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Unbalanced N:P Ratio: On Phytoplankton Abundance and Survival Rate of the Whiteleg Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

This study aimed to investigate the impact of an unbalanced N:P ratio on the planktonic ecosystem and the growth of the whiteleg shrimp (*Litopenaeus vannamei*) in aquaculture systems. Conducted at the Marine Aquaculture Laboratory, Faculty of Fisheries and Marine Science (FPIK), IPB University, the research utilized a completely randomized design (CRD) with four treatments (Control = 16:1, A = 20:1, B = 25:1, C = 30:1) and three replications. Shrimp seeds weighing 3.51 ± 0.07 grams were stocked at a density of 23 shrimp per unit and fed four times daily with a diet comprising 7% of their biomass per day, using crumble feed with 36% protein content over a 30-day maintenance period. Results revealed that increasing the N:P ratio led to higher phytoplankton abundance, with the highest levels observed in treatment C (30:1) from days 7 to 28. The analysis of water quality parameters revealed fluctuations in total ammonia nitrogen (TAN), nitrite, nitrate, and phosphate levels throughout the study. On day 0, significant differences in TAN, nitrite, and nitrate levels were noted between treatments (P<0.05). TAN levels decreased by day 7, with the control treatment showing significant differences from treatments A and B, excluding treatment C (P>0.05). From days 21 to 28, TAN levels continued to decrease without significant differences among treatments (P>0.05). Nitrite levels increased on day 7 and stabilized until day 28, showing no significant differences (P>0.05). Nitrate levels decreased on day 7, with the control treatment significantly differing from treatments A, B, and C (P<0.05). Phosphate levels fluctuated throughout the observation period, with significant differences only on day 28 between treatment A and C (P<0.05). Histopathological examination of shrimp pancreases revealed tissue damage, including vacuoles and necrosis across all treatments. While temperature, salinity, and dissolved oxygen (DO) levels remained within the optimal range, pH levels were not optimal, likely due to organic matter decomposition in the water. This study highlights the complex interactions between nutrient ratios and aquatic health, emphasizing the need for careful management in shrimp aquaculture.

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INTRODUCTION

The whiteleg shrimp (*Litopeneus vannamei*) is one type of fishery biota that is quite popular. This is supported by data on vanamel shrimp production in the world in 2022, which reached 6.7 million tons **(FAO, 2024)**. Success in aquaculture activities must be supported by good environmental conditions viz. water quality, since water quality is the key to the life of aquatic organisms and one of the aspects that determines the success of aquaculture activities. **Snieszko (1974)** stated that environmental quality can affect the health of aquatic biota and the abundance of pathogens in a water body. One of the commonly measured water quality parameters in aquaculture activities is the measurement of plankton. **Widyo *et al*. (2022)** elucidated that one of the steps that can ensure the success of shrimp farming in ponds is to regulate and control the stability of plankton in the waters and the level of shrimp demand for plankton. Aquaculture water quality and nutrient availability affect the growth and health of the whiteleg shrimp greatly.

Plankton are microorganisms that live and float in water **(Nybakken, 1992)**. Plankton is important in the trophic level of water energy flow and food chain cycle **(Faiqoh *et al*. 2015)**. Plankton consists of two major groups, namely phytoplankton (plants) and zooplankton (animals). This group of plankton has a role in various aquatic ecosystems **(Triyawan & Arisandi 2020)**. In the food chain cycle in aquatic ecosystems, zooplankton plays an important role as a source of nutrients or natural food for larger organisms including the whiteleg shrimp (*L. vannamei*). To maintain the abundance of zooplankton as natural food, farmers need to keep the balance of phytoplankton populations in the waters. According to **Faiqoh *et al*. (2015)**, phytoplankton play an important role in the food chain as producers, and zooplankton are a level 1 consumer. Phytoplankton have many types, such as diatoms, green algae, dinoflagellates, etc. Some phytoplankton that are commonly used as natural food in aquaculture are diatoms, green algae, and dinoflagellates. Some phytoplankton commonly cultivated in pond waters are green algae and diatoms. However, several types of phytoplankton are avoided in pond culture, namely the dinoflagellate group and cyanobacteria, because they can affect shrimp growth **(Widyo *et al*. 2022)**.

The growth and abundance of phytoplankton are strongly influenced by the availability of nutrients in the waters. Nutrients are elements or chemical compounds that are essential to an organism's metabolic or physiological processes. Nutrients with an essential role in the growth and metabolism of phytoplankton are nitrogen and phosphate **(Risamasu & Prayitno, 2011)**. Nitrogen (N) is the nutrient needed by phytoplankton to synthesize proteins. At the same time, phosphate (P) is essential for the life of aquatic organisms because it functions in the storage and the transfer of energy in cells and functions in the genetic system to accelerate the growth of algae **(Alianto, 2011)**. Nitrogen and phosphate needs can be covered through utilizing phytoplankton in the form

of inorganic nutrients such as nitrate, ammonium (nitrogen), and orthophosphate (phosphate).

One of the problems in whiteleg shrimp farming is the high nutrient content in the rearing water due to the accumulation of waste (feed residue, feces) which can trigger an increase in nitrogen and phosphate levels in the water. This can potentially trigger harmful algae bloom (HAB), which can cause mass mortality of shrimp and economic losses for farmers **(Alonso & Osuna, 2003)**. Conversely, nitrogen and phosphate deficiencies can inhibit the growth of desirable phytoplankton, reduce dissolved oxygen (DO) levels, and disrupt the food chain cycle, which ultimately has a negative impact on shrimp production. Therefore, in addition to maintaining water quality, farmers also need to understand the N:P ratio in the water to preserve the abundance of beneficial phytoplankton and suppress the growth of undesirable species, such as cyanobacteria. **Arrigo (2005)** stated that the N:P ratio requirement varies depending on the type of phytoplankton. The development of Chlorophyceae (green algae) requires an N:P ratio of 30:1, Dinophyceae requires 12:1, Bacillariophyceae (diatoms) and red algae require 10:1. At the same time, cyanobacteria (BGA) tend to thrive when the N:P ratio is below 10:1.

