Shift from P to N limitation of phytoplankton growth across the Pearl River estuarine plume during summer

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ABSTRACT: Anthropogenic loading of nutrients in rivers often increases disproportionally among N, P, and Si, and thus may shift the type of phytoplankton nutrient limitation in the coastal receiving waters. The effect of anthropogenic nutrient loading has rarely been addressed in the Pearl River estuary along the southern coast of China, even though it is one of the largest rivers in the world. We conducted a cruise along the Pearl River estuary and adjacent coastal waters south of Hong Kong during July 17 to 18, 1999. Samples were taken for salinity and nutrients (NO_3, SiO_4, PO_4, NH_4 and urea) and nutrient addition experiments were conducted on board. Vertical profiles of salinity showed a salt-wedge estuary and the coastal plume covering the waters south of Hong Kong. Concentrations of NO_3 were very high (ca 90 µM) upstream of the Pearl River estuary, and much of the riverine NO_3 was not utilized in the estuary until depletion at the edge of the coastal plume on the east side of Hong Kong. SiO_4 was 120 µM upstream and its utilization was similar to that of NO_3. PO_4 was low in surface waters (<0.5 µM) and higher below the halocline in the estuary. NH_4 and urea were generally <4 and 1.5 µM, respectively. In the estuary, N:P ratio was 200:1, indicating potential P limitation, while N:Si was below 1:1. Beyond the coastal plume to the east of Hong Kong, N:P and N:Si ratios were <5:1 and 1:0.3, respectively, indicating potential N limitation. Nutrient limitation was shown in nutrient addition experiments and was consistent with the ratios of nutrients. Therefore, nutrient limitation shifted across the coastal plume from P limitation in the estuary to N limitation in the oceanic waters. Potential P limitation was observed in the estuary; P and Si co-limiting occurred at the edge of the coastal plume, and N was limiting in the oceanic side. This spatial shift in nutrient limitation has great implications for nutrient pollution control and coastal management of Hong Kong waters.

KEY WORDS: Nutrient ratios · N:P · N:SI · Nutrient limitation · Pearl River estuary · Hong Kong · Northern South China Sea

INTRODUCTION

Anthropogenic loading of nutrients in rivers has increased over the last few decades (Jickells 1998). Concentrations of nutrients such as N and SiO_4 are often several times higher in rivers than in receiving coastal seawater in the vicinity of the largest rivers in the world such as the Amazon River (Ryther et al. 1967, Gibbs 1970, Edmond et al. 1981), the Mississippi River (Turner & Rabalais 1991, Rabalais et al. 1996), and the Yangtze River (Edmond et al. 1985, Shen 1993). The increased nutrients promote primary productivity in receiving coastal waters and cause algal blooms. Eventually they result in eutrophication if the algae are not grazed by zooplankton. An alarming example is the

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'dead zone' in the northern Gulf of Mexico caused by the increased input of nutrients from the Mississippi River (Malakoff 1998). Accompanying the increase in nutrients is an alteration in nutrient ratios such as N:Si and N:P as a result of excess N relative to Si and P, which can have an impact on phytoplankton biomass and species composition (Smayda 1990, Jickells 1998). In the Black Sea, the increasing N and P relative to Si resulted in the dominance of other phytoplankton such as coccolithophores and flagellates, which displaced the diatoms (Humborg et al. 1997). In the area where the Rhine River flows into the southern North Sea, the nuisance alga *Phaeocystis* has been reported to bloom frequently as a result of shifts in nutrient ratios in addition to elevated nutrient concentrations (Cadée & Hegeman 1986, Riegman et al. 1992).

In spite of high fluxes of nutrients from rivers to estuaries, phytoplankton biomass may be ultimately limited by a nutrient that is low relative to other nutrients in the vicinity of an estuary. P is often the most limiting nutrient in freshwater (Schindler 1977, Hecky & Kilham 1988) while in marine waters N is usually the most critical (Ryther & Dunstan 1971, Hecky & Kilham 1988). In an estuary, there may be a shift from P to N limitation, potentially controlling phytoplankton species and biomass when surface water moves from rivers to oceans. In the northern Gulf of Mexico, there was evidence of a shift from P to N limitation (Lohrenz et al. 1999) or N and Si co-limitation (Dortch &

Whitledge 1992). In Chesapeake Bay, the shift from P to N limitation is not only spatial, but also seasonal (Fisher et al. 1999). The possible consequences of a shift in nutrient limitation for phytoplankton species and biomass have not been studied for the Pearl River estuary and adjacent waters.

The Pearl River estuary is a semi-enclosed area located in the south China coast, bounded by Hong Kong to the east and Guangdong Province of Mainland China to the west (Fig. 1). The Pearl River is the second largest river in China next to the Yangtze River, and it is the 13th largest river in the world by discharge volume (Lerman 1981). The Pearl River stretches for 2214 km and drains an area of 452 000 km^2 (Zhao 1990). The Pearl River consists largely of 3 rivers: the Xi Jiang (West River), Bei Jiang (North River), and the Dong Jiang (East River). The 3 rivers branch into many small rivers in a network and merge into 8 river gates before entering the South China Sea. Four of the river gates open to the Pearl River estuary between Hong Kong and Macau (Fig. 1). The yearly average river discharge is 10 524 m^3 s^{-1}, with 20% occurring during the dry season in October to March and 80% during the wet season in April to September (Zhao 1990). The monthly maximum of the Pearl River discharge is in July, reaching as high as 22 200 m^3 s^{-1} (Zhao 1990). Shenzhen River is a small river between Hong Kong and Shenzhen, and its water is black. It may contain high nutrients that have a local influence on the Pearl River estuary.

Freshwater from the 4 rivers flows into the estuary and mixes with seawater. The mixture of freshwater and seawater forms a plume, which will be referred to as the estuarine plume when it is in the estuary (geographically bounded by the land margin between Hong Kong and Macau). The estuarine water (estuarine plume) flows into the coastal waters in the northern South China Sea and covers a large area of the South China Sea including Hong Kong coastal waters (Watts 1971). This water will be referred to as the coastal Pearl River estuarine plume or the coastal plume. The estuarine plume tends to flow out to the west side of the estuary and then turns to the east and becomes the coastal plume. The coastal plume is driven by the southeast monsoon and it continues to move eastwards across waters south of Hong Kong.

