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Changes in the nutrient ratios and phytoplankton community after declines in nutrient 1 2 concentrations in an eutrophic semi-enclosed bay in Hong Kong 3 Alle A. Y. Lie<sup>1</sup>, C. Kim Wong<sup>1</sup>, Jackie Y. C. Lam<sup>2</sup>, J.H. Liu<sup>2</sup> & Y. K. Yung<sup>2</sup> 4 5 <sup>1</sup>Department of Biology, The Chinese University of Hong Kong, Shatin, Hong Kong 6 <sup>2</sup>Water Policy and Science Group, Hong Kong Government Environmental Protection 7 Department, Revenue Tower, 5 Gloucester Road, Wan Chai, Hong Kong 8 9 <sup>^</sup>Current address: Department of Biological Sciences, University of Southern 10 California, 3616 Trousdale Parkway, Los Angeles, 90089-0371, CA, USA 11 \*Corresponding author: 12 13 email: alie@usc.edu Tel.: +852 26096773; Fax: +852 26035391 14

15	Abstract
1)	- ADSITACI

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17 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 18 19 nutrient concentrations, accompanied by lower N:P ratios, higher algal biomass and 20 shifts in the phytoplankton community. We studied the effects of nutrient loading reduction measures on nutrient concentrations, nutrient ratios and phytoplankton 21 22 dynamics in Tolo Harbour by comparing data collected before the full implementation 23 of nutrient loading reduction measures (1986–1997) to those after the implementation 24 (1998–2008). Such measures led to declines in nutrient concentrations, changes in 25 N:P and N:Si ratios, lower chlorophyll-a concentrations and fewer algal blooms. 26 Diatoms were the most abundant phytoplankton group in Tolo Harbour both before 27 and after declines in nutrient concentrations. The density of dinoflagellates did not 28 change, but substantial increase in other algal group abundance was recorded.

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

# 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

51 studies, with long-term monitoring data, are relatively scarce in marine coastal 52 ecosystems (Examples summarized by Smith et al., 1999). 53 Hong Kong is one of the most densely populated cities in the world. Intensive 54 urbanization and industrialization during the past few decades has dramatically 55 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 56 program by the Hong Kong Environmental Protection Department in the 1980s (Lee 57 58 et al., 2006). One of the intensely monitored zones is Tolo Harbour, a landlocked bay 59 in the northeastern corner of Hong Kong. 60 Tolo Harbour consists of a shallow inner bay, with a mean depth of 6-7 m, and a 61 narrow tidal channel which opens into the neighboring Mirs Bay. Urban development and large-scale reclamation around Tolo Harbour began in the 1970s. Sewage 62 63 discharge from the expanding population caused a sharp increase in nutrient input 64 (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 65 Harbour. In addition to nutrient inputs, the bottleneck topography of the Tolo Harbour 66 leads to poor tidal exchange rates and persistence of high nutrient concentrations (Lee 67 68 et al., 2006). Increased nutrient concentrations produced algal blooms, with 69 dinoflagellates such as *Noctiluca scintillans* being the most common causative species

70 during the 1980s and 1990s (Lam and Ho, 1989; Liu and Wong, 2006). Although 71 Noctiluca are generally considered as heterotrophic (Kiørboe and Titelman, 1998), 72 strains of Noctiluca miliaris with phytosynthetic symbionts have been found in 73 Southeast Asia (Sweeney, 1971; Hansen et al., 2004), which may benefit from 74 increased nutrient concentrations (Sriwoon et al., 2008). In addition, the increased 75 nutrient concentrations would also favor the growth of diatoms and other prey for N. 76 scintillans (Liu and Wong, 2006). 77 Wear et al. (1984) reported mean concentrations of NO<sub>x</sub>-N and PO<sub>4</sub>-P in the inner Tolo Harbour to be 0.05 µM and 0.01 µM respectively during 1974–1976. By 78 79 early 1990s, annual mean concentrations have increased to 19.99 µM for inorganic 80 nitrogen and 1.94 µM for PO<sub>4</sub>-P (Hong Kong Environmental Protection Department, 81 1991). Moreover, PO<sub>4</sub>-P values as high as 10.65 µM have been reported in Tolo 82 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 83 al., 1997), and was considered to be caused by excessive P input (Hodgkiss and Chan, 84 1983, Hodgkiss and Yim, 1995). Some researchers noted that the observed changes in 85 86 nutrient parameters had caused a shift in the species composition of the phytoplankton 87 community, with diatoms gradually replaced by dinoflagellates, (Chan and Hodgkiss, 88 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<sup>-1</sup> 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.

