

## Water quality and growth of *Litopenaeus vannamei* (Boone, 1931) in culture tanks supplemented with blue-green algae

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**Abstract**. Harmful blue-green algae had a remarkable impact on the growth of *Litopenaeus vannamei* (Boone, 1931) (whiteleg shrimp). The study objective was to determine varying water quality elements and the effects of different algal densities on the growth and survival rate of whiteleg shrimp under laboratory conditions. The experiment was set up with four treatments, including (1) control treatment (T1) without algae addition, (2) treatment 2 (T2) (6,000 ind. mL<sup>-1</sup>), (3) treatment 3 (T3) (16,000 ind. mL<sup>-1</sup>), and treatment 4 (T4) (26,000 ind. mL<sup>-1</sup>). Blue-green algae (*Planktothrix pseudagardhii*) was added to shrimp tanks for 63 days of the culture cycle. Each treatment was repeated three times. The results indicated that water environment parameters were desirable for shrimp growth, except levels of NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, and chlorophyll-a. The shrimp length and weight were not significantly different (p>0.05) among treatments. Shrimp survival in T4 was the lowest and significantly different (p<0.05) compared to other treatments. The FCR and FER indices showed a significant difference (p<0.05) between treatments of T2, T3, T4, and T1.

**Key Words**: blue-green algae (*Planktothrix pseudagardhii*), whiteleg shrimp, survival rate, water quality parameters.

**Introduction**. Whiteleg shrimp (*Litopenaeus vannamei* (Boone, 1931)) is one of the most popular farming species in the Mekong Delta, Vietnam's coastal province, accounting for about 45% of the total shrimp farming area in the country. In recent years, whiteleg shrimp have been farmed at different levels of intensification, such as semi-intensive, intensive, and super-intensive (Thanh 2022). During the shrimp farming process, managing the water quality in shrimp ponds is essential to the culturing model's success. For the super-intensive shrimp cultivation model, shrimp are stocked at a very high density, so the amount of food supplied to the shrimp ponds is very large. The amount of excess food, as well as shrimp waste products, especially at the end of the crop, often makes the water environment rich in nutrients in the shrimp ponds (Arifin et al 2018). This is a favorable condition for harmful algae groups to develop and dominate at this stage. Cyanobacteria were significantly higher in control compared to treated ponds during the final phase of the shrimp culture cycle (Yusoff et al 2002). However, the overgrowth of algae in shrimp ponds will degrade the aquatic habitat, which will negatively impact the shrimp.

Nowadays, whiteleg shrimp can be commonly farmed in many different forms, such as farming in earthen ponds with plastic sheets placed at the bottom of the pond. In addition, a popular form today is farming shrimp in round tanks made of iron frames placed on the ground. Research by Lien et al (2022) indicated that vannamei shrimp was cultured in circular tanks constructed of iron frames placed on the ground and covered with tarpaulins at the pond bottom. During the commercial shrimp farming and the shrimp nursery, the typical water levels kept in the shrimp tanks were around 1.5 and 1.2 meters, respectively. The economic effectiveness of the farming model will increase with effective management of the water quality in shrimp ponds. Poor water quality, on the other hand, can promote the growth of toxic algae, particularly blue-green algae.

Factors favoring blue-green algae in ponds are high concentrations of nutrients (Boyd 1990). Because of changes in the ecology, deteriorating water quality inhibits shrimp growth and raises mortality rates (Kumar 2023).

In general, the water environment plays a very important role and affects the availability of phytoplankton and the growth of the whiteleg shrimp. According to Lien et al (2023), the amount of algae tended to increase from the middle to the end of the whiteleg shrimp farming cycle (42-70 days). Especially at these stages, there was an increase in the density of cyanobacteria and dinoflagellates, with the predominance of M. aeruginosa (Cyanobacteria) and Alexandrium sp. (Dinophyta). PH, nitrate, and total organic matter significantly influenced phytoplankton abundance in the pond (Musa et al 2023). In addition, Zebek & Szymańska (2017) stated that Cyanobacteria is found in waters rich in nutrients. Blue-green algae can fixate  $N_2$  from the air and is very easy to grow in high-phosphate conditions. The toxins from blue-green algae pose a threat to animal health and potentially to consumers if they are present in aquaculture products. Microcystins are prevalent cyanotoxins generated by various taxa of cyanobacteria, including Microcystis, Dolichospermum, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, and Anabaenopsis. In shrimp ponds dominated by cyanobacteria, particularly Microcystis and Oscillatoria, microcystin-leucine arginine levels were high. In pond water, Microcystis and Oscillatoria levels increased along with NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, clarity, and salinity (Soegianto et al 2025). The development of blue-green algae in whiteleg shrimp ponds greatly affects the health of the shrimp. Therefore, this study aimed to evaluate the variation in water quality factors as well as the impact of blue-green algae density on the survival rate and growth of white-leg shrimp. The findings of the study will provide basic data for further studies to find measures to control harmful algae in shrimp ponds.

## Materials and Methods

**Study time and location**. The study was conducted from January to May 2024 at the College of Aquaculture and Fisheries, Can Tho University (Figure 1).



Figure 1. Study site of the experiment.

