

## Impact of Super Intensive Pond Waste on Plankton Dynamics and Abundance

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### ABSTRACT

Super-intensive *L. vannamei* cultivation produces waste that causes pollution, while simultaneously an appropriate water source, meeting water quality standards, is required for its sustainability. Hence, aquaculture waste poses a significant challenge in minimizing the source of water pollution. Therefore, this study aimed to determine the physicochemical and biological properties of the quality of water source, used for the super-intensive *L. vannamei* farming. The experiment was conducted at 18 points of the water source of super-intensive *L. vannamei* cultivation. Samples were categorized into three groups: vertical at distances of 0, 100, and 200 meters, while horizontal consisted of 6 points. The study found 21 plankton genera comprising 13 phytoplankton and 8 zooplankton with low diversity ( $H'$ ) and stability. Plankton uniformity ( $E$ ) below  $0.4 \leq E \leq 0.6$  indicates low uniformity, and no dominant individuals ( $D$ ) were detected. The temperature, pH, TSS, and BOT correlation showed a moderate relationship. The variables  $NO_3$ ,  $NO_2$ ,  $PO_4$ , and DO were in the low category, while salinity,  $NH_4$ , and  $NH_3$  showed a very low correlation. In terms of distance, a significant difference ( $P > 0.05$ ) was detected in water quality based on Permen KP. No. 75 of 2016. Thus, the seawater quality standards for super-intensive vannamei shrimp ponds are in appropriate conditions, and the seawater quality in front of the super-intensive pond as a quality standard is suitable for cultivation.

### INTRODUCTION

*L. vannamei* shrimp farming is rapidly developing due to its ability to meet the global need for protein (Bondad-Reantaso *et al.*, 2005; Madani *et al.*, 2018; Zhang *et al.*, 2024). However, *L. vannamei* cultivation produces solid and liquid waste, polluting seawater as a source of clean water. Suwoyo *et al.* (2019) confirmed that the solid waste produced reached a weight of 18.2, 20.3 and 21.9 tons, respectively, at a density of 750-1,250 ind/m<sup>2</sup>, with high N and P contents. Meanwhile, liquid waste has TN and TP concentrations that exceed the quality standards for shrimp cultivation waste (Fahrur *et al.*, 2015). Waste is discharged into seawater, directly impacting seawater quality and can cause eutrophication.

The need for clean water to produce 1kg of shrimp is between 1.60-2.75L at a density of 750-1,250 ind/m<sup>2</sup>. Meanwhile, shrimp farming in Indonesia is a commercial activity along coastal areas with high production levels, reaching 7-12 tons per 1,000m<sup>2</sup> (Syah *et al.*, 2017;Khoa *et al.*, 2020; Rakhmanda *et al.*, 2021; Braga *et al.*, 2023). It requires clean water between 1.60-2.75L/kg of shrimp, with higher density showing the need for good water quality, so the higher the density, the higher the water demand.

The seawater quality standards for shrimp are essential to be measured continuously as initial information for making appropriate water management decisions for *L. vannamei* (Liu *et al.*, 2023). The decline in water quality can be identified from physical, chemical, and biological water quality parameters (Luhede *et al.*, 2024; Oliveira *et al.*, 2024; Song *et al.*, 2024). Physical parameters include total suspended solids (TSS) causing turbidity, while ammonia can lead to the death of aquatic organisms. In comparison, the composition of bacteria and plankton can cause blooms which impact marine biota and cultivation (Sgarzi *et al.*, 2019; Vinothkumar *et al.*, 2021; Satanwat *et al.*, 2023). Therefore, monitoring the abundance of plankton and water quality in seawater used for cultivating *L. vannamei* shrimp is very important, therefore this research aimed to address the composition and dynamics of plankton in *L. vannamei* cultivation water source.

