Spatio-temporal variability of phytoplankton assemblages in the Pearl River estuary, with special reference to the influence of turbidity and temperature

Ping-Ping Shen, Gang Li, Liang-Min Huang, Jian-Lin Zhang, Ye-Hui Tan

Key Laboratory of Marine-Bioreresources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China

1. Introduction

Estuarine ecosystems, recognized as transitional zone linking fresh- and marine-water, are becoming increasingly affected by near-shore human activities (Spatharlis et al., 2007). One of the major characteristics of these areas is environmental gradients associated with mixing of turbid and nutrient-rich freshwater with clear and nutrient-poor seawater, especially the sharp salinity and turbidity clines that are usually used to establish apparently objective distinct zones in estuaries worldwide (Elliot and McLusky, 2002). The environmental gradients often strongly influence the species composition, distribution and abundance of estuarine biological communities (Muylaert et al., 2000; Giberto et al., 2007; Shen et al., 2010). Phytoplankton assemblages are among the most important components of estuarine ecosystems, as they provide a vital link between environment and organic matters. Their abundance and composition vary with environmental changes, such as temperature, salinity, light and nutrient levels (e.g. Blanco et al., 2008). Many studies have demonstrated that nutrient enrichment significantly affect the phytoplankton community by altering its composition and diversity (Piehler et al., 2004; Buyukates and Roelke, 2005). In some extreme cases, nutrient discharges often lead to the development of massive algal blooms (Anderson et al., 2002; Beman et al., 2005). It is particularly true for estuarine ecosystems, which are the most frequent sites of phytoplankton blooms due to the high river nutrients-load (Smith and Demaster, 1996; Lohrenz et al., 1999).

The Pearl River Estuary (PRE) is located in subtropical areas, the northern part of the South China Sea (Fig. 1). The plume of Pearl River presents a distinct monsoonal behavior due to the influences of South–west Monsoon in summer (wet season, April–September) and North–east Monsoon in winter (dry season, October to next March) (Zhao, 1990), which also influences the hydrographic characteristics nearby (Dong et al., 2004; Su, 2004). As a consequence, the annual rainfall ranges from 1600 to 2300 mm and the annual water flow rate of the Pearl River is about 11,100 m³ s⁻¹, with 20% of the discharge occurring during the dry season and 80% during the wet season (Zhao, 1990; Wong et al., 1995). On the other hand, the rapid development of the PRE district has brought about environmental pressures on this estuary ecosystem during the last three decades. The annual discharges of industrial waste- and sewage-water have risen to as much as 200
and 40 million tons, respectively (Wong et al., 1995), resulting in the frequent occurrence of the harmful algal blooms (HABs) in this estuary (Qi et al., 2004). Therefore, many studies have so far been carried out to characterize its hydrodynamics, environmental factors as well as biological features, such as Dong et al. (2004) depicted the seasonal variation and dynamics of the Pearl River plume; Lin et al. (2003) described the Chemical Oxygen Demand (COD) distribution during wet season and Yin et al. (2006) mainly investigated the environmental changes during dry season. The features of phytoplankton bulk biomass and primary productivity in PRE were also involved by Huang et al. (1995, 1997, 2004), as well as the distributions of geochemical parameters by Huang et al. (2003), Lin et al. (2003) and Yin et al. (2001, 2004). Some other studies are also concerned with the relationships of the biotic and abiotic properties in the PRE (e.g. Yin et al., 2000; Qiu et al., 2010). However, most of previous studies were rather descriptive and poorly interpreted, and also badly off the robust statistical analyses (e.g. Huang et al., 2004; Qiu et al., 2010). It is generally regarded that there is more than one single source of nutrients and physical disturbance in estuaries and the biotic integrity is thus under a cumulative effects of all physico-chemical stressors; that is, the effect of eutrophication may be disturbed by physical factors, such as freshwater and sediment discharge or tidal currents. In this case, analysis of community structural changes seem to be more sensitive than merely geochemical parameters in detecting environmental disturbance; however, little has so far been documented on such analyses (Anderson and Gribble, 1998; Clarke and Gorley, 2006), especially for the Pearl River estuary.

The objective of this study is therefore to investigate the spatial and temporal variabilities of phytoplankton assemblages and analyze the cumulative effects of various physical and chemical factors in both dry and wet seasons from the Pearl River estuary. The key factors or their combinations would be found to explain the regulation of the changes in phytoplankton community through the multivariate analyses.

