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Short-term Assessment of Phytoplankton Composition and Abundance in Cebu and Subic Bay Ports, Philippines

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ABSTRACT

A short-term study to evaluate the composition and abundance of marine diatoms and dinoflagellates in two major ports in the country was conducted in May 2015 and July 2015. *Pseudo-nitszchia* spp. bloom comprising about 42.3% of the total phytoplankton population was observed in Cebu International Port in May 2015. Furthermore, *Chaetoceros* spp. comprised about 53.58% of the total phytoplankton population in Naval Supply Depot (NSD) terminal in May 2015, while *Thalassionema* spp. and *Leptocylindrus* spp. accounted for 50.16% and 34.78%, respectively, of the total phytoplankton population in July 2015. Bloom-forming and potentially harmful species including diatoms, such as *Coscinodiscus* spp., *Nitzschia* spp., and *Pseudo-nitzschia* spp., and dinoflagellates, such as *Ceratium* spp., *Ceratium furca, Gonyaulax* spp., *Gymnodinium* spp., *Linguludinium* spp., *Phalacroma* spp., *Prorocentrum micans, Prorocentrum* spp., and the IOC-UNESCO listed Harmful Algal Bloom (HAB) species *Dinophysis caudate*, were also recorded. The results of this study contribute to the establishment of baseline data for phytoplankton composition and abundance, which are necessary for the identification of potentially toxic/harmful microalgae which pose risks of ballast water inclusion and transport.

Keywords: Phytoplankton, ports, harmful algal blooms, diatoms, dinoflagellates

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INTRODUCTION

Phytoplankton are predominant aquatic microalgae and play important role in the marine and estuarine ecosystem. They significantly contribute to biological productivity (Arumugam et al. 2005; Sharma et al. 2015) and are the first biological community to respond to environmental changes (Kozak et al. 2015). However, excessive growth and accumulation of phytoplankton in response to increased favorable conditions attributed to anthropogenic and chemical pollutions have caused recurrent phytoplankton blooms known as "Red Tides," which have had negative impacts in many coastal waters (Corrales and Maclean 1995; Azanza and Taylor 2001; Wong and Wong 2003; Yap et al. 2004; Chang et al. 2009; Furio et al. 2012; Yñiguez et al. 2012). The inevitable impacts of the worldwide Harmful Algal Bloom occurrences on biodiversity, economics, and human health, have drawn the attention of the scientific community and have prompted the use of mitigation and control methods (Padilla et al. 2010).

Harmful Algal Blooms (HABs) have been a recurring phenomenon causing havoc in the many affected areas in the Philippines and the rest of the Southeast Asian Region (Corrales and Maclean 1995; Azanza and Taylor 2001; Hansen et al. 2004; Wang et al. 2008; Yñiguez et al. 2012). Associated with Paralytic Shellfish Poisoning (PSP) and even fatalities, HAB events in the country have been primarily caused by the toxic dinoflagellates *Pyrodinium bahamense* var. *compresum* and *Alexandrium* species, which greatly affected Manila Bay and other 31 bays/coastal areas in the country from 1983 to 2013 (Azanza et al. 2004; Sombrito et al. 2004; Azanza and Benico 2013). Microalgal researches in the country have been mainly focused on major bays affected by HABs and less focalized on major ports with high vessel traffic where risk of ballast water inclusion and transport of HAB species are more likely.

The inclusion and survival of phytoplankton in ballast tanks of vessels have already been well documented in many studies all over the world. In this study, two of the country's major ports were studied to evaluate the composition and abundance of marine diatoms and dinoflagellates, which are necessary baseline data for the identification of potentially toxic/harmful microalgae that pose risks of being transported from one port to another via ballast water movement.

MATERIALS AND METHODS

Study Areas

Samples were collected from Cebu International Port (CIP) on May 14, 2015 and July 8, 2015, and from Naval Supply Depot (NSD) Terminal on May 27, 2015 and July 22, 2015. Three different randomly selected sampling stations with over 200-meter distance were established (Stations 1-3) in both ports (Figures 1B-1C). In terms of size, both CIP and NSD terminal were comparable, and both ports are single-wharf structures; hence, the two were chosen as sampling sites for the study. CIP (Figure 1C) is located between Cebu and Mactan Island, approximately 10°18'33.11 N 123°55'24.81 E, comprising 495 meters of quayside (international zone only). The NSD Terminal in Subic Bay, Zambales (Figure 1B), on the other hand, is located approximately 14°48'12.46 N, 120°15'43.03 E, with a 28-hectare terminal area comprising a 560-meter quayside and 13.5-meter actual berthing depth.

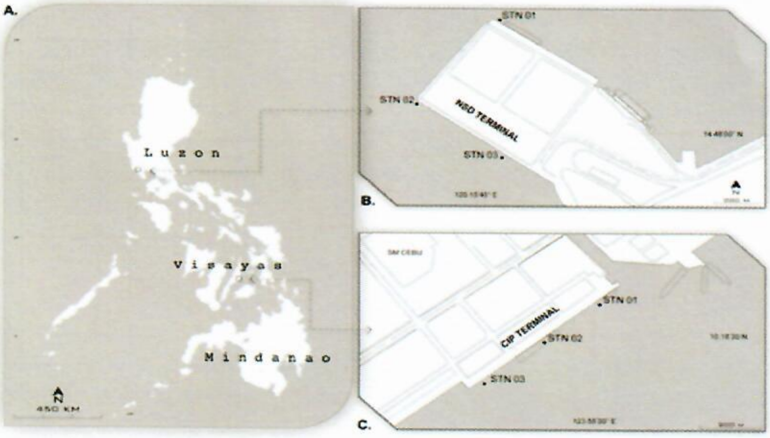

Figure. 1. Sampling sites location: (A) Philippine Map; (B-C) sampling sites, each with three sampling stations labeled STN01 – STN03; (B) NSD Terminal, Subic; (C) CIP, Cebu. (Illustrated using Google Earth Pro (ver. 7.1.2.2041) and Google Maps 2016 as reference materials).

