HAYATI
Journal of Biosciences

Vol. 29 No. 6, November 2022 851-862
DOI:10.4308/hjb.29.6.851-862
ISSN: 1978-3019
EISSN: 2086-4094

Zooplankton Composition in Super-Intensive Whiteleg Shrimp, *Litopenaeus vannamei* (Boone, 1931) Culture Tanks

Nguyen Thi Kim Lien[1], Phan Thi Cam Tu[1], Vo Nam Son[1], Doan Xuan Diep[2*]

[1]*College of Aquaculture and Fisheries, Can Tho University, Vietnam*
[2]*Medicinal Chemistry, Hi-tech Agriculture and Bioactive Compounds Research Group, School of Engineering and Technology, Van Lang University, Ho Chi Minh City, Vietnam*

ARTICLE INFO

Article history:
Received June 11, 2022
Received in revised form July 27, 2022
Accepted August 1, 2022

KEYWORDS:
Zooplankton,
whiteleg shrimp,
species composition,
density

ABSTRACT

This study aimed to determine the zooplankton species composition in super-intensive whiteleg shrimp, *Litopenaeus vannamei* (Boone, 1931) tanks. The research was conducted from January to May 2021 in Bac Lieu City, Bac Lieu province, Vietnam. Eleven sampling times were divided into two periods, the nursery phase (six times) and the grow-out phase (five times) of shrimp culture. The results showed that water quality parameters fluctuated dramatically during the culture period, in which some nutrient concentrations tended to increase at the end of the shrimp culture period. Nine zooplankton species were recorded, of which five species belonged to Protozoa, three Rotifera species, and one Copepoda species. The number of zooplankton species did not differ significantly among the sampling periods. Protozoa had the highest species composition and density during most of the shrimp culture period. Copepoda was only identified in the nursery stage of shrimp culture. The species component of zooplankton had a close positive correlation with temperature, but their abundance did not have a significant relationship with water quality parameters because each species was affected by the different water quality parameters. *Zoothamnium* sp. had significantly positive correlations with total ammonia nitrogen (TAN), nitrate (NO_3)-, total phosphorus (TP), and total nitrogen (TN) concentrations. The rotifer *Brachionus plicatilis* had a strong relationship with TP content, whereas *Dartintinnus alderae* had a strong relationship with alkalinity. Protozoa dominance in shrimp tanks could affect shrimp growth, decreasing the economic efficiency of shrimp farming. Therefore, the results of this study contribute to water quality and natural food management to improve shrimp productivity.

1. Introduction

In recent years, aquaculture, particularly brackish shrimp farming, has grown rapidly in Vietnam's Mekong Delta. With varying degrees of intensification, The black tiger shrimp (*Penaeus monodon* Fabricius 1798), and whiteleg shrimp (*Penaeus vannamei* (Boone. 1931)), are the main species of brackish water aquaculture in Vietnam (Quyen *et al.* 2022). As a result, the role of shrimp farming has become increasingly vital for the socioeconomic development of Vietnam's coastal areas (Tran *et al.* 2015). In 2021, the brackish water shrimp farming area was estimated at 740,000 hectares (an increase of 0.5% from 2020), of which the area for farming black tiger shrimp was 630,000 hectares, and whiteleg shrimp was 110,000 hectares. Estimated farming shrimp production in 2021 reached 970,000 tonnes (an increase of 4.3% from 2020), of which the production of black tiger shrimp comprised 277,500 tonnes and whiteleg shrimp 642,000 tonnes (D-Fish 2021).

However, the sector suffers from various sustainability issues that hinder development and contribute to soil and water pollution (Nguyen *et al.* 2021), directly impacting the aquaculture industry. In a polluted environment, disease outbreaks are always a possibility, and shrimp productivity suffers as a result. In addition, investment costs, such as seed, materials, veterinary drugs, and feed, are quite volatile, and the price of commercial shrimp is not stable, affecting the shrimp culture system's economic efficiency. For these reasons, shrimp farmers have gradually improved their farming techniques and tested new models to increase

* Corresponding Author
E-mail Address: diep.dx@vlu.edu.vn

farmed shrimp productivity and income. As a result, the model of super-intensive shrimp farming has become increasingly appealing and is widely used by many shrimp farmers.

The advantages of super-intensive shrimp farming models include a high success rate, a reduction in disease penetration, and the ability to save and reuse water, all of which contribute to environmental protection. Environmental factors are strictly controlled during the culture period, including natural food sources like zooplankton. They are an important link in the trophic web since they can control the phytoplankton community and describe as a good bioindicator with high sensitivity to different environmental stresses (Almeidal *et al.* 2020). Several groups of zooplankton have been identified as indicator organisms at various levels of pollution. Furthermore, zooplankton contributes to the nutrition of shrimp postlarvae immediately after stocking (Coman *et al.* 2003).

Zooplanktons are frequently used as a starting food for many aquatic species. They contribute to increased growth rates and higher survival rates in other groups of organisms because of their high nutrient content, small size, and ease of digestion. The natural zooplankton in the shrimp ponds represents a potential food source for the shrimp larvae and juveniles during the first months of culture (Cardozo *et al.* 2007). In addition, the stomach contents of shrimp revealed their higher consumption of zooplankton when these organisms are abundant in the whiteleg shrimp culture ponds (Porchas-Cornejo *et al.* 2013). Shrimp consume more zooplankton when their density in the shrimp ponds is high (Chen and Chen 1992). Furthermore, the contents of shrimp stomachs indicate shrimps' preference for copepods, polychaetes, and protozoans and reveal their higher consumption of zooplankton when these organisms are abundant in the culture (Porchas-Cornejo *et al.* 2013). Therefore, this study aims to determine zooplankton's species composition and density to develop appropriate methods for natural food source management and stable development of super-intensive whiteleg shrimp farming.