This study aimed to understand and describe how an imbalance in the N:P ratio can affect the planktonic ecosystem and growth of vanname shrimp in the culture system. The results of this study are expected to contribute to the field of aquaculture, especially vaname shrimp farming, by enriching knowledge about the dynamics of unbalanced N:P ratios on phytoplankton abundance and shrimp survival, and become a basis for consideration in the preparation of policies related to sustainable aquaculture management.

MATERIALS AND METHODS

The research was conducted for 30 days, from May 2024 until June 2024. The research was conducted at the Marine Culture Laboratory, Environmental Laboratory, and Aquatic Organism Health Laboratory, Faculty of Fisheries and Marine Science, University IPB.

1. Research design

The study used a completely randomized design (CRD) with four treatments and three replicates. The treatment design can be seen in Table (1).

Table 1. Research design

Treatment	N : P	
Control (K)	16:1	(1,6 N mg L^{-1}: 0,1 P mg L^{-1}) (Mayers *et al.* 2014)
A	20:1	(2,0 N mg L^{-1}: 0,1 P mg L^{-1})
B	25:1	(2,5 N mg L^{-1}: 0,1 P mg L^{-1})
C	30:1	(3,0 N mg L^{-1}: 0,1 P mg L^{-1})

2. Test preparation

The test animals used are whiteleg shrimp (*Litopenaeus vannamei*) obtained from PT Suri Tani Pemuka (STP), Anyer, Banten, which are certified pathogen-free. The shrimp were reared until they reached an average size of 3.51 ± 0.07 grams. The water used is sourced from the waters of PIK III, which contain various types of phytoplankton that have been previously identified.

3. Tool sterilization

Before the research began, the maintenance media and equipment were sterilized. The initial step involved washing the tubs and tools with clean running water, followed by cleaning them with soap and rinsing again with clean water. The seawater used in this study was not sterilized to preserve the natural abundance of phytoplankton and to avoid phytoplankton mortality caused by chemical exposure. This approach also allows for the observation of the effects of the nitrogen-to-phosphorus (N:P) ratio on the abundance and composition of phytoplankton species.

4. Preparation of maintenance media

The maintenance media used were plastic containers measuring 40 x 60 x 35cm, as many as 12 pieces, and arranged into two rows. The containers that have been placed are filled with seawater with a salinity of 25-26ppt, with a water level of 25cm. The maintenance media is equipped with oxygen supply in the form of additional aeration, a heater with a temperature of 28^{o}C, and a net at the top so that shrimp do not get out of the maintenance media, as well as aquascape lights with a wavelength of 1,000 lux which aims to help the photosynthesis process of phytoplankton.

5. Preparation and addition of nitrogen and phosphate

Before the treatment, the concentrations of nitrogen and phosphate in the water were measured first. This measurement aims to determine the initial concentration of nitrogen and phosphate in the water medium. Based on the results of these measurements, the researchers then made nitrogen and phosphate mother solutions. The nitrogen mother liquor was made using urea fertilizer as the nitrogen source, while the phosphate mother liquor was made using Triple Super Phosphate (TSP) fertilizer as the phosphate source. Both types of fertilizer were dissolved in distilled water. Urea and TSP fertilizers were obtained from the Environmental Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB).

This study set phosphate as the threshold nutrient, with a concentration range of 0.09-1.8 mg L^{-1} **(Mackenthun, 1969)**. Nitrogen mother liquor was prepared by dissolving urea fertilizer with distilled water to a concentration of 10,000ppm. Based on the calculation results, the urea needed to make the 10,000ppm mother liquor was 21.739 grams L^{-1}. The phosphate mother liquor was prepared using the same method, dissolving TSP fertilizer with distilled water to a concentration of 1,000ppm. Based on the

calculation results, the amount of TSP needed to make the 1,000ppm mother liquor is 3.125 grams L^{-1}.

Next step, phosphate mother liquor was tested to determine the dosage that could produce phosphate concentrations within the target range (0.09-1.8mg L^{-1}). The test was conducted directly on the water medium, with a dose of 0.1-1ppm of phosphate mother liquor. The mother liquor was diluted gradually, and then the phosphate concentration in the water was measured using a spectrophotometer. Based on the test results, the phosphate dose used in this study was set at 0.1ppm. In applying the test solution for treatment, researchers made nitrogen and phosphate solutions according to the research design, and the solution was multiplied by the volume of media water used to determine what dose would be used according to the treatment design.

6. Application of treatments

Before applying the fertilizer, shrimp were acclimatized for ± 12 hours in a plastic container filled with seawater, with the number of shrimp in each container as many as 23 shrimp with a size of 3.51 ± 0.07 grams. Acclimatization is done to reduce stress on test animals due to differences in their native environment. Furthermore, the fertilizer was added to the plastic container where the shrimp were acclimatized. Fertilizer was applied only once, at the beginning of the study. During maintenance, feeding was done four times daily at 07:00 a.m., 12:00 p.m., 05.00 p.m., 10.00 p.m. Feeding was given as much as 7% of biomass/day using crumble-shaped feed with a protein content of 36% **(SNI, 2014)**. The container and treatment water was cleaned and replaced during the study. Lights used were supported with the on-off method and an intensity of 12 hours, from 06.00 a.m. to 06.00 p.m. **(Facta *et al*. 2006)**.

7. Test parameters

Plankton abundance

Plankton abundance measurements were performed weekly for each treatment. The abundance was calculated using a hemocytometer following the methods of **Helm *et al.* (2004)** and **Aziz (2015)**. Plankton abundance was determined using the following formula:

$$N\ (cell\ mL^{-1}) = \left(\frac{n}{4}\right) \times 10^4$$

Description

N : Plankton abundance (cells mL^{-1})

n : Number of plankton observed (cells)

Relative abundance

Relative abundance refers to the proportion of plankton species, indicating the diversity of organisms in a specific area. Measurements of relative abundance were conducted weekly for each treatment. Relative abundance was calculated using the method described by **Prescott (1984)**. Relative abundance was determined using the following formula:

$$Kr\ (\%) = \frac{ni}{N} x\ 100$$

Description

Kr : Relative abundance (%)
ni : Number of individuals in the genus
N : Total number of individuals

TAN (Total ammonia nitrogen)