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#### 2. Materials and Methods

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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 19plus 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<sub>5</sub>, chlorophyll-a concentration, suspended solids concentration, and the concentrations of NO<sub>3</sub>-N, NH<sub>4</sub>-N, total inorganic nitrogen (TIN), total nitrogen (TN), PO<sub>4</sub>-P, total phosphorus (TP) and SiO<sub>2</sub>. The data from the three depths were averaged into depth-averaged data. Values from the bottom depth were also analyzed for DO and BOD<sub>5</sub> to provide information on hypoxia at the water bottom. Measurement of BOD<sub>5</sub>, nutrient concentrations, chlorophyll-a concentration and suspended solids

127	concentration were carried out by the Hong Kong Government Laboratory using
128	procedures described by APHA (American Public Health Association et al. 1998).
129	Data on algal bloom occurrences were obtained from the Hong Kong Agriculture,
130	Fisheries and Conservation Department, and were independent from our data. Such
131	data on algal bloom occurrences was based on sighting reports of seawater
132	discoloration, and may therefore underestimate algal bloom occurrences. It also does
133	not contain taxonomic information regarding the algal bloom.
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135	(Insert Fig. 1 here)
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137	Water samples from 1 m below the water surface were preserved in Lugol's
138	iodine solution (1: 100 ml). In the laboratory, phytoplankton were examined in an
139	Utermöhl counting chamber under an inverted microscope, and the densities of
140	diatoms, dinoflagellates and other algal groups (Algae that were neither diatoms nor
141	dinoflagellate) were counted. Phytoplankton data for TM6 was only available after
142	1993, while data collection for other stations have began in 1986 (TM8) and 1988
143	(TM3 and TM4).
144	Two-way ANOVA was used to test for temporal differences before and after
145	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<sub>4</sub> increased at all stations after nutrient loading reduction, while pH, BOD<sub>5</sub>, chlorophyll-a concentrations, all nutrient concentrations and TIN/SiO<sub>2</sub> decreased. DO and SiO<sub>2</sub> 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.

#### 163 (Insert Table 1 here)

N (including NO <sub>3</sub> -N, NH <sub>4</sub> -N, total inorganic N and total N) and P (including
PO <sub>4</sub> -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 <sub>2</sub> concentrations did not appear to differ among stations before nutrient
loading reduction. NO <sub>3</sub> /NH <sub>4</sub> 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 <sub>4</sub>
and TIN/SiO <sub>2</sub> showed much clearer increasing and decreasing trends respectively (Fig
3). TIN/PO <sub>4</sub> 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 <sub>2</sub>
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 <sup>-1</sup> in
1996 and 1998, and decreased to < 1,000 cells ml <sup>-1</sup> in recent years. Other algal groups
occurred at densities of < 5000 cells ml <sup>-1</sup> before 1996, but increased rapidly and
considerably after 1996. Density at TM3 remained > 6,000 cells ml <sup>-1</sup> 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 general
common in all four stations and with average densities > 1,000 cells ml <sup>-1</sup> in TM3 and

203	> 600 ml <sup>-1</sup> in TM8 included Cerataulina, Chaetoceros, Pseudo-nitzschia,
204	Leptocylindrus and Thalassiosira. Prorocentrum and Scrippsiella were the most
205	abundant dinoflagellate genera in all stations. Average density was > 1,000 cells ml <sup>-1</sup>
206	in TM3 and > 250 cells ml <sup>-1</sup> in TM8 for <i>Prorocentrum</i> , and > 700 cells ml <sup>-1</sup> in TM3
207	and > 100 cells ml <sup>-1</sup> in TM8 for <i>Scrippsiella</i> . The dinoflagellates <i>Gymnodinium</i> ,
208	Gyrodinium and Protoperidinium were also frequently found at all stations, but their
209	average densities were much lower. Small flagellates represented the most abundant
210	and frequently recorded other algal group in the samples.

212 (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<sub>5</sub> were usually positive at TM3 and TM4,

222	but negative at TM6 and TM8, and vice versa with the results for SiO <sub>2</sub> . It is also not
223	uncommon to find contradicting results between phytoplankton cell densities and
224	chlorophyll-a concentrations due to the different trends exhibited by these two
225	parameters. Correlation between phytoplankton variables and nutrient variables were
226	mostly negative in all stations except TM6. Most phytoplankton variables at TM3 and
227	TM4 correlated significantly and negatively with nutrient concentrations. In TM6,
228	chlorophyll-a concentrations correlated negatively with NO <sub>3</sub> -N and NH <sub>4</sub> -N
229	concentrations, while dinoflagellate densities correlated positively with total N, PO <sub>4</sub> -P
230	and total P concentrations. NO <sub>3</sub> /NH <sub>4</sub> correlated negatively with the densities of all
231	groups of phytoplankton at TM3, and positively with diatom densities at TM6 and
232	TM8. TIN/PO <sub>4</sub> correlated positively with other algal group densities in all stations.
233	TIN/SiO <sub>2</sub> correlated negatively with phytoplankton densities in all stations, but
234	exhibited especially strong negative correlations with the densities of dinoflagellates,
235	other algal groups and total phytoplankton at TM3 and TM4. Kendall's correlations
236	between the number of algal blooms and all parameters were also determined.
237	Surprisingly, the number of algal blooms did not correlate significantly with
238	phytoplankton densities, nutrient concentrations and nutrient ratios. It correlated
239	weakly with pH (r = 0.313) and bottom DO (r = 0.305) ( $p < 0.05$ ), and strongly with
240	BOD <sub>5</sub> (r = 0.513) and chlorophyll-a concentration (r = 0.417) ( $p < 0.01$ ).

