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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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15 Abstract

16

17	Tolo Harbour is a landlocked bay with poor tidal flushings in the northeastern part of
18	Hong Kong. During the 1980s, excessive nutrient loading led to dramatic increase in
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
21	reduction measures on nutrient concentrations, nutrient ratios and phytoplankton
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.
29	

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

1. Introduction

34	Eutrophication is a common consequence of anthropogenic activities on water
35	bodies, and is widely regarded as the most wide-spread water quality problem
36	globally (Carpenter et al., 1999; Smith et al., 1999; Smith, 2003). The most common
37	effects of eutrophication are increases in phytoplankton productivity and biomass,
38	which in turn produce other adverse effects such as decreases in water transparency,
39	increases in the frequency of bottom hypoxia or anoxia and loss of various aquatic
40	organisms (Nixon, 1995; Smith et al., 1999; Smith, 2003). Numerous recent studies
41	have linked eutrophication to algal blooms which cause ecological and economical
42	damages (e.g. Anderson et al., 2008; Cloern, 2001; Smith et al., 1999). Nutrient
43	loadings may cause changes in the nutrient ratios (Justić et al., 1995; Turner, 2002),
44	and such changes may affect the species composition of the phytoplankton
45	community and higher trophic levels (Officer and Ryther, 1980; Philippart et al., 2000;
46	Smith, 1983; Turner et al., 1998; Turner, 2002). Although eutrophication in freshwater
47	systems has been studied extensively and intensively during the past few decades,
48	with many studies reporting on the effects of increased or decreased nutrient
49	concentrations in lakes (e.g. Battarbee et al., 2005; Carvalho and Kirika, 2003;
50	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
56	on the coastal water quality led to the initiation of a marine water quality monitoring
57	program by the Hong Kong Environmental Protection Department in the 1980s (Lee
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
62	and large-scale reclamation around Tolo Harbour began in the 1970s. Sewage
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
65	nitrate and phosphate loading of 32,460 kg and 3,190 kg respectively into Tolo
66	Harbour. In addition to nutrient inputs, the bottleneck topography of the Tolo Harbour
67	leads to poor tidal exchange rates and persistence of high nutrient concentrations (Lee
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_x -N and PO_4 -P in the
78	inner Tolo Harbour to be 0.05 μ M and 0.01 μ M respectively during 1974–1976. By
79	early 1990s, annual mean concentrations have increased to 19.99 μM for inorganic
80	nitrogen and 1.94 µM for PO ₄ -P (Hong Kong Environmental Protection Department,
81	1991). Moreover, PO ₄ -P values as high as 10.65 μ M have been reported in Tolo
82	Harbour in 1978–1979 (Hodgkiss and Chan, 1983). Decreasing trends in N:P ratio
83	were recorded during the 1980s and early 1990s (Hodgkiss and Chan, 1997; Yung et
84	al., 1997), and was considered to be caused by excessive P input (Hodgkiss and Chan,
85	1983, Hodgkiss and Yim, 1995). Some researchers noted that the observed changes in
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

89	collected by the Hong Kong Environmental Protection Department did not reveal such
90	a shift in the phytoplankton during 1982–1992 (Yung et al., 1997).
91	The Hong Kong Government initiated the Tolo Harbour Action Plan in 1986 to
92	halt the declining water quality of Tolo Harbour. Two of the most important
93	components of the action plan were the modification of existing sewage treatment
94	works processes (implemented in 1992) and the Tolo Harbour Effluent Export
95	Scheme (Chau, 2007). Commissioned in the mid-1990s and became fully operational
96	in 1998, the Tolo Harbour Effluent Export Scheme allows treated sewage from two
97	large sewage treatment works to be exported via pipelines for discharge outside of
98	Tolo Harbour. Nitrogen input into Tolo Harbour was reduced to ~1000 kg day ⁻¹ by
99	2000 (Hong Kong Environmental Protection Department, 2006).
100	In this study, data collected from four sampling stations in Tolo Harbour during
101	the period from 1986-2008 were analyzed to provide information on the long-term
102	changes in the hydrography and phytoplankton dynamics. Conditions before
103	(1986–1997) and after (1998–2008) the full implementation of the Tolo Harbour
104	Effleunt Export Scheme were compared to: (1) determine whether declines in nutrient
105	concentrations had led to changes in the nutrient ratios, and (2) identify shifts in the
106	phytoplankton community that may be related to changes in the nutrient
107	concentrations and ratios. Overall changes of the hydrography and phytoplankton