TAN (Total Ammonia Nitrogen) measurements were conducted weekly for each treatment. Water samples (10mL) were collected from each treatment and placed into test tubes. Each sample was treated with one drop of $MnSO_4$, followed by 0.5mL of Clorox and 0.6mL of Phenate. The samples were left to stand for approximately 15 minutes. After this period, the samples were analyzed using a spectrophotometer at 630nm. The total ammonia nitrogen concentration was calculated by inputting the absorbance value into the standard solution regression equation, as described by **APHA (2005)**:

$$y = ax + b$$

Nitrite

Nitrite measurements were performed weekly for each treatment. Water samples (10mL) were collected from each treatment and placed into test tubes. Each sample was treated with four drops of sulfanilamide and two drops of NED (n-(1-naphthyl)-ethylenediamine). The samples were then left to stand for approximately 15 minutes. Afterward, the samples were analyzed using a spectrophotometer at a wavelength of 543 nm. The nitrite concentration was determined by inputting the absorbance value into the regression equation of the standard solution **(APHA, 2005)**:

$$y = ax + b$$

Nitrate

Nitrate measurements were performed weekly for each treatment. Water samples (5mL) were collected from each treatment and placed into test tubes. Each sample was treated with 0.5mL of Brucin and 5mL of H_2SO_4. The samples were left to stand for approximately 15 minutes. After this period, the samples were analyzed using a spectrophotometer at a wavelength of 410nm. The nitrate concentration was determined

by inputting the absorbance value into the regression equation of the standard solution **(APHA, 2005)**.

$$y = ax + b$$

Phosphate

Phosphate measurements were performed weekly for each treatment. Water samples (25mL) were collected from each treatment and placed into test tubes. To each sample, 0.5mL of ammonium molybdate and four drops of $SnCl_2$ were added, and the mixture was homogenized. The samples were left to stand for approximately 10 minutes. Afterward, the samples were analyzed using a spectrophotometer at a wavelength of 690nm. The phosphate concentration was determined by inputting the absorbance value into the regression equation of the standard solution **(APHA, 2005)**.

$$y = ax + b$$

Survival rate

Survival rate is the ratio of the number of shrimp at the end of the treatment to the number of shrimp at the beginning of stocking. The survival rate was calculated using the following equation **(Effendi, 2004)**:

$$SR\ (\%) = \frac{\mathrm{Nt}}{\mathrm{No}} \times 100$$

Description

SR : Survival rate (%)
Nt : number of initial fish
No : number of final fish

Histopathology

Histopathological observations of hepatopancreas tissue were conducted at the end of the study, following the method described by **Munaeni *et al.* (2020)**. The hepatopancreas tissue from the test shrimp was fixed in Davidson's solution for 24 hours. The tissue was then cut into 3-5 mm thick sections, measuring 0.5 x 0.5cm, and underwent a series of steps, including dehydration, clearing, embedding, paraffin blocking, sectioning, staining, and microscopic examination at 100x magnification.

Total hemocyte count (THC)

THC is one of the measurement methods used to assess the level of crustacean stress **(Supriyono *et al*., 2023)**. Measurement of total hemocytes (THC) was performed at the end of the study. Total hemocyte count (THC) was measured following the method described by **Wang and Chen (2006)**. A volume of 0.2mL of hemolymph was collected

and mixed with an equal volume of anticoagulant solution (30 mM trisodium citrate, 0.34 M sodium chloride, 10 mM EDTA, 0.12 M glucose; pH 7.55) in a 1:1 ratio. The mixture was homogenized and then applied to a hemocytometer, which was subsequently covered with a cover glass. THC was calculated using a hemocytometer under a light microscope at 100× magnification.:

$$THC = \frac{\sum observed\ cells}{\sum observed\ box} X\ 25\ X\ (hemocytometer\ volume)^{-1} X\ dilution$$

Water quality in rearing containers

Water quality measurements were taken once a day in the morning. The parameters measured were DO, pH, temperature, and salinity.

Data analysis

All data collected are presented as mean. Plankton abundance, relative abundance, Shannon-Wiener diversity index, evenness diversity index, dominance index, water quality in the rearing container, and histological analysis were measured descriptively. Measurements of TAN, nitrite, nitrate, phosphate, survival rate, and total hemocyte count (THC) will be analyzed using SPSS 26.0 data software and Microsoft Excel 2016. Data were analyzed using analysis of variance (ANOVA). After analysis of variance and finding significantly different effects (P<0.05), it is necessary to conduct further tests using the Duncan test method with a 95% confidence interval.

RESULTS

1. Phytoplankton abundance

Adding different N:P ratios to the control (K) (16:1), treatments A (20:1), B (25:1), and C (30:1) significantly increased phytoplankton growth. The results of phytoplankton growth measurements during the maintenance period are shown in Fig. (1). Phytoplankton abundance increased significantly by day 7 and began to decline by day 14 across all treatments. In treatments K and B, phytoplankton abundance increased again by day 21, while treatments A (20:1) and C (30:1) continued to show a decline. By day 28, an increase in phytoplankton abundance was observed in all treatments.

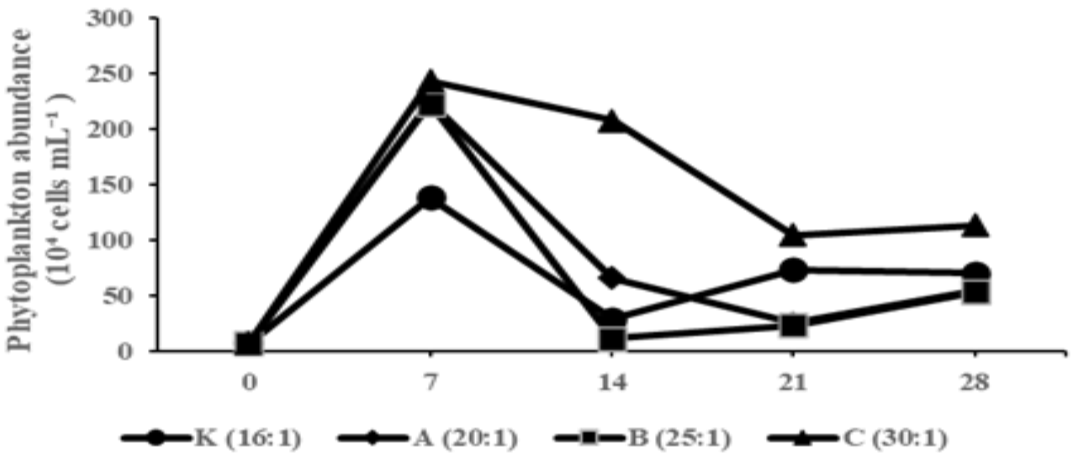

Fig 1. The abundance of phytoplankton treated with different doses (ratios) of nitrogen fertilizer