Our previous study showed that there was a region with a chlorophyll maximum in waters south of Hong Kong (Yin et al. 2000). To the estuarine side of the chlorophyll maximum, *in vivo* fluorescence increased during 24 h incubation while it decreased in water samples on the other side of the chlorophyll maximum. Concentrations of ambient nutrients suggest that P or SiO$_4$ limits
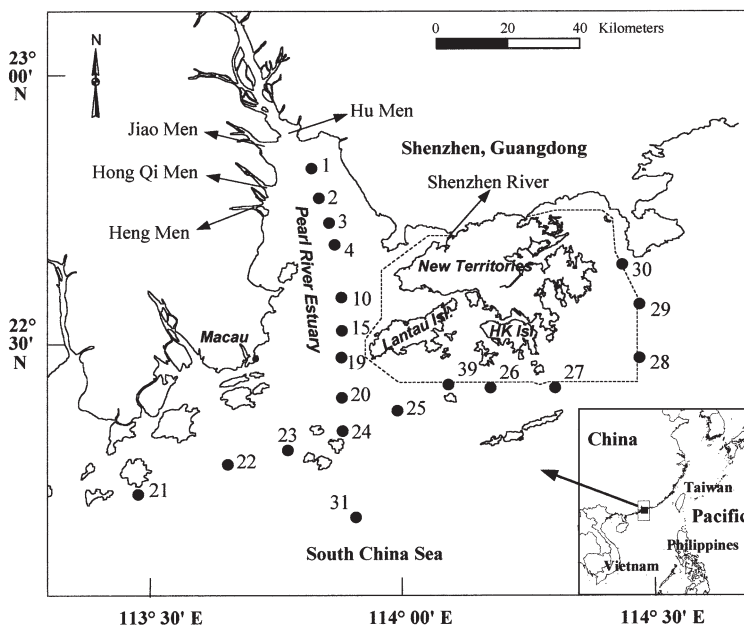

Fig. 1. Map of the Pearl River estuary and sampling stations during the cruise between July 17 and 28, 1999. The dashed line is the boundary of the Hong Kong territorial water. HK: Hong Kong; Isl: Island. The water depth for each station can be found in Fig. 2

phytoplankton growth (Yin et al. 2000). There is an urgent need to investigate what limits phytoplankton biomass in the Pearl River estuary and adjacent coastal waters. There is a debate in Hong Kong over whether the Hong Kong government should build secondary and tertiary sewage treatment facilities. The removal of N has been debated, but not of P (IRP 2000, see http://www.info.gov.hk/SSDS.review/). Therefore, the question of which nutrient should be removed from domestic sewage effluent is very important in economic terms. Spatial variability in nutrient limitation has implications for determining where effluent sites should be planned. For example, if P is found to be the nutrient most limiting to phytoplankton growth and biomass in the region of the sewage discharge point, the removal of N would have little effect on the consumption of oxygen. So far, our understanding of the dynamics of nutrients in this region is very limited. We know little about how the Pearl River estuary and South China Sea interact with each other with regard to the dynamics of nutrients, let alone the spatial variability of nutrient limitation. The objectives of this study were: (1) to examine how the distribution of nutrients is related to physical processes by investigating the horizontal and vertical distribution of nutrients; and (2) to determine which nutrient is limiting or co-limiting to phytoplankton growth along spatial gradients of salinity using nutrient addition bioassays. This research is part of the large project 'The Pearl River Estuary Pollution Project', which is investigating a wide array of environmental and oceanographic variables and processes.

MATERIALS AND METHODS

Sampling. A cruise during July 17 to 28, 1999, was conducted on the research vessel Haijian 74 along 2 transects in the Pearl River estuary and its vicinity. One north-south transect was along the axis of the estuary from an upstream region near the river mouth (Stn 1) to a station (Stn 31) 30 km offshore (Fig. 1). The other east-west transect crossed the coastal plume south of Hong Kong and terminated to the east of Hong Kong (Fig. 1). The 2 transects were intended to investigate the spatial structure of nutrients and to examine the eastern extent of the coastal plume.

A YSI® 6600 (YSI Incorporated, Yellow Spring, OH, USA) was used at a station to take vertical profiles of salinity, temperature, chlorophyll *a* (chl *a*) fluorescence, dissolved oxygen, pH, and turbidity. Water samples were taken using a 2.5 l PVC Go-flo sampler (General Oceanics, Miami, FL, USA). After the position of the halocline was determined, a few water samples were taken at depths from the surface to below the

halocline. The halocline referred to in this study is considered to have the largest gradient when there are multiple layers, which are often found in the water column in other estuaries (e.g., Yin et al. 1995a,b). Water samples were transferred to 10 l carboys cleaned before-hand in 10 % HCl for sub-sampling. Samples for nutrient determinations were taken with 60 ml syringes and filtered through a pre-combusted Whatman® GF/F filter (Whatman Inc., Clifton, NS, USA) mounted on Swinnex® (Swinnex Filter holders, Millipore, Bedford, MA, USA) into 30 ml Nalgene bottles (Nalgene, Nalge Nunc International, Rochester, NY, USA). All plastic wares were pre-cleaned with 10 % HCl. The filtered water samples were placed in a deep freezer at –20°C and frozen immediately. Nutrients were analyzed in the laboratory on shore within 2 wk.

Nutrient addition experiments. Nutrient addition experiments were conducted on board the ship at selected stations. Nutrients were added in 8 combinations: control (no addition), $+NO_3$, $+PO_4$, $+SiO_4$, $+NO_3+PO_4$, $+NO_3+SiO_4$, $+PO_4+SiO_4$, and $+NO_3+PO_4+SiO_4$. They will be referred to as Cont, +N, +P, +Si, +NP, +NSi, +PSi, and +NPSi, respectively. The final concentrations in the treatments after the nutrient addition were 30, 3, and 30 µM for NO_3, PO_4, and SiO_4, respectively. Duplicates of each treatment were incubated in 50 ml culture glass tubes for 48 h in a water bath cooled with surface seawater. The tubes were covered with 1 layer of neutral density screen in order to reduce ambient light by 35 %. The *in vivo* fluorescence of each treatment in the glass tubes was measured using a Turner Designs fluorometer (Model 10; Turner Designs, Sunnyvale, CA, USA) at 0, 24, and 48 h.

