#### Effect of Nutrient Over-enrichment on Spatio-temporal Variability of Phytoplankton in Manila Bay, Philippines

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#### Abstract

To understand Manila Bay's current condition, physicochemical parameters were correlated with net phytoplankton composition from 2012 to 2015. Nitrate concentrations reached 24.18  $\mu$ M, which is above the critical value (5  $\mu$ M) recommended by the Philippine Department of Environment and Natural Resources and ASEAN. Hypoxia has been observed with dissolved oxygen levels as low as 1.47 mg L<sup>-1</sup>. Phytoplankton composition varies but dominated by *Chaetoceros curvisetus, Skeletonema costatum, Thalassiosira* sp., and *Thalassionema nitzchiodes*. Phytoplankton densities also vary between seasons but mostly concentrated in stations near the tributaries and urban areas. Trends in both phytoplankton and physicochemical properties suggest that the ecosystem of the bay is highly dependent on rainfall. Shannon-Wiener diversity index does not go higher than 2.46 and based on monthly averages, the bay can be categorized as moderately heavy to heavy polluted. A watershed system approach is urgently needed since found heavy eutrophication generally occurs in estuaries near urban and industrial areas.

Keywords: eutrophication, Manila Bay, hypoxia, watershed, nutrients

## **I**NTRODUCTION

Manila Bay has long been significant to Philippine history and livelihood. It has been, and still is the venue for most of the domestic and international trades in the island of Luzon through the ports in Metro Manila. Recently, however, much industrialization around Manila Bay and its watershed has caused pollution in its waters. In 2008, the Supreme Court of the Philippines issued a *mandamus* to investigate the status of the Bay, in an attempt to restore and revitalize its ecosystem (Supreme Court General Registry Nos. 171947-98, 2008).

Despite the international importance of Manila Bay as an aquatic environmental pollution model (Chang, et al. 2009), most literature about its waters were either on Harmful Algal Species (HAS) (Azanza & Miranda, 2001; Azanza et al., 2004; Furio et al., 1996; Furuya, et al. 2006) or studies on its water quality and physical profiles (Hayashi, et al., 2006), which tend to focus on the near bottom layer of the bay (Jacinto, et al. 2006; Prudente, et al., 1994; Sotto, et al., 2014.). However, not much on the correlation among these factors has been reported. In 2009, Chang, et al., released a study that determined the effect of Manila Bay's polluted waters on its ecosystem function. It determined that the bay is already suffering from eutrophication, especially in ammonium and that the eutrophication is causing undesirable effects to the bay's ecosystem.

The study aimed to determine correlations of physicochemical parameters of the surface waters of Manila Bay with its phytoplankton community by (1) determining species composition and spatiotemporal trends in distribution, (2) determining if there are spatiotemporal trends in the bay's physicochemical properties, and (3) identifying the degree of pollution in the bay through assessment of phytoplankton species diversity.

## MATERIALS AND METHODS

The semi-enclosed Manila Bay has a surface area of 1,994 km<sup>2</sup> (769.9 sq. mi.), and a coastline of 190 km (118.1 mi.), surrounded by Metro Manila in the east and the provinces of Bulacan and Pampanga in the north, Bataan in the west, and Cavite in the southeast (Figure 10.1).

A total of 16 north to south transect stations were established in the entire bay for hydrobiological sampling. Sampling was conducted every two months from January 2012 to November 2013. Depth, temperature, salinity, dissolved oxygen, and chlorophyll *a* levels were determined using a YSI MDS 6600 CTD from surface to one meter above recorded bottom depth via an echo sounder. Only data from zero to one meter below the surface was used for this study. Water samples were collected using a 5L Niskin sampler and filtered through a 0.45 µm Whattman filter using an Eyela A-3S vacuum filter and were frozen until analysis. Afterward, inorganic nutrients content (NO<sub>3</sub>, NO<sub>2</sub>, PO<sub>4</sub>, SiO<sub>3</sub><sup>2-</sup>) were determined by methods modified from Parsons et al. (1974) using a Merck Spectroquant Pharo 300 UV-VIZ spectrophotometer.