Abiotic Factors

Water samples for the measurement of physico-chemical parameters were obtained from the near surface layer (1 m), middle layer (4 m), and near bottom layer (7 m) using a Niskin bottle sampler. Physico-chemical parameters like temperature and salinity were measured in situ using a hand-held YSI-85 Multi Meter. In addition, 250-mL seawater samples were also collected to measure chlorophyll-*a* values and nutrient (PO_4, SiO_3, NO_3, NO_2 and NH_3,) content using the modified method described by Strickland and Parsons (1972).

Biological Parameters

Phytoplankton samples were collected from 10-m depths by towing vertically from the water column using a plankton net with a 0.35-m mouth-diameter and 25-µm mesh size. To ensure the capture of rare taxa, the plankton net was towed three times (equivalent to three subsamples). For the quantitative analysis, a standard rotor flow meter was also attached to determine the filtered water volume. The samples were subsequently preserved with Lugol's solution and used for qualitative analysis. Enumeration and counting of phytoplankton was performed using Sedgwick-Rafter counting chamber based on microscopic methods for quantitative phytoplankton analysis following the techniques of Corrales et al. (1995) and Azanza (1997). Phytoplankton taxonomic identification was based on Tomas (1997) and Yamaji (1984) up to the lowest taxonomic level possible. Images of phytoplankton species were also captured using the Dinolite Eye-Piece and Carl Zeiss Axio-cam.

Statistical Analysis

Prior to various univariate and multivariate analysis, all data were tested for normality with Shapiro – Wilks Normality Test available in R v. 3.2.2. Non-normal data were transformed (e.g., sine, cosine, square root, etc.) (Table 1).

Table 1. Shapiro-Wilks Normality Tests

	Inorganic N	Transformations	ρ-value
CIP	Temperature	NONE	0.1819
	Salinity	NONE	0.09651
	Chl-*a*	LOG	0.07606
	Inorganic N	NONE	0.165
	PO_4	NONE	0.06551
	SiO_3	NONE	0.1794
NSD	Temperature	NONE	0.484
	Salinity	SIN	0.3055
	Chl-*a*	NONE	0.2549
	Inorganic N	NONE	0.7597
	PO_4	NONE	0.6599
	SiO_3	NONE	0.4319

Multivariate Analysis of Variance (MANOVA) was performed on physico-chemical parameters (e.g., temperature, salinity, nutrients, chlorophyll-*a*) in order to examine significant relationships. MANOVA can test all response variables simultaneously, and can detect differences among correlated variables that are too small to be perceived through individual and successive ANOVA. To further investigate the close associations and relationships among variables, Principal Component Analysis (PCA) was also performed for the most common and abundant taxa in relation to physico-chemical parameters. Diversity index analysis, on the other hand, was analyzed using PAST3 *ver.* 1.0 (Hammer et al. 2001) at genus level only.

RESULTS

Phytoplankton Abundance and Composition

Diatoms were the most dominant taxa found in both ports in the May 2015 and July 2015 sampling (Figure 2). There were a total of 53 phytoplankton taxa, comprising 36 diatoms, 16 dinoflagellates, and one blue green alga, identified in two ports. Phytoplankton taxa include bloom-forming and potentially harmful species (Figure 3) of diatoms, such as *Coscinodiscus* spp., *Nitzschia* spp., and *Pseudo-nitzschia* spp., and of dinoflagellates, such as *Ceratium* spp., *Ceratium furca, Gonyaulax* spp., *Gymnodinium* spp., *Linguludinium* spp., *Phalacroma* spp., *Prorocentrum micans, Prorocentrum* spp., and the IOC-UNESCO listed Harmful Algal Bloom (HAB) species *Dinophysis caudata* (Table 2).

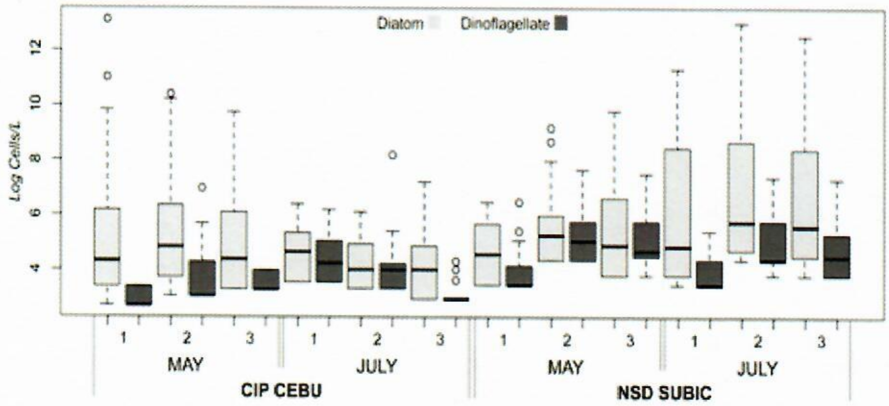

Figure 2. Diatom and dinoflagellate abundance in three sampling stations (1-3) in Cebu and Subic ports in May 2015 and July 2015.

