Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. ISSN 1110 – 6131 Vol. 29(3): 2527 – 2545 (2025) www.ejabf.journals.ekb.eg



## Phytoplankton Abundance in relation to Nitrate and Phosphate Dynamics in a Brackish Water Aquaculture Pond System in Marana, Maros Regency, South Sulawesi, Indonesia

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## **ARTICLE INFO**

Article History: Received: March 4, 2025 Accepted: May 18, 2025 Online: June 17, 2025

Keywords: Nutrient, Interactions, Pond, Ecosystem, Aquaculture

#### ABSTRACT

Phytoplankton is a primary food source in aquaculture systems, and the size fractions play distinct roles in nutrient cycling. This study aimed to evaluate the relationship between the abundance of size-fractionated phytoplankton and nutrient concentration in brackish water aquaculture systems in Marana Silvofishery, Maros Regency, South Sulawesi, Indonesia. Phytoplankton were categorized into three size fractions (25, 40, and 60µm), and their abundance was quantified alongside nutrient concentrations. Statistical analyses, including one-way ANOVA and Tukey's HSD test, revealed significant spatial variation in phytoplankton abundance and nutrient levels. Correlation and linear regression analyses revealed that phosphate had a stronger influence on larger phytoplankton size fractions (40 and 60µm) despite consistently higher nitrate concentrations. In contrast, smaller fractions (25µm), likely dominated by picophytoplankton, exhibited higher abundance and adaptability, reflecting their efficient nutrient uptake strategies. Environmental conditions were consistent across the stations, ensuring stable growth conditions. Nitrate concentrations were higher than phosphate levels, but phosphate had a stronger influence on the larger phytoplankton size fractions. Smaller fractions were more abundant, reflecting nutrient uptake efficiency. The results enhance the understanding of phytoplankton-nutrient interactions in brackish water ecosystems and contribute to sustainable aquaculture practices. This study provides insights into the ecological dynamics of phytoplankton in brackish water aquaculture systems, showing the need for balanced nutrient management to optimize natural feed availability.

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## **INTRODUCTION**

Phytoplankton comprise a diverse assemblage of microscopic autotrophic organisms that serve as primary producers in aquatic ecosystems. These organisms





convert inorganic nutrients into organic matter via photosynthesis, thereby forming the foundational base of aquatic food webs. The abundance and distribution of phytoplankton are governed by multiple environmental factors, particularly light availability and concentrations of essential nutrients such as nitrogen (typically in the form of nitrate) and phosphorus (as phosphate) (Karydis *et al.*, 2015; Hatta *et al.*, 2022; Rahmah *et al.*, 2022). In addition, other factors, including water temperature, salinity, hydrodynamics, and predation, significantly influence phytoplankton abundance (Umar & Hatta, 2021; Wu *et al.*, 2022; Chen *et al.*, 2023). Nitrogen and phosphorus are critical for cellular metabolism and growth, and their availability often constrains primary production in aquatic systems. Achieving a balanced ratio between these nutrients is thus essential. Nitrogen often limits marine productivity, while phosphorus tends to limit freshwater and estuarine systems (Xu *et al.*, 2022).

In brackish water ecosystems, salinity, temperature, and light influence phytoplankton dynamics. Salinity gradients create niches that influence the composition of phytoplankton communities, with specific groups thriving within specific ranges (**Zhu** *et al.*, **2023**). Temperature affects metabolic rate and nutrient uptake, favoring cyanobacteria under warmer conditions (**Rasconi** *et al.*, **2017**). These factors interact with nutrient availability, shaping phytoplankton communities and influencing higher trophic levels (**Sharoni & Halevy, 2020**).

Phytoplankton plays a crucial ecological role in aquaculture systems, especially as a natural feed for larvae and other organisms during the early stages. In brackish water aquaculture, where artificial feed may be scarce, the availability of phytoplankton directly affects the growth and survival of the farm species. The efficiency in nutrient assimilation and trophic transfers depends on size fractions, namely nanophytoplankton (2–20 $\mu$ m) (**Creswell, 2010**), picophytoplankton (0.2–2 $\mu$ m) (**Palupi** *et al., 2022*), and microphytoplankton (20–200 $\mu$ m) (**Indrayani** *et al., 2023*). Each fraction plays distinct roles in nutrient cycling, oxygen production, and as a food source. Smaller fractions excel in nutrient uptake and support microbial food webs, while larger fractions facilitate direct energy transfer to zooplankton and fish (**Sekino** *et al., 2007*; **Morán** *et al., 2010*).