108	community in Tolo Harbour after declines in nutrient concentrations were also
109	compared to those reported in other ecosystems after recovery from eutrophication.
110	
111	2. Materials and Methods
112	
113	All data were provided by the Hong Kong Environmental Department. Four
114	sampling stations in Tolo Harbour, TM3, TM4, TM6 and TM8 (Fig. 1), were sampled
115	frequently, mostly once each month, but irregularly from 1982–1992. Monthly means
116	were calculated for each station. Physico-chemical parameters, including temperature,
117	salinity, pH and dissolved oxygen concentration (DO), were recorded at 1 m below
118	the water surface, mid-depth in the water column and 1 m above the bottom by a
119	SEACAT 19plus CTD water profiler (Sea-BIRD Electronics). Water samples were
120	also collected at the three depths using Niskin-type water bottles for the determination
121	of BOD ₅ , chlorophyll-a concentration, suspended solids concentration, and the
122	concentrations of NO ₃ -N, NH ₄ -N, total inorganic nitrogen (TIN), total nitrogen (TN),
123	PO_4 -P, total phosphorus (TP) and SiO ₂ . The data from the three depths were averaged
124	into depth-averaged data. Values from the bottom depth were also analyzed for DO
125	and BOD ₅ to provide information on hypoxia at the water bottom. Measurement of
126	BOD ₅ , nutrient concentrations, chlorophyll- <i>a</i> 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.
134	
135	(Insert Fig. 1 here)
136	
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.

149

150 **3. Results**

151

In general, physico-chemical, biological and nutrient parameters showed similar 152 153 changes among the four sampling stations after nutrient loading reduction (Table 1). 154 Temperature, salinity, bottom DO and TIN/PO₄ increased at all stations after nutrient loading reduction, while pH, BOD₅, chlorophyll-a concentrations, all nutrient 155 156 concentrations and TIN/SiO₂ decreased. DO and SiO₂ concentration did not differ significantly between the two time periods and among the stations respectively (p >157 158 0.05). All other parameters showed significant differences both temporally and spatially, with most p values < 0.001. Comparison among sampling stations revealed a 159 160 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. 161 162

163 (Insert Table 1 here)

165	N (including NO ₃ -N, NH ₄ -N, total inorganic N and total N) and P (including
166	PO ₄ -P and total P) concentrations were high, especially at TM3 and TM4, prior to
167	nutrient loading reduction (Fig. 2). Concentrations decreased noticeably within a year
168	after the full implementation of the sewage export scheme, and have remained at
169	relatively low levels in recent years. N and P concentrations differed markedly among
170	stations before nutrient loading reduction, but the differences became less apparent
171	afterwards. SiO ₂ concentrations did not appear to differ among stations before nutrient
172	loading reduction. NO ₃ /NH ₄ fluctuated widely during the entire study period, but the
173	value was generally lowest at TM3 and highest at TM8 (Fig. 3). In contrast, TIN/PO ₄
174	and TIN/SiO ₂ showed much clearer increasing and decreasing trends respectively (Fig
175	3). TIN/PO ₄ decreased gradually from between 1986 and 1996, but have returned to
176	approximately the 16:1 Redfield N:P ratio after declines in nutrient concentrations.
177	Declines in nutrient concentrations reduced the among-station differences in TIN/SiO ₂
178	and the values at all stations were well below the 1:1 Redfield ratio for N:Si.
179	

180 (Insert Fig. 2 and Fig. 3 here)