## MATERIALS AND METHODS

The study was conducted on the Topek Jawa coast, which is part of the administrative area of Manggara Bombang sub-district, Takalar Regency, South Sulawesi, Indonesia, where there are super-intensive ponds that use seawater as a source of clean water and receive waste from aquaculture. Hence, sampling was conducted at three distance groups to see the impact on water quality and plankton dynamics. These included St1, St2, St3, St4, St5, and St6 as 0M or 0 meters from the shore at low tide, followed by St7, St8, St9, St10, St11, and St12 as 100M or 100 meters from the 0M point. Lastly, St13, St14, St15, St16, St17, and St18 are 200M or 200 meters from the 0M point, as shown in Fig. (1).



**Fig. 1.** Seawater sampling station in front of super-intensive ponds

## 1. Sample collection and preparation

Surface plankton sampling was conducted by filtering 100L of water using a plankton net with a diameter of 30cm and a mesh size of 25 $\mu$ m, equipped with a 100ml bottle. The sample was placed into a 100ml polyethene bottle and preserved with 6 drops of Lugol's solution.

## 2. Calculation of abundance and plankton index

The quantitative analysis of plankton was observed and calculated with the help of Sedwick-Rafter counting cells, which were calculated according to **Welech (1952)**. The sampling method and plankton abundance were calculated using the formula proposed by **Rosenberg (1976)**:

$$N = O_i/O_p \times V_r/V_o \times 1/V_s \times n/p$$

Where, N - Total individuals per liter;  $O_i$  - Area of the preparation cover glass ( $\text{mm}^2$ );  $O_p$  - Area of one field of view ( $\text{mm}^2$ );  $V_r$  - Volume of filtered water (mL);  $V_o$  - Observed water volume (mL);  $V_s$  - Volume of filtered water (L); n - Total plankton in the entire field of view; p - Total field of view observed.

The diversity index was calculated using the formulas by **Shannon and Weaver (1964)** and **Somerfield et al. (2008)**.

$$H' = - \sum P_i \ln P_i \quad P_i = n_i/N$$

Where,  $H'$  - Species diversity index;  $n_i$  - Total individuals of the  $i$ th taxa; N - Total individuals;  $P_i$  - Proportion of species. Classification of biota community conditions based on  $H'$  is as follows:  $H' < 2,30$  (Little diversity and low community stability),  $2,30 < H' < 6,91$  (Medium diversity and moderate community stability),  $H' > 6,91$  (High diversity and high community stability).

The uniformity index was calculated based on the formula:

$$E = H'/H_{\text{max}}$$

Where, E - Species uniformity index;  $H'$  - Species diversity index;  $H_{\text{max}}$  - maximum diversity index. The classification of the species diversity index (E) is as follows:  $E < 0,4$  (Low category),  $0,4 < E < 0,6$  (Medium category),  $E > 0,6$  (High category).

The dominance index was calculated using the formula:

$$D = \sum_{i=1}^s [n_i/N]^2$$

Where,  $D$  - Simpson's dominance index;  $N_i$  - Total  $i$ -th individual;  $N$  - Total Individual;  $S$  - Total types. Under the condition. Generally, the  $D$  value, which falls from 0 to 1, indicates population dominance. Plankton observations were made using an Olympus electronic microscope with 100x magnification. Subsequently, the obtained plankton abundance data were used as the basis for calculating the diversity index ( $H'$ ), the evenness index ( $E$ ), and the dominance index ( $D$ ).

### 3. Water quality

The *in-situ* water quality parameters measured were temperature, salinity, pH, and dissolved oxygen, while *ex-situ* ones were ammonia ( $\text{NH}_3$ ), ammonium ( $\text{NH}_4$ ), nitrite ( $\text{NO}_2$ ), nitrate ( $\text{NO}_3$ ), phosphate ( $\text{PO}_4$ ), total suspended solids (TSS), and total organic matter (TOM). Samples were taken from the water surface using 1000ml polyethene bottles.

### 4. Data analysis

Analysis of variance (ANOVA) was used to analyze seawater quality data, while the relationship between plankton abundance and physicochemical properties of water was conducted using Pearson correlation applying SPSS 25 and Excel tools. The results obtained from the analysis are presented in the form of Tables and Figs.

