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Distribution and controlling factors of phytoplankton assemblages associated with mariculture in an eutrophic enclosed bay in the East China Sea

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Abstract

The distribution of phytoplankton and its correlation with environmental factors were studied monthly during August 2012 to July 2013 in the Yantian Bay. A total of 147 taxa of phytoplankton were identified, and the average abundance was in the range of 0.57×10^4 to 7.73×10^4 cell/L. A total of 19 species dominated the phytoplankton assemblages, and several species that are widely reported to be responsible for microalgae blooms were the absolutely dominant species, such as *Skeletonema costatum*, *Navicula* sp., *Thalassionema nitzschioides*, *Pleurosigma* sp., and *Licmophora abbreviata*. The monthly variabilities in phytoplankton abundance could be explained by water temperature, dissolved oxygen, salinity, dissolved inorganic nitrogen (DIN), and suspended solids. The results of a redundancy analysis showed that pH and nutrients, including DIN and silicate (SiO_4), were the most important environmental factors controlling phytoplankton assemblages in specific months. It was found that nutrients and pH levels that were mainly influenced by mariculture played a vital role in influencing the variation of phytoplankton assemblages in the Yantian Bay. Thus, a reduction of mariculture activities would be an effective way to control microalgae blooms in an enclosed and intensively eutrophic bay.

Key words: Sansha Bay, phytoplankton, eutrophication, microalgae blooms, bioremediation, East China Sea

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1 Introduction

Many coastal regions are at risk from eutrophication, which is a process resulting from an increase in anthropogenic nutrient inputs and other biogenic elements in estuarine waters, intensive mariculture activities, and other serious disasters (Picart et al., 2015). Mariculture has expanded in recent years, with an increased output to meet the growing global demand for aquatic products (Halwart et al., 2007; Wilfart et al., 2013; Chen and Qiu, 2014; Ferreira et al., 2014). However, the intensive mariculture of fish, shrimp, shellfish, and other economically important aquatic animals can lead to dissolved inorganic nutrients being released directly into seawater, and can also result in feces and pseudo feces depositing into sediments, which would subsequently increase the nutrient concentration in sea water due to decomposition. Intensive mariculture could lead to changes in the structure of dissolved inorganic nutrients and the sedimentary environment in coastal waters, especially in semi-enclosed and enclosed marine areas (de Jonge et al., 2002; Neori et al., 2004). The high nutritional status can change the characteristics of the ecosystem and cause a series of adverse ecological events, including phytoplankton blooms, macroalgae blooms, and other serious disasters (Nagasoe et al., 2010; Glibert and Burkholder, 2011; Huo et al., 2012; Schumacher et al., 2014).

Phytoplankton blooms cause mass mortalities of wild and farmed animals worldwide, with catastrophic impact on aquaculture and local economies (Richlen et al., 2010). Harmful phytoplankton blooms also contaminate shellfish with toxins, making them unsafe for human consumption and can cause a variety of health problems (Flewelling et al., 2005; Peng et al., 2012). The distribution of phytoplankton assemblages is primarily influenced by environmental factors, such as water temperature and nutrient levels (Byun et al., 2007; Paerl et al., 2011). Several previ-

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ous studies have described the relationships between the phytoplankton distribution and environmental factors in coastal waters. Silicate (SiO_4) and soluble reactive phosphorus (SRP), which are both influenced by hydrodynamic conditions, were the most important environmental factors influencing the phytoplankton distribution in the semi-enclosed environment of the Bohai Bay (Peng et al., 2012). Dissolved nitrate and pH have been shown to control the seasonal fluctuations of phytoplankton along the eastern coast of the Gulf of Suez in Egypt (Nassar et al., 2015). Shen et al. (2011) reported that temperature and turbidity had significant effects on the pattern of phytoplankton assemblages during wet and dry seasons in the Zhujiang (Pearl River) Estuary in China. Therefore, understanding the relationship between phytoplankton and environmental factors is of fundamental importance in studies of the mechanisms that distribute phytoplankton assemblages and the establishment of control measures (Peng et al., 2012).

The Sansha Bay is a typical enclosed bay along the coast of the East China Sea and is one of the most important aquaculture bases in southeastern China. It is seriously impacted by both poor hydrological exchange and anthropogenic activities (Wu et al., 2015). There are 220 000 fish cages in the Sansha Bay, with *Pseudosciaena crocea* (Su, 2009). *Crassostrea gigas* and *Apostichopus japonicus* were also cultivated on a large scale in recent decades. The excess nutrients from mariculture activities, which enter the environment as dissolved ammonia, feces, and uneaten feed can stimulate rapid phytoplankton growth (Li et al., 2010). Yu et al. (2014) demonstrated that anthropogenic disturbance is the major controlling factor of habitat degradation in the Sansha Bay.

This study was conducted in the Yantian Bay, which has an area of approximately 405.86 km^2 (Wu et al., 2015). In recent years, the Yantian Bay has experienced serious eutrophication mainly due to intensive mariculture (Hu et al., 2014; Yu et al., 2014), which is a weak source of CO_2, resulting in low pH values (Wei et al., 2016). The rate of habitat degradation in the Yantian Bay, one of the six sub-bays of the Sansha Bay, is more severe (33.50%) than in the other sub-bays mainly due to mariculture activities. To the best of our knowledge, little information is available regarding the relationship between phytoplankton assemblages and environmental factors in enclosed and intensively eutrophic mariculture sea areas.

In this study, the composition and distribution of phytoplankton assemblages and the relationships between the environmental factors and phytoplankton were evaluated based on data obtained from 12 continuous cruises conducted during the period from August 2012 to July 2013 in the Yantian Bay. The aims of this study are to investigate phytoplankton assemblages and their relationship with environmental properties in intensively eutrophic mariculture areas and to identify the most important variables that determine the distribution of phytoplankton in these areas.

