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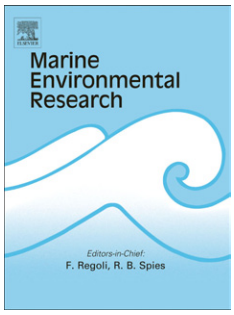

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Changes in the nutrient ratios and phytoplankton community after declines in nutrient concentrations in an eutrophic semi-enclosed bay in Hong Kong

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Abstract

Tolo Harbour is a landlocked bay with poor tidal flushings in the northeastern part of Hong Kong. During the 1980s, excessive nutrient loading led to dramatic increase in nutrient concentrations, accompanied by lower N:P ratios, higher algal biomass and shifts in the phytoplankton community. We studied the effects of nutrient loading reduction measures on nutrient concentrations, nutrient ratios and phytoplankton dynamics in Tolo Harbour by comparing data collected before the full implementation of nutrient loading reduction measures (1986–1997) to those after the implementation (1998–2008). Such measures led to declines in nutrient concentrations, changes in N:P and N:Si ratios, lower chlorophyll-*a* concentrations and fewer algal blooms. Diatoms were the most abundant phytoplankton group in Tolo Harbour both before and after declines in nutrient concentrations. The density of dinoflagellates did not change, but substantial increase in other algal group abundance was recorded.

Keywords: Nutrients, Nutrient ratios, Phytoplankton, Eutrophication, Coastal waters, Tolo Harbour.

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1. Introduction

Eutrophication is a common consequence of anthropogenic activities on water bodies, and is widely regarded as the most wide-spread water quality problem globally (Carpenter et al., 1999; Smith et al., 1999; Smith, 2003). The most common effects of eutrophication are increases in phytoplankton productivity and biomass, which in turn produce other adverse effects such as decreases in water transparency, increases in the frequency of bottom hypoxia or anoxia and loss of various aquatic organisms (Nixon, 1995; Smith et al., 1999; Smith, 2003). Numerous recent studies have linked eutrophication to algal blooms which cause ecological and economical damages (e.g. Anderson et al., 2008; Cloern, 2001; Smith et al., 1999). Nutrient loadings may cause changes in the nutrient ratios (Justić et al., 1995; Turner, 2002), and such changes may affect the species composition of the phytoplankton community and higher trophic levels (Officer and Ryther, 1980; Philippart et al., 2000; Smith, 1983; Turner et al., 1998; Turner, 2002). Although eutrophication in freshwater systems has been studied extensively and intensively during the past few decades, with many studies reporting on the effects of increased or decreased nutrient concentrations in lakes (e.g. Battarbee et al., 2005; Carvalho and Kirika, 2003; Jeppesen et al., 2005; Jeppesen et al., 2007; Mehner et al., 2001), similar kinds of

studies, with long-term monitoring data, are relatively scarce in marine coastal ecosystems (Examples summarized by Smith et al., 1999).

Hong Kong is one of the most densely populated cities in the world. Intensive urbanization and industrialization during the past few decades has dramatically increased nutrient input into Hong Kong's coastal seas (Lee et al., 2006). The concern on the coastal water quality led to the initiation of a marine water quality monitoring program by the Hong Kong Environmental Protection Department in the 1980s (Lee et al., 2006). One of the intensely monitored zones is Tolo Harbour, a landlocked bay in the northeastern corner of Hong Kong.

Tolo Harbour consists of a shallow inner bay, with a mean depth of 6–7 m, and a narrow tidal channel which opens into the neighboring Mirs Bay. Urban development and large-scale reclamation around Tolo Harbour began in the 1970s. Sewage discharge from the expanding population caused a sharp increase in nutrient input (Lam and Ho, 1989; Wear et al., 1984). Hodgkiss and Yim (1995) estimated a daily nitrate and phosphate loading of 32,460 kg and 3,190 kg respectively into Tolo Harbour. In addition to nutrient inputs, the bottleneck topography of the Tolo Harbour leads to poor tidal exchange rates and persistence of high nutrient concentrations (Lee et al., 2006). Increased nutrient concentrations produced algal blooms, with dinoflagellates such as *Noctiluca scintillans* being the most common causative species

during the 1980s and 1990s (Lam and Ho, 1989; Liu and Wong, 2006). Although *Noctiluca* are generally considered as heterotrophic (Kiørboe and Titelman, 1998), strains of *Noctiluca miliaris* with phytosynthetic symbionts have been found in Southeast Asia (Sweeney, 1971; Hansen et al., 2004), which may benefit from increased nutrient concentrations (Sriwoon et al., 2008). In addition, the increased nutrient concentrations would also favor the growth of diatoms and other prey for *N. scintillans* (Liu and Wong, 2006).

Wear et al. (1984) reported mean concentrations of NO_x-N and PO_4-P in the inner Tolo Harbour to be 0.05 µM and 0.01 µM respectively during 1974–1976. By early 1990s, annual mean concentrations have increased to 19.99 µM for inorganic nitrogen and 1.94 µM for PO_4-P (Hong Kong Environmental Protection Department, 1991). Moreover, PO_4-P values as high as 10.65 µM have been reported in Tolo Harbour in 1978–1979 (Hodgkiss and Chan, 1983). Decreasing trends in N:P ratio were recorded during the 1980s and early 1990s (Hodgkiss and Chan, 1997; Yung et al., 1997), and was considered to be caused by excessive P input (Hodgkiss and Chan, 1983, Hodgkiss and Yim,1995). Some researchers noted that the observed changes in nutrient parameters had caused a shift in the species composition of the phytoplankton community, with diatoms gradually replaced by dinoflagellates, (Chan and Hodgkiss, 1987; Hodgkiss and Ho, 1997; Lam and Ho, 1989), although a review of the data

collected by the Hong Kong Environmental Protection Department did not reveal such a shift in the phytoplankton during 1982–1992 (Yung et al., 1997).

The Hong Kong Government initiated the Tolo Harbour Action Plan in 1986 to halt the declining water quality of Tolo Harbour. Two of the most important components of the action plan were the modification of existing sewage treatment works processes (implemented in 1992) and the Tolo Harbour Effluent Export Scheme (Chau, 2007). Commissioned in the mid-1990s and became fully operational in 1998, the Tolo Harbour Effluent Export Scheme allows treated sewage from two large sewage treatment works to be exported via pipelines for discharge outside of Tolo Harbour. Nitrogen input into Tolo Harbour was reduced to ~1000 kg day^{-1} by 2000 (Hong Kong Environmental Protection Department, 2006).

In this study, data collected from four sampling stations in Tolo Harbour during the period from 1986–2008 were analyzed to provide information on the long-term changes in the hydrography and phytoplankton dynamics. Conditions before (1986–1997) and after (1998–2008) the full implementation of the Tolo Harbour Effleunt Export Scheme were compared to: (1) determine whether declines in nutrient concentrations had led to changes in the nutrient ratios, and (2) identify shifts in the phytoplankton community that may be related to changes in the nutrient concentrations and ratios. Overall changes of the hydrography and phytoplankton

community in Tolo Harbour after declines in nutrient concentrations were also compared to those reported in other ecosystems after recovery from eutrophication.

