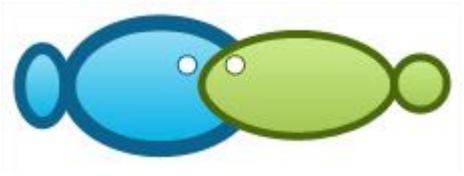

Influence of stocking density on survival and growth of larval and postlarval white leg shrimp (*Litopenaeus vannamei* Boone, 1931) applied biofloc technology

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Abstract. The aim of this study is to determine the optimal stocking density and production in larval rearing of whiteleg shrimp (*Litopenaeus vannamei*) applied biofloc technology (BFT). The experiment consisted of four treatments with triplicates each in different stocking densities (treatments, i.e. 150 larvae L^{-1}, 200 larvae L^{-1}, 250 larvae L^{-1} and 300 larvae L^{-1}, respectively). Larvae were stocked in 500 L^{-1} tanks at a salinity of 30 ‰ with continuous aeration. Sugar was provided daily as a carbon source to rearing tanks and supplemented from the Mysis 3 stage; C:N was manipulated at a ratio of 20:1. Results displayed the water quality, total bacteria, *Vibrio* spp., total bacteria count and biofloc parameters were in suitable range for larvae development. The highest length and survival rate of postlarvae-12 were found at stocking density of 150 larvae L^{-1} (i.e., 11.59±0.16 mm; 58.7±7.9%, respectively), and they were not significantly different ($p > 0.05$) from those at stocking density of 200 larvae L^{-1} (i.e. 11.49±0.38 mm; 55.0±3.0%, respectively). The highest production was found at stocking density of 200 larvae L^{-1} (i.e., 110±6 postlarvae L^{-1}) and it was significantly different ($p < 0.05$) from the remaining treatments. In short, rearing whiteleg shrimp larvae at a density of 200 larvae L^{-1} under BFT presented the best practice and to be recommended for application to the whiteleg shrimp hatchery protocols.
Key Words: biofloc, carbon source, sugar, whiteleg shrimp.

Introduction. Whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) is a crustacean with a palatable, tasty flavour and it can grow to a large size. It could tolerate a wide range of environmental conditions and can be cultured at high stocking density. Whiteleg shrimp could be engaged with intensive culture with high production and has high export value. For these reasons whiteleg shrimp is a targeted species for grow-out in Vietnam in intensive or even super intensive farming system (MARD 2015). In 2020, there were 112,000 ha of whiteleg shrimp were cultivated in Vietnam to produce approximately 900,000 tons; in total there were 687 whiteleg shrimp hatcheries to produce 98 billion postlarvae (PLs) as the need for the growers (MARD 2021). Currently, a number of constraints on whiteleg shrimp farming have been recorded e.g., weather change, environment deterioration, and especially shrimp diseases disaster, among those unsuitable and poor quality of PLs were the worse, which is one of the main issue for the big loss of shrimp farming (MARD 2021). Therefore, bio-security provisions for PLs are necessary with great attention from researchers and shrimp producers, as well. Several solutions have been tried to overcome those constraints and application of biofloc technology (BFT) in whiteleg shrimp hatcheries seems to be a promised solution. A number of studies have been convinced BFT has its important role in stabilizing the water quality, bio-security, and disease prevention, moreover floc particles with high nutrition content and suitable size being considered as directed food for several cultured species; it may also provide nutritional components and reduce water pollution (McIntosh et al 2000). So far, several studies have been performed on grow-out whiteleg shrimp applied BFT (Taw 2010; Phuong 2016; Santhana et al 2018) as well as in the hatchery phases (Tao et al 2015a; Hoa et al 2021). Similarly, studies on rearing of tiger shrimp (*Penaeus*

monodon) (Tao et al 2018; Tao et al 2019), and whiteleg shrimp (Tao et al 2020; Hoa et al 2020) applied BFT helped to cleaning up the environment and reducing considerably the feed used as its most advantages; however, determining an appropriate stocking density in the hatchery phase of whiteleg shrimp applied-BFT has not yet been considered, and thus such a study is necessary prior apply to larger production scale.

Material and Method

Location and the period of study. This study was conducted on College of Aquaculture, Cantho University, Vietnam during March to April 2019.