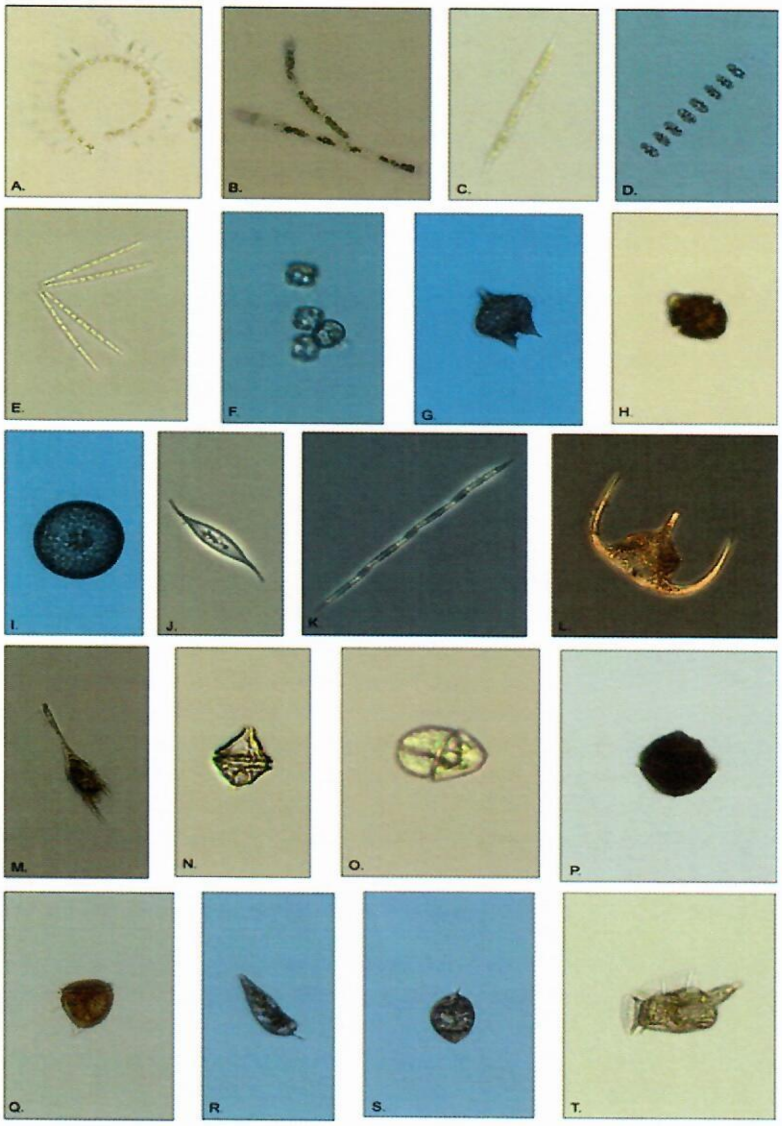

Figure 3. Representative micrographs/images of bloom-forming diatoms: (A) *Chaetoceros* sp., (B) *Leptocylindrus* sp., (C) *Rhizosolenia* sp., (D) *Skeletonema* sp. (E) *Thalassionema* spp., (F) *Thalassiosira* spp., and dinoflagellates: (G) *Protoperidinium* spp., (H) *Scrippsiella trochoidea* (Stein) Loeblich III 1976; potentially harmful microalgal species including diatoms: (I) *Coscinodiscus* spp., (J) *Nitszchia* sp., (K) *Pseudo-nitszchia* sp., and dinoflagellates: (L) *Ceratium* spp., (M) *Ceratium furca*, (N) *Gonyaulax* spp., O) *Gymnodinium* sp., (P) *Linguludinium* sp., (Q) *Phalacroma* sp., (R) *Prorocentrum* sp., (S) *P. micans*; and the IOC-UNESCO listed Harmful Algal Bloom (HAB) species: (T) *Dinophysis caudata* Saville-Kent 1881 observed in Cebu and Subic Ports.

Table 2. Abundance (cells/liter) and composition of phytoplankton taxa identified from port water sampled in CIP, Cebu and NSD Terminal, Subic in May 2015 and July 2015

Taxonomy	CIP Cebu		NSD Subic	
	May	July	May	July
Bacillariophyceae				
Amphora Ehrenberg ex Kutzing 1844		22		119
Auricula Castracane 1873				120
Bacillaria Gmelin 1791	4	13		310
Bacteriastrum Shadbolt 1854		119	43	3,494
Cerataulina Peragallo ex Schutt 1896	217	12	120	472
Chaetoceros Ehrenberg 1844*	1,606	242	8,198	17,247
Cocconeis Ehrenberg 1837	251	102	59	136
Corethron Castrane 1886	7			
Coscinodiscus Ehrenberg 1839**	15	266	98	1,651
Cyclotella (Kutzing) Brebisson 1838		77		
Cylindrotheca Rabenhorst 1859				15
Cymbella Agardh 1830		31		
Diploneis Ehrenberg 1854				4
Ditylum Bailey 1861	248	57		144
Fragilaria Lyngbye 1819		47		1,986
Ethmodiscus Castracane 1886	21			
Guinardia Peragallo 1892	128		96	419
Helicotheca Ricard 1987		54		93
Himiaulus Heiberg 1863			30	87
Leptocylindrus Cleve 1889*	4,232			149,426
Licmophora Agardh 1827	10	98	196	26
Lithodesmium Ehrenberg 1839	39			37
Melosira Agardh 1824	67	129	237	386
Navicula Bory de St. Vincent 1822	32	117	15	102
Nitszchia Hassall 1845**	25	193	105	41
Odontella Agardh 1832	147	33	26	21,792
Planktoniella Schutt 1892		6		
Pleurosigma Smith 1852	822	487		117
Pseudo-nitszchia Peragallo 1900**	42,246	179	15	119
Rhizosolenia Brightwell 1858*	627	71	52	707
Skeletonema Greville 1865*	3,788	296		237
Stephanopyxis Ehrenberg 1845	6			
Surirella Turpin 1828		34		11
Thalassionema Grunow ex Mereschkowsky	6	229	486	215,487
Thalassiosira Cleve 1873*	4,840	121	235	11,397
Triceratium Ehrenberg 1839		12		15
Dinophycea				
Ceratium Schrank 1793**	81	19	203	78
Ceratium furca Claparede and Lachmann 1859**	110	23	107	151
Dinophysis caudata Saville-Kent 1881***	15			62
Diplopsalis Berg 1881	42	51	814	495
Gonyaulax Diesing 1866**			361	253
Gonyaulax polygramma Stein 1883	16	21	232	61

Table 2. Abundance (cells/liter) and composition of phytoplankton taxa identified from port water sampled in CIP, Cebu and NSD Terminal, Subic in May 2015 and July 2015 (Cont'n.)

