Seasonal variation of size-fractionated phytoplankton in the Pearl River estuary

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To investigate the dynamics of phytoplankton size structure in the Pearl River estuary, concentrations of size-fractionated chlorophyll *a* (Chl *a*) were determined during four cruises carried out in 2008 and 2010. The distribution of Chl *a* in this geographical location showed a high degree of temporal variation. Chl *a* concentrations were highest in autumn, approximately three times higher than those in summer and winter. Microphytoplankton was the dominant contributor, accounting for 66.9% of the Chl *a* concentration in autumn 2008. In summer and spring 2008, nano-sized cells dominated the phytoplankton population throughout the study region. During the winter cruise, two different areas of water were found, characterized by (1) low salinity and high nutrient content and (2) high salinity and low nutrient content; nano- and picophytoplankton co-dominated the first area, while microphytoplankton dominated the second. It is arguable that grazing could have played a role in determining phytoplankton community size structure in winter. Nutrient concentrations were assumed not to limit phytoplankton growth during the investigation period. Size-differential capacity in competing for the resources available under different hydrodynamic conditions seemed to be the major factor in determining seasonal variation in the structure of the phytoplankton communities. High N:P ratios in the Pearl River estuary had major implications for nutrient pollution control. Our results indicated that studies of phytoplankton size structure provide greater insight into phytoplankton dynamics and are necessary to better manage water quality in the Pearl River estuary.

phytoplankton, dominant species, size-fractionated chlorophyll *a*, Pearl River estuary

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Phytoplankton are a critical component of coastal and estuarine ecosystem food webs and phytoplankton community dynamics remain the most important issue in marine ecology [1,2]. Studies of the biological community from a size-based perspective can lead to a better understanding of phytoplankton dynamics [3]. Furthermore, the size structure of marine phytoplankton assemblages reflects responses to environmental conditions [4–6]. Under oligotrophic or light-limiting conditions, small cells have a higher capacity for nutrient and light acquisition [7–9], whereas under favorable conditions for growth, namely high irradiance and nutrient concentrations, large-sized cells have a higher pho-

tosynthetic efficiency and a higher growth rate [3,6,10–12].

The Pearl River estuary is located in southern China. The Pearl River itself is the second largest river in China and the 13th largest river in the world in terms of discharge volume. The yearly average river discharge is $10524 \text{ m}^3 \text{ s}^{-1}$, with 20% occurring in the dry season (October–March) and 80% occurring in the wet season (April–September) [13]. The river water empties via eight major channels into the South China Sea. Four of these enter the Lingdingyang estuary, a main subestuary of the Pearl River estuary, accounting for about 50%–55% of the Pearl River water flow; the remaining 45%–50% discharges directly into the South China Sea [14]. With several economic and industrial centers around the Lingdingyang estuary, such as Hong Kong, Macau,

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Shenzhen and Guangzhou, the delta region of the Pearl River is one of the most densely populated areas in China [15]. Over the past few decades, excessive wastes have been released into the estuary due to massive economic growth and urban development in the region [15–17]. The increased wastes provide nutrients that promote primary productivity in the receiving coastal waters and cause algal blooms. Eventually they result in eutrophication if the algae are not grazed by zooplankton [18], and such eutrophication has been a major issue in terms of the Pearl River estuary. To obtain an insight into the ecological effects of nutrient enrichment, one approach is to fractionate phytoplankton assemblages into different size classes, since cell size influences the response of phytoplankton communities to perturbation in the environment [19,20]. There have been several studies on phytoplankton biomass and size distribution of phytoplankton in the Pearl River estuary [21–24]. In our study, we investigated the dynamics of phytoplankton biomass in terms of chlorophyll *a* (Chl *a*) content to reveal the spatial and temporal distribution patterns of total and size-fractionated phytoplankton biomass over an annual cycle.

1 Materials and methods

1.1 Sampling stations

The present study was based on four research cruises during the following time periods: April 2008, July 2008, October 2008 and January 2010. The sampling sites were located in the coastal water of Zhuhai (22.1°N–22.3°N, 113.5°E–114°E). A total of two transects were employed, with one transect (transect Z1–Z4) in the inner part of the estuary and the other (transect Z5–Z8) in the outer part of the estuary (Figure 1).

