

Nitrogen Concentrations in Surface Water and Bottom Water of Tolo Harbour, Hong Kong, & Its Potential Effect on Marine Life

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The industrialization of Hong Kong has caused eutrophication in water systems. Specifically, Tolo Harbor, Hong Kong, located just one km from industrial and wastewater treatment facilities, such as the Tai Po Sewage Treatment Works, makes it highly vulnerable to pollution. The sewage facilities can introduce nitrogen-rich effluent into the ocean, and because Tolo is an enclosed harbor, limited mixing occurs, and pollutants tend to concentrate (Environmental Protection Department, 2022). As a result, the eutrophication present in Tolo Harbor could lead to hypoxic conditions, or oxygen deficiency, in its water. Through monthly sampling at five different locations within Tolo Harbor, the study asks the question: what are the trends between surface and bottom water NO₂ and NO₃ concentrations near the coast, and farther from the coast in Tolo Harbour? The findings of this study not only allow us to understand nitrogen dynamics in Tolo Harbour, but the final part of the study will present different species of marine organisms in Tolo Harbour such as corals, bivalves, fish, and echinoderms, and go into depth on how they are negatively affected by hypoxic conditions.

Keywords: nitrogen, hypoxia, eutrophication, surface water, bottom water

INTRODUCTION

Excess nitrogen from human activities has altered and impacted many marine systems around the world. Furthermore, eutrophication is the gradual increase of nitrogen and other chemicals in an aquatic ecosystem. While eutrophication can be a natural process, it is more often a result of anthropogenic causes. Anthropogenic eutrophication, also known as cultural eutrophication, occurs when human water pollution speeds up the aging process of a lake (or other body of water) by introducing direct sewage discharge, detergents, and fertilizers. These discharges are high in nitrogen and introduce excess into the ecosystem.

Moreover, when it rains, water washes excess nitrogen into rivers through sewage effluents, which are ultimately discharged into coastal regions. Coastal regions are often urban centers and particularly susceptible to anthropogenically-produced nitrogen due to their proximity to urban wastewater treatment facilities (Menció *et al.* 2023). Nitrogen is a limiting nutrient in coastal surface water, which means that it largely controls the amount of algae and other plant life that is produced in the marine ecosystem. When there is an addition of nitrogen through anthropogenic activity, it will be exported to offshore bottom water (Voss *et al.* 2011). Atmospheric deposition can lead to high concentrations of nitrogen.

In addition, atmospheric deposition refers to particles collecting themselves on solid surfaces. Atmosphere deposition of nitrogen fertilizers in agriculture and sewage wastewater is a major source of nitrogen content in coastal regions. (Muralidhar *et al.* 2017).

The process of upwelling also brings nitrogenous compounds to the surface of coasts. When winds blow over the ocean surface and push water aside, deeper water rises and replaces it. As a result, nitrogenous compounds are brought up to the surface, increasing the N concentration near the surface of coasts (NOAA).

As a result of the excessive nitrogen content in a body of water, there is an overgrowth of algae. When the algae decompose, bacteria eat the algae and consume oxygen. This severely depletes oxygen levels in a marine ecosystem and can cause a phenomenon called hypoxia. Hypoxia is defined as low or depleted dissolved oxygen in a body of water (NOAA, 2021). Hypoxia has significant effects on marine organisms. This lack of oxygen in water systems is associated with increased mortality in fish, bivalves, and other marine organisms (Gaubler and Baumann, 2016).

This essay focuses on uncovering the nitrogen dynamics in Tolo Harbor. Tolo Harbor is located in the northeastern part of New Territories, Hong Kong. Also known as part of the Tai Po district, Tai Po has a population density of 2,137/km² (City Population, 2022). Its main industries include manufacturing and service industries, such as Tai Po Industrial Estates (Tai Po District Council, no date). Specifically, in Tolo Harbor chemicals and pollutants like $SiO₃$, PO₄, and NO³ are discharged into the harbor (Hodgkiss and Chan, 1983).

