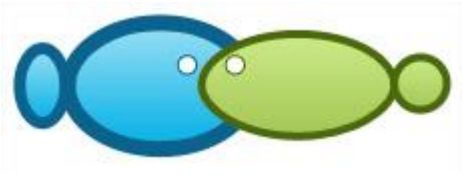

Effect of aeration on wild algae development in effluent from intensive whiteleg shrimp (*Penaeus vannamei*) farming

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Abstract. This study investigated the effects of aeration on wild algae growth in effluent produced from the intensive farming of whiteleg shrimp *Penaeus vannamei* (Boone, 1931). The analyzed effluent had been released following 54 days of shrimp culture, wherein shrimp were fed with pellets (30% N) at a feeding rate of 4-5% of total shrimp biomass per day. The experiment consisted of four treatments (NT1: no aeration (control); NT2: 12-h nighttime aeration; NT3: 12-h daytime aeration; NT4: 24-h (full-day) aeration) in a completely randomized design, with three replicates each. Wild algae development was continuously monitored over a 10-day period. Eleven genera of algae were detected across all the treatments, mostly comprising green algae (27.7 %) and Ochrophyta (57.9 %), of which *Nannochloropsis* sp. and *Chlorella* sp. represented 71.2% and 26.17%, respectively. Although treatments NT2, NT3, and NT4 differed in their algal density, these differences were not statistically significant ($p > 0.05$) from day 3 to day 6. However, significant differences ($p < 0.05$) in algal density were observed between NT1 and treatments NT3 and NT4. Overall, the highest density of algal growth and highest algal abundance over time occurred in treatment NT3, where the effluent had been aerated for 12 h per day during daytime hours.
Keywords: algal density, shrimp effluent, whiteleg shrimp, wild algae.

Introduction. The effluent produced in the intensive farming of whiteleg shrimp *Penaeus vannamei* (Boone, 1931) primarily consists of components including nutrients, organic matter, suspended solids, and microbial organisms, which arise from the metabolic activities of shrimp, uneaten feed, and decomposition processes within the culture environment. These effluents typically contain elevated levels of nitrogen and phosphorus, which, if unregulated, can lead to eutrophication - an over-enrichment of water bodies that triggers harmful algal blooms and disrupts aquatic ecosystems (Ding et al 2020; Sakomoto et al 2020). The negative impacts of whiteleg shrimp effluents have been well documented (Chang et al 2019; Ramos-Sotelo et al 2021), with research indicating that intensive culture of this species usually generates effluents rich in nutrients such as nitrogen and phosphorus, primarily in the form of ammonia and organic matter, which may cause eutrophication in receiving waters if not treated appropriately. Notably, an estimated 35% of nitrogen and 10% of phosphorus introduced into shrimp ponds can be exported through effluents during water exchanges and drainage activities (Priyadarsani & Abraham 2016; Hargan et al 2020) since the effluent is notably high in nitrogenous compounds that primarily present as total ammonia nitrogen (TAN), nitrite (NO_2^-), and nitrate (NO_3^-). Moreover, the concentrations of these compounds can vary significantly, with TAN levels averaging approximately 0.9 ppm, while nitrite concentrations are typically lower, and nitrates can be elevated due to the breakdown of organic matter (Priyadarsani & Abraham 2016; Qin et al 2021). These nutrient loads have been shown to stimulate algal growth. Additionally, such effluent contains significant phosphate (PO_4^{3-}) levels, which coincide with increases in organic matter derived from uneaten feed and shrimp excreta (Chowdhury et al 2011; Barraza-Guardado et al 2013). These phosphates contribute to nutrient richness in the water, providing benefits for certain types of aquatic organisms while potentially causing harm to others through the facilitation of algal blooms.

There are several methods of water treatment for shrimp pond effluent, one of which utilizes microalgal species as a resourceful bioremediation strategy. For example, the microalga *Chlorella vulgaris* has demonstrated significant efficacy in nutrient uptake from aquaculture wastewater, effectively removing nitrogen and phosphorus while contributing to biomass that can be repurposed as aquaculture feed or biofuels (Sakomoto et al 2020). Additionally, the genus *Ulva* has been noted for its heightened protein content when cultivated in aquaculture effluents, showcasing its potential as a high-value feed source in multitrophic systems (Favot et al 2019; Henares et al 2020). Diverse strains of microalgae have been found to thrive in shrimp effluents; several species, such as *Nannochloropsis* sp., *C. vulgaris*, and *Arthrospira platensis*, have also shown promising outcomes in nutrient absorption dynamics when grown in these conditions (Galindro et al 2016; Lopes et al 2021; Santanumurti et al 2022). Simultaneously, *Nannochloropsis* sp., *Tetraselmis* sp., and *Dunaliella* sp. can thrive in wastewater derived from whiteleg shrimp culture, facilitating nutrient removal and biomass production (Santanumurti et al 2022). It has also been reported that the biomass produced from these microalgal cultures can serve as a high-quality feed source for shrimp, leading to improved growth performance and enhanced resistance to diseases such as *Vibrio parahaemolyticus* (González-Meza et al 2022). Therefore, enhancement with the presence of wild algae in the wastewater treatment systems of whiteleg shrimp ponds is necessary to increase the efficiency of wastewater treatment and mitigate negative environmental impacts. Due to the nutrients available in shrimp effluent, moderate aeration can prolong favorable conditions for phytoplankton development (Rybak 1985). Furthermore, Johnston & Raven (1992) found that the rate of aeration significantly influences the growth rates of various algal species, underscoring the need for tailored aeration strategies that meet the specific physiological requirements of algae in shrimp farming. The specific focus on this study is to determine how different aeration rates influence algal growth dynamics and nutrient removal. The scope encompasses the treatment of nutrient-rich wastewater (specifically targeting nitrogenous and phosphorous compounds) to mitigate eutrophication risks, while simultaneously exploring the production of valuable algal biomass.

