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ARTICLE

Influence of environmental changes on phytoplankton pattern in Daya Bay, South China Sea

Influencia de los cambios ambientales sobre el patrón del fitoplancton en Bahía Daya, Mar de la China Meridional

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Resumen.- En este estudio, se analizaron datos ambientales (físicos y químicos) y biológicos (fitoplancton) obtenidos de Bahía Daya, Mar de China Meridional, durante 4 cruceros, llevados a cabo en enero, abril, agosto y octubre de 2001. El objetivo principal de este estudio fue identificar los factores ambientales clave que afectan la estructura del fitoplancton de la bahía. Los resultados sugieren que los factores ambientales como la temperatura y la proporción de nutrientes tienen una gran influencia en la sucesión del fitoplancton. Los factores ambientales son afectados por una combinación de actividades humanas y procesos naturales. El factor limitante pasó de N en invierno a P en primavera. Los procesos hidrodinámicos (mezcla en invierno y estratificación en verano) parecen ser otro factor forzante para el crecimiento del fitoplancton. Las diatomeas fueron el grupo dominante durante gran parte del año y las especies dominantes fueron *Chaetoceros affinis*, *Chaetoceros lorenzianus*, y *Nitzschia pungens* durante todo el año.

Palabras clave: Nutrientes, fitoplancton, factores limitantes, Bahía Daya, Análisis de componentes principales

Abstract.- In this study, we analyzed the environmental (physical and chemical) and biological (phytoplankton) data obtained from the Daya Bay, South China Sea, during 4 cruises, carried out in January, April, August and October, 2001. The main objective of this study was to identify the key environmental factors affecting phytoplankton structure in the bay. The results suggest that environmental factors like temperature and nutrients ratios have great influence on phytoplankton succession. Environmental factors are affected by the combination of human activities and nature processes. The limiting factor transited from N limiting in winter to P limiting in spring. Hydrodynamic processes (mixing in winter and stratification in summer) seem to be another main driving force for phytoplankton growth. The diatoms were the dominant group during most of the year and the dominant species were *Chaetoceros affinis*, *Chaetoceros lorenzianus*, and *Nitzschia pungens* all the year round.

Key words: Nutrients, phytoplankton, limiting factors, Daya Bay, Principal component analysis

INTRODUCTION

Many estuarine and coastal ecosystem worldwide are impacted by human activities, such as urban waste and sewage discharge, increasing use of agricultural fertilizers, freshwater runoff, riverine nutrient inputs, coastline construction, tourism, marine culture, etc., and natural processes such as monsoon, upwelling, and water well vertical mixing, which stimulates phytoplankton production, and having flow-on effects to biogeochemical processes and food webs (Jickells 1998). These material inputs produced by human activities may be from point source and nonpoint resource. The nutrient supply and its ratios have a decisive effect on the species composition of the phytoplankton since different algal species have different nutrient requirements (Hodgkiss & Lu 2004). The nutrient ratios are changing over time implying alterations in the ecosystem in Baltic Sea (Danielsson *et al.* 2008). Nutrient ratios may have led to the decrease of large diatoms and a shift of phytoplankton species composition in Jiaozhou Bay (Shen 2001). Phytoplankton biomass, production and species composition are therefore important indicators of eutrophication in coastal waters. Although any resource essential for algal growth in estuarine and coastal marine waters can be potentially limiting, the main drivers of change in phytoplankton communities are usually identified as nutrient availability (in particular C, N, P, and Si for diatoms) and light (Tilman *et al.* 1982). Phytoplankton is important primary producers and the basis of the food chain in open water. Because the variation of phytoplankton succession is strongly linked to seasonal change and hydrodynamics such as stratification and mixing, patterns in estuarine/coastal ecosystems differ considerably from those of open waters. The dynamics of phytoplankton are a function of many of the some environmental processes that affect species diversity. The coastal water is always exchanged with the open ocean; the coastal ecosystem appears to be more susceptible to nutrient concentration and their ratios. Thus, the limiting nutrient for phytoplankton growth may become an important controlling factor. The changes in nutrients have strongly influenced the phytoplankton community structure in this area. Coastal environments vary considerably in their physical and hydrographic properties, such as tidal stirring, depth, and freshwater runoff, which makes phytoplankton dynamics complex (Cloern 1996).

Daya Bay is an enclosed small bay in Shenzhen and Huizhou, bordering Shenzhen to the west, Huizhou to the north and east, with a coastal line of 92 km, and an area of 600 km^2. In the last decades, massive economic growth and urban development in the region have led to excessive release of waste into the bay. In addition, two nuclear power stations: Daya Bay Nuclear Power Plant (DNPP) and Lingao Nuclear Power Plant (LNPP) are being operated since 1993 and 2003, respectively. Human activities strongly affected aquatic environment. Many reports have focused on environment changes in the bay (Wu & Wang 2007, Wu *et al.* 2009b, c; Wu *et al.* 2010b, 2011). The marine aquaculture industry has been one of the important industries. Area of aquaculture increased rapidly from 540 m^2 in 1994 to 1300 m^2 in 1998 (Wu & Wang 2007). Anthropogenic activities were the main factor for impacting the ecological environment in Daya Bay. Daya Bay is facing pollution problems.

This study is the first attempt to understand both natural and anthropogenic influences on the coastal bay environment. Two major objectives were established in this study: (1) to detect seasonal variations of environmental factors and (2) to delineate general features of phytoplankton succession.

MATERIALS AND METHODS

SAMPLING STATIONS

In order to evaluate the anthropogenic and nature effects in this bay, the survey stations design as follows: These two monitoring stations (S1 and S2) locate in the mouth of the bay. Stations (S3 and S8) locate in Dapeng Cove, and Aotou, respectively. Stations (S4 and S5) are near the nuclear power plants (DNPP and LNPP). Stations (S6, S7, S9 and S12) are the areas from the central to eastern area of the bay for evaluating the South China Sea and anthropogenic influence. Stations (S10 and S11) locate in the northern or top part of the bay. Twelve monitoring stations are located in Daya Bay (Fig. 1).

SAMPLING AND ANALYTICAL METHODS

Water samples were taken at the surface and bottom layers of all stations in January (winter), April (spring), August (summer) and November (autumn) in 2001. A Quanta® Water Quality Monitoring System (Hydrolab Corporation, USA) was employed to collect the data for temperature (T), pH and salinity (S) in the surface and bottom layers. Seawater samples for analysis of nutrients, chlorophyll *a* (Chl-*a*), were taken using 5-L GO FLO bottles at surface and bottom layers, and other samples from the surface and bottom layers were also collected according to the methods and sampling tools of 'The specialties for oceanography survey' (GB12763-91, China). Water samples from the surface and bottom layers were analyzed for nitrate (NO_3-N), nitrite (NO_2-N) and silicate (SiO_3-Si) with a SKALAR auto-analyzer (Skalar Analytical B.V. SanPlus, Holand). Ammonium (NH_4-N), phosphorus (PO_4-P) was analyzed with methods of oxidized by hypobromite and molybdophosphoric blue, respectively. Two replicates of 1.5-L samples from the depths mentioned above were filtered through 0.45 μm GF/F filters and deep frozen immediately at -20ºC. At the end of the cruise, all filters were transported to the shore laboratory in liquid nitrogen. Within a week, the chlorophyll *a* was extracted in10 ml 90% acetone in the dark for 24 h in a refrigerator and its concentration determined with 10-AU Fluorometry (Turner Designs, USA).

