



# Succession of phytoplankton functional groups from spring to early summer in the central Bohai Sea using HPLC–CHEMTAX approaches

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## Abstract

Phytoplankton is a key component in the functioning of marine ecosystems, phytoplankton community structures are very sensitive to their environment. This study was conducted in the central Bohai Sea in the spring and early summer of 2015. Spatial variations in phytoplankton functional groups were examined through high-performance liquid chromatography pigment–CHEMTAX analysis. Results suggested that the phytoplankton biomass (chlorophyll *a* [Chl *a*]) in spring was mainly derived from the diatom community and was 3.5-fold higher than that in the summer. Meanwhile, the phytoplankton in the early summer sustained more diverse marker pigments than that in the spring. Despite the overwhelming predominance of micro-sized phytoplankton in the spring, some smaller phytoplankton (pico- or nanosized), including flagellates, such as prasinophytes, chlorophytes, and cryptophytes, highly contributed to the total Chl *a* in the summer. Various physico-chemical variables were recorded, and their correlations with phytoplankton density were established by redundancy analysis. Temperature, water stratification, nutrient availability, and even nutritive proportion influenced the succession of phytoplankton functional groups from diatom dominance in the spring to flagellate (mainly haptophytes and prasinophytes) dominance in the early summer. In conclusion, our work comprehensively evaluated the phytoplankton diversity and dynamics in the central Bohai Sea and suggests the need for long-term monitoring for further investigation.

**Keywords** Biodiversity · Dynamic · Diatom · Flagellate · Pigment

## 1 Introduction

Primary productivity in the sea accounts for ~ 50% of the total global annual production (Longhurst et al. 1995). Phytoplankton act as primary producers in bio-geochemical processes and serve as a direct food source for aquatic animals. Phytoplankton functional groups (PFGs) are indicators of primary production and are identified by their morphological, physiological, and ecological attributes rather than common phylogeny. Within particular groups, phytoplankton species possess similar morphologies, environmental sensitivities, and tolerances (Reynolds 2006). Changes in

the community structures and abundances of phytoplankton are largely influenced by environmental fluctuations, which may occur with variable frequency and intensity. Accordingly, biota vary in qualitative and quantitative aspects and hence show selected PFGs through competitive processes that favor these groups (Gemelgo et al. 2009). These features encourage interest in studying PFGs, biodiversity, and responses to environmental factors.

Traditionally, phytoplankton communities are studied by microscopy with identification, cell counting, cell size measurement, and volume-to-biomass conversion (Agirbas et al. 2015). However, phytoplankton diversity is low in microscopy-based research, which restricts species into limited functional groups, such as diatoms and dinoflagellates (Chen et al. 2016). In addition, microscopy is a laborious and time-consuming technique that requires high-level expertise because of the morphological similarities between phytoplankton species (Abad et al. 2016). High-performance liquid chromatography (HPLC), which relies on the relative concentration of phytoplankton pigments, serves as an alternative that would overcome the limitations

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of microscopy-based analysis (Mendes et al. 2015). HPLC is used to detect various other pigments characterized by distinct functional groups and accurately quantify chlorophyll *a* (Chl *a*). For example, fucoxanthin (Fuco), peridinin (Peri), Chl *b*, alloxanthin (Allo), and zeaxanthin (Zea) are typically produced by diatoms, dinoflagellates, chlorophytes, cryptophytes, and cyanobacteria, respectively (Madhu et al. 2014). However, pigment data interpretation can be difficult because some pigments are present in several algal groups (Mendes et al. 2011). A common solution is the statistical software CHEMTAX. By calculating the ratios of marker pigments to Chl *a*, CHEMTAX estimates the biomasses of various PFGs (Mackey et al. 1996). This software package has been widely used to determine the distribution of PFGs in different regions of the world oceans (Agirbas et al. 2015; Goela et al. 2014; Jiang et al. 2016; Mendes et al. 2011; Wright et al. 2010).

The Bohai Sea is the largest inner sea along Chinese coasts. In the past several decades, the marine environment of the Bohai Sea has changed greatly because of anthropogenic pressure (Jiang et al. 2005; Guo 1993; Guo et al. 2014). With increasing nitrogen and decreasing phosphorus loading, the N/P ratio rose significantly, and phytoplankton community changed accordingly. Previous studies suggested that diatoms dominated the phytoplankton before the 1990s (Guo et al. 2014). In recent years, a large shift from diatom dominance to diatom and dinoflagellate co-dominance was observed in the Bohai Sea (Guo et al. 2014; Wei et al. 2004). More recently, under the influence of anthropogenic disturbance (e.g., aquaculture activity), spring–summer blooms of a newly recorded picophytoplankton species (*Aureococcus anophagefferens*, ~ 2 μm) have been more frequently reported in the Qinhuangdao Sea area, northwest of the Bohai Sea (Kong et al. 2012; Zhang et al. 2012). These

phenomena suggest that non-diatom species may currently play important roles in the Bohai Sea.