2. Materials and methods

2.1. Description of the study area

The PRE is created by the inflow of freshwater from the Pearl River to the South China Sea (SCS). The Pearl River consists largely of 3 tributaries: the West River, the North River and the East River and forms 8 outlets before entering the SCS. Four of the outlets open to the Pearl River estuary and three of them (Jiaomen, Hongqimen and Hengmen) are all located in the north–west side of the PRE (Fig. 1). The river system covers an area of more than 8000 km2 during periods of peak discharge with a watershed of 230,000 km2. The annual rainfall ranges from 1600 to 2300 mm and the annual water flow rate of the Pearl River is about 11,100 m3 s⁻¹, with 20% of the discharge occurring during the dry season and 80% during the wet season (Zhao, 1990; Wong et al., 1995). The whole study area is within the subtidal zone with strong freshwater and marine water interactions and circulation currents along the west coast (Wong et al., 1995). It is profoundly influenced by three water regimes: the Pearl River, SCS and south China coastal current. Consequently, the dynamical variation and biogeochemical processes of nutrients and physical process in the estuary are very complicated (Zhang et al., 1999; Dong et al., 2004).

2.2. Field sampling and laboratory analysis

Field sampling was conducted during the spring (April 08–10, wet season) and autumn (October 31–November 03, dry season) in 2009. Eighteen stations along the north–south transect from...
upper estuary (freshwater dominated: FW) to estuary (mixing region: ES) and marine (MA) sectors were determined by Global Position System. Stations (1–4) were in FW sector, which was close to the river mouth. Stations (5–15) were in ES sector, which was in the mixing region of the estuary and stations (16–18) were in MA sector, which was in the oceanic waters. At each of the 18 stations, water samples at different depth (surface, sub-surface, middle-layer and bottom) were taken using a 2.5 l Niskin bottle sampler. Vertical profiles of temperature, salinity and turbidity of the water column were measured using an YSI instrument (Yellow Springs Instrument Co., USA). Sub-samples for phytoplankton (each 1000 ml) were immediately removed and preserved in approximately 1.5% Lugol’s and stored in plastic bottles before treated in the laboratory.

Sub-samples for dissolved nutrients (SiO₃, PO₄, NH₄, NO₃ and NO₂) were also taken at each station. After collection, the water samples were filtered through 0.45 µm cellulose filters for inorganic nutrients determination. All water samples were preserved at −20 °C in dark before further processing in the laboratory. The inorganic nutrients were measured by colorimetric methods and technique details were described earlier by Yin et al. (2001).

2.3. Phytoplankton analysis

Sample processing followed a method that was widely used for phytoplankton monitoring projects. For each sample a 50 ml-subsamples was concentrated using Utermöhl Settling Chamber for at least 24 h. Supernatant was aspirated so that the sample was concentrated to 1 ml. The concentrated sample was then examined under an Olympus BX51 microscope. Phytoplankton was identified to species or genus whenever possible, or class and family otherwise. For those species, which could not be quantified accurately, such as colony or filaments, estimations were made.

2.4. Statistical analysis

Multivariate analyses were used to detect any spatial and temporal differences in the species composition and abundance

Fig. 2. Contours of temperature (°C) along the estuary (Stations 1–18), (A) surface; (B) bottom in spring; (C) surface and (D) bottom in autumn.
of the phytoplankton assemblages, and to assess, which taxon mainly contributed to the spatial and temporal variability (Software PRIMER v6). Though collected at multiple depths, the phytoplankton abundance and composition were similar among depths \( P > 0.05 \). Therefore, the species abundances (after square root transformation) at multiple depths were treated as replicates for each station and the statistical analysis was performed between seasons and stations at one level. Similarity matrices were constructed using Bray–Curtis similarity (Clarke and Warwick, 1994). Non-metric multidimensional scaling (nMDS) was also applied to the similarity matrices to determine the similarity of sites with respect to phytoplankton composition. Following the cluster analysis, the species having the greatest contribution to the division of samples into cluster were determined using the similarity percentage program (SIMPER) (Clarke and Gorley, 2006).