2. Materials and Methods

2.1. Study Time and Location

This study was carried out from January to May 2021 in the super-intensive whiteleg shrimp tanks in Bac Lieu city, Bac Lieu province, Vietnam.

2.2. Research Design

The whiteleg shrimp culture tanks were circular and constructed of iron frames placed on the ground and covered with tarpaulins at the pond bottom. The tanks were aerated 24 h and were protected with anti-sunlight nets.

Pond preparation: Before being supplied to shrimp ponds, water in a reservoir pond was treated with chlorine at a concentration of 30 ppm for five days. The process of water color creation was carried out by incubating 5 liters of molasses combined with two types of probiotics-MP Biozyme I at a dosage of 2 L and MP Aqua-I at a dose of 4 L. This mixture was incubated anaerobically for 48 hours, poured into the shrimp tanks and subjected to combined aeration until the light brown water color was present (typically approximately 1-2 days). After that, shrimp stocking was initiated.

The study included two nursery tanks (256 m^2) and four commercial shrimp tanks (500 m^2). The average water levels maintained in the shrimp tanks during the nursery and commercial shrimp farming were approximately 1.2 m and 1.5 m, respectively. Water exchange was conducted once a day at a proportion of 25%. The shrimp ponds were supplemented with PondPlus probiotics periodically for 3-5 days at 8 am at the dosage specified by the manufacturer. Shrimp were fed commercial pellet feed (CP foods) dispensed by an automated system. Shrimp were raised according to a two-stage farming process.

Stage 1 (nursery stage): The postlarvae (PL11) were bought from hatcheries in Bac Lieu city, Bac Lieu province, and stocked into the nursery tanks at a density of 937 shrimp/m^2 (tank 1) and 1,796 shrimp/m^2 (tank 2). The nursery stage lasted approximately five weeks. At this stage, zooplankton samples were collected six times, one of which was one day before PL stocking and subsequent weekly sampling for five weeks (Figure 1A and Table 1).

Stage 2 (grow-out stage): After five weeks, the juvenile shrimp were transferred into four tanks for the grow-out period of 10 weeks. The stocking density was approximately 200 shrimp.m^{-2} (approximately 700 shrimp/kg). At this phase, zooplankton samples were collected every 2 weeks five times until the shrimp were harvested (Figure 1B). The sampling cycle of zooplankton throughout the study periods is shown in Table 1.

2.3. Analysis of Water Quality Parameters

The water quality parameters in the shrimp tanks studied included salinity, temperature, pH, alkalinity, total ammonia nitrogen (TAN), nitrate (NO_3^-), phosphate (PO_4^{3-}), total nitrogen (TN), total phosphorus (TP), and chlorophyll-a concentrations. The analysis and collection methods of these parameters are presented in Table 2.

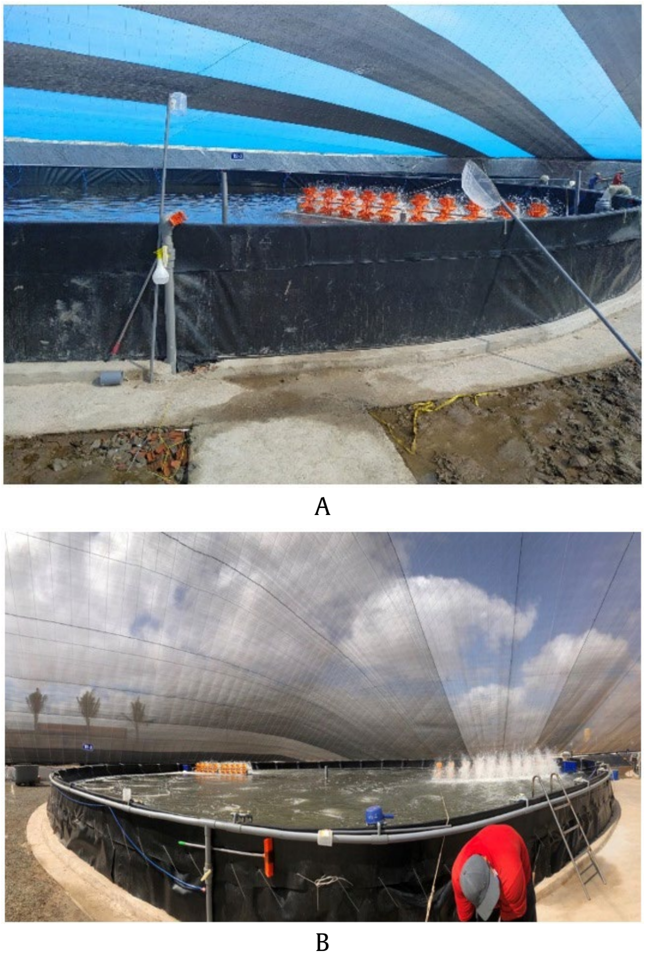
Figure 1. (A) Shrimp nursery tank, (B) shrimp grow-out tank

Table 1. During the study, the zooplankton sampling interval from the super-intensive whiteleg shrimp *Litopenaeus vannamei* culture tanks