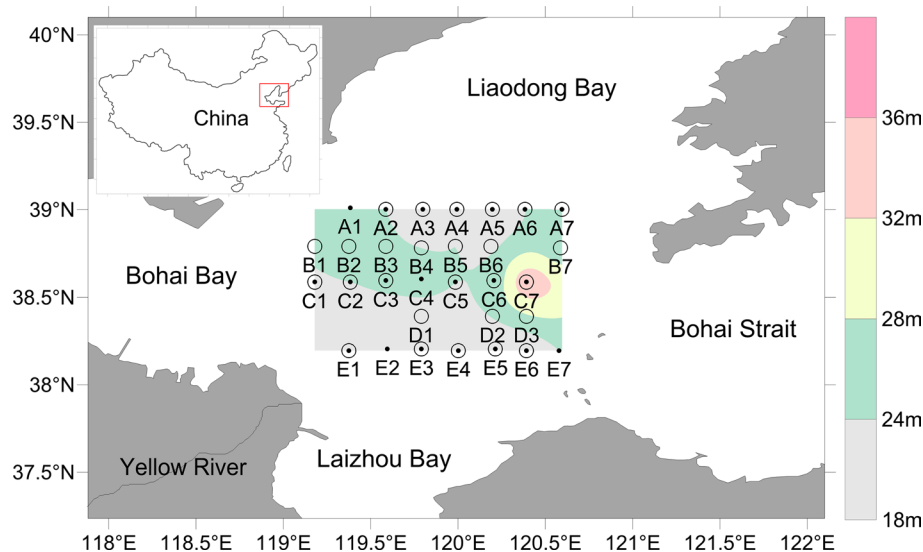
The abundance and species composition of phytoplankton in the Bohai Sea have been widely investigated by microscopy in previous studies (Fei et al. 1991; Sun et al. 2001; Zhang et al. 2004; Lin et al. 2006; Guo et al. 2014; Liu and Chen 2014). However, former investigations mainly focused on diatoms and dinoflagellates due to the limitations of the microscopic method. Some other important PFGs, for example prasinophytes, chlorophytes, and cyanobacteria, have been rarely reported in this area. The principal aim of present study was to elucidate succession of PFGs from spring to early summer in the central Bohai sea using HPLC pigments–CHEMTAX analysis, and the relationship between the phytoplankton community structure and physical–chemical processes.

## 2 Method

### 2.1 Study site

The Bohai Sea is composed of the following four parts: the Bohai Bay, Liaodong Bay, Laizhou Bay, and central Bohai Sea. The Bohai Sea covers  $7.7 \times 10^4$  km<sup>2</sup>, with an average depth of 18.7 m (Zhang et al. 2004). The study area (38.21°–39.01°N, 118.98°–120.83°E) is located at the Bohai Sea center (Fig. 1), which comprises a shallow basin with a deep center (~ 30 m) and some surrounding shallow areas (~ 18 m). Yellow River, the most turbid river in the world, carries large nutrient loads to the central Bohai Sea and significantly influences the phytoplankton structures in the area (Wei et al. 2004).

**Fig. 1** Map with contours showing the sampling locations in the central Bohai Sea. Solid dots and hollow circles indicate the sampling sites in the spring and early summer, respectively



## 2.2 Sample collection

Two cruises were undertaken in the spring (April 3–5) and early summer (June 4–6) of 2015. Surface seawater samples (0.5 m in depth) were collected into Niskin bottles from 21 stations in the spring and 27 stations in the early summer for nutrient and phytoplankton pigment analysis. Samples were not collected in some stations because of poor weather. Temperature, salinity, pH, and dissolved oxygen (DO) were recorded in situ by YSI 556 multiparameter (Yellow Springs Instruments, USA) at three depths (0.5 m, 10 m layer, and 1 m above the bottom floor).

## 2.3 Nutrient analysis

At each sampling station, 100 mL seawater was collected, filtered by a GF/F filter, and stored at  $-20\text{ }^{\circ}\text{C}$  to analyze the nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), ammonium ( $\text{NH}_4^+$ ), dissolved reactive phosphate (DRP), and dissolved silicate (DSi) levels. DIN refers to the sum of the nitrate, nitrite, and ammonium contents. The concentrations of these nutrients in each sample were determined in triplicate by using a Skalar San++ continuous flow analyzer (Strickland and Parsons 1972).

## 2.4 HPLC pigment analysis

Pigment extraction and determination were performed in accordance with a protocol adapted from Zapata et al. (2000). Exactly 1.0 L seawater samples were filtered through 0.7  $\mu\text{m}$  filters (GF/F, Whatman; 47 mm diameter). The filters were immediately deep frozen in liquid nitrogen and stored at  $-80\text{ }^{\circ}\text{C}$ . Phytoplankton pigments were extracted in dim light with 3 mL of 95% HPLC-grade methanol. Samples were ultrasonicated for 5 min in an ice-water bath and then centrifuged at 1500g for 3 min at  $4\text{ }^{\circ}\text{C}$ . The supernatants were filtered through 0.22  $\mu\text{m}$  PTFE membranes to remove the remains of filter and cell debris. A mixture of 200  $\mu\text{L}$  extracts and 67  $\mu\text{L}$  Milli-Q water were prepared for phytoplankton pigment analysis.

An Agilent series 1100 HPLC system fitted with a Waters Symmetry  $\text{C}_8$  column ( $150 \times 4.6\text{ mm}^2$ , 3.5  $\mu\text{m}$  particle size, 100  $\text{\AA}$  pore size) and with a G1314A variable wavelength detector was used to separate the phytoplankton pigments. Pigments were identified by comparing their absorbance spectra, retention times, and concentrations detected by the photodiode array detector (e.g., 440 nm) with those of authentic standards. A total of 22 commercially available pigment calibration standards were obtained from DHI Inc. (Hørsholm, Denmark). These standards included Chl *c*3, Mg-2,4-divinylpheoporphyrin (MgDVP), Chl *c*2, Peri, pheophorbide *a* (Pheide *a*), 19-but-fucoxanthin (But-Fuco), Fuco, neoxanthin (Neo), prasinoxanthin (Pras), violaxanthin

(Viola), 19'-hex-fucoxanthin (Hex), diadinoxanthin (Diadino), Allo, diatoxanthin (Diato), Zea, lutein (Lut), canthaxanthin (Cantha), gyroxanthin-diester (Gyro), Chl *b*, Chl *a*, pheophytin *a* (Phe *a*), and  $\beta$ -carotene ( $\beta$ -Car).

## 2.5 CHEMTAX analysis of pigment data

The relative abundance of the PFGs contributing to the total Chl *a* biomass were calculated from pigment concentration data by using version 1.95 of the CHEMTAX chemical taxonomy software (Mackey et al. 1996; Wright et al. 1996, 2009, 2010). Initial pigment ratios were obtained from a range of values given by Mackey et al. (1996). Meanwhile, calculation procedures and processes were fully described by Wright et al. (2009) and Higgins et al. (2011). A series of 60 pigment ratio matrices were generated by multiplying each ratio from the initial matrix by a random function to optimize the matrix. The average of best six output matrices (with the lowest residual or root-mean-square error) were considered as the optimized results. Data from the spring and early summer were run separately so as to take potential variations into account in optimization of CHEMTAX procedures. The initial and final ratios are given in Table 1. The output data are presented as absolute concentrations (ng/L) of Chl *a* and relative proportions attributed to each phytoplankton group.