Nutrient loading plays a crucial role in altering the composition of phytoplankton communities and overall primary productivity in aquatic ecosystems. Experimental bioassays by **Pinckney** *et al.* (2020) demonstrated rapid responses of phytoplankton to nutrient enrichment, indicating the potential for significant ecological shifts in estuarine environments. Among various environmental drivers, salinity has been identified as a primary determinant of phytoplankton composition in such transitional systems, often interacting with nutrient concentrations to modulate community structure (**Zhu** *et al.*, 2018). Under eutrophic conditions, imbalances in nutrient dynamics can facilitate harmful algal blooms (HABs), disrupting both benthic and pelagic food webs (Whitfield, 2023). Seasonal variability adds further complexity, as changes in temperature, salinity,

and light availability contribute to temporal shifts in phytoplankton size structure (Kim et al., 2023). High nutrient inputs, particularly from anthropogenic sources, tend to shift the dominance from smaller fractions, such as nanophytoplankton, to larger microphytoplankton, which alters trophic interactions and may reduce system resilience (Mo et al., 2023). In this context, understanding the distribution and abundance of sizefractionated phytoplankton in relation to nutrient concentrations is critical for informed aquaculture management. Smaller-size classes, such as picophytoplankton and nanophytoplankton, exhibit high nutrient uptake efficiency and form an essential food base for zooplankton and higher trophic levels. At the same time, micro phytoplankton can help regulate eutrophication by assimilating excess nutrients. However, empirical studies focusing on these relationships in brackish water aquaculture systems remain limited. This is a notable gap given the transitional nature of brackish ecosystems, where dynamic interactions among salinity, nutrient availability, and temperature strongly influence phytoplankton community structure and ecological function (Xu et al., 2022; Zhu et al., 2023).

Maros Regency, located in South Sulawesi, Indonesia, is recognized as one of the province's key centers for brackishwater aquaculture, contributing significantly to regional fish and shrimp production. Within this regency, the Silvofishery Marana area represents a prominent aquaculture zone where brackish water ponds have historically supported intensive farming practices reliant on natural feed sources. However, increasing anthropogenic influences, such as agricultural runoff, aquaculture intensification, and domestic waste discharge, have led to nutrient enrichment, particularly elevated concentrations of nitrate and phosphate, which can significantly alter phytoplankton dynamics and disrupt ecological balance (Kocer & Sen, 2014). In aquaculture ecosystems, shifts in nutrient availability can drive changes in phytoplankton community structure, influencing both natural feed availability and water quality. Understanding the responses of phytoplankton communities, especially across different size fractions, to such nutrient fluctuations is therefore critical for optimizing aquaculture productivity and ensuring ecological resilience. As emphasized by **Yusuf** et al. (2024), sustainable resource management is fundamental to achieving long-term utilization of marine and coastal resources in alignment with national goals under the blue economy framework. This study aimed to evaluate the abundance patterns of three phytoplankton size classes, picophytoplankton, nanophytoplankton, and microphytoplankton, in relation to nitrate and phosphate levels within the Marana pond system. By filling an important knowledge gap on how nutrients affect different sizes of phytoplankton in brackish water aquaculture systems, this study provides valuable insights for enhancing water quality monitoring, more sustainable nutrient management, and supporting the long-term health of aquaculture environments.

## MATERIALS AND METHODS

## 1. Study area

This study was conducted in brackish water aquaculture ponds at Marana Silvofishery, Maros Regency, South Sulawesi, Indonesia, specifically at the Marana Silvofishery Pond Installation, a component of the Smart Fisheries Village (SFV) program, which is an initiative based on Technical Implementation Units, as shown in Fig. (1). The study site is managed by the Center for Brackish Water Aquaculture Research and Fisheries Extension and is characterized by intensive aquaculture practices. Six sampling stations were selected to represent the variability in environmental conditions across the site (Fig. 1).