As Cullen (1982) pointed out, *in vivo* fluorescence is not necessarily a measure of chlorophyll, and chlorophyll (chl *a*) is not necessarily a measure of phytoplankton biomass in a situation of unbalanced growth induced by nutrient shift-up. Because the ratio of fluorescence to chl *a* increases with nutrient starvation (Blasco 1973, Kiefer 1973, Sakshaug & Holm-Hansen 1977, cf. Cullen 1982), the interpretation of the results from our nutrient addition experiments is not misleading. Since nutrient additions would decrease the ratio, an increase in *in vivo* fluorescence induced by a nutrient addition compared with the control indicates a real increase in chl *a* rather than remobilizing organic materials within the cells. As the ratio of carbon to chl *a* decreases with increasing nutrient limited growth rate (Cullen 1982), an increase in chl *a* may not necessarily indicate organic carbon production in a transient status or over a short time scale. As the growth rates of a natural phytoplankton community may reach 2.4 d^{-1} in the regional subtropical waters (30°C) (Yin et al. 2000), time scales of 24 and 48 h are long enough to balance the deficit of the first limiting nutrient and to reach bal-

anced growth. Thus, any change in chlorophyll would have been translated into phytoplankton biomass in terms of organic carbon production during 24 h.

Nutrient analysis. Nitrate (plus nitrite) and ammonium were determined manually following the procedures of Wood et al. (1967) and Slawyk & MacIsaac (1972), respectively. Manual analyses of phosphate and silicate were based on the methods by Hager et al. (1968) and Armstrong et al. (1967). Analysis of urea was based on the modified method by Price & Harrison (1987) and urea was expressed in µM (equivalent to 2 µg at N l^{-1}).

Phytoplankton species counts. Phytoplankton species samples were preserved with Lugol's solution according to Parsons et al. (1984). A subsample of 40 ml was concentrated to 5 ml by settling and siphoning the supernatant. A subsample of 0.5 ml was transferred to a 0.5 ml chamber on a glass slide using a pipette and covered with a thin cover slip. Phytoplankton species were identified and counted under a regular microscope for the whole 0.5 ml sample.

RESULTS

There was a typical estuarine gradient along the north-south transect from Stn 1 to Stn 31 (Fig. 2). Salinity at Stn 1 was nearly 0 at the surface, increased at Stn 10 (1.1) and reached 11 at Stn 31. The salt wedge was evident in the estuary and it progressed up river to Stn 1, very near the river mouth (Hu Men). The halocline was sharp (denser contour lines) inside the estuary (Stns 2 to 20), indicating strong outward flow of the surface layer and inward flow of the bottom layers. The halocline thickened at Stns 24 to 31, indicating more vertical mixing. The coastal plume outside the estuary, indicated by a brackish water lens (salinity of 15 to 20) in the top 4 m of the water column, was evident at Stn 24 and even Stn 31 (Fig. 2). There was a gradient of salinity between Stns 25 and 29, indicating the presence of the coastal plume (Fig. 2). Surface salinity was <15 at Stn 25 and increased to >33 at Stns 29 and 30. The gradient in salinity occupied almost the top 12 m of the water column. Apparently, the water column at Stns 29 and 30 had not been influenced by the coastal plume at the time of the cruise.
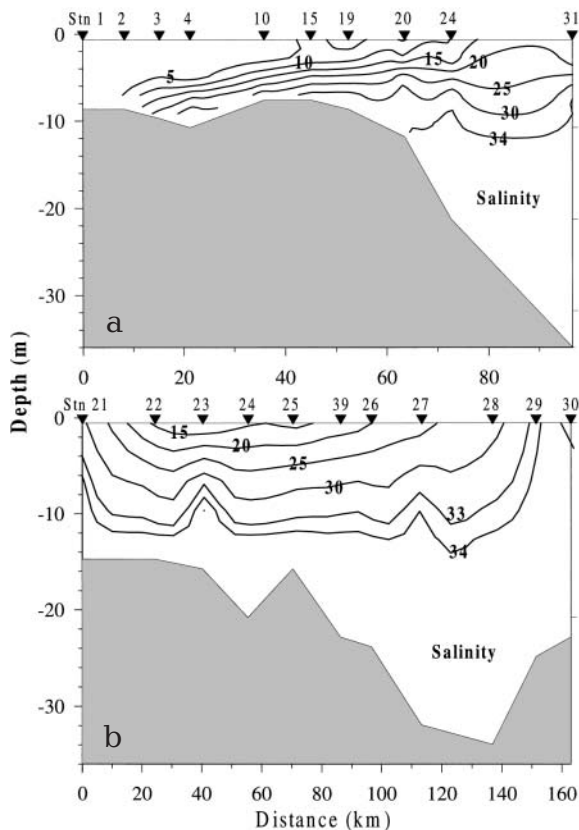

Fig. 2. Distance-depth contours of salinity (a) along the estuary (Stns 1 to 31) during July 20 and 21, and (b) across the coastal plume (Stns 21 to 30) during July 18 and 19. The contour interval is 5 unless a line is labeled specifically
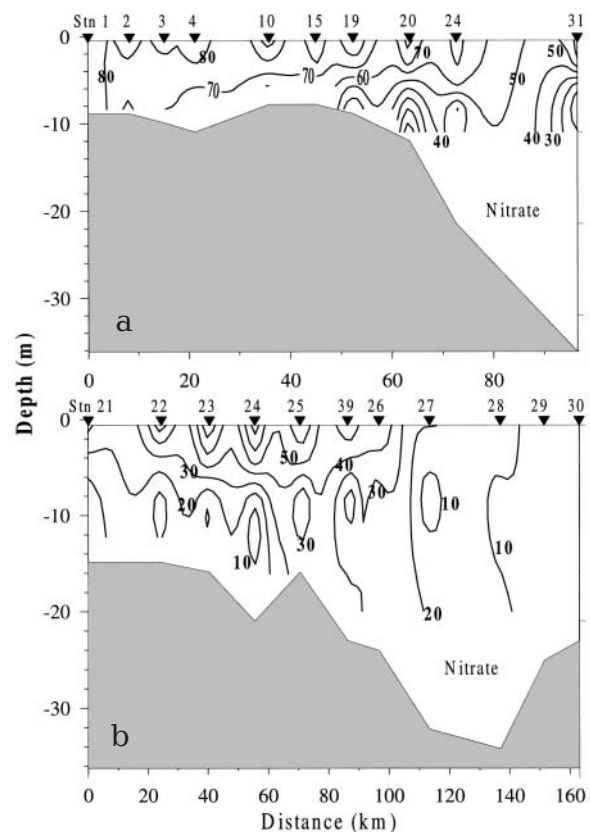

Fig. 3. Distance-depth contours of NO$_3$ (µM) (a) along the estuary (Stns 1 to 31) during July 20 and 21, and (b) across the coastal plume (Stns 21 to 30) during July 18 and 19. The contour interval is 10 µM