Phytoplankton samples were obtained by vertically towing a conical net (0.3 m mouth diameter, 20um mesh) from a depth of 3 m to the surface. These were then preserved in a 10% formaldehyde solution and stored in 25ml Nalgene bottles. Phytoplankton cell counts or density were performed using the formula recommended by the American Public Health Association (WEF & APHA, 2005). Morphological analysis of phytoplankton samples was performed on an Olympus CX41 equipped with Infinity Capture Digital Camera (Lumenera Corporation, Ontario, Canada) at 100x and 400x magnifications. Specimens were identified up to the lowest possible taxa. As an aid to the identification, taxonomic publications of Tomas (1978),



Figure 10.1. Study Site: Manila Bay (14°31′00″N120°46′00″E) showing 16 pre-established sampling stations.

Hasle (1978), Heimdal (1993), and Omura *et al*. (2012) were used.

Profile of water's physicochemical parameters and phytoplankton abundance and distribution were analyzed and interpreted using isopleths generated through Surfer 11 (Golden Software, 2012); multivariate canonical correspondence analyses in CANOCO 5 (Leps & Šmilauer, 2012). Phytoplankton species diversity was calculated using Shannon-Wiener Index and computed using the software PRIMER 5 (Clarke & Warwick, 2001).



#### **Physico-chemical parameters**

Sea surface temperature (SST) in Manila Bay peaked during May and July all years, as the summer transitions into the Southwest Monsoon (SWM). Lower temperature is observed during SWM across all years. SST in almost all sampling periods is generally uniform throughout the bay's surface water. Although 2012 had the highest average surface temperature among the sampling years (27.74±1.68°C), the highest temperature was recorded in May 2014 at 35.59°C. Lowest recorded SST was at 23.97°C on September 2013 (Figure 10.2).

Salinity profiles during the wet season when the SWM is prevalent for July and Septem-

ber vary across the surface of the bay. Across all years, the salinity is almost uniform from January to May. Surface salinity ranged from 2.36 parts per million (ppm) (November 2015) to 31.31ppm (March 2012). Surface salinity was lowest during September for the whole survey duration. Widest salinity range difference in minimum and maximum values were observed in the September surveys as well. The very low salinity in September may be explained by rain during the survey period. The salinity was lower near the coast particularly in the eastern (MB9, MB10), northern (MB14, and MB16) and western areas (Figure 10.3).

The surface of the bay is relatively welloxygenated, however, dissolved oxygen concentration in the surface greatly varies among years and seasons. Only one hypoxic (very low DO concentration) occurrence was observed on the eastern side for 2012 in March (4.61 mg L<sup>-1</sup>). For 2013, sections of the bay reached hypoxic levels, varying in location and size. There were instances wherein the entire surface of the bay had hypoxia (November 2013 and July 2014). Lowest DO level recorded was at 1.06 mg L<sup>-1</sup> on January 2015 (Figure 10.4).

#### Chlorophyll a

In general, the trend for chlorophyll *a* is that it reaches its highest levels in Manila Bay during the tradewinds or Southeast Monsoon (SEM), that prevails from March to June, or an intermonsoon especially when the climate starts to transition from tradewinds to the southwest monsoon. No data was collected on March 2012, July 2013 and September 2013. Across the surface, Chl *a* is commonly concentrated in either the northern, northeastern, or eastern parts of the bay, near the Meycauayan and Pasig River systems (Figure 10.5). The highest levels were observed in May 2013 reached 18.54 µg L<sup>-1</sup>. This was due to an algal bloom that lasted until July

2013, which was also reported in Chapter 8 of this volume.

#### Nutrients

Nitrates (N-NO<sub>3</sub>) on the surface of Manila Bay occasionally exceed the Association of Southeast Asian Nations (ASEAN) critical value of 4.29  $\mu$ M (McPherson, *et al.* 2001) especially in stations near the tributaries and urban areas surrounding the bay. In September and November 2013, N-NO<sub>3</sub> concentrations above the critical value were observed on the northern half of the Bay. Highest recorded amount of N-NO<sub>3</sub> on the surface water was on September 2013 at 24.18  $\mu$ M in Station 14. The highest average value, however, was noted on November 2013 at 8.39±7.38  $\mu$ M. September 2013 had an average N-NO<sub>3</sub> concentration of 7.99±7.41  $\mu$ M (Figure 10.6).