Although Tolo Harbor is susceptible to marine pollution, its marine ecosystem is still biodiversity. Between 2015 and 2018, 1473 marine species were cataloged in Tolo Harbor (Astudillo *et al.* 2019). As this research focuses on surface and bottom water nitrogen content and hypoxia, four different depths of water, and the marine organisms present in them, will be studied. In shallow water and the rocky shore of Tolo Harbor, both examples of surface water, the effect of hypoxia on corals and bivalves will be studied. In the demersal and sea bed area, both examples of bottom water, the effect of hypoxia on fish and echinoderms will be studied.

Thus, to study the nitrogen concentrations in Tolo Harbor, which could potentially cause hypoxic conditions, –and by association potential drivers of hypoxic conditions–surface and bottom water nitrogen samples were studied across five locations.

METHODS

To begin, samples of bottom water and surface water were collected every month for 12 months at the following five locations in June and July of 2023.

Surface water was collected and filtered through a 100 µm mesh, to sieve any sediment floating on the surface. Bottom water (ranging from 5 - 20 m depending on the site) was pumped up by a mechanical pump and then filtered through a 100 µm mesh. The water samples were then tested for NO₂ and NO³ colorimetrically. Because the absorbance of the Tolo Harbour samples is recorded and not their concentration, we need standard solutions of different concentrations. NO² and NO³ standard solutions of 8 different concentrations were created—40, 20, 10, 5, 1, 0.5, 0.1, and 0 μ mol L⁻¹. Then 200 μ L of the samples and standard solutions were pipetted in a 96-well plate. In this case, there were multiple technical replicates, with 2 or 3 samples taken from a single biological replicate. For $NO₂$ and $NO₃$ analysis, two indicators, Greiss Reagent, and VCl3, were added to the samples to identify the concentration of $NO₂$ and $NO₃$. 20 µL of Greiss Reagent was pipetted for NO₂ indication. And 20 μ L of VCl₃ was pipetted to determine $NO₃$ indication. If there is any $NO₂$ or $NO₃$ present, the samples will turn into a dark pink color. A darker pink color would yield a higher absorbance.

Image 1: Map of Tolo Harbour marked with sampling sites 1 through 6. The red dot is the location of Tai Po Sewage Treatment Works (*image courtesy of The Chinese University of Hong Kong)*

Figure 1: NO₃ standard absorbance curve

Then, using a Tecan Spark 10M Microplate Reader, the 96-well plate was read at an absorbance of 540 nm. After the samples are measured with a spectrophotometer, the absorbance values of the standard solutions are plotted to create a standard curve. With known concentrations and absorbance, Beer-Lambert Law is utilized to derive the constant values of (light path x molar absorptivity). In the equation below, A is absorbance, $\boldsymbol{\varepsilon}$ is the numerically unknown (light path length x molar absorptivity), and C is the known concentration:

$$
A = \varepsilon b C
$$

Below is the standard curve derived from using the Beer-Lambert law, where $0.015 = e b$.

Since the standard curve provides the linear relationship between absorbance and concentration, we can determine the otherwise unknown concentrations of our field samples using the known absorbance (A) and constant (eb) values.

RESULTS

Tolo Harbor Site 1: See Appendix A for a table of the data

Samples of all sites were taken on June 15th, 2023, and July 25th, 2023. Site 1, or TH01, is nearest to the docking point at the Simon F.S. Li Marine Science Laboratory. The surface water at site 1 was found to have an average $NO₂$ concentration of 0.376 µmol L⁻¹ and an average $NO₃$ concentration of 14.1 µmol L⁻¹. The surface water at site 1 was found to have an average NO₂ concentration of 0.373 μ mol L⁻¹ and an average NO₃ concentration of 3.49 µmol L⁻¹. While the NO₂ bottom water concentration in June was 0.400 µmol L-¹, and the NO₃ concentration was 2.16 μ mol L⁻¹. In July, the NO₂ bottom water concentration was 0.293 μ mol L⁻

¹. The bottom water concentration of $NO₃$ was 1.77 µmol L-1.