241	
242	(Insert Table 2 here)
243	
244	4. Discussion
245	
246	4.1. Temporal and spatial variations in marine water quality and phytoplankton
247	densities
248	
249	While spatially there is an apparent pattern of better water quality in TM6 and
250	TM8, all parameters show high temporal variations and do not exhibit an apparent
251	trend except changes due to nutrient loading reduction measures. Our data set is
252	unable to explain the cause of specific events such as the relatively lower nutrient
253	concentrations during 1989 (Fig. 2), and the rise in phytoplankton densities in 1995
254	(Fig. 5), which may be due to isolated algal bloom events that inflated the annual
255	mean value. Both of these parameters may also be affected by factors such as water
256	current, weather conditions and anthropogenic activities that are not available in our
257	data set.
258	Changes in various physico-chemical and biological parameters, especially

nutrient concentrations, after the implementation of the Tolo Harbour Action Plan

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 <sub>5</sub> . 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 <sub>3</sub> /NH <sub>4</sub> 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 <sub>4</sub> -N concentrations than that of NO <sub>3</sub> -N in TM8. The maximum release rate of
NH <sub>4</sub> -N from contaminated sediments into the water column in Tolo Harbour was
found to be more than sixfolds higher than that of NO <sub>3</sub> -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 <sub>4</sub> -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

298	nutrient concentrations was poor and usually insignificant at TM6 and TM8 in the
299	outer part of the harbour. In contrast, phytoplankton densities at TM3 and TM4 tended
300	to correlate strongly and negatively with nutrient concentrations, suggesting that
301	nutrients were removed rapidly by phytoplankton in the inner part of Tolo Harbour.
302	
303	4.2. Changes in nutrient ratios and shifts in the phytoplankton community
304	
305	Since it is believed that some phytoplankton prefer to use NH <sub>4</sub> over NO <sub>3</sub> (Dortch
306	1990), a decrease in NO <sub>3</sub> /NH <sub>4</sub> may favor phytoplankton growth. However, the lack of
307	consistent correlation between NO <sub>3</sub> /NH <sub>4</sub> and phytoplankton density suggests that the
308	phytoplankton community in Tolo Harbour did not have specific preference for either
309	NO <sub>3</sub> or NH <sub>4</sub> .
310	Decreases in nutrient concentrations following nutrient loading reduction
311	measures had altered the values of TIN/PO <sub>4</sub> and TIN/SiO <sub>2</sub> significantly. Hodgkiss and
312	Ho (1997) related the increased abundance of dinoflagellates in Tolo Harbour to a
313	decrease in N:P ratios. It must be noted that no evidence of a decrease in
314	dinoflagellate density has been detected in our data, despite a significant increase in
315	TIN/PO <sub>4</sub> 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 <sub>5</sub> , 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 <sub>4</sub> was very low (Figs. 3
& 4). The density of dinoflagellates reached another peak in 1998, due primarily to an
extremely dense population of <i>Prorocentrum</i> (122,467 cells ml <sup>-1</sup> ) at TM3 in April.
But dinoflagellate densities have remained relatively low since 2005, while TIN/PO <sub>4</sub>
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 <sub>4</sub> 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 <i>Noctiluca</i> blooms, Liu and Wong (2006)
showed that Noctiluca scintillans were still common in Tolo Harbour after declines in
nutrient concentrations, and <i>Noctiluca</i> blooms still persisted.

336	Compared to the decrease in $N$ concentrations, the decrease in $SiO_2$
337	concentrations was less dramatic. Although a significant decrease in depth-averaged
338	$SiO_2$ concentrations was recorded after declines in nutrient input ( $p = 0.045$ ), surface
339	$SiO_2$ concentrations did not changed significantly ( $p=0.849$ ). Overall, TIN/SiO <sub>2</sub>
340	tended to decrease at all sampling stations. Diatoms have a physiological N:Si ratio of
341	1:1 (Turner, 2002), and an environmental ratio of > 1 has been reported to limit
342	diatom growth and shift a diatom-dominated community to one dominated by
343	non-diatoms, particularly flagellates (Cloern, 2001; Egge and Aksnes, 1992; Paul et
344	al., 2008; Turner et al. 1998). While diatom densities did correlate weakly with
345	TIN/SiO <sub>2</sub> in TM3 and TM4, diatoms were the most abundant algal group in these two
346	stations and Tolo Harbour (Lam and Ho, 1989; Yung et al., 1997) before declines in
347	nutrient concentrations, when $TIN/SiO_2$ at $TM3$ and $TM4$ were frequently > 1. This
348	suggests that although N:Si ratios may have some effect on diatom densities, it is not
349	enough to shift diatom dominance in Tolo Harbour. In addition, the Si concentrations
350	in Tolo Harbour may not be limiting to diatom growth. Egge and Aksnes (1992)
351	reported that a Si concentration of $> 2 \ \mu M$ was enough to induce consistent
352	dominance by diatoms in Norway. While this low threshold concentration may not
353	apply in the warm subtropical waters of Hong Kong, the lowest SiO <sub>2</sub> concentration in
354	this study was $\sim 10~\mu M$ at TM8, fivefolds higher than the threshold concentration