Mean concentrations of N-NO<sub>2</sub> do not exceed the ASEAN (McPherson, *et al.* 2001) critical value of 3.95  $\mu$ M (Figure 10.7). The only instances where N-NO<sub>2</sub> exceeded the critical value took place on September 2013 (4.52  $\mu$ M) and July 2015 (4.86  $\mu$ M). Highest mean concentration on the surface was observed on November 2013 at 1.00±0.52  $\mu$ M.

Average concentrations of phosphates (P-PO<sub>4</sub>) have not exceeded the ASEAN (McPherson, *et al.* 2001) critical value of 0.48  $\mu$ M. However, concentrations slightly below this value have been observed in many of the sampling periods, particularly near the river tributaries and urban areas. P-PO<sub>4</sub> concentrations above the critical value were observed on January 2012 and September 2013 in the northern part, near the Pampanga River (Station 14) and the mouth of the Bay. Highest recorded P-PO<sub>4</sub> concentration on the surface water was at 0.72  $\mu$ M on May 2014 in Station 16 (Figure 10.8).

Silicate (S-SiO<sub>3</sub>) concentrations have the











highest average values during the months of July and September. High concentrations relative to each sampling period can again be observed near tributaries and urban areas. Highest average concentration was observed in September 2013 at  $53.20 \pm 31.42 \mu$ M. Highest S-SiO<sub>3</sub> concentration was recorded in the same month amounting to 96.83  $\mu$ M (Figure 10.9).

### Phytoplankton Distribution, Composition, and Abundance

The phytoplankton tends to be more abundant in stations near the coast of the Bay and, in many instances, can be found on the eastern side of the Bay. Average values range from  $21,108 \pm 18,841$  to  $95,323 \pm 83,178$  cells L<sup>-1</sup>. The highest concentration of 121,919 cells L<sup>-1</sup> was recorded near the mouth (Station 1) of the bay on September 2012. In 2013 the highest phytoplankton concentration of 282,989 cells L<sup>-1</sup> was recorded on November in the western part off Bataan coast (Station 6) (Figures 10.10).

A total of 135 phytoplankton species consisting of 72 Bacillariophyceae (diatoms), 62 Pyrrophyceae (dinoflagellates), and 1 Cyanophyceae (blue-green algae) were identified throughout the sampling periods. The following species, all of which are diatoms, dominated the population across all sampling years: *Chaetoceros curvisetus, Skeletonema costatum, Thalassiosira* sp., and *Thalassionema nitzchioides*. These are followed by the species which were dominant in three out of four years: *Chaetoceros* sp., *Skeletonema* sp., and the cyanobacteria *Trichodesmium* sp.

#### **Canonical Correspondence Analyses**

Only the 12 most abundant species per year are shown in the canonical correspondence analyses (CCA) as it comprises the majority of the phytoplankton communities (2012 – 85.90%;

2013 - 88.82%; 2014 - 87.62%; 2015 - 86.33%). The rest of the species were included in the analyses but omitted in the CCA triplots. Results of the multiple stepwise regressions by Monte Carlo simulation are presented in Table 2. Based on the *P*-values, the temperature is the most important parameter that affects the species composition. This is followed by both salinity and dissolved oxygen which posed a highly significant correlation to species composition from 2012-2014. Chlorophyll *a* also indicated *P*-values of high significance for 2013 and 2015, where relatively high chlorophyll levels were observed. Other nutrients showed *P*-values that show high to moderate correlation (Figures 10.11).

#### Species Diversity (Shannon-Weiner Index)

Relatively high species diversity index values can be found near the middle of the bay for most sampling periods. In July 2012 for most sampling periods. In July 2012 and 2013, high values were observed in the northern half of the bay. Throughout the sampling period, the Shannon-Weiner index values only had a range of 0.02 to 2.46 (Figures 10.12).

