WVSU RESEARCH JOURNAL

Vol. 13 No. 2 pp. 1-11 (December 2024)

Preliminary Investigation of the Marine Phytoplankton Species in a Semi-Enclosed Estuarine System in Central Philippines

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Abstract

Toxin-producing phytoplankton triggering harmful algal blooms (HABs) have plagued the province of Capiz in central Philippines since the 1980s. Hence, investigating marine phytoplankton species, their distribution, and the conditions fostering their bloom is imperative. This study provides a preliminary investigation of the diversity, abundance, and composition of marine phytoplankton in Tinagong Dagat, a shallow estuarine lagoon located in the central Philippines, and explored how they are influenced by oceanographic parameter variations. Water samples were collected in January and February 2018 from the four sampling stations of Tinagong Dagat, as specified by the Provincial Agriculturist Office - Capiz. Species identification employed conventional enumeration and morphological analysis of phytoplankton characteristics. Phytoplankton species diversity and evenness were determined using the Shannon-Wiener Index and the evenness index formulas, respectively. Repeated Measure Analysis was used to determine if there is a correlation between the species abundance of marine phytoplankton species and the oceanographic parameters. A total of 32 species of phytoplankton were recorded during the study period, with three of the species being harmful, as per the IOC-UNESCO Taxonomic Reference. Abundances of two harmful phytoplankton have a strong positive linear relationship with depth and salinity. There is no statistically significant difference in mean abundance of marine phytoplankton species between months ($p=0.122$), but there are statistically significant differences between stations ($p=0.007$). The results of this study provide preliminary data for environmental assessments and ecosystem analyses, which may guide interventions to mitigate effects of HABs. A thorough phytoplankton analysis and seasonal assessments of species abundance, composition, and optimal growth conditions are recommended.

Keywords: phytoplankton, HABs, Tinagong Dagat, Capiz

INTRODUCTION

The Tinagong Dagat in Northern Panay, Philippines receives fresh water from the mountains through several big rivers, and sea water from Pilar Bay and Jintotolo Channel, one of the major fishing grounds in the Philippines (Bombeo-Tuburan et al., 2001) through a narrow inlet. Located along Capiz's vast coastline, Tinagong Dagat is shallow, highly silted, turbid,

https://doi.org/10.59460/wvsurjvol13iss2pp1-11
E-ISSN: 2244-4345 | P-ISSN: 2651-6659
Published by the **West Visayas State University**

with slow-moving water, and minimal water exchange (Solis, 2008) and is crucial for supporting the fishing and aquaculture industry in the province. Local fisherfolk make a livelihood through catching mud crab (*Scylla serrata* var *paramamosain*), orange-spotted grouper (*Epinephelus coioides*), tilapia (*O. mossambicus*), green mussels (*Perna viridis*) and oysters (*Crassostrea* sp.). Over the years, the coastal waters of Tinagong Dagat have experienced recurrent harmful algal blooms (HABs), as per data of the Bureau of Fisheries and Aquatic Resources (BFAR) (2014). These events have caused adverse effects on human health, manifested through gastrointestinal and neurological illnesses, fatalities, and on marine resources and the regional economy.

HABs occur when species which produce potent toxins grow out of control (U.S. Department of Commerce, National Oceanic and Atmospheric Administration, 2022). Currently, there are alarming trends of increasing bloom incidence, affecting larger areas and causing higher economic losses (Woods Hole Oceanographic Institution, 2022). This is observed as well in the country, including Capiz, with occurrences now reported more frequently (Yñiguez et al., 2012). Anthropogenic processes like nutrient over enrichment, coupled with rising temperatures, and an increasing frequency of extreme hydrologic events contribute to the occurrence of HABs (Paerl et al., 2018; Borja et al., 2019).

HABs in Capiz can be traced back to the first documented case of Paralytic Shellfish Poisoning (PSP) in the Philippines in June 1983. The outbreak, due to blooms of harmful microalga *Pyrodinium bahamense* var. *compressum* (Estudillo, 1984), resulted in 20 fatalities and nearly 700 reported illnesses (San Juan, 2000) across the country. This event also significantly impacted the coastal waters and residents of Capiz. Fifteen years later, another bloom of Paralytic Shellfish Poisoning was recorded in the area (Azanza et al., 2024). The devastating occurrence has caused three cases of PSP and a single reported death (Hallegraeff, 1989).