- Station 1: Source water from the Marana River used for aquaculture.
- Station 2: Polyculture ponds for seaweed (*Gracilaria* sp.) and milkfish (*Chanos* chanos).
- Station 3: Mangrove-filtered water reservoir for aquaculture supply (mangroves serve as a biofilter system).
- Station 4: Monoculture milkfish production ponds.
- Station 5: Inlet and outlet channels of the aquaculture system.
- Station 6: Dock connected to canal; boat access for pond maintenance and sampling.

Sampling was conducted over a two-month period from March to May 2023, with data collected at biweekly intervals.



Fig. 1. Research area (sampling station) at Marana Silvofishery, Maros, Indonesia

## 2. Sampling design and data collection

## 2.1. Environmental parameters

Environmental parameters, including salinity, temperature, dissolved oxygen (DO), and pH, were measured *in-situ* using a YSI DO meter. Secchi disk measurements were used to determine the water transparency, and the parameters were recorded at each station during the sampling period.

## 2.2. Nutrient concentration

Water samples for nutrient analysis were collected using 100mL sample bottles and preserved in Lugol's solution. The concentrations of nitrate ( $NO_3^-$ ) and phosphate ( $PO_{4^{3^-}}$ ) were determined using spectrophotometric methods, following the standard procedures outlined by **APHA** (2017). Nitrate and phosphate ions were analyzed at wavelengths of 543 and 880nm, respectively. This method is based on the principle that these ions react with specific reagents to form colored compounds, the intensity of which is directly proportional to the concentration of the target ions in the sample, allowing for quantitative measurement through spectrophotometry.

## 2.3. Phytoplankton collection and fractionation

Phytoplankton samples were collected using plankton nets with mesh sizes of 25, 40, and 60µm to fractionate the size classes into picophytoplankton, nanophytoplankton, and microphytoplankton, respectively. Sample collection involved filtering 10 liters of water through each mesh, which was then preserved in 100mL bottles with 1% Lugol solution and transported to the laboratory for analysis. Phytoplankton abundance was quantified using a Sedgwick-Rafter cell counting system.

## 3. Data analysis

Statistical analyses were conducted to assess environmental parameters, nutrients, and phytoplankton abundance by size fraction in six sampling stations. One-way analysis of variance (ANOVA) was performed to identify significant differences in environmental variables, nutrients, and phytoplankton abundance by size fractions across stations. When significant effects were observed, Tukey's Honestly Significant Difference (HSD) post hoc test was applied to determine pairwise group differences at a 95% confidence level ( $\alpha = 0.05$ ). Data processing and analysis of variance (ANOVA) were conducted using IBM SPSS Statistics version 25. Data visualization, including annotated boxplots, was carried out using OriginPro 2023. Pearson correlation analysis and distribution plots were generated using RStudio version 4.2.2 to explore inter-variable relationships. Linear regression analysis was also applied to quantify the effect of nutrient concentrations (nitrate and phosphate) on phytoplankton abundance by size fraction using RStudio version 4.2.2.

## **RESULTS AND DISCUSSION**

#### 1. **Environmental parameters**

The environmental parameters measured at all stations (Table 1) indicated favorable conditions that support phytoplankton growth, as suggested by **Cloern (2001)**. These findings are also consistent with the seawater quality standards for marine life outlined in Decree No. 51 of 2004, issued by the Minister of State for the Environment. The analysis of variance (ANOVA) results for the environmental parameters are presented in Table (1).