Taxonomy	CIP Cebu		NSD Subic	
	May	July	May	July
Gymnodinium Stein 1878**			146	
Gyrodinium Kofoid and Swezy 1921	4	27		
Linguludinium Wall 1967**		48		15
Oxyphysis Kofoid 1926	211			
Peridinium Ehrenberg 1832	4	184	76	169
Phalacroma Stein 1883**	10	6	61	
Prorocentrum micans Ehrenberg 1833**	18	42	99	179
Prorocentrum Ehrenberg 1883**	10	140	1,002	237
Protoperidinium Berg 1881*	542	50	1,863	1,461
Pyrophacus Stein 1883	28	21	262	126
Scrippsiella Balech ex Loebelich III 1965			89	41
Scrippsiella trochoidea (Stein) Loeblich III 1976*		59	218	
Cyanophyceae				
Trichodesmium Ehrenberg ex Gomont 1892	298	22	26	
Total number of taxa				
Bacillariophyceae	23	24	17	30
Dinophyceae	13	13	14	13
Cyanophyceae	1	1	1	

Note: (*) bloom-forming species, (**) potentially HABs, (***) IOC UNESCO listed HABs.

In addition, CIP recorded 46 phytoplankton taxa comprising 31 diatoms, 15 dinoflagellates, and one blue green alga. Twenty-three diatoms and 13 dinoflagellates were identified in May, while 24 diatoms and 13 dinoflagellates were recorded in July. The most abundant taxa *Pseudo-nitzschia* spp. accounted for 42.3% of the total phytoplankton population during the month of May. Commonly known taxa identified include *Cerataulina* spp., *Chaetoceros* spp., *Dytilum* spp. *Leptocylindrus* spp., *Rhizosolenia* spp., *Skeletonema* spp., *Thalassiosira* spp.; common diatoms, such as *Chaetoceros* spp., *Coscinodsicus* spp., *Navicula* spp., *Nitzschia* spp., *Pleurosigma* spp., *Pseudo-nitzschia* spp., *Rhizosolenia* spp., *Thalassionema* spp.; and dinoflagellates *Prorocentrum* spp. and *Protoperidium* spp. (Table 2).

On the other hand, 47 phytoplankton taxa comprising 31 diatoms and 16 dinoflagellates were identified in the NSD terminal (Table 2). Seventeen diatoms and 14 dinoflagellates were identified in May, which consisted mainly of *Chaetoceros* spp. (53.58% of the total phytoplankton population). Diatoms *Melosira* spp., *Thalassionema* spp., *Thalassiosira* spp., and dinoflagellates *Ceratium* spp., *Diplopsalis* spp., *Gonyaulax* spp., *Pyrophacus* spp., *Prorocentrum* spp., *Protoperidinium* spp. were also present. Moreover, 30 diatoms and 13 dinoflagellates were identified in July. *Thalassionema* spp. accounted for 50.16%, while *Leptocylindrus* spp. accounted for 34.78% of the total phytoplankton population. Diatoms *Cerataulina* spp., *Chaetoceros* spp., *Coscinodiscus* spp., *Fragillaria* spp., *Odontella* spp., *Thalassiosira* spp., and dinoflagellates *Diplopsalis* spp. and *Protoperidinium* spp. were also present.

Shannon Diversity Index and Simpson Dominance Index

In CIP, the Shannon diversity index (H) value was highest in stations 2 and 3 (Kruskal, p= 1.83E-02), while the highest dominance (D) value was observed in station 1 (Kruskal, p= 1.83E-02) in May 2015. On the other hand, in July 2015, the H values measured from station 1 to station 3 are 3.12, 2.20, and 2.86, respectively (Table 2). In addition, high H values of 0.81, 0.69, and 0.64 for station 1 to station 3, respectively, were also observed in NSD Terminal during the month of May. In addition, the H values measured from station 1 to station 3 were 1.37, 1.21, and 1.06, respectively, in July. The highest D value was measured in station 3 (Table 3).

Table 3. Variation in phytoplankton diversity indices in different stations (STN01-STN03) in two sampling ports (CIP, Cebu and NSD, Subic) in May 2015 and July 2015

	Cebu International Port		Naval Supply Depot	
	May	July	May	July
Shannon (H)				
STN01	0.65	3.12	2.15	1.37
STN02	1.90	2.20	1.86	1.21
STN03	1.93	2.86	1.70	1.06
Simpson (D)				
STN01	0.72	0.06	0.19	0.34
STN02	0.22	0.27	0.31	0.39
STN03	0.24	0.09	0.36	0.59

Note: Diversity index values were measured using Past3 (ver. 1.0).

Principal Component Analysis (PCA)

In CIP, four of the five principal components (PCs) with eigenvalues greater than one account for 95.47% variability in May and July (Table 4). About 73.57% of the variation was explained by the first two axes (Figure 4). The first PC (Dim1) explains 58.69% of the variation that revealed high loadings for SiO_3, NH_3, NO_3 PO_4, temperature, and salinity with abundant taxa *Dytilum, Rhizosolenia, Thalassionema, Chaetoceros*, *Pleurosigma,* and *Cerataulina*. The second PC (14.88%) obtained high loadings for SiO_3 with *Pseudonitzschia, Skeletonema,* and *Odontella*. PC3 (12.41%)

had the highest loadings for NO_2 and SiO_3 with *Odontella*, whereas the highest loadings in PC4 (9.51%) was for *Chaetoceros*.

