

# Resistance of Hong Kong waters to nutrient enrichment: assessment of the role of physical processes in reducing eutrophication

Jie Xu · Kedong Yin · Joseph H. W. Lee · Donald M. Anderson · Yuelu Jiang · Xiangcheng Yuan · Alvin Y. T. Ho · Paul J. Harrison

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**Abstract** Hong Kong waters receive high nutrient loading from year-round sewage effluent and Pearl River discharge during the summer wet season. We assessed the role of physical processes in reducing eutrophication by calculating a eutrophication reduction index for four different hydrographical areas and four seasons. We used outdoor incubation experiments to assess the response of phytoplankton when physical (mixing and dilution) processes and mesozooplankton grazing were reduced. The primary regulator of phytoplankton growth in low nutrient eastern waters (reference site) shifted from nutrients in the wet season to increased vertical mixing in the dry season. In the highly flushed western waters and Victoria Harbour, the majority (>86 %) of the eutrophication impacts were reduced by strong hydrodynamic mixing (turbulence, vertical mixing, and flushing effects) all year. In southern waters, eutrophication effects were severe (chlorophyll *a* of up to  $\sim 73 \mu\text{g L}^{-1}$ ) and was regulated by the ambient phosphate ( $\text{PO}_4$ ) concentration ( $\sim 0.1 \mu\text{M}$ ) during summer.

In contrast, 62–96 % of the potential eutrophication impacts were reduced by physical processes during other seasons. Bioassays also revealed that the yield of chlorophyll from dissolved inorganic nitrogen (DIN) that was taken up by phytoplankton [ $1.1\text{--}3.3 \text{ g Chl (mol N)}^{-1}$ ] was not significantly different in both N- and P-limited cases. In contrast, the uptake ratios of DIN: $\text{PO}_4$  (26:1–105:1) and Chl:P ratios [ $42\text{--}150 \text{ g Chl (mol P)}^{-1}$ ] in the P-limited cases were significantly ( $p < 0.05$ , *t* test) higher than the N-limited cases [ $\sim 16\text{DIN:1P}$  and  $22\text{--}48 \text{ g Chl (mol P)}^{-1}$ ]. The C:Chl ratios ranged from 32 to  $87 \text{ g g}^{-1}$ . These potential ranges in ratios need to be considered in future nutrient models.

**Keywords** Eutrophication impacts · Hydrodynamics · Algal biomass · Pearl River discharge · Sewage effluent · Hong Kong · Phosphorus limitation · Chl:N, Chl:P, and C:Chl ratios

J. Xu (✉) · Y. Jiang · X. Yuan · A. Y. T. Ho · P. J. Harrison  
Division of Environment, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR, People's Republic of China  
e-mail: xujie@ust.hk

K. Yin  
Australian Rivers Institute, Griffith University, Nathan Campus, Brisbane, QLD 4111, Australia

J. H. W. Lee  
Department of Civil and Environmental Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR, People's Republic of China

D. M. Anderson  
Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

## 1 Introduction

The inputs of anthropogenic nutrients have increased dramatically over the last few decades in coastal waters (Nixon and Pilson 1983; Cloern 2001). Eutrophication is not simply a matter of nutrient enrichment, since the biological and physical processes through which the nutrients impact marine productivity are highly variable and need to be fully understood (Visser and Kamp-Nielsen 1996). The response of plankton communities to nutrient enrichment in coastal ecosystems is an issue of major global concern (Malone et al. 1988; Smith et al. 1999; Rabalais and Nixon 2002). Biological processes are often coupled with physical processes (Monbet 1992; Tada et al. 2001; Harrison et al. 2008). Many coastal and estuarine ecosystems that receive

high nutrient loading respond differently to such inputs because phytoplankton growth is controlled by hydrodynamic conditions, even at the community level (Ragueneau et al. 1996). The Bay of Brest showed high tolerance to nutrient enrichment (Le Pape et al. 1996), while Chesapeake Bay does not have the capability to withstand nutrient enrichment (Fisher et al. 1988). Hence, it is necessary to consider all the processes that reduce the phytoplankton response to nutrient enrichment when we assess the susceptibility of coastal ecosystems to nutrient enrichment.

Hong Kong is situated on the eastern side of the Pearl River estuary and its southern and eastern waters are influenced by the invasion of shelf waters from the northern region of the South China Sea. The Pearl River discharge is the second largest river next to the Yangtze River in China in terms of freshwater discharge (Zhao 1990). The annual average river discharge reaches  $10,500 \text{ m}^3 \text{ s}^{-1}$ , with 80 % of the discharge occurring in the wet season (April–September). The maximum river discharge occurs in July (Zhao 1990). In the summer wet season, the high volume Pearl River discharge ( $20,000 \text{ m}^3 \text{ s}^{-1}$ ) contains high nitrate ( $\text{NO}_3$ ) ( $>100 \text{ }\mu\text{M}$ ) and silicate [ $\text{Si}(\text{OH})_4$ ] ( $>100 \text{ }\mu\text{M}$ ) concentrations and it flows mainly into the western and southern waters of Hong Kong as a result of forcing by southwest monsoon winds. Furthermore, nutrients from the domestic sewage effluent with high ammonium ( $\text{NH}_4$ ) and phosphate ( $\text{PO}_4$ ) and low  $\text{Si}(\text{OH})_4$  are a major nutrient source, since over 2 million tons of sewage effluent are discharged daily into the coastal waters around Victoria Harbour.

In 2001, Hong Kong government implemented the Harbour Area Treatment Scheme (HATs), which collects 70 % of this sewage previously discharged into Victoria Harbour, treats it, and discharges it into waters west of Stonecutters Island after chemical enhanced primary treatment (CEPT). The relocation of sewage effluent from distributed sources in Victoria Harbour to a concentrated source at Stonecutters Island has had a significant effect on the dynamics of nutrients and phytoplankton biomass. The input of high nutrient loading from Pearl River and Hong Kong has resulted in nutrient over-enrichment in some local areas.

In addition to the Pearl River discharge, sewage effluent, monsoon-induced ocean currents, and tidal hydrodynamics play an important role in Hong Kong waters. Tides in Hong Kong are characterized as mainly semi-diurnal. The tidal ranges for spring and neap tides are typically 2.0 and 1.0 m, respectively (Lee et al. 2006). Previous studies (Yin 2002) have suggested that there was uncoupling between algal-based expressions of eutrophication (i.e. algal blooms) and high nutrient loading in Hong Kong waters.

The objective of this study was to quantify the importance of these physical factors in reducing eutrophication by using outdoor incubations experiments in which mixing, dilution and sinking were eliminated and grazing by mesozooplankton was reduced.

We calculated a eutrophication reduction index (ERI) which compares the potential maximum phytoplankton biomass (the maximum amount of algal biomass that can be produced when the limiting nutrient in a water body is converted to algal biomass in bioassay experiments, where physical effects such as vertical mixing and light limitation are eliminated) with the actual algal biomass (chlorophyll) for four areas with different hydrodynamics for four seasons. The potential maximum phytoplankton biomass is an algal-based expression of eutrophication potential supported by the ambient concentration of nutrients in terms of biological processes (that is, conversion of all the nutrients to algal biomass) alone when physical effects are eliminated, while the actual algal biomass in the water column was the result of both biological and physical processes, reflecting in situ eutrophication impacts. The difference between both parameters (expressed by ERI) allows one to determine whether biological factors (i.e. nutrients) or physical processes (i.e. flushing and mixing) are the major regulator of eutrophication impacts (expressed by algal biomass). If ERI is large, then physical processes may be the main controlling factor of phytoplankton biomass accumulation. Conversely, if ERI is small, then nutrients are the main factor that limit phytoplankton biomass yield. Our study will help to determine whether nutrients should be removed during sewage treatment in order to reduce eutrophication impacts resulting from high biomass algal blooms.

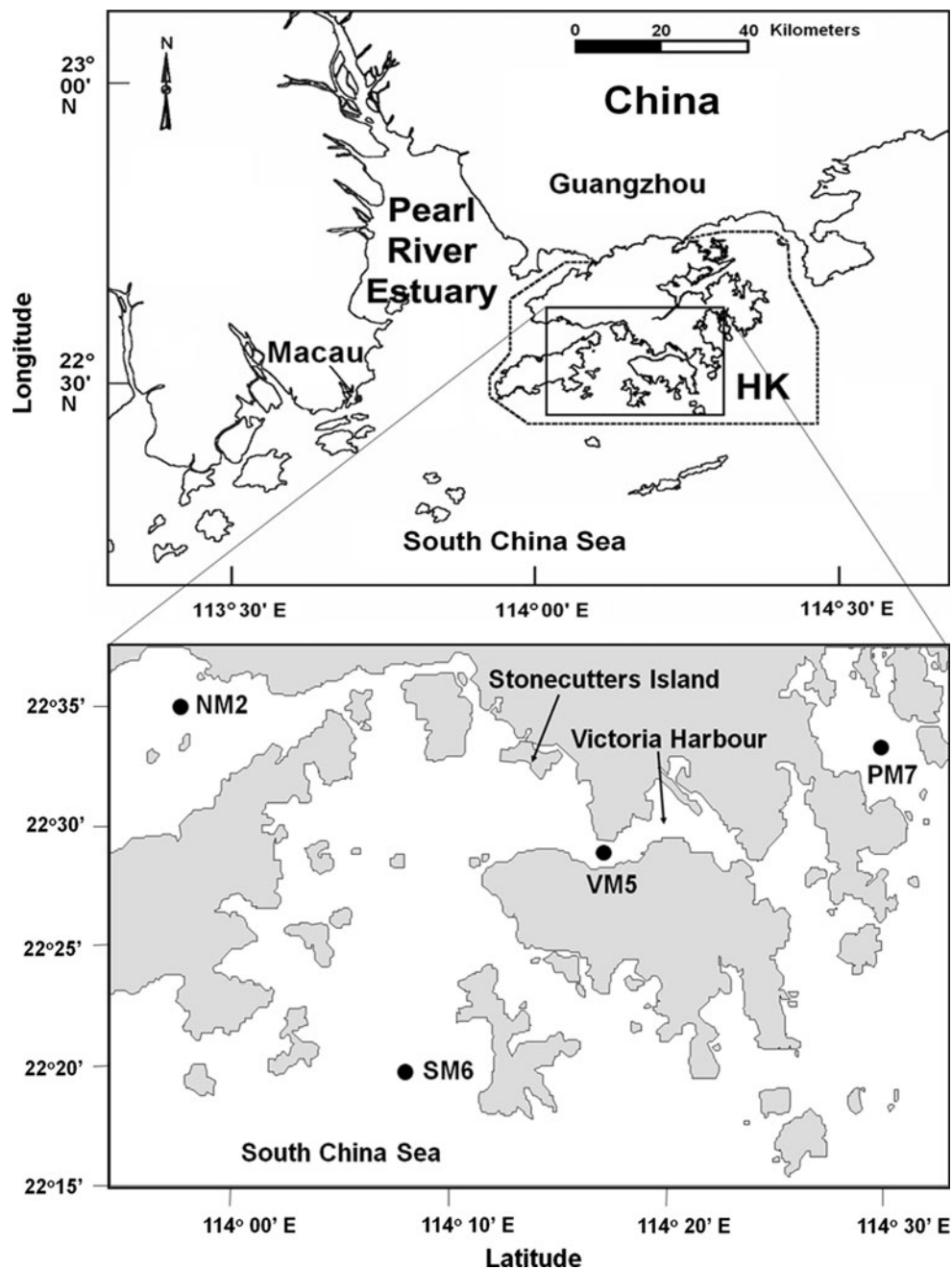
## 2 Materials and methods

### 2.1 Study sites

Four stations were selected to represent different geographical regions and water quality zones: NM2 in western waters, VM5 in Victoria Harbour, SM6 in southern waters, and PM7 in Port Shelter; the station locations and station numbers are the same as those sampled by the Hong Kong Environment Protection Department (EPD) (Fig. 1). They are representative of the estuarine influence (NM2 and SM6), local sewage effluent impacts (VM5), and coastal/oceanic conditions (PM7).