2. Materials and Methods

All data were provided by the Hong Kong Environmental Department. Four sampling stations in Tolo Harbour, TM3, TM4, TM6 and TM8 (Fig. 1), were sampled frequently, mostly once each month, but irregularly from 1982–1992. Monthly means were calculated for each station. Physico-chemical parameters, including temperature, salinity, pH and dissolved oxygen concentration (DO), were recorded at 1 m below the water surface, mid-depth in the water column and 1 m above the bottom by a SEACAT 19*plus* CTD water profiler (Sea-BIRD Electronics). Water samples were also collected at the three depths using Niskin-type water bottles for the determination of BOD_5, chlorophyll-*a* concentration, suspended solids concentration, and the concentrations of NO_3-N, NH_4-N, total inorganic nitrogen (TIN), total nitrogen (TN), PO_4-P, total phosphorus (TP) and SiO_2. The data from the three depths were averaged into depth-averaged data. Values from the bottom depth were also analyzed for DO and BOD_5 to provide information on hypoxia at the water bottom. Measurement of BOD_5, nutrient concentrations, chlorophyll-*a* concentration and suspended solids

concentration were carried out by the Hong Kong Government Laboratory using procedures described by APHA (American Public Health Association et al. 1998). Data on algal bloom occurrences were obtained from the Hong Kong Agriculture, Fisheries and Conservation Department, and were independent from our data. Such data on algal bloom occurrences was based on sighting reports of seawater discoloration, and may therefore underestimate algal bloom occurrences. It also does not contain taxonomic information regarding the algal bloom.

(Insert Fig. 1 here)

Water samples from 1 m below the water surface were preserved in Lugol's iodine solution (1: 100 ml). In the laboratory, phytoplankton were examined in an Utermöhl counting chamber under an inverted microscope, and the densities of diatoms, dinoflagellates and other algal groups (Algae that were neither diatoms nor dinoflagellate) were counted. Phytoplankton data for TM6 was only available after 1993, while data collection for other stations have began in 1986 (TM8) and 1988 (TM3 and TM4).

Two-way ANOVA was used to test for temporal differences before and after nutrient loading reduction, as well as the spatial differences among sampling stations

in various water quality and phytoplankton parameters. Tukey's test was used for multiple comparison between sites. Kendall's correlation was used to test the correlation between phytoplankton and water quality variables.

3. Results

In general, physico-chemical, biological and nutrient parameters showed similar changes among the four sampling stations after nutrient loading reduction (Table 1). Temperature, salinity, bottom DO and TIN/PO_4 increased at all stations after nutrient loading reduction, while pH, BOD_5, chlorophyll-*a* concentrations, all nutrient concentrations and TIN/SiO_2 decreased. DO and SiO_2 concentration did not differ significantly between the two time periods and among the stations respectively ($p > 0.05$). All other parameters showed significant differences both temporally and spatially, with most p values < 0.001. Comparison among sampling stations revealed a general trend of improvement in water quality, marked by changes such as decreasing nutrient concentrations and increasing DO and secchi depth, from TM3 to TM8.

(Insert Table 1 here)

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N (including NO_3-N, NH_4-N, total inorganic N and total N) and P (including PO_4-P and total P) concentrations were high, especially at TM3 and TM4, prior to nutrient loading reduction (Fig. 2). Concentrations decreased noticeably within a year after the full implementation of the sewage export scheme, and have remained at relatively low levels in recent years. N and P concentrations differed markedly among stations before nutrient loading reduction, but the differences became less apparent afterwards. SiO_2 concentrations did not appear to differ among stations before nutrient loading reduction. NO_3/NH_4 fluctuated widely during the entire study period, but the value was generally lowest at TM3 and highest at TM8 (Fig. 3). In contrast, TIN/PO_4 and TIN/SiO_2 showed much clearer increasing and decreasing trends respectively (Fig. 3). TIN/PO_4 decreased gradually from between 1986 and 1996, but have returned to approximately the 16:1 Redfield N:P ratio after declines in nutrient concentrations. Declines in nutrient concentrations reduced the among-station differences in TIN/SiO_2 and the values at all stations were well below the 1:1 Redfield ratio for N:Si.

(Insert Fig. 2 and Fig. 3 here)

Chlorophyll-*a* concentrations and densities of different phytoplankton groups exhibited varying trends temporally (Figs. 4 and 5 respectively). The lack of apparent

similar trends between chlorophyll-*a* concentrations and phytoplankton densities may be due to the fact that chlorophyll-*a* data were depth-averaged, while phytoplankton data were from the surface only. In general, the concentrations of chlorophyll-*a* and densities of all phytoplankton groups were highest at TM3 and lowest at TM8. Dinoflagellate density at TM3 increased to two major peaks of > 10,000 cells ml^{-1} in 1996 and 1998, and decreased to < 1,000 cells ml^{-1} in recent years. Other algal groups occurred at densities of < 5000 cells ml^{-1} before 1996, but increased rapidly and considerably after 1996. Density at TM3 remained > 6,000 cells ml^{-1} after declines in nutrient concentrations. As diatoms were the most abundant group, the density of total phytoplankton exhibited temporal and spatial patterns more similar to those of diatoms than those of dinoflagellates and other algal groups. Declines in nutrient concentrations did not affect the density of dinoflagellates significantly, but densities of diatoms, other algal groups and total phytoplankton all showed significant increase after declines in nutrient concentrations. Despite a significant increase in total phytoplankton density, the number of algal blooms recorded in Tolo Harbour dropped significantly from a mean of 15.8 before nutrient loading reduction to 7.6 (Paired t-test, $p = 0.02$) afterwards. No specific phytoplankton group or genus identified in the study could be concluded to be restricted to a particular station. Diatom genera common in all four stations and with average densities > 1,000 cells ml^{-1} in TM3 and

> 600 ml^{-1} in TM8 included *Cerataulina*, *Chaetoceros*, *Pseudo-nitzschia*, *Leptocylindrus* and *Thalassiosira*. *Prorocentrum* and *Scrippsiella* were the most abundant dinoflagellate genera in all stations. Average density was > 1,000 cells ml^{-1} in TM3 and > 250 cells ml^{-1} in TM8 for *Prorocentrum*, and > 700 cells ml^{-1} in TM3 and > 100 cells ml^{-1} in TM8 for *Scrippsiella*. The dinoflagellates *Gymnodinium*, *Gyrodinium* and *Protoperidinium* were also frequently found at all stations, but their average densities were much lower. Small flagellates represented the most abundant and frequently recorded other algal group in the samples.

(Insert Fig. 4 and Fig. 5 here)

Hong Kong is characterized by a warm wet season from April to September, and a relatively dry and cool season for the rest of the year. However, when plots of seasonal means of nutrient concentrations and phytoplankton densities were made, no apparent and general trend could be identified (Data not shown).

Kendall's correlation did not reveal any strong or obvious trends between phytoplankton variables and various parameters (Table 2). Results for TM3 and TM4 seemed to oppose those for TM6 and TM8 at times. For instance, correlation between phytoplankton variables and DO and BOD_5 were usually positive at TM3 and TM4,

but negative at TM6 and TM8, and vice versa with the results for SiO_2. It is also not uncommon to find contradicting results between phytoplankton cell densities and chlorophyll-*a* concentrations due to the different trends exhibited by these two parameters. Correlation between phytoplankton variables and nutrient variables were mostly negative in all stations except TM6. Most phytoplankton variables at TM3 and TM4 correlated significantly and negatively with nutrient concentrations. In TM6, chlorophyll-*a* concentrations correlated negatively with NO_3-N and NH_4-N concentrations, while dinoflagellate densities correlated positively with total N, PO_4-P and total P concentrations. NO_3/NH_4 correlated negatively with the densities of all groups of phytoplankton at TM3, and positively with diatom densities at TM6 and TM8. TIN/PO_4 correlated positively with other algal group densities in all stations. TIN/SiO_2 correlated negatively with phytoplankton densities in all stations, but exhibited especially strong negative correlations with the densities of dinoflagellates, other algal groups and total phytoplankton at TM3 and TM4. Kendall's correlations between the number of algal blooms and all parameters were also determined. Surprisingly, the number of algal blooms did not correlate significantly with phytoplankton densities, nutrient concentrations and nutrient ratios. It correlated weakly with pH ($r = 0.313$) and bottom DO ($r = 0.305$) ($p < 0.05$), and strongly with BOD_5 ($r = 0.513$) and chlorophyll-*a* concentration ($r = 0.417$) ($p < 0.01$).