Table 4. Important PC (Eigenvalues >1) from variables describing CIP, Cebu, with high component loadings shown in bold

Variables	Dim. 1	Dim. 2	Dim. 3	Dim. 4	Dim. 5
Eigenvalue	9.973	2.530	2.110	1.617	0.769
% of variation	58.666	14.8880	12.414	9.514	4.525
Cum. % or variation	58.666	73.546	85.961	95.475	100.00
Component loadings					
Temperature	**-0.90510**	-0.02672	-0.31188	-0.18208	0.22285
Salinity	**0.97106**	-0.12119	0.00888	0.20310	-0.03211
NO_2	-0.51611	0.34823	**0.74717**	-0.21159	0.09660
NO_3	**0.93345**	0.08498	-0.00419	-0.08903	0.23789
NH_3	**0.81853**	0.14612	-0.02007	0.3358	0.44382
SiO_3	0.27524	**0.62114**	**-0/69277**	-0.20753	-0.12422
Pseudo-nitzschia	-0.19484	**-0.74404**	0.44322	-0.26152	-0.37895
Leptocylindrus	**-0.91528**	-0.08780	-0.23796	0.31046	-0.03924
Skeletonema	-0.14042	**-0.86161**	0.18854	0.12861	0.43107
Chaetoceros	0.33054	-0.25133	-0.15389	**0.86103**	-0.25004
Thalassiosira	**0.91687**	-0.31079	-0.22079	-0.11489	-0.02828
Rhizosolenia	**0.92039**	0.11660	0.26496	-0.26136	0.02770
Pleurosigma	**-0.93959**	0.16256	0.07047	0.22928	0.18224
Dytilum	**0.86110**	0.28508	0.41990	-0.03577	-0.01979
Cerataulina	**-0.98431**	0.13291	0.01763	0.11202	0.02448
Odontella	-0.09194	**0.60-639**	**0.60186**	0.50209	-0.09752

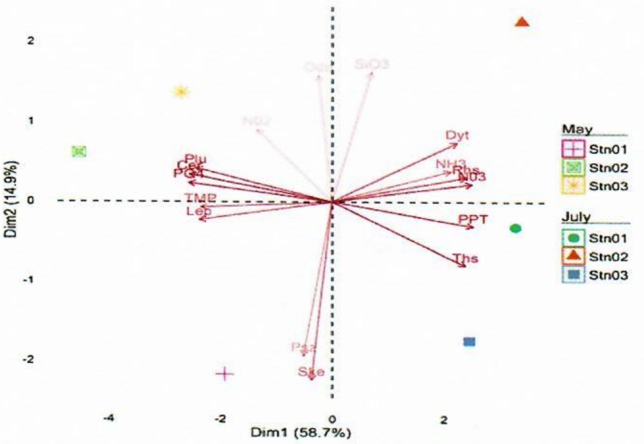

Figure 4. Ordination of variables PCA on physico-chemical parameters and abundant taxa in CIP, Cebu in May 2015 and July 2015. Note: TMP (Temperature), PPT (Salinity), Psz (*Pseudo-nitzschia*), Lep (*Leptocylindrus*), Ske (*Skeletonema*), Cha (*Chaetoceros*), Ths (*Thalassiosira*), Rhs (*Rhizosolenia*), Plu (*Pleurosigma*), Dyt (*Dytilum*), Cer (*Cerataulina*), Odo (*Odontella*).

In NSD, on the other hand, four of the five PCs that exhibited eigenvalues greater than one accounted for 96.58% variability in May and July (Table 5). Around 69.47% of the variation was explained by the first two PCs (Figure 5). The first PC (Dim1) explains 38.15% of the variation that revealed high loadings for temperature, salinity, NO_2, NH_3, PO_4, and SiO_3 with abundant taxa *Thalassionema* and *Coscinodiscus*. The second PC (31.35%) obtained high loadings for abundant taxa *Odontella, Chaetoceros, Protoperidinium, Fragillaria*, and *Thalassiosira*. PC3 (18.25%) had the highest loadings for *Leptocylindrus, Protoperidinium,* and *Cerataulina*, whereas the highest loadings in PC4 (8.86%) was for NO_3.

Table 5. Important PCs (Eigenvalues >1) from variables describing NSD, Subic with high component loadings shown in bold

Variables	Dim. 1	Dim. 2	Dim. 3	Dim. 4	Dim. 5
Eigenvalue	6.481	5.328	3.103	1.506	0.582
% of variation	38.125	31.345	18.250	8.861	3.421
Cum. % or variation	38.125	69.468	87.718	96.579	100.000
Component loadings					
Temperature	**0.86592**	-0.44676	0.14010	-0.16179	0.06922
Salinity	**-0.96457**	-0.04542	0.01823	-0.25923	0.00024
NO_2	**0.92640**	0.27654	0.10119	-0.21296	0.09854
NO_3	-0.57761	0.11300	0.20498	**-0.70465**	0.33917
NH_3	**0.67018**	0.45869	0.51562	-0.24921	-0.11173
PO_4	**0.81348**	-0.07143	0.47928	-0.18012	-0.04427
SiO_3	**-0.93228**	0.32153	0.14050	-0.07588	0.26644
Leptocylindrus	0.30362	0.59502	**-0.74174**	0.00518	0.05962
Thalassionema	**-0.84456**	0.35621	-0.33028	0.02809	-0.22352
Odontella	0.17453	**-0.93818**	0.20461	0.20198	-0.08181
Chaetoceros	-0.33899	**0.81073**	0.14595	0.42296	-0.16612
Coscinodiscus	**0.66902**	0.53795	-0.49476	-0.03567	0.13027
Protoperidinium	-0.18876	**0.65360**	**0.71367**	0.09521	-0.13705
Diplopsalis	0.24206	0.41979	0.46947	0.64451	-0.35971
Cerataulina	0.27101	0.41564	**-0.86572**	0.05977	0.02739
Fragillaria	-0.01500	**-0.97752**	-0.01239	0.18260	-0.10355
Thalassiosira	-0.25074	**-0.82393**	0.26151	0.29603	0.31975

Environmental Conditions

Summary of the multivariate analysis of variances (MANOVA) of physico-chemical parameters, such as temperature, salinity, chlorophyll-*a*, and nutrients, between months in CIP are shown in Table 6.