Table 1. Environmental parameters across six stations in Marana Silvofishery											
Station	Temperature	Oxygen	Salinity	pН	Transparency						
	(°C)	(Mg L <sup>-1)</sup>	(%)		( <b>cm</b> )						
1	$29.25 \pm 1.87$	$3.74\pm0.65^{a}$	$7.17 \pm 11.42$	$7.1\pm0.19^{\rm a}$	$60.5\pm13.18$						
2	$31.22\pm2.17$	$4.35\pm0.77^{ab}$	$5.64 \pm 6.85$	$7.42\pm0.15^{ab}$	$55.6\pm4.77$						
3	$30.1\pm1.25$	$3.87 \pm 1.63^{a}$	$5.55\pm5.88$	$7.34\pm0.58^a$	$63.4\pm5.9$						
4	$32.62\pm3.08$	$6.67 \pm 1.61^{b}$	$3.63 \pm 2.21$	$8.01\pm0.19^{b}$	$61.4\pm7.4$						
5	$29.86 \pm 1.65$	$3.89 \pm 1.21^{a}$	$4.07\pm5.16$	$7.47\pm0.35^{ab}$	$55.6 \pm 14.64$						
6	$29.78 \pm 1.56$	$4.15 \pm 1.32^{ab}$	$7.42 \pm 10.78$	$7.31\pm0.19^{ab}$	$67.0 \pm 21.3$						

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Note: Data are expressed as mean ± standard deviation. Tukey's Honest Significant Difference (HSD) post hoc test was employed for pairwise comparisons. Values sharing the same superscript letter are not significantly different at the 0.05 significance level (P>0.05).

Environmental parameters measured across the six sampling stations exhibited spatial variability in temperature, oxygen, salinity, pH, and water transparency (Table 1). The mean water temperature ranged from  $29.25 \pm 1.87^{\circ}$ C at Station 1 to  $32.62 \pm 3.08^{\circ}$ C at Station 4, with the highest values observed at Station 4. However, these differences were not statistically significant due to overlapping standard deviations. Oxygen concentrations showed considerable variation among stations (P < 0.05). Station 4 recorded the highest oxygen level  $(6.67 \pm 1.61 \text{ mg L}^{-1})$ , which was significantly different from stations 1, 3, and 5. In contrast, stations 2 and 6 showed intermediate values, not differing significantly from either of the two groups. Salinity levels were highly variable, particularly at Station 1  $(7.17 \pm 11.42\%)$  and Station 6  $(7.42 \pm 10.78\%)$ , indicating spatial heterogeneity likely influenced by tidal flux or freshwater intrusion. However, large standard deviations suggest considerable within-station variation. The pH also differed significantly among stations, with Station 4 exhibiting the highest value (8.01  $\pm$  0.19), which was significantly higher than those of stations 1 and 3. Other stations exhibited intermediate pH levels with no significant differences between them. Transparency values ranged from  $55.6 \pm 4.77$  cm at Station 2 to  $67.0 \pm 21.3$  cm at Station 6. Although no

statistical differences were noted for this parameter, stations 3, 4, and 6 tended to exhibit greater water clarity.

#### 2. Nutrient concentrations

The nutrients, including nitrate and phosphate, varied spatially across the six sampling stations, with nitrate consistently exhibiting higher mean values compared to phosphate (Fig. 2).



**Fig. 2.** Boxplot of nutrients across six sampling stations in Marana Silvofishery. Data are expressed as mean ± standard deviation. Tukey's Honest Significant Difference (HSD) post hoc test was employed for pairwise comparisons. Values sharing the same superscript letter are not significantly different at the 0.05 significance level (*P*>0.05).

The nutrients, including nitrate and phosphate, varied spatially across the six sampling stations, with nitrate consistently exhibiting higher mean values compared to phosphate (Fig. 2). Nitrate concentrations ranged from  $0.10\pm0.08$  (Station 3) to  $0.42\pm0.66$  mg/L (Station 4), while phosphate concentrations were markedly lower, ranging from  $0.01\pm0.01$  (Stations 2–3) to  $0.03\pm0.03$  mg/L (Station 4). The highest nitrate level was recorded at Station 4 ( $0.42\pm0.66$  mg/L), followed closely by Station 1 ( $0.41\pm0.49$  mg/L), indicating elevated nutrient inputs at these locations. In contrast, phosphate concentrations were substantial for nitrate, particularly at stations 1 and 4, suggesting heterogeneity in nutrient sources or temporal fluctuations.