(Insert Table 2 here)

4. Discussion

4.1. Temporal and spatial variations in marine water quality and phytoplankton densities

While spatially there is an apparent pattern of better water quality in TM6 and TM8, all parameters show high temporal variations and do not exhibit an apparent trend except changes due to nutrient loading reduction measures. Our data set is unable to explain the cause of specific events such as the relatively lower nutrient concentrations during 1989 (Fig. 2), and the rise in phytoplankton densities in 1995 (Fig. 5), which may be due to isolated algal bloom events that inflated the annual mean value. Both of these parameters may also be affected by factors such as water current, weather conditions and anthropogenic activities that are not available in our data set.

Changes in various physico-chemical and biological parameters, especially nutrient concentrations, after the implementation of the Tolo Harbour Action Plan

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suggested that nutrient loading reduction measures had led to water quality improvement in Tolo Harbour. While Hu et al. (2001) warned that recovery after nutrient loading reduction in Tolo Harbour may be slowed due to nutrients released into the water column from contaminated sediments, N and P concentrations appeared to decrease quite rapidly (Fig. 2) after the full implementation of the Tolo Harbour Effluent Export scheme. Surprisingly, although chlorophyll-*a* concentrations had decreased after declines in nutrient concentrations, the densities of both diatoms and other algal groups have increased. This was similar to the observation reported earlier by Yung et al. (1997) that there was an increase in the density of phytoplankton, especially that of other algal groups, when chlorophyll-*a* concentrations had not increased. As other algal groups in both Yung et al. (1997) and this study included mainly unidentified small flagellates and cryptomonads, an increase in the abundance of these small organisms may not be accompanied by an increase of chlorophyll-*a* concentrations. This is somewhat supported by the fact that correlation between density of other algal group and chlorophyll-*a* concentrations was recorded only at TM8, while diatoms and dinoflagellates correlated strongly and positively with chlorophyll-*a* concentrations at all stations.

Water quality measured in terms of nutrients and chlorophyll-*a* concentration has always been better in the channel region than in the inner part of the harbour (Hu et al.,

2001; Lam and Ho, 1989; Yung et al., 1997), and a seaward decrease in nutrient concentrations from the inner harbour to the channel was evident from our results. A similar spatial pattern, with water quality improving from TM3 to TM8, is recorded in other water quality indicators such as secchi depth, DO and BOD_5. Due to its proximity to the open sea and the much lower pollution levels, TM8 was less affected by eutrophication and subsequent declines in nutrient concentrations compared to stations in the inner part of the harbour. An interesting observation was that the mean values of NO_3/NH_4 decreased at TM3, TM4 and TM6, but increased at TM8 after declines in nutrient concentrations. This may be caused by a relatively higher decline of NH_4-N concentrations than that of NO_3-N in TM8. The maximum release rate of NH_4-N from contaminated sediments into the water column in Tolo Harbour was found to be more than sixfolds higher than that of NO_3-N (Hu et al. 2001), and although Hu et al. (2001) did not specify specific nutrient release rates in various parts of Tolo Harbour, it may be possible that comparatively less NH_4-N was released back into the water column from sediments in the less polluted outer parts of the Tolo Harbour.

Spatial differences in nutrient loading and tidal flushing may have led to the observed differences in how phytoplankton in different stations had responded to declines in nutrient concentrations. Correlation between phytoplankton densities and

nutrient concentrations was poor and usually insignificant at TM6 and TM8 in the outer part of the harbour. In contrast, phytoplankton densities at TM3 and TM4 tended to correlate strongly and negatively with nutrient concentrations, suggesting that nutrients were removed rapidly by phytoplankton in the inner part of Tolo Harbour.

4.2. Changes in nutrient ratios and shifts in the phytoplankton community

Since it is believed that some phytoplankton prefer to use NH_4 over NO_3 (Dortch 1990), a decrease in NO_3/NH_4 may favor phytoplankton growth. However, the lack of consistent correlation between NO_3/NH_4 and phytoplankton density suggests that the phytoplankton community in Tolo Harbour did not have specific preference for either NO_3 or NH_4.

Decreases in nutrient concentrations following nutrient loading reduction measures had altered the values of TIN/PO_4 and TIN/SiO_2 significantly. Hodgkiss and Ho (1997) related the increased abundance of dinoflagellates in Tolo Harbour to a decrease in N:P ratios. It must be noted that no evidence of a decrease in dinoflagellate density has been detected in our data, despite a significant increase in TIN/PO_4 after declines in nutrient concentrations. In general, the density of dinoflagellates correlate neither with TIN/PO_4 nor with most physico-chemical

parameters other than Secchi depth and BOD_5, both of which may be dependent variables affected by the dinoflagellate density. Hence, the density of dinoflagellate in Tolo Harbour may not be easily predicted from nutrient concentrations or N:P ratios alone. While our analysis has not established a strong link between nutrient concentrations and dinoflagellate densities, several investigators have reported an increasing dominance of dinoflagellates during the 1980s and early 1990s when nutrient concentrations in Tolo Harbour were at their highest (Chan and Hodkiss, 1987; Hodgkiss and Chan, 1983; Hodgkiss and Ho, 1997; Lam and Ho, 1989). The density of dinoflagellates was at a peak in 1996 when TIN/PO_4 was very low (Figs. 3 & 4). The density of dinoflagellates reached another peak in 1998, due primarily to an extremely dense population of *Prorocentrum* (122,467 cells ml^{-1}) at TM3 in April. But dinoflagellate densities have remained relatively low since 2005, while TIN/PO_4 increased. Hodgkiss and Ho (1997) also noted a decrease in N:P ratios and an increase in algal blooms from 1981–1990. Our data showed no significant correlation between these two variables, although an increase in TIN/PO_4 and a decrease in the number of algal blooms were found after declines in nutrient concentrations. Nevertheless, while our data did not contain information about *Noctiluca* blooms, Liu and Wong (2006) showed that *Noctiluca scintillans* were still common in Tolo Harbour after declines in nutrient concentrations, and *Noctiluca* blooms still persisted.

Compared to the decrease in N concentrations, the decrease in SiO_2 concentrations was less dramatic. Although a significant decrease in depth-averaged SiO_2 concentrations was recorded after declines in nutrient input (p = 0.045), surface SiO_2 concentrations did not changed significantly (p = 0.849). Overall, TIN/SiO_2 tended to decrease at all sampling stations. Diatoms have a physiological N:Si ratio of 1:1 (Turner, 2002), and an environmental ratio of > 1 has been reported to limit diatom growth and shift a diatom-dominated community to one dominated by non-diatoms, particularly flagellates (Cloern, 2001; Egge and Aksnes, 1992; Paul et al., 2008; Turner et al. 1998). While diatom densities did correlate weakly with TIN/SiO_2 in TM3 and TM4, diatoms were the most abundant algal group in these two stations and Tolo Harbour (Lam and Ho, 1989; Yung et al., 1997) before declines in nutrient concentrations, when TIN/SiO_2 at TM3 and TM4 were frequently > 1. This suggests that although N:Si ratios may have some effect on diatom densities, it is not enough to shift diatom dominance in Tolo Harbour. In addition, the Si concentrations in Tolo Harbour may not be limiting to diatom growth. Egge and Aksnes (1992) reported that a Si concentration of > 2 μM was enough to induce consistent dominance by diatoms in Norway. While this low threshold concentration may not apply in the warm subtropical waters of Hong Kong, the lowest SiO_2 concentration in this study was ~ 10 μM at TM8, fivefolds higher than the threshold concentration