Seasonal sampling was conducted at 1 m depth at all stations, except for PM7, in February, April, July, and November 2006, respectively. At PM7, samples were collected in October, December 2005, and April and July 2006. The dry season was defined as October–March and

**Fig. 1** The four sampling stations in Hong Kong waters. The depths for NM2, VM5, SM6, and PM7 are 11, 13, 15 and 17 m, respectively. These 4 stations are the same as the EPD monitoring stations



the wet season was from April–September. The four seasons were categorized as winter (December–February), spring (March–May), summer (June–August), and fall (September–November).

## 2.2 Salinity, temperature, nutrients, outdoor incubations and growth rate

Vertical profiles of salinity and temperature were measured with a YSI 6600 multi-parameter Water Quality Monitor equipped with temperature/conductivity, salinity, turbidity, chlorophyll, dissolved oxygen, and pH/ORP combination

sensors (YSI, Yellow Springs, OH, USA). At PM7, salinity and temperature data were obtained from monthly average data from the 20 years (1986–2005) monitoring dataset obtained from EPD. Photosynthetically available radiation (PAR) in the water column was measured with a Li-Cor underwater spherical quantum sensor (LI-193SA; LI-COR, Lincoln, NE, USA), while the solar radiation in the air was measured using a Li-Cor Pyranometer (LI-200SZ; LI-COR).

Inorganic nutrient concentrations [ $\text{NO}_3$ , nitrite ( $\text{NO}_2$ ),  $\text{NH}_4$ ,  $\text{PO}_4$  and  $\text{Si}(\text{OH})_4$ ] were determined colorimetrically with a SKALAR San Plus Continuous Flow analyser

(SKALAR, Breda, The Netherlands) following the protocols described by Strickland and Parsons (1968) and Grasshoff et al. (1983). Chlorophyll *a* (Chl) samples were extracted with 90 % acetone and analyzed using a fluorometer (Knap et al. 1996).

Outdoor incubations were conducted in triplicate under natural sunlight. Water samples were screened through a 200- $\mu\text{m}$  mesh nylon screen to remove large zooplankton, while microzooplankton (<200  $\mu\text{m}$ ) remained in the water samples. Triplicate water samples were incubated in 2-L acid-washed polycarbonate bottles at 30 and 50 % of natural sunlight during summer and winter, respectively. The light conditions in the incubators were likely somewhat higher than the average irradiance received by phytoplankton in vertically mixed waters during winter. Running seawater was used to maintain the in situ temperature during the four seasons and the bottles were manually stirred twice a day. Nutrient and Chl samples were taken daily or every other day, as well as particulate carbon (PC) samples for NM2, SM6, and VM5 in February, April, and July during the incubation.

Samples for PC were filtered onto precombusted (4 h at 460 °C) 25-mm Whatman GF/F filters (Whatman, Lawrence, KS, USA). The filters were frozen at 80 °C until analysis. Filters were dried at 60 °C in an oven overnight and then PC was measured with a Perkin-Elmer PE 2400 Series II CHN analyzer (Perkin-Elmer, Norwalk, CT, USA).

The specific growth rate ( $\text{day}^{-1}$ ) was calculated using the following equation:  $\mu = \ln(B_m/B_0)/\Delta t$ , where  $B_0$  and  $B_m$  are the initial and maximum in vitro Chl concentrations, respectively.  $\Delta t$  is the time interval between the beginning of the experiment and the time when Chl reached a maximum.

### 2.3 Eutrophication reduction index

Incubations were employed to evaluate the reduction in eutrophication by physical effects (such as mixing, dilution and sinking) and grazing. The ERI was calculated using the following equation:

$$\text{ERI} = (\text{PMB} - \text{AB})/\text{PMB} \times 100\%$$

where PMB is the potential maximum biomass (PMB) (expressed by Chl) that would be produced when all of the potentially limiting nutrient is utilized. AB represents the actual phytoplankton biomass (expressed by Chl) in the surface layer (the actual eutrophication) at the beginning of the incubation.

### 2.4 Stratification index (SI)

The SI was calculated according to Cloern (1991):

$$\text{SI} = \delta\sigma_t/h$$

where SI ( $\text{kg m}^{-4}$ ) is the stratification index,  $\delta\sigma_t$  ( $\text{kg m}^{-3}$ ) the difference between surface and bottom densities, and  $h$  is the depth (m) of the water column. Seawater density ( $\sigma_t$ ) was calculated based on Fofonoff and Millard (1983). In this study, the mixed layer depth was defined as a depth where  $\Delta\sigma_t$  is  $>0.2$  unit  $\text{m}^{-1}$  (Therriault and Levasseur 1985).

### 2.5 Yield of chlorophyll from DIN and $\text{PO}_4$ uptake and C:Chl in the incubations

The calculation of the yield of chlorophyll from dissolved inorganic nitrogen (DIN) or  $\text{PO}_4$  taken up during the incubations was as follows:

$$\text{Chl:DIN} = \Delta\text{Chl}/\Delta\text{DIN}, \quad \text{or} \quad \text{Chl:P} = \Delta\text{Chl}/\Delta\text{P},$$

where  $\Delta\text{Chl}$ ,  $\Delta\text{DIN}$ , and  $\Delta\text{P}$  represent the increase ( $\mu\text{g L}^{-1}$ ) in chlorophyll from the initial to maximum Chl and the amount ( $\mu\text{M}$ ) of DIN or  $\text{PO}_4$  taken up by phytoplankton respectively, during the incubations.  $\text{DIN} = \text{NH}_4 + \text{NO}_2 + \text{NO}_3$ . The estimation of phytoplankton C:Chl ratios was based on the linear regression of PC versus Chl for samples during the incubations.

### 2.6 Statistical analysis

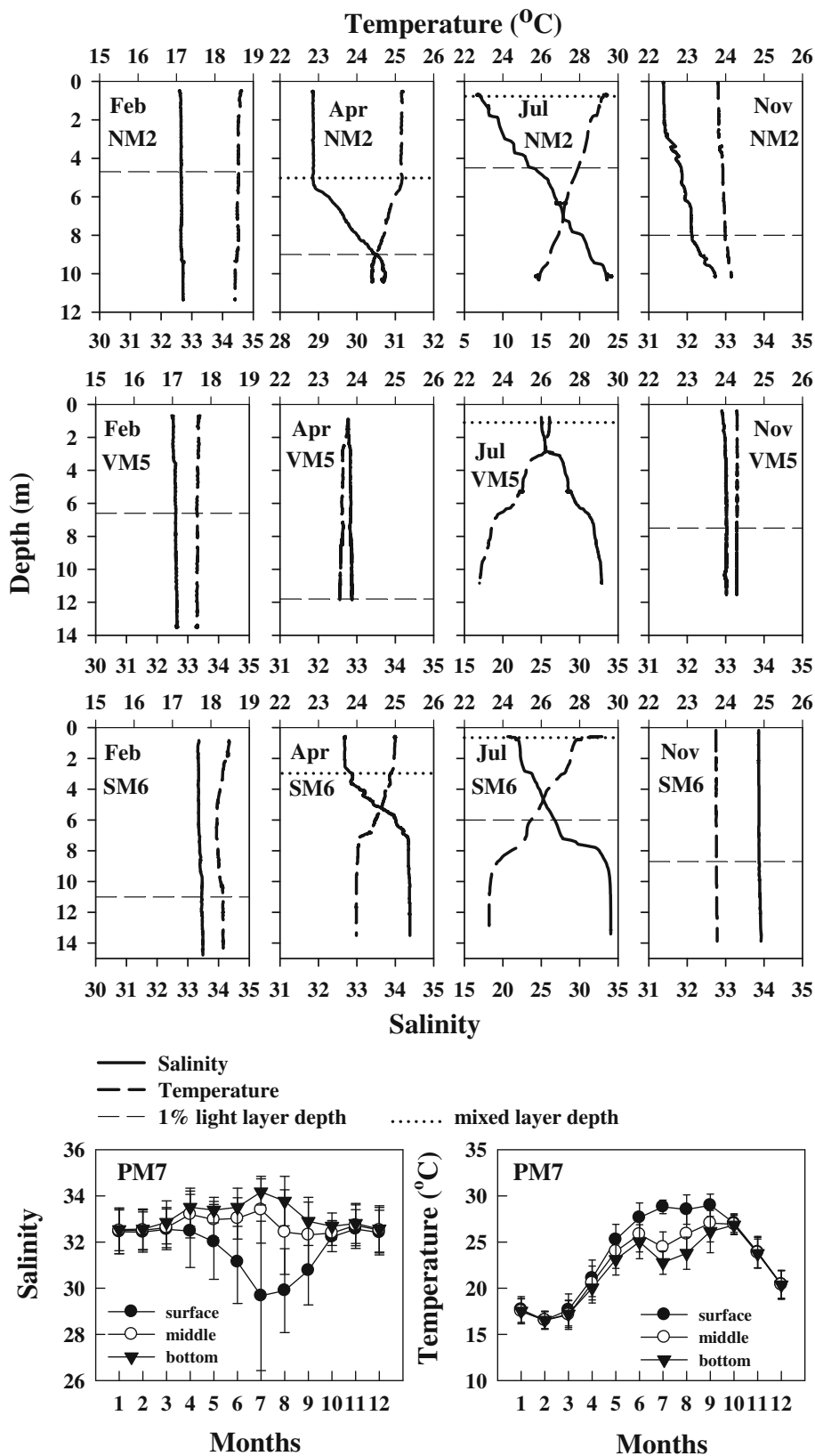
Statistical analyses were performed using Statistical Product and Service Solution (SPSS 11.0; SPSS, Chicago, IL, USA) software. An ANOVA with a least significant difference (LSD) multiple comparison technique was conducted to determine any significant difference between treatments ( $p = 0.05$ ) within each bioassay experiment. The error bars for the bioassay represent a pooled sample standard deviation of the mean. A *t* test analysis was conducted to determine any significant difference in Chl:N and Chl:P ratios between the N-limited and the P-limited cases ( $p < 0.05$ ).