## RESULTS

### 1. Abundance and plankton class

The calculation results of plankton in the water source of super intensive *L. vannamei* shrimp ponds identified 21 genera of plankton, consisting of 13 phytoplankton and 8 zooplankton. Different distances showed varying numbers of genera, with the 100M distance having the highest number of 19 genera, compared to the 0M (16) and the lowest at the 200M (13 genera). The resulted in the 50M distance having  $567 \pm 94.50$  Ind/L, higher than 309 Ind/L at the 0M and 375 Ind/L at the 100M distance. The phytoplankton with the highest numbers and present at all sampling distances was *Coscinodiscus* sp., while the lowest was *Thalassionema*, found only at the 0M distance. *Acartia* sp. was the zooplankton genus with the highest numbers at all sampling points, while *Copepod* sp. and *Polychaeta* sp. had the lowest numbers and were found only at the 100M distance.

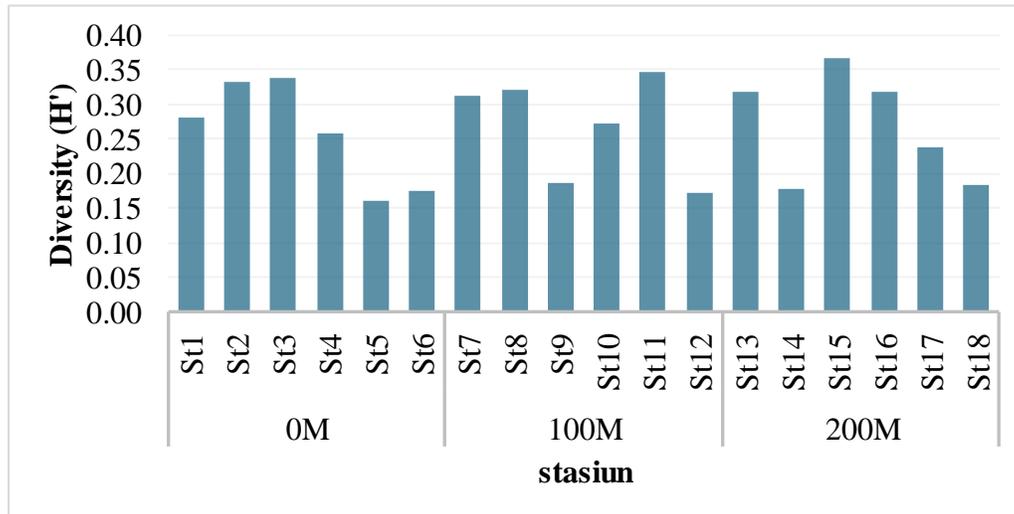
**Table 1.** Abundance and class of plankton in super intensive *L. vannamei* water source

Plankton class	Genus (ind/L)	0M	100M	200M
Fitoplankton				
<i>Diatom/Bacillarophyceae</i>	<i>Biddulphia</i> sp.	0	31	22
	<i>Cerataulina</i> sp.	20	10	0
	<i>Chaetoceros</i> sp.	10	143	0
	<i>Coscinodiscus</i> sp.	41	83	116
	<i>Gyrosigma</i> sp.	20	33	11
	<i>Licmophora</i> sp.	61	11	110
	<i>Melosira</i> sp.	10	11	0
	<i>Navicula</i> sp.	0	66	53
	<i>Nitzschia</i> sp.	72	22	0
	<i>Pleurosigma</i> sp.	11	74	21
	<i>Rhizosolenia</i> sp.	53	62	21
	<i>Thalassionema</i> sp.	11	0	0
	<i>Cyanophyceae</i>	<i>Oscillatoria</i> sp.	0	21
	<b>SUM</b>	<b>309.00</b>	<b>567.00</b>	<b>375.00</b>
	<b>AVG</b>	<b>51.50</b>	<b>94.50</b>	<b>62.50</b>
Zooplankton class				
<i>Crustacea</i>	<i>Acartia</i> sp.	476	527	207
	Copepoda sp.	0	11	0
	Nauplii copepoda sp.	111	182	91
	<i>Microsetella</i> sp.	10	30	32
	<i>Oithona</i> sp.	61	72	31
	<i>Temora</i> sp.	10	11	0
	<i>Gastropoda</i>	Larva mollusca sp.	10	0
<i>Polychaeta</i>	Polychaeta sp.	0	11	0
	<b>SUM</b>	<b>678.00</b>	<b>844.00</b>	<b>542.00</b>
	<b>AVG</b>	<b>113.00</b>	<b>140.67</b>	<b>90.33</b>