**Water, algae, and shrimp sources**. The water source for the experiment had a salinity of 10‰ and was treated with chlorine at a concentration of 30 mg L<sup>-1</sup> to ensure the water was free of pathogens and aerated vigorously to remove all chlorine. Blue-green algae (*Planktothrix pseudagardhii*) used in the experiment were isolated from white-leg shrimp ponds. Several *Planktothrix* species are reported as species of *Oscillatoria*. Shrimp in the post-12 period were purchased from a marine shrimp hatchery in Bac Lieu province, Vietnam, and were used for the experiment.

**Experiment design**. The experiment was randomly arranged in a 500 L tank containing 250 L, salinity of 10‰. Postlarvae shrimps were stocked in the tanks with 50 shrimps/tank. Shrimp were fed by industrial feed (Grobest) with a protein content of 38-42%. The average amount of food for shrimp was about 3-5% of body weight. Shrimp were fed 4 times/day at the following times: 6 am, 11 am, 5 pm, and 9 pm. Water was periodically changed every 3 days at a rate of 30-50%. The experiment was continuously aerated during the culture process. Blue-green algae were added to the shrimp tanks after 2 months of culture and were maintained for 7 days. The experiment was carried out for 70 days. The experiment consisted of 4 treatments; each treatment was repeated 3 times (3 tanks/treatment) as follows:

- Treatment 1: Control treatment (No blue-green algae was added)
- Treatment 2: Blue-green algae added at a density of 6,000 ind. mL<sup>-1</sup>
- Treatment 3: Blue-green algae added at a density of 16,000 ind. mL<sup>-1</sup>
- Treatment 4: Blue-green algae added at a density of 26,000 ind. mL<sup>-1</sup>

**Collecting and analyzing methods of water quality parameters**. Water environment elements were monitored during the culture period, including temperature, pH, and DO measured periodically every 3 days at 7 am and 2 pm using a multi-parameter. Alkalinity, ammonia (TAN), nitrite ( $NO_2^-$ ), nitrate ( $NO_3^-$ ), phosphate ( $PO_4^{3-}$ ), total nitrogen (TN), total phosphorus (TP), and chlorophyll-a contents were collected periodically every 7 days. The collecting and analyzing methods of water environment levels are presented in detail in Table 1.

Table 1

Collecting and analyzing the methods of water quality elements

No.	Parameters	Preservation	Analysis methods
1	Temperature (°C)		
2	рН	Measure directly	Multiparameter, ProQuatro-YSI
3	DO (mg L <sup>-1</sup> )		
4	Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )		2340 B-Titration method (APHA 2017)
5	TAN (mg $L^{-1}$ )		4500-NH3 F. Phenate (APHA 2017)
6	$NO_{2}^{-}$ (mg $L^{-1}$ )	Stored in plastic	4500-NO2 <sup>-</sup> B. Colorimetric (APHA 2017)
7	NO₃⁻ (mg L⁻¹)	bottle of 1 L at	ISO 7890-3:1988- Sulfosalicylic acid method
8	PO4 <sup>3-</sup> (mg L <sup>-1</sup> )	4°C	4500-P D. Stannous chloride (APHA 2017)
9	TN (mg L <sup>-1</sup> )		4500-Norg D. Persulfate method (APHA 2017)
10	TP (mg $L^{-1}$ )		4500-P B. Persulfate method (APHA 2017)
11	Chlorophyll-a (µg L-1)		10200 Acetone Chlorophyll (APHA 2017)

**Counting methods of blue-green algae**. Blue-green algae were identified by Sedgwick-Rafter counting chamber according to Boyd & Tucker (1992) as the following equation:

X (ind.  $L^{-1}$ ) = (T×1.000×V<sub>con.</sub>)/ (A×N×V<sub>sam.</sub>)

Where:

X - blue-green algae (ind. mL<sup>-1</sup>);

T - number of individuals counted by genus/species;

V<sub>con.</sub> - concentrated sample volume (mL);

V<sub>sam</sub>. - the collected sample volume (mL);

A - area of a counting cell  $(1 \text{ mm}^2)$ ;

N - the number of counted cells.

**Growth and survival evaluation of whiteleg shrimp**. Initial shrimp weight and length were determined by randomly measuring 30 shrimp with  $0.03\pm0.01$ g/shrimp and  $1.5\pm0.2$  cm, respectively. After stocking, the weight and length of shrimp were measured randomly on 10 shrimp/tank every 15 days, and restocking was done after

weighing. At the end of the experiment, the shrimp were weighed, measured, and counted to determine the growth rate and survival rate.

Feed conversion ratio (FCR) = Total feed consumed/Total weight gain

Food efficiency ratio (FER) = (body weight gain (g)/food intake (g))  $\times$  100

Survival rate (SR) (%) = (total number of shrimp at the end of experiment/ total number of initial shrimp) x 100

Daily weight gain (DWG) (g/day) = (final weight - initial weight)/days of culture

Specific growth rate (SGR) (%/day) = ((final weight - initial weight)/days of culture) x 100

**Data analysis**. Data were processed for maximum, minimum, mean, and standard deviation values using Excel software. Statistical analysis (One-way ANOVA with Duncan test) was applied to find the difference between treatments using SPSS 22.0 software at a significance level of p < 0.05.

## Results

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**Water quality parameters**. Temperature, pH, and DO concentrations during the experiment did not vary significantly between treatments as well as between morning and afternoon. These parameters in the morning and afternoon varied from  $25.4-28.7^{\circ}$ C and  $27.1-29.7^{\circ}$ C; 7.3-8.0 and 7.4-8.2; 4.1-5.5 mg L<sup>-1</sup> and 5.0-5.7 mg L<sup>-1</sup> for temperature, pH, and DO concentrations, respectively. The average values of temperature, pH, and DO concentrations of the treatments are presented in Table 2.