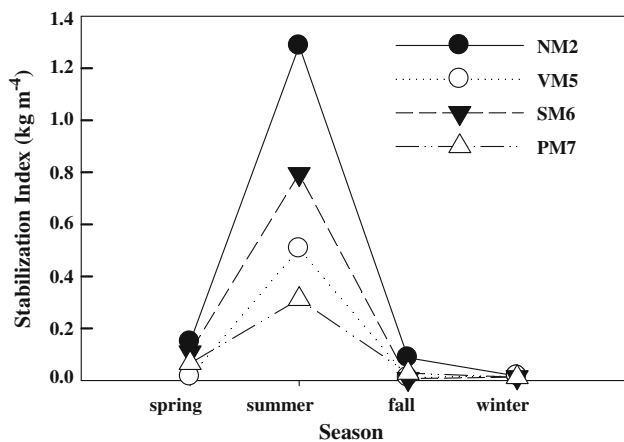
## 3 Results

### 3.1 Water column stratification

Surface temperature varied seasonally and was high (26.4–29.4 °C) in summer and low (16.5–18.7 °C) in winter (Fig. 2). The SI exhibited clear seasonal variability and was the highest (0.31–1.3  $\text{kg m}^{-4}$ ) in summer and the lowest (0.01–0.02  $\text{kg m}^{-4}$ ) in winter (Fig. 3). The water column was homogeneously mixed at all stations in the dry season. In summer, the SI varied spatially and was the highest (1.3  $\text{kg m}^{-4}$ ) in western waters that were strongly

**Fig. 2** Top three panels vertical profiles of salinity and temperature at three stations (NM2, VM5, SM6) in February, April, July and November 2006, respectively. The horizontal dashed line represents the 1% light depth and the dotted line is the mixed layer depth. Bottom panel monthly average salinity and temperature at the surface, middle, and bottom at PM7 from 1986 to 2005 (data from EPD, Hong Kong); vertical bars indicate  $\pm 1SD$  and  $n = 20$ . Note the mixed layer depth extended to the bottom at NM2 and SM6 in February and November 2006 and at VM5 in February, April, and November (from Xu et al. 2009)





**Fig. 3** Changes in the stabilization index in the water column at NM2, VM5, SM6, and PM7 in four seasons

influenced by the Pearl River discharge and the lowest ( $0.3 \text{ kg m}^{-4}$ ) in eastern waters that were not influenced by the Pearl River discharge (Fig. 3).

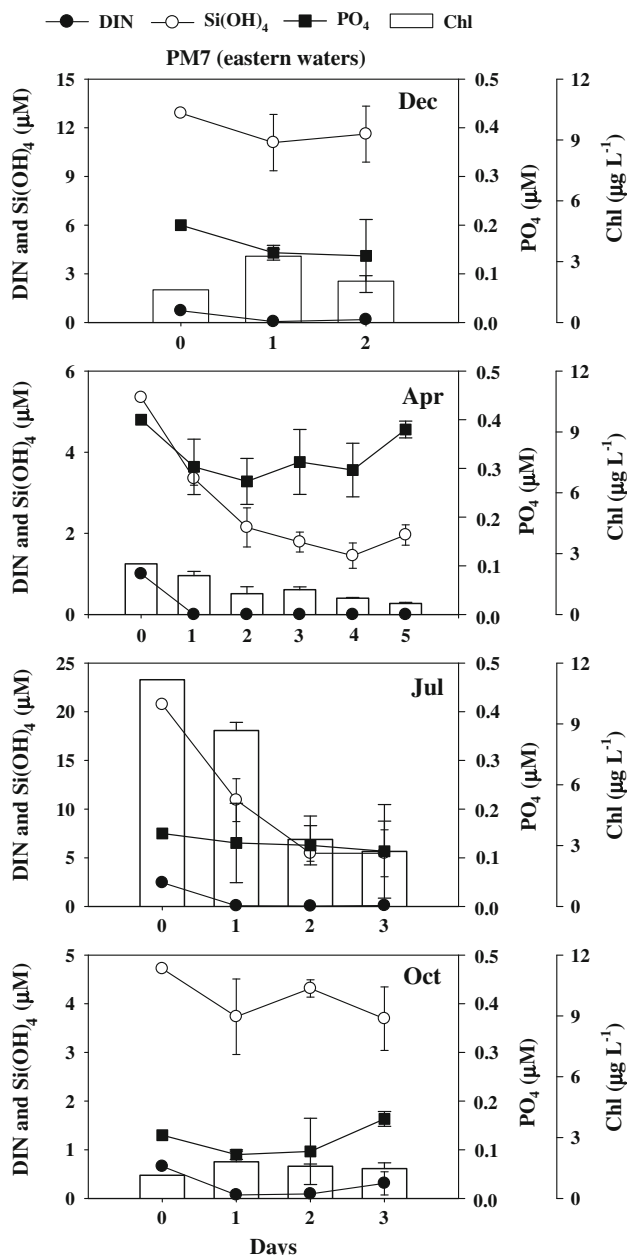
### 3.2 Dynamics of nutrients and phytoplankton biomass during incubations

At PM7 in eastern waters, DIN concentrations were always  $<2 \mu\text{M}$  during the whole study period except for June 2005. DIN:PO<sub>4</sub> ratios were  $<16:1$  and DIN:Si(OH)<sub>4</sub> ratios were  $<1:1$  all year. In contrast, Si(OH)<sub>4</sub>:PO<sub>4</sub> ratios were  $>16:1$  most of the year except for April (Xu et al. 2009).

During the incubation at PM7, DIN was depleted on day 1, while PO<sub>4</sub> and Si(OH)<sub>4</sub> remained at the end of the incubations in all seasons (Fig. 4), indicative of potential N limitation. The maximum algal biomass was achieved on day 1 and was relatively low ( $1.8\text{--}3.3 \mu\text{g L}^{-1}$ ) in October and December 2005, while the maximum algal biomass occurred at the start of the incubation in April and July 2006, indicating that N was already limiting on day 0 (Fig. 4). The uptake ratios of DIN:PO<sub>4</sub> were low (12–16) in the potential N-limited cases in October and December 2005 (Table 1).

At NM2 in the estuarine-influenced waters, high DIN and Si(OH)<sub>4</sub> concentrations [up to  $115 \mu\text{M}$  DIN and  $108 \mu\text{M}$  Si(OH)<sub>4</sub>] were observed in July, accompanied by high DIN:PO<sub>4</sub> (30–200:1) and Si(OH)<sub>4</sub>:PO<sub>4</sub> ratios (30–116:1). In February, nutrient concentrations [ $22 \mu\text{M}$  DIN and  $16 \mu\text{M}$  Si(OH)<sub>4</sub>] and nutrient ratios [DIN:PO<sub>4</sub>  $<10:1$  and Si(OH)<sub>4</sub>:PO<sub>4</sub>  $<10:1$ ] were lower relative to other seasons, and Chl was relatively low ( $<5 \mu\text{g L}^{-1}$ ) all year (Xu et al. 2009).

For incubations at NM2, phosphate always disappeared before nitrogen and silicate during April–November, indicating potential phosphorus (P) limitation, while nitrogen (N) was depleted before P and silicon (Si) in February,



**Fig. 4** Changes in nutrient concentrations (DIN, PO<sub>4</sub>, Si(OH)<sub>4</sub>) and Chl (bars) in the incubations at PM7 in October and December 2005, and April and July 2006. Note the different Y-axis scales. DIN = NO<sub>3</sub> + NH<sub>4</sub> + NO<sub>2</sub>. Error bars  $\pm 1\text{SD}$ ;  $n = 3$

suggesting potential N limitation (Fig. 5). The uptake ratios of DIN:PO<sub>4</sub> were low (12:1) in the N-limited cases in winter and high (26:1–105:1) in the P-limited cases in other seasons (Table 1). Nutrients were under-utilized and Chl concentrations increased dramatically ( $>15$ -fold) after a few days of incubation.

At VM5 in Victoria Harbour, DIN:PO<sub>4</sub> and Si(OH)<sub>4</sub>:PO<sub>4</sub> ratios were  $<16:1$  most of the year, except in July when relatively high DIN and SiO<sub>4</sub> concentrations [ $35 \mu\text{M}$  DIN and  $28 \mu\text{M}$  Si(OH)<sub>4</sub>] and DIN:PO<sub>4</sub> and

**Table 1** Uptake ratios and the amounts of NO<sub>3</sub>, NH<sub>4</sub>, NO<sub>2</sub>, PO<sub>4</sub>, and Si(OH)<sub>4</sub> taken up and the mean net specific growth rate (GR) during the growth period from the initial until the maximum Chl biomass was achieved in the incubations

Stations	Seasons	$\Delta t$ (days)	GR (day <sup>-1</sup> )	Amount of nutrient taken up ( $\mu\text{M}$ )			Molar uptake ratio		
				DIN	PO <sub>4</sub>	Si(OH) <sub>4</sub>	DIN/PO <sub>4</sub>	DIN/Si(OH) <sub>4</sub>	Si(OH) <sub>4</sub> /PO <sub>4</sub>
PM7	Winter	1	0.70 (0.10)	0.67 (0.06)	0.06 (0.02)	2.68 (1.3)	12.4 (3.3)	0.27 (0.12)	40 (15)
	Fall	1	0.43 (0.28)	0.59 (0.08)	0.04 (0.01)	0.99 (0.78)	15.4 (5.1)	0.84 (0.51)	23 (13)
NM2	Winter	7	0.58 (0.05)	21.7 (0.21)	1.80 (0.14)	14.8 (0.49)	12.0 (1.4)	1.45 (0.07)	8.00 (1.4)
	Spring	3	0.75 (0.01)	17.9 (6.23)	0.17 (0.01)	20.7 (0.25)	105 (39)	0.83 (0.29)	122 (6.1)
	Summer	3	1.44 (0.01)	60.3 (2.48)	1.38 (0.06)	46.3 (3.60)	45.0 (1.0)	1.30 (0.00)	34.3 (1.5)
	Fall	4	0.80 (0.01)	27.7 (1.65)	1.10 (0.10)	24.8 (10.6)	25.7 (1.2)	1.30 (0.67)	22.3 (8.1)
VM5	Winter	6	0.64 (0.04)	10.2 (0.00)	0.70 (0.00)	11.1 (1.27)	16.0 (0.0)	0.95 (0.07)	17.0 (1.4)
	Spring	3	1.26 (0.01)	17.8 (2.00)	1.30 (0.10)	9.67 (0.06)	14.0 (1.0)	1.83 (0.06)	7.70 (0.6)
	Summer	2	0.98 (0.03)	30.9 (0.88)	0.80 (0.00)	23.0 (3.44)	40.3 (0.0)	1.37 (0.29)	30.0 (4.4)
	Fall	5	0.63 (0.01)	23.9 (1.88)	1.92 (0.23)	22.3 (0.48)	15.3 (2.0)	1.30 (0.03)	11.8 (1.6)
SM6	Winter	6	0.53 (0.14)	10.7 (2.62)	0.70 (0.00)	9.45 (1.91)	15.5 (0.7)	1.15 (0.21)	13.5 (2.1)
	Spring	2	0.92 (0.04)	3.35 (1.39)	0.05 (0.06)	6.67 (0.95)	74.0 (58)	0.47 (0.15)	126 (91)
	Fall	4	0.45 (0.03)	8.43 (0.01)	0.52 (0.01)	12.7 (1.89)	16.2 (0.3)	0.70 (0.10)	24.7 (4.0)

$\Delta t$  is the number of days to achieve the maximum Chl concentration in the incubations. Numbers in parentheses represent  $\pm 1\text{SD}$ ;  $n = 3$ . DIN = NO<sub>3</sub> + NO<sub>2</sub> + NH<sub>4</sub>

Si(OH)<sub>4</sub>:PO<sub>4</sub> (~37:1) and high Chl (13  $\mu\text{g L}^{-1}$ ) were observed (Xu et al. 2009).