Nursery stage		Grow-out stage	
Sampling times (T)	Time interval	Sampling times (T)	Time interval
T1	Before 1 day stocking	T7	7 weeks
T2	1 week	T8	9 weeks
T3	2 weeks	T9	11 weeks
T4	3 weeks	T10	13 weeks
T5	4 weeks	T11	15 weeks
T6	5 weeks		

2.4. Collection and Analysis of Zooplankton
2.4.1. Qualitative and Quantitative Sampling Methods of Zooplankton

The filtration method was used to obtain quantitative and qualitative zooplankton samples. A conical (60 µm mesh size) plankton net was used with a 110 ml plastic bottle attached below to collect zooplankton samples. Water was scooped from various points in the shrimp pond with a 20 L plastic bucket and passed through the plankton net with a sample volume of 100 L. After collection, the sample was placed in a 110 ml plastic bottle and fixed with 4% formalin.

2.4.2. Analysis Method

The zooplankton species composition in whiteleg shrimp ponds was identified based on morphology and taxonomic documents by Shirota (1966), Dang *et al.* (1980), Boltovskoy (1999), Nguyen (2001), and Phan *et al.* (2015). First, the zooplankton density was determined using the Sedgwick-Rafter counting chamber according to the method of Boyd and Tucker (1992). Then, the zooplankton density was calculated using the following formula (Vu and Duong 2013):

$$X = \frac{T \times 1000 \times V_{con.}}{A \times N \times C_{sam.}} \times 10^6$$

Where X is the density of zooplankton (ind·m^{-3}), T is the number of individuals counted, V_{con}. is the volume of the concentrated sample (ml), A is the area of one cell (1 mm^2), N is the number of cells counted, and V_{sam}. is the volume of the collected sample (ml).

2.4.3. Data Analysis

Correlations (Pearson) between species composition and abundance of zooplankton and water quality parameters were analyzed using SPSS 22.0 software.

Table 2. Sample collection and analysis methods of water quality parameters in the super-intensive culture of whiteleg shrimp *Litopenaeus vannamei* culture tanks

Water parameters	Sample collection and storage	Analytical methods
Temperature (°C) pH Salinity (ppt)	Measure directly in shrimp ponds	Hana Multiparameter HI9828

Table 2. Continued

Water parameters	Sample collection and storage	Analytical methods
Alkalinity (mg/L)	These parameters (Alkalinity, TAN, NO_3, TN, PO_4^{3}-, TP, and chlorophyll-a) were collected and stored in a 1 liter plastic bottle. Then, all samples were preserved in the refrigerator at °C before analysis	Titration method (2320 B) (APHA 2017)
TAN (mg/L)		Indo-phenol Blue method (APHA 2017)
NO_3- (mg/L)		Salicylate method (ISO 7890-3:1988)
TN (mg/L)		Sample digestion by Macro-Kjeldahl method (4500-Norg B), then colorimetric by the Indo-phenol Blue method (APHA 2017).
PO_4^{3}- (mg/L)		$SnCl_2$ method (4500-P-D) (APHA 2017)
TP (mg/L)		Sample digestion by Kjeldahl method, then colorimetric by $SnCl_2$ method (4500-P-D) (APHA 2017)
Chlorophyll-a (µg.L^{-1})		Spectrophotometer, extraction with Acetone (APHA 2017)

3. Results

3.1. Water Quality Parameters

The water quality parameters at all sampling times in the super-intensive whiteleg shrimp tanks are presented in Figure 2. The water temperature and pH in the shrimp tanks ranged from 25.5±0.1°C to 29.5±0.1°C and 7.6±0.1 to 8.0±0.1, respectively. The shrimp pond salinity ranged from 18 to 34 ppt. In the early stages after initial PL stocking, salinity ranged from 18.5±0.7 to 19.5±0.7 ppt (T1, T2, and T3). The salinity gradually increased near the end of the grow-out stage to 32.0±1.4 ppt. The alkalinity in the whiteleg shrimp ponds varied greatly during sampling, ranging from 104.0±0.7 to 151.3±1.5 mg/L.

Throughout the study period, the nutrient contents in water, including TAN, NO_3−, and PO_4^{3}−, ranged from 2.2±0.0 to 5.7±1.9 mg/L, 0.5±0.2 to 1.6±0.2 mg/L, and 0.1±0.01 to 0.3±0.02 mg/L, respectively. These parameters tended to be low in the nursery stage and high during the grow-out period, particularly near the end of the culture period. The TN and TP concentrations ranged from 3.7±0.3 to 7.6±2.0 mg/L and 0.1±0.03 to 0.9±0.6 mg/L, respectively. The concentration of TN did not change significantly during the nursery stages but tended to increase in the middle of the grow-out period. The highest TP concentration was found in T2 of the nursery period, which was a favorable condition for developing undesirable algae groups. The chlorophyll-a content varied greatly between sampling periods, ranging from 9.1±8.3 to 141.6±140.0 µg/L. The highest chlorophyll-a concentration was found at T3 of the PL nursery stage.

3.2. Zooplankton Composition in the Super-intensive Whiteleg Shrimp Tanks

3.2.1. The Species Composition of Zooplankton

Nine zooplankton species in the super-intensive shrimp ponds were recorded, including the Protozoa, Rotifera, and Copepoda groups. Protozoa had the most diverse species composition, with five species accounting for 56% of the zooplankton, followed by Rotifera with three species (33%) and Copepoda with one species (11%) (Figure 3).