Water resources. Brine with a salinity of 80% was obtained from the Vinh Chau saltworks (Soc Trang province) and tap water was used to make brackish water for whiteleg shrimp hatcheries. These were mixed to produce salinities of 30 ‰ as commonly required in marine shrimp hatcheries. Then the saline water was treated with chlorine at 50 g m^{-3}; chlorine residue was removed through strong aeration during 24-48 h. Sodium bicarbonate ($NaHCO_3$) was applied to stabilize the alkalinity to 160 mg $CaCO_3$ L^{-1} (Tao et al 2015b) throughout the culture; and the water was then passed through a 1 µm mesh size filter prior to fill to larval tanks.

Larvae. Healthy whiteleg nauplli were released by SIS broodstocks (Hawaii, USA, which were purchased from Chau Phi Company, Ninh Thuan province). They were acclimatized in prepared seawater as described for approximately 3 h and were then treated with formalin 200 ppm within 30 seconds prior to release in the rearing set-up.

Biofloc creation. Bien Hoa refined sugar (i.e. 55.54% C and 0.19% N) was used as a carbon source to create biofloc in larval tanks (Tao et al 2020). Carbon source provided was recommended by Avnimelech (2015) in order to maintain C:N = 20:1 in BFT system; refined sugar was supplied into larval tanks at the Mysis 3 stage. Per day estimated C as sugar added and one gram of commercial probiotic (i.e. *Bacillus subtilis*: $0.22x10^9$ CFU, *Bacillus licheniformis*: $0.24x10^9$ CFU, *Bacillus polymyxa*: $0.24x10^9$ CFU, *Bacillus circulans*: $0.5x10^9$ CFU, *Bacillus laterosporus*: $0.22x10^9$ CFU, *Bacillus megaterium*: $0.24x10^9$ CFU, *Bacillus mesentericus*: $0.24x10^9$ CFU, *Nitrosomonas* spp.: $0.5x10^9$ CFU, *Nitrobacter* spp.: $0.54x10^9$ CFU, *Saccharomyces boulardii*: $0.36x10^9$ CFU) per cubic meter of rearing water were achieved; these mixtures were incubated 24 h with strong aeration thoroughly prior to applying to larval tanks (Hoa et al 2020).

Experimental setup. There were four density treatments with three replicates each and in a randomized setup, of which:
- treatment 1: stocking density 150 nauplii L^{-1};
- treatment 2: stocking density 200 nauplii L^{-1};
- treatment 3: stocking density 250 nauplii L^{-1};
- treatment 4: stocking density 300 nauplii L^{-1}.

The rearing tanks (Figure 1) used had a volume of 500-L each and salinity of 30 ‰ .

Figure 1. Composite tanks were used for whiteleg shrimp larval rearing set-up.

Larvae and postlarvae management. Feeding protocol applied for treatment 1 as displayed in Table 1; and feeding rates to the other stocking densities were adjusted as a function of stocking density, accordingly.

Table 1

Feeding regime for whiteleg shrimp at stocking density of 150 ind L^{-1} throughout hatchery phases (Tao et al 2020)

	Zoea 1	*Zoea 2-3*	*Mysis*	*PL 1-6*	*PL 7-12*
Chaetoceros sp.	60,000-120,000 cells mL^{-1}; 8 feedings day^{-1}; every 3 h				
50% Lansy ZM[(4)] + 50% Frippak-1[(1)]		0.4 g m^{-3} feeding; 8 feedings day^{-1}; every 3 h			
50% Lansy ZM[(4)] + 50% Frippak-2[(2)]		1-1.5 g m^{-3} feeding; 4 feedings day^{-1}; every 3 h	0.4 g m^{-3} feeding; 4 feedings day^{-1}; every 3 h		
Artemia			2 g *Artemia* m^{-3} feeding; 4 feedings day^{-1}; *Artemia* at umbrella stage	2-4 g *Artemia* m^{-3} feeding; 4 feedings day^{-1}; newly hatched nauplii *Artemia*	4-6 g *Artemia* m^{-3} feeding; 4 feedings day^{-1}; newly hatched nauplii *Artemia*
Frippak-150[(3)]				2-3 g m^{-3} feeding; 4 feedings day^{-1}	
Lansy PL[(5)]					3-4 g m^{-3} feeding; 4 feedings day^{-1}

*Commercial feeds have been used in whiteleg shrimp hatchery: [(1)] Frippak 1: protein 52%, lipid: 14.5%; [(2)] Frippak 2: Protein 52%, lipid: 14.5%; [(3)] Frippak 150: protein 42%, lipid: 14.5%; [(4)] Lansy ZM: protein 48%, lipid: 9%; [(5)] Lansy PL Protein 48%, lipid: 9%.