Tolo Harbor Site 2: See Appendix A for a table of the data

Site 2, or TH02, is further out from the dock and is located in the middle of Tolo Harbour. The surface water at site 2 had an average NO₂ and NO₃ concentration of 0.0472 μ mol L⁻¹ and 0.131 μ mol L⁻¹, respectively in June. The bottom water concentration of $NO₂$ and $NO₃$ were 0.0482 μ mol L⁻¹ and 0.0462 μ mol L-1, respectively. In July, the surface water at site 2 had an average NO₂ and NO₃ concentration of 0.0458 µmol L⁻¹ and 0.0454 µmol L⁻¹, respectively. And, the $NO₂$ and NO³ concentration of the bottom water was 0.0482 μ mol L⁻¹ and 0.0491 μ mol L⁻¹, respectively.

Figure 2: Tolo Harbour June surface vs bottom water NO₂ concentrations for 5 different sites

Figure 3: Tolo Harbour July surface vs bottom water NO₂ concentrations for 5 different sites

Tolo Harbor Site 3: See Appendix A for a table of the data

Site 3, or TH03, is located near Double Cove, which is near the middle of Tolo Harbour but more towards its outlet. In June, the surface water at this site had an average $NO₂$ concentration of 0.0449 µmol L⁻¹, while the bottom water had an average $NO₂$ concentration of 0.116 μ mol L⁻¹. In July, the surface water at this site had an average $NO₂$ concentration of 0.0443 µmol L⁻¹ and an average bottom water concentration of 0.0477 umol L^{-1} . For NO₃ in June, the surface water at this site had an average concentration of 0.0559 µmol L⁻¹, while the bottom water concentration was 0.0661 µmol L^{-1} . In July, the average surface water concentration of $NO₃$ was 0.0443 µmol L ¹, and the average bottom water concentration of NO_3 was 0.0513 µmol L⁻¹.

Tolo Harbor Site 5: See Appendix A for a table of the data

Site 5, or TH05, is the farthest away from the dock, and is the outlet of Tolo Harbour as it connects to Mirs Bay. The average $NO₂$ concentrations in June were 0.0447 μ mol L⁻¹ and 0.103 μ mol L⁻¹ for surface and bottom water respectively. In July, the average $NO₂$ concentrations were $4.243E-06$ µmol L ⁻¹ and 0.0113 µmol L-1 for surface and bottom water respectively. For NO3, the average concentrations in June were 0.0553 μ mol L⁻¹ and 0.0881 μ mol L⁻¹ for surface and bottom water respectively. In July, the average concentrations were 0.0574 µmol $L¹$ and 0.0816 µmol $L¹$ for surface and bottom water respectively.

Figure 4: Tolo Harbour June surface vs bottom water NO₃ concentrations for 5 different sites

Figure 5: Tolo Harbour July surface vs bottom water NO₃ concentrations for 5 different sites

	Surface Water - Bottom Water T-TEST				
	June $NO2$	July NO ₂	June NO ₃	July NO ₃	
TH01	0.68	2.10	$9.73***$	$5.83***$	
TH02	1.90	1.00	$7.81***$	0.76	
TH03	15.94***	$6.75***$	$2.98*$	$4.50**$	
TH05	$21.19***$	$9.02***$	0.40	1.73	
TH06	$5.62***$	28.14***	15.60***	$4.46**$	

Chart 1: Alpha <0.05 *, Alpha < 0.025**, Alpha < 0.01***. T-Test comparing surface water and bottom water concentrations at 5 different sites in Tolo Harbour for June NO₂, July NO₂, June NO₃, and July NO₃ concentrations.

Tolo Harbor Site 6: See Appendix A for a table of the data

Site 6, or TH06, is located in the innermost corner of Tolo Harbour, near Tai Po. The average $NO₂$ surface water concentration in June was 0.0575 µmol L⁻¹, while the bottom water concentration was 0.0479 μ mol L⁻¹. In July, the average NO₂ surface water concentration was 0.0581 μ mol L⁻¹, while the bottom water concentration was 0.0449 µmol L⁻¹. For NO₃, the average surface concentration in June was 0.358 µmol L-1, while the bottom water concentration was 0.0832 μ mol L⁻¹. In July, the average NO₂ surface concentration was 0.125 µmol L⁻¹, while the bottom water concentration was 0.0587 µmol L⁻¹.