2 Materials and methods

2.1 *Study area and survey methods*

The Yantian Bay (26.72°–26.84°N, 119.76°–119.83°E), which is an enclosed sub-bay inside the Sansha Bay at the coast of the

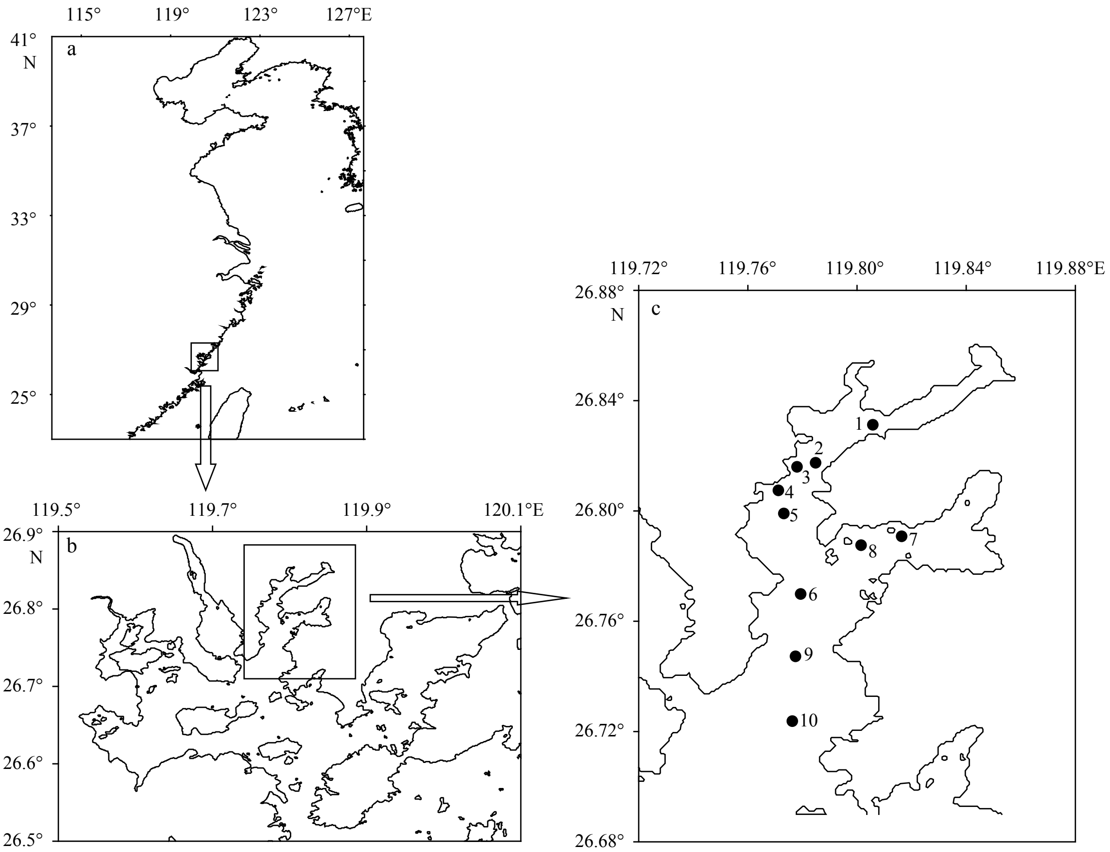

Fig. 1. The locations of the Sansha Bay on the coast of the East China Sea (a), the Yantian Bay inside the Sansha Bay (b) and ten sampling sites in the Yantian Bay during the period of August 2012 to July 2013 (c).

East China Sea (Fig. 1). During our investigations, *P. crocea*, *C. gigas* and *A. japonicus* were cultured all year round. In this area, the macroalgae, *Gracilaria lemaneiformis*, is most commonly cultivated during September and December, and *Laminaria japonicais* typically cultivated during January and May. There are no macroalgae cultivated during June and August in the Yantian Bay. Ten sampling sites were selected in the Yantian Bay, and samples were taken monthly during the period of August 2012 to July 2013 (Fig. 1). Sampling Site 1 was located in a non-mariculture area; sampling Site 3 was located in an area where *C. gigaswas* cultured; sampling Site 4 was located in an area where *P. crocea* was cultured; while the other sampling sites were distributed in seaweed cultivation areas. A total of 12 cruises were conducted in this study. Sampling activities were conducted onboard a local fishing boat. Water samples and phytoplankton samples were collected using Niskin bottles controlled by a hand-operated winch (Widco, Fort Lauderdale, FL, USA) in the middle of every month.

2.2 *Physico-chemical properties*

During each survey, the surface temperature, salinity, suspended solids (SS), pH and dissolved oxygen (DO) were measured in the field using a multi-parameter kit (MS5, HACH. Loveland, CO, USA). Seawater samples were taken at each sampling site during the slack tide period at a depth of 15–20 cm below the surface, and were analyzed for the determination of ammonium (NH_4-N), nitrite (NO_2-N), nitrate nitrogen (NO_3-N), soluble reactive phosphorus (PO_4-P), silicate (SiO_4), chemical oxygen demand (COD) and chlorophyll *a* (Chl *a*). Seawater samples for the measurement of dissolved inorganic nutrients were filtered through cellulose membranes (0.45 μm), which were pre-immersed in 10% HCl for at least 10 h and rinsed with distilled water many times before use, and one to two drops of mercury (II) chloride was also added. Water samples used for Chl *a* determination were filtered onto GF/F glass-fiber filters onboard and wrapped in tinfoil. All seawater samples were preserved in a freezer at –30°C onboard and then transported to the laboratory under cold conditions and preserved in a freezer at –30°C in the laboratory. NH_4-N, NO_2-N, NO_3-N, PO_4-P and SiO_4 concentrations were measured according to the GB/T 12 763.4 (2007) protocol. COD was measured directly according to the GB/T17378.4 (2007) protocol. Chl *a* was extracted from samples using 90% aqueous acetone, and the concentration of Chl *a* was determined using a fluorometer (Turner Designs, San Jose, CA, USA) (Parsons et al., 1984).