## 2.6 Statistical analysis

To explore the relationships between the environmental conditions and PFGs variability, two data matrices were used for each season. One included CHEMTAX derived Chl *a* concentrations of each phytoplankton group, and a second one included the environmental variables that may affect PFGs. The data in phytoplankton matrix were log transformed and centred by species prior to analysis. The environmental matrix included temperature, salinity, DO, pH, DIN,  $\text{PO}_4$ ,  $\text{SiO}_3$ , N/Si, N/P and Si/P. The data were first analyzed by detrending correspondence analysis (DCA). Because the longest lengths of gradient for the two seasons were both less than 3.0, we then performed redundancy analysis (RDA) by using the CANOCO 4.5 software (ter Braak and Smilauer 2002). Statistical significances were evaluated by Monte Carlo permutation test. Only those variables with  $P < 0.05$  were included in the analyses.

## 3 Results

### 3.1 Oceanographic conditions

In the spring, the sea surface temperature ranged from 3.9 to 6.3  $^{\circ}\text{C}$  with an average of 5.0  $^{\circ}\text{C}$  (Table 2). This surface

**Table 1** Input and output ratios of marker pigments to chlorophyll *a*

Taxonomic group	Peri	But-Fuco	Fuco	Hex-Fuco	Neo	Pras	Viol	Allo	Lut	Zea	Chl <i>b</i>	Chl <i>a</i>
Initial ratios												
Diatoms	0	0	0.754	0	0	0	0	0	0	0	0	1
Dinoflagellates	1.062	0	0	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0	0	0	0	0	0	0.229	0	0	0	1
Prasinophytes	0	0	0	0	0.142	0.295	0.058	0	0.009	0	0.886	1
Chlorophytes	0	0	0	0	0.064	0	0.056	0	0.202	0.010	0.263	1
Haptophytes (T3)	0	0	0	1.702	0	0	0	0	0	0	0	1
Haptophytes (T4)	0	0.246	0.585	0.538	0	0	0	0	0	0	0	1
<i>Synechococcus</i>	0	0	0	0	0	0	0	0	0	0.348	0	1
Final ratios (Arp.)												
Diatoms	0	0	1.386	0	0	0	0	0	0	0	0	1
Dinoflagellates	1.260	0	0	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0	0	0	0	0	0	0.250	0	0	0	1
Prasinophytes	0	0	0	0	0.157	0.365	0.062	0	0.010	0	0.681	1
Chlorophytes	0	0	0	0	0.068	0	0.053	0	0.207	0.010	0.255	1
Haptophytes (T3)	0	0	0	1.704	0	0	0	0	0	0	0	1
Haptophytes (T4)	0	0.514	0.992	0.088	0	0	0	0	0	0	0	1
<i>Synechococcus</i>	0	0	0	0	0	0	0	0	0	0.328	0	1
Final ratios (Jun.)												
Diatoms	0	0	1.131	0	0	0	0	0	0	0	0	1
Dinoflagellates	1.091	0	0	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0	0	0	0	0	0	0.861	0	0	0	1
Prasinophytes	0	0	0	0	0.314	0.522	0.151	0	0.018	0	0.226	1
Chlorophytes	0	0	0	0	0.062	0	0.056	0	0.168	0.010	0.283	1
Haptophytes (T3)	0	0	0	1.767	0	0	0	0	0	0	0	1
Haptophytes (T4)	0	0.688	1.766	1.420	0	0	0	0	0	0	0	1
<i>Synechococcus</i>	0	0	0	0	0	0	0	0	0	0.373	0	1

temperature exhibited a decreasing gradient from the south to the north of the sampling area, with minima recorded at the upper northwest stations (Fig. 2a). Severe convection occurred in the water column of every station and resulted in vertical differences between the surface and bottom layers of no more than 0.5 °C (Fig. 2e). In the early summer, the sea surface temperature ranged from 13.7 °C to 19.5 °C with an average of 16.5 °C (Fig. 2b; Table 2). The surface temperature exhibited a decreasing gradient from southwest to northeast of the sampling area, with minima recorded in the same upper northeast stations as that in the spring. A summer stratification was noted in most stations, and the temperature differences between the surface and bottom layers reached as high as 7.5 °C (Fig. 2f).

In the spring, the freshwater discharge of the Yellow River did not influence the study area. Sea surface salinity showed slight variations and ranged from 30.9 to 31.7 psu (Fig. 2c; Table 2). No obvious vertical difference (less than 0.06 psu) was observed between the surface and bottom layers (Fig. 2g). In the early summer, the sea surface salinity decreased from the northeastern to southern parts of the

study area because the influence of the Yellow River plume. The values ranged from 28.7 to 31.4 psu, with an average of 30.9 psu (Fig. 2d; Table 2). Given the summer stratification, the differences in salinity between the surface and bottom layers reached as high as 2.4 psu (Fig. 2h).