2. Relative abundance

The relative abundance of phytoplankton during the observation period showed differences in phytoplankton species abundance in each treatment and observation time. On day 0, before treatment, the highest relative abundance was observed in Cyanophyceae (cyanobacteria) and Bacillariophyceae (diatoms) in each treatment. Day 7, there was a significant increase in the relative abundance of Bacillariophyceae in each treatment. On day 14, the abundance of Bacillariophyceae started to decline, while Cyanophyceae began to increase. On day 21, the relative abundance of Cyanophyceae species increased significantly, while Bacillariophyceae species continued to decrease. On the 28th day, the relative abundance of Bacillariophyceae species decreased further, while Cyanophyceae species continued to increase and became the dominant phytoplankton species group in each treatment. The relative abundance during the rearing period is presented in Fig. (2).

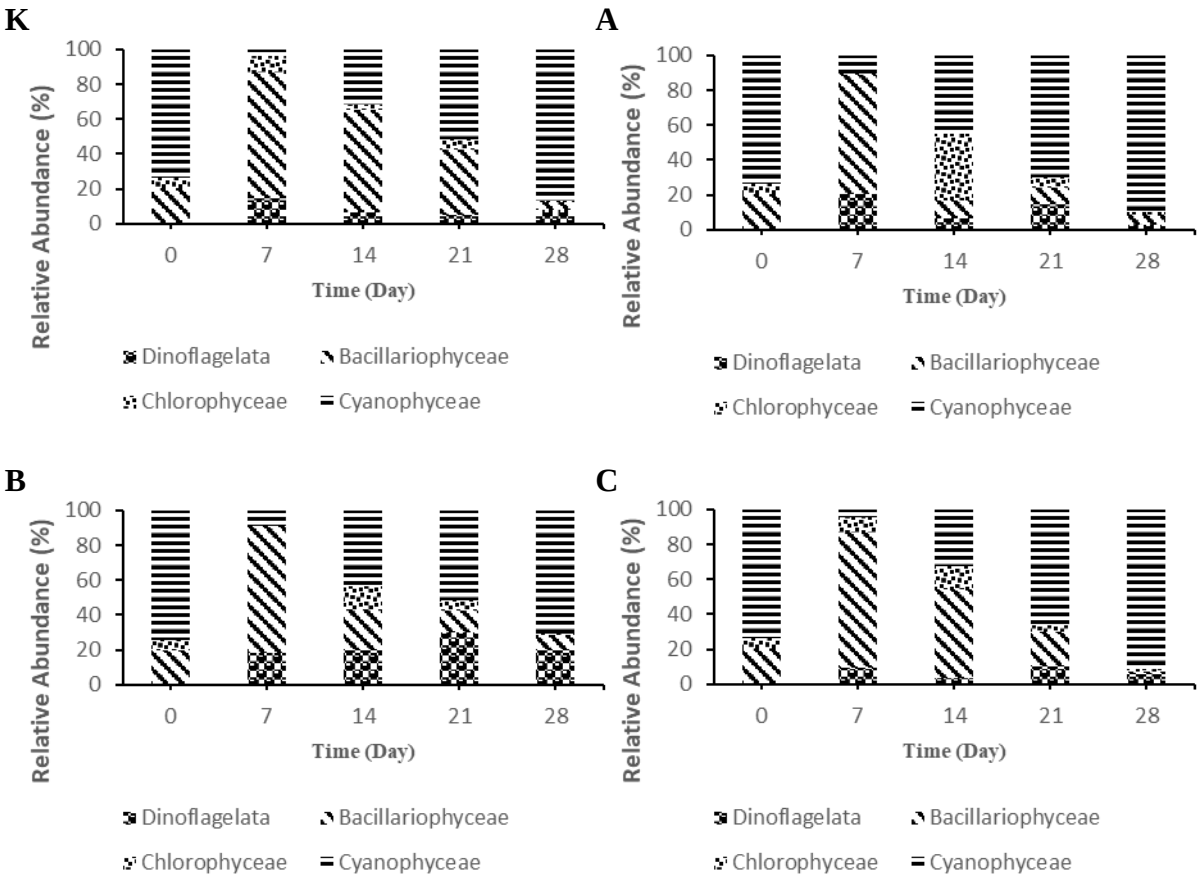

Fig. 2. Relative abundance treated with different doses (ratios) of nitrogen fertilizer

3. TAN (Total ammonia nitrogen)

Total ammonia nitrogen (TAN) consists of NH_3 (ammonia) and NH_4^+ (ammonium). TAN measurement results on day 0 showed significant differences between treatments (*P*-value <0.05), indicating that different N:P ratios affect the TAN content in the water. On day 7, TAN levels decreased in each treatment, where treatment K (16:1) showed a significant difference compared to treatment A (20:1) and B (25:1), but not significantly different from treatment C (30:1) (*P*-value < 0.05). On day 14, TAN levels increased without a significant difference between treatments (*P*-value > 0.05). Observations from day 21 to day 28 showed decreased TAN levels, with no significant differences between treatments (*P*-value > 0.05). TAN values during the rearing period are illustrated in Fig. (3). In addition, TAN values were further analyzed by separating ammonia (NH_3) and ammonium (NH_4^+). The ammonium (NH_4^+) value is presented in Fig. (4), while the ammonia (NH_3) value is shown in Fig. (5).

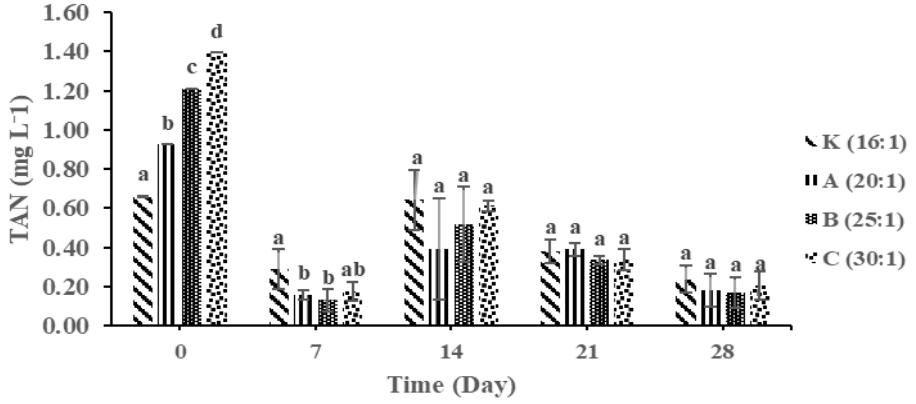

Fig. 3. Total ammonia nitrogen (TAN) content during the study fed with fertilizer at different N:P doses (ratios).