In October 2015, the coastal waters of Capiz tested positive for paralytic shellfish poison (DA-BFAR, 2015). Subsequently, two shellfish advisories, addressing the presence of paralytic shellfish poison in various bays of Capiz, were issued within the initial three months of 2016. The repercussions of these advisories were widespread, affecting nearly 2,000 fisherfolk in Western Visayas, as reported by the Philippine Information Agency.

This study assessed the diversity, evenness, abundance, and composition of marine phytoplankton in Tinagong Dagat, Northern Panay, Philippines, investigating their responses to variations in oceanographic parameters. Given the expanding spatial and temporal occurrence of HABs, it is crucial to examine the causative species, their distribution, and the conditions fostering their bloom. Phytoplankton, besides their pivotal role in aquatic food webs, serve as essential indicators of marine ecosystem change (Canini & Metillo, 2017). They are associated with changes in biological, physical, and chemical hydrologic conditions, offering potential early warning signals for deteriorating conditions and ecological variations, particularly amid threats to fishery resources (Biswas et al., 2010; Kannan & James, 2009).

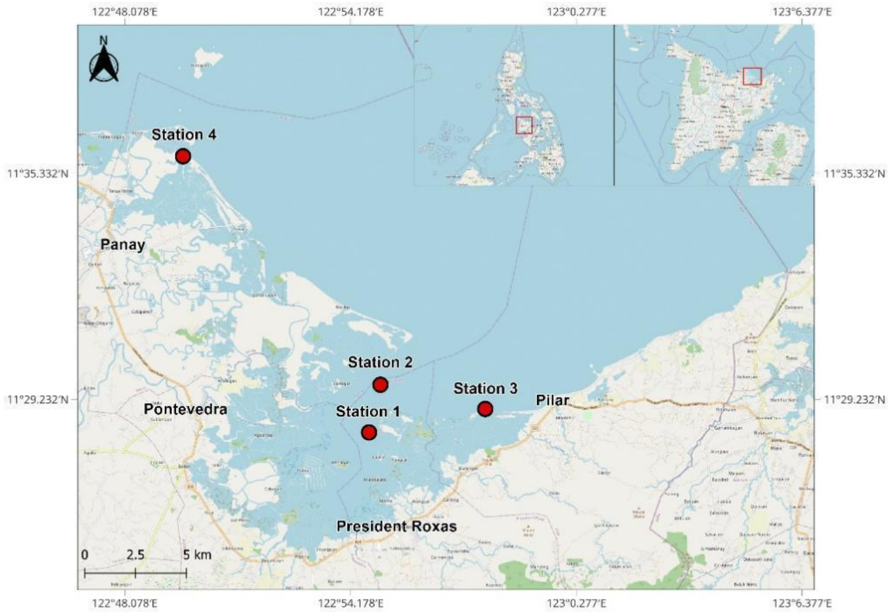
The results of this study can offer preliminary data that may serve as an ecological reference for assessing the phytoplankton and fisheries status and productivity in the area. They may also be used to inform targeted interventions that may be helpful in mitigating the effects of or in preventing HABs.

METHODS

Study area and sampling period

This study was conducted in Tinagong Dagat, Northern Panay, Philippines. Tinagong Dagat is an inlet in the Capiz Province and is at the mouth of Panay River. The residents heavily rely on the fish pen and fish cage operations in the coastal waters of Tinagong Dagat as their source of livelihood. The bay has shallow waters and is turbid, highly silted, highly eutrophic, and with minimal water exchange (Bombeo-Tuburan et al., 2001). Daytime collection of phytoplankton samples and determination of environmental conditions were done on January 24, 2018 and February 21, 2018. Four sampling stations (Fig. 1) were established throughout the bay by the Red Tide Monitoring Team of the Provincial Agriculturist Office in Roxas City, Capiz on the basis of shellfish growing sites or production areas in Capiz. They are: Station 1 at Brgy. Pandan, President Roxas (11° 28' 21" N, 122° 54' 41" E), Station 2 at Brgy. Pinamihagan, President Roxas (11° 29' 37" N, 122° 55' 01" E), Station 3 at Brgy. Binaobawan, Pilar (11° 28' 59.2" N, 122° 57' 47.0" E), and Station 4 at Brgy. Buntod, Panay (11° 35' 46.4" N, 122° 49' 39.0" E).