Material and Method

Experimental design. The experiment was conducted from January to March 2023 at the Artemia Experimental Farm - Vinh Chau - Soc Trang – College of Aquaculture and Fisheries - Can Tho University. The experiment was conducted indoors using a 50-liter tank. It included four treatments: NT1: no aeration (control); NT2: 12-h nighttime aeration; NT3: 12-h daytime aeration; NT4: 24-h (full-day) aeration. Treatments were conducted over a 10-day period, and the same effluent source was used for all treatments. The experimental design was completely randomized, with three replicates per treatment. The effluent used in the study was collected from a 54-day shrimp pond with a salinity of 23‰ and stocking density of 200 shrimp m^{-3}. The shrimp in the pond had been fed with pellets (30% protein) at a feeding rate of 4-5% per day according to the shrimps' body mass. Additionally, the shrimp pond operated with a water recirculation system, with a water exchange of 50-75% per day. The experiment was conducted outdoors under similar conditions to those of commercial shrimp ponds.

Water quality analysis. Water temperature, pH, and dissolved oxygen (DO) were monitored in situ twice per day at 7 am and 2 pm, and salinity was monitored after water exchange, while light intensity (in lux) was measured at 7 am, 10 am, 1 pm, and 4 pm daily. The parameters were checked using a pH and temperature meter (Hanna Instruments pH and temperature tester HI98128, Italy), a portable digital DO meter (Yieryi Oxygen Meter), a refractometer (Atago MASTER-S/Millα 2491, Japan), and a light meter (Testo 545 digital lux meter, Germany), respectively. Beside, turbidity/water transparency was determined using Secchi disks (Carlson 1977). Samples were taken from the experimental tanks on days 1, 4, 7, and 10 to monitor NH_4^+, NO_3^-, and PO_4^{3-} concentrations, which were measured using a multi-spectrophotometer (Hanna Instruments aquaculture photometer HI83303, Italy).

Algae sampling and preservation. For qualitative sampling, a 40-µm plankton net was used to collect algae samples. The net was placed approximately 10-15 cm below the surface of the water, with a 5 L volume of water passing through the filter. After collection, each sample was preserved in a 180-mL bottle and fixed with 2-4% formalin. For quantitative sampling, algae were collected using the sedimentation method, with a 180-mL glass being used to collect water from different points in the tank. The samples were mixed well, and then a quantitative sample was taken, placed into a plastic bottle, and fixed with 2-4% formalin.

Algae identification. Algae identification was performed according to Shaari et al (2011). Algae were preserved in Lugol's solution, examined under a light microscope (Nikon Eclipse E200, 40x magnification), and identified based on the morphological characteristics used in Shirota (1966). Algal density was counted using a Sedgwick–Rafter counting chamber according to the following equation from Boyd & Tucker (1992):

$$X = \frac{T \text{ x} 1000 \text{ x } V\, condensed \text{ x } 1000}{A \text{ x } N \text{ x } V\, sampled}$$

where: X is the density of algae (individuals L^{-1}), T is the number of individuals counted per phylum, $V_{condensed}$ is the volume of the condensed sample (mL), A is the area of one counting cell (1 mm^2), N is the number of counted cells (180 cells), and $V_{sampled}$ is the volume of sample collected through the filter (mL). The appearance frequency was based on Scheffer & Robinson (1939). Algae were marked as "high density" (+++) at a frequency of > 60%, "medium density" (++) at 30-60%, and "low density" (+) at < 30%.

Statistical analyses. The means and standard deviations of the data were calculated using Microsoft Excel, and the data were analyzed using a one-way ANOVA. A significance level of 0.05 was used, followed by a confirmation of normality and homogeneity of variance. Additionally, Duncan's post hoc procedures were performed to detect differences between the groups for multiple comparisons. All statistical tests were performed in SPSS Statistics version 20.

Results

Physical parameters. The temperature ranged between 24.6 and 25.1°C in the morning, and between 31.9 and 32.0°C in the afternoon (Table 1). The average pH among treatments ranged from 7.6 to 7.8 in the morning and from 8.5 to 8.7 in the afternoon (Table 1), which is considered suitable for algal growth.

Table 1

Fluctuation of temperature, pH, and DO throughout the experiment

Treatments	*Temperature (°C)*		*pH*		*DO (mg L^{-1})*	
	7 am	*2 pm*	*7 am*	*2 pm*	*7 am*	*2 pm*
NT1	25.1±0.18	32.0±1.2	7.6±0.18	8.5±0.27	5.3±0.11	8.2±0.13
NT2	24.7±0.64	32.0±1.3	7.7±0.20	8.6±0.30	5.4±0.08	8.3±0.10
NT3	24.6±0.47	32.0±1.3	7.7±0.14	8.6±0.31	5.6±0.08	8.5±0.12
NT4	24.8±1.57	31.9±1.2	7.8±0.14	8.7±0.23	5.6±0.07	8.6±0.13

The average DO remained stable among treatments, except for NT 1, which exhibited lower DO levels when compared to the other treatments. Simultaneously, due to the lack of aeration (i.e., since there was no water disturbance), the water temperature in NT1 was slightly higher than that of the other treatments; However, the salinity in this treatment was similar to the other treatments, resulting in its lower DO level.