DISCUSSION

While the data was collected meticulously, utilizing the Beer-Lambert law proved imperfect. Typically, each month's (June and July) samples each have their standard solution. However, when data points for the standard solution had to be removed to plot a standard curve with the most accurate linear regression, some concentrations became negative. To

fix this, the June and July $NO₂$ and $NO₃$ concentrations were both compared to the same standard solution. Furthermore, the ratio between the concentration average and standard deviation had to be above 10 for the concentration data to be considered accurate. But, some data points fell below the ratio of 10. However, there are still meaningful insights to be gleaned from this analysis. As well established in the literature, increased nitrogen levels can cause hypoxia as well as adversely affect local marine ecosystems.

Firstly, site 1 and site 6 have consistently higher surface water $NO₂$ and $NO₃$ concentrations. The June SW NO² concentrations for site 1 and site 6, were the highest among the other 3 sites, being 0.376 umol L^{-1} and 0.671 µmol L⁻¹ respectively. The July SW NO₂ concentrations for sites 1 and 6 were again the highest amongst the other 3 sites, being 0.373 µmol $L⁻¹$ and 0.698 umol L^{-1} respectively. Again in June, the SW NO₃ concentrations were the highest, with 14.1 µmol L ⁻¹ being the concentration for site 1, and 21.1 μ mol L⁻¹ being the concentration for site 6. In July the $NO₃$ concentration of the SW was 3.49 µmol L ⁻¹ for site 1, and for site 6 the concentration was 5.57 umol L^{-1} , the two highest concentrations.

	Surface Water - Bottom Water T-TEST				
	June $NO2$	July NO ₂		July NO ₃	
TH01	0.68	2.10	$9.73***$	$5.83***$	
TH06	$5.62***$	$28.14***$	15.60***	$4.46**$	

Chart 2: Alpha <0.05 *, Alpha < 0.025**, Alpha < 0.01***. T-Test comparing surface water and bottom water concentrations at sites 1 and 6 in Tolo Harbour for June NO2, July NO2, June NO3, and July NO³ concentrations.

	Surface Water - Bottom Water T-TEST				
	June NO ₂	July NO ₂	June $NO3$	July NO ₃	
TH03	15.94***	$6.75***$	$2.98*$	$4.50**$	
TH05	$21.19***$	$9.02***$	0.40	1.73	

Chart 3: Alpha <0.05 *, Alpha < 0.025**, Alpha < 0.01***. T-Test comparing surface water and bottom water concentrations at sites 3 and 5 in Tolo Harbour for June NO2, July NO2, June NO3, and July NO³ concentrations.

The t-test results indicate that the differences in $NO₂$ and $NO₃$ concentration in June and July between SW and BW are statistically significant for different sites. In sites 1 and 6 specifically, the SW concentration of $NO₂$ and $NO₃$ is significantly greater than the BW concentration. Site 1 and 6 are both closest to the land and could explain why their surface water nitrogen concentration is so high.

These sites are directly impacted by runoff from urban and industrial areas. Moreover, the atmospheric deposition of nitrogenous compounds explains why there are higher concentrations of nitrogen in coastal surface water areas (Voss *et al.* 2013). As this industrial discharge and factories, such as the Tai Po Sewage Treatment Works, are in closer proximity to the coast, there is a higher concentration of nitrogen in coastal surface water rather than the surface water of the open sea.

Moreover, the process of upwelling is a potential explanation for the high nitrogenous concentrations in surface water near the coast. When the nitrogen-rich water is brought to the surface, it can lead to an oxygen depletion in surface water, otherwise known as hypoxia (Voss *et al.* 2013). These phenomena can explain a potential reason why nitrogen surface water concentration is greater than bottom water concentration.

In contrast, as seen in the T-Test below, sites 3 and 5 had on average statistically significant higher concentrations of $NO₃$ and $NO₂$ in BW in comparison to SW, a trend not observed in sites 1 and 6. Specifically, the NO² SW-BW concentration, with the concentration for site 3 being 15.94 µmol L ¹ in June and 6.75 µmol L ¹ in July. Site 5 concentrations were 21.19 µmol L⁻¹ in June and 9.02 μ mol L⁻¹ in July.