reported in Norway, and may be sufficient to support diatom growth regardless of TIN/SiO_2. It should be noted that the maintenance of dominance by diatoms may not necessarily be beneficial both ecologically and economically. Although centric diatoms have been suggested to be the more desirable phytoplankton group when compared to non-diatom groups, such as dinoflagellates, due to their higher nutritional value and low toxicity (Hecky and Kilham, 1988; Officer and Ryther, 1980), pennate diatoms, including those belonging to the genus *Pseudo-nitzschia*, are also common in Tolo Harbour (Lam and Ho, 1989). Some species of *Pseudo-nitzschia* have been known to release the toxin domoic acid (Lefebvre et al., 2002; Sekula-Wood et al., 2009), and a connection between *Pseudo-nitzschia* blooms and eutrophication has been proposed (Parsons and Dortch, 2002; Schnetzer et al., 2007). Fortunately, *Pseudo-nitzschia* spp. may prefer high nitrate waters (Paul et al., 2008; Carter et al., 2005), so declines in nutrient concentrations in Tolo Harbour should discourage *Pseudo-nitzschia* blooms.

Although our data does not contain information on the size structure of the phytoplankton community, changes in nutrient concentration or ratios may cause a shift from larger phytoplankton species or cells to smaller ones. Stolte and Riegman (1995) had theorized that eutrophication favors large phytoplankton species because their larger storage capacity allows them to better compete under high nutrient

concentrations. Indeed, a shift towards an eutrophic environment has been shown to favor large diatoms in the western Wadden Sea (Stolte el al., 1994; Philippart et al., 2000), and Qiu et al. (2010) reported strong negative correlations between ≤ 3 µm chlorophyll-*a* and nutrient concentrations. The half-saturation constant for P uptake has been reported to be as high as 4.85–6.67 µM at 20°C for certain large diatom species (Nishikawa et al. 2009 and 2010), much higher than the means of TP in Tolo Harbour after nutrient loading reduction (Table 2). Declines in nutrient concentrations in Tolo Harbour may therefore favor smaller cells and species that are more competitive in a less eutrophic condition. Qiu et al. (2010) reported significant positive correlation between < 20 µm chlorophyll *a* concentrations and N/P ratios. Our data also show a strong and positive correlation between TIN/PO_4 and other algal group densities, caused mainly by an increase in the density of small flagellates and *Cryptomonas*. Recent studies in Tolo Harbour have revealed that cells < 5 µm accounted for > 25% of the chlorophyll-*a* biomass (Lie and Wong, 2010; Wong et al., 2010). In addition, phytoplankton size structures in many aquatic ecosystems have also been known to be affected by climate change, particularly by increases in water column stratification due to increased temperature (Finkel, 2007; Smol et al., 2005; Winder et al., 2010). If indeed such a shift to smaller phytoplankton is occurring in Tolo Harbour, it would help to explain the increase in phytoplankton density as

chlorophyll-*a* concentrations decrease.

4.3. Comparison with the recovery from eutrophication of other ecosystems

The effects of declines in nutrient concentrations had been intensively studied in freshwater lakes (e.g. Jeppesen et al., 2002; Jeppesen et al., 2005; Søndergaard et al., 2002), and most have reported improvements in water quality. Water quality improvement is mainly achieved through the decrease in phytoplankton biomass. This decrease can occur as a direct response to declines in nutrient concentrations, but it can be further facilitated through increased grazing pressure from zooplankton grazers (Edmondson, 1994) and the recolonization of submerged macrophytes, which has been shown to substantially affect the success rates of water quality improvement in lakes (Mehner et al., 2002). While there may be differences between freshwater and marine systems in their responses to declines in nutrient concentrations (Cloern, 2001; Smith et al., 1999), examples of dramatic improvements in once eutrophicated marine ecosystems such as Hillsborough Bay, Florida (Johansson and Lewis, 1992) and the coastal waters of Denmark (Rask et al., 1999) had both involved the recolonization of macrophytes such as seagrasses (Hillsborough Bay) and eelgrass (Denmark). However, an early study in Tolo Harbour has shown that most of the harbour was

covered by extremely fine sediments that support little plant life (Trott, 1972). Therefore, even with the decline of nutrient concentrations and increase in water transparency, the spread of macrophytes in Tolo Harbour is unlikely.

In regards to the zooplankton grazers in Tolo Harbour, a study comparing the copepod community before and after declines in nutrient concentrations in Tolo Harbour revealed a decrease in copepod density, from 6,392 ind. m^{-3} in 1987–1991, to 4,810 ind. m^{-3} in 2003–2004 (Wong et al., 1993; Zhang and Wong, submitted). The mesozooplankton community in Tolo Harbour was found to be heavily preyed upon by larval and juvenile stages of the black seabream, *Acanthopagrus schlegeli*, and the Japanese seaperch, *Lateolabrax japonicus*, which use Tolo Harbour as a nursery habitat (Nip et al. 2003). While presently there is no detailed description on the fish population in Tolo Harbour, the abundance of small planktivorous fishes will most likely increase when increased fishing pressure from overfishing of larger piscivorous fishes reduces the predation pressure on the small fishes. Such changes will in turn increase the predation pressure on mesozooplankton grazers, reducing their abundance and delaying any further decrease of phytoplankton abundance.

Although every water mass may have different responses to declines in nutrient concentrations (Carvalho et al., 1995; Smith et al., 1999), it is obvious that trophic interactions play a significant role in determining the outcome (Carpenter et al., 1995,

Manca and Ruggiu, 1998; Proulex et al., 1996; Smith et al., 1999). Increase in macrophytes and zooplankton grazers can further facilitate the decrease in phytoplankton biomass (Edmondson, 1994; Mehner et al., 2002), but the presence of planktivores and piscivores can affect the abundance of zooplankton grazers. Therefore, an appropriate balance between piscivores, planktivores and zooplankton grazers should be maintained to ensure the long-term stability of the recovered ecosystem (Mehner et al. 2002). Unfortunately, there is a lack of long-term monitoring on other communities, such as zooplankton and nekton, in Tolo Harbour. As there is insufficient information on the responses of various communities to changes in nutrient loading, the phytoplankton community alone is not a reliable indicator of the trophic status of the whole ecosystem (Philippart et al. 2007). Future efforts in water quality monitoring and eutrophic coastal restoration should therefore focus not only on nutrient concentrations and phytoplankton biomass and densities, but also other important components of the aquatic ecosystem such as zooplankton and nekton communities.

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Figure Legends

Fig. 1. A map of Hong Kong and Tolo Harbour showing the location of the four sampling stations.

Fig. 2. Temporal variations in the annual means of total inorganic nitrogen, total nitrogen, total phosphorus and SiO_2 concentration at the four sampling stations from 1986–2008. The shaded area represents the period before nutrient loading reduction .

Fig. 3. Temporal variations in the annual means of NO_3/NH_4, TIN/PO_4 and TIN/SiO_2 at the four sampling stations from 1986–2008. The shaded area represents the period

before nutrient loading reduction. The dashed lines indicate the Redfield N:P and N:Si ratios of 16:1 and 1:1 respectively.

Fig. 4. Temporal variations in the annual means of chlorophyll *a* at the four sampling stations from 1986–2008. The shaded area represents the period before nutrient loading reduction.

Fig. 5. Temporal variations in the annual means of diatoms, dinoflagllates, other algal groups and total phytoplankton densities at the four sampling stations from 1986–2008. The shaded area represents the period before nutrient loading reduction. Monitoring began in 1988 for TM3 and TM4, 1994 for TM6 and 1986 for TM8.