Faeces as well as solid wastes were siphoned in late of Zoea 3 stage and replaced with fresh seawater; however, there were no siphoning required during Mysis stage towards the end of the experiment except seawater was supplied to compensate for the evaporation loss.

Recorded parameters

Water quality. Temperature and pH were measured twice per day at 8 am and 2 pm using a thermometer and pH meter pH (HI-98127 Multi-Parameter Waterproof Meter, HANNA Instruments, Ltd.). Alkalinity was measured with a test kit (SERA test, Germany) during the periodic sampling every 3 days. Total ammonia nitrogen (TAN) and nitrite (NO_2^-) were also measured during the periodic sampling every 3 days; TAN was measured using Indophenol Blue, while NO_{2-} was measured by colometric method 4500-NO_2^-B (APHA 2005).

Bacteria parameters. Sampling water to determine the total bacteria and *Vibrio*, and the latter implied pathogenetic *Vibrio* which may cause shrimp infections (Goarant et al 1999), was performed twice per week and at the end of the experiment; similarly, bacteria and *Vibrio* were analyzed in the hepatopancreas of PL12 samples when

experiment terminated. The samples were defined by dilution method in which initial sample (i.e. concentration of 10^0) was diluted in physiology solution 0.86% into different concentration i.e. 10^{-1}, 10^{-2}, 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 TSA (trypticase soy agar) for *Vibrio* examination (Huys 2002). The total bacterial count (or the *Vibrio*) was calculated as:

Bacterial count (CFU mL^{-1}) = colony c ounted x dilution factor x 10

Biofloc parameters. Floc volume (FV) was monitored weekly by collecting a 1 L water sample and then settling in 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, bioflocs were also collected for particle measurement. Floc sizes of 30 floc samples per tank were observed and measured under a Nikon ECLIPSE Ti2 microscope with a DS-Qi2 camera (Nikon Corporation, Tokyo, Japan). Biofloc parameters were analyzed at PL4, PL8 and PL12 stages, respectively. Besides, approximate composition (i.e. protein, lipid, ash) of flocs were analyzed at the end of the experiment (AOAC 2016).

Zootechnical parameters

Growth of larvae and postlarvae. The length of larvae and postlarvae (PL) was measured at Zoea 3, Mysis 3, PL4, PL8, and PL12 stages, respectively and 30 randomized samples per tank were measured; length was measured under Nikon ECLIPSE Ti2 microscope with a DS-Qi2 camera (Nikon Corporation, Tokyo, Japan).

Survival: to be defined at PL12 stage by quantified method and estimated as:

Survival rate (%) = (harvested shrimps/initial stocked shrimps) x 100

Productivity: to be defined at the end of the experiment, as:

Productivity (ind L^{-1}) = number of shrimp collected/tank volume

Stress tests: the PL quality was evaluated by application of stress tests for PL12 (TCVN 10257: 2014 (MOST 2014), in which:

- stress test by submerging into formalin: randomized collecting of 100 PL12 from rearing tank and to stock into 1 L beaker, then formalin is dropped into the beaker until to reach 100 ppm in concentration. PL12 were suffering and counted after 30 minutes submerged; good PL quality are those displayed with 100% survival rate only;
- stress test by sudden change in salinity into freshwater (salinity 0‰): randomized collecting of 100 PL12 from rearing tank to stock into 1 L beaker of freshwater. PL12 were counted after soaking 30 minutes into freshwater; good PL quality are those only obtained of 100% survival rate recorded.

Data analysis. Recorded data were used to estimate the mean and standard deviation in Microsoft Excel (Office 2010). Differences among treatments were analyzed by one-factor ANOVA (Duncan test) when the dependent variable is normally distributed in each group with SPSS 20.0 package at a significant difference level of 5% ($p < 0.05$).

Results and Discussion

Water quality. Average temperatures recorded in the morning (8 am) and afternoon (2 pm) were rather stable throughout experiment in all treatments; the morning temperature fluctuated in the range of 29.4-29.9ºC while in the afternoon it varied in the range of 30.2-30.6^{o}C (Table 2). It was indicated the optimal temperature range for whiteleg shrimp growth stays within the range of 28-32°C (Ho & Lu 2003).