Table 2

		•				•	
la Traat	Trootmont	Temperature (°C)		pН		$DO (mg L^{-1})$	
lo.	Treatment	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
1	T1	26.9±0.80	28.4±0.81	7.60±0.23	7.78±0.18	5.14±0.21	5.28±0.12

Water temperature, pH, and DO of treatments in the experiment

-		-0.5 0.00				0.2. 0.22	0.20 0.22			
2	T2	27.0±0.72	28.5±0.80	7.62±0.21	7.80±0.20	5.23±0.23	$5.30 \pm 0.11$			
3	Т3	27.3±0.74	28.4±0.72	7.64±0.25	7.81±0.21	$5.22 \pm 0.12$	5.32±0.21			
4	T4	27.4±0.76	28.5±0.75	7.68±0.21	7.83±0.20	$5.32 \pm 0.14$	5.34±0.21			
Durino	g the ex	periment, alka	alinity in the	treatments	ranged fror	n 68.5 to 1	58 mg L <sup>-1</sup>			
		, eral, alkalinity								
	-									
experiment but was still within the appropriate range and recorded an average value in the treatments of $142.3\pm0.8$ mg L <sup>-1</sup> CaCO <sub>3</sub> in T1; $146.2\pm0.8$ mg L <sup>-1</sup> CaCO <sub>3</sub> in T2;										
			9	,		9	,			
1/6 2	+1 1 ma	$1^{-1}$ CaCO <sub>2</sub> in T	[2 and 1/15 (	)+00 mal <sup>-1</sup>	$C_{2}CO_{2}$ in T/	$\int On day 70$	) alkalinity			

146.2 $\pm$ 1.1 mg L<sup>-1</sup> CaCO<sub>3</sub> in T3 and 145.0 $\pm$ 0.9 mg L<sup>-1</sup> CaCO<sub>3</sub> in T4. On day 70, alkalinity decreased sharply in the three treatments supplemented with harmful algae and had the lowest value in T4 with 69.4 $\pm$ 0.7 mg L<sup>-1</sup> CaCO<sub>3</sub>. However, in the control treatment (T1), alkalinity still decreased but not significantly (Figure 2).

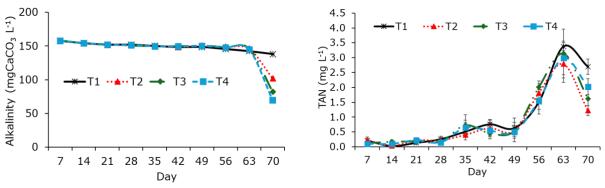


Figure 2. Alkalinity and TAN concentration in the experiment.

TAN content in the treatments during the experiment ranged from 0.01-3.79 mg L<sup>-1</sup>. TAN content tended to increase from day 7 to day 63 after shrimp release. The highest average values in the treatments were  $3.38\pm0.14$  mg L<sup>-1</sup> in T1;  $2.79\pm0.62$  mg L<sup>-1</sup> in T2;  $3.14\pm0.82$  mg L<sup>-1</sup> in T3 and  $2.99\pm0.57$  mg L<sup>-1</sup> in T4. On day 70 (end of the experiment), TAN content in all 4 treatments decreased, of which 3 treatments supplemented with blue-green algae decreased lower than treatment T1. Treatment T2 decreased the lowest with a determined TAN value of  $1.22\pm0.16$  mg L<sup>-1</sup>. The average TAN content in T2 was lower than that in the other three treatments and was recorded as  $1.02\pm1.12$  mg L<sup>-1</sup> in T1;  $0.81\pm0.87$  mg L<sup>-1</sup> in T2;  $0.91\pm1.02$  mg L<sup>-1</sup> in T3; and  $0.89\pm0.97$  mg L<sup>-1</sup> in T4 (Figure 2).

Through the study periods, the NO<sub>2</sub><sup>-</sup> level in the experiment changed from 0.01 to 5.78 mg L<sup>-1</sup>, with the lowest in T3 at 2.36±1.59 mg L<sup>-1</sup> and the highest in T4 at 2.45±1.48 mg L<sup>-1</sup>. The NO<sub>2</sub><sup>-</sup> content in all treatments reached low values in the early stages of the experiment, then gradually increased until day 35 and decreased sharply until day 49, continuing to increase again on day 63. At this stage, NO<sub>2</sub><sup>-</sup> element had very high values, notably in treatments T1 and T2. The mean NO<sub>2</sub><sup>-</sup> content of the treatments recorded were 5.11±0.21 mg L<sup>-1</sup> in T1; 5.14±0.26 mg L<sup>-1</sup> in T2; 4.44±1.05 mg L<sup>-1</sup> in T3; and 4.18±0.80 mg L<sup>-1</sup> in T4. This content decreased remarkably at the end of the experiment (Figure 3).

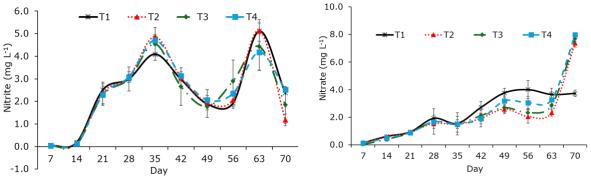


Figure 3.  $NO_2^-$  and  $NO_3^-$  concentrations in the experiment.