1.2 Environmental factors

Surface seawater was sampled to determine various environmental factors. A YSI 6600 multi-parameter water quality monitor (Yellow Springs Instrument Co., USA) was used to measure salinity and temperature. Surface water samples for the determination of inorganic nutrients (nitrite, nitrate, ammonium, phosphate and silicate) were filtered through 0.7 μm Whatman GF/F filters and stored at -20°C until analyses were performed. The determination was performed using a SKALAR Continuous Flow Analyzer, using spectrometric methods described previously [25].

1.3 Size-fractionated Chl *a*

The phytoplankton was divided into three classes: micro-(>20 μm), nano-(2–20 μm), and picophytoplankton (<2 μm), and size fractions of Chl *a* were derived from sequential filtrations. Water samples taken from the surface water were first filtered through 20 μm Nitex via gravity. Filtrates were

Figure 1 Sampling stations in the Pearl River estuary.

then sequentially filtered through 2 μm Millipore Polycarbonate Nuclepore and 0.7 μm Whatman GF/F filters under low vacuum pressure (<100 mmHg). Chlorophyll retained by the 20- μm filters was considered microphytoplankton biomass, and that retained by the 2 and 0.7 μm filters constituted the nano- and picophytoplankton fractions, respectively. To determine the total Chl *a* concentration, 500 mL of water was directly filtered through a GF/F filter. After filtration, pigments were extracted in 10 mL of 90% acetone for 16–24 h in the dark at 4°C . The fluorescence was then measured using a Turner Designs Fluorometer (BWT2X7200) [26] both before and after acidification with 10% HCl.

1.4 Microscopic analysis of phytoplankton composition

Subsamples of 1000 mL of seawater were distributed in Pyrex bottles and fixed immediately with Lugol's Solution, yielding a final concentration of 4%. For microscopic observation, 10 mL of each fixed sample was settled in a sedimentation chamber for at least 24 h. The bottom area of the whole chamber was examined under an inverted microscope (Nikon E200) to identify species and to enumerate the abundance of phytoplankton, as previously performed [27].

1.5 Statistical analysis

Pearson correlation analysis was used to determine relationships between the chlorophyll content of each phyto-

plankton size class and the various physical and biological variables reported within each cruise and across all cruises. Analysis was performed using the SPSS 13.0 statistical package for Windows.

2 Results

2.1 Environmental factors

Temperature, in the range 17.06–27.56°C, varied seasonally

in the Pearl River estuary, with the lowest value occurring in winter (Figure 2(d)). In summer and autumn, temperatures were higher, averaging 26.68 and 27.11°C, respectively. Surface temperature varied little across stations (Figure 2(a)–(d)). In the case of salinity, values were consistently lower in the inner part of the study area than those in the outer part (Figure 2(a)–(d)), especially during the autumn cruise. The average salinity of the inner part of the study area was 9.55. This was almost three times lower than that found in the outer part of the study area, reflecting the

Figure 2 Spatial distributions of (a)–(d) temperature and salinity, and (e)–(h) nutrient concentrations during the four cruises.

strong interaction between fresh water and seawater.

Dissolved inorganic nitrogen (DIN) concentrations ranged from 6.35 to 123.08 $\mu\text{mol L}^{-1}$ during the investigation period. The seasonal variability revealed that DIN concentrations in summer and autumn were higher than those in spring and winter (Figure 2(e) and (f)). Phosphate (PO_4) concentrations varied between 0.11–2.52 $\mu\text{mol L}^{-1}$, with 87.5% of the values being lower than 1.0 $\mu\text{mol L}^{-1}$ (Figure 2(e) and (f)). Higher PO_4 concentrations ($>1 \mu\text{mol L}^{-1}$) were found in autumn in the inner part of study area (Figure 2(g)). Silicate (SiO_3) concentrations varied significantly from 14.11–327.37 $\mu\text{mol L}^{-1}$. Unlike phosphate concentrations, higher silicate concentrations were found in spring (Figure 2(e)). The lowest PO_4 and SiO_3 concentrations were observed during the winter cruise (Figure 2(h)). In winter, two different areas were identified: (1) An area of higher nutrient content and lower salinity (<30) (Z1–Z4, Z8); and (2) an area of lower nutrient content and higher salinity (>30) (Z5–Z7) (Figure 2(d) and (h)).