### 3.2 Nutrients

The difference between the DIN concentrations in the two seasons ( $9.91 \pm 5.15 \mu\text{M}$  in the spring vs.  $9.71 \pm 6.71 \mu\text{M}$  in the early summer) was not significant ( $P > 0.05$ ) (Table 2). The areas with high DIN values shifted over time from the central part to the southern part of the sampling region close to LaiZhou Bay because of the freshwater discharge of the Yellow River (Figs. 2d, 3b). During the sampling periods, the lowest DIN concentration was obtained in the spring (2.10  $\mu\text{M}$ ) and the highest in the early summer (29.82  $\mu\text{M}$ ). The mean DRP concentration in the spring ( $0.70 \pm 0.30 \mu\text{M}$ ) was significantly higher than that of the early summer ( $0.33 \pm 0.10 \mu\text{M}$ ) ( $P < 0.01$ ; Table 2). Meanwhile, the lowest DRP concentration

**Table 2** Concentrations of environmental factors and main pigments obtained in this study (average and minimum–maximum values)

Abbreviations	Average and range of concentrations	
	April	June
Temp (°C)	5.01 (3.91–6.37)	16.48** (12.80–19.52)
Salinity	31.36 (30.92–31.66)	30.90* (28.74–32.60)
DO	13.20 (12.10–14.04)	10.75** (9.04–14.01)
pH	8.00 (7.18–8.25)	8.17* (7.72–8.45)
DIN (μmol/L)	9.92 (2.10–17.76)	9.71 (2.84–29.82)
DRP (μmol/L)	0.70 (0.27–1.30)	0.33** (0.17–0.64)
DSi (μmol/L)	6.56 (1.72–11.38)	4.03** (2.08–9.68)
N/P	14.42 (3.76–43.48)	30.09** (6.54–78.10)
N/Si	1.62 (0.25–3.21)	2.43** (0.81–5.52)
Si/P	10.08 (3.45–31.49)	12.57 (5.76–29.72)
Chl <i>a</i> (μg/L)	0.91 (0.01–3.22)	0.26** (0.04–0.76)
Fuco (μg/L)	0.89 (0.11–2.77)	0.35** (0.07–1.13)
Chl <i>b</i> (ng/L)	1.86 (0–7.15)	26.76** (0–140.21)
Allo (ng/L)	6.05 (0–21.18)	65.13** (9.25–171.06)
But-Fuco (ng/L)	4.76 (0–18.34)	73.49** (10.69–316.27)
Hex-Fuco (ng/L)	0.57 (0–4.14)	108.46** (3.21–369.10)
Lut (ng/L)	0.19 (0–1.13)	7.78** (0.88–29.76)
Neo (ng/L)	1.12 (0–3.48)	32.12** (14.06–72.44)
Peri (ng/L)	33.89 (0–186.13)	34.45 (0–230.59)
Pras (ng/L)	2.52 (0–5.55)	31.78** (0–125.18)
Viola (ng/L)	2.80 (0–7.45)	24.38** (6.57–64.26)
Zea (ng/L)	2.04 (0–6.24)	16.30** (3.92–46.16)

Asterisks denote the difference between seasons, \* $P \leq 0.05$ , \*\* $P \leq 0.01$  by *t* test

was observed in the early summer (0.17 μM), whereas the highest was noted in the spring (1.30 μM). The mean DSi concentration was significantly higher in the spring (6.56 ± 3.03 μM) than in the early summer (4.03 ± 1.88 μM) ( $P < 0.01$ ; Table 2). The lowest and highest DSi concentrations (1.72 and 11.37 μM, respectively) were recorded in the spring.

The nutrient ratios (DIN:P, Si:P, and Si:DIN) exhibited significant variations between the spring and early summer. According to Justić et al. (1995), the criteria for defining nutrient limitation were (a) N limitation of DIN:P < 10 and Si:DIN > 1, (b) P limitation of Si:P > 22 and DIN:P > 22, and (c) Si limitation of Si:P < 10 and Si:DIN < 1. The N:P ratios were higher in the early summer than in the spring in almost all of the sampling stations, with mean values of 14.43 ± 7.98 and 30.09 ± 18.11, respectively. P limitation occurred in two stations in the early summer, whereas N limitation was noted occasionally in either season. The relative proportion of available nutrients revealed a severe Si limitation in both seasons (Fig. 4).

### 3.3 Phytoplankton pigment concentrations

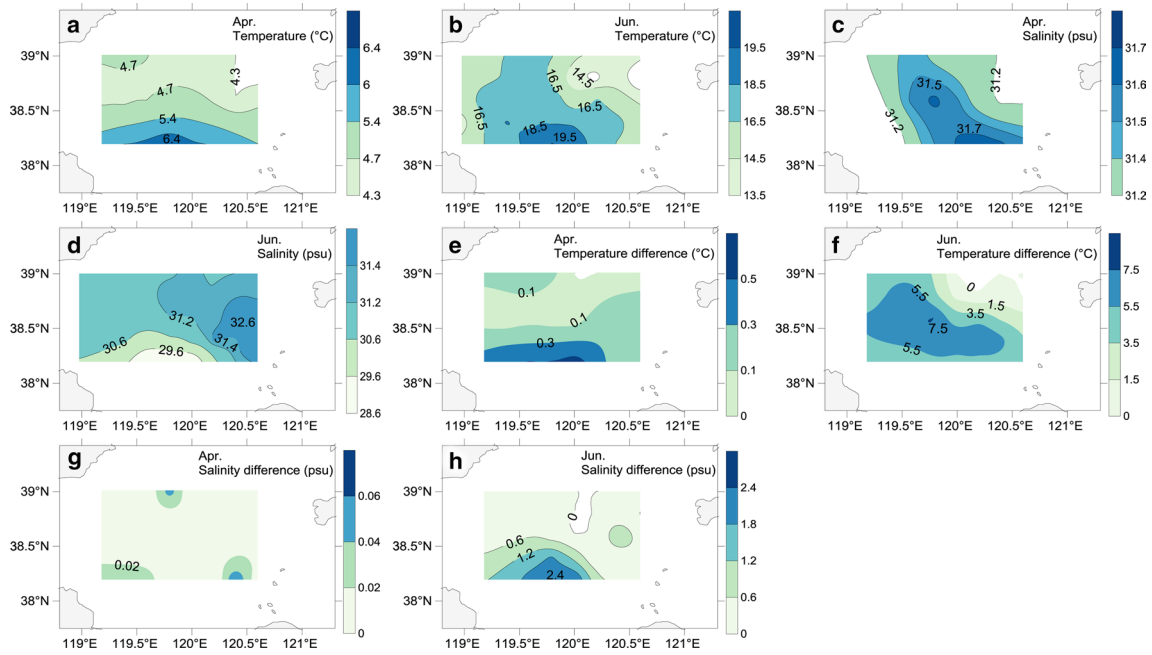
A total of 21 pigments were identified in the central Bohai Sea (Table 2). The average Chl *a* concentration in the spring was 0.91 μg/L, which was significantly higher than that in the early summer (0.26 μg/L) (Table 2). The peak Chl *a* level in the spring was recorded in the western and northeastern parts of the study area (Fig. 5a). Fuco was the dominant accessory pigment at concentrations ranging from 0.11 to 2.77 μg/L and 0.07 to 1.13 μg/L in the spring and early summer, respectively. Similar to mean Chl *a* concentrations, the mean Fuco concentration in the spring was significantly higher than that in the early summer (Table 2). A high-Fuco region was noted at the western part of the sampling region, and a clear gradient of decreasing levels toward the eastern part was observed in the spring. The Fuco distribution in the early summer shared a similar pattern as that in the spring except for the high value area that shifted northward. The Peri concentrations were generally uniform throughout the two seasons at average concentrations of 33.89 and 34.45 ng/L, respectively. Moreover, significantly higher increases in Allo, Hex-Fuco, Pras, and Zea levels were found in the early summer than in the spring ( $P < 0.01$ ; Table 2).