Recorded pH was stable throughout the experiment, the pH varied from 8.08 to 8.13. According to Hai et al (2017), the suitable pH range for the growth and development of marine shrimp is 7.5-8.5; therefore, these pH values were considered as the optimal range for whiteleg shrimp to develop.

Alkalinity throughout the experiment is displayed in Table 2, in which it varied in the range of 144.8-149.6 mg $CaCO_3$ L^{-1}. However, alkalinity tended to decrease towards

the end of the experiment; according to Tao et al (2015b), the optimal alkalinity range for whiteleg shrimp growth and development was 140-160 mg $CaCO_3$ L^{-1}; therefore, the recorded range of alkalinity in this experiment is appropriate for whiteleg shrimp to develop.

The average TAN in the experiment varied from 0.62 to 1.19 mg L^{-1} (Table 2). The lowest TAN was recorded in treatment 1 and was not significantly different ($p > 0.05$) from treatment 2 but significantly different ($p < 0.05$) to the others. TAN has a positive relationship to rearing density, i.e. the higher the density the higher the recorded TAN as nitrogen released from shrimps and un-eaten feeds left over, consequently.

The average concentration of NO_2^- varied from 0.07 to 0.35 mg L^{-1} (Table 2). The highest value was recorded in the treatment 3 but was not significantly different ($p > 0.05$) to the treatment of treatment 4; however, it was significantly different ($p < 0.05$) to the rest. According to Tinh (2004), Hai et al (2017) the suitable range of NO_2^- for whiteleg shrimp larvae to develop when it less than 1 mg L^{-1}, and thus the above recorded NO_2^- were safe for shrimp hatchery conditions.

Table 2

Environmental parameters

Parameters		*Treatments*			
		1	*2*	*3*	*4*
Temperature (^oC)	8 am	29.9±0.6	29.8±0.8	29.5±0.5	29.4±0.5
	2 pm	30.6±0.3	30.5±0.5	30.2±0.5	30.4±0.3
pH	8 am	8.13±0.01	8.09±0.03	8.10±0.01	8.08±0.03
	2 pm	8.12±0.04	8.10±0.04	8.11±0.01	8.09±0.04
Alkalinity (mg $CaCO_3$ L^{-1})		144.8±3.2[a]	146.8±2.9[a]	147.8±1.9[a]	149.6±2.3[a]
TAN (mg L^{-1})		0.62±0.02[a]	0.79±0.09[a]	1.13±0.19[b]	1.19±0.06[b]
NO_2^- (mg L^{-1})		0.07±0.02[a]	0.17±0.02[a]	0.35±0.08[b]	0.33±0.09[b]

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

In general, water quality parameters recorded throughout current experiment were maintained in suitable range for larvae/postlarvae of whiteleg shrimp to survive and develop, except TAN contents were a bit higher in treatment 250 ind L^{-1} and 300 ind L^{-1}, which had a negative impact on larval survival, accordingly.

Total bacteria count. There were significant fluctuations in total bacterial count among treatments (i.e. the values recorded at day 7, day 14 and day 21, respectively) in water medium (Table 3); the highest density, found at treatment 4 (3.30±0.44 CFU mL^{-1}), was not significantly different ($p > 0.05$) compared to treatment 3 but significant different ($p < 0.05$) to the rest. The lowest total bacterial count was found in treatment 2 (1.20±0.10 CFU mL^{-1}); according to Anderson (1993), good water quality corresponds to the total bacteria count of less than 10^3 CFU mL^{-1}, while as the total bacteria count is higher than 10^7 CFU mL^{-1}, it will cause negative impacts on shrimp/fish farming (Ngan & Hiep 2010). Again, the fluctuation of total bacterial count was considered as a function of nitrogen released from shrimps and un-eaten feeds left over and possibly the input from the atmosphere; however, total bacterial count in the current study was appropriate for shrimp development as similar recording by Tao et al (2020) when shrimp larvae were stoked at 150 ind L^{-1} and sugar applied as carbon source.