2.3 *Phytoplankton community structure*

At each sampling site, 1 000 mL seawater was sampled at a depth of 15–20 cm below the surface using Niskin bottles. These samples were immediately preserved with neutralized formaldehyde at a final concentration of 4% for the determination of phytoplankton. The samples were transported back to the laboratory under cool conditions, and 250 mL seawater samples were then placed in an Utermöhl counting chamber. Phytoplankton cells of greater than 5 μm diameter were identified and counted using an inverted microscope (Eclipse 100, Nikon, Tokyo, Japan) at 200× and 400× magnification. The entire chamber was examined and each cell was counted as a unit. At least 400 individuals of the more abundant species were counted from each sample with a 10% error. Diatoms were identified to species level if possible. The density of phytoplankton was recorded as cell/L and was calculated based on the volume of seawater examined. The dominance index (Y) of the phytoplankton species, species diversity index (H'), richness index (D), Margalef index (d), and evenness index (J) were calculated according to Wang et al. (2005), Shannon and Wiener (1963), Margalef (1968), and Pielou (1975). A species with $Y>0.02$ was considered to be a dominant phytoplankton species.

2.4 *Statistical analysis*

Based on the results of a test of normality, Pearson correlation analyses were used to determine correlations between environmental factors and total phytoplankton abundance, total diatom abundance and total dinoflagellate abundance in the Yantian Bay throughout the whole period investigated. Significant and highly significant correlations were defined at $P<0.05$ and $P<0.01$, respectively. Statistical analyses were conducted using SPSS 19.0. Multivariate ordination techniques were used to determine the relationship of environmental factors with the phytoplankton community in each month investigated using CANOCO for Windows 4.5 (Lepš and Šmilauer, 2003). Selected environmental parameters (sea surface temperature, pH, salinity, DO, Chl *a*, COD, DIN, dissolved inorganic phosphorus (DIP), SiO_4, DIN/SRP ratio and DIN/SiO_4 ratio) were adopted as the explanatory variables. Three forms of nitrogen were integrated as dissolved inorganic nitrogen (DIN), whereas PO_4-P was regarded as DIP in this study. All environmental parameters were transformed ($\log_{10}x$) before analysis except for pH. In the data matrix of phytoplankton species abundance, only those species with an abundance greater than 5% in at least one sample were used in the analysis (Peng et al., 2012). The phytoplankton species data were transformed ($\log_{10}(x+1)$) before analysis to obtain consecutive distributions. A detrended correspondence analysis (DCA) was applied to the phytoplankton species data to determine whether linear or unimodal ordination methods should be applied. The DCA revealed that the maximum gradient length of the four axes was less than three; therefore, linear methods were considered to be suitable ordination techniques in this study and a redundancy analysis (RDA) was applied to assess the relationships between phytoplankton and environmental parameters (Lepš and Šmilauer, 2003). A Monte Carlo simulation was used to test the significance of the environmental parameters when explaining the phytoplankton data by the RDA using CANOCO for Windows 4.5 (Lepš and Šmilauer, 2003).

3 Results

3.1 *Physico-chemical properties*

During the study period, the spatio-temporal distribution of the environmental factors varied among the investigation months. The results observed for these environmental factors were reported by Wu et al. (2015).

3.2 *Phytoplankton composition*

A total of 147 taxa of phytoplankton belonging to six taxonomic groups, were identified in the Yantian Bay during the investigation period. Diatoms and dinoflagellates accounted for 78.23% and 14.97% of the phytoplankton taxa, respectively, and there were 10 phytoplankton taxa belonging to Cyanophyta, Chlorophyta, Myzozoa and Ochrophyta (Table 1). The number and abundance of phytoplankton taxa varied in the different investigation months. Based on the Y values, 18 diatoms and only one dinoflagellate were identified as the dominant species throughout the whole year in the Yantian Bay (Table 2), and the dominant species and their abundance varied substantially in the different investigation months. Only *Skeletonema costatum*

Table 1. The number and average abundance (×10^4 cell/L) of phytoplankton taxa in specific taxonomic groups across ten sampling sites

Taxonomic group	Item	2012					2013						
		Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.
Diatom	*n*	54	53	48	48	59	50	46	35	41	38	42	47
	N	7.71	1.40	2.58	0.76	2.1	6.47	0.63	0.53	0.85	5.18	2.92	4.76
Dinoflagellate	*n*	9	5	3	4	3	2	4	3	5	9	9	4
	N	0.029	0.039	0.027	0.017	0.009 6	0.047	0.007 8	0.026	0.006 7	0.069	0.072	0.007 8
Cyanophyta	*n*								1	1			1
	N								0.000 4	0.000 4			0.018
Chlorophyta	*n*		2		1			1	1	2	1	1	2
	N		0.007 4		0.003 0			0.002 2	0.007 4	0.004 8	0.005 6	0.001 5	0.054
Myzozoa	*n*						1	1	1	1		1	
	N						0.002 6	0.001 5	0.005 6	0.002 2		0.000 4	
Ochrophyta	*n*				1				1	1		1	
	N				0.000 4				0.000 4	0.000 7		0.000 4	
Total	*n*	63	60	51	54	62	53	52	42	51	48	54	54
	N	7.73	1.45	2.60	0.78	2.10	6.52	0.64	0.57	0.87	5.26	3.00	4.84

Note: *n* indicates the number of phytoplankton species in specific taxonomic groups and *N* the average abundance of phytoplankton in specific taxonomic groups across ten sampling sites.