181

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

184	similar trends between chlorophyll-a concentrations and phytoplankton densities may
185	be due to the fact that chlorophyll-a data were depth-averaged, while phytoplankton
186	data were from the surface only. In general, the concentrations of chlorophyll-a and
187	densities of all phytoplankton groups were highest at TM3 and lowest at TM8.
188	Dinoflagellate density at TM3 increased to two major peaks of $> 10,000$ cells ml ⁻¹ in
189	1996 and 1998, and decreased to $< 1,000$ cells ml ⁻¹ in recent years. Other algal groups
190	occurred at densities of < 5000 cells ml ⁻¹ before 1996, but increased rapidly and
191	considerably after 1996. Density at TM3 remained > $6,000$ cells ml ⁻¹ after declines in
192	nutrient concentrations. As diatoms were the most abundant group, the density of total
193	phytoplankton exhibited temporal and spatial patterns more similar to those of
194	diatoms than those of dinoflagellates and other algal groups. Declines in nutrient
195	concentrations did not affect the density of dinoflagellates significantly, but densities
196	of diatoms, other algal groups and total phytoplankton all showed significant increase
197	after declines in nutrient concentrations. Despite a significant increase in total
198	phytoplankton density, the number of algal blooms recorded in Tolo Harbour dropped
199	significantly from a mean of 15.8 before nutrient loading reduction to 7.6 (Paired
200	t-test, $p = 0.02$) afterwards. No specific phytoplankton group or genus identified in the
201	study could be concluded to be restricted to a particular station. Diatom genera
202	common in all four stations and with average densities $> 1,000$ cells ml ⁻¹ in TM3 and

203	> 600 ml ⁻¹ 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 ⁻¹
206	in TM3 and > 250 cells ml ⁻¹ in TM8 for <i>Prorocentrum</i> , and > 700 cells ml ⁻¹ in TM3
207	and > 100 cells ml ⁻¹ 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.
211	
212	(Insert Fig. 4 and Fig. 5 here)
213	
214	Hong Kong is characterized by a warm wet season from April to September, and
215	a relatively dry and cool season for the rest of the year. However, when plots of
216	seasonal means of nutrient concentrations and phytoplankton densities were made, no
217	apparent and general trend could be identified (Data not shown).
218	Kendall's correlation did not reveal any strong or obvious trends between
219	phytoplankton variables and various parameters (Table 2). Results for TM3 and TM4
220	seemed to oppose those for TM6 and TM8 at times. For instance, correlation between
221	phytoplankton variables and DO and BOD ₅ were usually positive at TM3 and TM4,

222 but negative at TM6 and TM8, and vice versa with the results for SiO₂. 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 parameters. Correlation between phytoplankton variables and nutrient variables were 225 226 mostly negative in all stations except TM6. Most phytoplankton variables at TM3 and TM4 correlated significantly and negatively with nutrient concentrations. In TM6, 227 chlorophyll-a concentrations correlated negatively with NO₃-N and NH₄-N 228 229 concentrations, while dinoflagellate densities correlated positively with total N, PO₄-P 230 and total P concentrations. NO₃/NH₄ correlated negatively with the densities of all groups of phytoplankton at TM3, and positively with diatom densities at TM6 and 231 232 TM8. TIN/PO₄ correlated positively with other algal group densities in all stations. 233 TIN/SiO₂ correlated negatively with phytoplankton densities in all stations, but 234 exhibited especially strong negative correlations with the densities of dinoflagellates, other algal groups and total phytoplankton at TM3 and TM4. Kendall's correlations 235 between the number of algal blooms and all parameters were also determined. 236 Surprisingly, the number of algal blooms did not correlate significantly with 237 238 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 239 240 BOD₅ (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 TM8, all parameters show high temporal variations and do not exhibit an apparent 250 251 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 252 253 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 current, weather conditions and anthropogenic activities that are not available in our 256 257 data set.