For incubations at VM5 in February, DIN disappeared before PO<sub>4</sub> and Si(OH)<sub>4</sub> (Fig. 6). In April, DIN and Si(OH)<sub>4</sub> were completely depleted by day 3 when Chl increased by 50  $\mu\text{g L}^{-1}$ , while some PO<sub>4</sub> remained ~0.1  $\mu\text{M}$ , indicative of modest P sufficiency relative to N and Si (Fig. 6). In July, PO<sub>4</sub> disappeared before N and Si (Fig. 6). The high Chl of 90  $\mu\text{g L}^{-1}$  was obtained on day 2. In November, all nutrients disappeared almost simultaneously on day 5 (Fig. 6). The uptake ratios of DIN:PO<sub>4</sub> were ~16:1 in the N-limited cases during February and April and in the Si-limited case in November, and 40:1 in the P-limited case in July (Table 1).

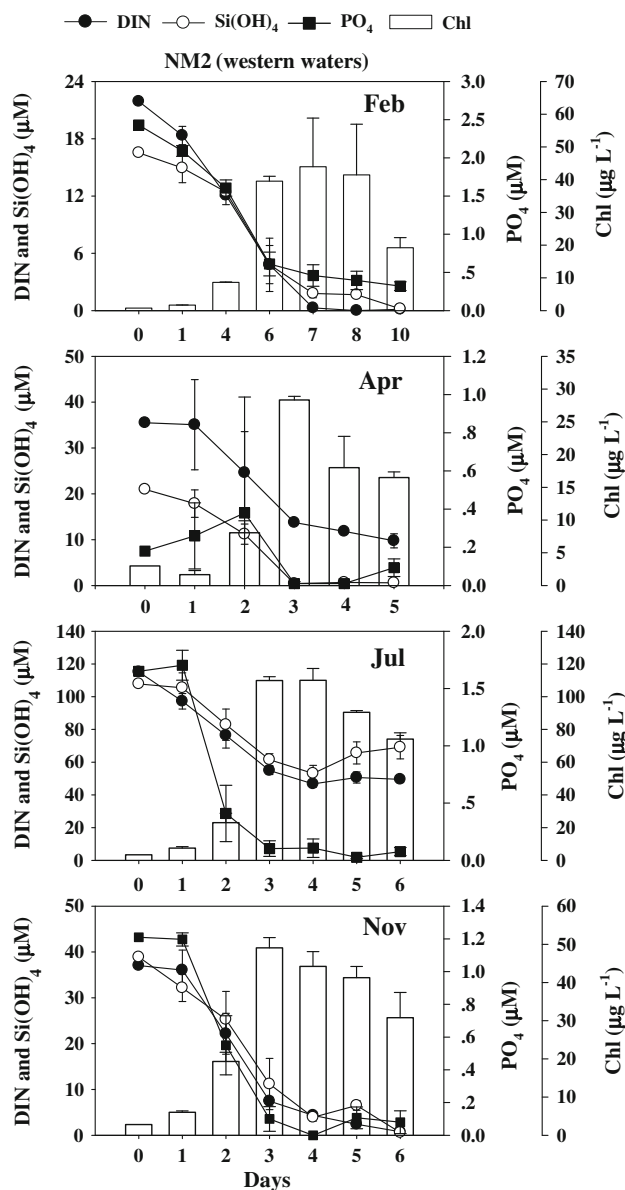
At SM6 in southern waters in February and November, nutrient concentrations were relatively low (DIN <12.3  $\mu\text{M}$  and PO<sub>4</sub> ~0.8  $\mu\text{M}$ ), along with low DIN:PO<sub>4</sub> ratios (<16:1) and DIN:Si(OH)<sub>4</sub> ratios (<1:1). In April, DIN and PO<sub>4</sub> were ~8 and 0.26  $\mu\text{M}$ , respectively, and DIN:PO<sub>4</sub> and Si(OH)<sub>4</sub>:PO<sub>4</sub> ratios were >30:1. In July, DIN and Si(OH)<sub>4</sub> concentrations were >30  $\mu\text{M}$ , while PO<sub>4</sub> was 0.12  $\mu\text{M}$  and DIN:PO<sub>4</sub> and Si(OH)<sub>4</sub>:PO<sub>4</sub> ratios were ~300:1. Chl concentration reached 73  $\mu\text{g L}^{-1}$  in July, a sharp contrast to <5  $\mu\text{g L}^{-1}$  in other seasons (Xu et al. 2009).

For incubations at SM6 in February, all nutrients disappeared at the end of the 8-day incubation (Fig. 7). DIN and Si(OH)<sub>4</sub> disappeared before PO<sub>4</sub> in February and April, and DIN and PO<sub>4</sub> before Si(OH)<sub>4</sub> in November, indicating that nitrogen was the potential limiting nutrient during this period. PO<sub>4</sub> was gone before N and Si in July, suggesting

potential P limitation. The pattern of the uptake ratios of DIN:PO<sub>4</sub> was the same as for NM2 and VM5, with a low ratio (~16:1) in the N-limited cases during fall and winter and high (74:1) in the P-limited case during spring (Table 1). In the field, nutrients were not fully utilized, and, therefore, in the incubations, Chl concentration increased markedly (up to 20-fold) most of the year. However, at SM6, in July, an algal bloom (73  $\mu\text{g L}^{-1}$ ) occurred along with actual P depletion.

### 3.3 Maximum net growth rates of phytoplankton communities

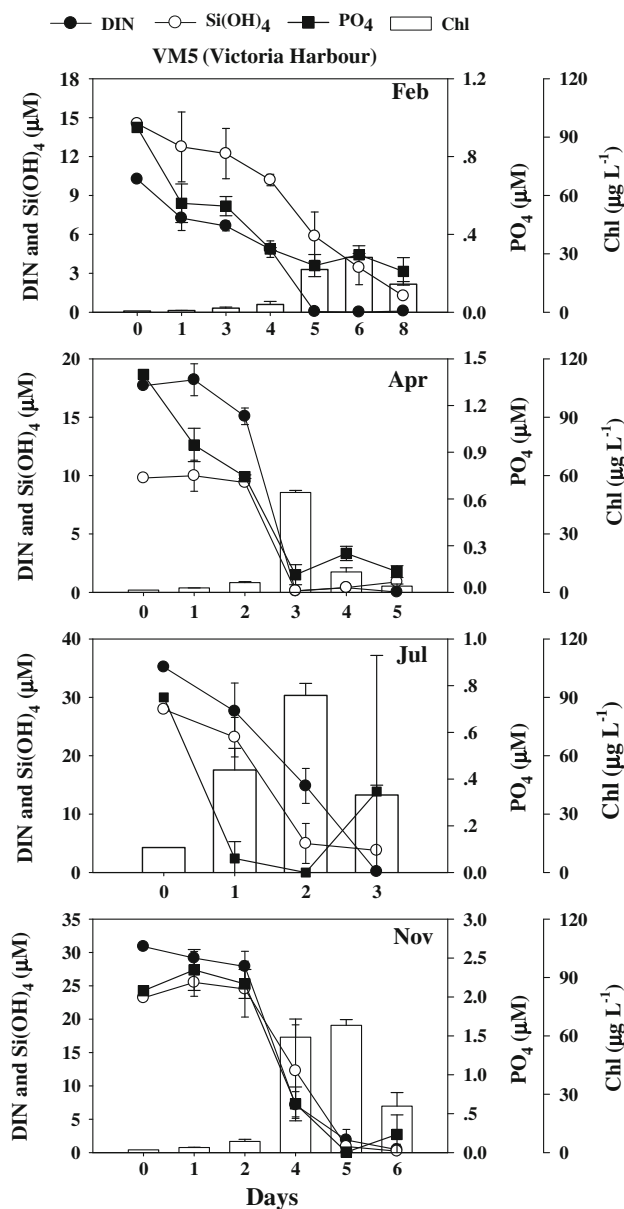
In the eastern waters (PM7), the maximum net specific growth rate was <1 day<sup>-1</sup> in October and December 2005. In contrast, in April and July 2006, there was no growth during the incubation due to actual nutrient limitation occurring already on day 0 (Fig. 8). At NM2, the maximum net growth rate exhibited clear seasonal variability, with the highest rate (2.4 day<sup>-1</sup> or 3.4 doublings day<sup>-1</sup>) in July and the lowest (0.87 day<sup>-1</sup>) in February (Fig. 8). At VM5, there was no seasonality in the maximum net growth rate. The maximum net growth rate was the highest (2.3 day<sup>-1</sup>) in April and the lowest (1.2 day<sup>-1</sup>) in November (Fig. 8). In contrast, the maximum net growth rate was relatively low (1.4 day<sup>-1</sup>) in July, due to a relatively low PO<sub>4</sub> concentration, compared to April (Fig. 8). At SM6, in February, the maximum net growth rate (0.87 day<sup>-1</sup>) was comparable to NM2. The maximum growth rate was 1.1 and 1.5 day<sup>-1</sup> in April and November, respectively. By comparison, in July, the maximum net growth rate was the lowest due to P limitation (Fig. 8).



**Fig. 5** Changes in nutrient concentrations [DIN,  $\text{PO}_4$ ,  $\text{Si(OH)}_4$ ] and Chl (bars) in the incubations at NM2 in February, April, July, and November 2006. Note the different Y-axis scales. DIN =  $\text{NO}_3 + \text{NH}_4 + \text{NO}_2$ . Error bars  $\pm 1\text{SD}$ ;  $n = 3$

### 3.4 Estimation of Chl:DIN, Chl:P and C:Chl during the incubations

The yield of chlorophyll from DIN taken up varied from 1.1–3.3 g Chl (mol N)<sup>-1</sup> in all cases (Table 2). The maximal Chl:DIN ratios always occurred in winter when N was the potential limiting nutrient except for VM5 where the ratio was maximal [2.83 g Chl (mol N)<sup>-1</sup>] in April. There was no significant ( $p > 0.05$ ,  $t$  test) difference in Chl:DIN ratios between the N-limited and P-limited cases. In contrast, the Chl:P ratios varied over a wide range [22–150 g Chl (mol P)<sup>-1</sup>] (Table 2). The maximal Chl:P