Fig. 4. Distance-depth contours of SiO_4 (µM) (a) along the estuary (Stns 1 to 31) during July 20 and 21, and (b) across the coastal plume (Stns 21 to 30) during July 18 and 19. The contour interval is 10 µM

Fig. 5. Distance-depth contours of PO_4 (µM) (a) along the estuary (Stns 1 to 31) during July 20 and 21, and (b) across the coastal plume (Stns 21 to 30) during July 18 and 19. The contour interval is 0.1 µM
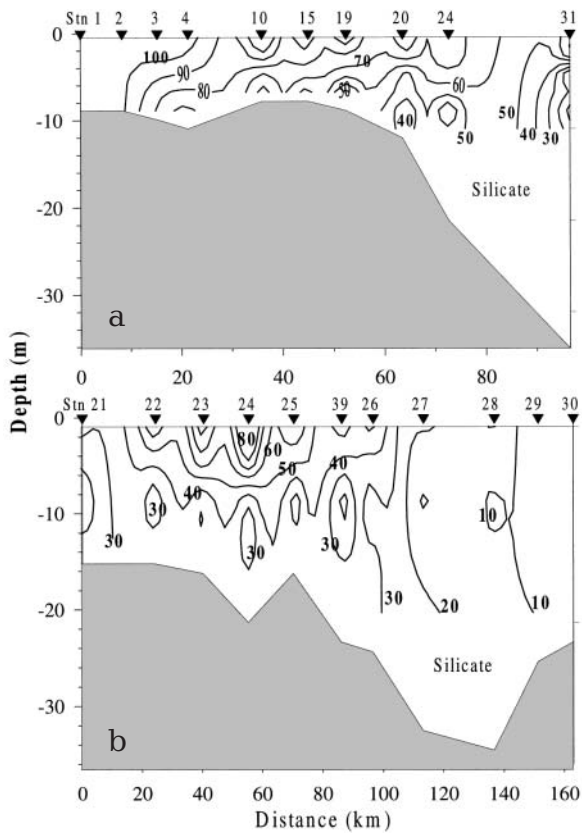
NO_3 concentration was >80 µM at Stns 1 to 3 (Fig. 3a). NO_3 concentration was slightly higher at Stns 4 to 20 (e.g., 95 µM at Stn 10, Fig. 3a), which was probably caused by freshwater sources from the Shenzhen River (Fig. 1). At Stns 24 and 31, the concentrations decreased to 73 and 63 µM, respectively (Fig. 3a). Near the bottom, the NO_3 concentration was <10 µM at Stn 31 and increased upstream. Across the coastal plume, NO_3 concentration decreased to the east and was undetectable at Stns 29 and 30 (Fig. 3b). SiO_4 concentration was almost parallel to the distribution of NO_3, although it was generally higher than that of NO_3 (120 µM at Stn 10) (Fig. 4). SiO_4 concentration was only <10 µM at Stns 29 and 30 (Fig. 4b). PO_4 concentration was <0.6 µM at the surface at Stns 1 to 10, increased at Stns 15 and 20 and decreased downstream to Stn 31 (Fig. 5a). PO_4 concentration was higher in the bottom water than at the surface along the estuary (Fig. 5a). Moving eastwards across the coastal plume, PO_4 concentration was low at the surface (Fig. 5b). PO_4 concentration was <0.2 µM below the halocline at all these stations except for Stn 39 (Fig. 5b). NH_4 concentration
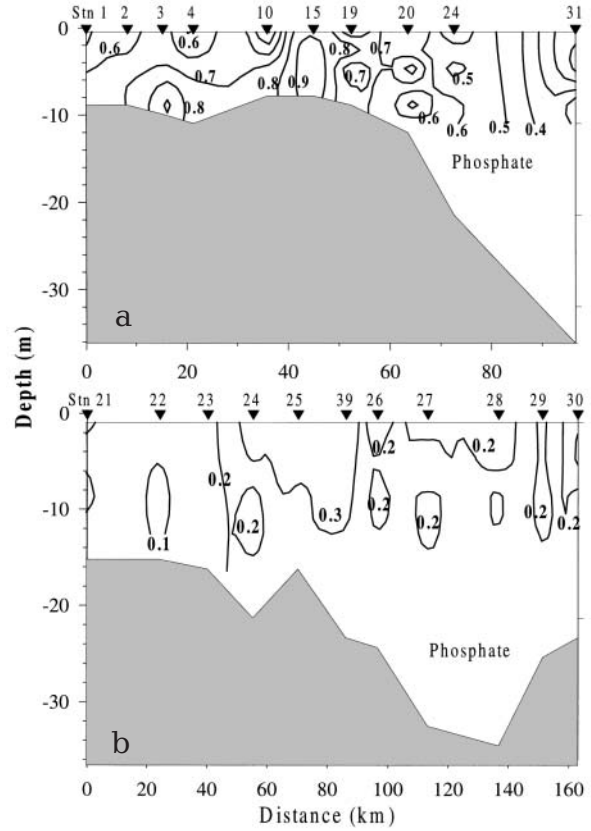
was variable and usually <4 µM at the surface (Fig. 6). There was a tendency for NH_4 to be higher in the tip of the salt wedge (Stns 2 to 4, Fig. 6a). Urea concentration varied as NH_4 in the estuary (Fig. 7a) and was less than 0.5 µM at stations (Stns 25 to 30) across the coastal plume (Fig. 7b).

The N:P ratio was >128:1 (7 times greater than the Redfield (1958) ratio of 16:1) in the Pearl River estuary, except for Stns 15 and in the coastal plume south of Hong Kong (Table 1). In contrast, the N:P ratio was only <5:1 in the oceanic waters at Stns 29 and 30. The ratio of N:P was much lower in the subsurface than at the surface (Table 1). The N:P ratio was <16 at Stns 24 to 31 and Stns 27 to 30, suggesting a potential N limitation of the oceanic waters. The N:Si ratio was between 0.7:1 and 1:1 at the surface in the estuary and in the waters south of Hong Kong. At Stns 29 and 30, N:Si ratios fell below 0.3 (Table 1). The lower ratios of N:P and N:Si were indicative of the limitation of N at these 2 stations.