355	reported in Norway, and may be sufficient to support diatom growth regardless of
356	TIN/SiO <sub>2</sub> . It should be noted that the maintenance of dominance by diatoms may not
357	necessarily be beneficial both ecologically and economically. Although centric
358	diatoms have been suggested to be the more desirable phytoplankton group when
359	compared to non-diatom groups, such as dinoflagellates, due to their higher nutritional
360	value and low toxicity (Hecky and Kilham, 1988; Officer and Ryther, 1980), pennate
361	diatoms, including those belonging to the genus Pseudo-nitzschia, are also common in
362	Tolo Harbour (Lam and Ho, 1989). Some species of Pseudo-nitzschia have been
363	known to release the toxin domoic acid (Lefebvre et al., 2002; Sekula-Wood et al.,
364	2009), and a connection between <i>Pseudo-nitzschia</i> blooms and eutrophication has
365	been proposed (Parsons and Dortch, 2002; Schnetzer et al., 2007). Fortunately,
366	Pseudo-nitzschia spp. may prefer high nitrate waters (Paul et al., 2008; Carter et al.,
367	2005), so declines in nutrient concentrations in Tolo Harbour should discourage
368	Pseudo-nitzschia blooms.
369	Although our data does not contain information on the size structure of the
370	phytoplankton community, changes in nutrient concentration or ratios may cause a
371	shift from larger phytoplankton species or cells to smaller ones. Stolte and Riegman
372	(1995) had theorized that eutrophication favors large phytoplankton species because
373	their larger storage capacity allows them to better compete under high nutrient

374	concentrations. Indeed, a shift towards an eutrophic environment has been shown to
375	favor large diatoms in the western Wadden Sea (Stolte el al., 1994; Philippart et al.,
376	2000), and Qiu et al. (2010) reported strong negative correlations between $\leq$ 3 $\mu m$
377	chlorophyll-a and nutrient concentrations. The half-saturation constant for P uptake
378	has been reported to be as high as $4.85{-}6.67~\mu M$ at $20^{\circ} C$ for certain large diatom
379	species (Nishikawa et al. 2009 and 2010), much higher than the means of TP in Tolo
380	Harbour after nutrient loading reduction (Table 2). Declines in nutrient concentrations
381	in Tolo Harbour may therefore favor smaller cells and species that are more
382	competitive in a less eutrophic condition. Qiu et al. (2010) reported significant
383	positive correlation between $< 20 \mu m$ chlorophyll $a$ concentrations and N/P ratios.
384	Our data also show a strong and positive correlation between TIN/PO <sub>4</sub> and other algal
385	group densities, caused mainly by an increase in the density of small flagellates and
386	Cryptomonas. Recent studies in Tolo Harbour have revealed that cells $< 5 \mu m$
387	accounted for $> 25\%$ of the chlorophyll- $a$ biomass (Lie and Wong, 2010; Wong et al.,
388	2010). In addition, phytoplankton size structures in many aquatic ecosystems have
389	also been known to be affected by climate change, particularly by increases in water
390	column stratification due to increased temperature (Finkel, 2007; Smol et al., 2005;
391	Winder et al., 2010). If indeed such a shift to smaller phytoplankton is occurring in
392	Tolo Harbour, it would help to explain the increase in phytoplankton density as

393 chlorophyll-*a* concentrations decrease.

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4.3. Comparison with the recovery from eutrophication of other ecosystems

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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

412	covered by extremely fine sediments that support little plant life (Trott, 1972).
413	Therefore, even with the decline of nutrient concentrations and increase in water
414	transparency, the spread of macrophytes in Tolo Harbour is unlikely.
415	In regards to the zooplankton grazers in Tolo Harbour, a study comparing the
416	copepod community before and after declines in nutrient concentrations in Tolo
417	Harbour revealed a decrease in copepod density, from 6,392 ind. m <sup>-3</sup> in 1987–1991, to
418	4,810 ind. m <sup>-3</sup> in 2003–2004 (Wong et al., 1993; Zhang and Wong, submitted). The
419	mesozooplankton community in Tolo Harbour was found to be heavily preyed upon
420	by larval and juvenile stages of the black seabream, Acanthopagrus schlegeli, and the
421	Japanese seaperch, Lateolabrax japonicus, which use Tolo Harbour as a nursery
422	habitat (Nip et al. 2003). While presently there is no detailed description on the fish
423	population in Tolo Harbour, the abundance of small planktivorous fishes will most
424	likely increase when increased fishing pressure from overfishing of larger piscivorous
425	fishes reduces the predation pressure on the small fishes. Such changes will in turn
426	increase the predation pressure on mesozooplankton grazers, reducing their
427	abundance and delaying any further decrease of phytoplankton abundance.
428	Although every water mass may have different responses to declines in nutrient
429	concentrations (Carvalho et al., 1995; Smith et al., 1999), it is obvious that trophic
430	interactions play a significant role in determining the outcome (Carpenter et al., 1995,

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

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684	
685	Figure Legends
686	Fig. 1. A map of Hong Kong and Tolo Harbour showing the location of the four
687	sampling stations.
688	
689	Fig. 2. Temporal variations in the annual means of total inorganic nitrogen, total
690	nitrogen, total phosphorus and SiO <sub>2</sub> concentration at the four sampling stations from
691	1986–2008. The shaded area represents the period before nutrient loading reduction .
692	
693	Fig. 2. Temporal variations in the annual manns of NO /NH TIN/DO and TIN/SiO
	<b>Fig. 3.</b> Temporal variations in the annual means of NO <sub>3</sub> /NH <sub>4</sub> , TIN/PO <sub>4</sub> and TIN/SiO <sub>2</sub>

695	before nutrient loading reduction. The dashed lines indicate the Redfield N:P and N:Si
696	ratios of 16:1 and 1:1 respectively.
697	
698	Fig. 4. Temporal variations in the annual means of chlorophyll $a$ at the four sampling
699	stations from 1986-2008. The shaded area represents the period before nutrient
700	loading reduction.
701	
702	Fig. 5. Temporal variations in the annual means of diatoms, dinoflagllates, other algal
703	groups and total phytoplankton densities at the four sampling stations from
704	1986–2008. The shaded area represents the period before nutrient loading reduction.
705	Monitoring began in 1988 for TM3 and TM4, 1994 for TM6 and 1986 for TM8.