The average NO<sub>3</sub><sup>-</sup> element in the treatments varied highly, from 0.03 to 8.09 mg L<sup>-1</sup>. The average NO<sub>3</sub><sup>-</sup> value was lowest in T2 with a content of 2.09±1.95 mg L<sup>-1</sup> and highest in T4 with a content of 2.40±2.17 mg L<sup>-1</sup>. The NO<sub>3</sub><sup>-</sup> level tended to increase gradually from day 7 until the end of the experiment for all treatments. However, the treatments supplemented with toxic algae had much higher NO<sub>3</sub><sup>-</sup> values than the T1 treatment. At the experiment end, the recorded NO<sub>3</sub><sup>-</sup> levels were 3.73±0.24 mg L<sup>-1</sup> in T1; 7.38±0.30 mg L<sup>-1</sup> in T2; 7.69±0.18 mg L<sup>-1</sup> in T3; and 7.98±0.10 mg L<sup>-1</sup> in T4 (Figure 3).

TN and TP contents for all treatments of the experiment fluctuated from 0.05-12.66 mg L<sup>-1</sup> and 0.02-3.74 mg L<sup>-1</sup>, respectively (Figure 3). In general, TN content tended to increase from the beginning to the end of the experiment for all treatments and reached the highest average value, recorded as  $7.68\pm0.22$  mg L<sup>-1</sup>; 10.66±0.82 mg L<sup>-1</sup>; 11.53±0.23 mg L<sup>-1</sup> and 12.25±0.36 mg L<sup>-1</sup> for treatments T1, T2, T3 and T4, respectively (Figure 3). TP content tended to increase from the beginning of the experiment to day 56, then decreased sharply on day 63, and tended to increase again at the end of the experiment. The average value of TP content during the sampling stages was  $1.36\pm0.94$  mg L<sup>-1</sup> in T1;  $1.46\pm1.14$  mg L<sup>-1</sup> in T2;  $1.51\pm1.10$  mg L<sup>-1</sup> in T3 and  $1.32\pm0.94$  mg L<sup>-1</sup> in T4 (Figure 4).

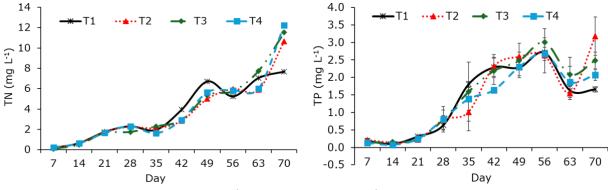


Figure 4. TN and TP concentrations in the experiment.

The PO<sub>4</sub><sup>3-</sup> content of the treatments ranged from 0.02-3.22 mg L<sup>-1</sup>. The highest average PO<sub>4</sub><sup>3-</sup> value recorded in T2 was 1.17±0.92 mg L<sup>-1</sup>, and the lowest in T1 was 1.02±0.72 mg L<sup>-1</sup>. This content in the treatments increased from day 7 to day 56 and reached an average level of  $2.39\pm0.14$  mg L<sup>-1</sup>,  $2.46\pm0.64$  mg L<sup>-1</sup>,  $1.87\pm0.48$  mg L<sup>-1</sup>, and  $2.21\pm0.25$  mg L<sup>-1</sup> for treatments T1, T2, T3, and T4, respectively. The average PO<sub>4</sub><sup>3-</sup> concentration decreased at day 63 and tended to increase at day 70. At this time, the mean PO<sub>4</sub><sup>3-</sup> content in T2 was  $2.68\pm0.24$  mg L<sup>-1</sup>, higher than that of the other three treatments. Treatment T1 had the lowest PO<sub>4</sub><sup>3-</sup> content at the end of the experiment (Figure 5).

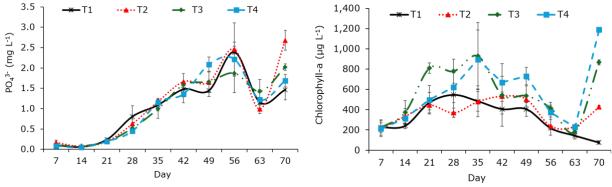


Figure 5.  $PO_4^{3-}$  and chlorophyll-a concentrations in the experiment.

The chlorophyll-a content of the treatments ranged from 59.7 to 1,341.3  $\mu$ g L<sup>-1</sup>. The chlorophyll-a content of the treatments supplemented with harmful algae tended to increase from day 7 to day 35, then decreased until day 63. On day 70, the chlorophyll-a content in treatments T3 and T4 increased, while treatment T1 reached the lowest value. For the control treatment (T1), the chlorophyll-a content increased from day 7 to day 28 and then decreased until the end of the experiment. The average chlorophyll-a content in the experiment recorded values of 321.1±184.1  $\mu$ g L<sup>-1</sup> in T1; 378.0±129.4  $\mu$ g L<sup>-1</sup> in T2; 565.7±288.1  $\mu$ g L<sup>-1</sup> in T3 and 573.1±324.7  $\mu$ g L<sup>-1</sup> in T4 (Figure 5).

**Growth and survival rate of whiteleg shrimp**. The length of white-leg shrimp from the period of 14-56 days was not statistically different (p>0.05) between treatments. After adding harmful blue-green algae, statistical differences in the shrimp length was also not found at the experiment end (p>0.05). Wherein, treatment T1 had the highest length ( $10.2\pm1.4$  cm) and treatment T2 had the lowest once ( $9.8\pm1.1$  cm) (Table 3).