Average ambient water temperature (29.50°C and 27.88°C, p=0.0009643) (Figure 6a), salinity (34.38 ppt and 35.14 ppt, p=0.0007995) (Figure 6b), chlorophyll-*a* (0.43 µg/L

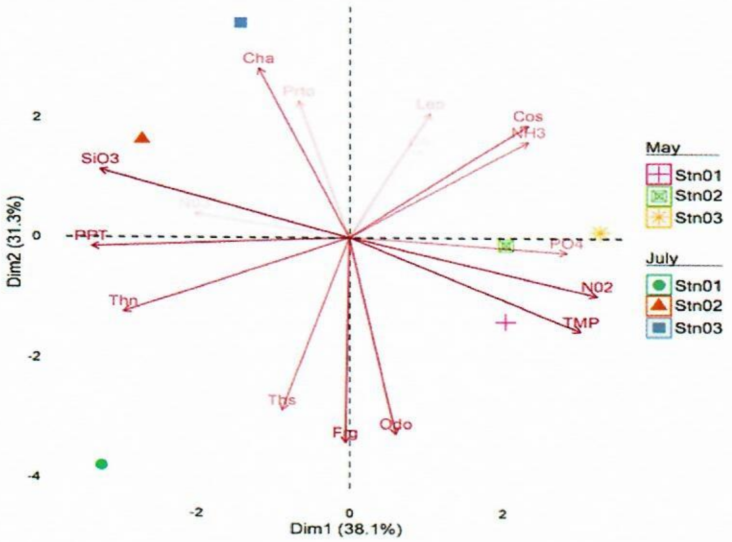

Figure. 5. Ordination of variables for PCA on physico-chemical parameters and abundant taxa in NSD, Subic in May 2015 and July 2015. (Note: TMP (Temperature), PPT (Salinity), Lep (*Leptocylindrus*), Thn (*Thalassionema*), Odo (*Odontella*), Cha (*Chaetoceros*), Cos (*Coscinodiscus*), Cer (*Cerataulina*), Frg (*Fragilaria*), Ths (*Thalassiosira*).

Table 6. Summary of MANOVA among variables in CIP, Cebu in May 2015 and July 2015

Variables	Df	Sum Sq	Mean Sq	F value	ρ-value
Temperature	1	3.9366	3.9366	75.558	0.0009643
Salinity	1	0.88167	0.88167	83.307	0.0007995
Chl-*a*	1	9.2142	9.2142	36.261	0.003831
Inorganic N	1	217.081	217.081	10.369	0.03228
PO_4	1	4.5067	4.5067	76.688	0.0009373
SiO_3	1	0.928	0.928	0.1137	0.753

and 0.04 µg/L, p=0.003831) (Figure 6c), inorganic nitrogen (6.11 µM/L and 18.14 µM/L, p=0.03228) (Figure 6d), and phosphates (1.93 µM/L and 0.19 µM/L, p=0.0009373) varied between May and July sampling periods (Figure 6e). On the other hand, silicate (5.52 µM/L and 6.31 µM/L) (Figure 6f) concentrations did not show significant variances between the May and July sampling months.

In the NSD terminal, on the other hand, MANOVA of physico-chemical parameters, such as temperature, salinity, chlorophyll-*a*, and nutrients, between months are shown in Table 7.