## 3. Phytoplankton abundance by size fraction

Phytoplankton abundance showed noticeable variation across stations and among size fractions (25, 40, and  $60\mu m$ ), as presented in Fig. (3).





Data are expressed as mean  $\pm$  standard deviation. Tukey's Honest Significant Difference (HSD) post hoc test was employed for pairwise comparisons. Values sharing the same superscript letter are not significantly different at the 0.05 significance level (P>0.05).

The 25µm fraction consistently displayed the highest mean abundances across all stations, reaching a peak of 1780.5  $\pm$  697.3 cells/L at Station 1 and remaining above 1000 cells/L in most locations. The 40 and 60µm fractions showed comparatively lower mean values, with the lowest abundance recorded in the 60µm fraction at Station 2 (527.0  $\pm$  118.6 cells/L). Station 4 had the highest variability, particularly in the 25µm fraction (2403.0 $\pm$ 2621.8 cells/L), suggesting episodic blooms or input-driven variability. Conversely, Station 2 showed the lowest overall abundances across all fractions, indicating consistently suppressed phytoplankton growth. Notably, the 25µm fraction was significantly different (*P*< 0.05). At multiple stations, while the 60µm fraction was not significantly different (*P*> 0.05), this emphasizes its relatively lower abundance and different ecological response.

#### 4. Correlation and distribution across size fractions

To further explore the relationships among nutrient availability and phytoplankton abundance across various size fractions, a Pearson correlation analysis was conducted, as presented in Fig. (4).

Correlation Matrix: Nutrients and Phytoplankton Abundance											
Nitrate (mg/L) –	1.00	0.44	0.94	0.91	0.88		- 0.9				
Phosphate (mg/L) -	0.44	1.00	0.52	0.48	0.51		- 0.8 5				
Phyto_25 (cell/L) -	0.94	0.52	1.00	0.99	0.94		rrelatio				
Phyto_40 (cell/L) –	0.91	0.48	0.99	1.00	0.97		ى 0.6 -				
Phyto_60 (cell/L) –	0.88	0.51	0.94	0.97	1.00		- 0.5				
	Nitrate (mg/L) -	Phosphate (mg/L) -	Phyto_25 (cell/L) -	Phyto_40 (cell/L) -	Phyto_60 (cell/L) -		-				

Fig. 4. Pearson correlation coefficients on nutrient-phytoplankton across size fractions Significance at 95% confidence level ( $\alpha = 0.05$ ).

The correlation matrix presented in Fig. (4) illustrates strong and statistically consistent relationships between nutrient concentrations (nitrate and phosphate) and phytoplankton abundance across three size fractions (25, 40, and 60 $\mu$ m). Nitrate exhibited a particularly strong positive correlation with all phytoplankton size classes, with coefficients of r = 0.94 for  $25\mu$ m, r = 0.91 for  $40\mu$ m, and r = 0.88 for  $60\mu$ m, suggesting a dominant and uniform influence of nitrate enrichment on phytoplankton growth across the size spectrum. Phosphate also showed moderate to strong correlations, with r values of 0.52, 0.48, and 0.51 for the 25, 40, and 60 $\mu$ m fractions, respectively. While slightly weaker than nitrate, the consistent phosphate-phytoplankton relationships confirm its relevance as a secondary growth factor, likely complementing nitrate uptake in co-limitation scenarios.

In addition, inter-fraction correlations among phytoplankton size classes were remarkably high (r = 0.94-0.99), reflecting a synchronized growth response to environmental drivers, most notably nutrient availability. The near-perfect correlation (r = 0.99) between the 25 and 40µm fractions may indicate shared ecological niches or similar physiological strategies for nutrient acquisition.



Fig. 5. Scatterplot matrix on nutrient-phytoplankton across size fractions

The scatterplot visualization provides an integrative overview of the relationships between nutrient concentrations (nitrate and phosphate) and phytoplankton abundance across three size fractions (25, 40, and  $60\mu$ m), highlighting both distribution patterns and pairwise associations. The histograms along the diagonal depict the distribution of each variable, showing moderate variability in phytoplankton abundance and nutrient concentrations. Notably, the scatterplots reveal positive linear trends between both nitrate and phosphate concentrations with all phytoplankton size classes.