Table 3

Contents	Traatmont	Day					
Contents	Treatment	1	14	28	42	56	70
	T1	1.5±0.2	3.5±0.3ª	5.1±0.6ª	6.7±0.8ª	9.0±1.1ª	10.2±1.4ª
Length	T2		3.5±0.3ª	5.1±0.8ª	6.7±0.7ª	9.2±0.9ª	9.8±1.1ª
(cm)	Т3		3.6±0.3ª	5.3±0.6ª	6.6±0.8ª	9.4±1,1ª	$10.1 \pm 1.4^{a}$
	T4		3.5±0.3ª	5.1±0.7ª	6.8±0.7ª	8.9±1.2ª	$10.0 \pm 1.5^{a}$

The values with different superscript letters in a column are significantly different (p < 0.05). Data are presented as mean±standard deviation.

The initial shrimp weight was  $0.03\pm0.01$  g. After 14 days of culturing, the shrimp weight in treatment T3 ( $1.0\pm0.1$  g) was higher than that in the other treatments ( $0.9\pm0.1$  g). The shrimp weight trended to increase among the treatments through the sampling periods, but a statistically significant difference was not found between treatments (p>0.05). The shrimp weight at the end of the experiment reached the values of  $11.7\pm2.7$  g in T1,  $11.3\pm2.5$  g in T2,  $11.8\pm3.3$  g in T3, and  $11.1\pm3.9$  g in T4, respectively (Table 3).

The growth rate in shrimp weight at 14 days of culture in treatment T3  $(25.2\pm0.9\% \text{ day}^{-1})$  tended to be higher than in the others. During the study stages, SGR was not a significant (p>0.05) difference among treatments. The SGR gradually decreased from a period of 14 days until the end of the experiment. The growth rate in shrimp weight was not significantly different at the harvest and reached values of  $8.5\pm0.3\% \text{ day}^{-1}$  in T1;  $8.4\pm0.3\% \text{ day}^{-1}$  in T2;  $8.5\pm0.4\% \text{ day}^{-1}$  in T3 and  $8.4\pm0.5\% \text{ day}^{-1}$  in T4 (Table 3). In addition, shrimp weight growth increased gradually through sampling periods, but the difference was not statistically significant (p>0.05) between treatments. The DWG index at the experiment's end reached values of  $0.17\pm0.04$  g day<sup>-1</sup>;  $0.16\pm0.04$  g day<sup>-1</sup>;  $0.17\pm0.05$  g day<sup>-1</sup> and  $0.16\pm0.06$  g day<sup>-1</sup> for treatments T1, T2, T3 and T4, respectively (Table 3).

Table 3

Elements	Treatment	Day						
		1	14	28	42	56	70	
	T1	0.03±0.01	$0.9 \pm 0.1^{a}$	2.0±0.4 <sup>a</sup>	4.2±1.1ª	8.9±2.8ª	11.7±2.7ª	
Weight	T2		$0.9 \pm 0.1^{a}$	2.0±0.6 <sup>a</sup>	4.1±0.9 <sup>a</sup>	9.7±2.0 <sup>a</sup>	11.3±2.5ª	
(g)	Т3		$1.0 \pm 0.1^{a}$	2.1±0.6 <sup>a</sup>	4.1±1.2ª	9.7±2.6ª	11.8±3.3ª	
	T4		0.9±0.1ª	2.1±0.6 <sup>a</sup>	4.2±1.2ª	9.3±2.9ª	11.1±3.9ª	
	T1		24.9±0.8 <sup>a</sup>	$14.9 \pm 0.8^{a}$	11.7±0.7ª	10.1±0.6ª	8.5±0.3ª	
SGR	T2		24.8±0.7ª	14.9±1.2ª	$11.7 \pm 0.6^{a}$	10.3±0.4ª	8.4±0.3ª	
(%/day)	Т3		25.2±0.9 <sup>a</sup>	15.1±1.1ª	11.6±0.7ª	10.2±0.6ª	8.5±0.4ª	
	T4		24.7±0.8 <sup>a</sup>	15.1±1.1ª	11.7±0.7ª	$10.1 \pm 0.6^{a}$	8.4±0.5ª	
	T1		0.07±0.01ª	0.07±0.02 <sup>a</sup>	0.10±0.03ª	0.16±0.05ª	0.17±0.04 <sup>a</sup>	
DWG	T2		0.07±0.01ª	0.07±0.02 <sup>a</sup>	0.10±0.02 <sup>a</sup>	0.17±0.03ª	$0.16 \pm 0.04^{a}$	
(g day⁻¹)	Т3		0.07±0.01ª	0.08±0.02 <sup>a</sup>	0.10±0.03ª	0.17±0.05ª	0.17±0.05ª	
	T4		0.07±0.01ª	0.07±0.02ª	0.10±0.03ª	0.17±0.05ª	$0.16 \pm 0.06^{a}$	

Growth in the weight of shrimp

The values with different superscript letters in a column are significantly different (p < 0.05). Data are presented as mean±standard deviation.

The survival rate of white-leg shrimp in the treatments ranged from 34.7 to 88.0%. The highest survival rate was in the control treatment (T1) with 88.0%, followed by T2 with 66.7% and T3 with 58.0%, and T4 had the lowest survival rate with 34.7%. The survival rate between treatments was a statistically significant difference (p<0.05) at the end of the experiment (Figure 6).