Table 2. Monthly dominant phytoplankton species and the average abundance (×10^4 cell/L) of each dominant species across ten sampling sites

Species	2012					2013						
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.
Skeletonema costatum	3.51	0.41	0.60	0.24	1.39	5.62	0.24	0.035	0.13	4.94	2.41	0.52
Navicula sp.		0.054		0.08	0.29	0.59	0.15	0.15	0.12		0.075	
Pleurosigma sp.		0.086	0.074	0.11	0.063		0.063	0.067	0.054		0.063	
Cylindrotheca closterium				0.021				0.035	0.043			0.13
Licmophora abbreviata			0.064	0.018	0.13				0.13			
Detonula pumila			0.11	0.038								1.82
Bacillaria paxillifera	0.45	0.12	0.18									
Pseudo-nitzschia delicatissima	2.45	0.20	1.19									
Thalassionema nitzschioides				0.021			0.018		0.32			
Ditylum brightwellii		0.029										0.15
Rhizosolenia setigera		0.031		0.033								
Paralia sulcata							0.030				0.086	
Climacosphenia moniligera								0.030	0.046			
Cerataulina pelagica												1.19
Chaetoceros abnormis												0.18
Thalassiosira rotula												0.22
Nitzschia sigma		0.058										
Grammatophora undulata				0.021								
Prorocentrum gracile		0.033						0.024				

dominated the phytoplankton community in May 2013, while nine species dominated the phytoplankton community during September and November in 2012. *Skeletonema costatum* dominated the phytoplankton community in all investigation months, and accounted for 6.75%–93.98% of the total phytoplankton abundance. *Navicula* sp. and *Pleurosigma* sp. were the dominant species in eight investigation months, and accounted for 2.58%–27.30% and 2.11%–14.67% of the phytoplankton abundance, respectively. *Pseudo-nitzschia delicatissima* accounted for 29.39%, 14.41% and 46.91% of the phytoplankton abundance during August, September and October in 2012, respectively. *Detonula pumila*, *Thalassionema nitzschioides*, *Cerataulina pelagica* and *Licmophora abbreviate* accounted for 37.67%, 36.94%, 25.12% and 15.12% of the phytoplankton abundance during July, April, July and April in 2013, respectively. The other ten dominant diatoms accounted for less than 8% of the phytoplankton abundance in the investigation months. *Prorocentrum gracile*, the only dominant dinoflagellate, accounted for 2.30% and 4.25% of the phytoplankton abundance in September 2012 and March 2013, respectively.

3.3 *Phytoplankton diversity*

The average Shannon–Wiener index (H') value varied from 0.89 to 3.54 among the different investigation months throughout the study period (Table 3). The average H' was as low as 0.89 in January 2013, increased to 3.00 in March 2013, and decreased gradually to 1.52 in June 2013. The average H' was maintained at a relatively high value of 2.66–3.54 from September to November in 2012. The Pielou index (J) was in the range of 0.21–0.75 during the study period, and the J values varied in the same way as the

Table 3. Three indexes of the phytoplankton community in the different investigation months in the Yantian Bay

Month	Shannon-Wiener index (H')			Pielou index (J)			Margalef index (d)		
	Min.	Max.	Average	Min.	Max.	Average	Min.	Max.	Average
2012									
Aug.	1.41	3.07	2.14	0.32	0.60	0.44	1.83	3.15	2.35
Sep.	2.88	4.24	3.54	0.59	0.86	0.75	2.27	3.25	2.62
Oct.	2.09	3.77	2.66	0.46	0.80	0.57	1.84	3.11	2.34
Nov.	2.58	3.84	3.25	0.61	0.84	0.74	1.73	2.94	2.27
Dec.	1.11	2.45	1.77	0.25	0.57	0.40	1.60	2.96	2.16
2013									
Jan.	0.58	1.17	0.89	0.14	0.27	0.21	1.39	1.86	1.71
Feb.	1.72	3.73	2.74	0.41	0.84	0.70	1.20	2.58	1.74
Mar.	2.67	3.46	3.00	0.65	0.80	0.73	1.59	2.52	1.91
Apr.	1.97	3.54	2.67	0.54	0.74	0.67	0.83	3.03	1.79
May	0.31	3.26	1.49	0.07	0.81	0.37	1.25	1.90	1.75
Jun.	1.19	2.09	1.52	0.25	0.51	0.33	1.59	2.56	2.26
Jul.	2.35	3.28	2.81	0.51	0.71	0.62	1.75	2.60	2.14

H' values among the different investigation months (Table 3). The lowest value of the Margalef index (d) of 1.71 was recorded in January 2013, and was maintained at 1.74–1.91 from February to May in 2013. The d values were in the range of 2.14–2.62 during the period of August to December in 2012 and June to July in 2013.

3.4 *Phytoplankton distribution*

The average phytoplankton density was 3.00×10^4 cell/L throughout the whole year. The highest average density of 7.73×10^4 cell/L was recorded in August 2012, and the lowest average density of 0.57×10^4 cell/L was recorded in March 2013. The average phytoplankton density across all sampling sites was in the range of 0.78×10^4 cell/L to 6.52×10^4 cell/L in the other ten investigation months.

The geographical distribution of phytoplankton during the study period is shown in Fig. 2. In the spring months of March and April, the phytoplankton were distributed relatively uniformly throughout the Yantian Bay, but in May there was an increase from sampling Site 1 with 0.27×10^4 cell/L inside the bay to sampling Site 10 with 18.76×10^4 cell/L at the mouth of the bay. During summer, the phytoplankton were distributed relatively uniformly between different sampling sites in June, and the phytoplankton abundance decreased from inside the bay to the mouth of the bay in July and August, with the highest density of 16.23×10^4 cell/L at sampling Site 7 in August 2012. During autumn, the phytoplankton were also distributed relatively uniformly in September and November, but the density was as high as 5.10×10^4 cell/L at sampling Site 2 and continuously decreased to 1.04×10^4 cell/L at sampling Site 10 in October 2012. During winter, the phytoplankton abundance was relatively high at sampling sites inside the bay and at the mouth of the bay compared with the abundance at sampling sites in the middle of the bay in December 2012. The density increased to 4.22×10^4–9.75×10^4 cell/L, with no obvious pattern in the spatial distribution in January 2013. It then decreased to 0.16×10^4–2.00×10^4 cell/L, with the lowest phytoplankton abundance occurring at the mouth of Yantian Bay in February 2013.