Statistically, Pearson correlation coefficients derived from the corresponding dataset support these visual trends, with nitrate showing strong correlations with phytoplankton abundance (r = 0.94 for 25 µm, r = 0.91 for 40 µm, r = 0.88 for 60 µm), while phosphate demonstrates moderate but meaningful associations (r = 0.52, 0.48, and 0.51, respectively). These results are consistent with the observed clustering of data points along positively sloping trajectories in the scatter matrices. Additionally, intercorrelations among the phytoplankton size classes (e.g., r = 0.99 between 25 and 40µm) suggest consistency in size fraction responses to nutrient inputs. The plots also reveal that the distributions of phytoplankton abundance are right-skewed, indicating that

higher cell densities are less frequent but present in specific samples, potentially due to localized nutrient enrichment. Overall, this multivariate visualization reinforces the interpretation that nitrate serves as the dominant driver of phytoplankton, especially in smaller-size fractions.

## 5. Relationship between phytoplankton and nutrients

To better understand the relationships between nutrient availability and phytoplankton abundance across different size fractions, a multiple regression analysis was performed, as presented in Fig. (6).



**Fig. 6.** Linear regression plots illustrate the relationships between nutrient concentrations and phytoplankton abundance across three size fractions Significance at the 95% confidence level ( $\alpha = 0.05$ ).

Based on the regression analysis of phytoplankton abundance across three size classes (25, 40, and 60 $\mu$ m) with nutrient concentrations (nitrate and phosphate), the results demonstrate consistent positive relationships between nutrient availability and phytoplankton growth. Statistically, all six regression models showed increasing trends, indicating that as nitrate and phosphate concentrations rise, phytoplankton abundance tends to increase, albeit with varying degrees of strength. For the smallest phytoplankton size (25 $\mu$ m), both phosphate and nitrate exhibited strong positive relationships with abundance, with R<sup>2</sup> values of 0.58 and 0.76, respectively. These values suggest that nutrient concentrations account for a substantial portion of the variability in phytoplankton abundance within this size class. Similarly, for the 40 $\mu$ m phytoplankton, the regression lines remained upward trending with slightly lower R<sup>2</sup> values (phosphate: 0.54; nitrate: 0.72), still indicating a significant influence of nutrient availability. The 60 $\mu$ m class, although displaying positive regression trends, showed the weakest correlations (phosphate: R<sup>2</sup> = 0.39; nitrate: R<sup>2</sup> = 0.31), which may be attributed to greater physiological or ecological variability in larger phytoplankton.

## 6. Overview of findings

The environmental conditions (Fig. 1) in the Marana Silvofisheries were generally conducive to the growth of phytoplankton. Salinity, temperature, dissolved oxygen (DO), and pH levels were all within acceptable ecological ranges for supporting brackish water ecosystems (Danavaro *et al.*, 2025). Similar observations were made by Liu *et al.* (2007), who reported stable nutrient dynamics in hydrologically connected estuarine systems. Nevertheless, the limited variability in these parameters may obscure localized nutrient inputs or biological interactions. The nutrient profiles (Fig. 2) reveal a consistent dominance of nitrate over phosphate across stations, a characteristic feature of nitrogenrich but phosphate-limited estuarine and semi-enclosed marine systems (Cloern, 2001). Elevated nitrate levels at stations 1 and 4 could result from land runoff, anthropogenic inputs, or hydrodynamic processes that concentrate nutrients in certain zones. Meanwhile, the consistently low phosphate concentrations across all stations suggest potential limitations in primary productivity.

The observed variability in nitrate, indicated by high standard deviations, may reflect episodic nutrient pulses or stratification events driven by rainfall, as documented in similar tropical estuarine settings (Quiqq *et al.*, 2025). Chaikaew *et al.* (2019) reported nitrate concentrations ranging from 1.08 to 2.10mg/ L, with peaks during harvesting periods. Similarly, **Rao** *et al.* (2019) observed elevated nitrate alongside consistently lower phosphate levels in estuarine environments, indicating a potential nutrient imbalance. The spatial uniformity of phosphate across stations further suggests that

phosphorus inputs are minimal or rapidly assimilated, highlighting the importance of integrated nutrient management in coastal ecosystems (Fulford *et al.*, 2022).