However, all treatments had similar salinity (i.e., 23‰) throughout the first three days, during which time the experiment was conducted indoors. Thereafter, the salinity increased toward the end of the experiment (to 26‰) due to evaporation; however, no significant differences ($p > 0.05$) in salinity were observed among treatments.

Turbidity and light intensity. Figure 1 presents the water transparency among treatments, which ranged from 17 to 30 cm. Although turbidity levels differed slightly between treatments due to different aeration regimes, they showed a general trend of gradually algae rising until the seventh day and then algae declining toward the end of the experiment. This turbidity pattern reflects the development cycles and proliferation of algae; when algae grow quickly, the water turbidity was increased, while at low levels of algae proliferation, the water becomes clearer and the turbidity was decreased.

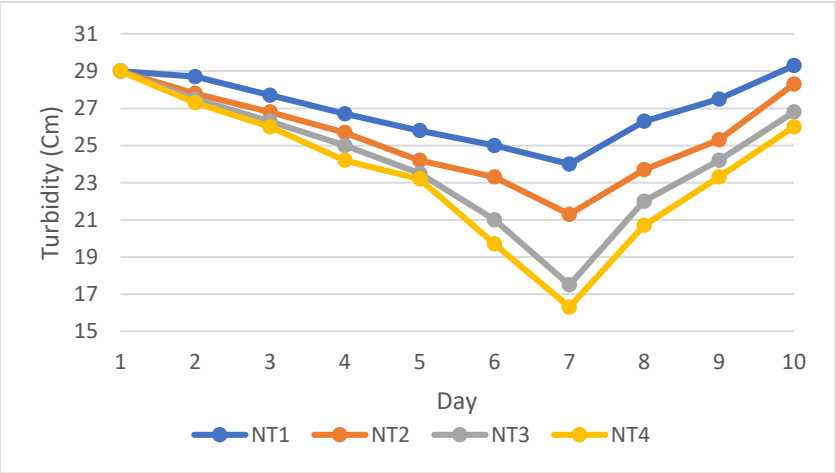

Figure 1. Fluctuation in turbidity among treatments.

The average light intensity measured during the experiment ranged from 9,773 to 14,954 lx and varied slightly over the experimental period (Figure 2). Light intensity increased toward the end of the experiment, which coincided with the natural sunlight intensity in the area where the experiment was performed.

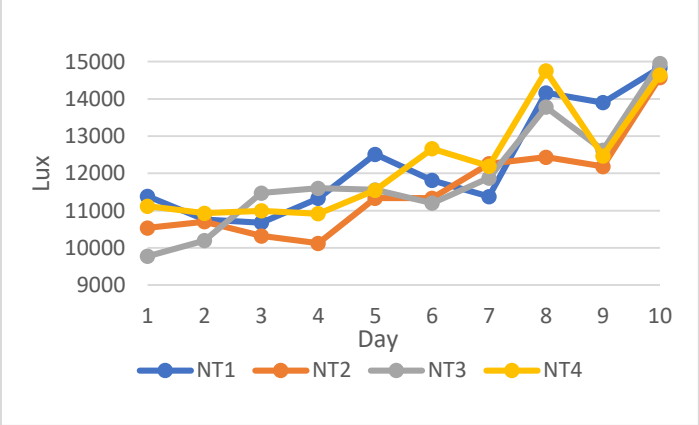

Figure 2. Variation in light intensity among treatments.

Chemical parameters. Ammonium (NH_4^+) concentrations decreased across all treatments during the experiment (Figure 3, Table 2). There was no significant difference ($p > 0.05$) in NH_4^+ among the treatments in the first four days. However, on day 7, the NH_4^+ levels of NT1 and NT2 were found to be significantly different ($p < 0.05$) from those of NT3 and NT4. Despite this, on day 10, no significant difference ($p > 0.05$) in NH_4^+ was detected among treatments, although NH_4^+ had declined toward the end of the experiment across all treatments. The observed decrease in NH_4^+ implied that it had been consumed by wild algae proliferating in the treatment environments.

Similarly, NO_3^- content decreased significantly within the initial four days across all treatments, with significant differences ($p < 0.05$) detected among treatments. However, a slow decrease in NO_3^- occurred in the control, while sharper decreases were observed in the other treatments - the longer the aeration (NT4), the lower the NO_3^- recorded. Additionally, although the aeration duration was the same (i.e., 12 h), daytime aeration

(NT3) showed a stronger decrease in NO_3^- content ($p < 0.05$) when compared to nighttime aeration (NT2). This can be attributed to the rapid increase in algal density since NO_3^- is essential for its growth and development.