A possible explanation for the statistically different bottom and surface water concentration could be because sites 3 and 5 are further offshore, compared to sites 1 and 6 which are near the coast. The high concentration of nitrogenous content in open sea bottom water could be due to reduced mixing and stratification. This barrier is formed by the temperature change and changes in salt content, which acts as a barrier to prevent bottom and surface water mixing (NOAA). Furthermore, the decomposition of sinking organic nitrogenous matter as it flows out from the coast, adds nutrients to the bottom water, explaining why nitrogen concentration in offshore bottom water is greater than surface water concentration (Webb).

Potential Hypoxic Conditions in Tolo Harbour & How They May Affect Marine Organisms

Sites 1, 6, 3, and 5 have relatively higher levels of $NO₂$ and NO3, posing a higher risk of hypoxia, which could negatively impact marine life in Tolo Harbour. Tolo Harbour is home to 1,000 different species and accounts for around 25% of Hong Kong's marine species (Agriculture, Fisheries, and Conservation Department of Hong Kong).

In shallow water, near site 6, there are many species of corals present. Some of these species include *Oulastrea crispata* and *Dipsastraea speciosa*. *Oulastrea crispata* is a type of encrusting coral, and *Dipsastraea speciosa* is a type of stony coral. Research has indicated that oxygen is a critical factor for coral reefs (Nelson and Altieri, 2019). Oxygen allows for respiration, photosynthesis, and calcification in corals (Nelson and Altieri, 2019). In hypoxic conditions, when corals are under oxidative stress, they are unable to respirate, grow, and eat properly (Nelson and Altieri, 2019). As a result, they can bleach and die off. The dependence of many marine organisms on coral reefs for shelter and food means that bleached corals from hypoxia may indirectly lead to a decline in marine organisms.

Image 2: Map of Tolo Harbour showing species present in shallow water (*image courtesy of Agriculture, Fisheries, and Conservation Department of Hong Kong:*

[https://www.arcgis.com/apps/webappviewer/index.html?id=7cfcd50ad17f4ddd9a1546010364465c\)](https://www.arcgis.com/apps/webappviewer/index.html?id=7cfcd50ad17f4ddd9a1546010364465c)

Similarly, on the rocky shore near sites 1 and 6, there are many species of bivalves present. Species include the *Perna viridis* which is present in sites 1 and 6, as well as the *Saccostrea cuccullata* which is present in site 6. Bivalves require oxygen for food consumption, assimilation efficiency, as well as increased respiration (Song *et al.* 2024). Surface water at sites 1 and 6 have high concentrations of nitrogen. In these potential hypoxic conditions, respiration is slowed. Bivalves have less energy allocated for growth, shell formation, and reproduction, making them more vulnerable to death (Song *et al.* 2024).

In the demersal area of Tolo Harbor, many different species of fish are present. The most common species are *Callionymus curvicornis* and *Apogonichthyoides niger*, which are both present at sites 3 and 5. The Callionymus for example, is a small fish, and therefore its heart needs to beat more frequently, to balance its oxygen capacities and blood and water flow rates (Hughes and Umezawa, 1968). In hypoxic conditions, respiratory movements and the heart rate rhythm can be affected. This causes bradycardia, a decrease in heart rate (Hughes and Umezawa, 1968).

This could cause Callionymus to die off if they are unable to recover from the change in oxygen concentration and bradycardia. The *Apogonichthyoides niger*, on the other hand, utilizes a behavior called mouthbrooding, where male fish will carry and protect their eggs in their mouth until they hatch (Nilsson and Nilsson, 2004). Mouthbrooding has been found to limit the fish's ability to respire and swim for long periods, especially in hypoxic waters (Nilsson and Nilsson, 2004). In hypoxic waters, the male fish will spit out the eggs from their mouths (Nilsson and Nilsson, 2004). By doing this male fish can save themselves from hypoxia-induced energy deficiency, which can kill them, but on the other hand, they leave their eggs unprotected (Nilsson and Nilsson, 2004).