Table 3

Total bacteria count in water column and in shrimp biomass in different treatments (10^4 CFU mL^{-1})

Day	*Treatments*			
	1	*2*	*3*	*4*
7	1.93±0.15[a]	2.13±0.21[a]	2.79±0.15[b]	3.30±0.44[b]
14	1.40±0.26[a]	1.33±0.15[a]	2.53±0.40[b]	2.80±0.40[b]
21	1.23±0.15[a]	1.20±0.10[a]	2.23±0.12[b]	2.37±0.21[b]
In shrimp	1.77±0.25[a]	2.00±0.26[a]	3.07±0.75[a]	4.37±2.56[a]

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

In shrimp, an average total bacterial count varied in the range of $1.77x10^4$ - $4.37x10^4$ CFU mL^{-1}; the highest value was found in treatment 4 but not significantly different ($p > 0.05$) from the rest. These are, moreover, lower values compared to the recording of Hoa et al (2020) when rearing at the same stocking density of 150 ind L^{-1} (i.e. 0.90 ± 0.20 to $13.44\pm0.82x10^5$ CFU mL^{-1} during day 4 to day 21, respectively) without BFT application.

Vibrio. In the 1st week the highest *Vibrio* level was found in the water column of treatment 4 but this value was not significantly different ($p > 0.05$) to the others (Table 4). In the 2nd and 3rd week, again *Vibrio* was recorded highest in the treatment 4, however it was not significantly different ($p > 0.05$) to the treatment 3 but significantly different ($p < 0.05$) to the other treatments.

Vibrio detected in shrimp was lowest in the treatment 1, which was not significantly different ($p > 0.05$) from the treatment 2, but significant difference ($p < 0.05$) to the other treatments. Remarkably, *Vibrio* concentration is lower compared to Tao et al (2020) when shrimp larvae were stoked at 150 ind L^{-1} and sugar applied as carbon source. Tao et al (2018) indicated *Vibrio* concentration in rearing medium and in shrimp biomass recorded as $30.83x10^3$ CFU mL^{-1} and $30.9x10^3$ CFU g^{-1} did not harm to the tiger shrimp (*Penaeus monodon*) in hatchery.

Table 4

Vibrio in water column (10^3 CFU mL^{-1}) and in shrimp biomass in different treatments (10^3 CFU g^{-1})

Day	*Treatments*			
	1	*2*	*3*	*4*
7	0.40 ± 0.30^a	0.57 ± 0.15^a	0.47 ± 0.38^a	0.60 ± 0.20^a
14	0.53 ± 0.15^a	0.77 ± 0.21^a	1.20 ± 0.10^b	1.30 ± 0.20^b
21	0.77 ± 0.15^a	0.80 ± 0.10^a	1.37 ± 0.12^b	1.43 ± 0.23^b
In shrimp	0.30 ± 0.26^a	0.40 ± 0.20^a	2.57 ± 0.21^b	3.20 ± 0.81^b

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

Floc volume. Floc volume (FV) recorded in the PL4 stage ranged from 0.57 to 0.70 mL L^{-1}, and there were no significant differences ($p > 0.05$) among treatments. In PL8 stage FV was highest in treatment 4, but again there were no significant differences ($p > 0.05$) among treatments. At PL12 stage floc volume continued to be highest in treatment 4 and significant differences ($p < 0.05$) to other treatments (Table 5); the lowest FV was found in treatment 1 and was not significant different ($p > 0.05$) to the treatment 2, but it was significant different ($p < 0.05$) to other treatments. In general, FVs increased by rearing time and in correspondence with the stocking density, i.e., the higher the rearing density the higher the expected FV. In practice, floc particles are defined as including of phytoplankton, bacteria, aggregates of living and dead particulate organic matter (Avnimelech 2015) and hence at higher stocking densities, higher floc particles could be enhanced and thus to level up the FV.

Table 5

Floc volume (mL L^{-1}) per treatment

Day	*Treatments*			
	1	*2*	*3*	*4*
PL-4	0.57 ± 0.06^a	0.63 ± 0.06^a	0.67 ± 0.06^a	0.70 ± 0.10^a
PL-8	1.40 ± 0.10^a	1.43 ± 0.21^a	1.50 ± 0.26^a	1.77 ± 0.45^a
PL12	1.77 ± 0.15^a	2.10 ± 0.20^{ab}	2.57 ± 0.23^b	3.80 ± 0.44^c

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

The width of biofloc. Variation of the width of biofloc particles among treatment was negligible (Table 6), the narrowest width of floc particles being found in treatment 1 but it was not significant differences ($p > 0.05$) to the other treatments. At PL8 and PL12 stages the largest width of floc particle was recorded in the treatments 4, and the largest

width of floc particles were also occurred in other treatments, therefore they were not significant different ($p > 0.05$) from each other.

