Research Article

Spatial Distributions of Picoplankton and Viruses in the Changjiang Estuary and Its Adjacent Sea Area during Summer

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Simultaneous determination of picoplankton (i.e., *Synechococcus* spp., *Prochlorococcus* spp., picoeukaryotes, and heterotrophic bacteria) and viruses in the Changjiang (Yangtze) River estuary and its adjacent sea area was made using flow cytometry during a cruise in June 2006. The results show that *Prochlorococcus* in all samples was below detectable level. The abundances of *Synechococcus*, picoeukaryotes, heterotrophic bacteria, and viruses ranged from 0.00 to 1.22×10^8 cell L⁻¹, 0.01×10^6 to 1.42×10^7 cells L⁻¹, 8.40×10^7 to 4.29×10^9 cells L⁻¹, and 1.20×10^7 to 1.06×10^{10} particles L⁻¹, respectively. The determined picoplankton groups and viruses distinctly increased with the distance off the estuary and where the maximum abundance that occurred in these groups was different somewhat due to the individual sensitivity to environmental changes. Viral abundance showed a positive correlation with salinity and negative correlations with turbidity and inorganic nutrient concentrations. Positive linear relations were found between *Synechococcus*, heterotrophic bacteria, and viruses.

1. Introduction

Autotrophic *Synechococcus* spp., *Prochlorococcus* spp., and picoeukaryotes, together with heterotrophic bacteria, are the principal components of marine picoplankton communities (0.2–2 μ m in diameter) [1–4]. These picoplankton groups in the Changjiang estuary have been the subject of research in the past [5–14]. However, few data have been published on picoplankton community structure after the Three Gorges Dam construction. Previous studies were mainly focused on only one picoplankton group rather than simultaneously observing all picoplankton groups, and most of the data in the literature were obtained by epifluorescence microscope, which was not as efficient, sensitive, and precise as flow cytometry (FCM) adopted in this study [12, 13]. Marie et al. [15] reported the method of FCM measurement on marine viruses, providing a glorious prospect on studying natural viral community. Compared with other oceanic regions, the field investigation on viruses in the Changjiang estuary was much less, and, up to now, only a few reports were found [12, 14, 16, 17].

With the construction of the huge Three Gorges Dam (height 180 m, width 2 km) in the middle reaches of Changjiang River, both the amount and the timing of the terrigenous input into the East China Sea will be different [18]. It has been suggested that after the water storage of the Three Gorges reservoir, light availability required for photosynthesis enhanced in the river mouth area due to less discharge of water and sediments from the river [7, 8]. Studies indicate that picoplankton and viruses are more sensitive to environmental changes than larger organisms because of their small size [19, 20]. Therefore, the quantifications of picoplankton and viruses are of great importance as it may reflect the changes in estuarine water quality associated with the construction of the dam.

In this study, a simultaneous determination of picoplankton groups and viruses was made in June 2006. The main objective is to observe the spatial distributions of picoplankton and viruses in the Changjiang estuary and its adjacent sea area and to discern the relationships between them and the environmental factors.

TABLE 1: List of the measured environmental parameters in the Changjiang estuary and its adjacent sea area in June 2006.