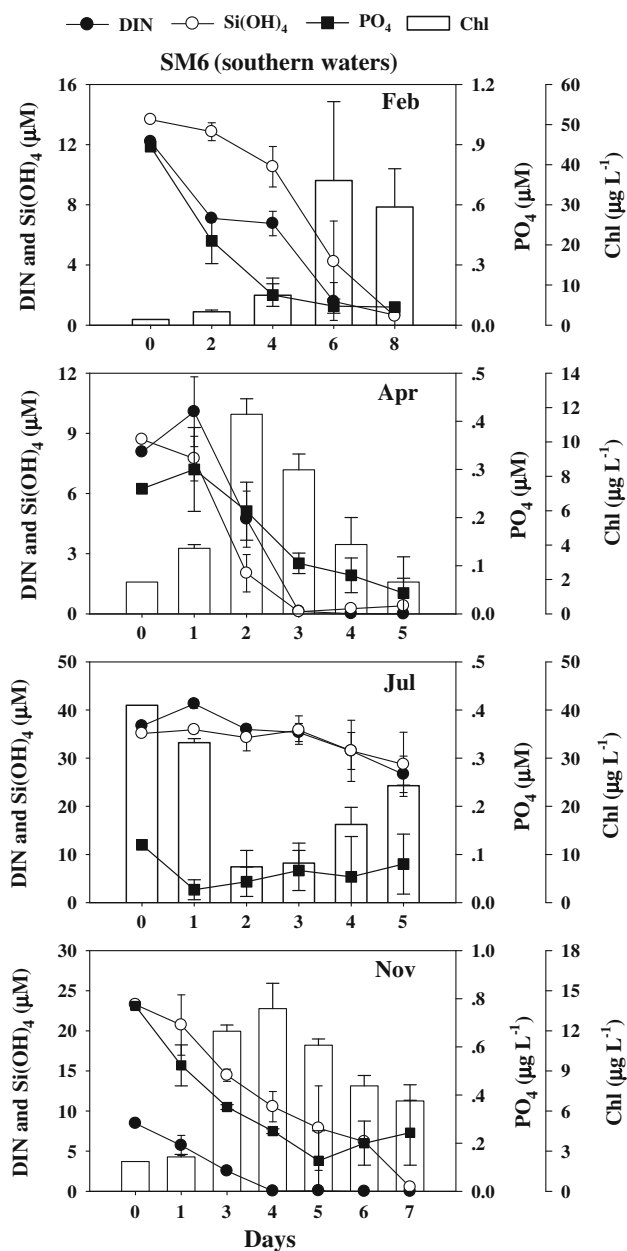


**Fig. 6** Changes in nutrient concentrations [DIN,  $\text{PO}_4$ ,  $\text{Si(OH)}_4$ ] and Chl (bars) in the incubations at VM5 in February, April, July, and November 2006. Note the different Y-axis scales. DIN =  $\text{NO}_3 + \text{NH}_4 + \text{NO}_2$ . Error bars  $\pm 1\text{SD}$ ;  $n = 3$

ratios occurred in the wet season when P was the potential limiting nutrient. The Chl:P ratios exhibited a significantly different pattern for the N-limited cases compared to the P-limited cases. The Chl:P ratios of 22–48 g Chl (mol P)<sup>-1</sup> for the N-limited cases were significantly ( $p < 0.05$ ,  $t$  test) lower than the values of 42–150 g Chl (mol P)<sup>-1</sup> for the P-limited cases. The Chl:DIN ratios varied by threefold, while Chl:P ratios varied by sevenfold.

The C:Chl ratios varied from 32 to 87 g g<sup>-1</sup> for the whole study area during four seasons (Fig. 9; Table 2). Overall, the C:Chl ratios were the lowest (32–50 g g<sup>-1</sup>) in summer (July) and the highest (61–87 g g<sup>-1</sup>) in winter (February). The





**Fig. 7** Changes in nutrient concentrations [DIN, PO<sub>4</sub>, Si(OH)<sub>4</sub>] and Chl (bars) in the incubations at SM6 in February, April, July, and November 2006. Note the different Y-axis scales. DIN = NO<sub>3</sub> + NH<sub>4</sub> + NO<sub>2</sub>. Error bars ±1SD; n = 3

C:Chl ratios (50–87 g g<sup>-1</sup>) in southern waters were higher than those (32–61 g g<sup>-1</sup>) in Victoria Harbour and western waters (Table 2).

## 4 Discussion

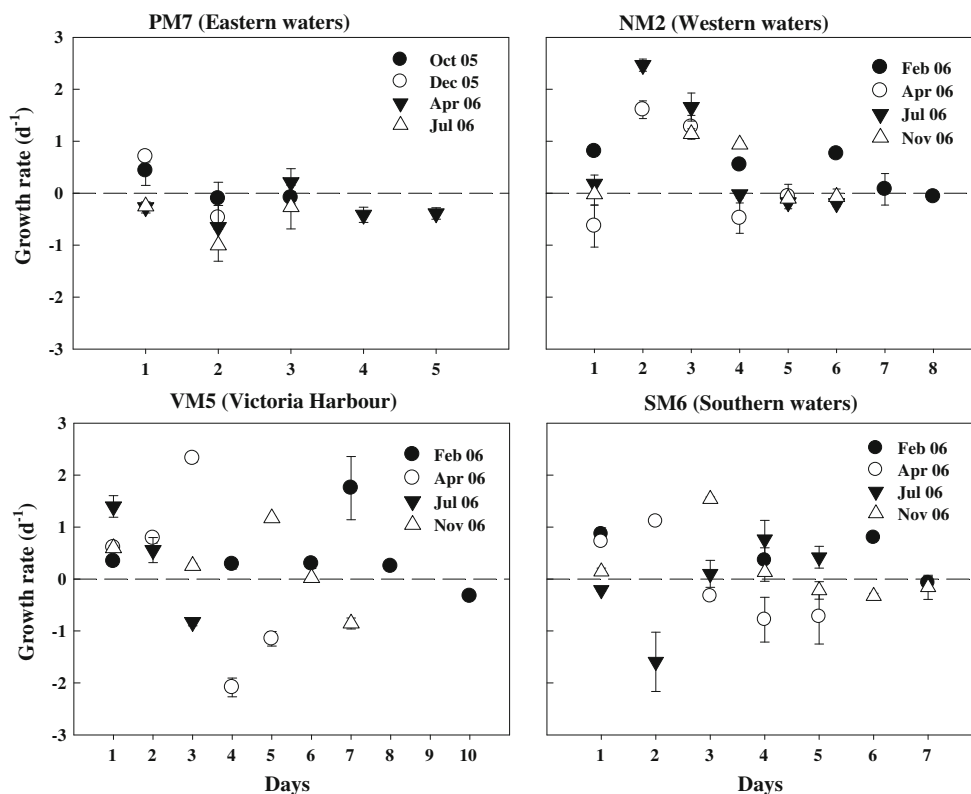
### 4.1 Actual versus potential eutrophication impacts

An important step in the assessment of the ecological status of a water body is the selection of a reference station

(Andersen et al. 2006). Eastern waters (e.g., PM7) are relatively far away from the Pearl River discharge and little influenced by the sewage effluent and, hence, they exhibit more coastal/oceanic characteristics such as low nutrients and relatively high surface salinity (generally >29) during most of the year (Lee et al. 2006). In eastern waters, nutrient concentrations were low and nitrogen was the potential primary limiting nutrient, and below the half-saturation constant for growth (DIN = 2 μM; Fisher et al. 1988) during the whole study period except for summer. Hence the net phytoplankton growth rate was relatively low (<1 day<sup>-1</sup>) due to nitrogen limitation, and PMB was much lower than in other areas (Table 3). Bioassays indicated that the PMB was normally <5 μg L<sup>-1</sup>, except for summer when the PMB was <12 μg Chl L<sup>-1</sup> due to the input of nutrients from rainfall and some runoff. In eastern waters, the flushing rates range from 0.02 day<sup>-1</sup> in the dry season to 0.05 day<sup>-1</sup> in the wet season (J. Lee, unpublished data), which had little effect on the accumulation of algal biomass. The primary regulator for phytoplankton growth varied seasonally as indicated by the seasonal differences in the ERI value (Table 3). In the wet season, thermohaline stratification restricts nutrient replenishment from the deep water. As a result, phytoplankton growth was N or N and P co-limited (Xu et al. 2009). In comparison, in the dry season, the northeast monsoon winds induced strong vertical mixing, as indicated by a low SI (0.02 kg m<sup>-4</sup>) and this mixing resulted in light limitation. Consequently, the primary regulator for phytoplankton growth shifted from nutrients in the wet season to physical processes and light limitation due to vertical mixing in the dry season, as indicated by the ERI value of 37 % in fall and 51 % in winter. Therefore, the eastern waters represent an appropriate baseline for algal biomass and nutrient concentrations in Hong Kong waters with little anthropogenic nutrient input.

In contrast, the other three sites (NM2, VM5, and SM6) were clearly nutrient enriched due to anthropogenic nutrient input. Western (NM2) and southern (SM6) waters are mainly influenced by the Pearl River discharge in the wet season, which contributes high DIN (~115 μM) and Si(OH)<sub>4</sub> (>100 μM) concentrations (Xu et al. 2008a). Victoria Harbour is subjected to the impact of the Pearl River discharge in summer plus year-round sewage effluent input that delivers high NH<sub>4</sub> and PO<sub>4</sub> concentrations (Xu et al. 2008a). The potential maximum phytoplankton biomass was extremely high (Table 3) when all of the ambient nutrient was converted to algal biomass in these waters, even in the dry season with little influence of the Pearl River discharge. In subtropical Hong Kong waters, the temperature fluctuates from 17 °C in winter to 27 °C in summer, which is partially responsible for the high growth rates in all seasons in our bioassays (Table 3). In particular, Chl exploded from 13 to 90 μg L<sup>-1</sup> in 2 days in Victoria

**Fig. 8** Changes in daily net specific growth rate at PM7 in October and December 2005, April and July 2006, and at NM2, VM5, and SM6 in February, April, July, and November 2006. Error bars  $\pm 1SD$ ;  $n = 3$ . Dashed line represents net growth rate equal to zero



**Table 2** *Chl:DIN*, *Chl:P* (means  $\pm SD$ ), and *C:Chl* ratios during the growth period from the initial until the maximum chlorophyll was achieved at NM2, VM5, and SM6 in February, April, July, and November 2006 in the incubations

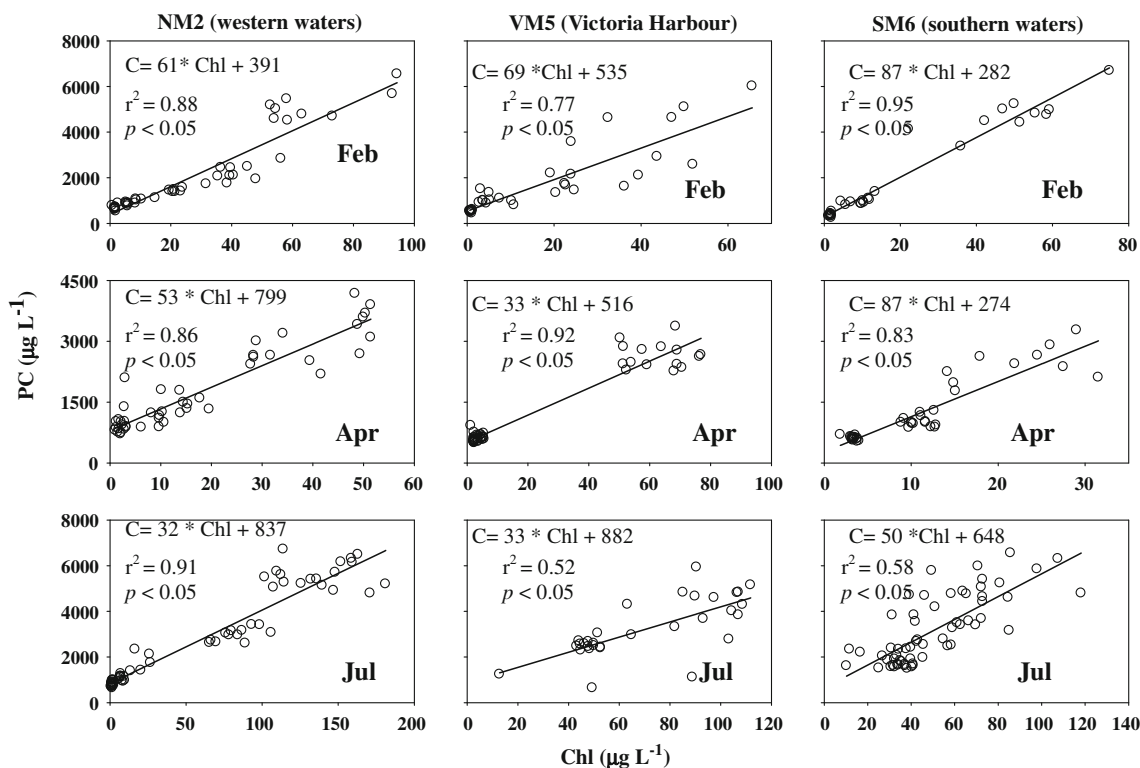
Stations	Seasons	<i>Chl:DIN</i> [ $\text{g} (\text{mol})^{-1}$ ]	<i>Chl:P</i> [ $\text{g} (\text{mol})^{-1}$ ]	<i>C:Chl</i> ( $\text{g g}^{-1}$ )	Potential limiting nutrient
PM7	October	$1.10 \pm 0.71$	$16.2 \pm 7.6$	NA	N
	December	$2.46 \pm 0.32$	$30.1 \pm 5.8$	NA	N
NM2	February	$2.07 \pm 0.61$	$24.1 \pm 5.1$	61	N
	April	$1.18 \pm 0.02$	$150 \pm 12$	53	P
	July	$1.80 \pm 0.03$	$80.6 \pm 2.6$	32	P
	November	$1.84 \pm 0.10$	$42.4 \pm 0.7$	NA	P
VM5	February	$2.70 \pm 0.58$	$39.3 \pm 11.5$	61	N
	April	$2.83 \pm 0.06$	$39.1 \pm 1.9$	32	N
	July	$2.53 \pm 0.22$	$104 \pm 8.2$	33	P
	November	$2.21 \pm 0.03$	$30.9 \pm 1.6$	NA	Si
SM6	February	$3.30 \pm 2.00$	$48.5 \pm 25.9$	87	N
	April	$1.35 \pm 0.13$	$60.1 \pm 7.8$	87	N and P
	July	NA	NA	50	
	November	$1.36 \pm 0.23$	$22.0 \pm 3.3$	NA	N