2.2 Total Chl *a* and size-fractionated Chl *a* concentrations

During the spring cruise, total Chl *a* concentration ranged from 4.40 to 15.60 $\mu\text{g L}^{-1}$. The highest Chl *a* concentration of 15.60 $\mu\text{g L}^{-1}$ was found at station Z4 (Figure 3(a)). The concentration of microphytoplankton Chl *a* ranged from 1.50 to 8.05 $\mu\text{g L}^{-1}$, and the highest microphytoplankton Chl *a* concentration was observed at station Z4. The proportion of the micro-sized fraction concentration to the total Chl *a* concentration varied between 16.4% and 51.6%. The concentration of nanophytoplankton Chl *a* concentration was in the range 2.15–8.75 $\mu\text{g L}^{-1}$, accounting for more than 50% at most stations (Figure 3(e)). Picophytoplankton Chl *a* concentrations in the spring cruise were generally less than 1.0 $\mu\text{g L}^{-1}$, with the exception being at Z6. The percentages of the pico-sized fraction were less than 20% (Figure 3(e)).

In summer, the total Chl *a* concentration ranged from 0.31 to 4.52 $\mu\text{g L}^{-1}$, with Chl *a* distribution gradually increasing seaward (Figure 3(b)). The average Chl *a* concentration in the inner part of the study area (0.74 $\mu\text{g L}^{-1}$) was almost four times lower than that in the outer part (3.04 $\mu\text{g L}^{-1}$). Size-fractionated Chl *a* concentrations showed similar trends to the total Chl *a* concentration, with high levels in the outer part of the river mouth. Nanophytoplankton was predominant in summer, making up over 80% of the phytoplankton biomass in half of the sampling stations (Figure 3(f)).

In autumn, Chl *a* concentration was significantly higher than that in the other three seasons. The range of total Chl *a* concentration was 3.03–18.01 $\mu\text{g L}^{-1}$. Unlike that of the summer and spring cruises, the Chl *a* level near the shore was relatively higher than that more offshore, with the highest value at station Z8 (Figure 3(c)). Similar trends were exhibited by the micro- and nano-Chl *a* concentrations.

Pico-Chl *a* concentrations were in the range 0.30–2.01 $\mu\text{g L}^{-1}$. High picophytoplankton biomass was only observed at station Z2 and in the rest of the study area, the Chl *a* concentration of picophytoplankton was generally in the range 0.30–0.57 $\mu\text{g L}^{-1}$. The phytoplankton community was dominated by microphytoplankton during the autumn cruise. The proportion of micro-sized fractions to total Chl *a* content ranged between 46.7% and 81.7%, with a mean of 66.9%. The nanophytoplankton was another important group, which was observed to occupy 15.3%–56.0% of the total Chl *a* content (Figure 3(g)).

In winter, the Chl *a* concentration varied from 2.27 to 3.87 $\mu\text{g L}^{-1}$. Unlike the other three seasons, there was not much spatial variation in the whole region (Figure 3(d)). Relatively high Chl *a* concentrations were found at station Z6, Z7 and Z2. Micro-Chl *a* concentration was in the range 0.39–2.22 $\mu\text{g L}^{-1}$ and increased seaward. Nano-Chl *a* concentration had the opposite distribution pattern, with higher levels in the inner part of estuary. It can be seen that nano- (35.9%–48.6%) and picophytoplankton (22.9%–44.6%) collectively dominated in stations Z1–Z4 and Z8 (Figure 3(h)), whereas microphytoplankton was significant at stations Z5–Z7 (Figure 3(h)). It should be noted that picophytoplankton increased its importance in January 2010, its contributions during that cruise being much higher than those of the other three cruises.