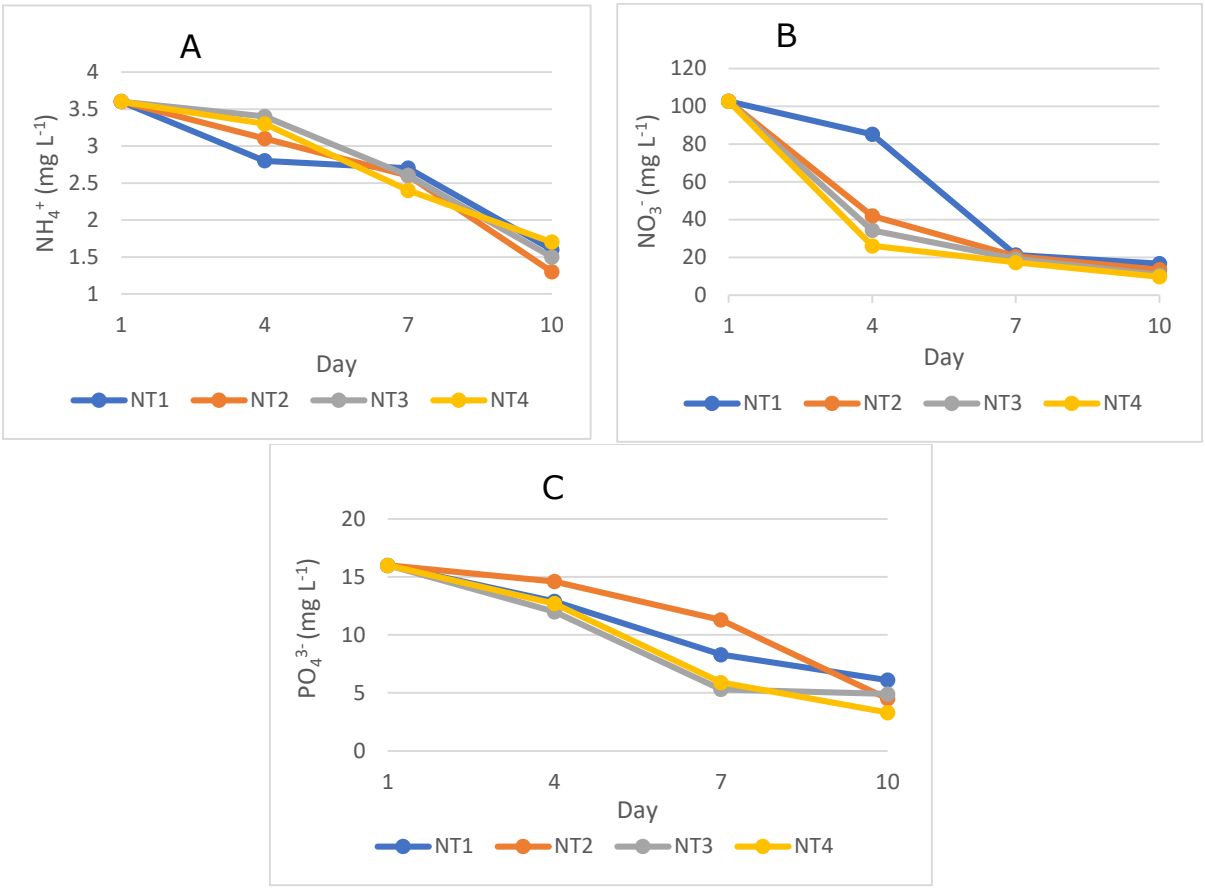

Figure 3. Differences in nitrogen and phosphorus among treatments (A: NH_4^+, B: NO_3^-; C: PO_4^{3-}).

The PO_4^{3-} content (Table 2) exhibited a decreasing trend throughout the experimental period, with values ranging from 3.3 to 16 mg L^{-1}. Although there was no significant difference ($p > 0.05$) among the treatments, PO_4^{3-} content tended to be lower in NT3 than in NT2. Additionally, NT4 had the lowest PO_4^{3-} content when compared to the other treatments on the last day of the experiment (day 10).

Table 2

Variation in NH_4^+, NO_3^-, and PO_4^{3-} (mg L^{-1})

Parameters	*Treatment*	*Day 1*	*Day 4*	*Day 7*	*Day 10*
NH_4^+	NT1	3.6±0.0^a	2.8±0.2^a	2.7±0.4^a	1.6±0.2^a
	NT2	3.6±0.0^a	3.1±0.1^a	2.6±0.3^a	1.3±0.3^a
	NT3	3.6±0.0^a	3.4±0.3^a	2.6±0.2^b	1.5±0.3^a
	NT4	3.6±0.0^a	3.3±0.1^a	2.4±0.3^b	1.7±0.5^a
NO_3^-	NT1	102.7±0.0^a	85.2±0.5^a	21.3±0.2^a	16.7±0.2^a
	NT2	102.7±0.0^a	42±0.2^b	34.3±0.2^a	26.1±0.8^b
	NT3	102.7±0.0^a	34.3±0.2^c	19.2±0.5^a	10.7±0.3^c
	NT4	107.7±0.0^a	26.1±0.7^d	17.7±0.3^a	9.7±0.5^c
PO_4^{3-}	NT1	16±0.0^a	12.9±0.4^a	8.3±0.1^a	6.1±0.2^a
	NT2	16±0.0^a	14.6±0.9^a	11.3±0.3^a	4.5±0.7^a
	NT3	16±0.0^a	12±0.3^a	5.3±0.8^a	4.9±0.2^a
	NT4	16±0.0^a	12.7±0.3^a	5.9±0.3^a	3.3±0.1^a

Values in the same column with different letters (a, b, c) indicate significant differences ($p < 0.05$).

Algal composition and abundance. Qualitative analysis identified 11 algal genera belonging to five phyla across all treatments: Chlorophyta (three species), Cyanobacteria (four species), Bacillariophyta (one species), Charophyta (two species), and Ochrophyta (one species) (Table 3).