PHYTOPLANKTON

Phytoplankton were sampled with a standard Shallow III Microplankton Net (diameter 37 cm, mesh fiber JF62,mesh size 0.077 mm) that was hauled vertically from the bottom to the surface layer, strictly following the standard method (China State Bureau of Technical Supervision). Samples were mixed with buffered formaldehyde to obtain a final concentration of 2.5%. Enumeration and identification of phytoplankton were performed using an Olympus BX51 Inverted Microscope (Olympus, Japan).

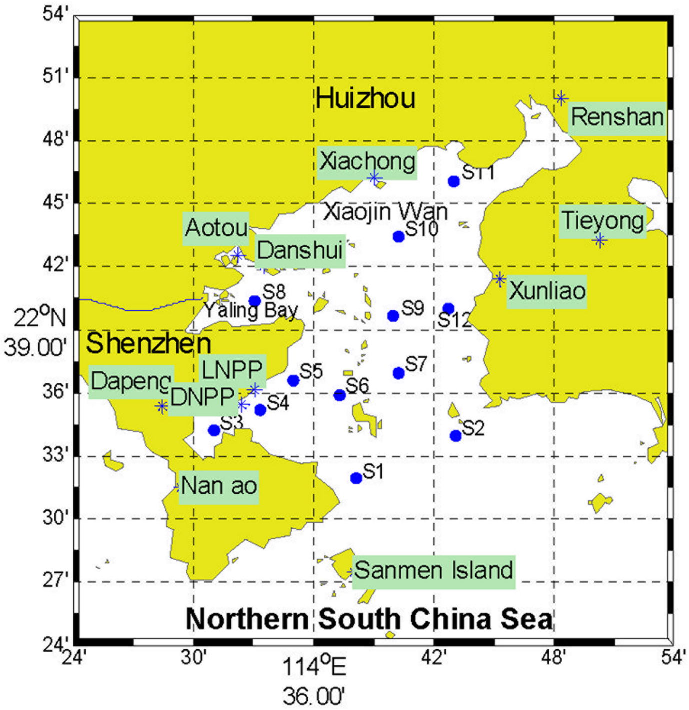

Figure 1. Monitoring stations in Daya Bay (Wu *et al.* 2010) / Estaciones de monitoreo en Bahía Daya (Wu *et al.* 2010)

Data analysis

Principal component analysis (PCA) is designed to transform the original variables into new, uncorrelated variables (axes), called the principal components, which are linear combinations of the original variables. The new axes lie along the directions of maximum variance (Shrestha & Kazama 2007, Wu & Wang 2007). It reduces the dimensionality of data set by explaining the correlation amongst a large number of variables in terms of a smaller number of underlying factors (principal components or PCs) without losing much information (Vega *et al.* 1998, Helena *et al.* 2000, Alberto *et al.* 2001, Li *et al.* 2009, Wu *et al.* 2010b). Canonical corresponding analysis: Physical, chemistry, and biological variables of Daya Bay water were analyzed by CCA to identify their relative contribution indiscriminating phytoplankton communities.

Statistical analysis -all the statistical analyses described below were performed with principal component analysis (PCA) procedures of the MATLAB software (Mathworks, USA).

Results

Environmental factors

Hydrologic conditions varied markedly in Daya Bay (Table 1). Temperature varied markedly seasonal changes ranged from 18.70°C to 32.40°C during the studying year, with the highest value (32.40°C) in summer, and the lowest value (18.70°C) in winter. Seasonal averages of temperature were over 19.00°C in all the four seasons. Temperature was low in January, and dropped to less than 20.00°C. On the contrary, there was comparatively high more than 30.00°C in summer. Salinity varied from 26.60 to 32.84 in the sampling year. Salinity exhibited strong seasonal fluctuation, with the highest value (32.84) in spring, and the lowest value (26.60) in summer.

Nutrient concentrations and their ratios

Higher SiO_3-Si concentrations (mean: 29.57 µmol L^{-1}) were detected in spring, and the low SiO_3-Si (16.14 µmol L^{-1}) was in winter. The SiO_3-Si concentration was higher in the western coastal region than in the eastern part in

Table 1. Descriptive statistics of physical-chemical and biological parameters in Daya Bay / Estadística descriptiva de parámetros físico-químicos y biológicos en Bahía Daya