The length of biofloc. Average length of floc particles during PL4, PL8 and PL12 stages (Table 6) were not significantly different ($p > 0.05$) among treatments; the longest length was recorded in treatment 4, and shortest length was found in the treatment 1 for PL4 and PL12. In PL8 the longest length recorded in treatment 3, then to treatment 2; however they were not significant difference ($p > 0.05$).

In short, floc particles in the current study were not different in dimension (i.e. width and length). During the rearing period floc particles intended to increase by size gradually. According to Logan et al (2010) bacteria composition in a shrimp-farming environment are very diverse, and together with other elements accumulated (Avnimelech 2015) by time, they could aggregate to form floc particles in various shapes and dimensions.

Table 6

Biofloc dimension

	Shrimp stages	*Treatments*			
		1	*2*	*3*	*4*
Width (mm)	PL4	0.13 ± 0.02^a	0.14 ± 0.01^a	0.14 ± 0.01^a	0.15 ± 0.02^a
	PL8	0.18 ± 0.01^a	0.19 ± 0.03^a	0.21 ± 0.02^a	0.22 ± 0.02^a
	PL12	0.24 ± 0.01^a	0.24 ± 0.01^a	0.25 ± 0.02^a	0.26 ± 0.02^a
Length (mm)	PL4	0.24 ± 0.01^a	0.25 ± 0.01^a	0.25 ± 0.01^a	0.26 ± 0.02^a
	PL8	0.32 ± 0.01^a	0.34 ± 0.03^a	0.35 ± 0.01^a	0.33 ± 0.01^a
	PL12	0.36 ± 0.01^a	0.37 ± 0.02^a	0.39 ± 0.05^a	0.40 ± 0.02^a

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

Proximate composition. Average protein content in floc particles collected in different treatments varied in the range of 28.9 to 29.2%, with a minor difference among treatments (Table 7). The highest protein content was recorded in treatment 2 and the lowest found in treatment 1, however there were no significant differences ($p > 0.05$) among treatments. These figures are, however, a bit higher than the protein contents obtained in flocs from Khoa et al (2020), of which protein levels in floc particles varied in the range of 22.2 to 25.1%. Different approximate composition of bioflocs were convinced due to type of carbon sources used (Hosain et al 2021), and possibly the PLs were fed with commercial pellets which had lower protein content (i.e. 40%) compare to larval and postlarvae feeds (i.e. 42-52% protein, Table 1) using in current study.

Lipid contents in floc particles varied in the range of 5.4 to 5.7%. There was a bit low lipid content in the treatment 3 was recorded but there were not significant differences ($p > 0.05$) among treatments; previous studies have indicated the highest lipid contents (i.e. $4.02\pm0.14\%$) recorded in floc particles when maize starch was used as carbon source, however these are lower compared to the current study (i.e. 5.4-5.7 %) which had similar lipid content (i.e. 5.3%) obtained by Bakhshi et al (2018); it was stated different nutritional composition of floc particles as an effect of different carbon sources applied (YanFang et al 2016). The ash contents were similar (approximately 29.1%) for all treatments and without significant differences ($p > 0.05$); previous study implied different ash contents in flocs as the function of carbon sources used (Hosain et al 2021), as well.

Table 7

Proximate composition of biofloc

Parameter	*Treatments*			
	1	*2*	*3*	*4*
Protein (%)	28.9 ± 0.4^a	29.2 ± 0.4^a	29.1 ± 0.3^a	29.0 ± 0.3^a
Lipid (%)	5.5 ± 0.4^a	5.7 ± 0.2^a	5.4 ± 0.1^a	5.6 ± 0.2^a
Ash (%)	29.1 ± 0.3^a	29.2 ± 0.2^a	29.0 ± 0.4^a	29.1 ± 0.2^a