The dominance of phosphate in larger phytoplankton size fractions underscores its critical role as a limiting nutrient, particularly in estuarine systems. This finding supports previous work by **Biggs** et al. (2013), who emphasized the importance of phosphorus availability for the growth of large-sized phytoplankton. In contrast, smaller phytoplankton fractions showed more balanced responses to both nitrate and phosphate, suggesting a degree of functional adaptability and flexible nutrient uptake mechanisms. In this study, although nitrate concentrations were relatively elevated, consistently low phosphate levels resulted in N:P ratios that frequently exceeded the canonical Redfield ratio of 16:1 (**Redfield**, 1958), a widely recognized optimal molar ratio for balanced phytoplankton growth. When ambient N:P ratios surpass this threshold, phosphorus typically becomes the limiting nutrient, even when its absolute concentration is not the lowest. This observation aligns with the findings of Howarth et al. (2011), who demonstrated that phosphorus limitation can suppress primary productivity in nitrogenrich systems, and Ikpi et al. (2013), who reported that low phosphate availability significantly restricted phytoplankton biomass despite adequate nitrate levels in estuarine environments.

The predominance of the 25µm size fraction across most stations (Fig. 3) suggests that smaller phytoplankton are better adapted for nutrient assimilation and light capture in brackish and coastal environments. This is particularly relevant in nitrogen-rich, phosphate-limited conditions, where smaller cells exhibit higher surface-area-to-volume ratios that facilitate efficient nutrient uptake under scarcity. Station 1, which recorded the highest phytoplankton abundance, may represent an area with enhanced nutrient availability or hydrodynamic conditions conducive to phytoplankton retention, such as water column stability or turbidity gradients (**Zhang** *et al.*, **2022**). Conversely, Station 2, which exhibited the lowest abundance across all fractions, may be influenced by nutrient depletion, higher grazing pressure, or hydrological flushing mechanisms that restrict the persistence of phytoplankton.

Fig. (3) clearly shows a stratification pattern, with the highest abundance of  $25\mu$ m phytoplankton at stations 1 and 4, locations also associated with elevated nitrate concentrations. This spatial co-occurrence supports the interpretation that nitrogen availability promotes growth in small phytoplankton fractions. However, despite elevated nitrate levels, the generally low phosphate concentrations appear to constrain the biomass of larger phytoplankton, such as the 60µm fraction, whose abundance remained relatively low across all stations. These findings align with observations in other tropical estuarine systems, where spatial heterogeneity and size-selective pressures shape phytoplankton assemblages (**Cloern, 2001; To** *et al., 2024*). The intermediate abundance of the 40µm fraction across stations likely reflects a transitional group composed of both smaller

diatoms and larger flagellates, organisms that may tolerate moderate phosphate levels but are outcompeted under extreme nutrient imbalances.

The nutrient-phytoplankton relationships illustrated in Figs. (4-6) reinforce the broader ecological narrative that both nitrate and phosphate jointly shape phytoplankton abundance, although their effects may be size-fraction-specific. The observed correlations suggest that nitrate primarily drives abundance in smaller fractions, while phosphate becomes increasingly critical for larger cell sizes. This is consistent with the broader literature emphasizing temperature (Deng et al., 2014), nutrient availability (Teixeira et al., 2018), light (Mena et al., 2019; Chen et al., 2021), mixing (Dung, 2019) and grazing (Umar & Hatta, 2021; Butts et al., 2022; Hatta et al., 2022), as key determinants of phytoplankton dynamics. Figs. (4, 6) suggest that intermediate-sized phytoplankton  $(40\mu m)$  are exceptionally responsive to nitrate enrichment, potentially due to their reliance on dissolved inorganic nitrogen (DIN) for sustained growth. The smaller 25µm fraction also benefits from elevated nitrate levels, likely due to its high surface-area-tovolume ratio, which facilitates efficient nutrient absorption (Tascón-Peña et al., 2025). However, the weaker slope in the 60µm fraction implies that larger cells may be nitrateindependent or are limited by other factors, such as light, mixing depth, or phosphorus availability.