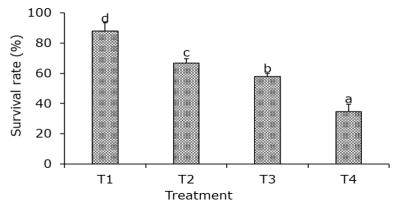


Figure 6. Survival rate (%) of shrimp in the experiment. The values with different superscript letters in a column are significantly different (p<0.05). Data are presented as mean±standard deviation.

The average feed conversion ratio (FCR) of shrimp in the treatments varied from 1.3 to 2.3. Of which, the highest FCR index in T4 was  $2.3\pm0.20$ , statistically significantly different (p<0.05) compared to the other treatments. However, the FCR index in T2 was not statistically significantly different (p>0.05) compared to T3 (Figure 7). Similarly, the average FER index of the treatments ranged from 0.44 to 0.79. The average FER index in T1 was the highest at  $0.79\pm0.07$  and statistically significantly different (p<0.05) compared to the other treatments. In which, the FER index in T2 was not statistically significant difference (p>0.05) compared to T3. The lowest FER coefficient was recorded at T4 with 0.44±0.04 (Figure 7).

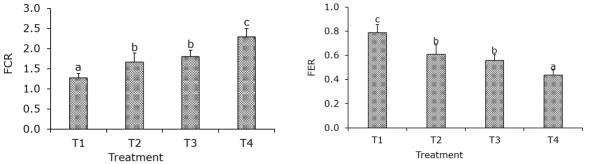


Figure 7. FCR and FER of treatments in the experiment. The values with different superscript letters in a column are significantly different (p<0.05). Data are presented as mean±standard deviation.

**Discussion**. Temperatures from 23 to 30°C were suitable for the growth of whiteleg shrimp, but around 26 to 33°C was considered their appropriate temperature (Abdelrahman et al 2019). Moreover, water temperature in *L. vannamei* shrimp ponds at different places of Andhra Pradesh, India ranged from 25 to 32°C (Bharathi et al 2017). According to the study of Hai et al (2017), the suitable pH range for whiteleg shrimp farming was from 7.5 to 8.5. The average pH value of all treatments in this experiment varied from  $7.60\pm0.2$  to  $7.83\pm0.20$ . Because the experiment was conducted in a covered experimental farm, pH did not vary greatly between treatments. The water pH was 7.4 to 7.9 in shrimp farms in the salinity-prone areas of Bangladesh (Rahman et al 2015). In addition, the optimal pH range for whiteleg shrimp farming is 7.5 - 8.5, with a fluctuation range of 0.5 (Reddy & Mounika 2018). The DO content in the whiteleg shrimp tanks reached a very high value through the sampling periods because the shrimp tanks were equipped with a continuous aeration system, so the DO content was always maintained stable, and there was an insignificant difference (p>0.05) between the treatments. The DO value in the treatments reached the regulation value according to MARD (2014) (DO  $\geq$ 3.5 mg L<sup>-1</sup>) and was desirable for shrimp development. According to Boyd & Green (2002), the dissolved oxygen content for brackish water shrimp farming was around 5.0-6.0 mg  $L^{-1}$ . Additionally, MST (2023) stated that the limited value of alkalinity for the

growth and development of whiteleg shrimp was from 100 to 200 mg  $CaCO_3 L^{-1}$ . In addition, Boyd & Green (2002) also stated that the favorable alkalinity for shrimp progress was from 80 to 160 mg  $CaCO_3 L^{-1}$ . Besides, Tao et al (2021) revealed that alkalinity tended to decrease towards the end of the experiment in larval and postlarval whiteleg shrimp tanks that applied biofloc technology, and the optimal alkalinity range for whiteleg shrimp growth and development was 140-160 mg  $L^{-1} CaCO_3$  (Tao et al 2015). In general, temperature, pH, DO, and alkalinity in this study were within the suitable range for shrimp production.