Figure 1. Geographical location of the four sampling stations (red dots) distributed throughout Tinagong Dagat, Northern Panay, Philippines. Inset: map of the Philippines with Tinagong Dagat enclosed in a square (left) and map of Panay Island (right). Base map from OpenStreetMap.



Procedures

Phytoplankton samples were collected using a 20-L water sampler with calibrated meter rope that was lowered to a depth of 1 m for each designated station in each study site and was poured to conical plankton net (mesh size: 20µm) thrice. Triplicate samples were collected in each station. The collected water samples were poured into the low-density Polyethylene bottles and were properly labeled. Samples were then preserved by using neutralized formaldehyde (2 mL per 100 mL water sample).

A total of six abiotic parameters were considered in the study for oceanographic parameters analyses. These include the chemical parameters (1) dissolved oxygen (DO), (2) salinity, (3) water ammonia and (4) nitrate, and the physical parameters (5) surface water temperature, and (6) column depth. DO, salinity, and temperature data of the water samples were obtained by using a YSI EcoSense ODO200 Optical Dissolved Oxygen Meter. Depth was measured using a water depth meter. Frozen 1-L water samples from each station were submitted to the Centralized Analytical Laboratory of the Southeast Asian Fisheries Development Center (SEAFDEC), Tigbauan, Iloilo for the Ammonia-Nitrogen content analysis using Phenate Method (APHA AWWA WEF 4500 – NH_3) and the Nitrite-Nitrogen content analysis using Colorimetry (APHA AWWA WEF 4500 – NO_2).

Phytoplankton were identified to species level using the phytoplankton species identification guidebook by Omura et al. (2012). Abundance of each species was estimated by counting cells in a gridded 1 mLSedgewick Rafter counting chamber cell (depth: 1 mm; length: 50 mm; width: 20 mm) using a compound light microscope. Four replicates of 1-ml sub-sample from the concentrated sample from each station were drawn using a pipette and were scanned thoroughly ensuring that all cells were counted. Densities in cells l-1 of different phytoplankton species were determined by dividing the number of cells from the concentrated sample by the volume of water filtered by the net.

Repeated Measure Analysis was used to determine if there is a correlation between the species abundance of marine phytoplankton species and the oceanographic parameters, $p<0.05$. IBM SPSS Statistics 19 was used for computation. The species diversity and evenness of phytoplankton were determined using the Shannon-Wiener Index (H') and the Evenness Index formulas (H'/ln(S)). The abundance of each phytoplankton species was expressed as cells per liter following the formula of Sournia (1981): Abundance (cells per liter) = average of the phytoplankton cell count (from the 4 sub-samples) x N; where: N = (1/L) x (n/v); L = original total volume of the sub-samples, n = total volume of concentrated sample (mL), v = volume of one drop, which is 1 mL.

RESULTS

Table 1 shows a total of 32 species of phytoplankton recorded during the study period. These species belong to three phytoplankton groups: diatoms, dinoflagellates, and cyanobacteria. In total, there are 26 diatom species, five dinoflagellate species, and one cyanobacteria species identified. Also, there are unidentified species in all stations. The most abundant phytoplankton species are diatoms. *Skeletonema* spp. is the most abundant in January, while *Coscinodiscus granii* is the most abundant in February of the same year. Three species are listed in the IOC-UNESCO Taxonomic Reference List of Harmful Marine Microalgae: *Dinophysis caudata* (diarrhetic shellfish poisoning [DSP]-causing species), *Nitzschia* spp. (amnesic shellfish poisoning [ASP]-causing species), and *Cochlodinium polykrikoides* (Fish kill-causing species).