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

Growth in length of whiteleg shrimp. There was no significant difference ($p > 0.05$) in lengths during Zoea 3 to Mysis 3 stages. In PL4 the greatest length found in the treatment 1, however there were no significant differences ($p > 0.05$) among treatments (Table 8). In PL8 and PL12 stages again the greatest length of PLs found in treatment 1; their lengths were not significantly different ($p > 0.05$) to PLs in treatment 2 but they were significant difference ($p < 0.05$) compared to other treatments. There were similar growth in length of whiteleg shrimp larvae and postlarvae by Hoa et al (2020) as they recorded the shrimp grow from Zoea 3, Mysis 3, PL4, PL8 and PL12 were 2.54±0.01 mm, 4.68±0.01 mm, 6.21±0.11 mm, 7.54±0.06 mm and 11.42±0.67 mm, respectively when both to have the same stocking density (i.e. 150 ind L^{-1}) and rearing period (i.e. 20 days). Possibly, retarded growth of whiteleg shrimp larvae found in the current study is a consequence of stocking density; it was indicated at higher stocking density of tiger shrimp (*Penaeus monodon*) larvae, slowly growth of PLs occurred at stocking densities of 200 ind L^{-1}, 250 ind L^{-1} and 300 ind L^{-1} compared to 150 ind L^{-1} was recorded (Tao et al 2019).

Table 8

Length (mm) of whiteleg shrimp larvae and postlarvae

Stages	*Treatments*			
	1	*2*	*3*	*4*
Zoea 3	2.43±0.03[a]	2.42±0.06[a]	2.41±0.03[a]	2.38±0.02[a]
Mysis 3	3.65±0.19[a]	3.51±0.03[a]	3.47±0.14[a]	3.40±0.12[a]
Postlarvae 4	5.96±0.37[a]	5.68±0.25[a]	5.58±0.16[a]	5.58±0.05[a]
Postlarvae 8	9.11±0.06[b]	9.09±0.34[b]	8.69±0.13[a]	8.57±0.21[a]
Postlarvae 12	11.59±0.16[b]	11.49±0.38[b]	10.83±0.19[a]	10.81±0.09[a]

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

Survival and productivity. The highest survival rate of PL12 was found in treatment 1 (i.e., 58.7±1.8%); this was not significant different ($p > 0.05$) to treatment 2 (i.e., 55.0±3.0%), but significant differences ($p < 0.05$) to remained treatments (Table 9). Previous studies by Tao et al (2020) at stocking density of 150 ind L^{-1} indicated PLs could survive in the range of 33.2 to 52.0% and the highest survival engaged with sugar as carbon source used in BFT system. Sugar dissolved easily in medium and to facilitate for bacteria to develop quickly which is considered as the main role for good water quality maintenance.

The highest productivity of PL12 was obtained in treatment 2 (i.e., 110 PL12 L^{-1}) and not significant different ($p > 0.05$) to treatment 1; but they were significantly different ($p < 0.05$) to the remained treatments. Similar survival and productivity were found in tiger shrimp (Tao et al 2019) when stocking density varied from 150 to 200 ind L^{-1}, at stocking higher than 250 ind L^{-1} the environment usually engaged with poor water quality and higher *Vibrio* density, which may concern to the mortality and hence low productivity in the hatchery phase.

Table 9

Survival rates and productivity of whiteleg shrimps per treatments

Items	*Treatments*			
	1	*2*	*3*	*4*
Survival (%)	58.7±1.8[b]	55.0±3.0[b]	35.1±2.6[a]	30.9±1.0[a]
Productivity (ind L^{-1})	88±3[a]	110±6[b]	88±6[a]	93±3[a]

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

Postlarvae quality. Quality of PLs produced were evaluated by different stress tests (i.e., formalin as well as salinity shock) to ensure their quality prior to the grow-out phase. PLs subjected to these stress tests displayed their 100% survival rate without significant differences ($p > 0.05$) among treatments implied the PLs quality are appropriate. Further, they met the Vietnamese National standard for PLs quality (TCVN 10257: 2014) prior stocking for grow-out phase.

Conclusions and recommendations. Rearing whiteleg shrimp in hatchery applied biofloc technology we found both initial stocking densities of 150 ind L^{-1} and 200 ind L^{-1} are suitable for larvae and postlarvae to develop throughout the rearing phases; while the former ended up with higher survival and growth, the latter displayed their higher productivity. Therefore, rearing of whiteleg shrimp larvae at stocking density of 200 ind L^{-1} applied BFT should be considered to expand for production scale.

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