According to Reddy & Mounika (2018), the optimum level of TAN for shrimp farming ponds was lower than 1 mg L<sup>-1</sup>. In general, the TAN concentration in all treatments was suitable for shrimp development, except for the 63rd day when the TAN concentration was higher than 2 mg  $L^{-1}$  in all treatments, which can be detrimental to shrimp. The mean nitrite level was  $4.70\pm0.58$  mg L<sup>-1</sup> in intensive whiteleg shrimp ponds in Ba Ria-Vung Tau province, the southeast delta of Vietnam (Dung et al 2024). Furthermore, research by Chen & Chin (1988) showed that the acceptable concentration of NO<sub>2</sub><sup>-</sup> for brackish shrimp was <4.5 mg L<sup>-1</sup>. The standard optimum of TAN and NO<sub>2</sub><sup>-</sup> concentrations, according to Edhy et al (2010) was 0.10 mg  $L^{-1}$  and 0.2 mg  $L^{-1}$ , respectively. Nitrite is toxic to shrimp, and exposure to high concentrations may cause retarded growth and mortalities. Exposing shrimp to a nitrite concentration of 4 mg/L for 2 d reduced their growth but did not affect their survival (Gross el al 2024). Thus, the  $NO_2^{-}$  level in the treatments was within the allowable limit for shrimp growth and did not cause adverse effects on shrimp health, except on days 35 and 63 of the experiment. The results obtained in a study by Furtado et al (2015) indicated that concentrations of  $NO_3^$ up to 177 mg  $L^{-1}$  were acceptable for the rearing of *L. vannamei* in systems with bio flocs, without renewal of water, at a salinity of 23 ppt. Nitrate levels in the superintensive whiteleg shrimp culture tanks in Bac Lieu city, Bac Lieu province, Vietnam changed from  $0.5\pm0.2$  to  $1.6\pm0.2$  mg L<sup>-1</sup> (Lien et al 2023). Additionally, shrimp can survive nitrate levels as high as 200 ppm, but it is not known if levels this high affect growth or disease resistance. Ideally, nitrate levels should be maintained at less than 60 mg  $L^{-1}$  (Van Wyk & Scarpa 1999), and from 0.2 to 10 mg  $L^{-1}$  was the desired level for shrimp culture ponds (Whetstone et al 2000). Besides, Mulis & Habibie (2022) reported that nitrate content was recorded in the *L. vannamei* ponds on the coast of Tomini Bay, Mootilango Village, Gorontalo, Indonesia, and was lower than 1 mg  $L^{-1}$ . The present study showed that the NO<sub>3</sub><sup>-</sup> concentration in the whiteleg shrimp tanks in all treatments tended to increase high at the close of the experiment. The reason is that the decomposition of blue-green algae after being added to shrimp tanks increased the nitrate content. Research by Lien et al (2023) revealed that blue-green algae abundance in intensive shrimp ponds was positively correlated with NO<sub>3</sub>- concentration. High nitrate concentration will cause eutrophication. Nitrate contents in this study were within acceptable values for shrimp mature progress. The mean  $PO_4^{3-}$  value in intensive shrimp ponds was 2.48 $\pm$ 0.20 mg L<sup>-1</sup> (Dung et al 2024). Moreover, Lien et al (2022) reported that mean  $PO_4^{3-}$  values in the super-intensive whiteleg shrimp culture tanks varied from  $0.1\pm0.01$  to  $0.3\pm0.02$  mg L<sup>-1</sup>. According to SCEM (2024), the limit value of PO<sub>4</sub><sup>3-</sup> element in the black tiger shrimp and whiteleg shrimp ponds was less than 0.15 mg L<sup>-1</sup>. The PO4<sup>3-</sup> concentration in all treatments in this study exceeded the allowable threshold, which proved that shrimp tanks had high nutrient contents. The factor of TN in the whiteleg shrimp fluctuated in the range of 2.289-2.993 mg  $L^{-1}$  (Lien & Giao 2020). Moreover, the content of TN in the water in the shrimp ponds in the coastal province of Quang Tri was from 10.25 to 18.42 mg L<sup>-1</sup> (Giang & Quyen 2018). The TN content in the shrimp tanks of this research was similar to the studies above. The analytical results showed that the total phosphorus in the super-intensive shrimp pond fluctuated between the survey locations, reaching from 0.34 to 6.12 mg  $L^{-1}$ , and the average was 1.27 mg  $L^{-1}$  (Giao 2021). Most of the shrimp tanks in the experiment had high nutrient content, especially after 35 days of the experiment.

Chlorophyll-a content can be used to assess the nutritional level and biomass of algae, so the variation of chlorophyll-a content is related to the change of algal quantity and nutrient content in water. According to Boyd (1990), the suitable chlorophyll-a

content in shrimp ranged from 50 to 200  $\mu$ g L<sup>-1</sup>. In this study, the chlorophyll-a level in all treatments mostly exceeded the appropriate threshold due to the high contents of  $NO_{3}$ ,  $PO_{4}^{3}$ , TN, and TP, providing nutrient resources for algae to grow strongly in shrimp tanks. Hoogenhout & Amesz (1965) reported that the growth rate of blue-green algae was always slower than that of other groups of algae, so Cyanobacteria usually predominate at the end of the culture cycle. The addition of blue-green algae at the end of the culture cycle affected the water environment parameters. The parameters of  $NO_2$ and TAN tended to decrease, while the factors of TN, TP,  $NO_3^-$  and  $PO_4^{3-}$  tended to increase. The NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, TN, and TP contents in the treatments supplemented with bluegreen algae rose at the 70-day stage compared to the 63-day stage as a result of the additional blue-green algae breakdown. Furthermore, waste products from shrimp and extra feed were among the factors that contributed to the increase in these parameters at the close of the study. The findings by Lien et al (2023) recorded that the nutrient contents in the shrimp ponds, including TAN,  $NO_3^-$ , and  $PO_4^{3-}$  concentrations, had a significant positive correlation (p<0.05) with the blue-green algae *M. aeruginosa*. According to Patrick (1965), blue-green algae (Oscillatoria) were highly tolerant to pollution and were, therefore, a strong indicator of eutrophication. In addition, Humm & Wicks (1980) suggested that blue-green algae can thrive in both fresh, brackish, and salt water, but their common habitats are large lakes and saltwater environments, so bluegreen algae can still thrive when salinity increases. This is a toxic algae group for aquatic life if it grows at too high a density, which is often recorded with blue-green algae such as Oscillatoria and Phormidium. When they developed at high density, toxins were produced from these species that can affect the growth of shrimp. A high abundance of blue-green algae indicated that the water environment in shrimp ponds was rich in nutrients (Palmer 1969). Furthermore, the toxins produced by blue-green algae species could degrade water quality (Okechukwu 2009). When blue-green algae were added at different densities, the survival rate of shrimp was significantly different (p < 0.05) between treatments. This showed that blue-green algae had a great influence on the survival rate of shrimp. The survival rate of shrimp decreased rapidly when blue-green algae were added at high densities. High blue-green algae abundance will make the shrimp smell awful and block the shrimp's carapace because algae produce mucus in the cell membrane. Shrimp may get white feces as a result of cyanophytes because algae in the shrimp intestinal tract are not digested. Both filamentous and granular algae are poisonous. Nonetheless, the filamentous form is frequently thought to be more harmful since it is easier to obstruct the carapace of shrimp and more difficult to digest if consumed by shrimp. The presence of filamentous blue-green algae (Oscillatoria brevis) reduced the survival rate of shrimp. The lowest survival rate was recorded when the abundance of blue-green algae O. brevis varied between 1,000 and 10,000 ind.  $L^{-1}$ . Tests of dead shrimps revealed that O. brevis was also present in the shrimp's digestive system, blocking the gills and adhering to the head and mouth parts (Massaut & Orti 2003). In addition, when algae bloom in the pond, it can cause partial hypoxia at night, leading to hypoxia in the blood, and causing mass mortality of shrimp (Alonso-Rodríguez & Páez-Osuna 2003). According to Smith (1996), the toxicity of the blooms of Oscillatoriales was the primary cause of prawn disease. Pond water and extracts from a tank culture of benthic Oscillatoriales caused mortalities when injected into P. monodon and P. japonicus. It is proposed that sub-lethal levels of toxin weaken the prawns, causing reduced feeding behavior and an impaired immune system. In addition, the cyanobacteria Limnothrix strain LmTK01 showed a negative direct and indirect effect on the shrimp survival rate and production. It was found that shrimp died due to the molting of shrimp and the filament of algae attached to the appendages, gills, and digestive tract (Maa-iad et al 2023).