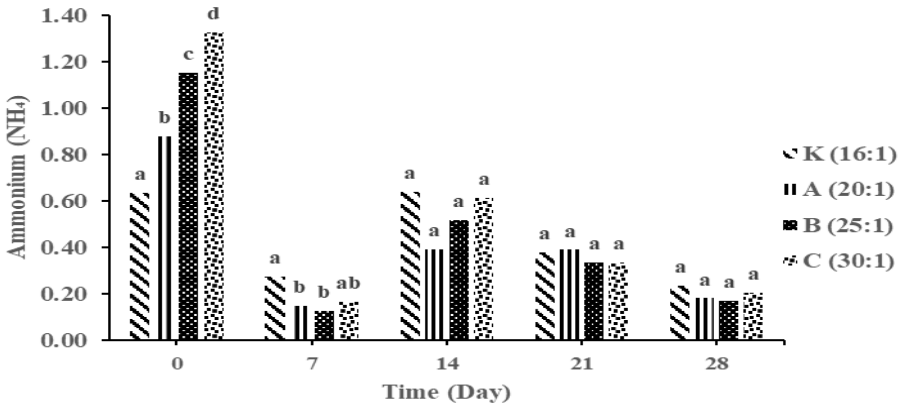

Fig. 4. Ammonium (NH_4^+) content during the study fed with fertilizer at different N:P doses (ratios)

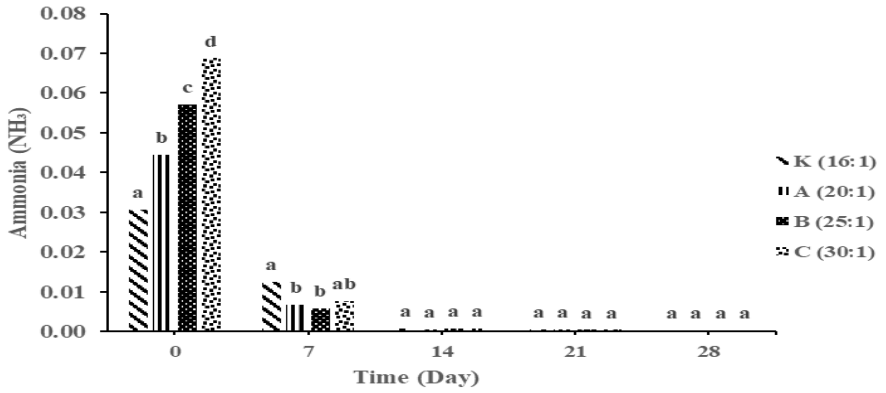

Fig. 5. Ammonia (NH_3) content during the study fed with fertilizer at different N:P doses (ratios)

4. Nitrite

Nitrite measurements on day 0 showed significant differences between treatments (*P*-value < 0.05), indicating that different N:P ratios affect the nitrite content in the water. On day 7, nitrite levels increased, but the differences between treatments were not statistically significant (*P*-value > 0.05). Observations from day 14 to day 28 showed that nitrite levels remained consistent across treatments, with no significant differences (*P*-value > 0.05). The nitrite content throughout the rearing period is illustrated in Fig. (6).

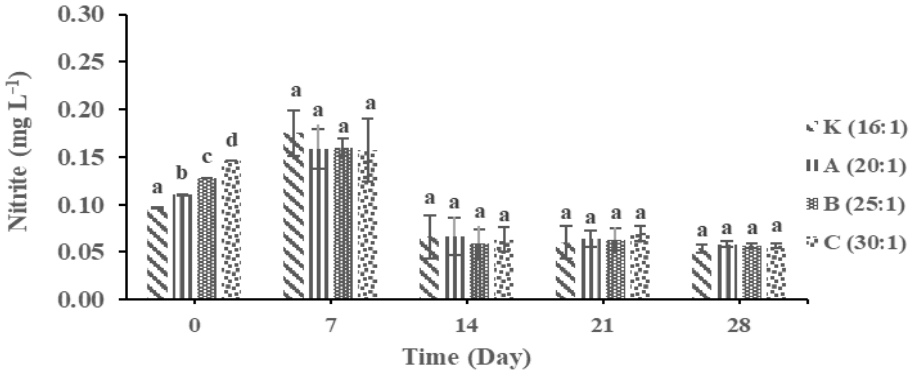

Fig. 6. Nitrite (NO_2^-) content during the study fed with fertilizer at different N:P doses (ratios)

5. Nitrate

Nitrate measurements on day 0 showed significant differences between treatments (*P*-value < 0.05), indicating that different N:P ratios affect nitrate content. On day 7, nitrate levels decreased, with treatment K (16:1) showing a significant difference compared to treatments A (20:1), B (25:1), and C (30:1) (*P*-value < 0.05). Observations on day 14 revealed an increase in nitrate levels, where treatment K (16:1) was significantly different from treatment C (30:1) but not significantly different from treatments A (20:1) and B (25:1) (*P*-value < 0.05). Observations on days 21 and 28 showed that nitrate content across all treatments did not differ significantly (*P*-value > 0.05). The nitrate content throughout the rearing period is illustrated in Fig. (7).