### 3.4 PFGs

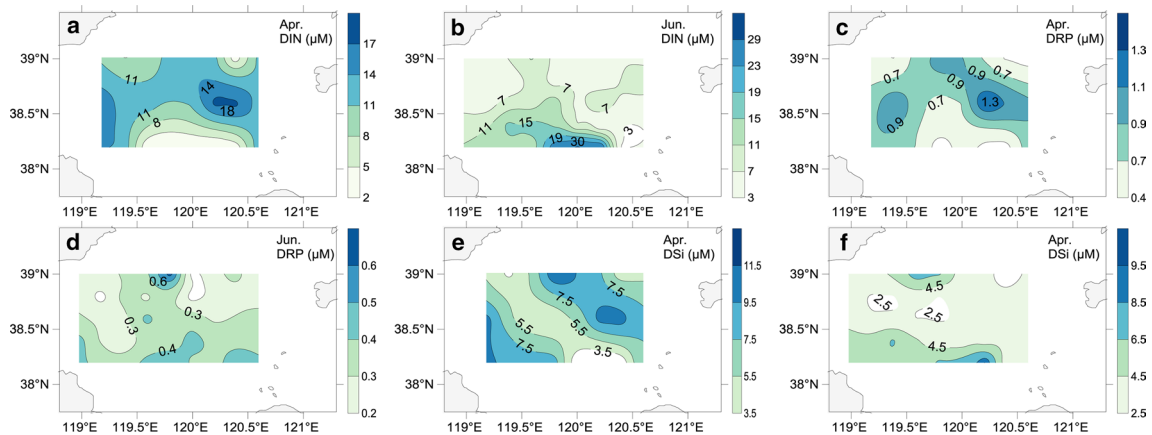
The CHEMTAX analysis identified eight major PFGs contributing to the Chl *a* biomass in the central Bohai Sea. These PFGs included diatoms, dinoflagellates, cryptophytes, prasinophytes, chlorophytes, haptophytes (T3), haptophytes (T4), and *Synechococcus* (Fig. 6). In the spring, the diatoms were the major contributors (83.31%) to the total Chl *a*, followed by the dinoflagellates (5.95%) and cryptophytes (5.08%) (Fig. 7). However, in the early summer, a sharp decrease was observed in the diatom biomass, which accounted for 15.03% of the total biomass. High diatom biomasses were confined to the northeast part of the investigated region (st.A5-7 and B5-7) (Fig. 6b). By contrast, the biomasses of the haptophytes (T4) and prasinophytes increased to become the largest PFGs, with proportions of 22.38 and 19.06%, respectively. The dinoflagellates showed similar relative contributions during both cruises. The contributions of other PFGs, including cryptophytes, chlorophytes, haptophytes (T3), and *Synechococcus*, increased in different degrees from the spring to the early summer (Fig. 7).

### 3.5 Influence of environmental parameters on PFGs

Redundancy analysis was conducted to investigate the response of PFGs to environmental parameters (Fig. 8). A Monte Carlo test showed that seven environmental variables (temperature, salinity, DIN, PO<sub>4</sub>, SiO<sub>3</sub>, N/Si, and