The diminished nitrate responsiveness in the 60µm class may also reflect sizedependent physiological constraints or sinking losses, which reduce residence time in the photic zone and limit nitrate uptake. In many estuaries, phosphorus commonly occurs in lower concentrations than nitrogen, establishing it as a primary growth-limiting factor. Empirical findings support this. For instance, Ikpi et al. (2013) demonstrated that phosphate availability directly affects phytoplankton biomass and productivity in estuarine systems. Similarly, Howarth et al. (2011) found that phosphorus limitation can constrain primary production and delay phytoplankton blooms in nitrogen-rich waters, emphasizing the critical role of nutrient stoichiometry. Moreover, abiotic factors such as salinity can modulate phosphorus availability. Increased salinity in brackish environments can reduce the solubility and bioavailability of phosphate. Flower et al. (2017) noted that saltwater intrusion may enhance phosphorus release from sediments, thereby altering nutrient cycling in coastal aquifers. Therefore, the high nitrate and low phosphate levels support the growth of small-sized phytoplankton. This suggests that the balance of nutrients, particularly between nitrate and phosphate, plays a crucial role in regulating phytoplankton abundance and should be closely monitored for optimal aquaculture.

## 7. Limitations and future directions

The limited temporal sampling may not capture seasonal variability, which could influence the nutrient dynamics and phytoplankton abundance. Future studies should focus on the key ecological processes that influence phytoplankton abundance and community dynamics. A critical area is the role of zooplankton predation in regulating phytoplankton abundance across size fractions, as grazing pressure may shape population structures. Exploring nutrient saturation on phytoplankton growth and community composition could also provide insights into the effects of excess nutrients on ecosystem balance. Seasonal studies are necessary to assess the temporal variability in nutrient dynamics and phytoplankton abundance, thereby explaining patterns driven by changing environmental conditions. These research directions will further enhance the understanding of complex interactions within aquatic ecosystems.

## 8. Practical implications for aquaculture

The results of this study provide valuable insights for enhancing aquaculture management in brackish water systems. Effective monitoring and regulation of nutrient inputs, particularly nitrate and phosphate, are important for optimizing phytoplankton growth, which serves as a natural feed source. Maintaining balanced nutrient levels can promote the abundance of desirable phytoplankton size fractions, enhance feed efficiency, and support the growth of cultured phytoplankton. These results provide a scientific basis for adopting sustainable aquaculture practices that enhance productivity, contribute to better water quality management, and the efficient use of natural resources in aquaculture systems.

## CONCLUSION

In conclusion, the environmental conditions in the Marana Silvofishery brackish water aquaculture system, including temperature, salinity, dissolved oxygen, and pH, remained within suitable ranges to support phytoplankton growth. Phosphate played a more critical role than nitrate in influencing the abundance of larger phytoplankton fractions (40 and  $60\mu$ m) despite consistently higher nitrate concentrations. Smaller fractions, particularly small phytoplankton (25µm), dominated across stations. Overall, both nutrients significantly shaped phytoplankton dynamics, but phosphate showed stronger effects on the larger size groups, suggesting its role as a key limiting nutrient in brackish water ecosystems.

This study offers important implications for the sustainable management of brackish water aquaculture. Effective monitoring and regulation of nutrient inputs, particularly nitrate and phosphate, are crucial for optimizing the composition and productivity of phytoplankton communities, which serve as natural feed sources for cultured aquatic species. Specifically, smaller phytoplankton fractions ( $25\mu$ m), such as picophytoplankton, are typically consumed by microzooplankton, which serve as an essential food source for the larval stages of aquaculture species. In contrast, larger

phytoplankton fractions (40–60 $\mu$ m) may be directly ingested by juvenile or adult filterfeeding organisms, thereby supporting later stages of the food chain. A balanced nutrient regime not only enhances natural feed efficiency but also contributes to improved water quality and overall ecosystem resilience.

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