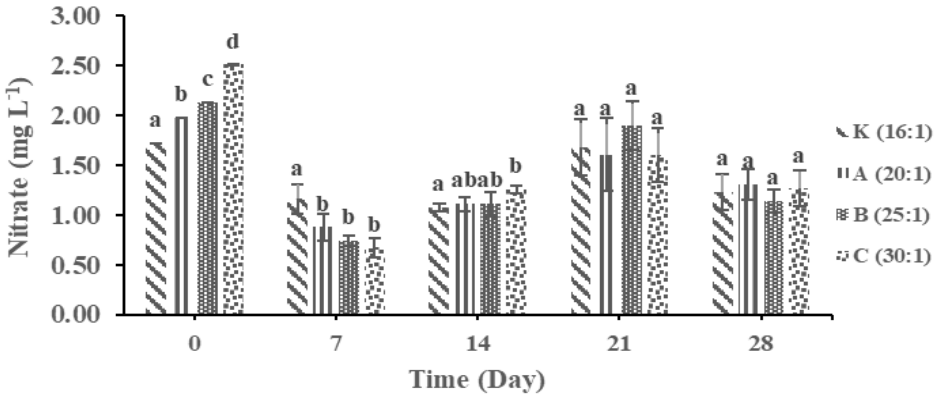

Fig. 7. Nitrate (NO_3^-) content during the study fed with fertilizer at different N:P doses (ratios)

6. Phosphate

The results showed that phosphate content on day 0 did not differ significantly between treatments (*P*-value > 0.05). On day 7, phosphate levels increased, with no significant differences observed across treatments. Observations on day 14 indicated a decrease in phosphate levels, which then increased again on day 21. By day 28, phosphate content in the water had decreased, with treatment A (20:1) showing a significant difference compared to treatment C (30:1) and no significant difference compared to treatments K (16:1) and B (25:1) (*P*-value < 0.05). The phosphate levels throughout the rearing period are presented in Fig. (8).

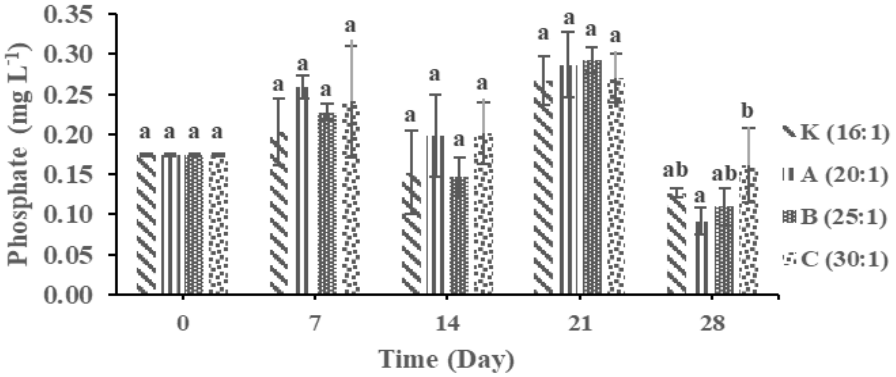

Fig. 8. Phosphate (PO_4) content during the study fed with fertilizer at different N:P doses (ratios)

7. Survival rate (SR)

The survival rate of the whiteleg shrimp (*L. vannamei*) during the rearing period was recorded with the highest average value in treatment K (40.58 ± 1.08). The analysis showed that treatment K obtained significantly different results from treatments A, B, and

C (*P*-value<0.05). The survival rate of the whiteleg shrimp (*L. vannamei*) during the rearing period can be seen in Fig. (9).

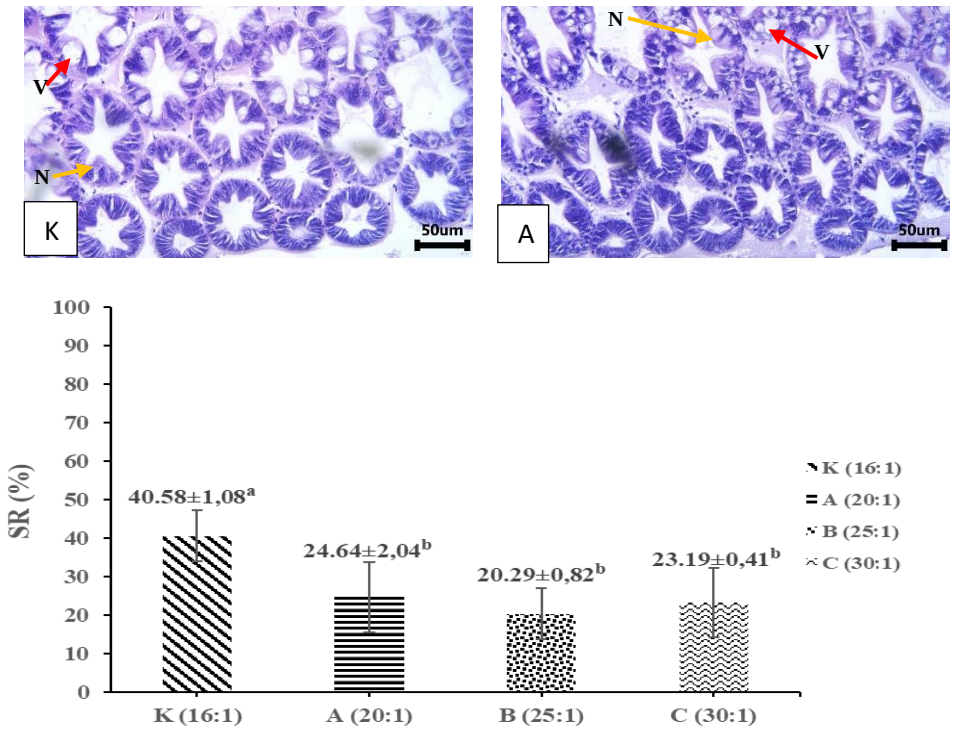

Fig. 9. Survival rate (SR) content during the study fed with fertilizer at different N:P doses (ratios)

8. Histopathology

Observations of the histopathology of the pancreas in the vannamei shrimp revealed necrosis and vacuolization of the hepatopancreas in all treatments. The hepatopancreas in treatments K (16:1), A (20:1), B (25:1), and C (30:1) showed damage characterized by tubular necrosis. The histological results are presented in Fig. (10).