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

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

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

279	2001; Lam and Ho, 1989; Yung et al., 1997), and a seaward decrease in nutrient
280	concentrations from the inner harbour to the channel was evident from our results. A
281	similar spatial pattern, with water quality improving from TM3 to TM8, is recorded in
282	other water quality indicators such as secchi depth, DO and BOD ₅ . Due to its
283	proximity to the open sea and the much lower pollution levels, TM8 was less affected
284	by eutrophication and subsequent declines in nutrient concentrations compared to
285	stations in the inner part of the harbour. An interesting observation was that the mean
286	values of NO ₃ /NH ₄ decreased at TM3, TM4 and TM6, but increased at TM8 after
287	declines in nutrient concentrations. This may be caused by a relatively higher decline
288	of NH ₄ -N concentrations than that of NO ₃ -N in TM8. The maximum release rate of
289	NH ₄ -N from contaminated sediments into the water column in Tolo Harbour was
290	found to be more than sixfolds higher than that of NO ₃ -N (Hu et al. 2001), and
291	although Hu et al. (2001) did not specify specific nutrient release rates in various parts
292	of Tolo Harbour, it may be possible that comparatively less NH ₄ -N was released back
293	into the water column from sediments in the less polluted outer parts of the Tolo
294	Harbour.

295 Spatial differences in nutrient loading and tidal flushing may have led to the 296 observed differences in how phytoplankton in different stations had responded to 297 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_4 over NO_3 (Dortch
306	1990), a decrease in NO ₃ /NH ₄ may favor phytoplankton growth. However, the lack of
307	consistent correlation between NO ₃ /NH ₄ and phytoplankton density suggests that the
308	phytoplankton community in Tolo Harbour did not have specific preference for either
309	NO ₃ or NH ₄ .
310	Decreases in nutrient concentrations following nutrient loading reduction
311	measures had altered the values of TIN/PO ₄ and TIN/SiO ₂ 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 ₄ after declines in nutrient concentrations. In general, the density of
316	dinoflagellates correlate neither with TIN/PO4 nor with most physico-chemical

317	parameters other than Secchi depth and BOD ₅ , both of which may be dependent
318	variables affected by the dinoflagellate density. Hence, the density of dinoflagellate in
319	Tolo Harbour may not be easily predicted from nutrient concentrations or N:P ratios
320	alone. While our analysis has not established a strong link between nutrient
321	concentrations and dinoflagellate densities, several investigators have reported an
322	increasing dominance of dinoflagellates during the 1980s and early 1990s when
323	nutrient concentrations in Tolo Harbour were at their highest (Chan and Hodkiss,
324	1987; Hodgkiss and Chan, 1983; Hodgkiss and Ho, 1997; Lam and Ho, 1989). The
325	density of dinoflagellates was at a peak in 1996 when TIN/PO ₄ was very low (Figs. 3
326	& 4). The density of dinoflagellates reached another peak in 1998, due primarily to an
327	extremely dense population of <i>Prorocentrum</i> (122,467 cells ml ⁻¹) at TM3 in April.
328	But dinoflagellate densities have remained relatively low since 2005, while TIN/PO ₄
329	increased. Hodgkiss and Ho (1997) also noted a decrease in N:P ratios and an increase
330	in algal blooms from 1981–1990. Our data showed no significant correlation between
331	these two variables, although an increase in TIN/PO ₄ and a decrease in the number of
332	algal blooms were found after declines in nutrient concentrations. Nevertheless, while
333	our data did not contain information about Noctiluca blooms, Liu and Wong (2006)
334	showed that Noctiluca scintillans were still common in Tolo Harbour after declines in
335	nutrient concentrations, and Noctiluca 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 ₂
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 ₂ 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 μ 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 ₂ concentration in
354	this study was ~ 10 μ 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 ₂ . 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 Pseudo-nitzschia 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.

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

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 μ 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 μ M at 20°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 <i>a</i> concentrations and N/P ratios.
384	Our data also show a strong and positive correlation between TIN/PO ₄ 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 μ 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.