The total number of identified zooplankton species did not differ significantly between the nursery and grow-out stages, with seven species found at each stage (Table 3). The number of zooplankton species in the nursery stage varied from three to six, and copepods were not recorded at this stage. By contrast, the number of zooplankton species ranged from 4-6 in the culture period, and Copepoda appeared in some samples but at a low density (Figure 4). Common Protozoa species were *Euplotes* sp., *Zoothamnium* sp., and *Dartintinnus alderae*.

The number of species found ranged from 3 to 6 species, with protozoa consistently accounting for

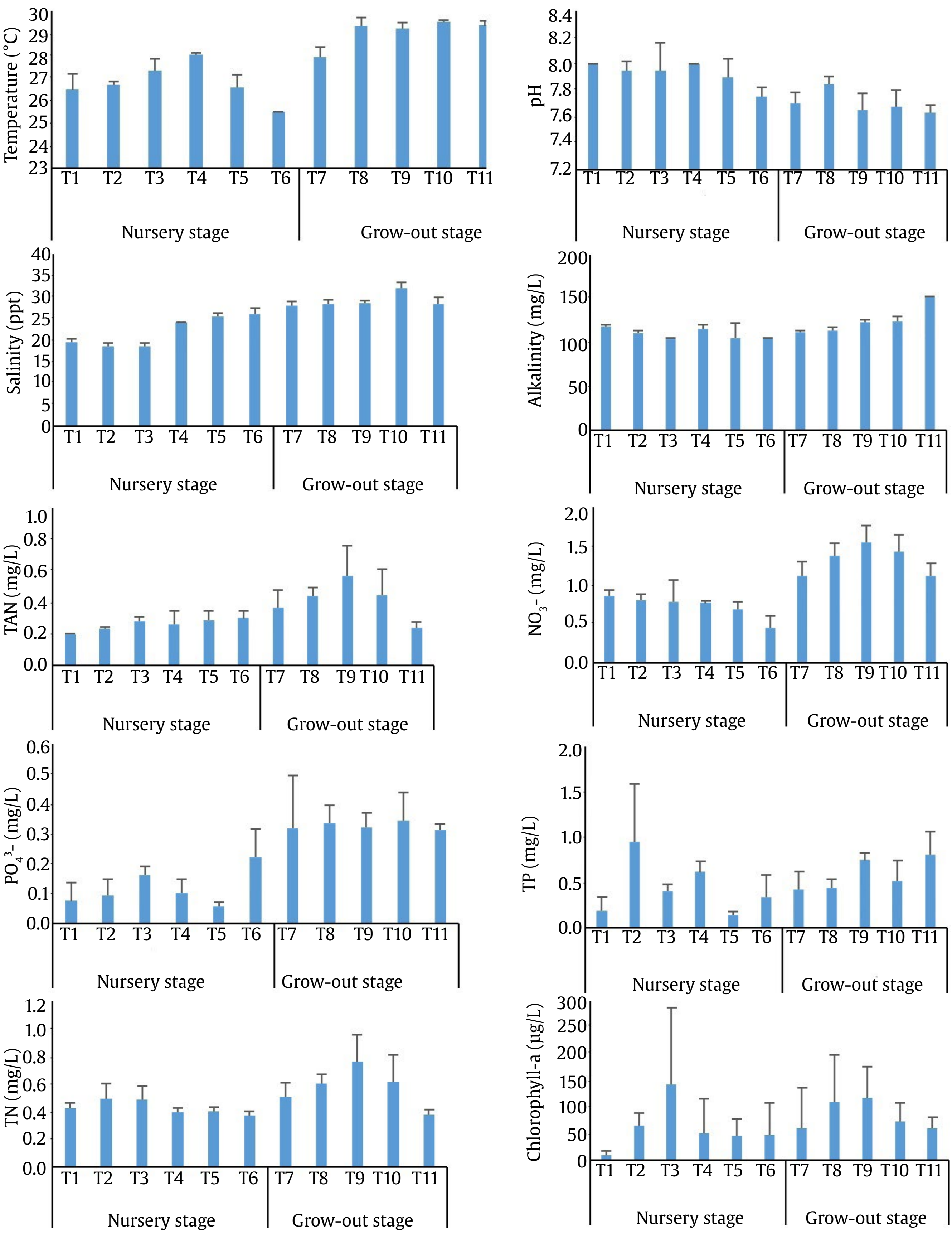

Figure 2. Water quality parameters (mean ± standard deviation) in super-intensive whiteleg shrimp *Litopenaeus vannamei* culture tanks during the nursery and grow-out stages

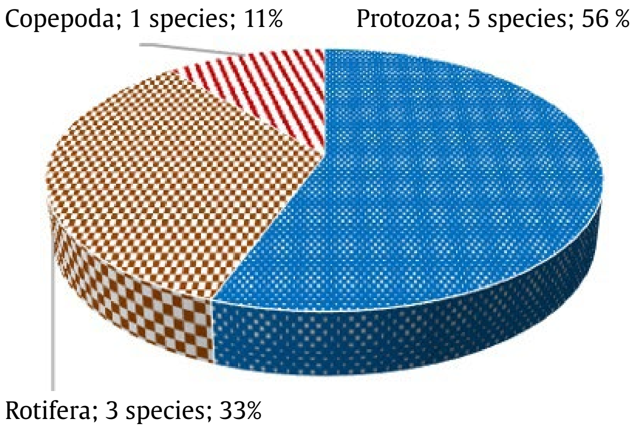

Figure 3. General zooplankton composition in the super-intensive whiteleg shrimp *Litopenaeus vannamei* culture tanks