Most of the dominating species seem to be resilient towards any changes in the measured water quality parameters. The maximum value for nitrates is higher than those from previous studies (Chang, et al., 2009; Sotto, et al., 2012). However, Sotto, et al. (2012) observed higher values in August 2012 for silicates and phosphates at 2.42  $\mu$ M and 14.5  $\mu$ M, respectively. The same sampling period in their study showed a lower maximum nitrite value as compared to the rest of the values obtained for 2012 in this study. A higher value for nitrites, however, was recorded in 2010 and 2011. This demonstrates the high variation of nutrient input and cycling in the bay (Hayashi et al., 2010) as well as the ongoing overenrichment.









	2012	2013	2014	2015
Species				
Diatoms				
Bacteriastrum delicatulum			6.99	
Bacteriastrum furcatum			-	17.80
Chaetoceros compressus		()	•	2.56
Chaetoceros curvisetus	14.80	8.17	1.98	14.10
Chaetoceros decipiens			1.25	5.28
Chaetoceros pseudo-curvisetus			1.70	
Chaetoceros sp.	6.03	2.23	6.40	
Coscinodiscus sp.	2.24	-	-	1.29
Dictyocha fibula	5.75		-	
Lauderia annulata	10.00	6.48	-	-
Navicula sp.	2.40			
Nitzschia sp.		2.20	1.30	-
Pseudo-nitzschia sp.				2.00
Rhizosolenia alata	- 1 <b>-</b> 5	3.40	1.20	-
Skeletonema costatum	16.60	3.22	11.40	13.50
Skeletonema sp.	9.03	11.00	3.27	-
Thalassionema frauenfeldii			1.00	
Thalassionema nitzschoides	1.72	14.10	6.26	3.91
Thalassiosira rotula	-	5.30	-	-
Thalassiosira sp.	9.53	29.64	41.73	18.25
Cyanobacteria				
Trichodesmium sp.	1.87		4.84	5.12
Dinoflagellates				
Ceratium furca	3.87			1.10
Ceratium teres			-	1.30
Protoperidenium sp.		1.40	-	

Table 10.1. Dominant species in Manila Bay. The 12 most abundant species per year were noted, with percentages in relation to annual population indicated.



#### Phytoplankton Density Manila Bay 2012

Figure 10.10a. Phytoplankton density in Manila Bay for 2012.



#### Phytoplankton Density Manila Bay 2013

Figure 10.10b. Phytoplankton density in Manila Bay for 2013.



#### Phytoplankton Density Manila Bay 2014

Figure 10.10c. Phytoplankton density in Manila Bay for 2014.



#### Phytoplankton Density Manila Bay 2015

Figure 10.10d. Phytoplankton density in Manila Bay for 2015.



Figure 10.12a. Shannon-Weiner diversity index for phytoplankton for 2012



### Phytoplankton Diversity in Manila Bay, 2013

Figure 10.12b. Shannon-Weiner diversity index for phytoplankton for 2013



Figure 10.12c. Shannon-Weiner diversity index for phytoplankton for 2014



Figure 10.12d. Shannon-Weiner diversity index for phytoplankton for 2015



Figure 10.11a. Canonical Correspondence Analysis of Phytoplankton to Physico-chemical parameters for 2012.



Figure 10.11b. Canonical Correspondence Analysis of Phytoplankton to Physico-chemical parameters for 2013.



Figure 10.11c. Canonical Correspondence Analysis of Phytoplankton to Physico-chemical parameters for 2014.



Figure 10.11d. Canonical Correspondence Analysis of Phytoplankton to Physico-chemical parameters for 2015.

Temperature, dissolved oxygen (DO), silicates, salinity, and chlorophyll *a*, are the significant environmental factors affecting the phytoplankton species variability for 2012, with DO being the most significant as it was able to account for 17.8% of the variability in species data. The second most important parameter is temperature, accounting for 9.5% of the variability. Most of the phytoplankton species that were dominant for 2012 and 2013 were reported to flourish in waters of very high nutrient content (Santiago et al., 2010; Carli, et al., 1994). Particularly, Skeletonema costatum is reported as an indicator species for pollution (Lo, 1998, as discussed in Lo, 2004; Larsen, et al. 2004). Trichodesmium thiebautii, dominant in May 2012, is different from the other species as it prefers oligotrophic waters and that particular sampling period experienced waters of relatively low nutrient concentrations. It may even have contributed to the reduction in N-NO<sub>3</sub> and N-NO<sub>2</sub> levels as it is a nitrogen fixator (Carpenter & McCarthy, 1975). In our redundancy analysis, nitrate and phosphate were not significant factors for both 2012 and 2015. This suggests that these eutrophication-linked macronutrients may no longer be limiting factors for phytoplankton growth as their concentration levels are generally very high throughout Manila Bay in 2012 and 2015.