Mixing diagrams of nutrients versus salinity are often a tool to indicate whether biological utilization or
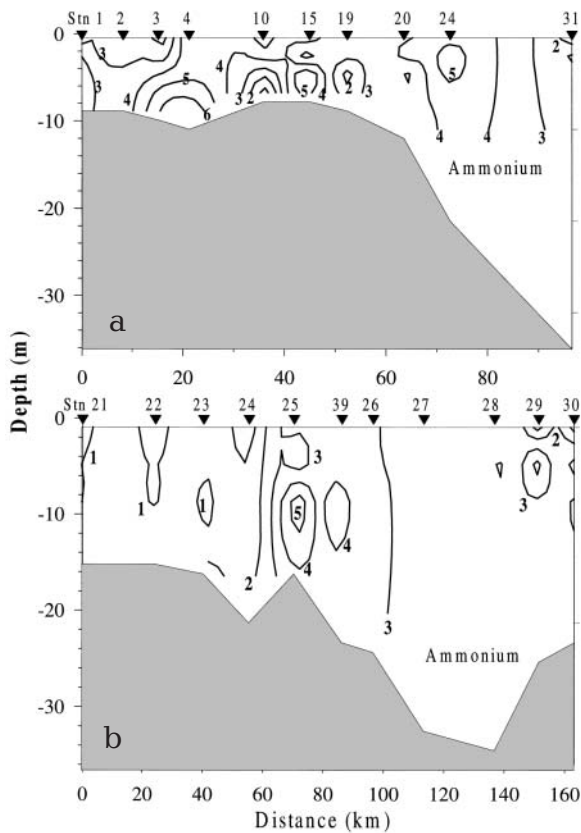
Fig. 6. Distance-depth contours of NH₄ (µM) (a) along the estuary (Stns 1 to 31) during July 20 and 21, and (b) across the coastal plume (Stns 21 to 30) during July 18 and 19. The contour interval is 1 µM

Fig. 7. Distance-depth contours of urea (µM) (a) along the estuary (Stns 1 to 31) during July 20 and 21, and (b) across the coastal plume (Stns 21 to 30) during July 18 and 19. The contour interval is 0.25 µM

physical mixing processes are dominant along the estuary. But the validity of the mixing diagrams depends on whether 2 end members are in the same mixing regime, how constant the concentration of nutrients and salinity of the 2 end members are and whether there are internal sources of nutrients. A separate mixing diagram was used for each transect to show differences in these processes between the 2 transects.

Mixing diagrams for surface NO_3 and SiO_4 versus salinity showed linear relations for both transects (Fig. 8). However, the slopes were –3.4 and –3.1 for NO_3 and SiO_4, respectively, across the coastal plume, which were larger than the slopes (–2.0 and –2.8, respectively) along the estuary (significant at p < 0.02). A steeper slope for a nutrient in the mixing diagrams for 1 zone means that the nutrient in the zone decreases more rapidly than in other zones along the same gradient of salinity. The differences should be caused by biological utilization of these nutrients. NO_3 and SiO_4 at the bottom deviated from the linear lines along the estuary (Fig. 8) and the deviation was

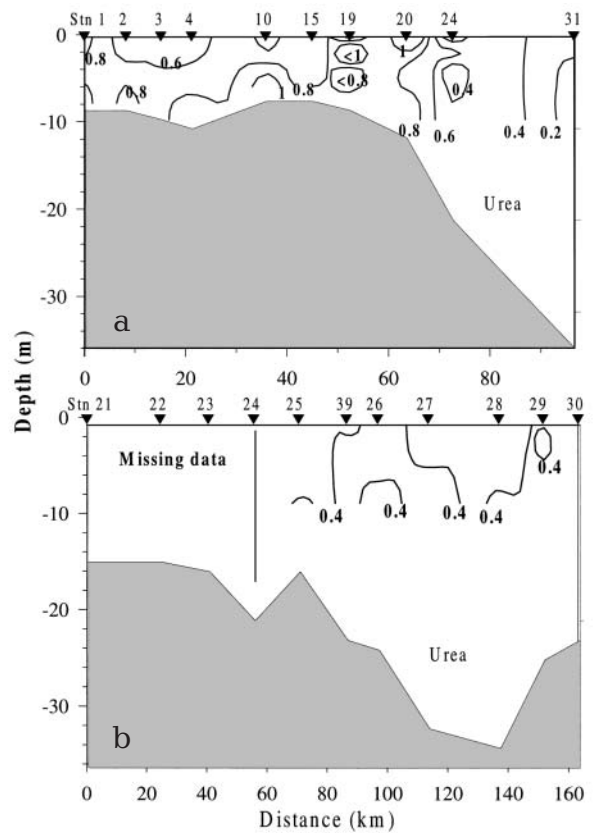greater for NO_3. Mixing diagrams of surface PO_4 versus salinity showed an increase in PO_4 when salinity was between 2 and 7 along the estuary (Fig. 8). The increase in PO_4 was more evident for the bottom layer between salinities of 23 and 35.

Nutrient addition experiments were conducted at 8 stations (Table 2, Fig. 9). Responses in the *in vivo* fluorescence to nutrient additions exhibited a spatial shift across the coastal plume to oceanic waters. First, an increase in light during the 48 h incubation revealed that the turbid waters in the middle of the estuarine plume would become P limited if light increased (less vertical mixing in the water column) (Stns 24 and 31) second, P limitation was most evident at the edge of the coastal plume (Stns 27 and 28), and third, N was most stimulating with P co-limitation beyond the plume (Stns 29 and 30). For example, there was obviously a time lag of 24 h before phytoplankton responded to the addition of nutrients at Stns 24, 31, 39, and 26 (Table 2, Fig. 9). At Stn 31, phytoplankton fluorescence increased in most P added treatments compared with the control (Fig. 9). Moving eastwards

Table 1. Ratios of nitrate to phosphate (N:P) and to silicate (N:Si) by atoms at the surface and at a subsurface depth along the estuary and the coastal plume