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Research highlights

Our study investigates the long-term changes in physico-chemical parameters, including nutrient concentration, nutrient ratios, and phytoplankton before and after nutrient reduction in an eutrophic bay in Hong Kong. Such study with long-term data relating environmental factors and phytoplankton is rather uncommon in marine ecosystems. We believe that such study of how anthropogenic causes led to changes in the phytoplankton community fits the aims and scope of your journal.

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Table 1. Mean values (± standard deviation) of various physico-chemical, biological and nutrient ratios. "All stations" refers to the mean of all four sampling stations. "86–97" indicates the period before nutrient loading reduction (i.e. 1986–1997), and "98–08" indicates the period after nutrient loading reduction (i.e. 1998–2008). "Overall" refers to the entire study period of 1986–2008. Values are depth-averaged values except for "Bottom DO" and "Bottom BOD_5".

Parameter		Station				
		TM3	TM4	TM6	TM8	All stations
Temperature (^oC)	86–97	23.06 ± 4.33	22.88 ± 4.32	22.55 ± 4.14	22.20 ± 4.02	22.67 ± 4.19
	98–08	23.93 ± 4.13	23.68 ± 4.13	23.36 ± 4.01	22.92 ± 3.87	23.47 ± 4.02
	Overall	23.47 ± 4.25	23.26 ± 4.24	22.93 ± 4.09	22.55 ± 3.96	23.06 ± 4.12
pH	86–97	8.31 ± 0.20	8.29 ± 0.23	8.26 ± 0.19	8.24 ± 0.17	8.27 ± 0.18
	98–08	8.21 ± 0.20	8.16 ± 0.21	8.16 ± 0.20	8.16 ± 0.15	8.17 ± 0.17
	Overall	8.26 ± 0.20	8.23 ± 0.23	8.21 ± 0.20	8.20 ± 0.17	8.22 ± 0.19
Salinity	86–97	30.75 ± 1.55	30.77 ± 1.66	31.44 ± 1.51	31.96 ± 1.06	31.22 ± 1.39
	98–08	31.18 ± 1.56	31.29 ± 1.48	31.78 ± 1.21	32.31 ± 1.07	31.64 ± 1.28
	Overall	30.96 ± 1.57	31.02 ± 1.60	31.61 ± 1.38	32.13 ± 1.08	31.42 ± 1.35
DO (mg l^{-1})	86–97	6.86 ± 1.93	6.85 ± 1.86	6.45 ± 1.61	6.36 ± 1.65	6.63 ± 1.60
	98–08	6.98 ± 1.37	6.70 ± 1.33	6.26 ± 1.33	6.06 ± 1.34	6.50 ± 1.22
	Overall	6.92 ± 1.68	6.78 ± 1.62	6.36 ± 1.48	6.22 ± 1.51	6.57 ± 1.43
Bottom DO (mg l^{-1})	86–97	4.97 ± 2.44	4.78 ± 2.42	4.83 ± 2.34	5.10 ± 2.56	4.92 ± 2.18
	98–08	6.30 ± 1.86	5.86 ± 1.88	5.30 ± 1.92	5.19 ± 2.02	5.66 ± 1.73
	Overall	5.61 ± 2.28	5.29 ± 2.24	5.05 ± 2.16	5.15 ± 2.31	5.28 ± 2.01
BOD_5 (mg l^{-1})	86–97	2.55 ± 0.99	2.44 ± 0.89	1.81 ± 0.72	1.18 ± 0.60	2.00 ± 0.65
	98–08	1.98 ± 0.75	1.73 ± 0.56	1.38 ± 0.47	0.89 ± 0.56	1.49 ± 0.45
	Overall	2.28 ± 0.93	2.10 ± 0.83	1.60 ± 0.65	1.04 ± 0.60	1.76 ± 0.62
Bottom BOD_5 (mg l^{-1})	86–97	1.33 ± 0.65	1.38 ± 0.79	0.95 ± 0.56	0.71 ± 0.40	1.09 ± 0.50
	98–08	1.35 ± 0.66	1.24 ± 0.60	0.89 ± 0.56	0.56 ± 0.42	1.01 ± 0.41
	Overall	1.34 ± 0.66	1.32 ± 0.71	0.92 ± 0.56	0.63 ± 0.42	1.05 ± 0.46

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Secchi Depth (m)	86–97	1.83 ± 0.66	1.82 ± 0.67	2.23 ± 0.79	3.49 ± 1.39	2.35 ± 0.63
	98–08	1.96 ± 0.69	2.10 ± 0.67	2.59 ± 0.88	3.41 ± 1.27	2.51 ± 0.69
	Overall	1.89 ± 0.67	1.89 ± 0.67	2.40 ± 0.85	3.45 ± 1.33	2.43 ± 0.66
Suspended solids ($mg\ l^{-1}$)	86–97	3.41 ± 2.62	3.63 ± 3.11	3.03 ± 2.37	3.20 ± 3.20	3.32 ± 2.38
	98–08	3.20 ± 3.97	2.42 ± 1.36	2.07 ± 1.01	2.26 ± 1.39	2.49 ± 1.30
	Overall	3.31 ± 3.33	3.05 ± 2.51	2.57 ± 1.90	2.75 ± 2.54	2.92 ± 1.98
Chl *a* ($\mu g\ l^{-1}$)	86–97	9.63 ± 6.87	9.82 ± 7.43	6.25 ± 4.80	3.38 ± 6.21	7.27 ± 4.80
	98–08	9.33 ± 6.28	7.58 ± 4.37	5.20 ± 2.90	3.13 ± 2.17	6.31 ± 3.15
	Overall	9.49 ± 6.58	8.75 ± 6.25	5.75 ± 4.03	3.26 ± 4.72	6.81 ± 4.12
NO_3-N (μM)	86–97	3.21 ± 3.17	3.40 ± 3.74	2.29 ± 2.15	1.52 ± 1.43	2.60 ± 2.30
	98–08	1.37 ± 2.36	1.42 ± 2.28	1.27 ± 1.95	1.45 ± 1.75	1.38 ± 1.87
	Overall	2.33 ± 2.96	2.45 ± 3.28	1.80 ± 2.11	1.48 ± 1.59	2.02 ± 2.19
NH_4-N (μM)	86–97	10.13 ± 6.37	8.27 ± 5.49	5.61 ± 3.95	3.56 ± 3.24	6.89 ± 4.25
	98–08	4.94 ± 4.39	4.40 ± 4.67	3.26 ± 2.53	2.57 ± 1.76	3.79 ± 3.09
	Overall	7.65 ± 6.08	6.42 ± 5.46	4.48 ± 3.54	3.08 ± 2.68	5.41 ± 4.04
Total inorganic N (μM)	86–97	14.31 ± 8.79	12.65 ± 8.00	8.82 ± 5.27	5.76 ± 3.69	10.38 ± 5.86
	98–08	6.77 ± 6.27	6.37 ± 6.16	5.10 ± 3.64	4.64 ± 2.49	5.72 ± 4.28
	Overall	10.71 ± 8.55	9.64 ± 7.82	7.04 ± 4.92	5.22 ± 3.22	8.15 ± 5.66
Total N (μM)	86–97	51.53 ± 19.03	48.58 ± 18.83	41.39 ± 19.00	35.10 ± 26.40	44.15 ± 18.05
	98–08	28.08 ± 20.20	25.98 ± 19.14	22.25 ± 16.91	19.04 ± 15.90	23.84 ± 17.64
	Overall	40.32 ± 22.81	37.77 ± 22.06	32.23 ± 20.39	27.42 ± 23.40	34.44 ± 20.52
PO_4-P (μM)	86–97	1.32 ± 0.78	1.32 ± 0.72	0.93 ± 0.49	0.59 ± 3.69	1.04 ± 0.54
	98–08	0.30 ± 0.23	0.31 ± 0.24	0.30 ± 0.16	0.33 ± 0.16	0.31 ± 0.17
	Overall	0.84 ± 0.77	0.84 ± 0.74	0.63 ± 0.49	0.46 ± 0.30	0.69 ± 0.55
Total P (μM)	86–97	3.59 ± 2.13	3.43 ± 1.58	2.75 ± 1.37	2.23 ± 1.42	3.00 ± 1.43
	98–08	1.14 ± 0.61	1.03 ± 0.53	0.94 ± 0.38	0.87 ± 0.31	0.99 ± 0.38
	Overall	2.42 ± 2.01	2.28 ± 1.69	1.89 ± 1.37	1.58 ± 1.25	2.04 ± 1.47