Dinophysis caudata is among 10 species of *Dinophysis* known to produce diarrhetic toxins, causing Diarrhetic Shellfish Poisoning (DSP). Symptoms, including incapacitating diarrhea, nausea, vomiting, and abdominal pain, typically manifest within 30 minutes to a few hours after consuming toxic shellfish (Barceloux, 2008, as cited in Lloyd et al., 2013). *Dinophysis caudata* is one of three *Dinophysis* species identified by Roxas in 1941, alongside *Dinophysis miles* f. *indica* Cleve and *D. hastata* Stein (Roxas, 1941). In 1993, *Dinophysis caudata* Kent was found in unusually high density in Sapian Bay, Western Visayas (Marasigan et al. 2001).

Borja et al. (2019) cited that there are two diatom species causing amnesic shellfish poisoning (ASP) that has been identified in Manila Bay: *Pseudonitzschia pungens* and *Nitzschia navis-varingica*. ASP results from the neurotoxin domoic acid (DA), which the genus *Nitzschia* may produce (Suriyanti et al., 2017). To date, ASP has not been confirmed in either the Philippines or Malaysia (Yñiguez et al., 2021).

The marine dinoflagellate *Cochlodinium polykrikoides* is a highly harmful blooming species, causing mass mortalities in both wild and farmed fish. It forms rusty brown discolorations and triggers massive fish kills, notably along the western coast of Palawan in the Philippines (Azanza et al., 2008).

Table 1. Phytoplankton species recorded in four sampling stations in Tinagong Dagat, Northern Panay, Philippines and their presence (+) and absence (-) in January and February 2018. Percentage relative abundance of the five most abundant species in every month is in parenthesis.

Species	Abundance (cells/L)									
	January					February				
	S1	S2	S3	S4	Total	S1	S2	S3	S4	Total
Diatoms										
Asterionellopsis glacialis	-	-	+	+	13	+	+	+	+	251 (2.68%)
Bacillaria paxillifera	-	-	-	+	6	+	+	+	+	22
Bacteriastrum sp.	-	-	-	+	1	+	+	+	+	8
Chaetoceros spp.	-	-	-	+	11	-	-	+	+	4
Cocconeis costata	-	-	+	+	9	+	+	+	+	194 (2.07%)
*Cochlodinium polykrikoides****	-	-	-	-	-	-	-	+	+	2
Coscinodiscus granii	+	+	+	+	20	+	+	+	+	2240 (23.89%)
Cylindrotheca closterium	-	-	+	-	25 (0.07%)	+	-	+	+	9
Detonula pumila	-	+	-	+	3	+	-	-	-	1
Ditylum brightwellii	+	+	+	+	355 (1.00%)	+	+	+	+	171 (1.82%)
Ditylum sol	-	-	-	1	1	-	-	-	+	1
Guinardia flaccida	-	+	-	1	2	-	-	+	-	1
Meuniera membranacea	-	-	-	-	-	-	-	+		3
Navicula sp.	3	-	-	-	3	+	-	-	+	3
Nitzschia spp.**	-	-	-	+	1	+	-	-	-	1
Odontella mobiliensis	-	+	+	+	10	+	+	+	+	115
Odontella sinensis	-	-	+	+	2	+	+	+	+	11
Pleurosigma angulatum	+	-	+	+	5	+	-	+	+	7
Pleurosigma normanii	+	+	+	+	98 (0.28%)	+	+	+	+	84
Pseudonitzschia spp.	-	-	-	-	-	+	-	-	-	2
Rhizoselenia crassispina	-	-	-	+	3	-	-	-	-	-
Rhizosolenia setigera	-	-	+	+	8	+	-	+	+	21
Skeletonema spp.	+	+	+	+	34875 (98.24%)	+	+	+	+	6064 (64.67%)
Thalassionema baccilare	-	-	-	+	3	+	-	+	+	51
Thalassionema nitzschiodes	-	-	-	+	8	-	-	-	-	-
Thalassiosira sp.	-	-	-	-	-	+	-	-	-	4
Dinoflagellates										
Ceratium furca	-	-	-	+	3	-	-	+	-	1
*Dinophysis caudata**	-	-	-	+	2	-	-	-	-	-
Gonyaulax striata	-	-	-	+	1	-	-	-	-	-
Prorocentrum micans	-	-	-	-	-	-	-	-	+	1
Peridinium quinquecorne	-	-	-	-	-	-	-	+	+	2