Parameter	Section I	Section II	Section III	Total station
Number of sample	39	45	15	96
Salinity (psu)				
Range	0.00–33.92	14.86–34.42	29.79–34.13	0.00–34.42
Mean $\pm$ SD	20.49 $\pm$ 14.09	30.13 $\pm$ 4.41	32.41 $\pm$ 1.58	26.62 $\pm$ 10.69
CV	69%	15%	5%	40%
Turbidity (FTU)				
Range	0.07–122.13	0.45–122.13	0.00–32.43	0.00–122.13
Mean $\pm$ SD	57.84 $\pm$ 57.09	22.77 $\pm$ 27.97	3.68 $\pm$ 8.29	31.78 $\pm$ 44.77
CV	99%	123%	225%	141%
Nitrate ($\mu\text{g L}^{-1}$)				
Range	11.89–1645.36	19.47–342.04	11.41–128.93	11.41–1645.36
Mean $\pm$ SD	493.43 $\pm$ 503.65	162.73 $\pm$ 84.10	45.65 $\pm$ 38.18	275.06 $\pm$ 372.35
CV	103%	52%	84%	135%
Nitrite ($\mu\text{g L}^{-1}$)				
Range	0.11–34.44	0.95–12.69	0.11–11.55	0.11–34.44
Mean $\pm$ SD	5.70 $\pm$ 6.86	4.90 $\pm$ 2.64	3.31 $\pm$ 3.21	5.11 $\pm$ 5.48
CV	120%	54%	97%	107%
Ammonia ($\mu\text{g L}^{-1}$)				
Range	3.62–63.93	26.31–130.22	17.97–123.74	3.62–130.22
Mean $\pm$ SD	39.23 $\pm$ 17.28	55.09 $\pm$ 17.83	56.20 $\pm$ 25.69	50.01 $\pm$ 20.37
CV	44%	32%	46%	41%
Phosphate ($\mu\text{g L}^{-1}$)				
Range	2.53–43.57	2.21–30.96	0.98–12.45	0.98–43.57
Mean $\pm$ SD	17.90 $\pm$ 11.91	13.28 $\pm$ 7.06	5.34 $\pm$ 3.14	13.63 $\pm$ 10.20
CV	67%	53%	59%	75%
Silicate ($\mu\text{g L}^{-1}$)				
Range	55.22–3027.20	157.57–1144.37	33.43–243.76	33.43–3027.20
Mean $\pm$ SD	936.83 $\pm$ 898.12	405.36 $\pm$ 195.53	151.69 $\pm$ 69.83	576.29 $\pm$ 659.66
CV	96%	48%	46%	114%

2. Materials and Methods

2.1. Field Sampling. The cruise was conducted from June 12 to 22, 2006, on board R/V *Dong Fang Hong 2*. Thirty-two stations, between 29°00' N and 32°19' N and 120°46' E and 124°01' E, were located along three different but representative sections identified as I, II, and III (Figure 1). Section I extended from the freshwater river to the saltwater sea covering the whole salinity gradient. Section II ran across the edge of the bar area and covered the whole river mouth. Section III represented the area off the river mouth. Water sample collections were guided by a Sea-Bird CTD (conductivity-temperature-depth) probe and carried out with Niskin bottles at three different depths (one meter below the surface, a few meters above the sea bed, and midway between, according to the depth at each station). Salinity and turbidity were recorded in the water column using the CTD assembly.

FIGURE 1: Location of sampling stations in June 2006.

FIGURE 2: Spatial distribution of *Synechococcus* ($\times 10^6$ cells L^{-1}) in the Changjiang estuary and its adjacent sea area in June 2006.

2.2. Inorganic Nutrients Analysis. Water samples were filtered immediately after collection through precleaned $0.45 \mu m$ pore-size cellulose filters. Subsequently, 1–2 drips of saturated $HgCl_2$ were added to the filtrate, which was preserved at room temperature in brown bottles for later analysis. Inorganic nutrients (nitrate, nitrite, ammonia, phosphate, and silicate), were determined using spectrophotometry with a SKALAR San^{plus} Segmented Flow Analyzer (Breda, The Netherlands).