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SiO_2 (μM)	86–97	13.56 ± 8.41	13.96 ± 7.86	13.90 ± 6.40	12.97 ± 5.03	13.60 ± 6.47
	98–08	12.64 ± 7.87	13.24 ± 7.38	12.68 ± 6.26	12.44 ± 5.84	12.75 ± 6.27
	Overall	13.12 ± 8.16	13.61 ± 7.63	13.32 ± 6.35	12.72 ± 5.43	13.19 ± 6.38
NO_3/NH_4	86–97	0.35 ± 0.33	0.75 ± 3.50	0.72 ± 1.44	0.73 ± 0.93	0.64 ± 1.08
	98–08	0.30 ± 0.56	0.41 ± 0.72	0.69 ± 2.10	1.16 ± 2.73	0.64 ± 1.34
	Overall	0.32 ± 0.45	0.58 ± 2.58	0.71 ± 1.78	0.94 ± 2.01	0.64 ± 1.21
TIN/PO_4	86–97	12.12 ± 6.06	10.39 ± 4.67	10.44 ± 5.13	11.69 ± 7.30	11.16 ± 5.01
	98–08	24.99 ± 18.58	23.16 ± 16.93	18.69 ± 12.95	15.44 ± 10.09	20.57 ± 12.91
	Overall	18.27 ± 15.00	16.49 ± 13.94	14.39 ± 10.52	13.49 ± 8.93	15.66 ± 10.70
TIN/SiO_2	86–97	1.37 ± 1.26	1.13 ± 1.00	0.74 ± 0.64	0.47 ± 0.26	0.93 ± 0.71
	98–08	0.73 ± 0.78	0.62 ± 0.65	0.49 ± 0.53	0.41 ± 0.27	0.56 ± 0.52
	Overall	1.07 ± 1.11	0.89 ± 0.89	0.62 ± 0.60	0.44 ± 0.27	0.75 ± 0.65

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Table 2. Kendall's correlation between phytoplankton variables and various physico-chemical, biological and nutrient ratios parameters at four sampling stations in Tolo Harbour. "Dino" represents dinoflagellates, "Others" represents algal groups other than diatoms or dinoflagellates, "Total" represents total phytoplankton cell counts. "+/-" represents statistical significance at the 0.05 level; "++/--" represents statistical significance at the 0.01 level. Tested values were depth-averaged values except for "Bottom DO" and "Bottom BOD_5".

Parameter	Station																			
	TM3					TM4					TM6					TM8				
	Diatom	Dino	Others	Total	Chl-*a*	Diatom	Dino	Others	Total	Chl-*a*	Diatom	Dino	Others	Total	Chl-*a*	Diatom	Dino	Others	Total	Chl-*a*
Temperature	++	-		++		++			++		++			++		++		+	++	
pH			--		++			--	-	++					++			--		+
Salinity					-					--		-	-	-	--		--			-
DO		++			++					+	--			-		--			--	
Bottom DO		++	++	+		-	+	++			--			--		--			--	-
BOD_5		++	--		++		++	--		++		++		+	++		++	-		++
Bottom BOD_5		+			++	-	++		-	+					+	--		--	--	
Secchi Depth	--	--		--	--	--		+	--	--	--	-		--	--	--	--	-	--	--
Suspended solids		++		+	++		+			++					++		++			++
NO_3-N	--	--	--	--	-	--		--	--	-						+				
NH_4-N	--		--	--		--	-	--	--		-				--	--			-	--

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Total inorganic N	--	-	--	--	--	--	-	--	--	--				--					--
Total N	--		--	--	+	--		--	--	++		++		++			--		
PO_4-P	--	-	--	--	-	--	-	--	--			+					-		-
Total P	--		--	--		--		--	--	++		++				-	--	--	
SiO_2	--				-	--						+					+	+	
NO_3/NH_4	-	--	--	--					-		+					++		+	
TIN/PO_4			++	++				++	-				++				+	+	
TIN/SiO_2	-	--	--	--		-	--	--	--					-				--	

ACCEPTED MANUSCRIPT

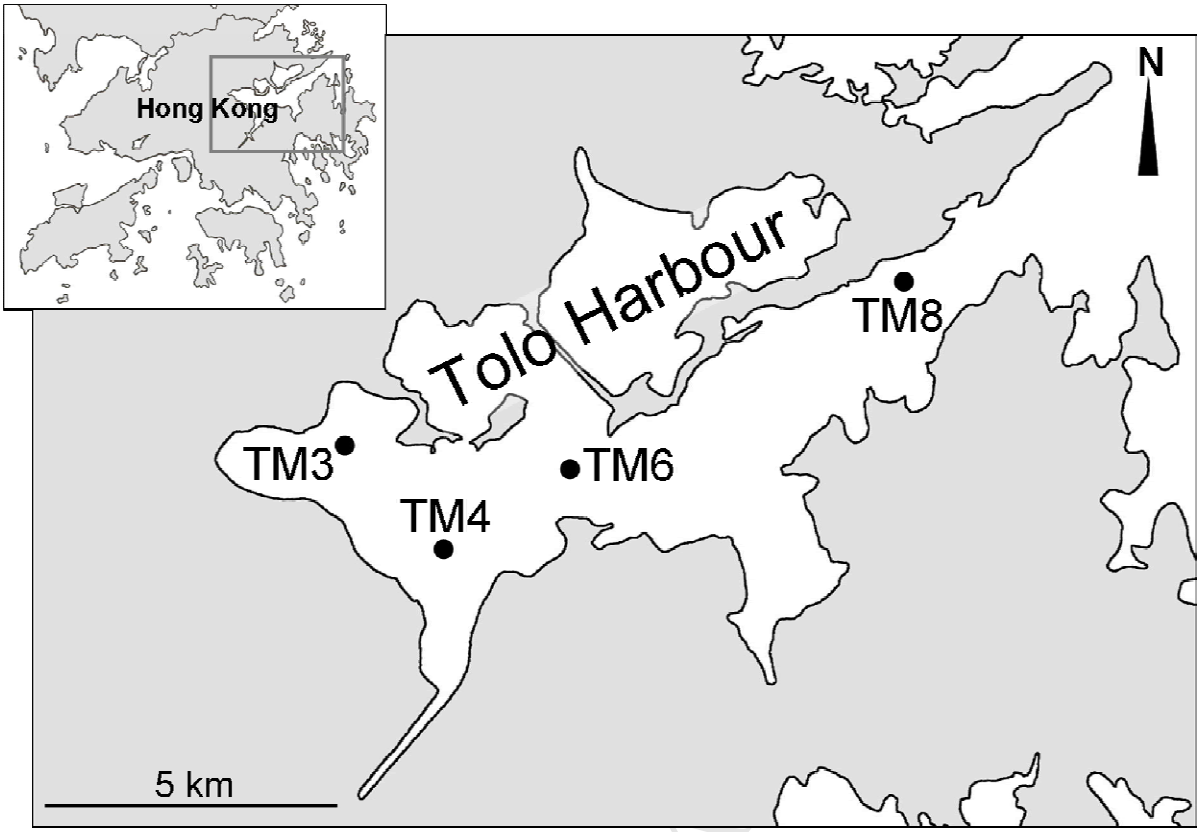

Fig. 1.