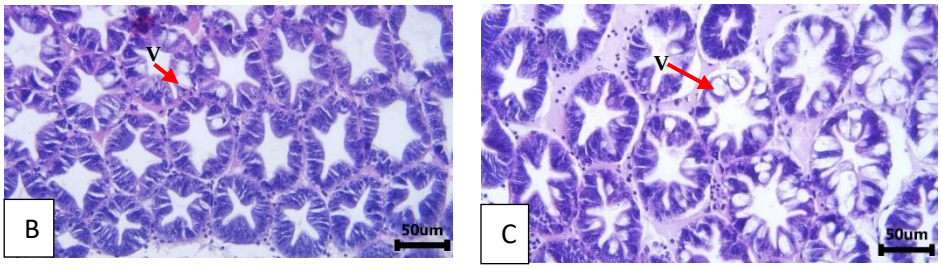

Fig. 10. Histology of hepatopancreas content during the study fed with fertilizer at different N:P doses (ratios)

Description: K (16:1), A (20:1), B (25:1), C (30:1), V= Vacuolization, N= Necrosis.

9. Total hemocyte count (THC)

The observation of total hemocyte count (THC) showed no significant difference in THC value between treatments (*P*-value > 0.05). The highest to lowest THC values are as follows: treatment C (36.11 ± 5.32), treatment B (34.33 ± 3.89), treatment A (31.33 ± 4.63), and treatment K (27.56 ± 4.76). THC values are presented in Fig. (11).

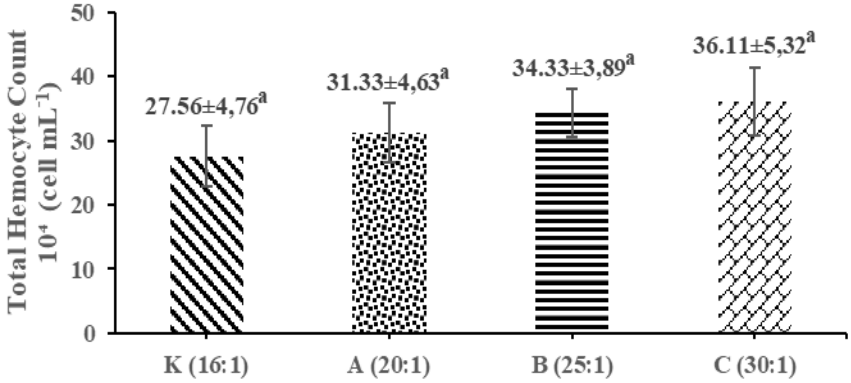

Fig. 11. Total hemocyte count content during the study fed with fertilizer at different N:P doses (ratios)

10. Water quality in maintenance containers

The observed water quality parameters include temperature, salinity, Dissolved Oxygen (Do), and pH. The results of the water quality observations throughout the rearing period are presented in Table (2).

Table 2. Water quality in rearing containers treated with different N:P ratios

Treatment	Parameters			
	temperature (ºC)	**Salinity (g/l)**	**pH**	**Do (g/l)**
K (16:1)	28-28.2	25-26	7,76-6.70	6,8-7.4
A (20:1)	28-28.2	25-26	7,77-6.62	6,8-7.2
B (25:1)	28-28.1	25-26	7,75-6.71	6,8-7.3
C (30:1)	28-28.1	25-26	7,78-6.72	6,8-7.3
Standard (SNI 8037.1:2014)	27-33	10-35	7,5-8,5	>4.0

DISCUSSION

The results of phytoplankton abundance measurements showed an increase on day 7, which can be attributed to the treatment applied on day 0, specifically the addition of nitrogen and phosphate. Adding these nutrients caused an increase in nutrient levels (nitrogen and phosphate) in the water, leading to a significant rise in phytoplankton growth. **Fachrul *et al.* (2005)** stated that nitrogen and phosphate are essential for phytoplankton or microalgae growth. Nitrogen is crucial in the formation of amino acids, nucleic acids, and metabolic cofactors during phytoplankton metabolism **(Alianto, 2011; Kumar & Bera, 2020)**. Phosphate (PO_4) is another vital nutrient for aquatic organisms, playing a key role in energy transfer from ADP (Adenosine Diphosphate) to ATP (Adenosine Triphosphate) in cell mitochondria **(Lin *et al.*, 2016)**. The decrease in phytoplankton abundance on day 14 indicates that they have entered the death phase, as their nutrient needs are no longer being met. This occurs when phytoplankton enter the stable phase and nutrient availability declines after being depleted during the exponential growth phase. **Tamama and Asadi (2024)** suggested that environmental conditions significantly affect phytoplankton growth, with factors such as water quality, nutrient availability, light intensity, and water flow likely contributing to variations in phytoplankton abundance

The results of the relative abundance study showed that on day 0, the relative abundance of phytoplankton was dominated by Cyanophyceae species. By day 7, there was a shift in the relative abundance of phytoplankton in each treatment, with Bacillariophyceae species becoming more prominent. On day 14, Bacillariophyceae species continued to dominate the relative abundance of phytoplankton in treatments K, A, and C, while in treatment B, Cyanophyceae species became dominant again. By day 21, all treatments showed a similar dominance of Cyanophyceae species. The supremacy of Bacillariophyceae species on days 7 and 14 can be attributed to their ability to adapt to environmental changes, particularly in polluted conditions, as well as their faster reproductive rate compared to other phytoplankton groups **(Wahyuningsih, 2024)**. The

decline in Bacillariophyceae abundance on days 14 and 21 was likely due to insufficient nitrogen (N) content in the water, leading to a nutrient competition between organisms until phytoplankton entered the death phase. Without new phytoplankton or water exchange, Bacillariophyceae species decreased in the treatment media. In contrast, Cyanophyceae species were able to survive because they can acquire nitrogen from outside the water. When nutrient competition occurs, Cyanophyceae are less affected because some species of this group, such as the blue-green algae (BGA), can fix nitrogen (N_2) from the air **(Supono, 2015)**. However, not all Cyanophyceae species can fix N_2 directly from the air. Generally, these species prefer to take up nitrogen in the form of NH_4^+. According to **Harris *et al.* (2016)**, cyanobacteria (Cyanophyceae) that cannot fix N_2 are favored when NO_3^- levels are depleted in the water, but NH_4^+ is simultaneously supplied or recycled at adequate levels for phytoplankton growth. The preference of Cyanophyceae species for NH_4^+ is due to the fact that nitrate/nitrite is more efficiently utilized by eukaryotic plankton species (diatoms, dinoflagellates) than by prokaryotes (Cyanobacteria), while prokaryotes assimilate reduced forms of nitrogen, such as NH_4^+, more efficiently than eukaryotes. This supports previous studies showing that cyanobacteria exhibit a high preference for NH_4^+ uptake, while diatoms show a greater preference for NO_3^- uptake **(Swarbrick *et al.* 2019)**.