The survival rate was significantly affected and correlated with FCR and FER values, thereby causing a sharp decrease in shrimp productivity. FCR in the treatment supplemented with the highest density of blue-green algae had higher values and was significantly different from the remaining treatments. However, the opposite trend was observed for FER. The FCR in the treatment supplemented with the highest density of algae had a higher value and was significantly different compared to the control

treatment. On the contrary, the FER in the control treatment was the highest and was significantly different from the treatment supplemented with harmful algae. According to Mustafa et al (2024), FCR changed from 1.44 to 1.59 in intensive brackish whiteleg shrimp ponds. The shrimp productivity of the treatments recorded was  $1.98\pm0.21$  kg m<sup>-3</sup> in T1; 1.52±0.19 kg m<sup>-3</sup> in T2; 1.28±0.13 kg m<sup>-3</sup> in T3; and 0.90±0.08 kg m<sup>-3</sup> in T4, respectively. Because the survival rate of shrimp was recorded to be lower in the treatments supplemented with higher densities of blue-green algae, the shrimp productivity achieved also had a similar trend. The research by Suwoyo & Hendrajat (2021) stated that the final weight of vaname shrimp in a controlled tank in three different stocking densities, 100, 200, and 300 ind. m<sup>-3</sup> was 13.70 g ind.<sup>-1</sup>, 12.27 g ind.<sup>-</sup> <sup>1</sup>, and 10.90 g ind.<sup>-1</sup>, respectively. In this study, the survival rate and production of shrimp ranged from 80-95% and 1.24-2.42 kg m<sup>-3</sup>, respectively. Shrimp productivity in super-intensive whiteleg shrimp farming tanks studied by Lien et al (2022) was higher than that in the current study, ranging from 2.31-2.67 kg m<sup>-2</sup>. Therefore, the investment cost was higher in treatments with higher blue-green algae density, thereby affecting the economic efficiency of the farming model. Cyanobacteria Limnothrix LmTK01 showed a significantly bad effect on the survival rate of shrimp, resulting in loss of shrimp production (Maa-iad et al 2023). Additionally, blue-green algae and dinoflagellates also increased in abundance at the end of the cycle, which can affect shrimp growth (Lien et al 2023). In general, the growth in length and weight of shrimp did not differ significantly among treatments. However, the survival rate of shrimp in treatments supplemented with blue-green algae decreased significantly. The higher the added density of algae in treatments, the lower the survival rate of shrimp was recorded, thereby affecting the FCR and FER indices.

**Conclusions**. The water environment parameters in the experiment were within the appropriate range for shrimp growth, except for  $NO_2^-$ ,  $PO_4^{3-}$ , and chlorophyll-a, which exceeded the allowable threshold at certain sampling stages. The addition of blue-green algae at the end of the culture cycle did not significantly affect shrimp length and weight. However, the survival rate of shrimp decreased significantly when adding *Planktothrix pseudagardhii* algae to shrimp tanks at higher densities. The greater the abundance of filamentous blue-green algae, the more they attach to the shrimp's gills and restrict its respiratory system, which is one of the main reasons for the shrimp's lower survival rate. Therefore, during the shrimp culture process, it is necessary to implement measures to limit the increase in nutrient levels to prevent the development of blue-green algae at the close of the culture cycle. Cyanobacterial toxin research is required to discover the types of toxins produced by harmful algal groups to develop techniques to control the spread of blue-green algae in brackish water shrimp ponds.

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**Conflict of interest**. The authors declare no conflict of interest.

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