For details for the estimation of *C:Chl*, *Chl:DIN*, and *Chl:P* ratios, see “Materials and methods”. Amounts of DIN and P taken up are from Table 1. *Chl:DIN* and *Chl:P* ratios at PM7 in April and July 2006 and at SM6 in July 2006 were not included because the maximum chlorophyll occurred at the start of experiment. *C:Chl* ratios are from regressions of PC versus Chl in Fig. 10.  $\text{DIN} = \text{NO}_3 + \text{NO}_2 + \text{NH}_4$ . The determination of the potential limiting nutrient is from Xu et al. (2009)

NA not available

Harbour during summer (Fig. 6). In Hong Kong waters, fast-growing diatoms, such as *Skeletonema costatum*, *Thalassiosira* spp., and *Nitzschia* sp., dominated the

phytoplankton community all year except in April when the dinoflagellate *Scrippsiella* sp. was the dominant species in Victoria Harbour (Xu et al. 2009).



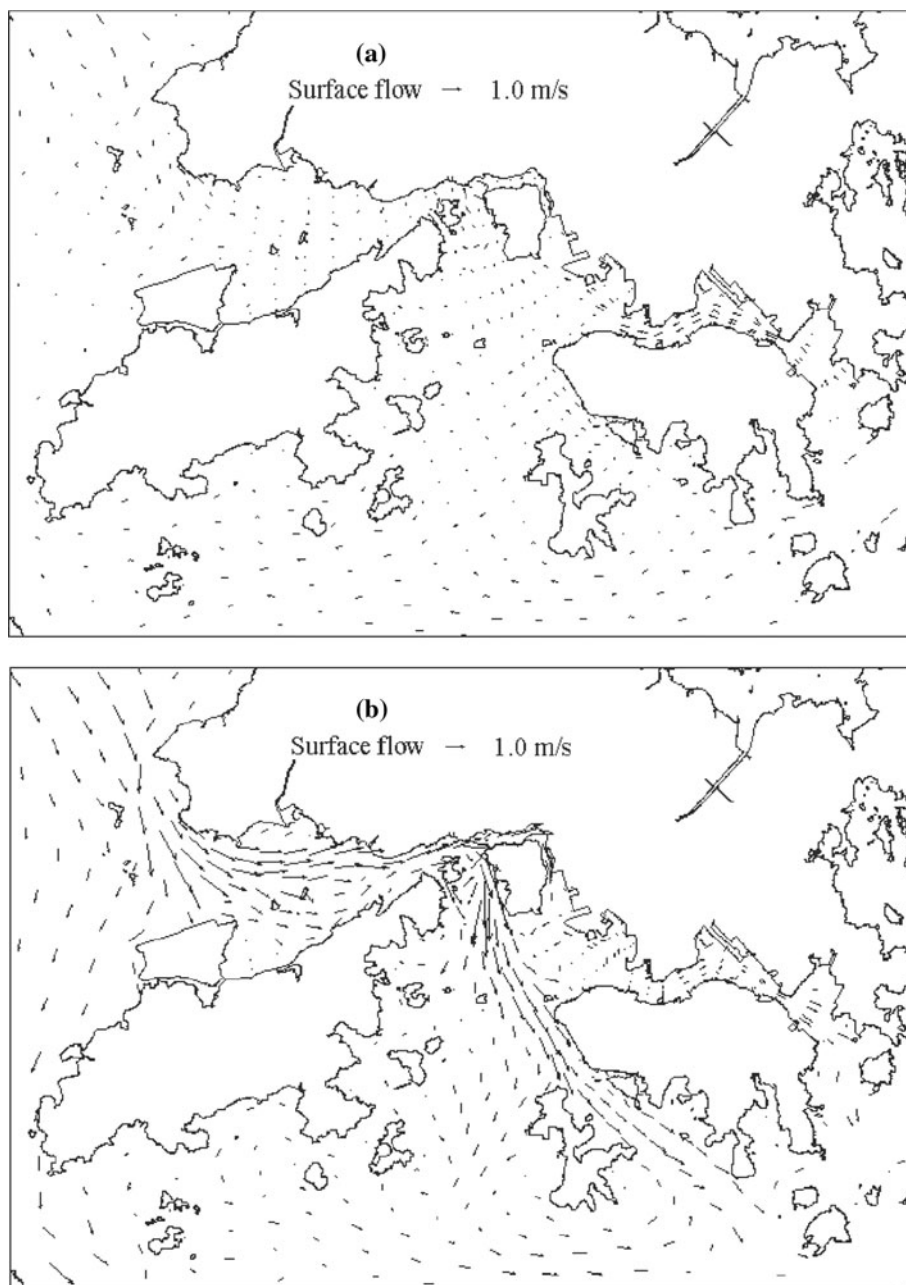
**Fig. 9** Correlation of surface Chl concentrations versus particulate carbon (PC) for three stations (NM2, VM5, and SM6) in three seasons (spring, summer, and winter)

**Table 3** Initial actual biomass (*AB*) and potential maximum biomass (*PMB* ±1SD), eutrophication reduction index (*ERI*) during the incubations

Stations	Seasons	AB (µg Chl L <sup>-1</sup> )	PMB (µg Chl L <sup>-1</sup> )	ERI (%)	Data from EPD (1986–2005)			
					Chl (µg L <sup>-1</sup> )	Mean (min–max) (µM)		
						DIN	PO <sub>4</sub>	Si(OH) <sub>4</sub>
PM7	Winter	1.61	3.27 ± 0.35	51	1.45 (0.20–4.70)	5.13 (0.71–19)	0.43 (0.06–2.52)	12.9 (1.50–65.0)
	Spring	2.50	2.50	0	2.14 (0.20–23.0)	3.10 (0.50–10.1)	0.32 (0.06–0.70)	6.34 (0.83–20.0)
	Summer	11.2	11.2	0	1.92 (0.20–9.60)	2.99 (0.5–14.1)	0.34 (0.06–3.55)	7.84 (0.83–36.7)
	Fall	1.14	1.81 ± 0.53	37	1.50 (0.20–5.60)	2.55 (0.50–10.8)	0.33 (0.06–1.20)	9.42 (1.00–20.0)
NM2	Winter	0.75	43 ± 15	98	3.18 (0.20–23)	17.5 (3.21–36.4)	1.75 (0.43–3.79)	14.2 (1.33–26.7)
	Spring	6.33	28 ± 0.55	77	3.58 (0.30–14)	33.8 (8.14–82.5)	1.91 (0.14–63.6)	26.3 (2.17–71.7)
	Summer	3.48	110 ± 7.2	97	3.95 (0.20–18)	54.0 (20–98)	2.24 (0.71–3.79)	71.4 (2.00–117)
	Fall	3.21	49 ± 2.7	93	2.61 (0.20–30.0)	25.4 (6.39–95.0)	1.94 (0.29–4.00)	30.1 (5.50–96.7)
VM5	Winter	0.63	28 ± 6.0	98	1.27 (0.20–8.20)	31.7 (17.9–68.4)	1.89 (0.55–4.19)	16.4 (2.00–26.7)
	Spring	1.16	51 ± 1.0	98	1.36 (0.20–10.0)	29.4 (10.9–51.6)	1.68 (0.52–2.65)	14.3 (2.00–36.7)
	Summer	12.8	91 ± 6.1	86	10.5 (0.20–43)	22.5 (2.57–56.2)	0.95 (0.06–3.10)	17.3 (0.83–61.7)
	Fall	1.46	65 ± 2.9	98	3.65 (0.20–36.0)	29.7 (0.86–70.6)	1.69 (0.06–3.58)	16.3 (0.83–28.3)
SM6	Winter	1.60	36 ± 20	96	1.77 (0.20–10.0)	8.30 (0.86–17.4)	0.56 (0.06–1.32)	13.8 (3.17–36.7)
	Spring	4.61	12 ± 0.9	62	2.75 (0.30–18)	11.2 (1.36–22.7)	0.57 (0.06–1.23)	12.3 (3.50–28.3)
	Summer	73.0	73.0	0	8.58 (0.50–33)	21.6 (0.93–49)	0.50 (0.06–1.61)	26.1 (1.46–67.3)
	Fall	3.92	14 ± 1.9	72	2.55 (0.50–13)	9.51 (1.07–31.4)	0.61 (0.16–1.10)	17.7 (9.00–38.3)

Mean (range in parentheses) surface values of Chl, DIN (=NO<sub>3</sub> + NO<sub>2</sub> + NH<sub>4</sub>), PO<sub>4</sub> and Si(OH)<sub>4</sub> during the last two decades (1986–2005) for four stations in Hong Kong waters are from Environmental Protection Department (EPD) of Hong Kong

**Fig. 10** General surface flow in the dry season (a) and wet season (b) in Hong Kong waters (from Lee et al. 2006)



Nevertheless, long-term (>20 years) water quality monitoring data provided by Environmental Protection Department of Hong Kong and our data showed that the actual biomass was always low ( $<5 \mu\text{g L}^{-1}$ ) in western waters most of the year, except in summer (Yin 2002; Xu et al. 2008a), comparable to that in eastern waters, indicating that phytoplankton did not respond to the very high nutrient inputs, and that eutrophication effects were not as severe as expected. The extremely high ERI (up to 98 %) suggested that physical processes (i.e. flushing and light availability) that were not included in the bioassays played a key role in regulating the eutrophication effects in Hong Kong waters, especially in the dry season.