Table 3. List of zooplankton phyla and species in the super-intensive whiteleg shrimp (*Litopenaeus vannamei*) culture tanks

Zooplankton phyla and species	Nursery stage	Grow-out stage
Protozoa		
Acineta sp. Ehrenberg, 1834	++	++
Dartintinnus alderae Smith *et al.* 2018	++	+++
Euplotes sp. O.F. Müller, 1786	++++	++++
Strombidium sp. Claparède and Lachmann, 1859	+	++
Zoothamnium sp. Bory de St. Vincent, 1824	+++	++++
Rotifera		
Brachionus plicatilis Müller, 1786	+	-
Colurella adriatica Ehrenberg, 1831	-	+
Synchaeta sp. Ehrenberg, 1832	+	-
Copepoda		
Schmackeria dubia Kiefer, 1936	-	+

++++: abundant (76-100%), +++: common (51–75%), occasional (26-50%), +: rare (1-25%), absent (0%) (Ismail and Adnan 2016)

a higher number of species than other groups in most sampling periods (3-6 species). The defined species were mainly adapted to high salinity. Rotifera primarily appeared in the nursery stage of 1-3 species. Furthermore, only one Copepoda species (*Schmackeria dubia*) was discovered during the culture period.

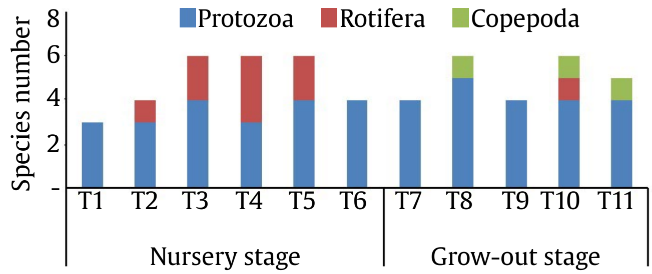

Figure 4. Species composition of zooplankton in the super-intensive whiteleg shrimp *Litopenaeus vannamei* culture tanks during the nursery and grow-out stages

3.2.2. The Abundance of Zooplankton in the Super-intensive Whiteleg Shrimp Ponds

The average density of zooplankton varied greatly between sampling periods, ranging from 61,772±22,823 ind./m^3 to 1,803,922±1,495,184 ind./m^3 (Figure 5). Zooplankton abundance fluctuated relatively high in the nursery stage between the sampling periods. The highest density was found with the dominant protozoa in T2. This result demonstrated that the water environment had a higher level of organic pollution. The Rotifera density varied from 14,212 to 752,350 ind./m^3, with the highest value in T5 (accounting for 82%), indicating that the water environment was rich in nutrients. Copepoda was not recorded during the nursery stage, whereas Protozoa dominated most of the survey periods.

During the grow-out period, the average density of zooplankton varied from 191,381±108,355 to 1,396,205±708,434 ind./m^3. The zooplankton abundance in the shrimp ponds tended to be high in T7, T9, and T11 and low in T8 and T10. Protozoa also dominated during the grow-out stages, with a proportion of 99-100%; *Euplotes* sp. species had the highest density.

3.2.3. Correlation between Zooplankton Species Composition and Abundance and Water Quality Factors

The correlation between the species composition and density of zooplankton with water environmental factors is shown in Table 4. The results revealed that the total number of zooplankton species had a statistically significant positive correlation ($p<0.05$) with temperature, but it was not strongly correlated with other water quality factors or chlorophyll-a content ($p>0.05$).

In addition, the densities of Protozoa, Rotifera, Copepoda, and zooplankton's total density did not strongly correlate with water quality parameters ($p>0.05$). Similar trends were found for zooplankton genera (such as *Acineta*, *Strobilidium*, *Synchaeta*, *Colurella*, *Schmackeria*, and nauplius larvae of Copepoda) with water quality parameters ($p>0.05$).

The abundance of *Euplotes* sp. had a significantly negative correlation ($p<0.05$) with temperature. *Zoothamnium* sp. also had a significantly negative correlation with pH ($p<0.05$) and a positive relationship with TAN ($p<0.01$), NO_3^- ($p<0.05$), TP ($p<0.05$), and TN ($p<0.01$). In addition, *Dartintinnus alderae* had a significantly ($p<0.01$) positive relationship with alkalinity in shrimp ponds. The Rotifer, *Brachionus plicatilis* was negatively correlated ($p<0.05$) with salinity but positively correlated ($p<0.01$) with TP content. Moreover, the results showed that chlorophyll-a concentration was not strongly correlated with the species composition or density of zooplankton.

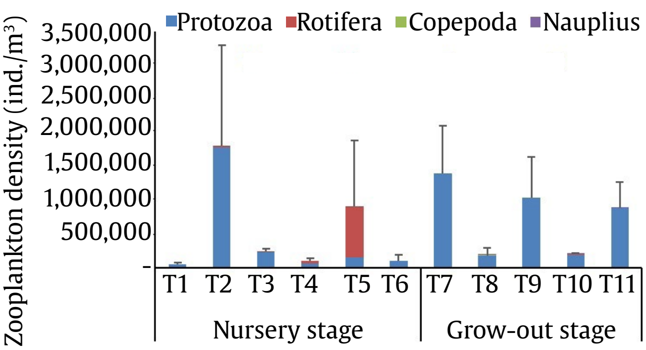

Figure 5. Mean zooplankton density in the super-intensive whiteleg shrimp *Litopenaeus vannamei* culture tanks during the nursery and grow-out stages

3.2.4. The Shrimp Harvest Yield

Shrimp production in the super-intensive system ranged from 23,086 to 32,000 kg/ha (Table 5). The mean shrimp weight varied from 17.5 to 21.4 g/shrimp, corresponding to 46-57 shrimp/kg.