### 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.

**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<sub>5</sub>".

Parameter				Station	/	
rarameter		TM3	TM4	TM6	TM8	All stations
	86–97	$23.06 \pm 4.33$	$22.88 \pm 4.32$	$22.55 \pm 4.14$	$22.20 \pm 4.02$	$22.67 \pm 4.19$
Temperature (°C)	98–08	$23.93 \pm 4.13$	$23.68 \pm 4.13$	$23.36 \pm 4.01$	$22.92 \pm 3.87$	$23.47 \pm 4.02$
	Overall	$23.47 \pm 4.25$	$23.26 \pm 4.24$	$22.93 \pm 4.09$	$22.55 \pm 3.96$	$23.06 \pm 4.12$
	86–97	$8.31 \pm 0.20$	$8.29 \pm 0.23$	$8.26 \pm 0.19$	$8.24 \pm 0.17$	$8.27 \pm 0.18$
pН	98–08	$8.21 \pm 0.20$	$8.16 \pm 0.21$	$8.16 \pm 0.20$	$8.16 \pm 0.15$	$8.17 \pm 0.17$
	Overall	$8.26 \pm 0.20$	$8.23 \pm 0.23$	$8.21 \pm 0.20$	$8.20 \pm 0.17$	$8.22 \pm 0.19$
	86–97	$30.75 \pm 1.55$	$30.77 \pm 1.66$	$31.44 \pm 1.51$	$31.96 \pm 1.06$	$31.22 \pm 1.39$
Salinity	98–08	$31.18 \pm 1.56$	$31.29 \pm 1.48$	$31.78 \pm 1.21$	$32.31 \pm 1.07$	$31.64 \pm 1.28$
	Overall	$30.96 \pm 1.57$	$31.02 \pm 1.60$	$31.61 \pm 1.38$	$32.13 \pm 1.08$	$31.42 \pm 1.35$
	86–97	$6.86 \pm 1.93$	$6.85 \pm 1.86$	$6.45 \pm 1.61$	$6.36 \pm 1.65$	$6.63 \pm 1.60$
$DO (mg l^{-1})$	98–08	$6.98 \pm 1.37$	$6.70 \pm 1.33$	$6.26 \pm 1.33$	$6.06 \pm 1.34$	$6.50 \pm 1.22$
	Overall	$6.92 \pm 1.68$	$6.78 \pm 1.62$	$6.36 \pm 1.48$	$6.22 \pm 1.51$	$6.57 \pm 1.43$
	86–97	$4.97 \pm 2.44$	$4.78 \pm 2.42$	$4.83 \pm 2.34$	$5.10 \pm 2.56$	$4.92 \pm 2.18$
Bottom DO (mg l <sup>-1</sup> )	98–08	$6.30 \pm 1.86$	$5.86 \pm 1.88$	$5.30 \pm 1.92$	$5.19 \pm 2.02$	$5.66 \pm 1.73$
	Overall	$5.61 \pm 2.28$	$5.29 \pm 2.24$	$5.05 \pm 2.16$	$5.15 \pm 2.31$	$5.28 \pm 2.01$
$\mathrm{BOD}_5$	86–97	$2.55 \pm 0.99$	$2.44 \pm 0.89$	$1.81 \pm 0.72$	$1.18 \pm 0.60$	$2.00 \pm 0.65$
- i	98–08	$1.98 \pm 0.75$	$1.73 \pm 0.56$	$1.38 \pm 0.47$	$0.89 \pm 0.56$	$1.49 \pm 0.45$
$(\text{mg l}^{-1})$	Overall	$2.28 \pm 0.93$	$2.10 \pm 0.83$	$1.60 \pm 0.65$	$1.04 \pm 0.60$	$1.76 \pm 0.62$
Pottom POD	86–97	$1.33 \pm 0.65$	$1.38 \pm 0.79$	$0.95 \pm 0.56$	$0.71 \pm 0.40$	$1.09 \pm 0.50$
Bottom BOD <sub>5</sub>	98–08	$1.35 \pm 0.66$	$1.24 \pm 0.60$	$0.89 \pm 0.56$	$0.56 \pm 0.42$	$1.01 \pm 0.41$
(mg l <sup>-1</sup> )	Overall	$1.34 \pm 0.66$	$1.32 \pm 0.71$	$0.92 \pm 0.56$	$0.63 \pm 0.42$	$1.05 \pm 0.46$