## 2. Diversity of plankton

The diversity index at a distance of 0M was 0.26, while distances of 100M and 200M showed diversity values of 0.27 and 32, respectively. Based on the station, the highest diversity was at St3, and the lowest was observed at St5 (0M). At 100M, St11 had the highest, while the lowest diversity was at St12. Furthermore, at a distance of 200M, St 15 was the highest, and the lowest value was found at St14, as shown in Fig. (2). Plankton abundance data were used to calculate index diversity ( $H'$ ). Based on categorization,  $H' < 2.30$  has low community stability,  $2.30 < H' < 6.91$  medium diversity, and  $H' > 6.91$  high

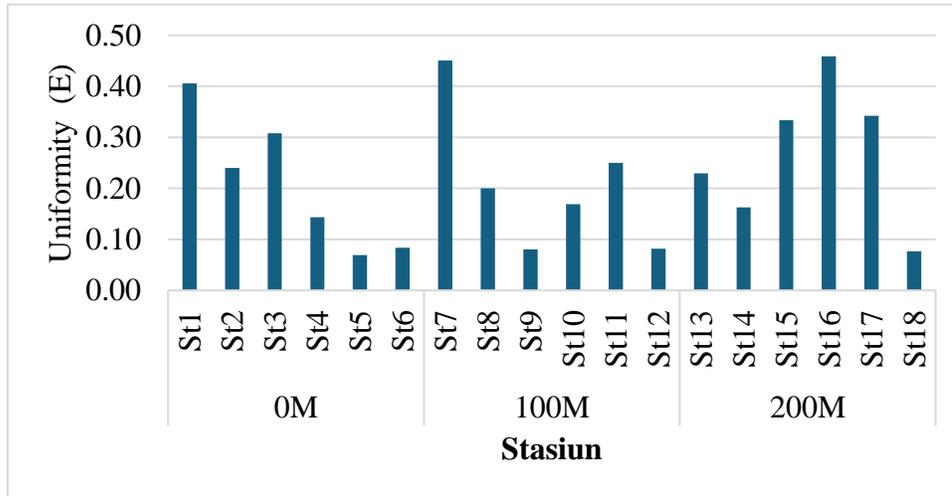
diversity and high community stability (Krebs, 2014). This showed low diversity and stability at distances of 0M, 100M, and 200M.



**Fig. 2.** Diversity of plankton at each station

### 3. Uniformity of plankton

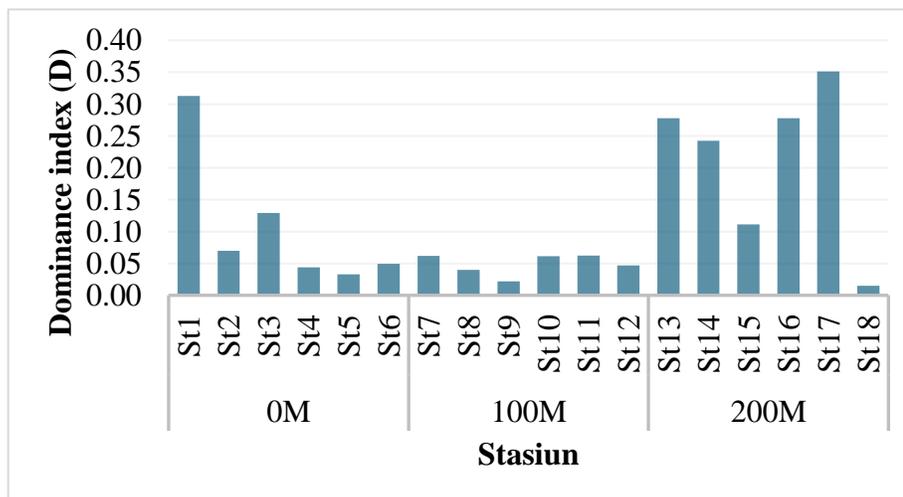
The uniformity within each observation distance group followed a similar pattern, showing high and low uniformity. Stations St1, St7, and St13, sampling points on a straight horizontal line at distances of 0M and 100M, showed higher uniformity than the 200M distance. At St6 (0M), St12 (100M), and St18 (200M) on the vertical line, there was low uniformity, while a horizontal line of each distance showed fluctuating values. At the 0M distance, St5 had the lowest uniformity, and St1 had the highest. Meanwhile, at the 100M distance, St7 has the highest, with St9 and St12 showing low values. At the 200M distance, St18 had the lowest uniformity, and St16 had the highest, as shown in Fig. (3). Plankton uniformity shows an even distribution, where values are within the  $0.4 \leq E \leq 0.6$  range, assuming that no species dominates each population within the community (Krebs, 2014).