Station	Layer	Temperature (℃)			DO			pH			Salinity			Chl-*a* (μg L^{-1})			NO_2-N (μmol L^{-1})			NO_3-N (μmol L^{-1})			NH_4-N (μmol L^{-1})			SiO_3-Si (μmol L^{-1})			PO_4-P (μmol L^{-1})		
		min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean
S1	Surface	21.30	30.00	25.77	5.90	7.00	6.53	7.85	8.26	8.10	27.90	32.76	30.80	0.87	3.44	1.86	0.03	0.13	0.07	1.69	6.05	3.57	0.61	1.85	1.38	14.30	25.20	18.69	0.06	0.68	0.28
S1	Bottom	21.40	28.90	25.60	5.31	6.88	6.33	7.91	8.30	8.14	28.75	32.76	31.15	0.59	2.59	1.53	0.03	0.09	0.06	1.35	4.37	2.91	0.66	1.83	1.44	12.83	24.70	16.84	0.04	0.42	0.18
S2	Surface	21.40	30.40	25.93	5.66	6.66	6.33	7.94	8.25	8.13	28.39	32.84	31.07	0.65	1.10	0.86	0.06	1.08	0.42	1.03	4.69	2.37	0.61	2.90	1.60	17.00	34.20	24.68	0.09	0.86	0.37
S2	Bottom	21.30	29.50	25.77	5.60	6.78	6.30	7.97	8.26	8.14	28.31	32.81	31.05	0.22	1.56	0.96	0.04	1.24	0.50	1.49	5.45	2.94	0.57	4.25	2.00	26.17	36.20	29.96	0.09	0.58	0.26
S3	Surface	20.20	31.40	24.75	6.80	7.30	6.97	8.02	8.34	8.15	26.73	31.74	30.26	1.43	6.05	2.75	0.09	0.48	0.22	1.00	3.59	2.32	0.74	4.67	3.00	15.20	29.92	21.02	0.02	0.71	0.24
S3	Bottom	20.30	31.30	24.80	6.78	7.32	6.95	7.89	8.34	8.12	27.07	31.87	30.38	0.24	3.68	2.43	0.04	0.35	0.16	1.25	3.21	1.89	0.75	4.65	2.62	13.30	33.25	20.01	0.09	0.42	0.21
S4	Surface	20.00	31.20	25.05	6.51	7.78	7.09	8.02	8.37	8.17	27.08	32.24	30.57	1.14	6.19	3.09	0.05	0.11	0.08	1.11	2.04	1.59	0.67	5.07	2.31	13.30	22.42	19.07	0.05	0.46	0.17
S4	Bottom	19.90	30.80	25.05	3.61	7.75	6.31	8.05	8.36	8.17	27.56	32.60	30.77	0.25	4.41	1.85	0.05	0.17	0.10	1.19	2.80	1.93	1.10	5.80	2.59	17.70	25.75	21.56	0.08	0.58	0.26
S5	Surface	19.60	31.60	25.20	6.88	7.98	7.41	7.98	8.37	8.16	28.21	32.72	31.07	0.46	10.44	4.43	0.04	0.21	0.09	0.97	2.06	1.50	0.65	1.72	1.26	8.30	32.00	21.95	0.09	0.42	0.18
S5	Bottom	19.30	30.10	24.73	5.22	7.91	6.70	7.96	8.37	8.15	28.44	32.73	31.24	0.58	2.21	1.70	0.04	0.25	0.12	0.74	3.17	1.74	0.73	6.55	2.80	15.80	30.33	22.94	0.07	0.65	0.25
S6	Surface	18.80	30.20	24.55	5.40	7.97	6.64	7.98	8.34	8.19	27.41	32.67	30.81	0.58	6.95	2.79	0.02	0.34	0.14	0.97	4.53	2.56	0.75	3.31	2.11	19.00	30.33	24.73	0.09	0.64	0.32
S6	Bottom	18.80	27.80	23.83	5.04	7.79	6.54	7.82	8.37	8.11	31.35	32.66	31.84	0.62	8.34	2.75	0.04	0.77	0.28	0.71	3.57	1.85	0.68	2.05	1.22	18.24	36.50	28.75	0.17	0.60	0.37
S7	Surface	18.70	29.80	24.33	6.47	7.85	7.19	7.99	8.40	8.17	27.51	32.57	30.78	0.85	8.91	3.30	0.04	0.20	0.09	1.34	3.34	2.26	0.80	2.55	1.78	20.59	33.25	26.76	0.08	0.34	0.23
S7	Bottom	18.70	29.40	24.08	6.58	7.75	7.12	7.99	8.39	8.16	28.81	32.56	31.11	0.56	6.80	2.76	0.05	0.39	0.15	1.12	1.63	1.35	0.72	1.65	1.08	13.65	36.58	23.61	0.08	0.37	0.26
S8	Surface	19.10	31.30	24.98	7.07	7.88	7.43	7.99	8.64	8.32	25.43	31.89	30.05	1.67	4.81	3.60	0.03	0.11	0.06	1.01	3.28	2.19	1.18	4.04	2.51	13.70	33.25	22.73	0.07	0.25	0.12
S8	Bottom	19.10	31.10	24.80	6.84	7.68	7.38	7.98	8.35	8.17	26.60	31.90	30.34	0.91	5.74	3.69	0.04	0.09	0.07	1.25	2.65	1.73	0.90	8.00	4.04	15.20	29.30	20.86	0.09	0.35	0.18
S9	Surface	19.00	30.20	24.85	6.77	7.83	7.24	8.00	8.43	8.17	27.39	31.91	30.59	0.58	1.37	0.96	0.01	0.11	0.07	0.08	1.90	0.97	1.11	1.80	1.57	10.12	41.17	26.57	0.08	0.30	0.21
S9	Bottom	18.90	29.50	24.33	6.23	7.84	6.98	8.00	8.40	8.17	28.08	32.14	30.89	1.26	6.22	3.26	0.08	0.09	0.08	0.60	1.80	1.12	1.08	6.40	2.57	8.94	31.17	19.95	0.06	0.38	0.23
S10	Surface	18.90	32.40	25.38	6.68	8.20	7.35	7.96	8.45	8.17	27.05	32.10	30.47	0.34	5.43	2.40	0.05	0.09	0.06	0.85	2.88	1.70	1.16	8.20	3.43	11.06	32.30	22.89	0.13	0.27	0.19
S10	Bottom	18.80	31.00	24.75	6.71	7.79	7.19	7.97	8.41	8.17	27.54	32.14	30.67	0.77	1.97	1.27	0.05	0.10	0.07	0.77	3.51	1.80	1.06	6.30	3.69	15.65	29.50	20.29	0.06	0.26	0.19
S11	Surface	19.00	32.00	25.08	6.85	7.58	7.23	7.90	8.31	8.09	27.15	31.72	30.16	0.38	2.61	1.57	0.06	0.27	0.16	0.78	2.47	1.44	0.66	9.63	3.48	15.18	50.70	32.25	0.11	0.45	0.22
S11	Bottom	18.90	31.00	24.70	6.78	7.47	7.03	7.92	8.29	8.08	28.08	31.73	30.44	0.70	2.46	1.65	0.02	0.22	0.11	1.07	2.43	1.71	0.83	7.45	2.75	10.71	43.70	28.57	0.11	0.41	0.24
S12	Surface	19.00	30.80	24.95	6.44	8.58	7.32	8.00	8.65	8.32	27.63	32.18	30.67	1.00	2.62	1.73	0.01	0.09	0.05	1.22	3.43	2.30	0.65	1.95	1.34	10.71	32.42	19.83	0.09	0.27	0.20
S12	Bottom	18.90	29.40	24.35	6.13	7.84	7.07	7.96	8.40	8.15	29.11	31.92	31.00	0.68	4.23	2.11	0.09	0.21	0.12	0.85	3.10	1.62	0.88	2.10	1.44	9.88	31.20	21.82	0.11	0.28	0.17

Season	Layer	Temperature (℃)			DO			pH			Salinity			Chl-*a* (μg L^{-1})			NO_2-N (μmol L^{-1})			NO_3-N (μmol L^{-1})			NH_4-N (μmol L^{-1})			SiO_3-Si (μmol L^{-1})			PO_4-P (μmol L^{-1})		
		min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Winter	Surface	18.70	20.20	19.23	7.30	8.58	7.85	8.31	8.45	8.38	31.33	31.89	31.53	0.34	8.91	2.71	0.09	0.34	0.16	0.78	4.53	1.77	0.65	4.16	2.32	10.12	21.88	16.40	0.25	0.71	0.41
	Bottom	18.70	20.30	19.16	7.32	7.91	7.71	8.29	8.41	8.37	31.35	31.90	31.59	0.24	8.34	2.91	0.09	0.25	0.14	1.12	3.17	1.68	0.72	5.87	2.30	8.94	23.41	15.87	0.25	0.65	0.43
Spring	Surface	21.30	22.70	22.08	6.47	7.24	6.83	8.13	8.26	8.19	31.36	32.84	32.24	0.46	4.81	1.34	0.03	0.48	0.10	1.03	3.59	2.08	0.61	1.18	0.86	16.58	42.83	30.13	0.06	0.13	0.09
	Bottom	21.30	22.50	21.98	6.49	7.39	6.81	8.12	8.26	8.18	31.36	32.81	32.28	0.22	3.84	1.13	0.03	0.35	0.10	0.71	3.21	1.76	0.57	1.30	0.90	12.83	41.58	29.01	0.06	0.17	0.11
Summer	Surface	29.80	32.40	30.94	5.40	7.88	7.02	7.85	8.02	7.98	25.43	28.39	27.32	0.58	10.44	3.13	0.01	0.09	0.05	0.08	3.54	2.06	1.10	2.75	1.92	8.30	32.30	20.12	0.09	0.24	0.14
	Bottom	27.80	31.30	29.98	3.61	7.68	6.30	7.82	8.05	7.95	26.60	31.39	28.31	0.79	6.22	2.19	0.02	0.77	0.15	1.00	3.57	2.06	1.18	5.80	2.06	13.00	36.50	20.34	0.04	0.33	0.18
Autumn	Surface	25.50	28.00	26.92	6.44	7.07	6.77	7.90	8.65	8.21	30.44	31.97	31.44	0.84	6.19	2.82	0.03	1.08	0.18	0.77	6.05	2.15	1.42	9.63	3.62	13.70	50.70	26.20	0.02	0.86	0.27
	Bottom	26.00	27.00	26.71	6.13	6.90	6.70	7.92	8.30	8.11	30.61	32.24	31.54	1.15	5.74	2.70	0.05	1.24	0.19	0.60	5.45	1.82	0.87	8.00	4.24	14.20	43.70	25.24	0.07	0.58	0.26
Average	Surface	18.70	32.40	25.26	5.40	8.58	7.07	7.85	8.65	8.17	25.43	32.84	30.55	0.34	10.44	2.55	0.01	1.08	0.12	0.08	6.05	2.03	0.61	9.63	2.13	8.30	50.70	23.71	0.02	0.86	0.21
	Bottom	18.70	31.30	24.90	3.61	7.91	6.81	7.82	8.41	8.13	26.60	32.81	30.87	0.22	8.34	2.29	0.02	1.24	0.15	0.60	5.45	1.86	0.57	8.00	2.38	8.94	43.70	23.01	0.04	0.65	0.22

winter (Fig. 2). The summer distribution of SiO_3-Si showed the spatial variation with a decrease from the northern region to the mouth of the bay. PO_4-P concentration showed seasonal change characterized by higher (mean: 0.42 µmol L^{-1}) in winter and lower (mean: 0.10 µmol L^{-1}) in spring. The PO_4-P concentration was higher in the western coastal region than in the eastern part in winter (Fig. 3).