4.3. Comparison with the recovery from eutrophication of other ecosystems

397	The effects of declines in nutrient concentrations had been intensively studied in
398	freshwater lakes (e.g. Jeppesen et al., 2002; Jeppesen et al., 2005; Søndergaard et al.,
399	2002), and most have reported improvements in water quality. Water quality
400	improvement is mainly achieved through the decrease in phytoplankton biomass. This
401	decrease can occur as a direct response to declines in nutrient concentrations, but it
402	can be further facilitated through increased grazing pressure from zooplankton grazers
403	(Edmondson, 1994) and the recolonization of submerged macrophytes, which has
404	been shown to substantially affect the success rates of water quality improvement in
405	lakes (Mehner et al., 2002). While there may be differences between freshwater and
406	marine systems in their responses to declines in nutrient concentrations (Cloern, 2001;
407	Smith et al., 1999), examples of dramatic improvements in once eutrophicated marine
408	ecosystems such as Hillsborough Bay, Florida (Johansson and Lewis, 1992) and the
409	coastal waters of Denmark (Rask et al., 1999) had both involved the recolonization of
410	macrophytes such as seagrasses (Hillsborough Bay) and eelgrass (Denmark).
411	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 ⁻³ in 1987–1991, to
418	4,810 ind. m ⁻³ 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,

Manca and Ruggiu, 1998; Proulex et al., 1996; Smith et al., 1999). Increase in 431 432 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 grazers should be maintained to ensure the long-term stability of the recovered 436 ecosystem (Mehner et al. 2002). Unfortunately, there is a lack of long-term 437 438 monitoring on other communities, such as zooplankton and nekton, in Tolo Harbour. As there is insufficient information on the responses of various communities to 439 changes in nutrient loading, the phytoplankton community alone is not a reliable 440 441 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, but also other important components of the aquatic ecosystem such as zooplankton 444 and nekton communities. 445

446

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685 Figure Legends

- Fig. 1. A map of Hong Kong and Tolo Harbour showing the location of the foursampling stations.
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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 .

692

Fig. 3. Temporal variations in the annual means of NO₃/NH₄, TIN/PO₄ and TIN/SiO₂

at the four sampling stations from 1986–2008. The shaded area represents the period

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	e annual	means	of	chlorophyll	a at	the fo	our	sampl	ing

- 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
702	
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.

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Table 1. Mean values (\pm 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 BOD₅".

Parameter				Station	/	
Parameter		TM3	TM4	TM6	TM8	All stations
	86–97	23.06 ± 4.33	22.88 ± 4.32	22.55 ± 4.14	22.20 ± 4.02	22.67 ± 4.19
Temperature (°C)	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
	86–97	8.31 ± 0.20	8.29 ± 0.23	8.26 ± 0.19	8.24 ± 0.17	8.27 ± 0.18
pН	98–08	8.21 ± 0.20	8.16 ± 0.21	8.16 ± 0.20	8.16 ± 0.15	8.17 ± 0.17
L	Overall	8.26 ± 0.20	8.23 ± 0.23	8.21 ± 0.20	8.20 ± 0.17	8.22 ± 0.19
	86–97	30.75 ± 1.55	30.77 ± 1.66	31.44 ± 1.51	31.96 ± 1.06	31.22 ± 1.39
Salinity	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
	86–97	6.86 ± 1.93	6.85 ± 1.86	6.45 ± 1.61	6.36 ± 1.65	6.63 ± 1.60
DO (mg l^{-1})	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
	86–97	4.97 ± 2.44	4.78 ± 2.42	4.83 ± 2.34	5.10 ± 2.56	4.92 ± 2.18
Bottom DO (mg l^{-1})	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	$5.28\pm\!2.01$
	86–97	2.55 ± 0.99	2.44 ± 0.89	1.81 ± 0.72	1.18 ± 0.60	2.00 ± 0.65
BOD ₅	98–08	1.98 ± 0.75	1.73 ± 0.56	1.38 ± 0.47	0.89 ± 0.56	1.49 ± 0.45
$(mg l^{-})$	Overall	2.28 ± 0.93	2.10 ± 0.83	1.60 ± 0.65	1.04 ± 0.60	$1.76\pm\!0.62$
	86–97	1.33 ± 0.65	1.38 ± 0.79	0.95 ± 0.56	0.71 ± 0.40	1.09 ± 0.50
Bottom BOD_5	98–08	1.35 ± 0.66	1.24 ± 0.60	0.89 ± 0.56	0.56 ± 0.42	1.01 ± 0.41
$(mg l^{-})$	Overall	1.34 ± 0.66	1.32 ± 0.71	0.92 ± 0.56	0.63 ± 0.42	1.05 ± 0.46