2.3 Phytoplankton biomass and dominant phytoplankton species

The phytoplankton in the Pearl River estuary exhibited a high species biodiversity. There were 178 phytoplankton taxa identified: 65% belonged to the Bacillariophyta, 16.4% to the Pyrrophyta, 9.0% to the Cyanophyta, and 9.7% to other species. Phytoplankton biomass ranged from 0.27×10^5 to $1.12 \times 10^8 \text{ cells L}^{-1}$, with a mean of $0.91 \times 10^7 \text{ cells L}^{-1}$. The maximum phytoplankton abundance occurred during autumn at station Z8, whereas the minimum occurred during summer at station Z1. The abundance of phytoplankton during the autumn cruise was two or three orders of magnitude higher than those of the other three seasons (Figure 4(a)–(d)).

Diatoms dominated the phytoplankton community during the winter, spring and autumn cruises (Figure 4(a), (c), and (d)), while blue-green algae dominated during the summer cruises (Figure 4(b)). The dominant population changed with an obvious seasonal succession. During the spring and autumn cruises, *Skeletonema costatum* dominated almost all of the sampling stations, accounting for 54.8%–97.0% (averaging 72.2%) of the total abundance in spring (Figure 4(e)) and 54.5%–99.6% (averaging 87.6%) in autumn (Figure 4(g)). However, abundance of *S. costatum* in spring was one or more orders of magnitude lower than in autumn (Figure 4(a) and (c)). In winter, *Chaetoceros curvisetus* was dominant in all stations (Figure 4(h)). Other major species

Figure 3 Spatial distribution of (a)–(d) total Chl *a* and (e)–(h) percentages of the micro-, nano- and pico-size fractionated Chl *a* concentration to total Chl *a* concentration.

included *Thalassiosira* spp., *Chaetoceros debilis* and *Chaetoceros socialis* (Figure 4(h)). Blue-green algae dominated the community during the summer cruise in most stations (Z3–Z8), and the dominant species varied with different stations (Figure 4(b) and (f)). *Pseudoanabaena* sp. was an important species in stations Z3–Z5, accounting for more than 30% of the total abundance. Comparably, *Merismopedia tenuissima* became the dominant species at stations Z6–Z8, representing at least 22.2% of the total abundance (Figure 4(f)). Diatoms were the most abundant taxa only in stations Z1 and Z2, with *Melosira* sp. being dominant in station Z1 and *Skeletonema tropicum* being dominant in station Z2.

2.4 Relationships between environmental variables and Chl *a* distribution

Table 1 shows the results of Pearson relation analyses of relationships between the Chl *a* concentrations of various phytoplankton size classes and various environmental properties. In spring and autumn, environmental variables were not correlated with any size class of Chl *a* (Table 1). Micro- and nano-Chl *a* classes were negatively correlated with phosphorus concentrations in summer. In winter, a significantly negative correlation between the micro-Chl *a* size class and nutrient concentrations (DIN, PO₄, SiO₃) was observed, whereas nano-Chl *a* was positively correlated with DIN and PO₄ concentrations. Nano-Chl *a* was negatively correlated with temperature in winter. We also observed negative correlations of pico-Chl *a* with temperature and silicate concentrations when values from all seasons and stations were pooled.

3 Discussion

Seasonal variation in the structure of phytoplankton communities is common in estuaries such as Chesapeake Bay [28], and contributions of size-fractionated Chl *a* in the Pearl River estuary showed a large seasonal fluctuation (Figure 3(e)–(h)). In spring and summer, nanophytoplankton formed the bulk of the phytoplankton biomass, which is consistent with results previously reported [23,24]. In autumn, the microphytoplankton was dominant in our study. However, picophytoplankton ($\leq 3 \mu\text{m}$) was most dominant in autumn 2005 [24]. We have no clear explanation for these differing observations. However, about ten days before our investigation in autumn 2008, tropical storm Higos arrived at Guangdong. As reported in previous studies, typhoons or tropical cyclones cause temporal influences on the phytoplankton. For example, remote sensing studies revealed that Typhoon Kai-Tak in 2000 triggered a thirtyfold increase in surface Chl *a* concentrations over the South China Sea [29]. Blooms of phytoplankton were also observed after cyclonic disturbances in subtropical coastal waters, and species