Cyanobacteria										
Oscillatoria lutea	-	-	+	+	21 (0.06%)	+	+	+	+	96
Unidentified species	+	+	+	+	9	+	-	+	+	7
Total number of species*****	6	7	12	25	26	20	11	21	21	29

**DSP-causing species produce marine biotoxin that can be accumulated in shellfish that when eaten, may cause consumers to fall ill (Nielsen et al., 2020).*
***ASP-causing species are responsible for ASP, an illness which results from the ingestion of shellfish that have accumulated domoic acid, a neurotoxin generated by certain phytoplankton strains (Jeffery et al., 2004).*
****Fish kill-causing species cause reductions in dissolved oxygen in water, subsequently resulting in algal blooms and subsequent death of the algae (The Pennsylvania State University, 2022).*
*****Some cells are unidentifiable through morphological analysis. The researchers and consulted experts were unable to confirm the identification of the cells through the photographs of specimens visualized through a compound light microscope.*
******The total number of species excludes cells that were unidentifiable. It is also not the sum of the species found per station, as some species were found in various stations.*

Station 2 had the highest H’ (1.246) and H’/ln(S) (0.472.) With this, there is higher diversity in Station 2, compared with Station 1 (H’=0.898 and H’/ln(S)=0.290), Station 3 (H’=0.745 and H’/ln(S)= 0.241), and Station 4 (H’=0.15 and H’/ln(S)= 0.044).

Table 2 shows that the abundances of *Dinophysis caudata* and *Nitzschia* spp., two harmful species found only in the month of January, have a strong positive linear relationship with DO (r=0.855) and salinity (r=0.979), weak negative linear relationship with depth (r=-0.333) and ammonia (r=-0.389) and moderate positive linear relationship with temperature (r=0.503). The abundances only have a significant relationship with salinity (p=0.021). Meanwhile, the abundance of *Cochlodinium polykrikoides*, a fish kill-causing species found only in the month of February, has a weak negative linear relationship with depth (r=-0.447) and ammonia (r=-0.352), moderate positive linear relationship with DO (r=0.541), temperature and (r=0.691), strong positive relationship with salinity (r=0.905), and no linear relationship with nitrite (r=0). It has no significant relationship with any of the parameters.

Significant Interaction Tests on the species abundance of marine phytoplankton in Tinagong Dagat, Northern Panay, Philippines between the four stations and the months of January and February showed that there is no statistically significant difference in mean abundance of marine phytoplankton species between the months of January and February (p= 0.122), but there are statistically significant differences between station (p=0.007).

Table 2. Correlation of the species abundances of the three species listed in the IOC-UNESCO Taxonomic Reference List of Harmful Marine Microalga and recorded in four sampling stations in Tinagong Dagat, Northern Panay, Philippines in January and February 2018, with oceanographic parameters, or their absence (-). The said species are: *Dinophysis caudata* (DSP-causing species), *Nitzschia* spp. (ASP-causing species), and *Cochlodinium polykrikoides* (fish kill-causing species).

Species		Depth		DO		Temperature		Salinity		Ammonia		Nitrite	
		Jan	Feb	Jan	Feb	Jan	Feb	Jan	Feb	Jan	Feb	Jan	Feb
D. caudata and *N.* sp.	Pearson Correlation	-0.333	-	0.855	-	0.503	-	0.979	-	-0.389	-	ND*	-
	Sig. (2-tailed)	0.667	-	0.145	-	0.497	-	0.021	-	0.611	-	ND*	-

C. polykrikoides	Pearson Correlation	-	-0.447	-	0.541	-	0.691	0.905	0.000	-	0.000
	Sig. (2-tailed)	-	0.553	-	0.459	-	0.309	0.095	1.000	-	1.000

**"ND" denotes "not detected" to signify that nitrite concentrations are so minute that the water samples appear to lack any nitrite content. Concentrations are reported solely up to parts per million (ppm). However, it is conceivable for nitrite to be present at levels as low as parts per billion (ppb) or even parts per trillion (ppt).*

CONCLUSION

This study assessed the diversity, evenness, abundance, and composition of marine phytoplankton in Tinagong Dagat, Northern Panay, Philippines, investigating their responses to variations in oceanographic parameters. The results revealed the presence of 32 phytoplankton species throughout the study period. Notably, the occurrence of phytoplankton species in January did not necessarily replicate in February, and vice versa. Despite this temporal variability, there was no statistically significant difference in the mean abundance of marine phytoplankton species between January and February ($p= 0.122$). However, significant differences were observed between various stations ($p= 0.007$).