An exceptional case was southern waters in summer. In summer, the Pearl River plume often reaches the southern waters and increases stratification (Yin 2002). Southern waters were nutrient enriched due to the input of high DIN and  $\text{Si(OH)}_4$  concentrations from the Pearl River discharge. However, the flushing is weaker in southern waters than western waters and Victoria Harbour (Fig. 10; Lee et al. 2006). Favorable physical factors and high nutrient concentrations trigger algal blooms. Actual phytoplankton biomass reached the PMB, implying that the ambient nutrients were often fully utilized by phytoplankton, and that the yield of the phytoplankton biomass was terminated by the depletion of  $\text{PO}_4$  in southern waters. During this

period, actual P limitation occurred (Xu et al. 2009). The extremely high actual phytoplankton biomass ( $73 \mu\text{g L}^{-1}$ ) indicated that eutrophication impacts were more severe in this region than other waters (e.g., western and Victoria Harbour). An increasing trend in Chl concentrations was observed in this region, possibly due to the relocation of sewage effluent discharge after HATS (Ho 2007; Xu et al. 2011). According to the Redfield equation ( $138\text{O}_2:106\text{C}:16\text{N}:1\text{P}$ , molar ratio; Redfield et al. 1963) for organic matter decomposition and a C:Chl ratio of  $50 \text{g g}^{-1}$  in southern waters (SM6) during summer (Fig. 9),  $5 \mu\text{mol O}_2$  is needed to decompose  $1 \mu\text{g Chl}$ . As a result, hypoxia or anoxia could occur if  $\text{O}_2$  could not be replenished by mixing processes. Therefore, the removal of P in the sewage effluent and the Pearl River discharge is critical in order to reduce further eutrophication impacts in southern waters. However, in other seasons, strong vertical mixing (low SI of  $0.01\text{--}0.13 \text{kg m}^{-4}$ ) was the primary regulator of eutrophication impacts, as indicated by the low ambient actual biomass ( $<5 \mu\text{g L}^{-1}$ ) and high ERI ( $62\text{--}96 \%$ ) (Table 3).

#### 4.2 Pearl River discharge, vertical mixing and eutrophication effects

Hong Kong waters are subjected to strong vertical mixing induced by winds and tides most of the year, especially in winter when SI was very low ( $0.02 \text{kg m}^{-4}$ ). Vertical mixing was a major constraint of phytoplankton growth in terms of light limitation and dilution since nutrients were not limiting most of the year in Hong Kong waters. A significant correlation between the SI and the ambient surface Chl concentrations for all samples except for western and southern waters in summer (Fig. 11), suggested that the ambient phytoplankton biomass was primarily dependent on the vertical mixing intensity most of the year, rather than nutrient concentrations. In the dry season, the mixed layers at four stations were deeper than the euphotic zone, which reduced the mean daily water column irradiance and consequently led to light limitation (Ho et al. 2010). On the other hand, strong vertical mixing restricted the accumulation of algal cells by evenly distributing cells over the entire water column. As a result, Hong Kong waters were resistant to nutrient enrichment during the dry season.

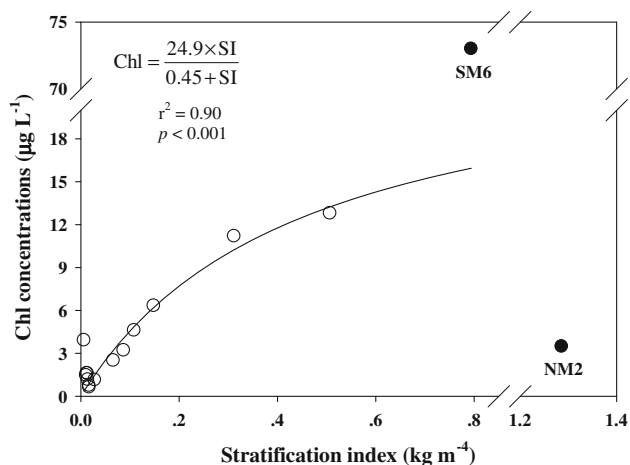
In contrast, the Pearl River discharge input would increase stratification and decrease vertical mixing in the water column and promote phytoplankton growth, especially in summer. This was the case for Victoria Harbour where an intrusion of the riverine freshwater results in stratification that helps to retain phytoplankton in the euphotic zone. The favorable physical factors (high temperature, light intensity, and stratification) and high

nutrients trigger algal blooms with relatively high actual algal biomass ( $13 \mu\text{g L}^{-1}$ ) (Xu et al. 2008a) and episodic large algal blooms ( $\sim 43 \mu\text{g L}^{-1}$ ) were recorded in summer (Table 3). On the other hand, the invasion of the Pearl River discharge enhanced the flushing rate (up to  $0.7 \text{day}^{-1}$ ) in Victoria Harbour (Lee et al. 2006), which accounted for up to 70 % of the growth rate observed in our bioassays. In summer, the in situ net growth rate was  $\sim 0.3 \text{day}^{-1}$  in Victoria Harbour if the flushing rate was taken into account. Hence, it takes a long time to develop an algal bloom in these waters. However, it is difficult to maintain stratification since tidal mixing destroys the stratification.

A good illustration of the importance of the Pearl River discharge in diluting phytoplankton biomass was found in western waters in summer (Fig. 10). Freshwater flushing and strong dilution were the primary factors regulating phytoplankton biomass, rather than vertical mixing despite high SI ( $1.3 \text{kg m}^{-4}$ ) during summer. The ERI was always very high ( $>77 \%$ ), with the highest values (98 %) in winter, suggesting that only a very small amount of nutrients was utilized and converted into biomass due to strong physical processes. High freshwater discharge from the Pearl River and narrowness of this region leads to a high flushing rate ( $0.5 \text{day}^{-1}$ ), high current speeds for the surface layer (Fig. 10; Lee et al. 2006), and a short water residence time, preventing algal biomass accumulation. Our results suggest that few nutrients are assimilated in western waters and, consequently, much of the surplus nutrients that are not utilized by the phytoplankton are transported to southern waters and Victoria Harbour and offshore, which enhanced algal biomass and altered nutrient composition in the river-impacted area in the northern South China Sea (Xu et al. 2008b).

#### 4.3 Meso- and macro-zooplankton grazing effects

In our bioassays, only large zooplankton ( $>200 \mu\text{m}$ ) such as meso- and macrozooplankton were removed and hence microzooplankton ( $<200 \mu\text{m}$ ) remained. Previous studies have shown that meso- and macrozooplankton grazing consumed 8 % of the primary production in the dry season and 20 % in the wet season in the Pearl River estuary around Hong Kong waters (Tan et al. 2004). However, a recent study revealed that mesozooplankton preferentially feed on microzooplankton rather than phytoplankton (Chen and Liu 2011). Hence, the removal of meso- and macrozooplankton in our incubations probably reduced phytoplankton production through a trophic cascade effect since microzooplankton are normally consumed by mesozooplankton. There is low microzooplankton biomass in the river-impacted western waters in June and July during the period of the maximum Pearl River discharge, and



**Fig. 11** Correlation of surface Chl concentrations versus stratification index for four stations (NM2, VM5, SM6, and PM7) in four seasons (spring, summer, fall, and winter) except for summer at NM2 and SM6 (solid circles). In summer at NM2, the high flushing rate regulates algal biomass, rather than the stratification index, and at SM6, a massive algal bloom occurred that was considered a special case

microzooplankton reduces the phytoplankton production by  $\sim 30\%$  (Chen et al. 2009). In February (winter), microzooplankton grazing makes a smaller contribution ( $<20\%$ ) to the reduction in algal production in Hong Kong waters due to lower microzooplankton metabolic rates, relative to the dilution and light limitation induced by strong vertical mixing (Chen et al. 2009), since maximum growth rates of the herbivorous protists decreases more sharply than those of phototrophic protists in response to decreasing temperature (Rose and Caron 2007). In contrast, in late spring (May), the microzooplankton grazing rate is comparable to algal growth rate (Chen et al. 2009), suggesting that grazing may be one of the factors responsible for the lack of algal blooms in late spring.

#### 4.4 Prediction of potential biomass from dissolved inorganic nutrients

The yield of chlorophyll derived from the uptake of dissolved inorganic nutrients has been shown to be a potentially useful parameter for predicting algal-based expressions of eutrophication (Edwards et al. 2005). The yield of chlorophyll from DIN [ $1.1\text{--}3.3\text{ g Chl (mol N)}^{-1}$ ] in Hong Kong waters (Table 3) was within the range [ $0.25\text{--}4.4\text{ g Chl (mol N)}^{-1}$ ] reported by Gowen et al. (1992) in Scottish coastal waters, UK. The lack of a difference in the Chl:N ratios in both N-limited and P-limited cases suggested that nitrogen status had a limited effect on the changes in the Chl:N ratios in Hong Kong waters.

In contrast, the ratio of the maximum to minimum Chl:P ratios and DIN:PO<sub>4</sub> uptake ratios varied by 7 times, and

depended on the degree of phosphorus limitation, which indicated that phosphorus status had a significant effect on the Chl:P ratios and DIN:PO<sub>4</sub> uptake ratios. This suggestion agrees with previous laboratory results showing that the variation in the N and P cell quota expressed as the ratio of the maximum to minimum amount of P per cell (3–70) was much higher than the maximum to minimum N per cell (1–6) under different nutrient status (Harrison et al. 1990). This is related to the fact that N is involved in the synthesis of chlorophyll and protein, while P is involved in RNA synthesis and less in chlorophyll synthesis. The variability of cellular N and P contents could be linked to co-variation of N with cellular protein contents and P with cellular RNA (Leonardos and Geider 2004). Intracellular stores of P might be sufficient for several days of growth, but intracellular N pools can be exhausted faster (Elrifi and Turpin 1985; Andersen et al. 1991).

A recent study has shown that phytoplankton reduce their cellular phosphorus requirements by substituting non-phosphorus ‘betaine’ lipids for phospholipids in P-limited cases (Van Mooy et al. 2009). These results indicate that a small amount of P can produce a large amount of Chl if N is replete, since there is a larger variation in the P cell quota compared to the N quota. Therefore, it is difficult to predict potential biomass from dissolved inorganic nutrients in P-limited cases since the amount of chlorophyll that is produced can vary by 20-fold due to the highly variable P cell quota.

The carbon to chlorophyll ratio is a useful tool to estimate phytoplankton carbon from the measured chlorophyll (Cho and Azam 1990). A linear regression of PC versus chlorophyll has been used to estimate the phytoplankton fraction of PC from the slope of the fit (Wienke and Cloern 1987; Townsend and Thomas 2002; Behrenfeld et al. 2005). Our estimated C:Chl ratios ( $32\text{--}87\text{ g g}^{-1}$ ) in this study agreed well with those ( $27\text{--}67\text{ g g}^{-1}$ ) estimated by using simple standard extraction procedures for natural populations in the eutrophic waters in Roskilde Fjord, Denmark (Riemann et al. 1989). The average C:Chl ratio of  $56\text{ g g}^{-1}$  was comparable to that in San Francisco Bay ( $51\text{ g g}^{-1}$ ), derived from the regression of PC versus Chl (Wienke and Cloern 1987). The seasonal variations in the C:Chl ratios might be related a shift in light availability and the limiting nutrient from potential light and N limitation in winter to P limitation in summer. The estimate of Chl:N and C:Chl ratios obtained in this study provide useful locally derived ratios for different areas and seasons for future ecosystem modeling of Hong Kong waters.