| Stn	Surface N:P	N:Si	Depth (m)	Subsurface N:P	N:Si
Along the estuary					
1	173	0.78	7	132	0.71
2	148	0.73	7	114	0.78
3	130	0.74	8	74	0.93
4	183	0.81	9	73	0.91
10	242	0.82	6	73	1.03
15	103	0.88	6	49	0.94
19	148	0.87	7	38	0.80
20	119	0.89	8	45	0.66
24	198	0.69	19	12	0.15
31	399	0.93	8	4.5	0.32
Across the coastal plume					
21	143	0.99	13	7.2	0.06
22	398	0.82	13	89	0.24
23	358	0.79	17	9.6	0.05
24	185	0.96	8	44	0.89
25	181	1.02	8	70	0.80
39	163	1.10	8	27.0	0.80
26	2750	0.95	8	144	0.94
27	233	1.04	8	14.0	0.39
28	239	0.85	8	12.0	0.74
29	4.5	0.26	8	6.8	0.49
30	4.7	0.08	8	13.4	0.39
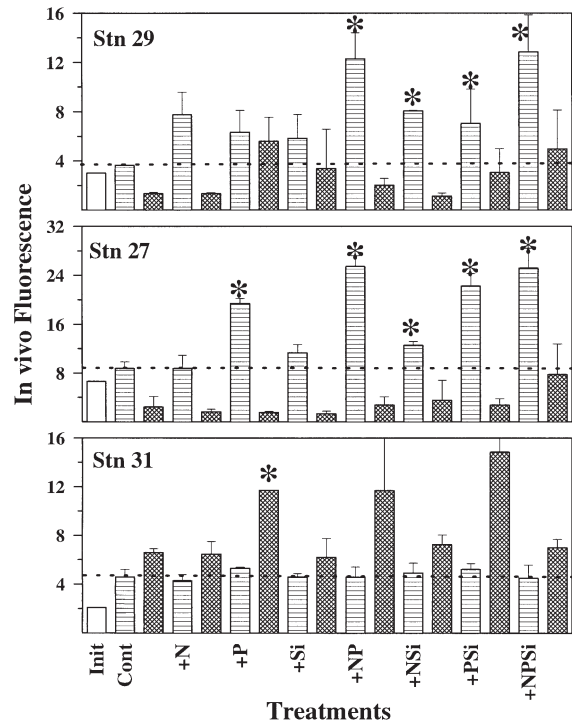
Fig. 9. Nutrient addition experiments at 3 stations. The horizontal dashed line on each graph indicates the fluorescence for the control at $T = 24$ h. Init is the initial reading taken at $T = 0$ h. The other labels on the x-axis are treatments as described in 'Materials and methods'. The vertical bars are standard errors. (✳) Treatment was significantly different ($p < 0.05$) from the control based on Student's t-test using the t-test in Microsoft® Excel 2000

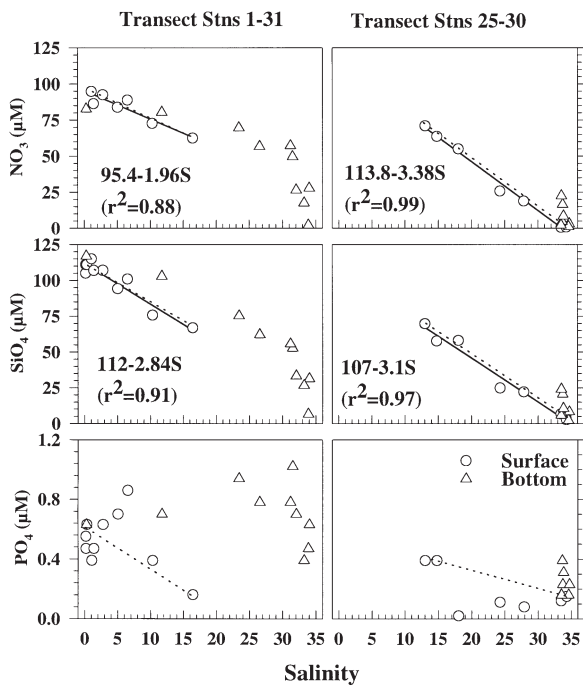Fig. 8. Mixing diagrams of NO_3, SiO_4, PO_4, N:P ratio, and N:Si ratio versus salinity along the estuary (Stns 1 to 31) during July 20 and 21 (left column) and across the coastal plume (Stns 25 to 30) during July 18 and 19 (right column). Only the surface (○) and bottom (△) data in Figs 2 to 5 were used if there were more than 2 data points in the vertical profiles

to Stn 27, P stimulation was most evident after 24 h in all P addition treatments (Fig. 9). Responses were very similar at Stn 28. At Stn 30, the northeasternmost station, the addition of +NP and +NPSi showed a stimulation (156 and 221 %, respectively) of fluorescence at 24 h; but not significantly different from that of the control (Table 2). At Stn 29, N additions stimulated an increase in all N-addition treatments, particularly in +NP (3.4 times) and +NPSi (3.5 times) (Fig. 9, Table 2). In contrast to responses at Stns 24, 31, and 39, phytoplankton fluorescence dramatically declined from the 24 h control at the eastern stations, Stns 27 to 29, after 48 h (Table 2). Phytoplankton were mainly diatoms that were alternately dominated by *Skeletonema costatum*, *Pseudonitzschia*, *Chaeotoceros* and *Rhizosolenia* in the estuary and coastal waters (data not shown). At Stn 27, the latter 3 species were dominant (Table 3). After 48 h incubation, total phytoplankton counts decreased to 0.12 million cells l⁻¹ from 3.54 million cells l⁻¹ in the beginning of the control culture (Table 3). Additions of NO_3 and PO_4 showed a similar result. This 30-fold decrease in the total phytoplankton counts was consistent with the decrease in the *in vivo*

Table 2. Responses (%) of *in vivo* fluorescence to additions of nutrients in 8 treatments at 8 stations during the cruise. The control at 24 h is taken as 100%, and the responses after 24 and 48 h incubation in all other treatments, including the initial one, are expressed as a percentage relative to the control. *Significant response at $p < 0.05$ based on Student's *t*-test using the *t*-test in Microsoft® Excel 2000

Stn	Initial	Control	+N	+P	+Si	+NP	+NSi	+PSi	+NPSi
After 24 h incubation									
30	80	100	90	112	69	156	112	112	221
29	83	100	213	174	160	338*	222*	194*	353*
28	107	100	122	233*	171	184*	174	148*	178*
27	76	100	100	221*	129	290*	143	254*	287*
26	60	100	92	126	98	167*	99	150	164
39	73	100	111	115	109	132	90	124	105
31	45	100	93	116	100	100	107	114	98
24	49	100	91	85	93	95	92	84	101
After 48 h incubation									
30		117	115	129	103	120	107	138	111
29		37	37	154	93	55	31	84	137
28		41	28	54	40	44	45	67	47
27		28	19	18	15	31	40	31	88
26		115	101	156	86	214	64	256	223
39		106	141	143	75	150	105	161	194
31		144	140	254	135	254	158	323	152
24		100	155	125	169	178	172	121	183

Table 3. Phytoplankton species composition and counts (cells l^{-1}) at the initial ($T = 0$ h) and final trials ($T = 48$ h) in the control (without addition of nutrients) and in the treatment (with addition of NO_3 and PO_4) groups at Stn 27