Secchi Depth (m)	86–97 98–08 Overall	$\begin{array}{c} 1.83 \pm 0.66 \\ 1.96 \pm 0.69 \\ 1.89 \pm 0.67 \end{array}$	$\begin{array}{c} 1.82 \pm 0.67 \\ 2.10 \pm 0.67 \\ 1.89 \pm 0.67 \end{array}$	$\begin{array}{c} 2.23 \pm 0.79 \\ 2.59 \pm 0.88 \\ 2.40 \pm 0.85 \end{array}$	$\begin{array}{c} 3.49 \pm 1.39 \\ 3.41 \pm 1.27 \\ 3.45 \pm 1.33 \end{array}$	$\begin{array}{c} 2.35 \pm 0.63 \\ 2.51 \pm 0.69 \\ 2.43 \pm 0.66 \end{array}$
Suspended solids (mg l ⁻¹)	86–97 98–08 Overall	$\begin{array}{c} 3.41 \pm 2.62 \\ 3.20 \pm 3.97 \\ 3.31 \pm 3.33 \end{array}$	3.63 ± 3.11 2.42 ± 1.36 3.05 ± 2.51	3.03 ± 2.37 2.07 ± 1.01 2.57 ± 1.90	3.20 ± 3.20 2.26 ± 1.39 2.75 ± 2.54	3.32 ± 2.38 2.49 ± 1.30 2.92 ± 1.98
Chl a (µg l ⁻¹)	86–97 98–08 Overall	9.63 ± 6.87 9.33 ± 6.28 9.49 ± 6.58	9.82 ± 7.43 7.58 ± 4.37 8.75 ± 6.25	$\begin{array}{c} 6.25 \pm 4.80 \\ 5.20 \pm 2.90 \\ 5.75 \pm 4.03 \end{array}$	3.38 ± 6.21 3.13 ± 2.17 3.26 ± 4.72	7.27 ± 4.80 6.31 ± 3.15 6.81 ± 4.12
NO3-N (µM)	86–97 98–08 Overall	3.21 ± 3.17 1.37 ± 2.36 2.33 ± 2.96	3.40 ± 3.74 1.42 ± 2.28 2.45 ± 3.28	$\begin{array}{c} 2.29 \pm 2.15 \\ 1.27 \pm 1.95 \\ 1.80 \pm 2.11 \end{array}$	1.52 ± 1.43 1.45 ± 1.75 1.48 ± 1.59	2.60 ± 2.30 1.38 ± 1.87 2.02 ± 2.19
NH4-N (µM)	86–97 98–08 Overall	$\begin{array}{c} 10.13 \pm 6.37 \\ 4.94 \pm 4.39 \\ 7.65 \pm 6.08 \end{array}$	$\begin{array}{c} 8.27 \pm 5.49 \\ 4.40 \pm 4.67 \\ 6.42 \pm 5.46 \end{array}$	5.61 ± 3.95 3.26 ± 2.53 4.48 ± 3.54	3.56 ± 3.24 2.57 ± 1.76 3.08 ± 2.68	$\begin{array}{c} 6.89 \pm 4.25 \\ 3.79 \pm 3.09 \\ 5.41 \pm 4.04 \end{array}$
Total inorganic N (μM)	86–97 98–08 Overall	$\begin{array}{c} 14.31 \pm 8.79 \\ 6.77 \pm 6.27 \\ 10.71 \pm 8.55 \end{array}$	$\begin{array}{c} 12.65 \pm 8.00 \\ 6.37 \pm 6.16 \\ 9.64 \pm 7.82 \end{array}$	$\begin{array}{c} 8.82 \pm 5.27 \\ 5.10 \pm 3.64 \\ 7.04 \pm 4.92 \end{array}$	5.76 ± 3.69 4.64 ± 2.49 5.22 ± 3.22	$\begin{array}{c} 10.38 \pm 5.86 \\ 5.72 \pm 4.28 \\ 8.15 \pm 5.66 \end{array}$
Total N (µM)	86–97 98–08 Overall	51.53 ± 19.03 28.08 ± 20.20 40.32 ± 22.81	$\begin{array}{c} 48.58 \pm 18.83 \\ 25.98 \pm 19.14 \\ 37.77 \pm 22.06 \end{array}$	$\begin{array}{c} 41.39 \pm 19.00 \\ 22.25 \pm 16.91 \\ 32.23 \pm 20.39 \end{array}$	35.10 ± 26.40 19.04 ± 15.90 27.42 ± 23.40	$\begin{array}{c} 44.15 \pm 18.05 \\ 23.84 \pm 17.64 \\ 34.44 \pm 20.52 \end{array}$
PO ₄ -P (µM)	86–97 98–08 Overall	$\begin{array}{c} 1.32 \pm 0.78 \\ 0.30 \pm 0.23 \\ 0.84 \pm 0.77 \end{array}$	$\begin{array}{c} 1.32 \pm 0.72 \\ 0.31 \pm 0.24 \\ 0.84 \pm 0.74 \end{array}$	$\begin{array}{c} 0.93 \pm 0.49 \\ 0.30 \pm 0.16 \\ 0.63 \pm 0.49 \end{array}$	0.59 ± 3.69 0.33 ± 0.16 0.46 ± 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 ± 2.13 1.14 ± 0.61 2.42 ± 2.01	3.43 ± 1.58 1.03 ± 0.53 2.28 ± 1.69	2.75 ± 1.37 0.94 ± 0.38 1.89 ± 1.37	2.23 ± 1.42 0.87 ± 0.31 1.58 ± 1.25	3.00 ± 1.43 0.99 ± 0.38 2.04 ± 1.47