ANOSIM was run to detect the effect of seasons and sampling sites (freshwater, estuary or marine sectors) on the composition and distribution of the phytoplankton in the PRE. For this test to be valid the data had to be normally distributed and homogeneity of variance. Where required, a \( \log(X+1) \) transformation function was applied to normalize the data and make the variance constant.

The BIOENV procedure was used to determine, which set of environmental variables (similarity calculated with the Euclidean distance coefficient) best explains the biological matrices (Clarke and Gorley, 2006). The environmental variables included temperature, salinity, turbidity and nutrients such as SiO\(_3\), PO\(_4\), NH\(_4\), NO\(_3\) and NO\(_2\). Prior to analysis, a draftsman plot (scatter plots between pairs of environmental variables) was used to assess the linearity of the data and the inter-correlation between variables. Variables with high degree of correlation (Spearman’s correlation coefficient \( p_s > 0.9 \)) were omitted from the BIOENV analysis. All parameters were transformed with the \( \log(X+1) \) and multivariate analysis were carried out using PRIMER software (Clarke and Gorley, 2006).

**Fig. 3.** Contours of salinity along the estuary (Stations 1–18), (A) surface and (B) bottom in spring; (C) surface and (D) bottom in autumn.
3. Results

3.1. Environmental factor gradients

There were significant differences in environment factors either between seasons ($R = 0.607, P < 0.01$, ANOSIM) or water locations ($R = 0.389, P < 0.01$, ANOSIM). The average temperature, salinity and turbidity of water column in spring were 20.74°C, 24.26 and 700.61 NTU, while in autumn were 25.77°C, 25.38 and 59.95 NTU, respectively. Big differences were observed especially in temperature and turbidity. Horizontal profiles of these parameters showed a relative low-temperature, low-salinity while high-turbid estuary plume covering the waters north–west of the PRE in spring, indicating the strong influence of freshwater discharge from 4 river gates on the north–west side of the PRE during the wet season. In autumn, however, high-temperature, high-salinity and clear oceanic water infiltrated from the south–east towards the north–west of the PRE, pushing the edge of a strong coastal plume to the intake of the Pearl River estuary (Figs. 2–4). As a consequence of low flow and high residence times in autumn, the difference between estuary (ES) and marine sectors (MA) was not on a significant level, indicating an oceanic water dominated estuary during the dry season. For instance, the surface salinity of FW, ES and MA sectors in autumn were 14.66, 25.99 and 30.86, and the bottom were 19.00, 27.47 and 32.84, respectively, which shows the mark difference in salinity between FW and ES/MA. Despite the extreme case in autumn, there was still significant variation between upper estuary and estuary/marine sectors (FW vs. ES: $R = 0.581, P < 0.01$; FW vs. MA: $R = 0.754, P < 0.01$, ANOSIM) which meant there was a typical estuarine gradient along the north–south transect from inner stations 1–4 (FW) to outer stations (ES and MA). Moreover, the halocline and turbidity degree between the surface and bottom was sharp inside the estuary, indicating apparent outward flow of the surface layer and inward flow of the bottom layer (Figs. 3 and 4).

Fig. 4. Contours of turbidity (NTU) along the estuary (Stations 1–18), (A) surface; (B) bottom in spring; (C) surface and (D) bottom in autumn.
Concentration of nutrients were associated with the physical gradient and also spatio-temporally varied in the estuary. Nutrient levels were decreasing from the inner part to the outside the estuary, indicating an apparent gradient from stations 1 to 18 with a potential influence of riverine plume. For example, both surface and bottom concentrations of N, P and Si were much higher in FW than those of ES and MA sectors (Table 1). In addition, except the concentration of silicate, the nutrient levels in spring were much higher than those of autumn, reflecting higher river discharge in wet season than in dry season. Though the average concentration of silicate were much higher in autumn than in spring, it is difficult to find a general pattern of silicate distribution among the sampling locations (Table 1). But interestingly, when comparing with the phytoplankton abundance, the higher concentration of silicate in the PRE was exactly coinciding with the lower abundance of phytoplankton, especially in the dry season.