3.5 *Relationships between phytoplankton abundance and environmental factors*

The results of the Pearson correlation analysis across the whole study period showed that total phytoplankton abundance and total diatoms were significantly correlated with water temperature, DIN, and DIN/SiO_4 (P<0.01, Table 4) and DO (P=0.016 and 0.017, Table 4). The total dinoflagellate abundance was significantly correlated with salinity and suspended solids (P=0.002 and P<0.001, Table 4).

The relationship between environmental factors and phytoplankton abundance in each month investigated was analyzed by RDA (Table 5 and Fig. 3). The results of Monte Carlo tests showed that only the first canonical axis was significant during these investigation months, which indicated that these environmental factors may be important for explaining the phytoplankton community compositions. The first axis explained 20.1% to 43.4% of the phytoplankton variations in the investigation months. There were different significant environmental factors that explained the variability of the phytoplankton composition in the investigation months according to the RDA with forward selection. Silicate levels were found to statistically explain the variation in the composition of phytoplankton in November 2012 and July 2013, while pH was the significant environmental factor in August 2012, September 2012, February 2013, and April 2013. In December 2012, March 2013 and May 2013, the significant environmental factors were DO, DIN and salinity, respectively. During the study period in October 2012, January 2013 and June 2013, no environmental factor was significantly correlated with phytoplankton abundance in the Yantian Bay.

4 Discussion

The composition and abundance of phytoplankton in the Sansha Bay in May, August and November in 1990, and in February 1991, sampled using a conical phytoplankton net, was reported by Lin (1993). It is difficult to compare the phytoplankton abundance in this study with the results reported by Lin (1993) because of the different sampling methods, but the difference in the dominant phytoplankton species over the 20 years can be evaluated. *Skeletonema costatum*, *Ditylum brightwellii* and *Nitzschia sigma*, which were the dominant species in the early 1990s also dominated the phytoplankton community in our study, while *Biddulphia sinensis*, *Biddulphia regia*, *Coscinodiscus centralis*, *Coscinodiscus oculusiridis*, *Chaetoceros decipiens*, *Bacillaria paradoxa*, *Thalassiothrix frauenfeldii*, *Ceratium fusus* and *Noctiluca scintinllans* were dominant species in the early 1990s, but not in the early 2010s. *Noctiluca scintinllans* was the dominant dinoflagellate in the early 1990s in the Sansha Bay (Lin, 1993),