greater than $5 \mu\text{m}$ were responsible for the rapid increase of phytoplankton following a cyclone [30]. The average NO₃, NO₂, PO₄ concentrations were 43.65 ± 35 , 8.43 ± 8.21 and $0.78 \pm 0.55 \mu\text{mol L}^{-1}$, respectively, in September 2005 [24] and were 66.75 ± 38.47 , 8.71 ± 4.91 and $1.04 \pm 0.65 \mu\text{mol L}^{-1}$ in October 2008, indicating the higher nutrient levels after tropical storms. The fact that the phytoplankton was dominated by microphytoplankton in October 2008 might have been due to wind event-induced elevation of nutrient levels, which in turn reduced the levels of competition for nutrients between larger and smaller phytoplankton species. Thus, the photosynthetic rate of the former group might have been enhanced [30–32].

Among the factors affecting phytoplankton size structure in different marine ecosystems, temperature, light and nutrient supply have been suggested to be the major ones [9,11,33]. According to the criteria of Dortch and Whitledge [34], Si limitation occurs if Si:N < 1 and Si < $2 \mu\text{mol L}^{-1}$; P limitation occurs if N:P > 30 and P < $0.2 \mu\text{mol L}^{-1}$; and N limitation occurs when N:P < 10 and DIN < $1 \mu\text{mol L}^{-1}$. In our study, N:P was always higher than 10 throughout the sampling period and the minimum value of N concentration was $6.35 \mu\text{mol L}^{-1}$. Thus, phytoplankton growth was not limited by N content. Furthermore, Si:N > 1 and the minimum observed value of Si concentration was $16.02 \mu\text{mol L}^{-1}$ during the spring, summer, and autumn cruises. In winter, although Si:N was less than 1 in most of the sampling stations, Si concentration was always higher than $10 \mu\text{mol L}^{-1}$. Therefore, Si was considered not to be a limiting factor in the area. As a result of higher inputs of N from the Pearl River in summer, P potentially limits the amount of algal biomass during that season [35–37]. In the case of our study, although N:P was always high and occasionally more than 200, P concentrations were almost always higher than $0.2 \mu\text{mol L}^{-1}$. Therefore, P was assumed not to be the limiting factor for phytoplankton growth during our study. Thus, nutrient content was assumed to be saturated in our study area.

Previous studies [38] conclude that microphytoplankton abundance increases and picophytoplankton decreases as latitude increases. In the present work, statistical analysis indicated a significantly inverse correlation between pico-Chl *a* concentration and temperature ($P < 0.01$) when values from all seasons and stations are pooled (Table 1). However, it was also reported that surface temperature does not exert a direct control on picophytoplankton variability [39]. In the natural ocean environment, temperature and nutrient content are strongly covariant [33]. The inverse relationship observed between picophytoplankton and surface temperature can be explained from the correlation analyses. Consequently, when considering the effects of temperature on phytoplankton size structure, it is recommended that the concentration of nutrients be considered at the same time.

Figure 4 Spatial distribution of (a)–(d) major phytoplankton abundance and (e)–(h) percentage of total abundance.

Table 1 Pearson correlation coefficients for each independent variable and significance between phytoplankton Chl *a* versus temperature, salinity and nutrients^{a)}