**Fig. 3.** Uniformity of plankton at each station

#### 4. Dominance index

Based on previous research, the dominance index (D) ranges from 0-1 (**Odum & Srigandono, 1993**). When the dominance index value is close to 0, no individual dominates the community, while a value close to 1 shows that individuals dominate. In super intensive *L. vannamei* shrimp ponds at distances of 0M, 100M, and 200M, no values exceed 1. This shows that no individual dominates, although St17 at the 200M distance has the highest value, as shown in Fig. (4).



**Fig. 4.** Dominance index at each station

## 5. Water quality

The results of statistical analysis of distance for water quality using the analysis of variance (ANOVA) showed no significant effect  $P>0.05$  between distances of 0 M, 100 M, and 200 M for temperature, salinity, pH, DO, PO<sub>4</sub>, NH<sub>4</sub>, NH<sub>3</sub>, NO<sub>2</sub>, NO<sub>3</sub>, TSS, and BOT. However, the concentration of water quality variables of super-intensive pond sources is below the threshold of standard requirements (Ministerial Regulation of Marine Affairs and Fisheries No. 75 of 2016). Thus, the water quality of super-intensive vannamei shrimp pond sources is suitable for shrimp cultivation.

**Table 2.** Water quality during the study

Variable	0 M		100 M		200 M		Super intensive Water Quality Standards
	Average	Stdv	Average	Stdv	Average	Stdv	
Temperature (°C)	30.95 <sup>a</sup>	0.46	30.79 <sup>a</sup>	0.28	30.90 <sup>a</sup>	0.29	28-30
pH	8.31 <sup>a</sup>	0.39	8.39 <sup>a</sup>	0.33	8.44 <sup>a</sup>	0.31	7,5-8,5
Salinity (‰)	32.63 <sup>a</sup>	0.14	32.65 <sup>a</sup>	0.13	32.57 <sup>a</sup>	0.07	26-32
DO (mg/L)	6.98 <sup>a</sup>	0.52	7.17 <sup>a</sup>	0.72	7.17 <sup>a</sup>	0.72	>4
PO <sub>4</sub> (mg/L)	0.0003 <sup>a</sup>	0.0004	0.0003 <sup>a</sup>	0.0004	0.0003 <sup>a</sup>	0.0004	0,1-5
NH <sub>4</sub> (mg/L)	0.0121 <sup>a</sup>	0.0060	0.0129 <sup>a</sup>	0.0038	0.0135 <sup>a</sup>	0.0069	-
NH <sub>3</sub> (mg/L)	0.0148 <sup>a</sup>	0.0073	0.0156 <sup>a</sup>	0.0046	0.0164 <sup>a</sup>	0.0084	≤0,1
NO <sub>2</sub> (mg/L)	0.0003 <sup>a</sup>	0.0005	0.0004 <sup>a</sup>	0.0008	0.0003 <sup>a</sup>	0.0004	≤1
NO <sub>3</sub> (mg/L)	0.2957 <sup>a</sup>	0.2442	0.2906 <sup>a</sup>	0.2419	0.2545 <sup>a</sup>	0.1806	0,5
TSS (mg/L)	45.17 <sup>a</sup>	18.20	36.33 <sup>a</sup>	13.08	42.83 <sup>a</sup>	19.24	-
TOM (mg/L)	57.45 <sup>a</sup>	7.95	58.42 <sup>a</sup>	7.49	56.83 <sup>a</sup>	7.50	≤90