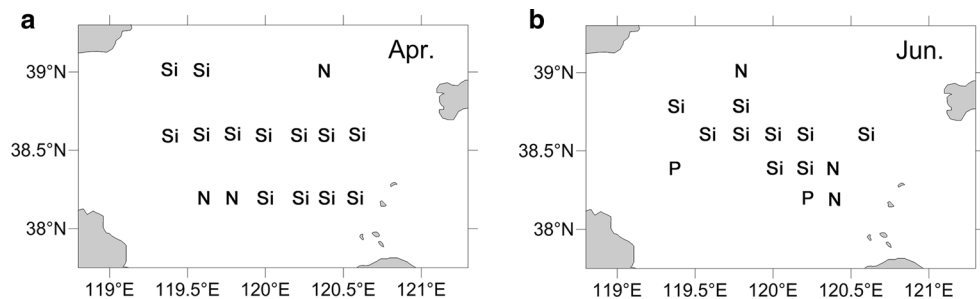


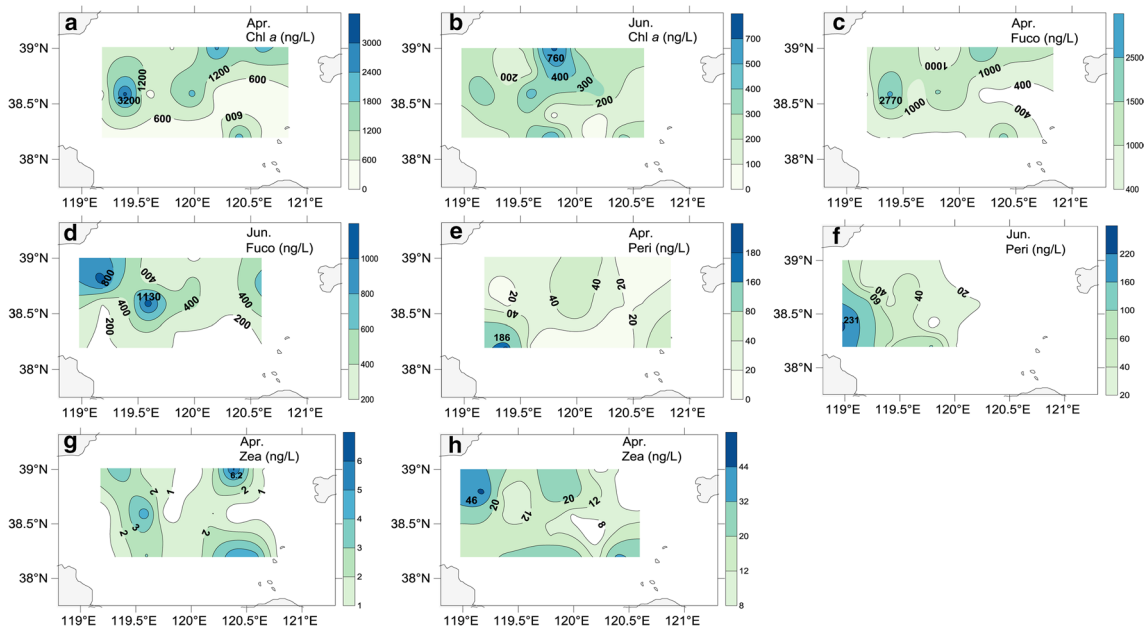
**Fig. 2** Distribution of surface temperature (**a, b**) and salinity (**c, d**), as well as the differences in temperature (**e, f**) and salinity (**g, h**) between the surface and bottom area, in the central Bohai Sea. The differences in temperature and salinity between the surface and bottom areas were obtained by subtracting the bottom values from the surface values, respectively



**Fig. 3** Distribution of DIN, DRP, and DSi across the surface waters of the central Bohai Sea

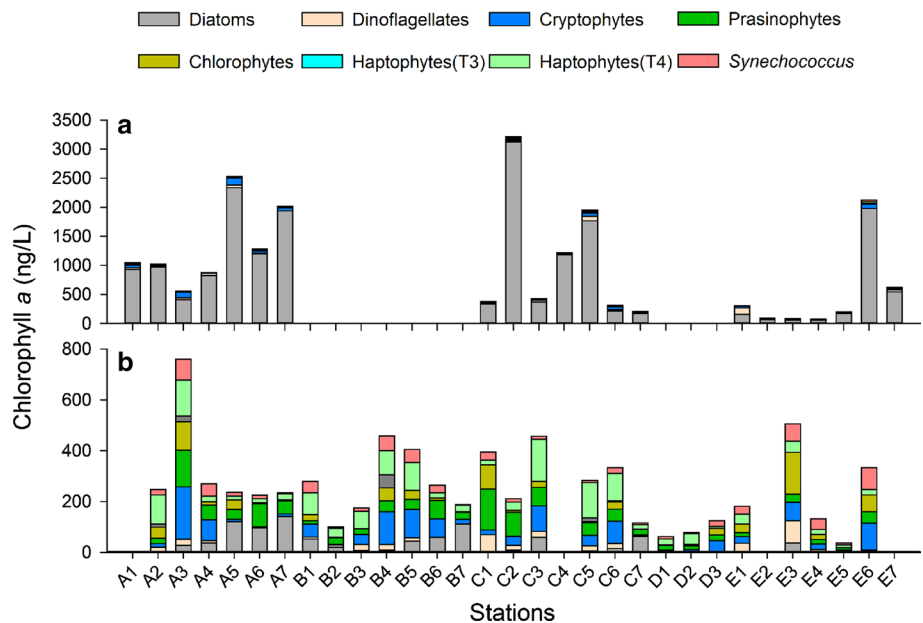
**Fig. 4** Nutrient limitations in the spring (**a**) and early summer (**b**) in the central Bohai Sea. *N* N-limited, *P* P-limited, *Si* Si-limited





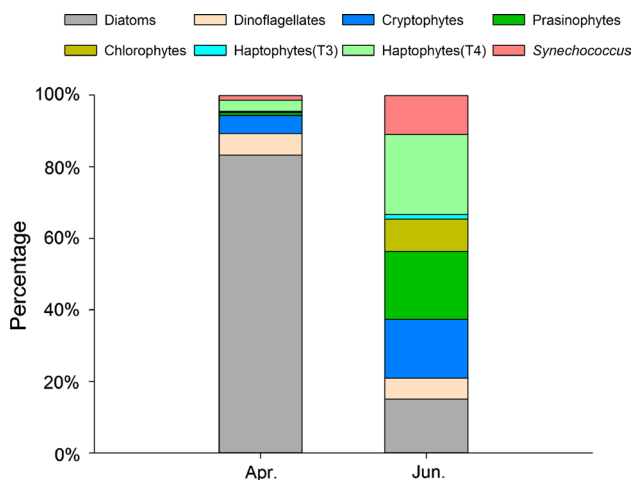
**Fig. 5** Distribution of major pigments (Chl *a*, Fuco, Peri, and Zea) across the surface waters of the central Bohai Sea

**Fig. 6** Absolute contributions of various phytoplankton functional groups to the total chlorophyll *a* in the spring (a) and early summer (b) in the central Bohai Sea



N/P) significantly contributed to the spatial distribution of PFGs. The RDA explained 93.0 and 93.8% of the variance associated with the phytoplankton–environment relationship in the spring and early summer, respectively. The first two canonical axes explained 68.7 and 72.6% of the total spatial distributions of PFGs in the spring and early summer, respectively. In the spring, the distribution of diatoms (*Diat*), prasinophytes (*Pras*), chlorophytes (*Chlo*), and *Synechococcus* (*Syne*) was positively associated with the N:Si and N:P ratios and characterized by low temperature. The two types

of haptophytes (*Hapt*) and cryptophytes (*Cryp*) were negatively associated with DIN, PO<sub>4</sub>, and SiO<sub>3</sub> concentrations. The dinoflagellates (*Dino*) were found to positively correlate with high nutrient concentrations. In the early summer, the diatoms were dominant in the northeast part of the sampling area (st.A5-7, B7), which was characterized by high salinity, as well as low temperature and nutrient concentration. Converse to the diatom abundance, the dinoflagellate abundance was positively associated with temperature and nutrient concentrations and negatively associated with



**Fig. 7** Relative contributions of various phytoplankton functional groups to the total Chl *a* in the central Bohai Sea

salinity. Most other flagellates (haptophytes, prasinophytes, chlorophytes, and cryptophytes), as well as *Synechococcus*, which were abundant in the central part of the study area, were intermediately positioned in relation to other variables.