4. Discussion

The temperature in the shrimp tanks did not change significantly and was within a range suitable for the development of zooplankton. According to Vu and Duong (2013), the suitable temperature for the growth of aquatic animals ranges from 20-30°C. Furthermore, Chanratchakool (1995) indicated that when the temperature was higher than 33°C or lower than 25°C, the ability of shrimp to catch

Table 5. The final harvest yield in shrimp ponds

Tank	Mean weight (g/shrimp)	Number of shrimps/kg	Production (kg/ha)	Production (kg/m²)
1	20.4	49	26,330	2.63
2	21.4	46	32,000	3.20
3	17.5	57	23,086	2.31
4	18.5	54	26,710	2.67

Table 4. Correlation (Pearson) between species composition and density of zooplankton and water quality parameters (n = 31)

Zooplankton groups or genus	Tem.	pH	Sal.	Alka.	TAN	NO_3-	PO_4^{3-}	TP	TN	Chlo.
Total species	0.38*	0.00	0.13	0.23	-0.06	0.11	0.12	0.12	-0.11	0.01
Protozoa	-0.22	-0.32	-0.07	0.18	0.12	0.14	0.23	0.27	0.23	0.09
Rotifera	-0.17	0.26	-0.07	-0.35	-0.15	-0.19	-0.29	-0.24	-0.18	-0.16
Copepoda	0.25	-0.07	0.15	0.15	0.13	0.20	0.25	0.06	0.14	0.27
Total density	-0.28	-0.20	-0.10	0.03	0.05	0.06	0.10	0.16	0.15	0.02
Euplotes	-0.38*	-0.21	-0.14	0.01	0.10	0.04	0.19	0.22	0.21	0.09
Zoothamnium	0.33	-0.41*	0.24	0.24	0.58**	0.52*	0.31	0.40*	0.63**	0.16
Dartintinnus	0.22	-0.26	0.07	0.49**	-0.20	0.07	0.11	0.09	-0.23	-0.01
Acineta	-0.23	0.16	-0.31	-0.12	-0.19	-0.13	-0.20	-0.13	0.00	-0.09
Strombidium	0.21	-0.13	0.21	0.26	-0.13	-0.03	-0.04	-0.09	-0.22	-0.06
Brachionus	-0.12	0.27	-0.45*	-0.18	-0.20	-0.20	-0.32	0.53**	-0.17	-0.13
Synchaeta	-0.16	0.26	-0.06	-0.35	-0.14	-0.18	-0.29	-0.26	-0.17	-0.15
Colurella	0.19	0.01	0.20	0.05	0.05	0.09	0.24	0.20	0.03	-0.15
Nauplius	0.26	-0.17	0.26	0.16	-0.12	0.09	0.00	-0.07	-0.14	0.10
Schmackeria	0.25	-0.07	0.15	0.15	0.13	0.20	0.25	0.06	0.14	0.27

*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed); Tem. is temperature; Sal. is salinity; Alka. is alkalinity and Chlo. is chlorophyll-a

prey decreased by 30-50%, which led to desirable conditions for attack by pathogens. Parasitic groups of protozoan are generally found in environmental conditions that experience instability in the water quality, especially temperature, as *Zoothamnium* sp. can breed faster in environmental conditions with a temperature above 30°C (Irvansyah *et al.* 2012). In addition, pH is critical and can directly or indirectly influence shrimp growth. The suitable pH for shrimp is 7.5 to 8.5, and the daily range should not exceed 0.5. Tran *et al.* 2017 observed that a euryhaline shrimp species could live in salinities ranging from 0.5–50 ppt, but the best conditions for growing shrimp are at 10 to 25 ppt. Furthermore, Chanratchakool *et al.* (1995) revealed that shrimp cultured at a salinity higher than 30% were often susceptible to disease, especially white spot and yellow head diseases. However, Saoud *et al.* (2003) also found an effect of age on postlarval survival after acclimation attempts to low salinity water. In addition to survival, growth rate might be affected by the age of acclimation to low salinity water (Davis *et al.* 2005).

In shrimp ponds, total alkalinity is an important variable in water for aquaculture systems (Boyd 2016). The alkalinity level should be maintained within an optimal range for whiteleg shrimp development of 75 to 150 ppm (Durai *et al.* 2021). In this study, the alkalinity tended to increase at the end of the culture period when the salinity was high, but it was still within the suitable range for the growth of whiteleg shrimp. Nutrient content at the grow-out stage tended to be higher than that in the nursery stage because when shrimp grow larger, their waste products increase and excess food and decomposed algae cause the nutrient content in the water to increase in the grow-out stage.

According to Boyd (1998), the appropriate TAN concentration for shrimp ponds is 0.2-2.0 mg/L, and the NH3 content should be less than 0.1 mg/L. Therefore, the TAN content in this study was much higher than the suitable level for shrimp growth, which could have affected shrimp survival at the end of the culture period. In addition, a study by Durai *et al.* (2021) indicated that the optimal nitrate level in whiteleg shrimp farming is <0.1 mg/L. Therefore, the nitrate concentration in most shrimp tanks in the present study was relatively high, which should supply the nutrient resource for the development of natural food.