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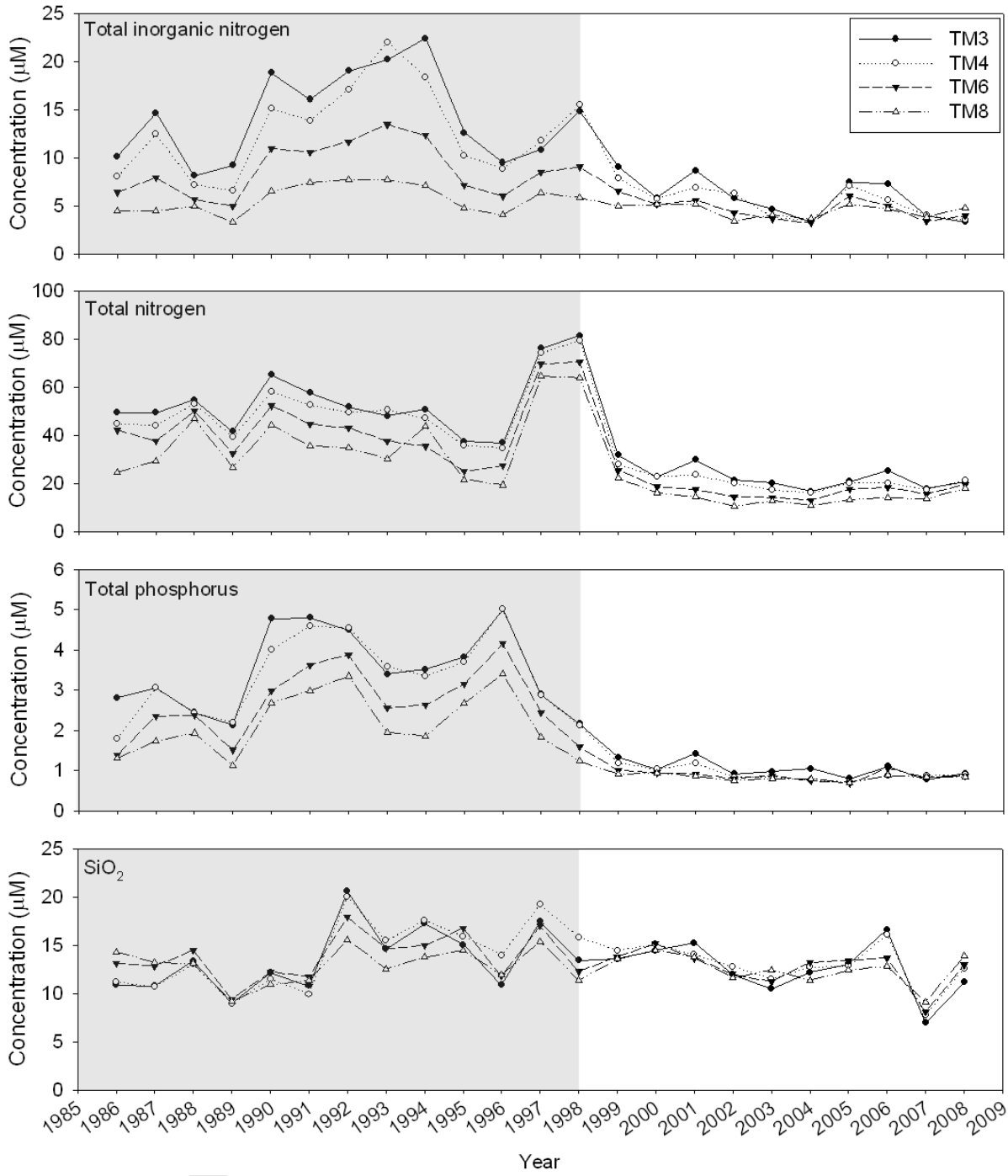
Total inorganic nitrogen
TM3
TM4
TM6
TM8
Concentration (µM)
Total nitrogen
Total phosphorus
SiO2
Year

Fig. 2.

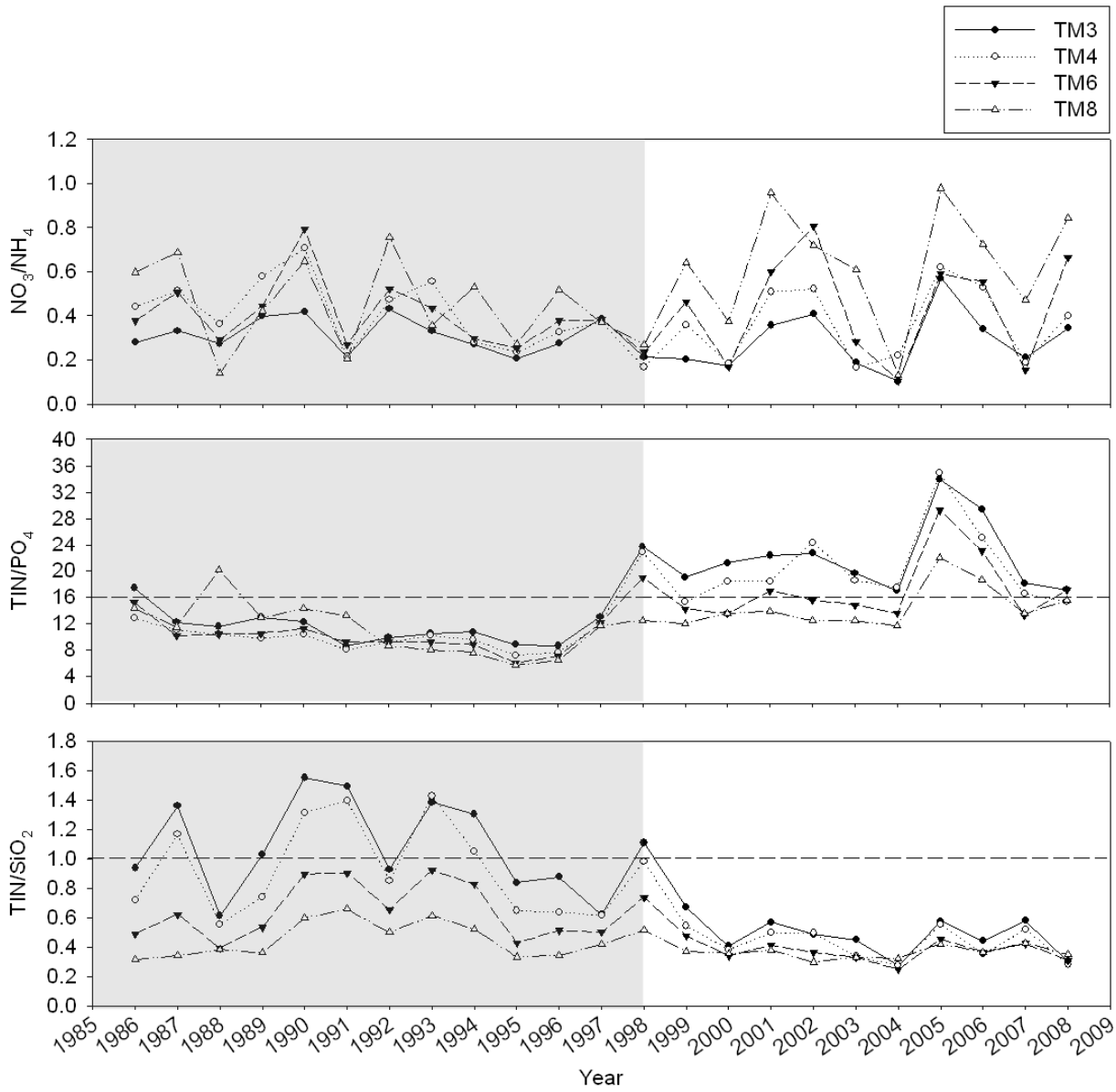

Fig. 3.