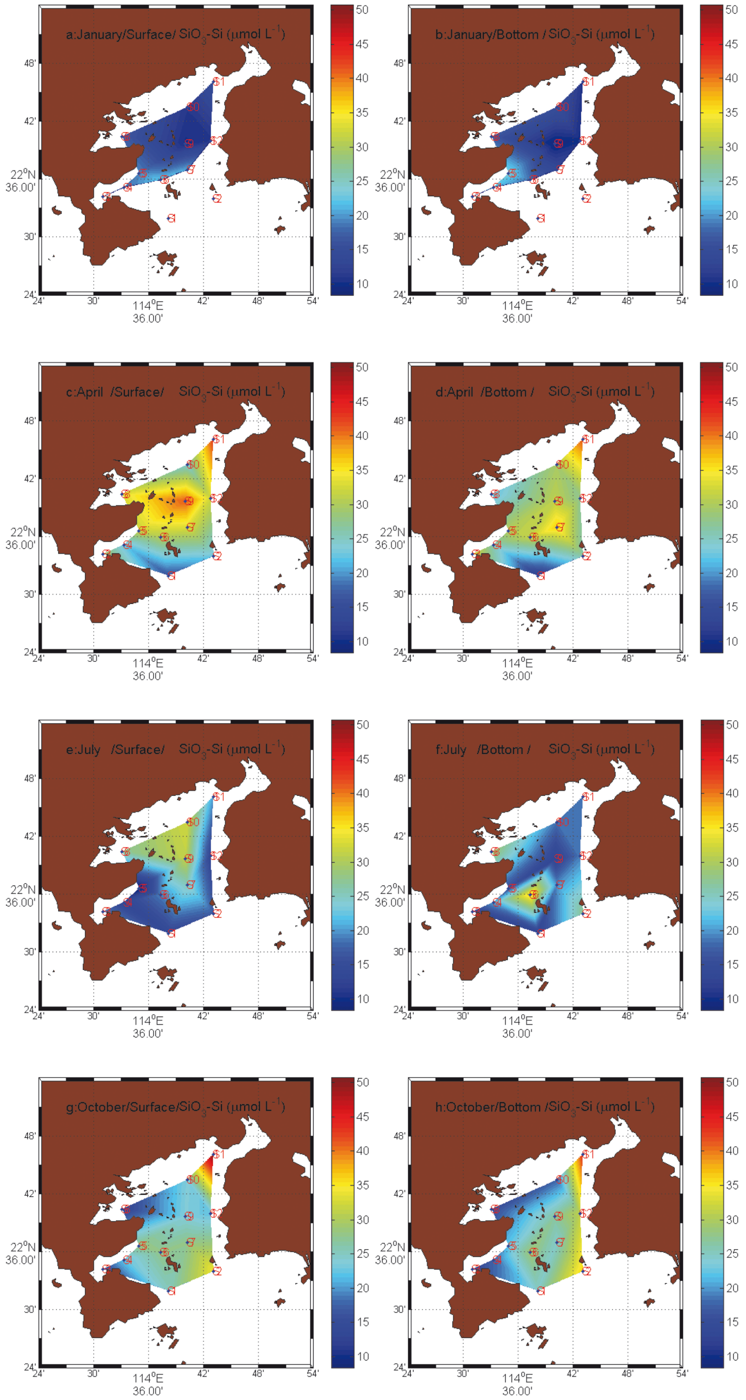

Figure 2. Seasonal horizontal distributions of SiO_3-Si concentrations in Daya Bay / Distribuciones estacionales de concentración de SiO_3-Si en Bahía Daya

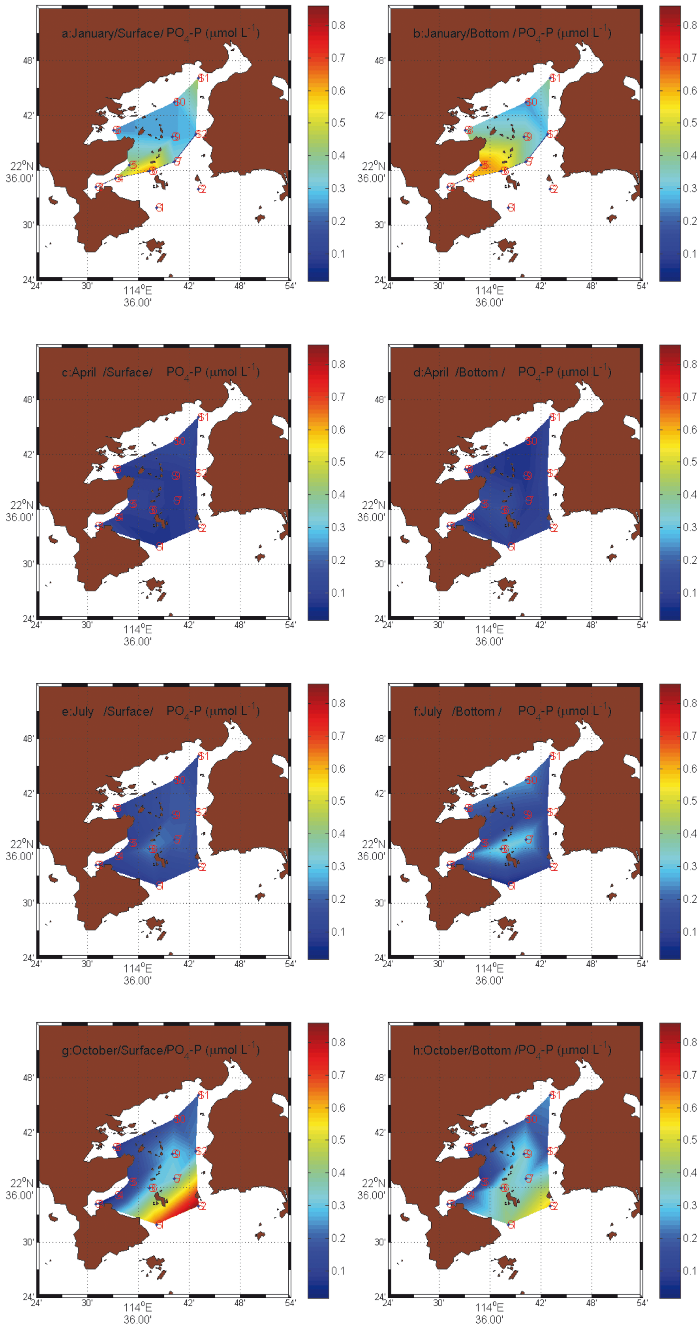

Figure 3. Seasonal horizontal distributions of PO_4-P concentrations in Daya Bay / Distribuciones horizontales estacionales de concentración de PO_4-P en Bahía Daya

NH_4-N concentration varied from 0.57 μmol L^{-1} to 9.63 μmol L^{-1} during the studying year, with the highest value (9.63 μmol L^{-1}) in autumn, and the lowest value (0.57 μmol L^{-1}) in spring.NO_3-N concentration varied from 0.08 μmol $L^{-1}$to 6.05 μmol L^{-1} during the studying year, with the highest value (6.05 μmol L^{-1}) in autumn, and the lowest value (0.08 μmol L^{-1}) in summer. NO_2-N concentration was less than 0.15 μmol L^{-1}in all stations. The horizontal distribution of NO_3-N surface concentration reveals that the NO_3-Nconcentration increases from the eastern part to the western part of the bay. NO_3-N is the main form of DIN (NH_4-N+NO_3-N+NO_2-N) in spring, and the concentration of NH_4-N is almost equal to that of NO_3-N in other seasons. The seasonal cycle in NO3-N concentration in surface water in Daya Bay was not in good agreement with surface water temperature (r^2= 0.001, P= 0.75 > 0.05) and salinity (r^2= 0.001, P= 0.76 > 0.05) during the studying periods, respectively.