## DISCUSSION

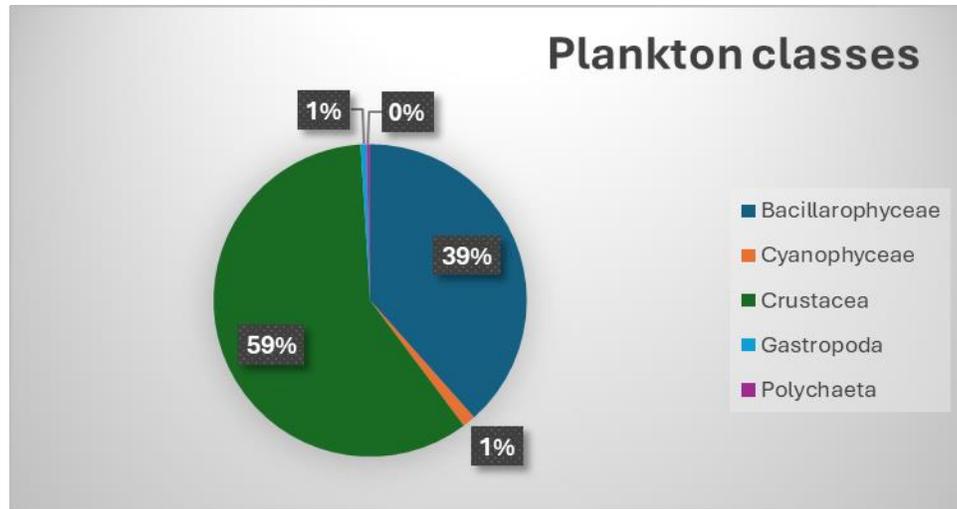
Based on the statistical analysis results, the correlation between water quality and plankton abundance shows that temperature, pH, TSS, and BOT have a close relationship between 0.40-0.59, classified as moderate. The variables NO<sub>3</sub>, NO<sub>2</sub>, PO<sub>4</sub>, and DO are included in the low and salinity categories, while NH<sub>4</sub> and NH<sub>3</sub> are included in the deficient categories. The impact of superintensive cultivation waste comes from wastewater containing high concentrations of N and P, causing eutrophication of seawater, while solid waste causes sediment buildup at the bottom of the waters, threatening micro and macrobenthos. A research study of *L. vannamei* waste with superintensive density discharged directly into waters at a density of 600 ind/m<sup>2</sup> obtained 10.31kg/ day N and 0.81kg/mday P (Paena *et al.*, 2020). While at the same density in this research location, it reached 362kg TN and 119 kg TP, impacting 43 hectares (Syah *et al.*, 2014). However,

these conditions do not directly impact water quality, ultimately impacting plankton abundance.