Secchi Depth (m)	86–97 98–08 Overall	$\begin{array}{c} 1.83 \pm 0.66 \\ 1.96 \pm 0.69 \\ 1.89 \pm 0.67 \end{array}$	$1.82 \pm 0.67$ $2.10 \pm 0.67$ $1.89 \pm 0.67$	$2.23 \pm 0.79$ $2.59 \pm 0.88$ $2.40 \pm 0.85$	$3.49 \pm 1.39$ $3.41 \pm 1.27$ $3.45 \pm 1.33$	$2.35 \pm 0.63$ $2.51 \pm 0.69$ $2.43 \pm 0.66$
Suspended solids (mg l <sup>-1</sup> )	86–97 98–08 Overall	$3.41 \pm 2.62$ $3.20 \pm 3.97$ $3.31 \pm 3.33$	$3.63 \pm 3.11$ $2.42 \pm 1.36$ $3.05 \pm 2.51$	$3.03 \pm 2.37$ $2.07 \pm 1.01$ $2.57 \pm 1.90$	$3.20 \pm 3.20$ $2.26 \pm 1.39$ $2.75 \pm 2.54$	$3.32 \pm 2.38$ $2.49 \pm 1.30$ $2.92 \pm 1.98$
Chl $a$ ( $\mu$ g $l^{-1}$ )	86–97 98–08 Overall	$9.63 \pm 6.87$ $9.33 \pm 6.28$ $9.49 \pm 6.58$	$9.82 \pm 7.43$ $7.58 \pm 4.37$ $8.75 \pm 6.25$	$6.25 \pm 4.80$ $5.20 \pm 2.90$ $5.75 \pm 4.03$	$3.38 \pm 6.21$ $3.13 \pm 2.17$ $3.26 \pm 4.72$	$7.27 \pm 4.80$ $6.31 \pm 3.15$ $6.81 \pm 4.12$
NO <sub>3</sub> -N (μM)	86–97 98–08 Overall	$3.21 \pm 3.17$ $1.37 \pm 2.36$ $2.33 \pm 2.96$	$3.40 \pm 3.74$ $1.42 \pm 2.28$ $2.45 \pm 3.28$	$2.29 \pm 2.15$ $1.27 \pm 1.95$ $1.80 \pm 2.11$	$1.52 \pm 1.43$ $1.45 \pm 1.75$ $1.48 \pm 1.59$	$2.60 \pm 2.30$ $1.38 \pm 1.87$ $2.02 \pm 2.19$
NH <sub>4</sub> -N (μM)	86–97 98–08 Overall	$10.13 \pm 6.37$ $4.94 \pm 4.39$ $7.65 \pm 6.08$	$8.27 \pm 5.49$ $4.40 \pm 4.67$ $6.42 \pm 5.46$	$5.61 \pm 3.95$ $3.26 \pm 2.53$ $4.48 \pm 3.54$	$3.56 \pm 3.24$ $2.57 \pm 1.76$ $3.08 \pm 2.68$	$6.89 \pm 4.25$ $3.79 \pm 3.09$ $5.41 \pm 4.04$
Total inorganic N (μM)	86–97 98–08 Overall	$14.31 \pm 8.79$ $6.77 \pm 6.27$ $10.71 \pm 8.55$	$12.65 \pm 8.00$ $6.37 \pm 6.16$ $9.64 \pm 7.82$	$8.82 \pm 5.27$ $5.10 \pm 3.64$ $7.04 \pm 4.92$	$5.76 \pm 3.69$ $4.64 \pm 2.49$ $5.22 \pm 3.22$	$10.38 \pm 5.86 \\ 5.72 \pm 4.28 \\ 8.15 \pm 5.66$
Total N (μM)	86–97 98–08 Overall	$51.53 \pm 19.03$ $28.08 \pm 20.20$ $40.32 \pm 22.81$	$48.58 \pm 18.83$ $25.98 \pm 19.14$ $37.77 \pm 22.06$	$41.39 \pm 19.00$ $22.25 \pm 16.91$ $32.23 \pm 20.39$	$35.10 \pm 26.40$ $19.04 \pm 15.90$ $27.42 \pm 23.40$	$44.15 \pm 18.05 23.84 \pm 17.64 34.44 \pm 20.52$
PO <sub>4</sub> -P (μM)	86–97 98–08 Overall	$\begin{array}{c} 1.32 \pm 0.78 \\ 0.30 \pm 0.23 \\ 0.84 \pm 0.77 \end{array}$	$\begin{array}{c} 1.32 \pm 0.72 \\ 0.31 \pm 0.24 \\ 0.84 \pm 0.74 \end{array}$	$0.93 \pm 0.49$ $0.30 \pm 0.16$ $0.63 \pm 0.49$	$0.59 \pm 3.69$ $0.33 \pm 0.16$ $0.46 \pm 0.30$	$\begin{array}{c} 1.04 \pm 0.54 \\ 0.31 \pm 0.17 \\ 0.69 \pm 0.55 \end{array}$
Total P (μM)	86–97 98–08 Overall	$3.59 \pm 2.13$ $1.14 \pm 0.61$ $2.42 \pm 2.01$	$3.43 \pm 1.58$ $1.03 \pm 0.53$ $2.28 \pm 1.69$	$2.75 \pm 1.37$ $0.94 \pm 0.38$ $1.89 \pm 1.37$	$2.23 \pm 1.42$ $0.87 \pm 0.31$ $1.58 \pm 1.25$	$3.00 \pm 1.43$ $0.99 \pm 0.38$ $2.04 \pm 1.47$