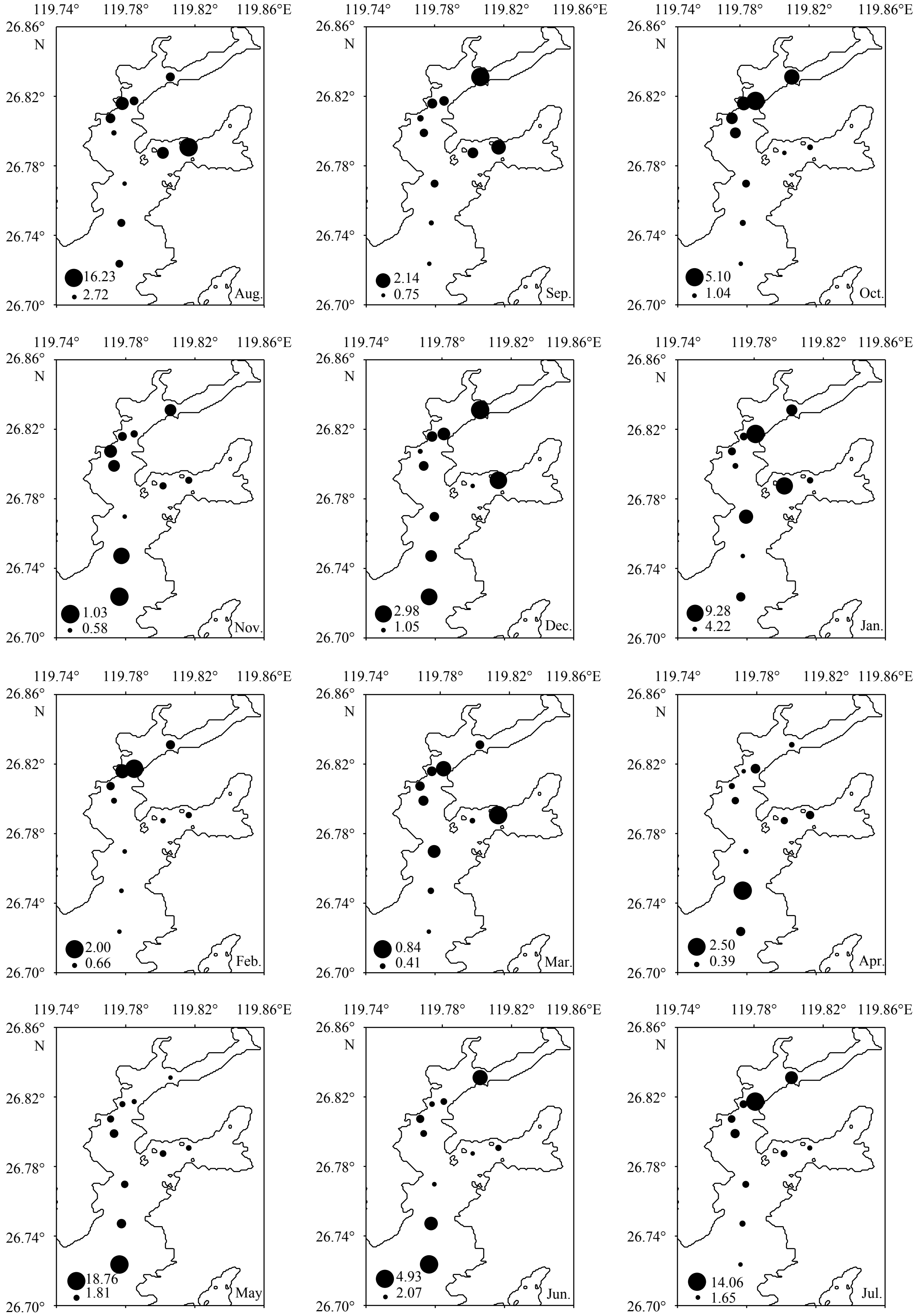

Fig. 2. Geographical distribution of phytoplankton abundance (×10^4 cell/L) across ten sites in the Yantian Bay during the period of August 2012 to July 2013.

but *P. gracile* was the only dominant dinoflagellate in September and March in the 2010s in the Yantian Bay. The *H*′ values in the current study were much smaller than in the corresponding months in the 1990s (Lin, 1993), which indicated that the phyto-

Table 4. Pearson correlation between total phytoplankton abundance, total diatom abundance, total dinoflagellate abundance and environmental factors across the whole study period in the Yantian Bay

Environmental factor	Total	Diatom	Dinoflagellate	*n*
Temperature	0.315 **	0.313 **	0.106	120
Salinity	0.164	0.167	−0.277 **	120
pH	−0.058	−0.059	−0.019	120
DO	−0.221*	−0.219 *	−0.046	120
SS/mg·L^{-1}	−0.157	−0.154	−0.336 **	120
COD	−0.079	−0.083	0.088	120
DIN	−0.399 **	−0.397 **	−0.176	120
PO_4-P	−0.150	−0.147	−0.079	120
SiO_4	−0.055	−0.055	−0.079	120
DIN/PO_4-P	−0.176	−0.177	−0.012	120
DIN/SiO_4	−0.402 **	−0.399 **	−0.155	120

Note: ** Highly significant correlation at the 0.01 level (2-tailed); * significant correlation at the 0.05 level (2-tailed).

Table 5. Apartitioning analysis of environmental factors influencing phytoplankton assemblages in the Yantian Bay during the period of August 2012 to July 2013

Month	Environmental factor	Eigenvalues	Variation explains solely/%	*F*	*p*
2012					
Aug.	pH	0.434	43.4	6.129	0.002
Sep.	pH	0.295	29.5	3.351	0.002
Nov.	SiO_4	0.201	20.1	2.015	0.042
Dec.	DO	0.227	22.7	2.355	0.030
2013					
Feb.	pH	0.264	26.4	2.872	0.002
Mar.	DIN	0.271	27.1	2.978	0.022
Apr.	pH	0.311	31.1	3.613	0.004
May	salinity	0.357	35.7	4.448	0.014
Jul.	SiO_4	0.256	25.6	2.756	0.020

plankton composition and abundance had changed substantially over the 20 years.

The phytoplankton abundance was significantly different among the different months investigated. The monthly variations of the phytoplankton community and their abundance in the Yantian Bay were greatly influenced by environmental factors (Table 4). The most important environmental factor influencing the monthly variations of phytoplankton abundance was water temperature. Water temperature can control the seasonal dynamics of phytoplankton successions (Dupuis and Hann, 2009). Some researchers have reported that the abundance of diatoms is inversely related to temperature, with a high abundance of diatoms at temperatures below 18°C (da Silva et al., 2005; Turner et al., 2009). In the Yantian Bay, the highest total diatom abundance probably occurs during periods with a relatively high water temperature, which differs from the situation in the Bohai Bay (Peng et al., 2012). The abundance of small-celled diatoms, such as *S. costatum*, *Navicula* sp. and *Pleurosigma* sp., increases during warm water periods, because warm water is well known to be less viscous than cold water, favoring a species with a small cell size (Tunin-Ley et al., 2007).

Nutrient availability is one of key factors influencing the monthly variations of the phytoplankton community (Llope et al., 2009; Ward et al., 2011; Peng et al., 2012). Under normal conditions, phytoplankton takes up N and P at the Redfield ratio (16:1). In the Yantian Bay, the observed molar ratio of N/P was higher than the Redfield ratio, indicating either a Nsurplus or a P-limited system (Wu et al., 2015). There was no significant correlation between phytoplankton abundance and the PO_4-P concentration, which was mostly higher than the eutrophication threshold value of 0.045 mg/L (Wu et al., 2015). These results indicated that phytoplankton abundance was not limited by P availability in the Yantian Bay during the study period. Total phytoplankton and total diatom abundance were significantly negatively correlated with the DIN concentration in this study. This may be explained by the increasing phytoplankton abundance, which led to nitrogen being rapidly depleted. These results differed from those results reported by Lin (1993), who reported a negative correlation between phytoplankton abundance and PO_4-P in the 1990s in the Sansha Bay.

Salinity is another important environmental factor that can influence the seasonal variations of phytoplankton (McQuoid, 2005). In this study, total dinoflagellate abundance was significant negatively correlated with salinity (Table 4). A relatively low salinity is favorable for the explosive growth of dinoflagellates, which increases the possibility of red tide outbreaks (Yuan et al., 2014). There was a significant correlation between total dinoflagellate abundance and the concentration of SS in this study. Dinoflagellates are unable to survive in very turbulent water (Margalef, 1978). Wang and Huang (2003) reported that low turbidity may also favor the growth of *Karenia mikimotoi* and *Prorocentrum dentatum*. The fine particles remain in suspension, resulting in high level so flight scattering and considerably reducing light penetration into the water column (Oliver et al., 2010), which would limit the growth and photosynthesis of phytoplankton (Shen et al., 2011).