## 4 Discussion

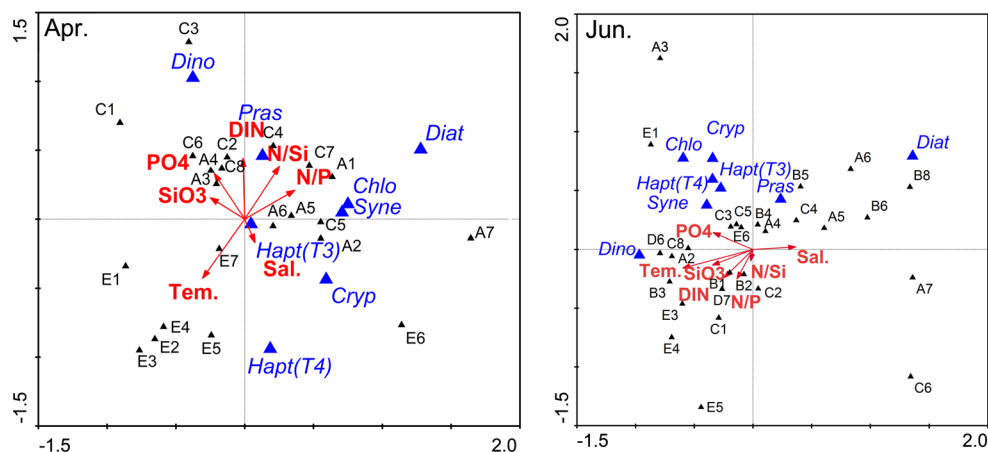
### 4.1 PFGs as revealed by pigment analysis in the central Bohai Sea

The temporal changes in Chl *a* levels are usually associated with changes in the phytoplankton community composition (Bouman et al. 2005b; Rodríguez et al. 1998) and variations in the relative concentrations of accessory pigments (Sathyendranath et al. 2006). Notably, the mean Chl *a* was 3.5 folds (Table 2) greater in the spring than in the early summer. Cooler temperatures and generally higher nutrient levels than those in the early summer prevailed in

the spring and led to elevated Chl *a* concentrations and the Fuco prominence over the other diagnostic carotenoids. However, the levels of marker pigments varied with the changing environment from spring to early summer. Fuco concentrations reduced significantly, whereas Chl *b*, Allo, But-Fuco, Hex-Fuco, Pras, and Zea levels increased markedly ( $P < 0.01$ ) (Table 2). The variations in these pigments corresponded with the increase in number of diatoms and the decrease in chlorophytes, cryptophytes, haptophytes, cryptophytes, prasinophytes, and cyanobacteria. The abundance of Zea in the early summer was related to the abundance of *Synechococcus* because divinyl Chl *a*, the exclusive biomarker of prochlorophytes, was not detected in this study. Therefore, the HPLC pigment analysis provided immediate insight into the phytoplankton community structure in the Bohai Sea.

Despite the convenience of HPLC methods, the quantitative composition of complex phytoplankton populations require further determination through other methods, such as CHEMTAX analysis (Agirbas et al. 2015). Previous studies documented that the phytoplankton community composition in the Bohai Sea was dominated by diatoms (99.8% in the spring and 38.5% in the summer) and dinoflagellates (61.5% in the summer), and only limited species of other PFGs were observed by optical microscopy (Chen et al. 2016). In the present study through CHEMTAX analysis, the PFGs varied widely between stations and seasons in the central Bohai Sea. The phytoplankton biomass was generally greater in the spring than in the early summer in the central Bohai Sea (Fig. 6). The diatoms largely dominated the phytoplankton community in the spring and accounted for 83.3% of the total Chl *a*. However, during the early summer, diverse PFGs coexisted and flagellates dominated (74.1% in total), followed by diatoms (15.0%) and *Synechococcus* (11.0%) (Figs. 6, 7). To our best knowledge, PFG determination by HPLC pigment–CHEMTAX analysis in the Bohai Sea has not been reported. CHEMTAX appears as an effective method to ascertain the PFG distribution in this sea area.

**Fig. 8** Redundancy analysis biplots of phytoplankton functional groups (PFGs) and major environmental variables in the central Bohai Sea. The following abbreviations were used for the different PFGs: *Diat*, Diatoms; *Dino*, Dinoflagellates; *Cryp*, Cryptophytes; *Pras*, Prasinophytes; *Chlo*, chlorophytes; *Hapt*, haptophytes; and *Syne*, *Synechococcus*





Since 1958, a few comprehensive investigations were conducted in the Bohai Sea and revealed marked changes in the phytoplankton composition (Fei et al. 1991; Wei et al. 2004; Guo et al. 2014; Chen et al. 2016). Obviously, the diatoms tended to be replaced gradually by dinoflagellates over decades. This change is presumed to be due to the changes in nutrient discharge caused by the intensification of the industry, agriculture, and aquaculture (Wei et al. 2004). However, previous studies mainly focused on the long-term shift of dinoflagellates because of the limitation of the microscopic method. This study suggests that other flagellated phytoplankton species, i.e., prasinophytes, haptophytes, chlorophytes, and cryptophytes, increased in abundance more than that of the dinoflagellates in the summer. The long-term changes in the abundances of these flagellated phytoplankton species cannot be achieved because lack of historical data on the last several decades. Further attention should be paid to acquire future data from the Bohai Sea.