The high chlorophyll-a content in most of the research reports demonstrated that the very high density of algae is an important food source for the development of zooplankton. Phytoplankton forms the sole base of the food chain in the aquatic system as they act as energy transducers and convert solar energy into chemical energy for food. Zooplankton passes this food energy to the higher trophic levels, thus providing a link between energy producers and consumers (Jakhar 2013). The highest chlorophyll-a concentration in T3 coincided with the highest PO_4^{3-} concentration in the nursery period. A high PO_4^{3-} content provides nutrients for the algae growth, causing the chlorophyll-a content to increase during this period. In addition, the peak algal density was also in T3 of the nursery stage in the same study (6,561,333 ind./L). According to Rubright *et al.* (1981), microzooplankton forms a crucial link between the phytoplankton and the shrimp. Zooplankton is a natural food for the larval stage, and as they grow, they can eat a combination of zooplankton and commercial feed. In general, the nutrient concentration in the super-intensive system was relatively high, indicating that the culture water was eutrophic.

The results showed that Protozoa accounted for a higher number of species (5 species) than the other groups (1-3 species). Most of the Protozoa species were found to be well adapted to brackish and saltwater and were widely distributed in the tanks. Rotifera is generally distributed in freshwater environments, but three species were found in tanks with high salinity ranging from 18-34 ppt.

In this study, the super-intensive white shrimp farming system was managed very closely. The tanks were designed in a circular shape, lined with canvas, placed on the ground, and covered with a net. This closed farming system limits diseases and intermediate hosts in spite of the relatively high shrimp stocking density. The shrimp were fed commercial food. Uneaten feed and waste products of the shrimp contributed to a water environment with high organic matter content, resulting in favorable conditions for protozoa development. Furthermore, the shrimp farming ponds were set up without substrates. Only the zooplankton species available in the pond could survive and develop without supplementation from other external sources or larvae present in the pond bottom mud. Therefore, the species composition of zooplankton was very low in the super-intensive shrimp ponds. Similarly, Gálvez *et al.* (2015) reported that about 13 genera of zooplankton belonging to the Rotifera phylum and the Copepoda, Protozoa, and Cladocera groups were identified in three tanks with a monoculture of shrimp and nine tanks with integrated biofloc systems containing *L. vannamei* and *Gracilaria* algae. Protozoan ectoparasite abundance varies greatly depending on the physicochemical conditions of bodies of water (Hafidloh and Sari 2019). The species composition of zooplankton identified in this study was significantly lower than that in the improved extensive black tiger shrimp ponds in Kien Giang province, Vietnam; in which a total of 45 zooplankton species were found, belonging to Protozoa, Rotifera, Cladocera, Copepoda, and other groups, such as Nematoda, Polychaeta larva, and Hydrozoa. Protozoa represented the most diverse species composition with 16 species (35%); Rotifera represented 11 species (24%), and the other groups varied from 3 to 11 species (Nguyen and Vu 2018). In addition, Huynh (2017) revealed that a total of 47 species of zooplankton belonged to five main groups (Protozoans, Rotifers, Cladocerans, Copepods, and a group of other taxa) in intensive whiteleg shrimp culture ponds under stimulating conditions (application of a commercial nutrient product as a fertilizer). The remarkable difference in the results of these studies was due to differences in nursery conditions and shrimp farming systems.

Several zooplankton species were identified in this study, including *Euplotes* sp., *Zoothamnium* sp., *Acineta* sp., *Dartintinnus alderae, Strombidium* sp. (Protozoa), and *Brachionus plicatilis* (Rotifera). The Protozoa predominant in shrimp ponds are often not beneficial to shrimp growth and were considered indicators of organic pollution (Dang *et al.* 2002). In addition, Biedenbach *et al.* (1989) indicated that Protozoa, such as flagellates and ciliates, contain sterols in their chemical composition and that many of these sterols were converted to cholesterol or other lipids. According to Loureiro *et al.* (2012), ciliates and rotifers were found in shrimp intestines in the early and middle stages of the culture cycle, which showed that shrimp preferentially used these groups of organisms, especially in the early stages after stocking. Moreover, Furuya (2001) demonstrated that Rotifera species were a useful food source for larval stage shrimp because of their small size and high protein content.

Although Copepoda is distributed mainly in brackish and saline environments, only one species was recorded because of the high organic matter in shrimp ponds, which limited their development in this study. By contrast, Porchas-Cornejo *et al.* (2013) indicated that copepods were the most abundant organisms in all treatments (>65%) in their study of zooplankton communities in shrimp earthen ponds with and without organic nutrient-enriched substrates, but their concentration decreased when shrimp were present in the ponds. The substrate of the shrimp farming ponds in this study was covered with tarpaulin, which is one of the reasons for the low species composition of copepods. The resting eggs of copepods can survive in sediments, but bottom mud was not present in shrimp tanks, which limited the development of copepods in this study.