Table 3

Algal species recorded throughout the experiment

No.	Treatment			
	NT1	NT2	NT3	NT4
			Chlorophyta	
1	*Chlamydomonas* sp.	*Chlamydomonas* sp.	*Chlamydomonas* sp.	*Chlamydomonas* sp.
2	*Chlorella* sp.	*Chlorella* sp.	*Chlorella* sp.	*Chlorella* sp.
3	*Oocystis* sp.	*Oocystis* sp.	*Oocystis* sp.	*Oocystis* sp.
			Cyanobacteria	
4	*Anabaena*	*Anabaena*	*Anabaena*	*Anabaena*
5	*Oscillatoria* sp.	*Oscillatoria* sp.	*Oscillatoria* sp.	*Oscillatoria* sp.
6	*Pseudanabaena* sp.	*Pseudanabaena* sp.	*Pseudanabaena* sp.	*Pseudanabaena* sp.
7	*Trichodesmium* sp.	*Trichodesmium* sp.	*Trichodesmium* sp.	*Trichodesmium* sp.
			Bacillariophyta	
8	*Thalassiosira* sp.	*Thalassiosira* sp.	*Thalassiosira* sp.	*Thalassiosira* sp.
			Charophyta	
9	*Closterium* sp.	*Closterium* sp.	*Closterium* sp.	*Closterium* sp.
10				*Cosmarium* sp.
			Ochrophyta	
11	*Nannochloropsis* sp.	*Nannochloropsis* sp.	*Nannochloropsis* sp.	*Nannochloropsis* sp.

The average algal density of NT2, NT3, and NT3 exhibited significant fluctuations over time, ranging from 56,524 to 1,392,950 cells mL^{-1}. These fluctuations exhibited a similar pattern, with maximal densities recorded at day 7, followed by a decline toward the end of the experiment (i.e., day 10; Figure 4, Table 4). Notably, the non-aeration treatment (NT1, control) exhibited the same trend as the others, but with less variation; its average algal density varied between 56,524 and 624,983 cells mL^{-1}. The control (NT1) exhibited a maximum algal density on day 7 that was only 60% of that observed in the 12-hour nighttime-aeration treatment (NT2), and 50% of that recorded for both the 12-hour day aeration (NT3) and full-day aeration (NT4) treatments. Additionally, NT3 exhibited an algal growth increase up to 36% greater than that of the nighttime-aeration treatment (NT2) despite having the same aeration duration. Therefore, daytime aeration was observed to promote algal growth to a level comparable to that of full-day aeration ($p > 0.05$), indicating that extended aeration at night may not be essential. The non-aeration treatment (NT1, control) exhibited a statistically significant difference ($p < 0.05$) (Table 4) in algal density when compared to the other treatments from day 2 afterwards. From day 3 to day 9, there was no statistically significant difference ($p > 0.05$) between NT3 and NT4 in algal density, while from day 7 to day 10, there were notable differences ($p < 0.05$) observed among the NT1 (control), NT2, NT3, and NT4 treatments. Table 4 indicates that treatments involving aeration resulted in better algal development when compared to the control. The green algae, which exhibited the highest density throughout the experimental period, were predominantly from the genera *Chlorella*, while brown algae presented by *Nannochloropsis* and among others. Subsequent observation indicated that blue-green algae exhibited the second-highest densities, followed by diatoms with the lowest (Figure 5). Species recorded at high density predominantly comprised the genera *Nannochloropsis* (+++), *Oocystis* (+), and *Chlorella* (+), which are classified as brown algae and green algae, respectively.

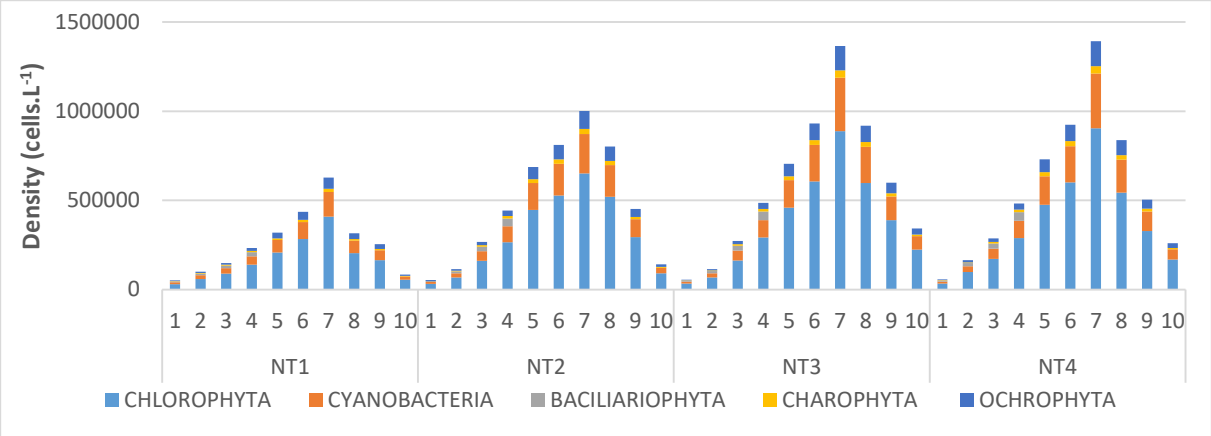

Figure 4. Algal abundance in different treatments.