The phytoplankton composition and abundance varied between different sampling sites, which were due to different

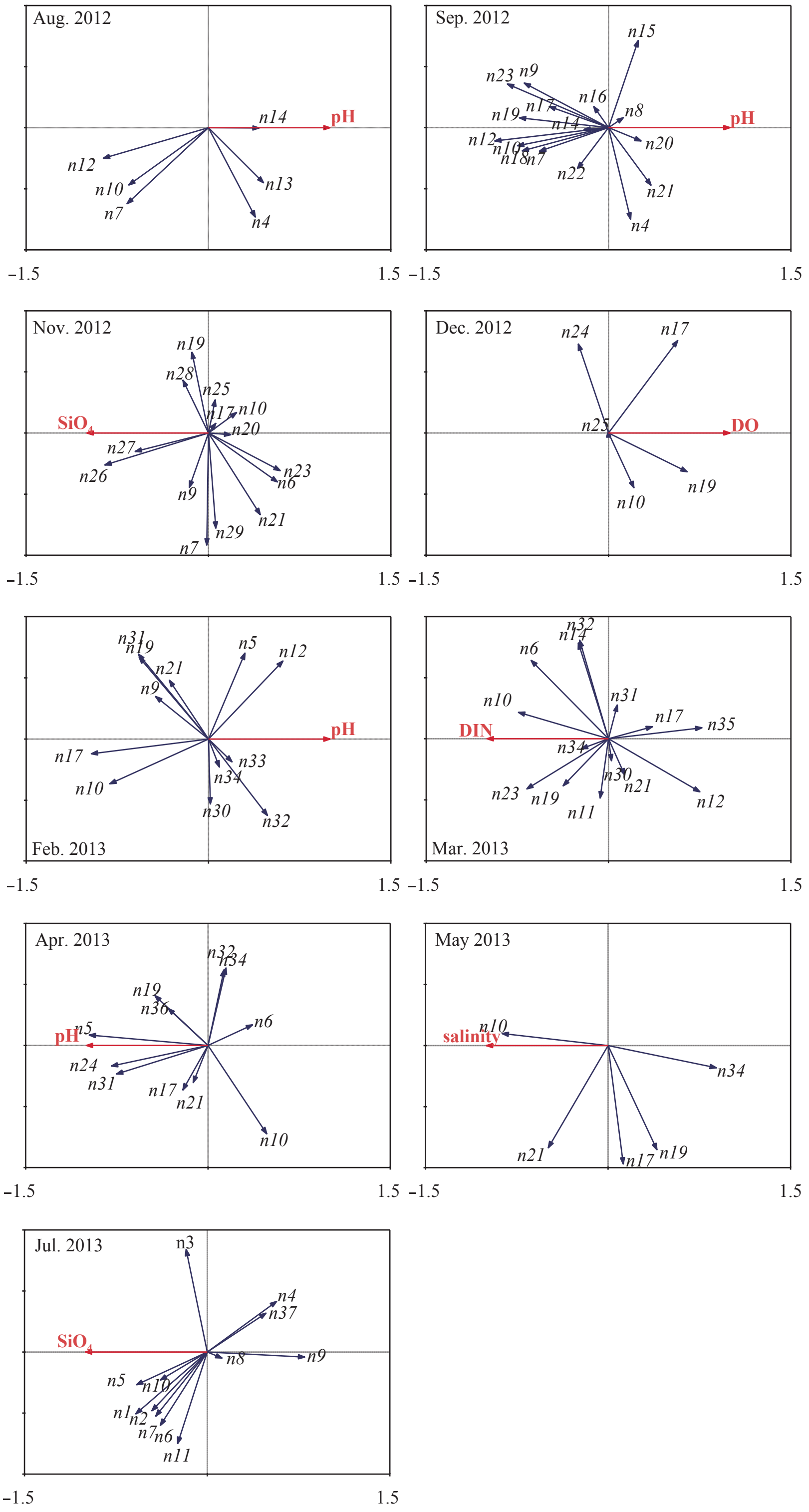

Fig. 3. Correlation plots of the redundancy analysis (RDA) for the relationship between the environmental variables and phytoplankton taxa. The numbers with letters represent the following species: *n1* represents *Cerataulina pelagica*, *n2 Chaetoceros abnormis*, *n3 Chaetoceros lauderi*, *n4 Chaetoceros* sp., *n5 Coscinodiscus* sp., *n6 Cylindrotheca closterium*, *n7 Detonula pumila*, *n8 Ditylum brightwellii*, *n9 Melosira nummuloides*, *n10 Skeletonema costatum*, *n11 Thalassiosira rotula*, *n12 Bacillaria paxillifera*, *n13 Chaetoceros curvisetus*, *n14 Pseudo-nitzschia delicatissima*, *n15 Chaetoceros debilis*, *n16 Melosira granulata*, *n17 Navicula* sp., *n18 Nitzschia sigma*, *n19 Pleurosigma* sp., *n20 Rhizosolenia setigera*, *n21 Thalassionema nitzschioides*, *n22 Thalassiosira* sp., *n23 Prorocentrum gracile*, *n24 Licmophora abbreviata*, *n25 Grammatophora undulata*, *n26 Melosira granulata*, *n27 Nitzschia lorenziana*, *n28 Nitzschia* sp., *n29 Pseudo-nitzschia pungens*, *n30 Asteroplanus karianus*, *n31 Climacosphenia moniligera*, *n32 Paralia sulcata*, *n33 Planktoniella blanda*, *n34 Scenedesmus quadricauda*, *n35 Coscinodiscus jonesianu*s, *n36 Triceratium alternans*, and *n37 Thalassiosira nordenskiöeldii*.

phytoplankton species adapting to changes in the physico-chemical environment in the specific investigation months. Although water samples were collected in different functional areas, all sampling sites were impacted by mariculture activities, as shown by the geographical distribution of dissolved inorganic nutrients (Wu et al., 2015).