The atomic Si:N:P ratio of marine diatoms is about 16:16:1,when nutrient levels are sufficient (Redfield 1963). Deviations from this ratio indicate potential for N, P, and Si limitation of phytoplankton growth. The N:P ratio ranged from 3.40 to 122.00 in all the stations during the studying period; the N:P ratios were 11.16, 15.89, 30.90 and 40.33 in winter, spring, summer and autumn, in 2001, respectively. The annual mean N:P ratio is 24.82. The Si:N ratio ranged from 2.21 to 22.12 in all the stations, and was more than 1.00. The Si:N ratios were 4.64, 5.98, 11.26 and 9.53 in winter, spring, summer and autumn, in 2001, respectively. Furthermore, the low concentration of SiO_3-Si (16.14 µmol L^{-1}) was found. A plot of N:P and Si:N ratios indicated that the limiting factor transited from N limiting in winter to P limiting in spring (Fig. 4). SiO_3-Si is unlikely to be the limiting factor for phytoplankton growth during the study period.

Phytoplankton community

Chl-*a* concentration exhibited seasonal variability, with the maximum value (10.44 µg L^{-1}) in summer, and the minimum value (0.22 µg L^{-1}) in spring. Average value of Chl-*a* concentration was 2.42 µg L^{-1}. The Chl-*a* concentration was higher in the eastern and northern coastal region than in the southern and eastern part of the bay.

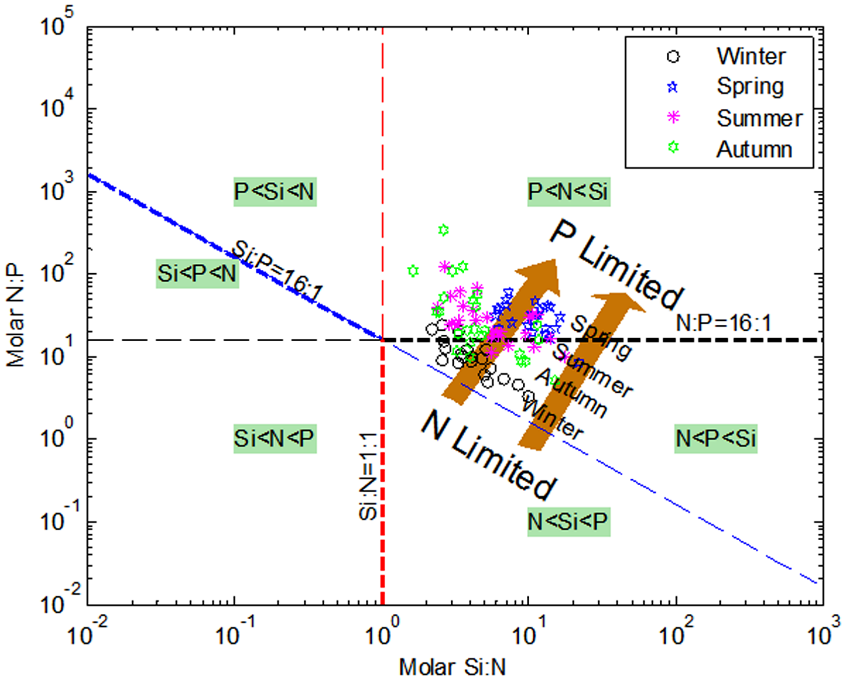

Figure 4. Scatter diagrams of the Si:N:P atomic nutrient ratios in the water column in 2001. Molar quotients between the concentrations of potentially limiting nutrients are delimited in this logarithmic plot (log Si:N *vs.* log N:P) by the Si:N= 1:1, N:P= 16:1 and Si:P= 16:1 lines, which define six different areas within the plot, with each one characterized by the potentially limiting nutrients in order of priority (Rocha *et al.* 2002; Wu & Wang 2007) / Diagramas de dispersión de las proporciones de nutrients Si:N:P en la columna de agua en 2001. Los cuocientes molares entre las concentraciones de nutrientes potencialmente limitante están delimitadas en el gráfico logarítmico (log Si:N *vs.* log N:P) por las líneas Si:N= 1:1, N:P= 16:1 y Si:P= 16:1, que definen 6 áreas diferentes dentro del gráfico, cada una caracterizada por los nutrientes potencialmente limitantes en orden de prioridad (Rocha *et al.* 2002; Wu & Wang 2007)

Table 2. Seasonal dominant phytoplankton / Fitoplancton dominante estacional

Winter	Spring	Summer	Autumn
Thalassiothrix fraenfeldii	*Ceratium trichoceros*	*Skeletonema costatum*	*Biddulphia sinensis*
Lauderia borealis	*Thalassiothrix fraenfeldii*	*Thalassiothrix fraenfeldii*	*Skeletonema costatum*
Bacteriastrum varians	*Ceratium farca*	*Nitzschia pungens*	*Dinophysis caudata*
Nitzschia pungens	*Dactyliosolen mediterraneus*	*Chaetoceros diversus*	*Chaetoceros coarctatus*
Chaetoceros curvisetus	*Nitzschia pungens*	*Chaetoceros curvisetus*	*Nitzschia pungens*
Chaetoceros lorenzianus	*Asterionella notata*	*Nitzschia delicatissima*	*Chaetoceros diversus*
Eucampia zoodiacus	*Chaetoceros lorenzianus*	*Chaetoceros lorenzianus*	*Asterionella japonica*
Chaetoceros affinis	*Chaetoceros eibenii*	*Chaetoceros affinis*	*Coscinodiscus asteromphalus*
Thalassiosira subtilis	*Chaetoceros affinis*	*Rhizosolenia styliformis*	*Nitzschia delicatissima*
	Rhizosolenia styliformis	*Ceratium fusus*	*Chaetoceros lorenzianus*
	Ceratium pulchellum	*Thalassionema nitzschioides*	*Chaetoceros affinis*
	Thalassionema nitzschioides		*Rhizosolenia styliformis*
			Thalassionema nitzschioides

From the analysis of phytoplankton, a total of 141 species including 110 species in 42 genera of diatoms, 26 species in 8 genera of dinoflagellates, and 5 species in 3 genera of other groups were identified (Fig. 5). Phytoplankton abundances were high, and the annual mean cell density was 4.39 X 10^5 cells m^{-3}. Phytoplankton abundances were higher in S3 and S8 than other studying stations (Fig. 6). The diatom species were dominant almost year round, usually making up more than 90% of the total abundance. The abundance of dinoflagellate was relatively higher in the spring and winter than in other seasons. The dominant phytoplankton groups in Daya Bay were diatoms, and then dinoflagellates. The Chrysophyceae and cyanobacteria were minor groups. The dominant taxa during the study period were shown in Table 2. *Chaetoceros* and *Rhizosolenia* were the most diversified genus in diatoms.