### 3.2. Species composition and diversity of phytoplankton

A total of 162 phytoplankton species belonging to 7 phyla were recorded during the two sampling efforts in 2009, including Bacillariophyta, Pyrrophyta, Chlorophyta, Cyanophyta, Haptophyta, Chrysophyta and Xanthophyta. In terms of species number, diatoms were the most dominant group with 124 taxa, followed by dinoflagellates with 30 taxa and other algal groups were totally 8 taxa. Among them, 97 species in 5 phyla were collected in spring while 115 species from 6 phyla were observed in autumn. Although the diatoms dominated in both seasons, the species number and abundance of dinoflagellates proliferated in autumn significantly, with a massive bloom of dinoflagellates *Cochlodinium geminatum*, coupled with diatom *Skeletonema costatum* and Haptophyta *Phaeocystis globosa* occurred from the end of October to the beginning of November, 2009. The percentage contribution of each microalgal group was illustrated in Fig. 5.

The species richness (S) and Shannon–Wiener diversity (H'(log_e)) were generally low in the PRE, which ranged from the highest 28 species at station (13) in autumn to the lowest 1 species at stations (4 and 11) in spring, and diversity varied between 0 at stations (4 and 11) and 2.37 at station (17) in spring (Fig. 6). Seasonally, species richness and diversity were much higher in autumn (average $S=17.35 \pm 5.42$; $H'=1.53 \pm 0.39$) than those of spring (average $S=6.81 \pm 4.43$; $H'=1.15 \pm 0.64$), while the evenness ($J'$) were much lower in autumn (average $J'=0.55 \pm 0.15$) than that of spring (average $J'=0.68 \pm 0.25$). The low evenness in autumn indicated that the phytoplankton community was dominated by a few species, coinciding with the observation of the blooms of *C. geminatum*, *S. costatum* and *P. globosa* in the study area during the autumn sampling. Spatially, species richness, diversity and evenness increased from the river mouth to outside of the estuary, with the lowest values was found in riverine side and the highest in marine region.

Moreover, marked changes of abundance were also found either between seasons or locations (Fig. 5). Due to the outbreak of the dinoflagellates bloom in the estuary, the total abundance of phytoplankton increased in autumn with orders of magnitude. The highest density ($2.93 \times 10^5$ cells l$^{-1}$) was recorded at the surface water of station (15) in autumn, where the center of bloom was observed during the sampling period. While in the normal status such as in spring, the phytoplankton abundance were much greater in upper estuary (stations 1–5) than those of mixing and ocean regions (stations 6–18, Fig. 5), reflecting the influence of freshwater discharge from the rivers.

<table>
<thead>
<tr>
<th>Season</th>
<th>Station</th>
<th>Surface (mg l$^{-1}$)</th>
<th>Bottom (mg l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NO$_3$</td>
<td>NO$_2$</td>
</tr>
<tr>
<td>Spring</td>
<td>FW average</td>
<td>1.871</td>
<td>0.184</td>
</tr>
<tr>
<td></td>
<td>ES average</td>
<td>0.894</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>MA average</td>
<td>0.401</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Total average</td>
<td>1.029</td>
<td>0.093</td>
</tr>
<tr>
<td>Autumn</td>
<td>FW average</td>
<td>0.735</td>
<td>0.134</td>
</tr>
<tr>
<td></td>
<td>ES average</td>
<td>0.278</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>MA average</td>
<td>0.168</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Total average</td>
<td>0.361</td>
<td>0.055</td>
</tr>
</tbody>
</table>

3.3. Phytoplankton community structure

Classification and ordination analysis separated sampling stations, at 8.9% of similarity, into two main groups, reflecting high heterogeneity between the wet and dry seasons (Figs. 7 and 8). Moreover, under each season cluster, three sub-clades corresponding to the FW, ES and MA sectors were defined with multivariate analysis (Figs. 7 and 8). The ANOSIM analysis based on the abundance of phytoplankton also confirmed the significant difference both in time and space (ANOSIM: season effect, $R=0.896$, $P<0.01$; station effect, $R=0.463$, $P<0.01$). Due to the homogeneity of ES and MA sectors and dominance of dinoflagellates in October and November (Fig. 8), the autumn group comprised higher similarities (19.2% average similarity) than the spring group (13.0% average similarity) (Fig. 7). Moreover, the FW or MA group comprised higher similarities than the ES group, showing that the phytoplankton communities in middle of the estuary were more variable due to the influence of diffusive mixing of the freshwater and oceanic water (Figs. 7 and 8). On the contrary, the FW or MA was dominated by relatively homogeneous oligo- or hyperhaline water and the phytoplankton community structure was rather stable and uniform. So according to the SIMPER results, the species contributing the greatest to the division of samples into different groups were *S. costatum*, *Coscinodiscus*, *Chaetoceros curvisetus*, *Lauderia borealis*, and *C. geminatum* in time and *S. costatum*, *P. globosa*, *Coscinodiscus* and *C. geminatum* in space (SIMPER, 50% cutoff).