Variable	Season	Number of measurements	Temperature	Salinity	DIN	PO ₄	SiO ₃
Micro-Chl <i>a</i>	Spring	8	0.431 ^{n.s.}	-0.401 ^{n.s.}	0.298 ^{n.s.}	0.410 ^{n.s.}	0.606 ^{n.s.}
	Summer	8	-0.536 ^{n.s.}	0.086 ^{n.s.}	-0.376 ^{n.s.}	-0.914**	-0.592 ^{n.s.}
	Autumn	8	-0.637 ^{n.s.}	0.413 ^{n.s.}	-0.456 ^{n.s.}	-0.669 ^{n.s.}	-0.526 ^{n.s.}
	Winter	8	0.644 ^{n.s.}	0.699 ^{n.s.}	-0.844*	-0.758*	-0.799*
	Across cruise	32	0.310 ^{n.s.}	0.233 ^{n.s.}	0.047 ^{n.s.}	0.044 ^{n.s.}	0.059 ^{n.s.}
Nano-Chl <i>a</i>	Spring	8	-0.241 ^{n.s.}	0.148 ^{n.s.}	-0.186 ^{n.s.}	0.062 ^{n.s.}	-0.171 ^{n.s.}
	Summer	8	-0.639 ^{n.s.}	0.182 ^{n.s.}	0.015 ^{n.s.}	-0.866**	-0.325 ^{n.s.}
	Autumn	8	0.052 ^{n.s.}	-0.297 ^{n.s.}	0.160 ^{n.s.}	-0.401 ^{n.s.}	0.076 ^{n.s.}
	Winter	8	-0.747*	-0.531 ^{n.s.}	0.726*	0.807*	0.641 ^{n.s.}
	Across cruise	32	0.211 ^{n.s.}	0.122 ^{n.s.}	-0.045 ^{n.s.}	-0.088 ^{n.s.}	0.344 ^{n.s.}
Pico-Chl <i>a</i>	Spring	8	-0.281 ^{n.s.}	0.330 ^{n.s.}	-0.527 ^{n.s.}	-0.536 ^{n.s.}	-0.637 ^{n.s.}
	Summer	8	-0.776*	0.254 ^{n.s.}	0.151 ^{n.s.}	-0.497 ^{n.s.}	0.025 ^{n.s.}
	Autumn	8	-0.131 ^{n.s.}	0.021 ^{n.s.}	-0.034 ^{n.s.}	0.001 ^{n.s.}	-0.034 ^{n.s.}
	Winter	8	-0.597 ^{n.s.}	-0.032 ^{n.s.}	0.152 ^{n.s.}	0.369 ^{n.s.}	0.034 ^{n.s.}
	Across cruise	32	-0.442*	0.512 ^{n.s.}	-0.308 ^{n.s.}	-0.219 ^{n.s.}	-0.363*

a) n.s., $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$.

In addition to nutrient concentrations and temperature, some authors suggest that phytoplankton size structure might be controlled primarily by hydrodynamics in the ocean [40]. Thus, water column stability determines the size-differential residence time of phytoplankton cells within the euphotic layer [9,40,41]. In the case of the Pearl River estuary, during the wet season, the whole estuary is highly stratified and greatly influenced by freshwater discharge. Due to strong stratification, high temperatures, and nutrient input from freshwater runoff, it is assumed that the micro-sized fraction is predominant in the phytoplankton biomass. However, as a result of high river discharge, especially during the period of maximal river discharge in June and July, the Pearl River estuary has a water residence time of less than three days [37,42], and the time might not be enough for phytoplankton growth, especially for microphytoplankton [24]. During the dry season, the Pearl River discharge is small, the water residence time is prolonged, and the water column is vertically homogeneous. Vertical mixing could have a favorable effect, e.g. upward motion could increase the residence time of large cells in the upper layer [41]. On the other hand, strong vertical motion could lift phytoplankton from the eutrophic zone, which would be in favor of the smaller cells that have an advantage under weak light conditions [3]. As a result, seasonal variations in the size structure of phytoplankton in the Pearl River estuary were, to a great extent, under the control of the size-dependent competitive abilities in a dynamic environment associated with physical processes such as river discharge, stratification and water vertical mixing.

Phytoplankton biomass and associated productivity may be dominated by large phytoplankton size fractions in waters supplied with high-nutrient concentrations [43]. Thus,

during the winter cruise, higher nutrient levels in Z1–Z4 and Z8 led to the expectation that the larger cells would exhibit greater abundance. On the contrary, phytoplankton Chl *a* was co-dominated by the nano- and pico-sized fractions, whereas in lower nutrient concentration conditions, microphytoplankton was dominant (Figures 2(h) and 3(h)). Light limitation of phytoplankton growth is suggested as an explanation for similarly observed phenomena [44]. This previous study revealed that growth of large cells in the York River estuary is dependent on nitrite, nitrate input, but only when light is unlimited. However, our study was based on data collected at the water surface. Thus, radiation was assumed to be saturated and non-limiting on phytoplankton growth. As previously discussed [32,45], zooplankton grazing also plays an important role in controlling the size distribution of phytoplankton communities. Large phytoplankton cells are more likely to be ingested by a mesozooplankton dominated by copepods [46–50]. Copepods are the most abundant mesozooplankton in winter in the Pearl River estuary [50,51], and salinity is one of the most important physical factors affecting zooplankton abundance and species richness in the Pearl River estuary [50–53]. In winter, mesozooplankton abundance and biomass are negatively related with salinity [51]. In <30 salinity areas, grazing pressure of zooplankton (especially copepods) is high [24,54]. Thus, in higher nutrient but lower salinity areas, the mesozooplankton might be abundant. Therefore, grazing pressure exerted on the microphytoplankton might be the reason that small-sized phytoplankton dominated in this area. This is the most likely reason why we observed that microphytoplankton Chl *a* was positively and negatively correlated with salinity and nutrient concentrations, respectively (Table 1).