2.3. Picoplankton and Viral Analysis. Samples were fixed for 15 min with paraformaldehyde (final concentration: 1% v/v) in the dark and then kept frozen in liquid nitrogen until they were analyzed in the laboratory. All of the

samples were analyzed with a FACScan instrument (Becton Dickinson, San Jose, CA) within a month of collection. The picophytoplankton was separated into groups according to their specific autofluorescence properties and side scatter differences and their abundance was recorded [12, 21–23]. For the enumeration of heterotrophic bacteria, samples were stained with 1/10000 (v/v) SYBR Green I (Molecular Probes, Inc.) and incubated in the dark for 15 min before FCM analysis [21]. For virus enumeration, natural samples were diluted using TE buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH 8) to 100–1000 virus s^{-1} using flow cytometry [24]. The diluted virus samples were stained with SYBR Green I (final concentration: 0.5×10^{-4} , v/v) for 10 min in the dark at

FIGURE 3: Spatial distribution of picoeukaryotes ($\times 10^6$ cells L^{-1}) in the Changjiang estuary and its adjacent sea area in June 2006.

80°C before FCM analysis [15, 24]. Bacteria and viruses were characterized according to their distinctive side scatter and green fluorescence signals, which are related to their size and nucleic acid content, respectively [15, 21].

Yellow-green fluorescent beads, 1.002–2.139 μm in diameter (Polysciences, Inc.) were added as internal references to calibrate cell fluorescence emissions and light scatter signals and to allow comparisons of fluorescence and cell sizes among samples. All stock solutions were prefiltered through a filter (0.2 μm pore size) before use to avoid contamination. The raw data were processed using CELLQuest software (Becton Dickinson, San Jose, CA). Samples were measured in triplicate to give an estimated precision greater than 8.0% (relative standard deviation).

2.4. Data Analysis. The data in tables are expressed as mean $\pm$ SD. Coefficient of variation (CV) was calculated by $CV = [SD/Mean] \times 100\%$. Regression analysis using the software SPSS 13.0 (SPSS Inc., Chicago, IL) was applied to test if there was any significant relationship between the detected biological groups and the environmental parameters. Pearson's correlation analysis was used to assess major linear relations between the detected biological groups.

3. Results

3.1. Environmental Parameters. High values of CV indicated strong changes in salinity, turbidity, and nutrients in the area studied (Table 1). Salinities ranged from 0 to

FIGURE 4: Spatial distribution of heterotrophic bacteria ($\times 10^6$ cells L^{-1}) in the Changjiang estuary and its adjacent sea area in June 2006.

34.42 psu (practical salinity unit) and obviously increased seaward. Turbidity and concentrations of inorganic nutrients decreased rapidly with the distance off the river mouth. The changes in these environmental parameters showed that freshwater from the Changjiang River dramatically affected the water quality in the estuary area, particularly at the stations near the river mouth.

3.2. Spatial Distributions of Picoplankton and Viruses. In the area studied, heterotrophic bacteria and viruses were ubiquitous. *Synechococcus* and picoeukaryotes could be detected except for a very few samples from stations in the river mouth

(Stations 16–18), while *Prochlorococcus* in all samples was below detectable level.

Average abundance of *Synechococcus* was 7.82×10^6 cells L^{-1} , with a range of $0\text{--}122.22 \times 10^6$ cells L^{-1} (Table 2). Its abundance exhibited distinct spatial differences (Figure 2). The abundance values were $<1 \times 10^6$ cells L^{-1} in the water around stations 17–21 and stations 5 and 6. At these stations near the river mouth, the abundance differences between the surface and bottom layers were relatively small because their shallow depth allows water to be mixed. At other stations outside the river mouth, the abundance was high at the surface layers and decreased with water depth.

FIGURE 5: Spatial distribution of viruses ($\times 10^6$ particles L^{-1}) in the Changjiang estuary and its adjacent sea area in June 2006.

Picoeukaryotes showed a lower average abundance (2.13×10^6 cells L^{-1}) than *Synechococcus* (Table 2). Its higher abundances ($>6 \times 10^6$ cells L^{-1}) were mostly recorded from the surface layers at stations 13–16 and 23–25 and the lower abundances ($<1 \times 10^6$ cells L^{-1}) in the water around stations 17–21 and 29–31 in the near bottom water around stations 9–13 and 22–28 (Figure 3). As a whole, the distribution of picoeukaryotes was uneven in different regions and water layers.