Principal component analysis was applied to identify the seasonal changes of environmental factors and phytoplankton groups (diatoms, dinoflagellates and others). Biplot (scores of sampling stations and loadings of environmental factors and phytoplankton groups) was shown in Figure 7. The scores of sampling stations during summer and autumn had positive values in PC1, and vice during winter and spring. The abundance of diatom and dinoflagellate had negative loadings in PC1, which indicated a higher influence on scores of sampling stations during winter and spring. Temperature had positive loading in PC1, which water temperature in summer was higher than other seasons. In addition, PCA was used to identify the seasonal change of the dominant species. Biplot showed the different dominant species in different seasons (Fig. 8). The dominant species were *Lauderia borealis, Bacteriastrum varians, Chaetoceros curvisetus, Eucampia zoodiacusm, Thalassiosira subtili* in winter*; Ceratium pulchellum, Asterionella notata, Dactyliosolen mediterraneus* in spring.

Discussion

Dynamics of nutrients

The importance of SiO_3-Si has been underrated in past studies because it was thought to rarely limit phytoplankton production and it was only assumed to cause a shift in species composition, but not necessarily productivity (Dortch & Whitledge 1992). However, interest in the importance of SiO_3-Si for marine ecosystems has increased. in the past several decades, SiO_3-Si concentration has decreased due to building irrigation in rivers, such as Jiaozhou Bay (Shen 2001). SiO_3-Si in seawater is mainly transport through rivers. Daya Bay is a semi-closed bay and has no major rivers but several seasonal rivers. The waters of Daya Bay exchange with coastal water adjacent to Pearl River Estuary in South China Sea. Therefore, the water in South China Sea has important effects on Daya Bay. SiO_3-Si concentration (*ca*., 23.70 μmol L^{-1}) in seawater was almost

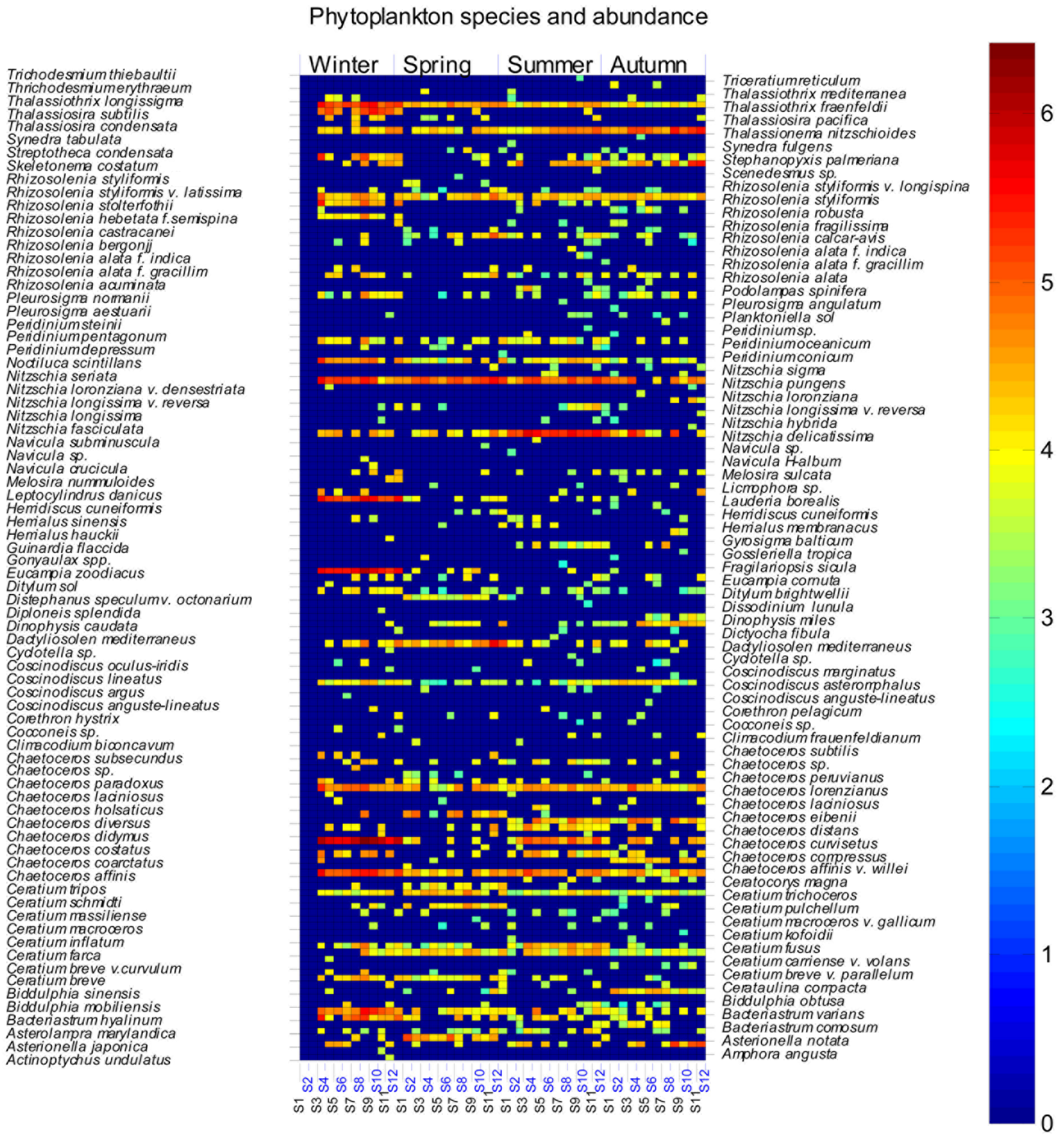

Figure 5. Phytoplankton species and abundance during the studying time in Daya Bay. Every species found had different abundance and its frequency in different stations and sampling time. Unit: log10 (Phytoplankton abundance + 1) / Especies de fitoplancton y abundancia durante el periodo de estudio en Bahía Daya. Cada especie tiene diferente abundancia y su frecuencia en diferentes estaciones y periodo de muestreo. Unidad: log10 (abundancia de fitoplancton + 1)

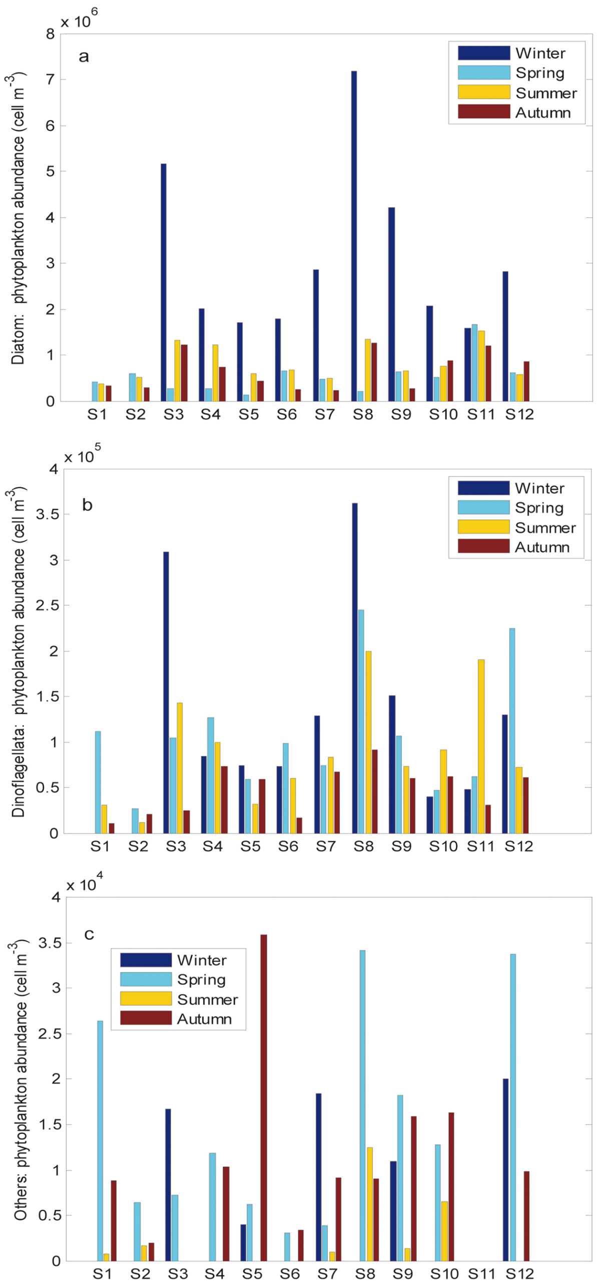

Figure 6. Diatom (a), dinoflagellates (b) and others (c) abundance during the studying time in Daya Bay / Abundancia de diatomeas (a), dinoflagelados (b) y otros (c) durante el periodo de estudio en Bahía Daya