Species	$T = 0$ h Control	$T = 48$ h Control	$T = 48$ h +NP
Asterionella japonica	61900	4350	2050
Bacteriastrum hyalinum	8500	175	100
B. mediterraneum	12800		
Cerataulina bergoni	32500		
C. compacta	15500		
***Chaeotoceros* sp.**	42700	7780	4380
Chaeotoceros abnormis	9600	1080	1130
C. affinis	5870	1580	1300
C. brevis	2100		
C. compressus	6900	650	275
C. lorenzianus	3200	2700	750
C. pelagicus	10100	425	250
C. subitils	4800		275
Leptocylindrus danicus	38900	725	500
***Pseudonitzschia* sp.**	993600	26400	21300
P. delicatissima	245900	21400	6580
P. pungens	747700	4980	14700
***Rhizosolenia* sp.**	51200	925	450
R. fragilissima	15470		
R. setigera	2130	50	50
R. stolterfothii	16000	250	150
Guinardia delicatula	17600	525	250
Thalassionema nitzschioides	8000	2550	1650
Thalassiothrix frauenfeldii	4800	675	700
Prorocentrum sp.	533	25	
Total	3537100	119000	89900

fluorescence in this experiment and supports the assumption that changes in *in vivo* fluorescence are tracking changes in phytoplankton biomass.

DISCUSSION

Nutrient loading and distribution

High nutrient loading from rivers has been found in many estuaries in North America and Northern Europe (Howarth et al. 1996) and has become a growing concern in causing eutrophication (Jickells 1998). During the season of high river discharge, NO_3 concentrations upstream of the Pearl River estuary were high (present study, Yin et al. 2000). NO_3 is certainly higher than in other large rivers such as the Amazon River (10 to 15 µM, Edmond et al. 1981), and the Sacramento and San Joaquin Rivers (<25 µM) discharging into San Francisco Bay (Schemel & Hager 1986) and even higher than the largest Chinese river to the north, the Yangtze River (Shen 1993). NO_3 concentrations in the Pearl River are lower than in the Rhine River (4 mg NO_3-N l^{-1} [270 µM], Schaub & Gieskes 1991) and comparable with those in the Mississippi River (60 to 120 µM, Dortch & Whitledge 1992, Rabalais et al. 1996), and Chesapeake Bay where NO_3 in the freshwater region ranged between 70 and 120 µM (Ward & Twilley 1986, Fisher et al. 1988). NH_4 in the Pearl River estuary (<4 µM) was similar to that in the Mississippi River estuary (Dortch & Whitledge 1992, Rabalais et al. 1996). Urea was low (<1 µM), comparable with that in the unpolluted Strait of Georgia off the west coast of Canada (Yin unpubl. data). PO_4 concentration was low (0.5 µM) in the Pearl River estuary and similar to that in the Amazon River (Edmond et al. 1981), the Yangtze River (Shen 1993), and Chesapeake Bay (Fisher et al. 1992). PO_4 in the Pearl River estuary was definitely much lower than in the Rhine River (0.35 PO_4-P mg l^{-1} [11 µM], Schaub & Gieskes 1991), and lower than in the Mississippi and Sacramento Rivers, where PO_4 concentrations were 3 µM (Dortch & Whitledge 1992, Rabalais et al. 1996) and 4 µM (Schemel & Hager 1986), respectively. In the northern Gulf of Mexico and in Chesapeake Bay, serious eutrophication effects of hypoxia or anoxia have been reported (Malone et al. 1988, Breitburg 1990, Rabalais et al. 1992, 1994, Dortch et al. 1994, Malakoff 1998). High concentrations of NO_3 and SiO_4 from the Pearl

River should exert an effect on the oligotrophic oceanic waters of the South China Sea. A low oxygen layer (<3 mg l^{-1}) below the halocline in the bottom water near the entrance of the estuary was observed during the same cruise (Yin unpubl. data).

The Pearl River estuary showed a typical salt-wedge circulation based on the salinity distribution along the estuary (Fig. 2). Saline water was pulled into the estuary at the bottom with fresher outflow at the surface. During this process, vertical mixing took place as a result of entrainment between the surface layer and the layer below with tidal cycles, as described by Geyer et al. (1989). Concentrations of both NO_3 and SiO_4 at depth increased upstream because of this vertical mixing. The PO_4 concentration at depth in the estuary was higher than that at the surface and even higher than in the oceanic waters at the same depth (Stn 31 or Stn 29, Fig. 4), suggesting an internal source of PO_4 at depth in the estuary. Desorption of PO_4 from particles and bacterial decomposition of organics in suspended particles settling from the surface layer may have played an important role in increasing the PO_4 concentration in the saline water as it moved upstream. Desorption of P from suspended particles is common (DeJonge & Engelkes 1993, Zwolsman 1994). P was also shown to be preferentially removed from dissolved organic matter during bacterial decomposition (Clark et al. 1998). In addition, benthic resuspension of overlying water at the sediment-water interface could also have contributed to the increase in PO_4 concentration. Both the processes, desorption and benthic flux, contributed to the increase in PO_4 as high turbidity was observed near the bottom (Yin et al. unpubl. data).

On the basis of the mixing diagrams, biological utilization of nutrients was not apparent along the estuary. It is likely that biological utilization of the nutrients would be more dominant further down stream. This could not be demonstrated as the cruise did not go far enough offshore to reach the boundary between the coastal plume and oceanic waters (salinity of 33 at the surface) in the South China Sea. However, moving eastward across the coastal plume south of Hong Kong, biological utilization of nutrients was evident as shown by the steeper slope for NO_3 in the mixing diagram along the coastal plume than along the estuary (Fig. 8). This is consistent with the initial high *in vivo* fluorescence reading at Stns 27, 29, and 31 (Fig. 9), which showed high chl *a* concentrations across the coastal plume compared with chl *a* in the estuary (Yin et al. unpubl. data). It is interesting to note that the decrease in NO_3 along the salinity gradient was 30% lower than the decrease in SiO_4 along the estuary, but 10% higher than SiO_4 across the coastal plume (Fig. 8). The former indicates more utilization of SiO_4 relative to NO_3, suggesting that other forms of N were being used

if the phytoplankton were utilizing N and Si in the typical 1:1 ratio.