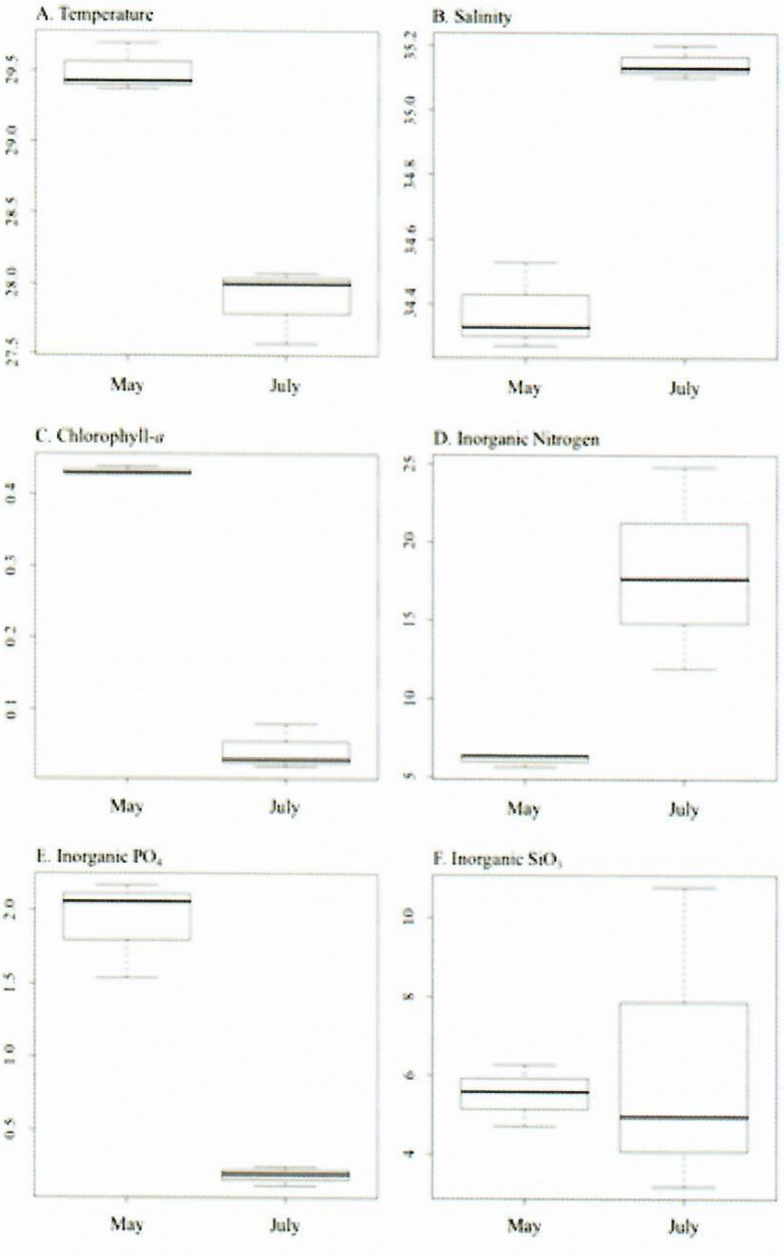

Figure. 6. Average physico-chemical parameters in CIP, Cebu: (A) temperature (°C); (B) salinity (ppt); (C) chlorophyll *a* (µg/L); (D)-(F) inorganic nutrients (µg/L) (nitrogen, PO_4^{-3}, and SiO_3^{-1}).

Average ambient water temperature (30.62 °C and 29.41 °C, p=0.003737) (Figure 7a), salinity (34.14 ppt and 32.41 ppt, p=0.009201) (Figure 7b), inorganic nitrogen (6.03 μM/L and 11.12 μM/L, p=0.0003957) (Figure 7d), and silicates (3.74 μM/L and 7.51 μM/L) (Figure 7f) varied between the May and July sampling periods. On the other hand, average chlorophyll-*a* (0.41 μg/L and 0.37 μg/L) (Figure 7c) and phosphates (0.87 μM/L and 0.61 μM/L) (Figure 7e) concentrations did not show significant variances between the May and July sampling months.

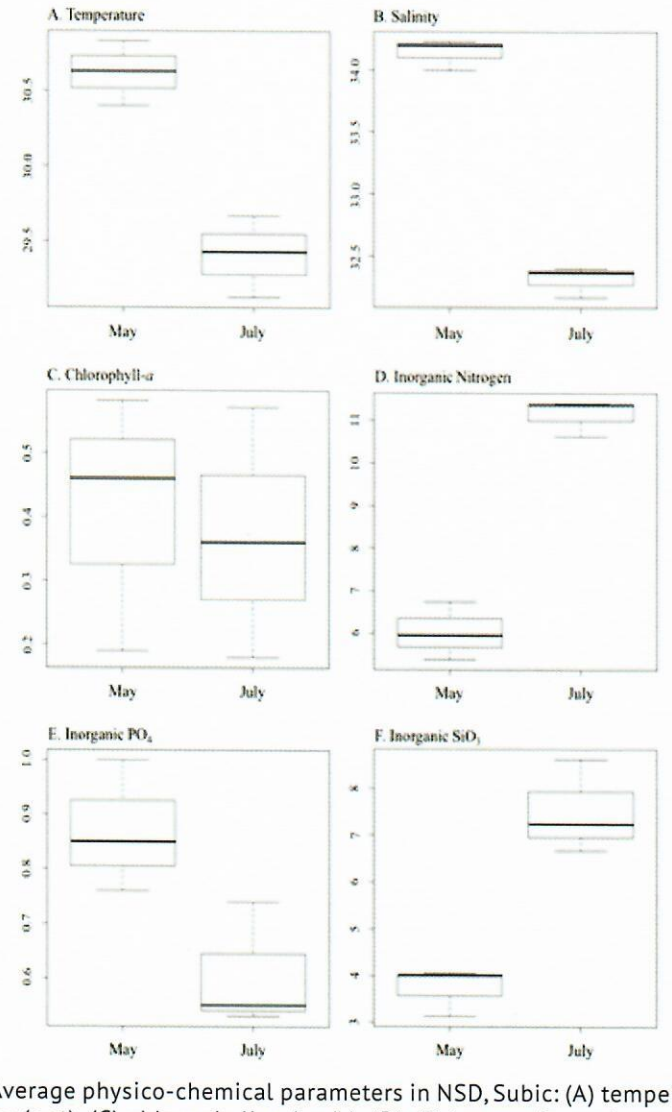

Figure 7. Average physico-chemical parameters in NSD, Subic: (A) temperature (°C), (B) salinity (ppt), (C) chlorophyll-*a* (μg/L), (D)-(F) inorganic nutrients (nitrogen, phosphates, and silicates).