The aeration of effluent from intensive whiteleg shrimp farming promoted the growth of not only green algae species but also other algal phyla. This suggests that effluent from intensive whiteleg shrimp farming can be regarded as a nutrient-rich medium containing accessible microalgae. Moreover, with aeration, microalgae in the effluent can be maximized (i.e., green-water) without altering nutrient levels, making them suitable as a food source for filter feeders such as *Artemia*, particularly those smaller than 50 µm.

Table 4

Differences in algal density (cells mL^{-1}) throughout the experiment

Day	*Treatment*			
	NT1	*NT2*	*NT3*	*NT4*
1	56,524±0.0^{a}	56,524±0.0^{a}	56,524±0.0^{a}	56,524±0.0^{a}
2	99,481±287^{a}	113,867±475^{b}	115,620±346^{b}	163,000±2,825^{c}
3	149,091±690^{a}	270,283±679^{b}	273,167±275^{b}	286,550±2,623^{b}
4	235,750±15,199^{a}	446,433±12,586^{b}	475,883±9,205^{b}	486,883±4,015^{b}
5	319,717±4,995^{a}	686,983±15,199^{b}	705,317±13,331^{b}	730,883±15,686^{b}
6	420,467±9,505^{a}	810,817±21,324^{b}	931,750±16,904^{b}	924,993±13,972^{b}
7	624,983±4,187^{a}	1,001,600±2,275^{c}	1,366,400±341,383^{b}	1,392,950±415,145^{b}
8	315,567±6,357^{a}	791,150±12,814^{c}	919,117±14,504^{b}	837,800±61,807^{b}
9	250,183±1,052^{a}	452,683±95,211^{c}	603,733±25,967^{b}	503,883±28,962^{b}
10	84,433±1,305^{a}	140,783±2,958^{b}	343,333±4,396^{c}	259,883±1,582^{c}

Note: Values in the same row with different letters (a, b, c) indicate significant differences ($p < 0.05$).

Discussion

Physical and chemical parameters. Most species of microalgae can tolerate temperatures between 16 and 27°C. Temperatures below 16°C will inhibit growth, while those above 35°C will be lethal to some species (Lavens & Sorgeloos 1996). Several studies have demonstrated that tropical marine algae flourish at temperatures between 26 and 34°C (Tseng et al 2018; Wang et al 2024). Additionally, higher nutrient levels can make algal blooms occur more often when the temperature is optimal, which shows that these two factors work together to allow marine algae to grow faster in tropical environments (Hay et al 2011). Consequently, the temperatures recorded in the present study (Table 1) fell within the optimal range for microalgal proliferation.

pH is an important environmental factor that influences aquatic life, and microalgae typically flourish in pH conditions ranging from slightly acidic to alkaline - generally between 7.0 and 9.0, although species-specific variations are present. *Microchloropsis salina*, a common marine microalga, exhibits optimal growth rates at a pH range of 7.5 to 8.0 and has demonstrated efficacy in reducing contaminants in open culture systems (Koruyucu et al 2024). Previous studies have suggested that a pH range of 7.5 to 8.5 is generally optimal for many marine microalgae species, allowing for maximum uptake of nutrients and photosynthetic efficiency (Radhakrishnan et al 2019; Bakky et al 2022). The pH

fluctuations observed in the present study, measured in the range of 7.6 to 8.7, are thus considered suitable for algal development.

The DO content in water is influenced by the water's physical and chemical properties, as well as by the biological activities present. For example, DO levels are influenced by flow disturbances and temperature. Elevated temperatures result in decreased DO, while increased salt concentration leads to an exponential decline in oxygen solubility, which is contingent upon the chloride content in water. The rate of oxygen dissolution in a static water source is influenced by the molecular diffusion process, resulting in a slow rate of dissolution. Keister et al (2000) demonstrated that phytoplankton abundance significantly increased when DO levels exceeded 2.0 mg L^{-1}. Notably, aeration significantly influences the growth rates of various algal species, thus underscoring the need for tailored aeration strategies that meet the specific physiological requirements of algae in shrimp farming (Johnston & Raven 1992). Moreover, the application of aeration not only influences growth rates but also affects the nutritional profiles of the algae produced; for example, Ronda et al (2012) determined that proper aeration supported the production of γ-linolenic acid, a valuable fatty acid for aquafeeds. The DO contents observed in the present study were measured at over 5 mg L^{-1} with or without aeration, which is higher than the optimal range for phytoplankton abundance and thus can be considered suitable for promoting algal development.

The concentration of salts in water, known as salinity, influences several physiological and metabolic functions in microalgae, including lipid synthesis, photosynthesis, and nutrient absorption. Although marine phytoplankton can tolerate variations in salinity, Coutteau (1996) asserted that salinity impacts the spread of algae. The ideal salinity range for algal growth is 20 to 24‰; however, they can thrive in environments with slightly lower salinity than that of their natural habitats. Another study concluded that different microalgal species can have somewhat different optimal salinities, noting that *Chlorella capsulata* and *Skeletonema costatum* prefer moderately saline conditions and that their growth is inhibited at greater salinities (Ebrahimi & Salarzadeh 2016). On the other hand, algae such as *Tetraselmis* sp. showed an increase in their biomass and lipid production at salinities between 18 and 30‰ (Adarme-Vega et al 2014). In the present study, salinity ranged from 23 to 26‰, which is ideal for algal growth.