Image 4: Map of Tolo Harbour showing species present at the demersal area (*image courtesy of Agriculture, Fisheries, and Conservation Department of Hong Kong:*

[https://www.arcgis.com/apps/webappviewer/index.html?id=7cfcd50ad17f4ddd9a1546010364465c\)](https://www.arcgis.com/apps/webappviewer/index.html?id=7cfcd50ad17f4ddd9a1546010364465c)

Taxa Groups

Image 5: Map of Tolo Harbour showing species present on the seabed (*image courtesy of Agriculture, Fisheries, and Conservation Department of Hong Kong:*

[https://www.arcgis.com/apps/webappviewer/index.html?id=7cfcd50ad17f4ddd9a1546010364465c\)](https://www.arcgis.com/apps/webappviewer/index.html?id=7cfcd50ad17f4ddd9a1546010364465c)

Image 6: Map of Tolo Harbour showing the Echinoderms present on the seabed (*image courtesy of Agriculture, Fisheries, and Conservation Department of Hong Kong:* [https://www.arcgis.com/apps/webappviewer/index.html?id=7cfcd50ad17f4ddd9a1546010364465c\)](https://www.arcgis.com/apps/webappviewer/index.html?id=7cfcd50ad17f4ddd9a1546010364465c)

The seabed of Tolo Harbour is the most biodiverse, as seen in Image 5. The seabed is home to many different species of snails, crabs, fish, bivalves, corals, echinoderms, and sea anemones. In particular, there are a large number of echinoderms, with the species Luidia hardwicki present at both site 3 and site 5. To avoid hypoxic bottom waters, some echinoderms will stand on their arm tips, elevating their bodies, and making their body immobile (Sunyer and Duarte, 2008). This results in depression of activity, reduced feeding activity, and reduced metabolic heartbeat rates (Sunyer and Duarte, 2008). Moreover, when echinoderms elevate themselves to get more oxygen, they are more prone to being preyed on by hypoxiatolerant predators (Li *et al.* 2019). Bivalves may also switch to anaerobic metabolism over time (Sunyer and Duarte, 2008).

CONCLUSION

This research provides insight into the presence of NO2 and NO³ concentrations in Tolo Harbour. In both June and July, the concentrations of $NO₂$ and $NO₃$ were significant between bottom water and surface water at both coastal and offshore regions, allowing for hypoxic conditions to form. As Tolo Harbour is a biodiverse marine ecosystem, work needs to be done to prevent effluent and waste from sewer treatment facilities from entering the water, so that the marine

biodiversity in Tolo Harbour can be preserved. This data was collected in partnership with The Chinese University of Hong Kong within the greater research project, "Seasonal biogeochemical dynamic of Tolo Harbour bottom water: Interactions between nitrogen and oxygen cycles." This author thanks The Chinese University of Hong Kong for having me as an intern.

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Appendix A:

Chart 5: July NO2 surface water and bottom water absorbance and concentration

NO ₃ DATA	June								
	Data 1	Data 2	Data 3	Average	Conc 1	Conc 2	Conc ₃	Conc Avg	Conc SD
SW1	0.228	0.243	0.289	0.254	12.459	13.475	16.508	14.147	2.106
SW ₂	0.143	0.110	0.142	0.131	6.760	4.556	6.692	6.002	1.253
SW ₃	0.056	0.056	0.055	0.056	0.982	0.997	0.939	0.973	0.030
SW ₅	0.054	0.058	0.054	0.055	0.847	1.086	0.859	0.930	0.135
SW ₆	0.352	0.331	0.390	0.358	20.731	19.312	23.253	21.098	1.996
BW1	0.069	0.073	0.079	0.074	1.854	2.114	2.523	2.164	0.337
BW ₂	0.045	0.048	0.045	0.046	0.263	0.458	0.251	0.324	0.117
BW ₃	0.072	0.060	0.066	0.066	2.062	1.273	1.630	1.655	0.395
BW ₅	0.007	0.003	0.254	0.088	-2.292	-2.525	14.168	3.117	9.571
BW ₆	0.079	0.080	0.090	0.083	2.529	2.612	3.232	2.791	0.384

Chart 6: June NO₃ surface water and bottom water absorbance and concentration

Chart 7: July NO³ surface water and bottom water absorbance and concentration