3.4. BIOENV analysis results

The subset of environmental variables that displayed the strongest correlation with phytoplankton patterns comprised of the temperature and turbidity (BIOENV, $r_w=0.49$, $P<0.01$), followed by a second subset with the same factors and the addition of PO$_4$ ($r_w=0.46$). This result indicated that temperature and turbidity were responsible for nearly 50% of variation in phytoplankton assemblage structure between the wet and dry
seasons in the PRE. Among single environmental factors, the temperature showed the highest correlation ($q_w = 0.45$), followed by turbidity ($q_w = 0.38$), NH$_4$ ($q_w = 0.23$), PO$_4$ ($q_w = 0.16$), salinity ($q_w = 0.12$), NO$_3$ ($q_w = 0.10$), NO$_2$ ($q_w = 0.04$) and silicate ($q_w = 0.04$). Thus, the best 2-variable combination (temperature and turbidity) showed a significant effect on the pattern of phytoplankton, the “proverbial” nutrients and salinity gradient here, however, were not important factors in the PRE neither in time nor in space.

### 4. Discussion

Both seasonal and spatial variabilities of phytoplankton community were observed in the Pearl River estuary, with two major groups (representing the wet and dry seasons) and several sub-clades (corresponding to the upper estuary, estuarine and marine sectors) were clearly defined at a statistical significant level (Cluster and nMDS ordination). In particular, BIOENV analysis identified the best combination of physical properties (water temperature and turbidity), which were most responsible for the observed structure of the phytoplankton community in the PRE. It was characterized by a continuum of phytoplankton assemblages along these major environmental gradients, as the temperature alone distinguished the samples between seasons and the turbidity, to some extent, separated the samples among water sections (Figs. 7 and 8). With the adding of other factors’ effect, such as the salinity and nutrients, the position of the samples would be displaced, reflecting the interactions among ambient elements. Multivariate statistical analysis thus provided a new point of view in studying the phytoplankton diversity.
dynamics in relation to environmental factors in the Pearl River estuary system (Clarke and Gorley, 2006).

As mentioned above, estuaries are the most impacted and highly eutrophicated habitats by human activities for decades, which result in high organic and inorganic nutrient concentrations in the system. A central challenge of estuary ecology is searching for the interacting spatial and temporal components of environmental variability that combine to drive changes in phytoplankton biomass (May et al., 2003). Numerous studies have been primarily devoted to nutrients, fluxes of organic constituents and phytoplankton within the estuaries and/or their associated plumes and coastal regions (Lohrenz et al., 1999; Yin et al., 2000, 2001; Ringuet and MacKenzie, 2005; Harrison et al., 2008). Despite occasionally P-limitation and/or Si-depleted during short periods, nitrogen and phosphorus are unlikely to limit the growth of phytoplankton populations due to the continuous high river discharge in the estuaries (Muylaert et al., 2000; Yin et al., 2000; Yin and Harrison, 2008). In present study, the massive dinoflagellates *C. geminatum* coupled with *P. globosa* blooms occurred in the PRE from October to November in 2009, when relatively lower nutrient concentrations were observed during the dry season (Table 1). In this case, physical or unusual climate conditions rather than the nutrient levels in water column might contribute to the development of the algal blooms. The results of multivariate analysis also supported this suggestion that the phytoplankton populations in the dry season were correlated best with salinity (BIOENV, $\rho_{w}=0.70$, $P < 0.01$). Therefore, numerical modeling analyses have been carried out to search for general principles that define phytoplankton population responses to physical dynamics characteristic of shallow, nutrient-rich coastal waters, which having complex bathymetry and influenced by tide, wind and river flow (Cloern, 1996; Yin et al., 2004).