The increase and decrease in total ammonia nitrogen (TAN) content are believed to be influenced by the nitrification process. Additionally, the decrease in TAN content in the water is attributed to the uptake of NH4+ by phytoplankton. One type of phytoplankton that utilizes NH4+ as a nitrogen source is Cyanophyceae. According to **Harris *et al.* (2016)**, Cyanophyceae phytoplankton prefer nitrogen in the form of NH4+, whereas they utilize NH3 less due to the nitrification process in the water, which converts NH3 into NO3-

Increased and decreased nitrite content is thought to be influenced by the nitrification process; besides that, nitrite can also affect the presence and diversity of phytoplankton. According to **He *et al.* (2022)**, based on BIOENV and VIF analysis testing, it can be concluded that nitrite is one of the critical environmental factors that influence the presence and diversity of phytoplankton species in waters.

The analysis of nitrate content during the rearing period indicated that the water media used fell within the criteria for mesotrophic waters in terms of fertility levels. According to **Kusumaningtyas (2010)** and **Mishbach *et al.* (2021)**, the classification of waters based on fertility levels includes oligotrophic waters with nitrate levels of 0–1mg/ L, mesotrophic waters with nitrate levels of 1–5mg/ L, and eutrophic waters with nitrate levels of 5–50mg/ L. Furthermore, fluctuations in nitrate content are believed to result from nitrate uptake by phytoplankton as a nutrient source. Algal cells can use nitrogen substrates such as NH4+ and NO3- to grow **(Glibert *et al.*, 2015)**.

The results of the phosphate content analysis during the rearing period showed that the phosphate content in the water media used was included in the criteria for waters with

fertile categories. According to **Patty *et al.* (2015)**, the classification of water fertility levels based on phosphate levels (mg/l) is divided into several fertility levels, namely 0 - 0.002 for the less fertile category, 0.0021 - 0.050 for the moderately fertile category, 0.051 - 0.100 for the fertile category, and 0.101 - 0.200 for the very fertile category, and > 0.201 for the very fertile category.

The shrimp survival rate observed during the study indicated that the N:P ratio treatments influenced shrimp survival. This effect is attributed to the abundance and composition of phytoplankton in each treatment. Certain types of phytoplankton can have negative impacts. For instance, harmful algal blooms (HABs) dominated by diatoms such as Chaetoceros, Ditylum, Guinardia, Odontella, Pseudo-nitzschia, Skeletonema, and Thalassiosira are potentially toxic, causing significant fish mortality and adversely affecting ecosystems **(Qi *et al.*, 2004)**. Nitzschia, globally widespread diatoms produce domoic acid (DA), a neurotoxin responsible for human illnesses and marine animal deaths **(Trainer *et al.*, 2012)**. In addition to diatoms, cyanobacteria and dinoflagellates also contribute to shrimp mortality. The dominance of cyanobacteria and dinoflagellates in water bodies is highly undesirable, as the toxins they produce can result in shrimp deaths or reduced growth rates **(Lyu *et al.*, 2021)**.

The histopathological examination of the shrimp pancreas in each treatment showed damage in the form of necrosis and vacuolization of tubules. **Dharmawan *et al.* (2020)** stated that the characteristics of vacuolization include the presence of round empty spaces that occur due to fat accumulation in hepatopancreatic tubules. Factors causing vacuolization include the accumulation of toxic materials, lack of oxygen, or excess fat consumption, which can interfere with metabolic processes. Necrosis is acute cell damage that can be focal or massive, causing the tissue to no longer form due to complete shrinkage or shrinkage of the nucleus. Cell necrosis can be caused by biological agents such as viruses, bacteria, fungi, and parasites **(Dharmawan *et al.*, 2020)**. One of the causes of hepatopancreas damage is toxins produced by phytoplankton such as cyanobacteria. According to **Wiegand and Pflugmacher (2005)**, cyanobacteria toxins are classified into five main groups: *hepatotoxins, neurotoxins, cytotoxins, dermatotoxins,* and *irritant toxins (lipopolysaccharides)*. Hepatotoxins are toxins that attack the liver, with microcystins being one type of hepatotoxin produced by cyanobacteria, such as *Microcystis, Anabaena, Planktothrix (Oscillatoria), Anabaenopsis, Nostoc, and Hapalosiphon*. Microcystins accumulate in vertebrate liver cells through an active transport mechanism by a highly expressed non-specific organic anion transporter (bile acid carrier transport system). **Vasconcelos *et al.* (2001)** reported that in the crayfish samples, microcystin accumulation was found in the hepatopancreas, but the toxin was not detected in muscle tissue.

The analysis of total hemocyte count (THC) values showed consistently high levels across all treatments, indicating that the vannamei shrimp experienced stress in each treatment. According to **Ekawati *et al.* (2012)**, an elevated number of hemocytes in the

hemolymph reflects the body's inability to respond effectively, making it an immune stressor that can weaken the immune system. Prolonged high-stress conditions can compromise shrimp health, increasing their susceptibility to pathogen attacks **(Djai *et al.* 2017)**.

The water quality measurements for temperature, salinity, and dissolved oxygen (DO) showed that the parameter values remained within the optimum range according to SNI. However, the pH values in each maintenance medium were outside the optimal range defined by SNI. This is believed to be due to the decomposition of organic matter in the water, which leads to a decrease in pH. **Araoye (2009)** and **Hastuti *et al.* (2023)** stated that a reduction in pH at the bottom of the water indicates increased microbial activity in the decomposition of organic matter, resulting in decreased O_2 levels and increased CO_2 concentrations.

CONCLUSION

An unbalanced N:P ratio in water can affect phytoplankton's number and relative abundance. The results showed that when phytoplankton were eutrophied on day 7, and the nutrient content in the water was no longer sufficient for the needs of the phytoplankton population, and this could cause phytoplankton mortality in the following days (days 14 to 21). In addition, fluctuating nutrient content can affect the N:P ratio in the water. Instability of the N:P ratio could potentially result in changes in the species composition and the relative abundance of phytoplankton in the water body. The lowest survival rate in this study was found in treatment B, which was 20.29%, while the highest survival rate was found in the control treatment, which was 40.58%.

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