Turbidity directly interacts with other growth factors such as light penetration and nutrient availability, influencing microalgal growth and biomass output. Light intensity in turbid microalgal cultures diminishes quickly due to suspended particles, which might hinder growth if light levels fall below a critical threshold for photosynthesis (Martínez et al 2018). Therefore, maintaining an ideal turbidity level for marine microalgal development in tropical conditions requires a complete approach that considers light availability, nutrient inputs, and water quality characteristics. In addition to temperature and pH, light influences algal development because it is the primary source of its energy during photosynthesis. Most algal growth processes require approximately one-tenth of direct sunlight (Gao et al 2007; Ma et al 2016). However, the turbidity measured in the present study indicates that the algae thrived under aeration, and algal development in NT3 and NT4 seemed to be similar, while the no-aeration or nighttime-aeration treatments were less effective at supporting algal growth. This finding aligns with research that found air circulation to positively influence the structure and dynamics of phytoplankton communities in treated effluents (Marchello et al 2015).

High NH_4^+ concentrations are correlated with increased phytoplankton density, particularly when cells preferentially utilize NH_4^+ over NO_3^-, as noted by Dugdale et al (2007). According to Varela & Harrison (1999), NH_4^+ is regarded as the preferred nitrogen source for most marine or brackish phytoplankton species. Farahin et al (2021) investigated the growth response of *Tetraselmis tetrathele* to different ammonium concentrations, determining that moderate NH_4^+ levels (0.31 to 0.87 g L^{-1}) enhanced biomass productivity while maintaining photosynthetic efficiency. Another study showed that the growth of microalgae, specifically *Chlorella stigmatophora*, achieves peak rates at approximately 1 mM $NaNO_3$, demonstrating considerable biochemical variability under different nutrient gradients (Fábregas et al 1987). Notably, NO_3^- is the final product of the decomposition of nitrogen-containing organic compounds and serves as a direct nutrient

source for algal development. Nitrogen and phosphorus are essential nutrients that enhance microalgal biomass production and can restrict phytoplankton growth in natural ecosystems (Richmond 2004). Phosphorus serves as an essential macronutrient for marine microalgae, influencing cellular processes such as growth rates, lipid accumulation, and fatty acid composition (García-Márquez et al 2023). Studies demonstrate that phosphorus thresholds are generally necessary for optimal biomass production in specific algal species; for example, phosphorus concentrations exceeding 0.3 mg L^{-1} are typically necessary for the optimal growth of species such as *Nannochloropsis* spp. and *Chaetocero*s spp., which serve important roles in aquaculture environments (Mahata et al 2022). In the present study, the medium was found to be rich in nitrate and phosphorus (Table 2) and thus rapidly increased algal growth up to day 7 in all treatments before declining thereafter. This pattern may have reflected the fact that 1) most of the experimental algae had a 7-day growth cycle and/or 2) the algae died off under suboptimal conditions despite the availability of nitrate and phosphorus after day 7. Moreover, phytoplankton can exhibit saturation behavior in nutrient uptake, so that once they reach maximal growth, their efficiency in using available nitrogen and phosphorus declines, leading to decreased productivity as they enter senescence (Oviatt et al 1995; Marañón et al 2018). Although nitrogen and phosphorus contents did not differ between treatments (Table 2), NT3 and NT4 showed better natural algal growth, which may be related to aeration activity because air circulation positively influences the structure and dynamics of phytoplankton communities in treated effluents (Marchello et al 2015). Given this, it is clear why the algae grew poorly in NT1 and when aeration only occurred at night (NT2): limited light for photosynthesis negatively impacted the algal growth and thus overall density.

Algal composition and abundance. Five algal phyla were previously identified (Lien et al 2023) in Bac Lieu, Vietnam; these are depicted in Figure 5 with their respective salinities. Dinophyta, particularly genera such as *Gonyaulax* and *Gymnodinium*, can proliferate under optimal water conditions, which are characterized by increased nutrient levels, including those of nitrogen and phosphorus, typically derived from shrimp feed and the decomposition of organic matter in the pond ecosystem (Prasetiyono et al 2024). Dinophyta are known to thrive in warm waters, specifically between 24 and 30°C, which is conducive to shrimp farming (Green 2008). Euglenoids are generally more resilient to varying salinity conditions when compared to dinoflagellates, which allows them to thrive in the suboptimal conditions frequently observed in variable shrimp pond environments (Abdelrahman et al 2018).

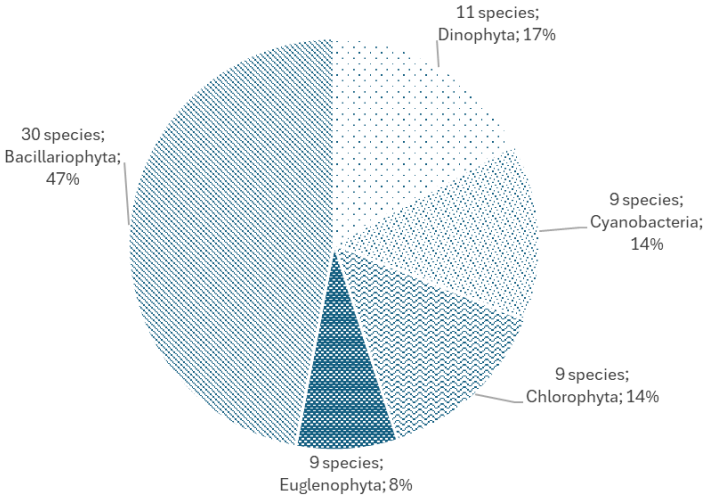

Figure 5. Algal composition in whiteleg shrimp effluent from Bac Lieu (Lien et al 2023).