During the whole investigation period, the phytoplankton community was mainly composed of neritic diatom species, such as the diatom *S. costatum*, *Navicula* sp., *P. delicatissima* and the dinoflagellate *P. gracile*. The concentration of SiO_4 could influence the structure of phytoplankton assemblages, with diatoms becoming the dominant species when the SiO_4 concentration was higher than 2 μmol/L and the levels of all other nutrients were sufficient (Egge, 1998). *Skeletonema costatum* dominated the phytoplankton community during the whole year. According to the results of the RDA analysis, its abundance was significantly correlated to high SiO_4 and DIN concentrations, suggesting that *S. costatum* prefers water with high levels of nutrients. The results of this study are consistent with other studies that have found that *S. costatum* prefers nutrient-rich seawater (Patil and Anil, 2011; Peng et al., 2012). *Navicula* sp., *Pleurosigma* sp., *C. closterium* and *P. sulcata* also have a preference for seawater containing high nutrient concentrations, and their abundance has been shown to be significantly correlated with levels of nutrients and/or SiO_4 (Du et al., 2016; El-Kassas and Gharib, 2016; Zhang et al., 2016). These diatom species could be considered as bioindicators for assessing environmental quality in specific ecosystems. *Detonula pumila* was one of the dominant species in the phytoplankton assemblage in November 2012 and July 2013 (Table 2 and Fig. 3), and its abundance was significantly correlated to the SiO_4 concentration in the RDA. The results of this study were consistent with those of Yuan et al. (2014) who found that the abundance of *D. pumila* was strongly related to silicate levels in the Sanggou Bay, Ailian Bay, and Lidao Bay at the coast of the Yellow Sea. *Pseudo-nitzschia delicatissima* was one of the dominant species in the summer period in the Yantian Bay, and is considered to be an estuarine species that is typically observed in the high flow season, which is characterized by a relatively high salinity (Zhang et al., 2014). In the RDA with forward selection, the abundance of *Chaetocerosabnormis*, *L. abbreviata*, *S. costatum* and *P. gracile*, were not only significantly correlated with nutrient levels, but also had a strong correlation with pH, dissolved oxygen and salinity in the specific months investigated in the Yantian Bay (Table 2 and Fig. 3). This was consistent with the results of Rai and Rajashekhar (2014) who found that the abundance of *C. curvisetus*, *L. abbreviata*, *S. costatum* and *P. micans* had significant positive correlation with pH and salinity in the Arabian Sea off Kerala along the southeast coast of India. *Prorocentrum gracile* was the only dinoflagellate that dominated the phytoplankton community in the Yantian Bay in September 2012 and March 2013, and is also considered to prefer the relatively low turbidity and low nutrient concentrations in the upper mixed layers of stratified seawater (Smayda, 1997; Wang and Huang, 2003; Wu et al., 2015). The results of the present study indicate that DIN, SiO_4, pH, and salinity are important environmental factors that regulate phytoplankton assemblages in specific months in the Yantian Bay.

During the study period, several phytoplankton species, which have been widely reported as microalgae-bloom-forming organism (Liu et al., 2005; Peng et al., 2012) dominated the phytoplankton community in the Yantian Bay. These species include *S. costatum*, *Navicula* sp., *T. nitzschioides*, *Pleurosigma* sp. and *L. abbreviata*. Although these species do not produce toxic chemical substances, they may cause fish mortality when blooms decay and produce high ammonia levels or entrainment within the gills (Engström-Öst et al., 2002). The results of present study show that pH and nutrient levels (DIN, SiO_4) are the most important environmental factors influencing the variations of phytoplankton assemblages. In the Yantian Bay, the annual output of *P. crocea* is approximately 9 670 t, and the annual output of *C. gigas* and *A. japonicus* is 5 790 t and 32.5 t, respectively. The total N production generated by the culturing of these aquatic animals is 305.12 t per year (Wu et al., 2015), which would maintain high concentrations of nutrients in the water column in the Yantian Bay. In seawater, the variation of pH is directly influenced by CO_2 levels because of the carbon dioxide–carbonic acid–bicarbonate system. The phytoplankton community structure was influenced by interactions between ocean acidification, solar radiation, and warming (Gao et al., 2012). In the seawater of the Yantian Bay, the annual concentration of CO_2 is in the range of 11.4–39.78 μmol/L, which makes it a weak source of atmospheric CO_2 (Wei et al., 2016) and keeps pH levels in the water column in the range of 7.53–7.89 (Wu et al., 2015). Carbon levels in the seawater can also be greatly impacted by the decomposition of fish feces and uneaten feed in mariculture sea areas as indicated by Mahmood et al. (2016). Wu et al. (2015) reported that the nutrient level in the Yantian Bay coming from rivers and tides was very low compared with other sources because of small rivers located upstream and weak exchange with open waters. So nutrients introduced by mariculture are a very important factor causing high concentrations of inorganic nutrient in the Yantian Bay (Wu et al., 2015).

The results of this study indicate that the nutrient and pH levels in the Yantian Bay were mainly greatly impacted by mariculture activities, and they play a vital role in influencing the growth and production of microalgae-bloom-forming phytoplankton. Based on the results of this study, there is a large potential for microalgae blooms to occur in the Yantian Bay, especially in seasons with a high water temperature. Thus, a reduction in the impact of mariculture activities is an effective way to control the occurrence of microalgae blooms in such an enclosed and intensively eutrophic bay.

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