	86–97	13.56 ± 8.41	13.96 ± 7.86	13.90 ± 6.40	12.97 ± 5.03	13.60 ± 6.47
SiO_2 (μ M)	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
	86–97	0.35 ± 0.33	0.75 ± 3.50	0.72 ± 1.44	0.73 ± 0.93	0.64 ± 1.08
NO ₃ /NH ₄	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
	86–97	12.12 ± 6.06	10.39 ± 4.67	10.44 ± 5.13	11.69 ± 7.30	11.16 ± 5.01
TIN/PO ₄	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
	86–97	1.37 ± 1.26	1.13 ± 1.00	0.74 ± 0.64	0.47 ± 0.26	0.93 ± 0.71
TIN/SiO ₂	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

1.07 ± 1.11 0.89 ± 0.89 0.62 ±

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 BOD₅".

	Station																				
	TM3						TM4					TM6						TM8			
Parameter	Diatom	Dino	Others	Total	Chl-a	Diatom	Dino	Others	Total	Chl-a	Diatom	Ċ	Dino	Others	Total	Chl-a	Diatom	Dino	Others	Total	Chl-a
Temperature	++	-		++		++			++		+	+)			++		++		+	++	
pH					++				-	++	5					++					+
Salinity					-								-	-	-						-
DO		++			++					+		-			-						
Bottom DO		++	++	+		-	+	++	\mathbf{Y}			-									-
BOD ₅		++			++		++			++			++		+	++		++	-		++
Bottom BOD ₅		+			++	-	++		-	+						+					
Secchi Depth						E		+				-	-						-		
Suspended solids		++		+	++		+			++						++		++			++
NO ₃ -N					_					-							+				
NH ₄ -N							-				-									-	

Total inorganic N		-					-											
Total N					+					++		++	- K	++				
PO ₄ -P		-			-		-					+				-		-
Total P										++		++	2		-			
SiO ₂					-							+				+	+	
NO ₃ /NH ₄	-								-		4				++		+	
TIN/PO ₄			++	++				++	-)	++			+	+	
TIN/SiO ₂	-					-							-					











Fig. 3.



Fig. 4.



Figure 5.