## 5 Summary

The eutrophication potential varied spatially and temporally in response to year-round sewage effluent, the

seasonal changes in the volume of the Pearl River discharge, and the intrusion of shelf/oceanic water. In summer, during the highest rainfall and the Pearl River discharge, different areas in HK waters responded differently to nutrient enrichment because phytoplankton growth was mainly controlled by hydrodynamic processes. In western waters, the eutrophication effects were dramatically restricted by high flushing effects with little increase in phytoplankton biomass in spite of the high nutrient inputs. Surplus nutrients that could not be utilized by phytoplankton in western waters were transported to the southern waters, Victoria Harbour, and offshore. In Victoria Harbour, although thermohaline stratification in summer favored the development of algal blooms to some extent, actual eutrophication effects were reduced by flushing and tidal currents. In southern waters that are frequently at the edge of the riverine plume during summer when discharge is high, stratification helped to trigger algal blooms that were ultimately terminated by the depletion of  $\text{PO}_4$ , and actual eutrophication effects were more severe than western waters and Victoria Harbour, due to the relatively persistent stable stratification. High net growth rates of  $>3$  doublings  $\text{day}^{-1}$  implies that very large summer algal blooms could occur quickly (a few days) and frequently in Hong Kong waters with stable conditions (e.g. southern waters).

The assessment of frequency, duration and magnitude of summer algal blooms in southern waters is needed to better document nutrient enrichment effects on the ecosystem. By comparison, in other seasons, eutrophication impacts were constrained by physical processes (e.g. flushing and mixing), especially in winter when strong vertically mixing was responsible for the lowest actual biomass and the highest ERI. Strong vertical mixing reduced phytoplankton growth rate due to light limitation, and the accumulation of algal biomass was constrained due to the dilution by oceanic waters. Hence hypoxia rarely occurs in Hong Kong waters because mixing transports oxygen to depth and much of the algal biomass is transported further offshore rather than sinking out.

The results from this research can assist in the development of coastal sewage management strategies. In summer, the removal of P should be the primary consideration in sewage treatment in order to reduce the accumulation of algal biomass and subsequent eutrophication impacts in the southern waters. Furthermore, the cost of P removal is much lower than N. In contrast, in winter, the Hong Kong coastal ecosystem is more resistant to eutrophication impacts due to strong hydrodynamic mixing, low temperatures, low light availability, and relatively lower ambient nutrients, and, hence, nutrient removal is less important in winter.

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## References

- Andersen T, Schartau AKL, Paasche E (1991) Quantifying external and internal nitrogen and phosphorus pools as well as nitrogen and phosphorus supplied through remineralization, in coastal marine plankton by means of a dilution technique. *Mar Ecol Prog Ser* 69:67–80
- Andersen JH, Schlüter L, Ærtebjerg G (2006) Coastal eutrophication: recent developments in definitions and implications for monitoring strategies. *J Plankton Res* 28:621–628
- Behrenfeld MJ, Boss E, Siegel DA, Shea DM (2005) Carbon based ocean productivity and phytoplankton physiology from space. *Glob Biogeochem Cycles* 19:GB1006. doi:10.1029/2004GB002299
- Chen MR, Liu H (2011) Experimental simulation of trophic interactions among omnivorous copepods, heterotrophic dinoflagellates and diatoms. *J Exp Mar Biol Ecol* 403:65–74
- Chen B, Liu H, Landry MR, Chen M, Sun J, Shek L, Chen X, Harrison PJ (2009) Estuarine nutrient loading affects phytoplankton growth and microzooplankton grazing at two contrasting sites in Hong Kong coastal waters. *Mar Ecol Prog Ser* 379:77–90
- Cho BC, Azam F (1990) Biogeochemical significance of bacterial biomass in the ocean's eutrophic zone. *Mar Ecol Prog Ser* 63:253–259
- Cloern JE (1991) Annual variations in river flow and primary production in the South San Francisco Bay Estuary. In: Elliot M, Ducrotoy JP (eds) *Estuaries and coasts: spatial and temporal intercomparisons*. ECSA 19 symposium, Olsen & Olsen, pp 91–96
- Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. *Mar Ecol Prog Ser* 210:223–253
- Edwards V, Icelly J, Newton A, Webster R (2005) The yield of chlorophyll from nitrogen: a comparison between the shallow Ria Formosa lagoon and the deep oceanic conditions at Sagres along the southern coast of Portugal. *Estuar Coast Shelf Sci* 62:391–403
- Elrifi IR, Turpin DH (1985) Steady-state luxury consumption and the concept of optimum nutrient ratios—a study with phosphate and nitrate limited *Selenastrum minutum* (*Chlorophyta*). *J Phycol* 21:592–602
- Fisher TR, Harding LW Jr, Stanley DW, Ward LG (1988) Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. *Estuar Coast Shelf Sci* 27: 61–93
- Fofonoff NP, Millard RC Jr (1983) Algorithms for computation of fundamental properties of seawater. UNESCO Technical Papers in Marine Science 44. UNESCO/SCPPOR/ICES/IAPSO Joint Panel on Oceanographic Tables and Standards and SCOR Working Group 51
- Gowen R, Tett P, Jones J (1992) Predicting marine eutrophication: the yield of chlorophyll from nitrogen in Scottish coastal waters. *Mar Ecol Prog Ser* 85:153–161
- Grasshoff KM, Ehrhardt M, Kremling K (1983) *Methods of seawater analysis*. Chemie, Weinheim
- Harrison PJ, Thompson PA, Calderwood GS (1990) Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *J Appl Phycol* 2:45–56

- Harrison PJ, Yin KD, Lee JHW, Gan JP, Liu HB (2008) Physical–biological coupling in the Pearl River Estuary. *Cont Shelf Res* 28:1405–1415
- Ho YT (2007) Dynamics of nutrients and phytoplankton biomass and production in Hong Kong waters. PhD thesis, The Hong Kong University of Science and Technology, Hong Kong
- Ho AYT, Xu J, Yin K, Jiang YL, Yuan XC, He L, Anderson DM, Lee JHW, Harrison PJ (2010) Phytoplankton biomass and production in subtropical Hong Kong waters: influence of the Pearl River outflow. *Estuar Coasts* 33:170–181
- Knap A, Michaels A, Close A, Ducklow H, Dickson A (1996) Protocols for the joint global ocean flux study (JGOFS) core measurements. JGOFS Report No 19, vi + 170. Reprint of the IOC Manuals and Guides No 29 UNESCO 1994
- Le Pape O, Del Amo Y, Ménesguen A, Aminot A, Queguiner B, Treguer P (1996) Resistance of a coastal ecosystem to increasing eutrophic conditions: the Bay of Brest (France), a semi-enclosed zone of Western Europe. *Cont Shelf Res* 16:1885–1907
- Lee JHW, Harrison PJ, Kuang C, Yin K (2006) Eutrophication dynamics in Hong Kong coastal waters: physical and biological interactions. In: Wolanski E (ed) *The environment in Asia Pacific harbour*. Springer, The Netherlands, pp 187–206
- Leonardos N, Geider RJ (2004) Responses of elemental and biochemical composition of *Chaetoceros muelleri* to growth under varying light and nitrate:phosphate supply ratios and their influence on critical N:P. *Limnol Oceanogr* 49:2105–2114
- Malone TC, Crocker LH, Pike SE, Wender BW (1988) Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. *Mar Ecol Prog Ser* 48:235–249
- Monbet Y (1992) Control of phytoplankton biomass in estuaries: a comparative analysis of microtidal and macrotidal estuaries. *Estuaries* 15:563–571
- Nixon SW, Pilson MEQ (1983) Nitrogen in estuarine and coastal ecosystems. In: Carpenter EJ, Capone DG (eds) *Nitrogen in marine environment*. Academic, New York, pp 565–648
- Rabalais NN, Nixon SW (2002) Preface: nutrient over enrichment of the coastal zone. *Estuaries* 25:639
- Ragueneau O, Quéguiner B, Treguer P (1996) Contrast in biological responses to tidally-induced vertical mixing for two macrotidal ecosystems of Western Europe. *Estuar Coast Shelf Sci* 42:645–665
- Redfield AC, Ketchum BH, Richards FA (1963) The influence of organisms on the composition of sea-water. In: Hill MN (ed) *The sea*, vol 2. Interscience, New York, pp 26–77
- Riemann B, Simonsen P, Stensgaard L (1989) The carbon and chlorophyll content of phytoplankton from various nutrient regimes. *J Plankton Res* 11:1037–1045
- Rose JM, Caron DA (2007) Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implication for algal biomass in cold waters. *Limnol Oceanogr* 52:886–895
- Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient input on freshwater marine and terrestrial ecosystems. *Environ Pollut* 100:179–196
- Strickland JDH, Parsons TR (1968) Determination of reactive nitrate. In: *A practical handbook of seawater analysis*. Fish Res Board Can Bull 167:71–75
- Tada K, Morishita M, Hamada K, Montani S, Yamada M (2001) Standing stock and production rate of phytoplankton and a red tide outbreak in a heavily eutrophic embayment, Dokai Bay, Japan. *Mar Pollut Bull* 42:1177–1186
- Tan Y, Huang L, Chen Q, Huang X (2004) Seasonal variation in zooplankton composition and grazing impact on phytoplankton standing stock in the Pearl River Estuary, China. *Cont Shelf Res* 24:1949–1968
- Therriault JC, Levasseur M (1985) Control of phytoplankton production in the lower St Lawrence Estuary: light and freshwater runoff. *Nat Can* 112:77–96
- Townsend DW, Thomas M (2002) Springtime nutrient and phytoplankton dynamics on Georges Bank. *Mar Ecol Prog Ser* 228:57–74
- Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblížek M, Lomas MW, Mincer TJ, Moore LR, Moutin T, Rappé MS, Webb EA (2009) Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature* 458:69–72
- Visser AW, Kamp-Nielsen L (1996) The use of models in eutrophication studies. In: Jørgensen BB, Richardson K (eds) *Eutrophication in coastal marine ecosystems*. American Geophysical Union, Washington DC, pp 221–242
- Wienke SM, Cloern JE (1987) The phytoplankton component of seston in San Francisco Bay. *Neth J Sea Res* 21:25–33
- Xu J, Ho AYT, Yin K, Yuan X, Anderson DM, Lee JHW, Harrison PJ (2008a) Temporal and spatial variations in nutrient stoichiometry and regulation of phytoplankton biomass in Hong Kong waters: influence of the Pearl River outflow and sewage input. *Mar Pollut Bull* 57:335–348
- Xu J, Yin K, He L, Yuan X, Ho AYT, Harrison PJ (2008b) Phosphorus limitation in the northern Southern China Sea during late summer: influence of the Pearl River. *Deep Sea Res I* 55:1330–1342
- Xu J, Ho AYT, Yin K, Lee JHW, Anderson DM, Harrison PJ (2009) Nutrient limitation in Hong Kong waters inferred from comparison of nutrient ratios bioassays and <sup>33</sup>P turnover times. *Mar Ecol Prog Ser* 388:81–97
- Xu J, Lee JHW, Yin K, Liu H, Harrison PJ (2011) Environmental response to sewage treatment strategies; Hong Kong's experience in long term water quality monitoring. *Mar Pollut Bull* 62:2275–2287
- Yin K (2002) Monsoonal influence on seasonal variations in nutrients and phytoplankton biomass in coastal waters of Hong Kong in the vicinity of the Pearl River estuary. *Mar Ecol Prog Ser* 245:111–122
- Zhao H (1990) *Evolution of the Pearl River estuary*. Ocean Press, Beijing (in Chinese)