Table 7. Summary of MANOVA among variables in NSD, Subic in May 2015 and July 2015

Variables	Df	Sum Sq	Mean Sq	F value	ρ-value
Temperature	1	2.1961	2.19615	36.756	0.003737
Salinity	1	0.21373	0.21373	22.237	0.009201
Chl-*a*	1	0.0024	0.0024	0.0615	0.8163
Inorganic N	1	38.913	38.913	119.82	0.0003957
PO_4	1	0.104017	0.104017	7.3945	0.05303
SiO_3	1	21.3948	21.3948	33.457	0.004438

DISCUSSION

The results of this study are essential for the establishment of the baseline data of the most common phytoplankton found in the two major ports in the Philippines. Results revealed diatoms to be the most common and dominant taxa in both Cebu and Subic ports as indicated by high chlorophyll-*a* concentrations. This result supports Wang and Wang (2011) who proposed that chlorophyll-*a* concentration is indicative of phytoplankton biomass in response to certain environmental factors. In Manila Bay, diatoms have also been observed as the most dominant taxa in all seasons from 1997 to 1998 (Azanza and Miranda 2001), while algal bloom of diatom *Stephanophyxis* was recorded in 2008 which could be attributed to the eutrophicated waters of the bay (Chang et al. 2009). Diatom abundance was also reported in Cape Bolinao, Pangasinan in all seasons from 1998 to 2000 (Yap et al. 2004), in 2001 before the onset of *Prorocentrum minimum* bloom in 2002 (Azanza et al. 2005), during the summer periods in 2004 (Azanza et al. 2006), and during the *Alexandrium* bloom events from 2010 to 2011 (Azanza and Benico 2013) associated with eutrophication (San Diego - McGlone et al. 2008). Other studies abroad also noted that diatoms, such as *Skeletonema costatum*, are most common in harbor areas, similar to what has been observed in Visakhapatnam Harbour, India (Rao and Mohanchand 1988) and Jiaozhou Bay, China (Hou and Shu 2005; Liu et al. 2005).

This abundance could be associated with several parameters, such as temperature, salinity, and nutrients, which influence phytoplankton diversity and community distribution (Shridhar et al. 2006; Shapoori and Gholami 2014). This was apparent in the study when, for example, diatom *Pseudo-nitzschia* spp., recorded the highest cell densities, while other diatoms, such as *Leptocylindrus, Pleurosigma, Thalassiosira,* and *Rhizosolenia*, were also common in CIP when temperature, salinity, and inorganic PO_4 were abundant in May. Previous studies have revealed that an increase in temperature enhances aggregation and optimal growth of diatoms at higher temperature and salinity in harbor and bay area (Hemalatha et al. 2012; Araujo and Garcia 2005; Thornton and Thake 1998; Mortensen et al. 1988). Shridhar et al. (2006) and Heneash et al. (2015) also mentioned salinity as one of the main physical parameters that could explain plankton diversity and community variations. In addition, using PCA, the algal abundance of the common taxa in CIP was revealed to have been greatly influenced by the ambient physico-chemical parameters at 58.7% and 14.9% variation. The PC loadings of physical parameters suggest that the abundance of *Leptocylindrus* and *Pleurosigma* were influenced by the highly correlated temperature and PO_4, whereas highly correlated salinity, NO_3, and NH_3 may also have significant impacts on the abundance of *Thalassiosira* and *Rhizosolenia*.

Moreover, the high cell abundance of *Thalassionema*, *Leptocylindrus*, *Thalassiosira, Coscnodiscus*, *Chaetoceros*, *Odontella*, and *Fragillaria* were also recorded in NSD terminal when temperature and inorganic nutrients, such as inorganic nitrogen, phosphates, and silicates, were higher in July. This result further demonstrates the essential influence of both temperature and nutrients to phytoplankton diversity and abundance (Nowrouzi and Valabi 2011; Shapoori and Gholami 2014). Aside from the fact that diatoms need inorganic silicates to form their shells (Turner et al. 1998), the experiments of Egge and Aksnes (1992) also indicate that diatoms dominate when SiO_3 is above 2 μM/L and tend to aggregate in areas with relatively high silicate concentration (Sommer 1998). More importantly, both inorganic phosphates and inorganic nitrogen are essential nutrients that stimulate the growth and activities of phytoplankton (Bizsel et al. 2001; Ho et al. 2008; Martin-Jezequel et al. 2015). PCA further supports the influence of these parameters in the variation of the algal abundance in NSD terminal. The loads of physical parameters suggest that *Coscinodiscus* was influenced by highly correlated parameters, such as temperature, NO_2, $NH_{3,}$ and PO_4, whereas the abundance of *Thalassionema* was greatly influenced by the highly correlated salinity, NO_3, and SiO_3.

Interestingly, *Pseudo-nitzschia* spp. was recorded as the most abundant diatom in CIP in May, whereas *Leptocylindrus* spp also exhibited high cell abundance in NSD terminal in July; however, both taxa did not respond to any physico-chemical parameters according to PCA. This result could either be species-specific or due to combined effects of other factors, thereby warranting further investigation. It is also important to note that phytoplankton diversity and other physico-chemical processes determine the distribution of inorganic nutrients (Webber et al. 2003; Longhi and Beisner 2009). Taking into consideration the combination of factors, such as land runoffs, nutrient uptake, excretion, and other physiological aspects of the dominant organisms, which are known to influence nutrient concentration and availability in the marine environment (Liu et al. 2005), will also be more essential in future studies.

In summary, the main findings of this study are the data of the assemblages and composition of diatoms and dinoflagellates in two of the major ports in the country. Diatoms were the most dominant and abundant component among all stations in both ports being investigated, which could be attributed to physical parameters, such as water salinity, temperature, and nutrients, as suggested by the PCA results. However, other factors, such as nutrient loadings from land runoffs, nutrient uptake, excretion, and other physiological aspects of dominant species that could influence nutrient availability, were not examined in this study. Thus, it is highly recommended to carry out a thorough research regarding those parameters in the future. Further studies on the temporal distribution of phytoplankton assemblages and composition in both ports should also be considered in the future. Lastly, the presence of bloom-forming, potentially harmful and toxic species in Cebu and Subic ports could impose risks in the possible uptake of these organisms in ballast tanks and transport. Results of this study would contribute to and serve an essential part in the creation of baseline data for port states, which could be utilized as an important tool in complying with the International Maritime Organization – Ballast Water Management Convention - 2004 (IMO-BWM-2004).

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