stable. The seasonal cycle in SiO_3-Si concentration in surface water in Daya Bay was not in good agreement with surface water temperature (r^2= 0.001, P= 0.94 > 0.05) and salinity (r^2= 0.04, P= 0.19 > 0.05) during the study periods, respectively. Freshwater runoff has little effect of on SiO_3-Si concentration in Daya Bay. The results may be different from temperate Jiaozhou Bay (Shen 2001), and tropical Sanya Bay (Wu *et al.* 2012). The riverine or / and land-resource loads of SiO_3-Si are small in Daya Bay. SiO_3-Siplays an important role in the ecosystem processes, by changes in diatom species and food web dynamics. SiO_3-Si concentration in Daya Bay had obvious seasonal variation, high concentration in spring and autumn and low in winter and summer. SiO_3-Si concentration in Daya Bay is mainly controlled by phytoplankton growth.

If we only take consumption by phytoplankton, regardless of their various origins (sediment and resuspension).The mean SiO_3-Si concentration in Daya Bay ranged from 16.14 µmol L^{-1} in winter to 29.57 µmol L^{-1} in spring. At the same time, ΔSiO_3-Si (maximum value-seasonal average values) represents the SiO_3-Si consumed by phytoplankton. The order of ΔSiO_3-Si is 13.43 µmol L^{-1}, 9.34 µmol L^{-1} and 3.85 µmol L^{-1} in winter, summer and autumn, respectively. SiO3-Si appeared to be predominantly consumed in winter more than other seasons. The phytoplankton abundance is higher in winter than other seasons. SiO_3-Si was consumed by diatoms and buried with dead diatoms, and the SiO_3-Si concentration was reduced to a minimum in winter. In other words, SiO_3-Si consumed was related to relatively higher phytoplankton abundance (2.61 x 10^6 cells m^{-3}) and a lower SiO_3-Siconcentration (*ca.*, 16.0 µmol L^{-1}) in winter; when relatively higher SiO_3-Si concentration (*ca.*, 30.0 µmol L^{-1}) occurred (Table 1), and lower phytoplankton abundance (0.54 x 10^6 cells m^{-3}) was found in spring (Fig. 6).

Surprisingly, when the lowest SiO_3-Si concentration appeared in winter, higher PO_4-P concentration also appeared. The seasonal cycle in PO_4-P concentration in surface water in Daya Bay was not related with surface water temperature (r^2= 0.04, P= 0.17) and salinity (r^2= 0.03, P= 0.26) during the studying periods, respectively. In other words, PO_4-P may be different ways for phytoplankton growth. In Daya Bay, diatom is a dominant group of phytoplankton (Fig. 6). Diatom may response to silicate recycles. Silica recycles much slower than both nitrogen and phosphorus, which means that phosphorus, can be used many times during its ‘life cycle’ whereas silica is usually only used once (Conley *et al.* 1988).

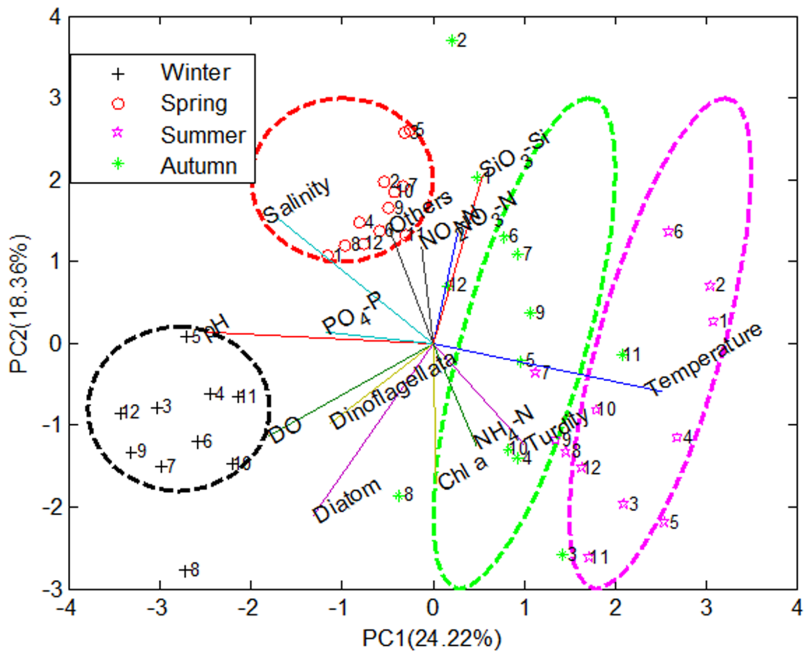

Figure 7. Biplot of scores of sampling stations and loadings of environmental factors and phytoplankton groups from the results of principal component analysis. Sampled stations are indicated by the number, phytoplankton groups and environmental variables are represented by lines / Puntajes de gráficos de las estaciones de muestreo y factores ambientales y grupos de fitoplancton como resultado del análisis de componentes principales. Las estaciones muestreadas se indican por el número, grupo de fitoplancton y variables ambientales son representadas por líneas

Currently, dissolved organic phosphorus (DOP) is found to be a very important species for phosphorus. In addition, the non-DIP sources might have played equal or an even more important role in production within the bay. If we take the nutrient (PO_4-P) as a whole, regardless of their various origins (sediment and resuspension), the mean PO_4-P concentration in Daya Bay ranged from 0.10 μmol L^{-1}in spring to 0.42 μmol L^{-1} in winter. The order of ΔPO_4-P is 0.32 μmol L^{-1}, 0.16 μmol L^{-1} and 0.06 μmol L^{-1} in winter, autumn and summer, respectively.

Factors driving seasonal changes of nutrient structure in Daya Bay

Our results provide plausible explanations for seasonal variations of nutrient structure in Daya Bay. The seasonal shift in N:P ratios seems to be associated with the environmental variables. The results of principal component analysis (PCA) applied for environmental variables (Fig. 7) suggested that Daya Bay under goes predictable seasonal changes. Four classes of environment and ecological status were identified by PCA. Temperature, salinity and pH were the most important variables in PC1. Temperature is significant indicator of climate change, and important forces in controlling the hydrodynamics in the bay. In this area, the Southeast Asian monsoons, northeasterly from October to April and southwesterly from May to September have important effects on biogeochemical cycles in Daya Bay waters (Wu & Wang 2007). During winter, waters are vertically mixed in Daya Bay under the influence of the northeast monsoon. By contrast, the stratified waters were distinct due to the water of lower temperature and high salinity intruded into the bay along the bottom from the South China Sea under the influence of the weak monsoons southwesterly from May to September in summer. In addition, there is plenty of rain from May to October, and there is less rain from November to the next April. The average precipitation is 1827 mm in Daya Bay, the maximum rainfall is 370 mm in June, and the minimum rainfall is 30 mm in December (Han 1998). Both the surface and bottom salinity are similar in winter. In summer, the bottom salinity is higher than the surface salinity; the rain diminishes the surface salinity.