In terms of the phytoplankton community, previous studies document that diatoms are always the main contributors in and near the Pearl River estuary [55,56]. Although the composition of dominant species varies in different years, *S. costatum* is always prominent in summer [62]. However, in the present study, fresh-water species of blue-green algae were dominant, representing 55.3% of the total phytoplankton abundance during the summer cruises (Figure 4(b) and (f)). The relative availability of nutrients plays an important role in structuring phytoplankton communities [57]. Diatoms have an absolute requirement for silicon [58]. Only after the depletion of nutrients (mainly silicate), would other phytoplankton species be expected to take over [59]. In our case, limitation of silicate did not seem to occur since silicate concentrations ($63.29\text{--}109.95\ \mu\text{mol L}^{-1}$) were high, with Si:N >1 and Si:P >16. As for PO_4 , it is suggested that a low concentration of phosphate limits the growth of diatoms in seawater [60], and that the growth of *S. costatum* in marine ecosystems can be limited when P concentration is less than $0.3\ \mu\text{mol L}^{-1}$ [61]. Within the range $0.39\text{--}0.94\ \mu\text{mol L}^{-1}$, our PO_4 values were comparable to those observed previously in several studies on the Pearl River estuary [23,62]. Hence, P concentrations during the summer cruise could not explain the replacement of diatoms by blue-green algae. Several recent studies note that there is a general trend of an increase in relative Cyanobacterial abundance with increasing water temperature, and decreased flushing rate [63,64]. During June 2008, cities located in the upper reach of the Pearl River drainage basin, including Guangzhou, Dongguan and Foshan, had the highest rainfall in the all-time record. Taking Guangzhou as an example, the average rainfall was 878.1 mm in June 2008, about 40% of the annual total precipitation. The air average temperature was 26.7°C . While in July, the precipitation decreased to 231 mm, accompanied by an increasing air temperature to 28.5°C (<http://www.gzstats.gov.cn>). In other years, the differential precipitation between two consecutive months was not as great as we found in June and July 2008. As we know, rainfall is generally positively related to river flow, air temperature can reflect water temperature, and massive river effluents may bring large amounts of blue-green algae. When the flushing rate was markedly decreased in July 2008, accompanied by increasing temperature, the blue-green algae might have responded prior to the diatoms to the dramatic changes in the environment. Thus, dominance of the blue-green algae probably suggested their quicker response relative to diatoms to the markedly decreased flushing rate in July 2008 relative to June 2008.

The Pearl River estuary is the principal receiving water body of land-based pollutants destined for the South China Sea. In the past decades, the large amounts of waste water coming from industrial and domestic sources were only partly treated, but were discharged into the river or coastal waters directly. In the Pearl River delta, domestic sewage is more than $6.0\times 10^8\ \text{m}^3$ per year (not including Hong Kong