The differences in species abundance and composition in Tinagong Dagat may be attributed to the fluctuations in oceanographic parameters, such as depth, temperature, salinity, dissolved oxygen, ammonia, and nitrite. Saltwater influx, rainfall, terrestrial runoff, anthropogenic activities, among others, may cause the changes in conditions. These variations induce shifts in the temporal patterns of phytoplankton species composition and abundance, consequently influencing the diverse growth of phytoplankton across different seasons (Lindsey & Scott, 2010).

Among the 32 phytoplankton species identified, three are considered harmful, as per the IOC-UNESCO Taxonomic Reference (2009): *Dinophysis caudata* (DSP-causing species), *Nitzschia* spp. (ASP-causing species), and *Cochlodinium polykrikoides* (fish kill-causing species). Notably, a strong positive linear relationship was observed between the abundances of two harmful phytoplankton species, *D. caudata and N.* spp., and the variables of depth and salinity. Comprehensive and regular assessment of species composition and diversity, along with their interactions with various oceanographic parameters, may predict conditions that are considered favorable for HABs to occur.

Low abundances may be linked to low nutrient content, particularly ammonia and nitrite. The overgrowth of blooms is highly impacted by the amount of specific nutrients crucial for phytoplankton growth, like excess nitrogen and phosphorus (United States Environmental Protection Agency, 2024). Another parameter known to have a relatively notable effect on phytoplankton community structure is salinity, particularly in estuarine and coastal environments (Peña & Pinilla, 2002; Lassen et al., 2004; Troccoli et al., 2004). In this study, harmful phytoplankton species identified in Tinagong Dagat demonstrated a strong positive relationship with salinity, emphasizing the need for further exploration of these interactions.

Moreover, the results also showed that Station 2 had the highest Shannon-Wiener Diversity index ($H'=1.246$) and Species Evenness and ($H'/\ln(S)= 0.472$). The three other stations with higher species richness and cell count did not have the highest diversity and evenness indices as the Shannon index is affected by both the number of species and their equitability, or evenness (Ohio University, 2011). A greater number of species and a more even distribution both increase diversity as measured by H'. The maximum diversity (H_{max}) of a sample is found when all species are equally abundant.

The outcomes of this study provide essential preliminary data for crafting interventions to mitigate or prevent HABs and lay the groundwork for future comprehensive assessments involving species identification, abundance, and optimal growth conditions in Tinagong Dagat, Northern Panay, and other major fishing grounds in the country. Further investigations could delve into phytoplankton analysis that explores diverse conditions such as different seasons, monsoons, or months. Broadening the analysis to incorporate additional oceanographic parameters, including chlorophyll, transparency, turbidity, and nutrient phosphorus, would yield a more extensive assessment of the trends in abundance and composition of harmful algae and other marine phytoplankton species. Furthermore, varying the sampling depths, encompassing surface, thermocline depth, and chlorophyll maximum depth, may offer a better understanding of the dynamics of these critical marine ecosystems.

Acknowledgments

The researchers would like to express their utmost gratitude and sincere appreciation to the WVSU Senior High School Department and Biological Sciences Departments of the College of Arts and Sciences, the Provincial Agriculturist Office – Capiz, Office of the Municipal Agriculturist of Panay, Pilar, and President Roxas, the University of the Philippines Visayas, and the Southeast Asian Fisheries Development Center. This research would not have come to fruition if not for the assistance and support of the people from the aforementioned offices.

Declarations

Source of Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this research work.

Data Availability

The authors are willing to share the data and materials based on relevant needs.

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