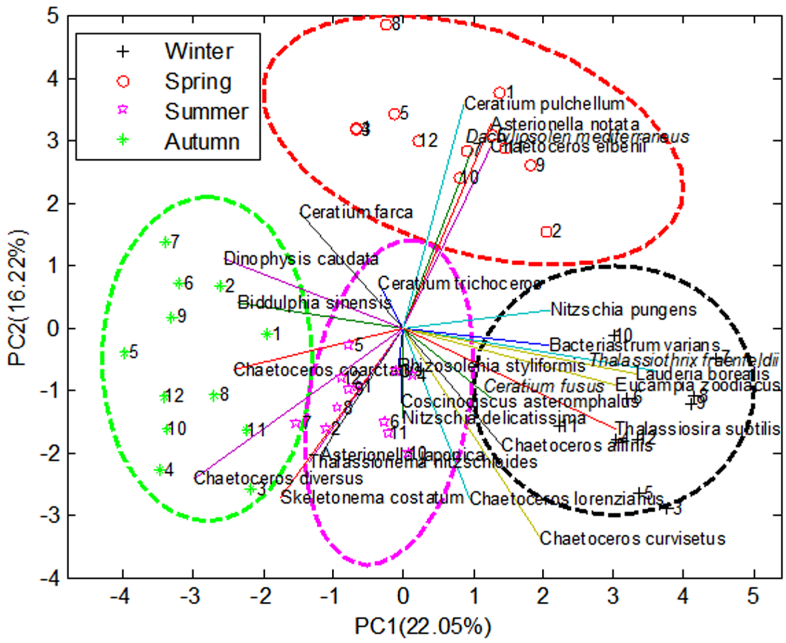

Figure 8. Biplot (scores of sampling stations and loadings of the different dominant species) from the results of principal component analysis. The number represents the sampling station. Diagram of the samples and the phytoplankton groups on the two first axes of the PCA, showing a clear separation depending on 4 seasons / Gráfico (puntajes de las estaciones de muestreo y las diferentes especies dominantes) de los resultados del análisis de components principales. El número representa la estación de muestreo. El diagrama de las muestras y grupos de fitoplancton sobre los dos primeros ejes del ACP, muestra una clara separación dependiente de las 4 estaciones

This study shows that temperature and salinity are important environmental factors.

The mean pH value during the southwest monsoon period (summer) are lower than that during northeast monsoon period, suggesting a high load of dissolved organic matter added from land-based resource, such as domestic wastewater, agricultural-related activities and industrial effluents. This results in anaerobic conditions in the bay, which, in turn, results in the formation of ammonia and organic acids. Hydrolysis of these acidic materials causes a decrease of pH (Shrestha & Kazama 2007).

A seasonal changing ratios of the key nutrients such as N:P and N:Si accompanied nutrient biogeochemistry in the bay. Recent studies indicated that not only human activity but also hydrodynamics (mixing and stratification) also lead to pronounced shifts in nutrients structure or their ratios (Wu & Wang 2007).

Factors driving phytoplankton seasonal succession in Daya Bay (hydrodynamics and nutrients)

Apparently, macronutrients availability is a crucial factor in phytoplankton communities. Nutrient structure and / or ratios maybe the main driving force for phytoplankton growth. The limiting factor transited from N limiting in winter to P limiting in spring (Fig. 4). The dominant species were the smaller chain-forming taxa *Skeletonema costatum* and *Thalassionema nitzschioides*, which also showed that nitrophilicity and tolerance to high temperature and lower salinity during the wet season (summer). Despite the differences in the ratios of N:P and Si:N among different seasons, phytoplankton community was dominated by diatoms, which were mostly diatoms such as *Nitzschia pungens, Chaetoceros lorenzianus, Chaetoceros affinis, Thalassiothrix fraenfeldii, Rhizosolenia styliformis*, and *Thalassionema nitzschioides* (Table 2 and Fig. 8). Since SiO_3-Si concentration is more than 2.0 µmol L^{-1} and the ratio of Si:N more than 1, it is unlikely to be the limiting factor for phytoplankton growth during the study period.

The ratio of Si:N is more than 1. In other words, the ratio of N:Si was less than 1:1 in the bay. From the point of view of algal stoichiometry (N:P= 16:1, Si:N= 1:1), there is some room for an increase in N flux from the exogenous to this bay ecosystem. Therefore, the phytoplankton community dominated by diatoms may not necessarily change in the bay when dissolved inorganic nitrogen (DIN) increases in the region. However, if DIN increases and N:Si ratio reaches more than 1:1 in the future, it may result in phytoplankton community change in the coastal waters influenced by human activities.

On the other hand, hydrodynamic processes (mixing in winter and stratification in summer) seem to be another main driving force for phytoplankton growth in the eastern coastal region of Guangdong Province, China. The eastern coastal region of Guangdong Province shows a complex hydrodynamic pattern at the end of the spring. Thus, the intrusion of bottom water of northern South China Sea into the coastal area of eastern coastal area causes low temperature and high salinity in the bottom in this coastal area, depicting a strong stratification. The typical continental shelf upwelling characteristics in the eastern coastal region of Guangdong Province are clearly shown in the surface and subsurface water of upwelling regions, such as low temperature, high salinity and high density (Gan *et al.* 2009, Jing *et al.* 2009, Han *et al.* 2012). The summer upwelling in eastern coastal region of Guangdong continental can intrude into the Daya Bay (Ji & Huang 1990).

The surface current system in Daya Bay was largely dominated by the South Asian Monsoon. The southeastern monsoon typically begins in April-May, which benefits phytoplankton to accumulate in the northwest coastal areas. It thus suggests that accumulation caused by winds and water currents might be an important way for outbreak of algal bloom in Daya Bay. Most of the red tides (sea water color changed bloom) in Daya Bay were reported to occur in the inshore areas.

The clockwise Euler Residual Current in spring, summer and autumn in Daya Bay (Xu 1989) carried the nutrients from land-based water from the west and north in the bay through this area, meanwhile, tidal current carried the nutrients from the South China Sea through this area as well, which may cause the higher concentration of Chl-*a* in this area (Qiu *et al.* 2005). In this study, phytoplankton abundance is higher in the west and north parts of the bay (S3, S8, S10 and S11) than other studying stations (Fig. 6). In Daya Bay, increasing cage culture makes the trend of eutrophication more distinct, and the phytoplankton biomass stays high in the west and north parts of the bay (Dapeng Ao Bay and Aotou Bay, Song *et al.* 2004). Recent studies find that the west and north parts of the bay is mainly related to anthropogenic activities such as fish-farming; the center, east and south parts of Daya Bay is mainly related to seawater input from South China China Sea (Wu *et al.* 2009a, b, c; 2011).

During the studying year, nutrients have obviously changed in the water column due to the influence of human activities and East Asian Monsoons. The nutrient concentration and its ratios play a decisive effect on the species composition of the phytoplankton since different algal species have different nutrient requirements. Nutrient structure and/or ratios maybe the main driving force for phytoplankton growth. In addition, the limiting factor transited from N limiting in winter to P limiting in spring. Hydrodynamic processes (mixing in winter and stratification in summer) seem to be another main driving force for phytoplankton growth in the eastern coastal region of Guangdong Province, China. The dominant phytoplankton groups in Daya Bay were diatoms, and then dinoflagellates. The dominant species were *Chaetoceros affinis, Chaetoceros lorenzianus*, and *Nitzschia pungens* all the year round. In conclusion, human activities and monsoon winds were shown to play important roles in controlling nutrients and phytoplankton community in Daya Bay, northern South China Sea.

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