Heterotrophic bacteria were quite abundant, with two to three orders of magnitude higher in average abundance (1387×10^6 cells L^{-1}) than those of small autotrophs and showed least variation in abundant (Table 2). Viruses

were nearly 2 times more abundance on average (2663×10^6 particles L^{-1}) than heterotrophic bacteria (Table 2). Spatial distribution of heterotrophic bacteria was similar to that of viruses (Figures 4 and 5). Commonly, their vertical distributions were almost even for the stations 17–21 near the river mouth, and for the other stations the abundances decreased with water depth.

4. Discussion

Spatial distributions of detected picoplankton groups and viruses featured distinct spatial variability, with different factors exerting control on their growths in different regions. In

FIGURE 6: Relationships between the determined biological groups and the environmental parameters (Syn: *Synechococcus*; Euk: picoeukaryotes; Bact: heterotrophic bacteria).

the turbid river mouth, picoplankton and viral abundances were low, although the concentrations of nutrients were high (Figures 6(a)–6(e)). Unfavorable light conditions because of high turbidity exerted strong limitations to picoplankton [14, 25]. Further offshore, the abundances increased gradually, with suspended matter settling out. The maximum abundances occurred at about 150 km (this distance differs somewhat in different groups due to the difference among the groups in the sensitivity to environmental changes) from the river mouth, where turbidity was less than 5 FTU (Formazan Turbidity Units, Figure 6(f)), salinity varied from 28 to 32 psu (Figure 6(g)), and nutrients largely depleted

(Figures 6(a)–6(e)). Ning et al. [25] pointed out that the front of microzooplankton just appears in these regions because of the availability of abundant preys, which are not tested in this study, however.

Positive linear relations were found between *Synechococcus*, heterotrophic bacteria, and viruses (Figure 7), and these relations have been reported in many previous studies. Heterotrophic bacteria are dependent on substrates produced by the small primary producers like picophytoplankton (e.g., *Synechococcus*) due to a tight link between picophytoplankton and dissolved organic matter (DOM) production [13, 26, 27]. Viral lysis of heterotrophic bacteria

FIGURE 7: Linear relations between *Synechococcus*, heterotrophic bacteria, and viruses.TABLE 2: List of the measured picoplankton ($\times 10^6$ cells L^{-1}) and viruses ($\times 10^6$ particles L^{-1}) in the Changjiang estuary and its adjacent sea area in June 2006.

Item	Section I	Section II	Section III	Total station
Number of sample	39	45	15	96
<i>Prochlorococcus</i>	Undetected	Undetected	Undetected	Undetected
<i>Synechococcus</i>				
Range	0.00–44.24	0.11–13.23	1.50–122.22	0.00–122.22
Mean $\pm$ SD	4.98 $\pm$ 10.03	2.35 $\pm$ 3.02	30.09 $\pm$ 38.02	7.82 $\pm$ 18.64
CV	201%	129%	126%	238%
Picoeukaryote				
Range	0.11–13.97	0.01–14.21	0.38–10.84	0.01–14.21
Mean $\pm$ SD	2.02 $\pm$ 3.11	2.81 $\pm$ 3.84	1.78 $\pm$ 2.61	2.13 $\pm$ 3.11
CV	154%	137%	147%	146%
Heterotrophic bacteria				
Range	84–3159	543–3706	626–2229	84–4287
Mean $\pm$ SD	1158 $\pm$ 567	1533 $\pm$ 637	1309 $\pm$ 541	1387 $\pm$ 694
CV	49%	42%	41%	50%
Virus				
Range	12–5762	85–10266	1479–10642	12–10642
Mean $\pm$ SD	1604 $\pm$ 1615	2523 $\pm$ 2498	4247 $\pm$ 2471	2663 $\pm$ 2574
CV	101%	99%	58%	97%

potentially benefits *Synechococcus* by supplying nutrients while simultaneously removing their potential competitors (heterotrophic bacteria) for inorganic and organic nutrients, which is consistent with the view that nutrient cycling by viral lysis of heterotrophic bacteria may control phytoplankton growth and ecosystem scale carbon production [28].