**Table 3.** Correlation of water quality and plankton abundance

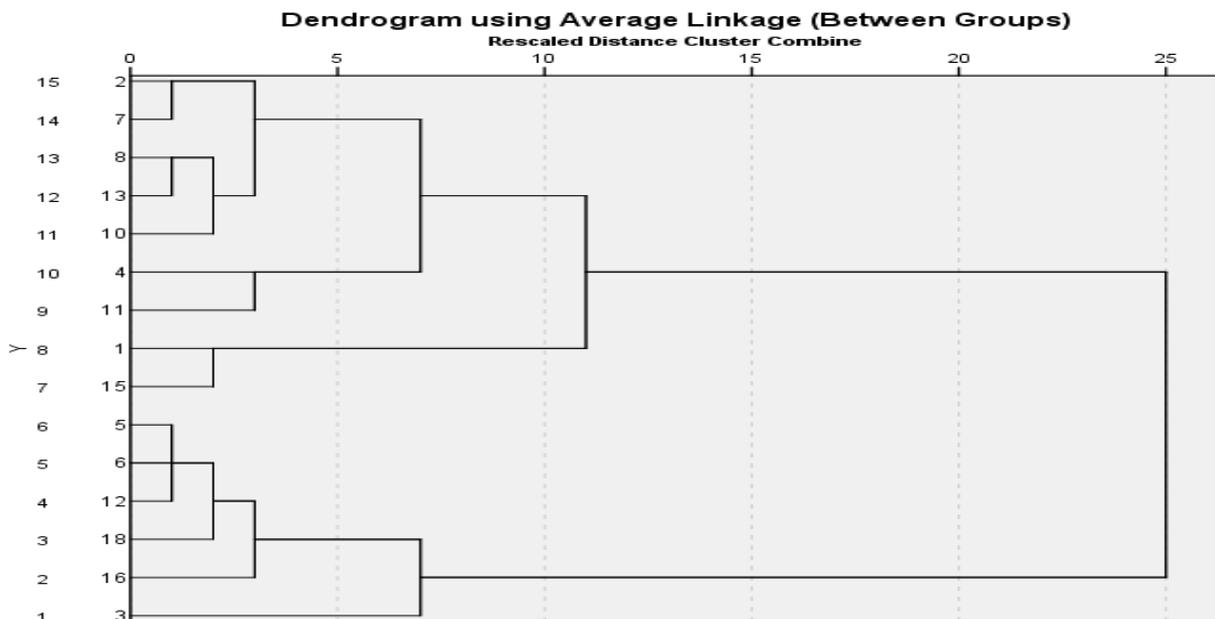
Factor	Correlation	Equality	R <sup>2</sup>
Suhu (°C)	0,524	Y= 9205,47+-292X	0,229
pH	0,468	Y= -2074,65+269,65X	0,171
Salinity (‰)	0,026	Y= -1179,09+41,793X	-0,062
DO (mg/L)	0,367	Y= 970,74+-110,69X	0,081
PO <sub>4</sub> (mg/L)	0,306	Y= 226,11+-167777,8X	0,037
NH <sub>4</sub> (mg/L)	0,037	Y= 167,35+1307,75X	-0,061
NH <sub>3</sub> (mg/L)	0,037	Y= 167,35+1076,97X	-0,061
NO <sub>2</sub> (mg/L)	0,266	Y=213,74+-85867,9X	0,013
NO <sub>3</sub> (mg/L)	0,195	Y=233,15+-174,79X	-0,022
TSS (mg/L)	0,533	Y=-69,46+6,11	0,240
BOT (mg/L)	0,505	Y=946,44+-13,24	0,209

The low correlation of NO<sub>3</sub>, NO<sub>2</sub>, PO<sub>4</sub>, and DO does not affect their role in phytoplankton growth. Moreover, the lower concentration of NO<sub>2</sub> compared to NO<sub>3</sub> shows that the nitrification process is proceeding normally. This is supported by sufficient oxygen availability, leading to aerobic nitrogen decomposition (**Gilbert *et al.*, 2010**). Water quality conditions in this research confirm that the diatom class of phytoplankton has the highest number of 12 genera, and the Crustacea class of zooplankton has 6 genera, as shown in Table (4). The total population of diatoms reaches 39%, and Crustacea reaches 59%. This happens if the NO<sub>3</sub> concentration is low (**Amengual-Morro *et al.*, 2012**). Diatoms grow and thrive at low concentrations of N and P, proliferating rapidly at high concentrations, potentially leading to blooming. The advantage of diatoms is the frequent availability of phytoplankton in freshwater environments, reaching 85.5% in estuarine to marine areas (**Saggiomo *et al.*, 2021; Oliveira *et al.*, 2022; Wang *et al.*, 2024; Zhao *et al.*, 2024**). In estuarine areas, N and P concentrations fluctuate (**Bermejo *et al.*, 2020**), and diatoms class phytoplankton thrive at high concentrations (**Satanwat *et al.*, 2023**). Diatoms are commonly used to detect various kinds of anthropogenic impacts on flowing areas, such as eutrophication (**Salomoni *et al.*, 2006**), acidification (**Olenici *et al.*, 2017**) and organic pollution (**Mbao *et al.*, 2020**).



