinity stress tolerance. Aquaculture International 19(5):891-901.
Emerenciano M., Gaxiola G., Cuzo G., 2013 Biofloc technology (BFT): a review for aquaculture application and animal food industry. In: Biomass now: cultivation and utilization. Matovic M. D. (ed), Intech Open, DOI: 10.5772/53902.
Emerenciano M. G. C., Martínez-Córdova L. R., Martínez-Porchas C. M., Miranda-Baeza A., 2017 Biofloc technology (BFT): a tool for water quality management in aquaculture. In: Water quality. Tutu H. (ed), Intech Open. DOI: 10.5772/66416
FAO, 2020 The state of world fisheries and aquaculture 2020. FAO, Rome, 224 pp.
Fischer H., Romano N., Renukdas N., Egnew N., Sinha A. K., Ray A. J., 2020 The potential for rearing juveniles of bluegill, *Lepomis macrochirus*, in a biofloc system. Aquaculture Reports 17:100398.
Fuller R., 1989 Probiotics in man and animals. Journal of Applied Bacteriology 66:365-378.
Hai T. N., Tao C. T., Phuong N. T., 2017 [Text-book on seed production and farming of crustaceans]. Cantho University Publishing House, 211 pp. [in Vietnamese]
Hai T. N., Day P. V., Tao C. T., 2018 [Study on nursing of larval giant freshwater prawn with different carbon sources following biofloc technology]. Ministry of Agriculture and Rural Development, Vietnam, 10:125-129. [in Vietnamese]
Hai T. N., Day P. V., An C. M., Tao C. T., 2019 [Rearing larvae of freshwater prawn (*Macrobrachium rosenbergii*) by biofloc technology at different stocking densities]. Scientific Journal of Can Tho University, 55(2B):79-87. [in Vietnamese]
Hoa T. T. T., Hang N. T. T., Oanh D. T. H., Phuong N. T., 2004 [Composition and pathogenicity of isolated virus from freshwater prawn (*Macrobrachium rosenbergii* De Man, 1879) hatchery]. Scientific Journal of Can Tho University, pp. 153-165. [in Vietnamese]
Huang J., Yang Q., Ma Z., Zhou F., Yang L., Deng J., Jiang S., 2017 Effects of adding sucrose on *Penaeus monodon* (Fabricius, 1798) growth performance and water quality in a biofloc system. Aquaculture Research 48(5):2316-2327.
Huys G., 2002 Preservation of bacteria using commercial cryopreservation systems. Standard Operation Procedure, Asia resist, 35 pp.
Khatoon H., Banerjee S., Yuan G. T. G., Haris N., Ikhwanuddin M., Ambak M.A., Endut A., 2016 Biofloc as a potential natural feed for shrimp postlarvae. International Biodeterioration and Biodegradation 113:304-309.
Mallasen M., Valenti W. C., 2006 Effect of nitrite on larval development of giant river prawn (*Macrobrachium rosenbergii*). Aquaculture 261:1292-1298.
Miao S., Sun L., Bu H., Zhu J., Chen G., 2017 Effect of molasses addition at C:N ratio of 20:1 on the water quality and growth performance of giant freshwater prawn (*Macrobrachium rosenbergii*). Aquaculture International 25(4):1409-1425.
Ministry of Agriculture and Rural Development (MARD), 2020 [Decision on master plan for production and exportation of freshwater prawn dated November 2, 2020]. 8 pp. [in Vietnamese]

Nghi L. T., Truyen P. M., Tao C. T., Hoa N. V., Hai T. N., 2020 [Effects of carbon sources on growth performance and survival rate of larval rearing of the freshwater prawn (*Macrobrachium rosenbergii*) by biofloc technology]. Journal of Vietnam Agricultural Science and Technology 5(114):117-123. [in Vietnamese]
Pérez-Fuentes J. A., Pérez-Rostro C. I., Hernández-Vergara M. P., 2013 Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with the biofloc system. Aquaculture 400-401:105-110.
Phuong N. T., Hai T. N., Hien T. T. T., Wilder M. N., 2003 [Principle and production technique for freshwater prawn (*Macrobrachium rosenbergii*)]. Agriculture Publishing House, Ho Chi Minh city, 127 pp. [in Vietnamese]
Phuong T. V. 2016 [Development of biofloc technology and application in white leg shrimp (*Liptopenaeus vannamei*)]. PhD thesis in aquaculture, Cantho University, Vietnam, 198 pp. [in Vietnamese]
Santhana K. V., Pandey P. K., Anand T., Bhuvaneswari G. R., Dhinakaran A., Kumar S., 2018 Biofloc improves water, effluent quality and growth parameters of *Penaeus vannamei* in an intensive culture system. Journal of Environmental Management 215:206-215.
Shirota A., 1966 The plankton of south Viet-Nam: freshwater and marine plankton. Overseas Technical Cooperation Agency, Japan, 162 pp.
Tao C. T., Phu T. M., 2015 [Effect of alkalinity on survival and growth performance of giant freshwater prawn (*Macrobrachium rosenbergii*) larvae and postlarvae]. Ministry of Agriculture and Rural Development, Vietnam, Vol. 3+4, pp. 93-99. [in Vietnamese]
Trang L. T., Doc L. Q., Ha V. T. T., Tu N. V., 2018 A case study of phytoplankton used as a biological index for water quality assessment of Nhu Y river, Thua Thien - Hue. Vietnam Journal of Science, Technology and Engineering 60(4):45-51.
Truyen P. M., Nghi L. T., Tao C. T., Hoa N. V, Hai T. N., 2020 [Rearing larvae of freshwater prawn by biofloc technology with different C/N ratio]. Journal of Vietnam Agricultural Science and Technology 1(110):102-108. [in Vietnamese]
Uno Y., Soo K. C., 1969 Larval development of *Macrobrachium rosenbergii* reared in the laboratory. J. Tokyo Univ Fish 55(2):79-90.
Vargas-Ceballos M. A., Badillo-Zapata D., Chong-Carrillo O., Ponce-Palafox J. T., Hernández-Hernández L. H., Vega-Villasante F., 2020 Intake of different food sources in the first zoeae stages of *Macrobrachium tenellum* (Decapoda: Palaemonidae). Latin American Journal of Aquatic Research 48(1):156-161.

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