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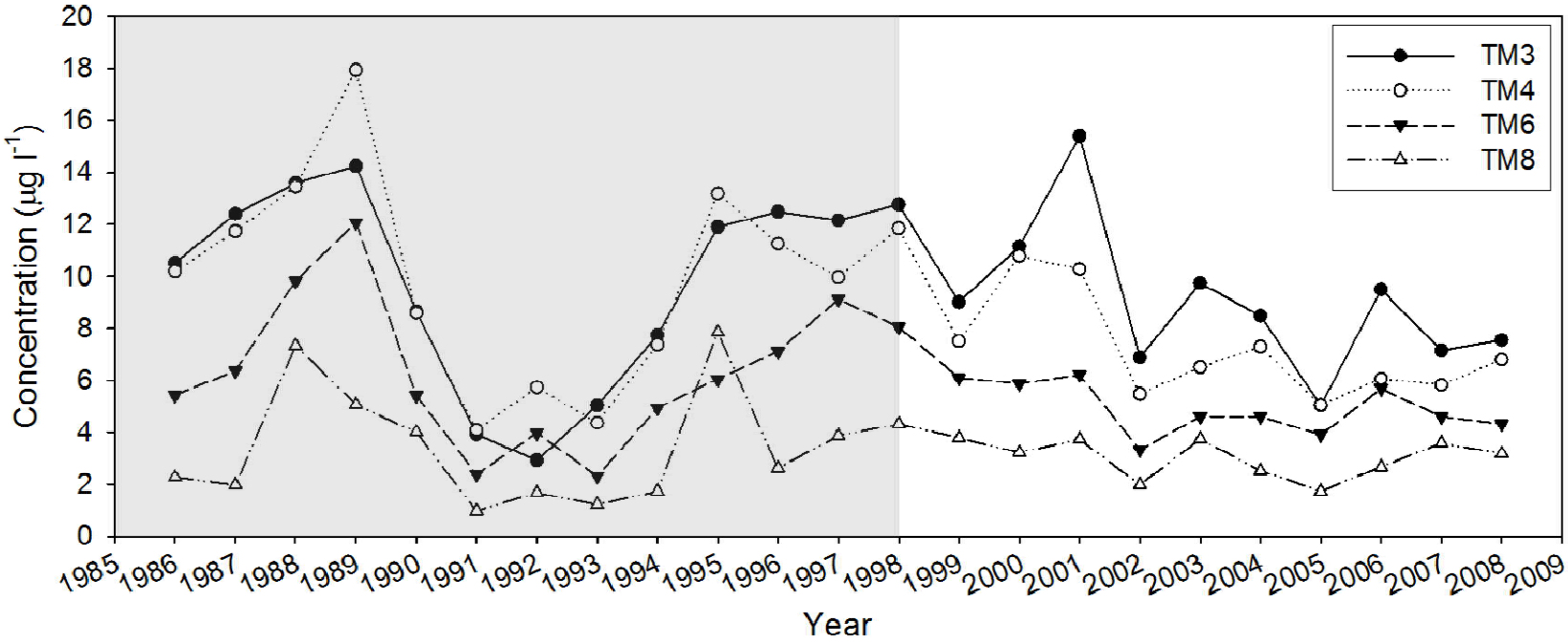

Fig. 4.

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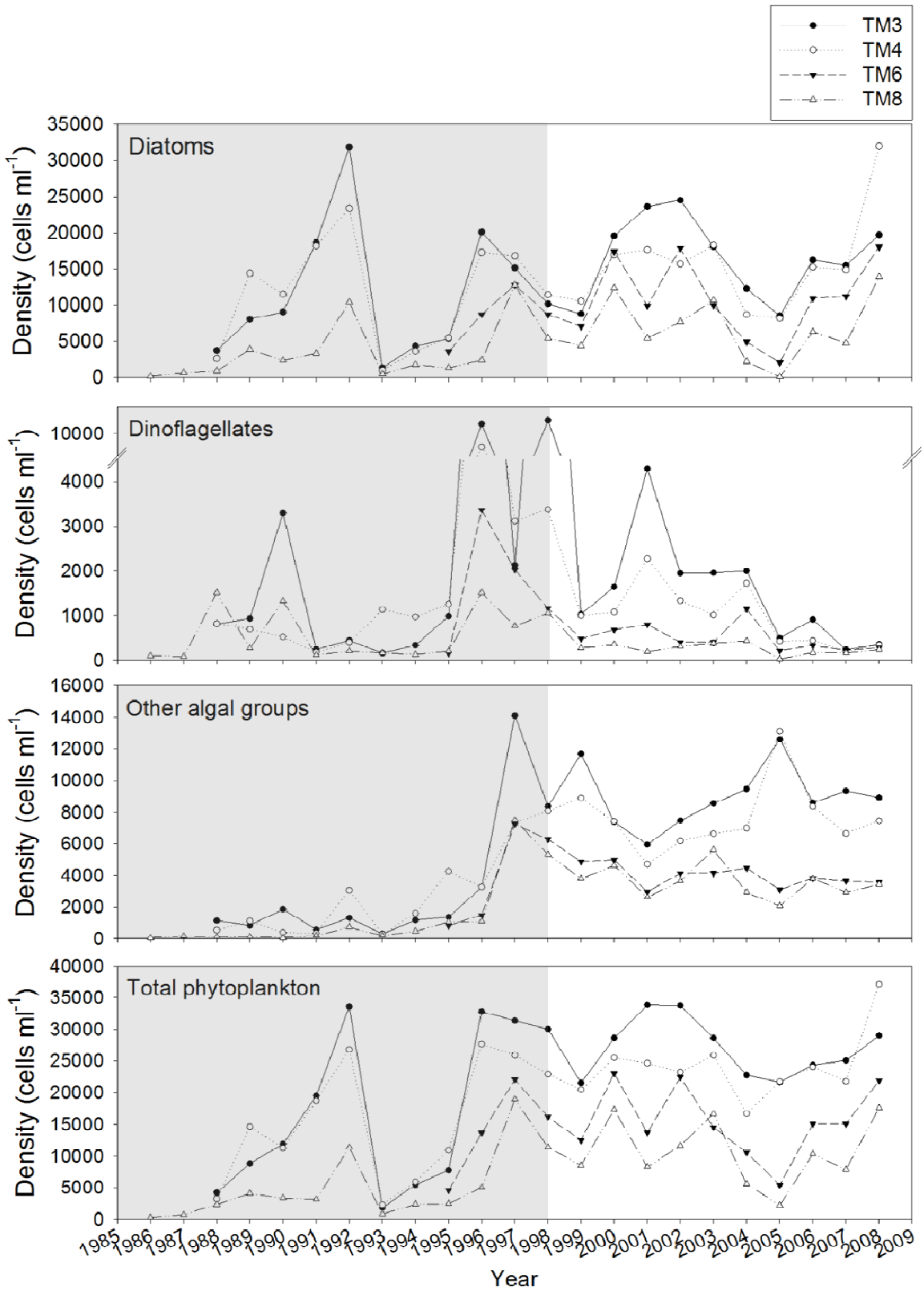
TM3
TM4
TM6
TM8
Diatoms
Dinoflagellates
Other algal groups
Total phytoplankton
Density (cells ml-1)
Year

Figure 5.