At the nursery stage, the dominance of *Euplotes* sp. (Protozoa) indicated that the water environment had a high level of organic pollution. Similarly, in the grow-out stage, *Euplotes* sp. also dominated and tended to increase at the end of the culture period. They swim slowly and are from 45–65 µm in length, oval or elongated shape with both ends rounded (Syberg-Olsen *et al.* 2016), making them suitable as food for shrimp. In addition, the density of the *Zoothamnium* sp. and *Acineta* sp. species tended to decrease at the end of the culture period. They often parasitize Copepoda and other crustaceans, including marine shrimp. Their high densities in shrimp ponds could cause disadvantages for shrimp growth. Typically, they do not cause direct harm to shrimp, but they have indirect effects by sticking to the gills, preventing water flow through the gills, blocking gas exchange, clinging to the shell, and making it difficult to molt and move. Parasites that live on shrimp will affect the prey catch of shrimp. However, Hafidloh and Sari (2019) indicated that *Zoothamnium* sp. is a normal living ciliate in shrimp culture ponds. Therefore, this parasite can still grow despite good water quality. An abundance of *Zoothamnium* sp. infested the vannamei shrimp but did not cause high mortality. Moreover, the *Strombidium* sp. and *Dartintinnus alderae* species were also detected in the shrimp ponds of the present study, but at a relatively low density. Therefore, they did not affect the growth of shrimp. In spite of the disadvantage of protozoa, ciliates were seen in the gut contents of shrimp mainly in the initial and intermediate phases of culture, indicating that the shrimp grazed preferentially on these microorganisms, especially in the early stages of life (Loureiro *et al.* 2012). Copepoda and nauplius larvae of copepods were also recorded but at very low densities. Due to this period, shrimp were cultivated at a lower density than at the nursery stage in the present study, and the organic matter content was also lower, which provided suitable conditions for the presence of copepods. However, the concentration of copepods seemed to be enhanced by the presence of substrates (Porchas-Cornejo *et al.* 2013). The close system shrimp tanks in this present study probably limited the development of copepods and zooplankton in general.

In general, the structure of zooplankton species did not change significantly during the study period. When the nutrient concentration in the water increases, it provides a nutrient source for algae development. Because of this, the abundance of zooplankton also increases. This condition was demonstrated by the positive correlation between total zooplankton density and water nutrient content ($p>0.05$). Moreover, Coman *et al.* (2003) also showed that changes in the abundance and biomass of the zooplankton assemblage were not correlated with physicochemical characteristics such as water quality parameters. The zooplankton densities fluctuated greatly throughout the culture period in the nursery and grow-out stages, with Protozoa dominating. Porto Neto *et al.* (2009) observed that Protozoa and Rotifera replaced Copepoda dominance as nutrient content increased with the culture period, indicating that eutrophic conditions can strongly influence zooplankton composition in the tank.

The low species diversity trend was indicative of a culture system with high levels of nutrients, which reduces water quality. Hence, one of the four harvested shrimp tanks in this study was highly productive and profitable (tank 2), whereas the other tanks were less productive and only broke even. The shrimp yield obtained in this study was higher than that obtained by Yeong-Rok *et al.* (2010). The authors indicated that the total production of super-intensive whiteleg shrimp in HDPE-lined ponds with no water exchange on the west coast of South Korea was between 2.33 and 2.48 kg/m^2 (equivalent to 23,300–24,800 kg/ha), with an initial stocking density of 272 ind./m^2. However, shrimp production in the present study was lower than the average productivity in super-intensive indoor shrimp farming in Bac Lieu, with a yield of 40-80 tonnes/ha/crop (Seafood-tip 2021). In this study, the highest shrimp yield was found in tank 2 (32,000 kg/ha), which brought profit to shrimp farmers. However, other shrimp tanks had lower production because of disadvantages from the water environment and inappropriate management.

According to the results of the present study, one of the reasons for the low shrimp yield was the relatively low water quality in the shrimp ponds. The concentrations of TAN, NO_3^-, PO_4^{3-}, TN, and TP were relatively high in the culture stage. The high organic matter content and predominant protozoa could affect shrimp growth. Therefore, shrimp farmers must increase the daily water change rate and limit uneaten feed to reduce the organic matter content in shrimp ponds. Water probiotics should be applied to eliminate algal blooms and maintain useful natural food for shrimp. The use of probiotics enhances shrimp growth by improving nutrient absorption, which benefits culture biomass (Xie *et al.* 2019). Probiotics such as *Bacillus* sp. can improve pond water quality by decomposing organic matter to CO_2, especially during intensive production. In Malaysia, use of probiotics such as photosynthetic bacteria and *Bacillus* sp. can improve water quality, survival and growth rates of shrimp juveniles on commercial farms (Loh 2017). They also need to maintain optimal water quality parameters throughout the culture period to improve shrimp productivity.

In conclusion, this study identified a total of nine zooplankton species in super intensive whiteleg shrimp, *Litopenaeus vannamei* culture tanks with a stocking density of 200 shrimp.m^{-2}. The number of zooplankton species did not differ greatly between the nursery and commercial shrimp grow-out stages. The dominance of Protozoa over the study period was unfavorable for shrimp growth. Temperature, pH, salinity, alkalinity, NO_3^-, TAN, PO_4^{3-}, TN, TP, and chlorophyll-a concentrations fluctuated considerably during the shrimp culture cycle. Some nutrient contents such as TP and NO_3- concentrations tended to increase at the end of the culture period. Water quality parameters substantially influenced zooplankton development in the super-intensive whiteleg shrimp ponds. Thus, this research contributes to the identification of zooplankton species composition and enables the recognition of a dominant zooplankton group in the super-intensive white shrimp tanks. The results of this study can also be used as a reference for water quality and natural food management in shrimp ponds.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

We sincerely thank Van Lang University, Vietnam, for funding this research. In addition, the authors would like to thank colleagues at Can Tho University, Vietnam, and anonymous referees of this journal for constructive comments.

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