$SiO_2 (\mu M)$	86–97	$13.56 \pm 8.41$	$13.96 \pm 7.86$	$13.90 \pm 6.40$	$12.97 \pm 5.03$	$13.60 \pm 6.47$
	98–08	$12.64 \pm 7.87$	$13.24 \pm 7.38$	$12.68 \pm 6.26$	$12.44 \pm 5.84$	$12.75 \pm 6.27$
	Overall	$13.12 \pm 8.16$	$13.61 \pm 7.63$	$13.32 \pm 6.35$	$12.72 \pm 5.43$	$13.19 \pm 6.38$
NO <sub>3</sub> /NH <sub>4</sub>	86–97	$0.35 \pm 0.33$	$0.75 \pm 3.50$	$0.72 \pm 1.44$	$0.73 \pm 0.93$	$0.64 \pm 1.08$
	98–08	$0.30 \pm 0.56$	$0.41 \pm 0.72$	$0.69 \pm 2.10$	$1.16 \pm 2.73$	$0.64 \pm 1.34$
	Overall	$0.32 \pm 0.45$	$0.58 \pm 2.58$	$0.71 \pm 1.78$	$0.94 \pm 2.01$	$0.64 \pm 1.21$
TIN/PO <sub>4</sub>	86–97	$12.12 \pm 6.06$	$10.39 \pm 4.67$	$10.44 \pm 5.13$	$11.69 \pm 7.30$	$11.16 \pm 5.01$
	98–08	$24.99 \pm 18.58$	$23.16 \pm 16.93$	$18.69 \pm 12.95$	$15.44 \pm 10.09$	$20.57 \pm 12.91$
	Overall	$18.27 \pm 15.00$	$16.49 \pm 13.94$	$14.39 \pm 10.52$	$13.49 \pm 8.93$	$15.66 \pm 10.70$
TIN/SiO <sub>2</sub>	86–97 98–08 Overall	$1.37 \pm 1.26$ $0.73 \pm 0.78$ $1.07 \pm 1.11$	$1.13 \pm 1.00$ $0.62 \pm 0.65$ $0.89 \pm 0.89$	$0.74 \pm 0.64$ $0.49 \pm 0.53$ $0.62 \pm 0.60$	$0.47 \pm 0.26$ $0.41 \pm 0.27$ $0.44 \pm 0.27$	$0.93 \pm 0.71$ $0.56 \pm 0.52$ $0.75 \pm 0.65$

**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<sub>5</sub>".

	Station																			
	TM3			TM4						TM6					TM8					
Parameter	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	++	-		++		++			++		++			++		++		+	++	
pН					++				-	++					++					+
Salinity					-							-	-	-						-
DO		++			++					+				-						
Bottom DO		++	++	+		-	+	++												-
$BOD_5$		++			++		++			++		++		+	++		++	-		++
Bottom BOD <sub>5</sub>		+			++	-	++		-	+					+					
Secchi Depth						-		+				-						-		
Suspended solids		++		+	++		+			++					++		++			++
NO <sub>3</sub> -N					_ >					-						+				
NH <sub>4</sub> -N							-				-								-	

Total inorganic N		-					-							
Total N					+					++	++ ++			
PO <sub>4</sub> -P		-			-		-				+		-	-
Total P										++	++	-		
$SiO_2$					-						+ /		+	+
NO <sub>3</sub> /NH <sub>4</sub>	-								-		+	++		+
TIN/PO <sub>4</sub>			++	++				++	-		++		+	+
$TIN/SiO_2$	-					-					<u>-</u>			

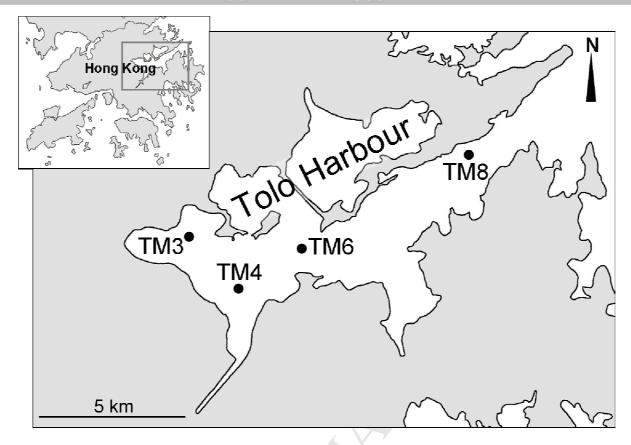


Fig. 1.