The present results also confirmed that small-sized phytoplankton, such as prasinophytes, chlorophytes, and cryptophytes, substantially contribute to the total Chl *a* pool, especially in the summer. Indeed, brown tides caused by the picophytoplankton *A. anophagefferens* have been regularly observed in the northwest part of the Bohai Sea (sea areas of Qinghuangdao) recently (Xu et al. 2017). However, the historical changes in the phytoplankton community composition of the central Bohai Sea remain unclear because of the inadequacy of the earlier results based on microscopic identification to account for the small-sized organisms. To date, we cannot ascertain whether this discovery is due to the restrictions of traditional methods or the tendency of the phytoplankton species to assume small sizes. This aspect thus merits further investigation.

## 4.2 Environmental factors influencing PFGs

Temperature is an important environmental parameter that influences biological processes in the ocean. Various studies have demonstrated that phytoplankton community structure varies on a regular, predictable pattern with temperature (Bouman et al. 2003, 2005a; Mendes et al. 2015). In general, microplankton, such as diatoms, usually flourish at low temperatures and low light intensity, which are typical of early spring in temperate latitudes (Nelson 1963). Meanwhile, cyanobacteria, such as *Synechococcus* (Agawin et al. 1998; Kemp et al. 2005; Marshall et al. 2009) and flagellates, such as haptophytes (Bouman et al. 2003, 2005a), are prevalent in the summer when the water temperature is high and the seasonal thermal stratification limits the nutrient supply to the upper layer (Agawin et al. 1998). The results presented here clearly showed the similar influence of seawater temperature on the community structure in the central Bohai Sea (RDA in Fig. 8).

Some other water temperature related abiotic factors, such as water stratification, nutrient availability, and structure, may have also played a significant part in regulating the distribution of phytoplankton communities along the study area. Several authors have proposed the general rule that sea areas with stratification are more favorable to the growth of flagellates than to that of diatoms (Smith et al. 1983; Mann 1993; Tilstone et al. 2000; Mendes et al. 2011). Additionally, flagellates can move vertically and hence have access to nutritive resources in deep water and light energy near the sea surface (Monaco and Prouzet 2014). These organisms are thus better adapted to low-mixing conditions than other organisms (Goela et al. 2014). Notably, in several stations (e.g., A5, A6, A7, B7, and C7) located near the center of a widely reported well-mixed warm water column (Fig. 2b, d) (Lin et al. 2006), the diatoms accounted for the largest proportion among all the PFGs in the summer (Fig. 7).

Similar to the present study, previous studies have suggested silicate as a limiting factor for diatom growth during the summer because of its strong consumption during the spring (Brown and Landry 2001; Drira et al. 2014; Erga et al. 2014). Likewise, a negative correlation between diatom biomass and silicate concentration was found in our study (Fig. 8). This result confirmed that silicate served as a limiting nutrient and caused the cessation of diatom prominence because of its importance to diatom growth (Lewin and Ralph 1962; Goela et al. 2014). The current result also showed that despite the diatom dominance in the spring, the diatom biomass did not correlate with silicate levels (Fig. 8). In fact, the sea is already under extensive silicate limitation in the spring (Fig. 4), and this limitation may explain the non-correspondence. The high diatom biomass was probably due to the vertical exchanges of the surface and bottom waters. These changes may have provided sufficient silicate levels to sustain the diatom growth. Despite the silicate limitation, the phosphorus limitation began to appear in some stations in the early summer. This P limitation may have promoted the succession from diatoms to flagellates. According to Goela et al. (2014), fluctuations in nutrient regimes are related to such succession pattern, with P limitation favoring flagellate dominance. Although the diatoms were proven to be most sensitive class to nutrient enrichment (Loureiro et al. 2008), they were more apparently affected than flagellates by the decreased nutrient supply (Egge 1998).

Numerous studies have demonstrated the succession of diatoms to flagellates under elevated N:P ratios. At high N:P ratios, diatom growth is more severely limited than flagellate growth (Egge 1998). Increased N:P ratios and P limitation promoted the P-related growth decline in diatoms (Skjoldal 1993). Other sea areas of China, such as the Yellow Sea (Lin et al. 2005), Changjiang River Estuary and East China Sea (Li et al. 2009), and the Pearl River Estuary and South China Sea (Yin et al. 2000),

experienced rising N:P ratios and subsequent increases of flagellates blooms and decrease of diatom dominance. Given the slump in Yellow River input, the present study area is undergoing the same process. Since 1960, the nitrate concentrations in the central Bohai Sea have increased by a factor of 10, whereas the phosphate concentrations have decreased by a factor of 2 (Zhang et al. 2004). The nitrogen limitation in the central Bohai Sea has gradually changed to phosphate and silicate deficiency (Yu et al. 2001). Such a nutrient status in the sea is of great importance in determining the structure and function of phytoplankton communities. The mesocosm experiments implemented by Schollhorn and Graneli (1993), Sommer (1995), Watanabe et al. (1995), and Escaravage et al. (1999) also showed that elevated N:Si ratios in the surroundings lead to elevated flagellate:diatom ratios, which is consistent with our results (Fig. 8). In the present study, phytoplankton succession was related to the nutrient variation patterns.

## 5 Conclusion

HPLC pigment–CHEMTAX analysis in the central Bohai Sea showed that the spring was almost invariably dominated by diatoms at the entire sampling area, whereas the early summer revealed a highly diverse phytoplankton population dominated by flagellates. These results indicated a relatively pronounced seasonal succession. The seasonal variation of PFGs was influenced by temperature, water stratification, nutrient availability, and even nutritive proportions. Compared with previous studies by microscopy, our work provided thorough understanding of the phytoplankton community in the central Bohai Sea. In particular, the small phytoplankton (pico/nanophytoplankton) comprised the major share of the phytoplankton community structure, especially in the summer. This research may help future investigations ascertain and evaluate the changes in structure of the central Bohai Sea ecosystem. Also, it is recommended that microscopy and flow cytometry data combined with HPLC phytoplankton pigments data be used in the future work to better understand the marine ecosystem in the central Bohai Sea.

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