The Shannon-Weiner index confirms the state of pollution in the bay. According to Mason (1998), the index should have a value above 3.0 for phytoplankton for the area to be considered as clean. An index value of 1.0 to 3.0 would indicate that the area is moderately polluted, while values lower than 1.0 would characterize the area as heavily polluted (Fig. 10.15) Monthly average values of the index indicate that the bay is experiencing moderately heavy to heavy pollution, with lowest average values in March, September, and November for both 2012 and 2015. There are, however, instances where the value almost reached a value of 3.0.

## CONCLUSION

Temperature, salinity, and dissolved oxygen are the most significant factors for the phytoplankton species composition in Manila Bay as they have consistently displayed high correlations across all years. However, it can be noted that nutrient levels, particularly of nitrates, may also affect the amount of dissolved oxygen present, making it a significant factor contributing to the changes in species composition. This has then affected the phytoplankton composition in the bay such that it already has a low diversity, with different species dominating in almost every sampling period. Consequently, this greatly lowered the Shannon-Weiner index values in the bay to as low as 0.02, indicating that certain ecosystems are being dependent on rainfall, as most of the observed trends coincide with either Northeast Monsoon or Southwest Monsoon. However, this has to be compared to the rainfall data during this sampling period before it can be confirmed. Observations on the nutrient levels have also determined the degree of pollution the bay is currently experiencing. Further analysis of the bay's deeper waters and the sediments need to be analyzed in order to fully grasped the severity of the pollution occurring in the bay.

Table 10.2. – Canonical Correspondence Analysis multiple stepwise regression by Monte Carlo permutation for physicochemical explanatory variables and phytoplankton species abundance. *P*-value of 0.002 indicates that Factor has a significant effect on species abundance.

2012 Factor	% Explained 10.0 8.2 4.9 1.8 1.2 1.1	pseudo-F	P 0.00 0.00 0.00 0.08 0.21 0.29				
Salinity		8.6					
Temperature Dissolved Oxygen P-PO4 <sup>-</sup> N-NO3 <sup>-</sup> Chlorophyll a		7.7					
		4.9					
		1.8 1.2 1.1					
				N-NO <sub>2</sub>	1.0	1.0	0.43
				Si-SiO4 <sup>4-</sup>	0.8	0.8	0.80
2013 Factor	% Explained	pseudo-F	Р				
Temperature	8.9	7.6	0.002				
Salinity	8.4 5.8 2.2 2.2 1.8	7.8 5.7 2.2 2.2 1.8	0.002 0.002 0.008 0.004 0.01				
Dissolved Oxygen							
Chlorophyll a N-NO2 <sup>-</sup> Si-Sio4 <sup>6-</sup>							
				P-PO4	1.7	1.7	0.014
				N-NO3 <sup>-</sup>	1.3	1.4	0.128
2014 Factor	% Explained	pseudo-F	Р				
Temperature	7.0	6.7	0.002				
Salinity	3.5	3.5	0.002				
Dissolved Oxygen	3.4	3.5	0.002				
P-PO4	1.8	1.9	0.006				
S-SiO44	1.8	1.8	0.016				
Chlorophyll a	1.4	1.4	0.112				
N-NO <sub>3</sub>	1.0	1.1	0.268				
N-NO <sub>2</sub> -	0.8	0.8	0.686				
2015 Factor	% Explained	pseudo-F	Р				
Temperature	9.2	6.1	0.002				
S-SiO4 <sup>4-</sup>	4.2	2.9	0.002				
Chlorophyll a	4.6	3.2	0.002				
Salinity	2.5	1.8	0.068				
N-NO <sub>3</sub> -	2.2	1.6	0.076				
P-PO <sub>4</sub> -	1.9	1.4	0.116				
Dissolved Oxygen	1.8	1.3	0.154				
N-NO <sub>2</sub>	1.4	1.0	0.416				

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