and Macau). Less than 30% is treated, and the remainder is discharged without any treatment [15,65]. Agricultural fertilizers and mariculture are also main sources of pollutants for the estuary [15]. DIN in the Pearl River estuary mainly comes from the four river channels in the main estuary [15]. However, relative to the PO_4 from the vertical mixing of deep water to the surface, the PO_4 load from the river does not enhance the PO_4 concentration [36]. Results from an earlier study indicate that the amounts of DIN inputted from four river channels (Humen, Jiaomen, Hongqimen, Hengmen) are about 30 times more than those of phosphate [15]. Due to the anthropogenic loading of nutrients in the rivers disproportionally increasing N and P, a 100N:1P ratio (about seven times higher than the normal Redfield ratio of 16N:1P that is optimal for phytoplankton growth) occurs [36]. In the present study, N:P ratios generally exceeded 16:1, with 62.5% greater than 100:1. A high N:P ratio has become a characteristic feature of coastal areas affected by freshwater discharge [60,66]. In the other large estuaries of China, such as the Yellow River estuary [67], and the Changjiang estuary [60], a higher N:P ratio is also observed. Alterations in nutrient ratios, such as N:Si and N:P as a result of excess N relative to Si and P, can change phytoplankton biomass and species composition [68,69]. In the Black Sea, the increasing N and P relative to Si results in the dominance of phytoplankton such as coccolithophores and flagellates instead of diatoms [70]. In the Bohai Sea, the replacement of diatoms by dinoflagellates is the major characteristic of variations in the phytoplankton community in recent years, which probably result from increasing N:P ratio and decreasing Si:N ratio [71]. In such a case, the result may have significant implications for local nutrient pollution control. The removal of N through sewage treatment as a means of controlling nutrient effluent discharge into the coastal waters in the vicinity of the Pearl River estuary should be considered.

In spring, *S. costatum* was most abundant (Figure 4(a) and (e)), accounting for 72.2% of the total phytoplankton cell count. It is worth noting that *S. costatum* dominated the phytoplankton assemblage in autumn as well (Figure 4(c) and (f)), and accounted for 87.6% of the phytoplankton biomass. This result is supported by a study [24], and similar results are shown in 1999–2000 in the same estuary, where *S. costatum* was observed as the dominant species in both summer and winter [62]. Other results [72] reveal that this chain-forming diatom is capable of tolerating extreme physico-chemical conditions, offering an explanation as to why it is dominant in the phytoplankton assemblages of both the spring and autumn cruises. However, the nano-sized fraction dominated the phytoplankton population in spring, whereas the micro-sized fraction was dominant in autumn. We might attribute this to the survival strategy of *S. costatum* adapting to changes of its environment. Phytoplankton changes its structure, composition and physiology

as an adaptation to altered environments [73] and, in a study on several species of epilithic diatoms, size variation in a natural population within a diatom species is described and they are found to respond to environmental conditions very differently [74]. From the results of a monitoring and subsequent mesocosm study [75], cells of *S. costatum*, of both larger (20–200 μm) and smaller fractions (2–20 μm and to a lesser extent 0.2–2 μm) are found to exist in the water column at the same time, and the photosynthetically active radiation quantum controlled the relative abundance of one size class over the other. Since our study was conducted at the water surface, light conditions might be ruled out as a controlling factor to determine the size class of *S. costatum*. On the other hand, the identification of phytoplankton <5 μm in size is difficult with an inverted microscope and, possibly, a large number of phytoplankton with cell size between 2 and 5 μm existed in the water column from the spring cruise, suggesting that analyses of phytoplankton size structure are necessary to better understand phytoplankton dynamics.

Physical fractionation using differential filtration is the most widely used method to group phytoplankton into different size classes. Despite its simplicity and widely accepted use, precise control of the filtration technique is difficult, since the size separation is influenced by a variety of factors, such as the filter type, vacuum, and characteristics of the algae (for instance, cellular shape) in the sample. In the present study conducted in the Pearl River estuary, the dominant species were nano-chain-forming species, such as *S. costatum*, *Ch. curvisetus*, and *Thalassiosira* spp. (except for in summer). These species are normally in chains with lengths more than 20 μm but comprised of many cells with sizes less than 20 μm . Therefore, they might pass through the 20 μm filter when their chain lengths were less than 20 μm or when the cell colonies were oriented in such a way that their diameters were less than 20 μm . However, the pass rate of *S. costatum* through the 20 μm mesh net was only 14.37% [76]. In summer, the dominant blue-green algae were colonial species with width or length larger than 20 μm , such as filamentous *Trichodesmium* spp., filamentous *Pseudoanabaena* sp., and rectangular *M. tenuissima*. Thus, micro-Chl *a* concentration measurements included some of those cells as part of the nano-fraction. This may be another possible explanation why the dominant species was *S. costatum* in both spring and autumn, while the dominant size classes were different.

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