Nutrient ratios, N:P, and N:Si

A change in the ratios of the key nutrients such as N:P and N:Si accompanied the increased fluxes of riverine nutrients. Apparently, the high load of N from the Pearl Rivers increased the N:P ratio to >7 times the Redfield ratio of 16:1 in the estuarine influenced waters. Despite the differences in the ratios of N:P and N:Si between Stns 29 and 30 and other stations, phytoplankton species composition did not show any apparent change in dominant species, which were mostly diatoms such as *Skeletonema costatum*, *Nitzschia pungens*, and *N. delicatissima* (Yin unpubl. data). Since the supply ratio of N:Si by both freshwater and seawater end members is $<1:1$ in the Pearl River estuary and its vicinity, there is some room for an increase in N flux from the Pearl River to this system from the point of view of algal stoichimetry (Justić et al. 1995a,b). In other words, the phytoplankton species composition that was dominated by diatoms may not necessarily change in this region when there is any further increase in N loading in the region. However, if N loading increases and N:Si ratio becomes $>1:1$ in the future, it may result in a species composition change in the coastal waters influenced by the Pearl River discharge.

Nutrient limitation

As high concentrations of riverine nutrients flow downstream, they are diluted and utilized by phytoplankton. Ultimately 1 nutrient becomes limiting to phytoplankton biomass. Because of the dynamics of the estuarine mixing, and chemical and biological processes, there is a shift in nutrient limitation or co-limitation along the estuarine waters, as observed in the Mississippi River-Gulf of Mexico (Dortch & Whitledge 1992, Lohrenz et al. 1999), in Chesapeake Bay (Fisher et al. 1999), and in the St. Lawrence River-Gulf of St. Lawrence region (Levasseur et al. 1990). A previous study showed that waters south of Hong Kong Island were on the edge of the coastal plume, and chl *a* was found to be maximal there in July (Yin et al. 2000). On the basis of depletion of ambient PO_4 and SiO_4 and excess N, co-limitation of P and Si was suggested (Yin et al. 2000). In this study, the edge of the coastal plume was found at least beyond Stn 28, a station east of Hong Kong, indicating that the coastal plume had moved across the entire Hong Kong coastal waters. The region of the chl *a* maximum was between Stns 26 and 28 (Yin et al. unpubl. data). Shipboard nutrient

addition experiments over 24 h showed that there was a shift in nutrient limitation from one area to another. P was the most limiting nutrient and Si was co-limiting at the edge of the coastal plume. Westward to the estuary, P was the only potential limiting nutrient over 48 h at Stns 26 and 31. Beyond the coastal plume, N was the most limiting nutrient with P co-limiting. The spatial shift in a nutrient limitation in estuarine regions is common in estuaries. For example, in the estuarine waters of the eastern Gulf of Finland, P limitation was found in the inner estuary, while the open eastern Gulf was mainly N limited (Pitkänen & Tamminen 1995). There was evidence of a shift from P to N limitation (Lohrenz et al. 1999), or N and Si co-limitation (Dortch & Whitledge 1992) in the Mississippi River influenced region of the northern Gulf of Mexico and in Chesapeake Bay (Fisher et al. 1999).

The results of the 48 h incubation were interesting. In the estuary, the large increase in phytoplankton *in vivo* fluorescence after 48 h might have resulted from a time lag after the light-limited phytoplankton in the turbid estuarine waters were incubated under saturating light. However, the decline in phytoplankton biomass at Stns 27 to 29 after 48 h cannot be explained with the present data. A similar phenomenon was also observed in our previous study (Yin et al. 2000). During July 1998, surface seawater samples were incubated for 24 h with no addition of nutrients in the same type of culture tubes. Phytoplankton chlorophyll increased on the estuarine (west) side of the regional chl *a* maximum and decreased on the eastern side (Yin et al. 2000). The difference between the 2 observations is that macro-nutrients were added to water samples in this study and the decline in phytoplankton fluorescence did not occur until after 24 h. The major phytoplankton species were diatoms in this study, including *Pseudonitzschia*, *Chaeotoceros*, and *Rhizosolenia* (Table 2). In the previous study (Yin et al. 2000), phytoplankton were mostly diatoms similar to those found in the present study (unpubl. data). Diatoms are usually not fragile when cultured in small tubes. Therefore, we do not believe that this phenomenon was an artifact. It is possible that a nutritional trace element such as a trace metal or even a vitamin may have become limiting because of high input of macro-nutrients from the river. Iron might be a good candidate as the limiting trace element, as observed for this region (Zhang et al. 1999). Iron binds to organic matter as a result of coagulation during the estuarine mixing (Sholkovitz et al. 1978, Bale & Morris 1981). P is absorbed onto iron oxide-organic matter and coagulates in significant amounts (Sholkovitz 1976). When P became limiting to the phytoplankton assemblages as shown in the P addition experiments at Stns 27 and 28, it is possible that iron could have become the next lim-

iting factor if other nutrients had still been abundant. This is somewhat analogous to a coastal upwelling regime where iron limitation has been reported (Hutchins & Bruland 1998). The interaction between the limitation by iron and by N has been observed in the tropical Pacific Ocean (DiTullio et al. 1993). High temperatures at the sea surface in our tropical waters (29 to 30°C) may have accelerated the rate of such a limitation.

Summary

The Pearl River is one of the largest rivers in the world and, therefore, its impact on the ecosystem of the Pearl River estuary and its receiving coastal waters around Hong Kong is large. In the present study, the coastal plume was found to cover a large area of the northern part of the South China Sea. The distribution of nutrients such as NO_3 and SiO_4 was coupled with this estuarine circulation. Much of the discharged riverine nutrients were not utilized in the estuary and not even in the coastal plume 30 km offshore in the downstream direction. The unique feature of the Pearl River estuary in terms of nutrients is that it is very low in PO_4 relative to N and SiO_4 when compared with other large estuaries such as the Amazon, Mississippi, Yangtze, and Rhine Rivers. Unfortunately, we do not know whether nutrient loading in the Pearl River has increased in the past as a result of the rapidly growing human population in the Pearl River delta in the last decade (Hills et al. 1998).

These results have significant implications for local nutrient pollution control. There is a debate in Hong Kong over whether the Hong Kong government should build secondary and tertiary sewage treatment facilities. The removal of N has been debated, but not that of P. However, our observations suggest that the removal of P through sewage treatment as a means of controlling nutrient effluent discharge into the coastal waters in the vicinity of the Pearl River estuary needs further consideration. With regard to oxygen, low oxygen waters become a concern in summer or during the wet season only for waters south of Hong Kong (EPD 2000). In this regard, P can be more important in limiting phytoplankton growth and biomass during summer. Coincidentally, chemically enhanced treatment of domestic sewage that is mostly deployed in Hong Kong removes much of the P. Thus, the objective of controlling the oxygen level may be achieved. However, the dilemma is the uncertainty that a further increase in the ratios of N:P and N:Si may change species composition of phytoplankton and cause a consequence of trophic transfers along the food chains. A comprehensive study is needed to solve this issue.

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