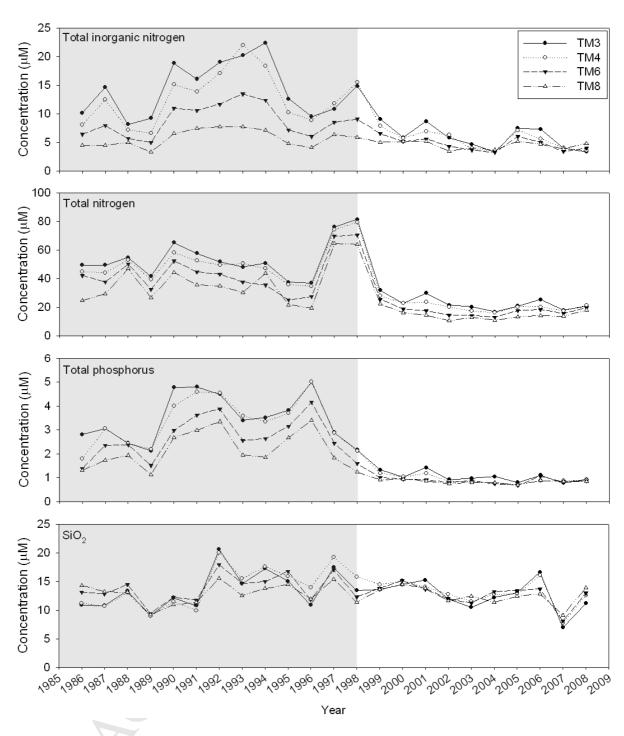


Fig. 2.

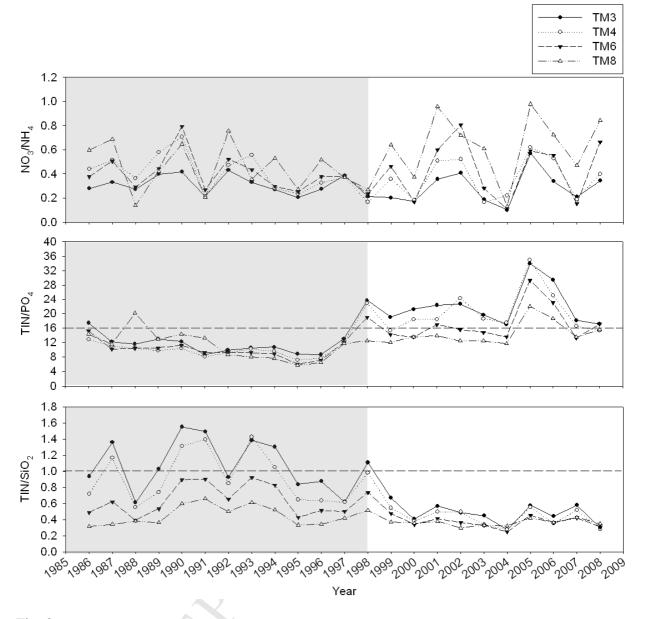


Fig. 3.

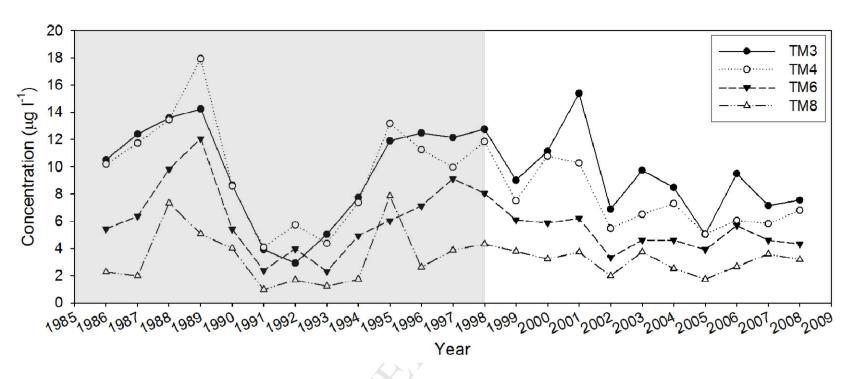


Fig. 4.

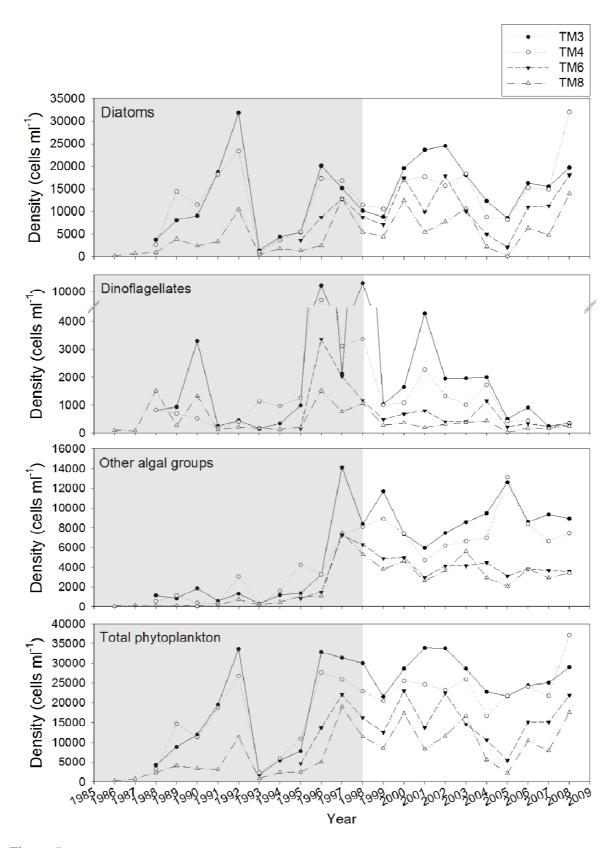


Figure 5.