**Fig. 5.** Plankton class is found in seawater sources

Based on cluster analysis, plankton sampling points are divided into two groups. Sampling points 1, 2, 4, 7, 8, 10, 11, and 15 are categorized into group one, forming new clusters, with the smallest consisting of two points. Meanwhile, group two has two clusters, forming one new cluster at point 3. The proximity of plankton abundance at each sampling point vertically and horizontally shows varying abundance, even within the same straight line. Plankton are microscopic plants and animals that are easily carried by currents, inhabit bodies of water, and reside in sediment (Huang *et al.*, 2023). Uneven abundance occurs even at the closest to the farthest distances (Pinel-Alloul, 1995; Mouritsen & Richardson, 2003; Alvain *et al.*, 2008).



**Fig. 6.** Relationship between plankton abundance and sampling points

The Minister of Marine Affairs and Fisheries Regulation No. 75 of 2016 (Permen KP. No. 75 Tahun, 2016) has regulated and outlined the source designated for biota. In this research, the standardized water quality criteria for *L. vannamei* shrimp farming meet the requirements regarding both physicochemical and biological aspects. Based on the results, the values obtained were below the threshold limits designated for shrimp farming. This means that water quality is in good condition and can support super intensive shrimp farming. However, regular observations throughout the year or specific seasons are essential to comprehensively assess water quality dynamics. This preventive measure ensures that plankton abundance remains within safe limits and prevents algal blooms. Higher plankton abundance is found at 100M compared to 0M and 200M, with a moderate correlation category influenced by temperature, pH, TSS, and BOT. The concentration of TSS at 100M is lower than 0M and 200M. TSS, consisting of suspended solids that cause water turbidity, obstructs sunlight penetration into water. This phenomenon causes lower temperatures at 100M compared to 0M and 200M, thereby affecting the photosynthesis process of phytoplankton. Generally, temperature in tropical regions is influenced by weather, rainfall (season), currents, and depth (Arai *et al.*, 2023).

Sunlight is also crucial, as clear weather can enhance light absorption by phytoplankton. Photosynthesis uses CO<sub>2</sub> and is influenced by the availability of N and P to produce O<sub>2</sub> (Haworth *et al.*, 2022; Zhang *et al.*, 2023). The availability of O<sub>2</sub> in nature is essential for bacteria to decompose total organic matter (TOM). When O<sub>2</sub> levels and pH are low, there is a tendency for a decrease in density and even the death of a genus (Piscoya *et al.*, 2022). This is because the decomposition process of ammonia produces H<sub>2</sub>S (Koch *et al.*, 2022). The neutral pH in this research shows that the water source in front of super intensive ponds is in good condition.

Moreover, excessively low or high pH can increase the toxicity of ammonia to biota (Cai *et al.*, 2024). Temperature also affects plankton abundance (Wang *et al.*, 2021), as higher values temperatures correlate with greater abundance (Gu *et al.*, 2021; Shaikh *et al.*, 2021). The low correlation between salinity and plankton abundance shows the potential to thrive and adapt to fluctuating salinity levels (Xu *et al.*, 2022).

## CONCLUSION

The source of water used in super intensive cultivation of *L. vannamei* found 21 plankton genera, consisting of 13 phytoplankton and 8 zooplankton, with low diversity (H') and stability. Plankton uniformity (E) below  $0.4 \leq E \leq 0.6$  indicates low uniformity, and there are no dominant individuals (D). The correlation results regarding temperature, pH, TSS, and BOT show a moderate relationship. The variables NO<sub>3</sub>, NO<sub>2</sub>, PO<sub>4</sub>, and DO are in the low category, while salinity, NH<sub>4</sub>, and NH<sub>3</sub> show a very low correlation. Therefore, the water quality and plankton in seawater as a source of super intensive *L. vannamei* ponds are still in good condition.

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