Meanwhile, viral abundance showed a positive correlation with salinity and negative correlations with turbidity

and inorganic nutrient (nitrate, phosphate, and silicate) concentrations (Figure 6), indicating that viruses are sensitive to environmental changes [29, 30] and their abundance and dynamics may be influenced by these parameters to a large extent [19]. Over the past 20 years, considerable progress has been made in revealing the ecological role of viruses in aquatic ecosystems, but further studies are needed for understanding of the environmental controls on viral

TABLE 3: Abundances of picoplankton groups and viruses in the Changjiang estuary and its adjacent sea area from different studies.

Location	Year	Month	Syn ^a	Pro ^a	Abundances ($\times 10^6$ cells L ⁻¹)		Bact ^a	Virus	Detection technique	Source
31–32°N, 121–124°E	1986	Jul	0.1–200						Epifluorescence microscope	[5]
25–33°N, 120–130°E	1998	Jul	<15	<120		<14	<540		Flow cytometry	[6]
27–32°N, 122–130°E	1998	Jul	0.6–19.7						Epifluorescence microscope	[9]
25–33°N, 120–128°E	2000	Oct–Nov	7.6–31.1				690 $\pm$ 1780		Epifluorescence microscope	[10, 11]
28–32°N, 122–129°E	2000	Oct–Nov	21 $\pm$ 45	6 $\pm$ 15		14 $\pm$ 15			Flow cytometry	[13]
	2003	Sep	97 $\pm$ 335	53 $\pm$ 98		5 $\pm$ 10	733 $\pm$ 583		Flow cytometry	
			23.5	11.3		2.62	1000	19000		
29–32°N, 122–124°E	2004	Sep–Oct	(1.83–270)	(0–210)		(0.14–9.34)	(411–2530)	(3440–45600)	Flow cytometry	[12]
			7.82			2.13	1387	2663		
29–32°N, 120–124°E	2006	Jun	(0–122.22)	0		(0.01–14.21)	(84–4287)	(12–10642)	Flow cytometry	This study

^a Syn: *Synechococcus*; Pro: *Prochlorococcus*; Euk: picoeukaryotes; Bact: heterotrophic bacteria.

abundance, impacts of viral infection upon host community structure, and roles of viruses in biogeochemical cycles [20].

Prochlorococcus was below detectable level, which may be because of high turbidity and nutrients in this area studied. *Prochlorococcus* has been found to be more abundant in oligotrophic waters than in eutrophic waters [31]. Pan [12] indicated that *Prochlorococcus* only presents in waters of salinity >32.6 psu and suspended sediment concentration <72 g m⁻³ during a summer cruise in the Changjiang estuary and its adjacent sea area. The key limiting factor for the coastward distribution of *Prochlorococcus* in the East China Sea was considered to be the movements of the warm water currents, the Kuroshio and the Taiwan Warm Water Currents [6, 7].

The abundances of different picoplankton groups obtained in this study were different to a certain extent in the other studies conducted in the Changjiang estuary and its adjacent sea area (Table 3). Particularly, the abundance of viruses remarkably decreased compared to that reported in the literature [12]. The virus-to-bacterium ratio (VBR) was only 1.92, which is very low given the Changjiang estuary is a eutrophic coastal ecosystem, and a high VBR of 8.7 has been reported in previous study [32]. Jiao et al. [8] suggested that, although causes for the changes in the microbial community structure in the Changjiang estuary could be multiple, the sudden decrease of river runoff and an ensuing intrusion of East China Sea ocean currents were postulated to be among the major ones. The information obtained from this study provides an important and valuable base for evaluating the effects of environmental changes in this area, associated with the construction of the Three Gorges Dam, and long-term monitoring of picoplankton and viruses is necessary.

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