In the present study, five phyla were detected (Figure 6) as Chlorophyta, Cyanophyta, Charophyta, Ochrophyta and Bacillariophyta. Multiple genera were recorded for Chlorophyta, Charophyta and Cyanophyta, but only one was recorded for Bacillariophyta (i.e., *Thalassiosira* sp.) and one for Ochrophyta (i.e. *Nannochloropsis* sp).

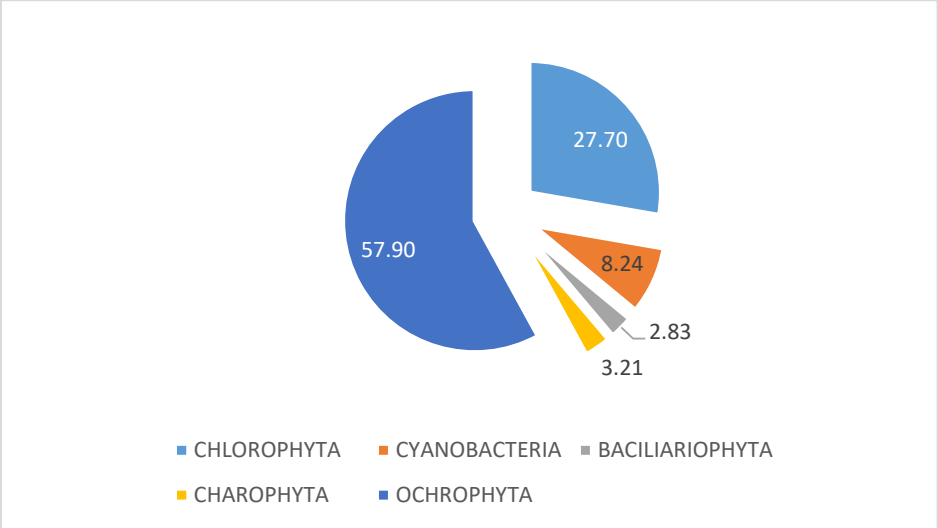

Figure 6. Algal composition of whiteleg shrimp effluent in current experiment.

The algal species composition did not differ across treatments, and green algae predominated in all treatments. High nutrient concentrations have been shown to encourage the dominance of green algae and diatoms in aquatic systems (Burford 1997). Shrimp culture effluents contribute substantial levels of organic and inorganic nutrients, particularly nitrogen and phosphorus, leading to eutrophic conditions that selectively favor certain algal species (Herbeck & Unger 2013; Cardozo & Odebrecht 2014). Moreover, shrimp may preferentially consume a particular algal taxon, such as the diatom, thus reducing the number of species recorded. In practice, frequent water changes designed to maintain water quality can adversely affect algal diversity by removing essential species from the ecosystem (Sani et al 2022); however, among the species existing in aquaculture ponds, green algae predominate. These are characterized by tiny species less than 50 µm in size, such as *Chlorella* sp., which measures 1-5 µm in both length and width, and *Nannochloropsis* sp., with dimensions of 1-2 µm in both length and width (Ut et al 2013). Unicellular algal species measuring less than 50 µm provide suitable feeding particles for filter feeders, especially *Artemia* (Sorgeloos et al 1986).

In summary, the algal density fluctuations in the present study followed the same pattern for all treatments over time, with all treatments reaching their maximum on day 7. Additionally, algae growth was higher in all aeration treatments than in the control treatment (NT1), as also noted by Marchello et al (2015). The daytime aeration (NT3) and full-day aeration (NT4) treatments had nearly 40% higher algal density when compared to the nighttime-aeration treatment (NT2), clearly demonstrating that aeration helps to evenly disperse nutrients and increase the access of algae cells to light, thus promoting photosynthetic activity. The study also found no difference in algal density between NT3 and NT4, suggesting that using only daytime aeration (although frequent mixing is necessary) would help to minimize the cost for green-water enhancement. Ultimately, all treatments indicated that algae can grow from whiteleg shrimp effluent without the need for additional nutrients for at least seven days after introduction into the tank.

Conclusions. Aeration regimes influenced algal growth in effluent from intensive whiteleg shrimp farming, with the highest algal densities observed on the seventh day for both full-day (NT4) and daytime (NT3) aeration treatments. Additionally, microalgae species such as *Nannochloropsis* sp. and *Chlorella* sp. (Ochrophyta and Chlorophyta, respectively) were present throughout the trial, rendering them appropriate nourishment for filter feeders, particularly *Artemia* species. Applying this protocol would reduce fertilizer expenses for *Artemia* farming and the environmental pollution resulting from shrimp wastewater. Consequently, it is advisable to establish an integrated system for farming whiteleg shrimp and *Artemia*, wherein the system components undergo technical and economic assessments to reduce effluent discharge into the environment and generate cost-effective *Artemia* cyst/biomass, as required for most shrimp and fish species.

Authors Contributions. LVT was responsible for designing the experiment and collecting data; TMK monitored the experiment and collected data; VNU discussed and proposed methods for data processing; NVH discussed the data, synthesized opinions, wrote the draft, and corrected/revised the paper according to the reviewer's feedback.

Conflicts of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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