Among the numerous physical gradients, salinity is thought to be the major factor responsible for diversity patterns in estuaries and has been intensively studied worldwide (Barron et al., 2002; Pilkaitytė et al., 2004; Muylaert et al., 2009). Those studies demonstrate that in most cases, salinity is closely correlated with the phytoplankton community as the salinity has a pronounced effect on the growth and distribution of microalgae (e.g. Pilkaitytė et al., 2004). While in some cases, changes in salinity associated with altered seasonal freshwater discharge have a limited impact on phytoplankton dynamics, such as in Swan River in Australia during 3-years study period (Chan, 2006). Similarly, though there was also a sharp gradient in salinity along the upstream to downstream of the Pearl River estuary, the effect is, however, not significant in changing the phytoplankton structure between the wet and dry seasons and high correlation between phytoplankton assemblages and salinity was observed only during the dry season when other factors, such as turbidity and temperature, were relatively stable. Thus, this short-term result should be interpreted with caution as salinity is not necessarily independent and is a function of riverine discharge, as well as the turbidity degree (Quinlan and Phlips, 2007).

The turbidity is due to suspended particles and colloidal matter in the water, and is extremely variable, with recorded values ranging from 19 NTU to nearly 3780 NTU in spring, and 1.8 to 977 NTU in autumn in the PRE. It is highly associated with river flow or re-suspended off the bottom by the tidal and wind
wave currents (Cloern, 1996). The fine particles remain in the suspension, resulting in high levels of light scattering and considerably reducing the light penetration into the water column (Oliver et al., 2010). Consequently, light limitation is a major control on phytoplankton growth and photosynthesis in the water column of the estuaries in spite of high nutrients and this is consistent with results from both theoretical studies and field investigations (Cloern, 1987; Soetaert et al., 1994; Desmit et al., 2005). Field examples also include present study that turbidity associated with the light availability has a strong correlation with the development of phytoplankton populations in the PRE, in particular during wet seasons (Yin et al., 2000, 2004). Thus, factors controlling the turbidity may have an indirect effect on light attenuation in the water column, and then phytoplankton assemblages. Besides the river runoff, other physical forces, such as wind events or human activities including over-exploitation, navigation and dredging, may consequently influence the spatial and temporal variabilities in phytoplankton community (Cloern, 1996; May et al., 2003; Yin et al., 2004). In light of the results from these studies, turbidity can be a model index incorporated all the effects of surrounding physical driving forces in the shallow estuarine ecosystem. Moreover, physical–biological coupling study indicates that the PRE exhibits fewer impacts from eutrophication than one would expect and shows a remarkable capacity to cope with excessive nutrients (Harrison et al., 2008). Therefore, physical process either natural or anthropogenic within the hydrologically distinct zone is a more important factor rather than inorganic nutrients in controlling the phytoplankton species composition, distribution and abundance in the Pearl River estuary and this result may also apply to other turbid estuaries, in particular in the subtropical areas of the world.

5. Summary

In summary, multivariate statistical analyses have been first applied on the wet and dry season's phytoplankton assemblages and corresponding environmental factors in the Pearl River estuary. Two distinct groups related to the wet and dry seasons, and sub-clusters representing the upstream, estuarine and marine sectors...
were defined based on the species composition and abundance. The wet season's phytoplankton was mainly dominated by an assemblage of diatoms throughout the estuary with lower temperature and higher turbidities. The dry season's composition had a more diverse representation of dominant taxa that included greater abundance from both diatoms and dinoflagellates with higher temperature while lower turbidities. The cosmopolitan diatom S. costatum dominated in both seasons throughout the transect, regardless of temperature, turbidity, salinity or water quality, while the dinoflagellates C. geminatum coupled with Haptophyta P. globosa bloomed in the dry season due to the relatively stable, hyperhaline and nutrient-rich conditions. Under the cumulative effects of both physical and chemical factors, multivariate analysis BIOENV procedure identified the best combination of variables (water temperature and turbidity), which were most responsible for the variability in the structure of phytoplankton community in the PRE. As the Pearl River is located in the subtropical area and is among the largest river systems in the world, the results of present study, on a worldwide basis, may provide a similar experience to the estuary ecology study, especially for the subtropical areas.

Acknowledgments

This study was supported by the Key Innovation Project of the Chinese Academy of Sciences (Grants no. KZCX2-YW-Q07, XDA05030403), and the National Natural Science Foundation of China (Grants no. 41006092, 40976022). We would like to thank our team members for logistic support on sampling transport and assistance.

References