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Determination of phytoplankton community structure and biomass with HPLC-CHEMTAX and microscopic methods during winter and summer in the Qinzhou Bay of the Beibu Gulf

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The Qinzhou Bay, a typical semi-enclosed bay, is facing environmental pressure from local fast-growing industrial and aquacultural development. Dominant species of phytoplankton community (based on microscopic examination) show a trend of miniaturization, while pico-phytoplankton (based on CHEMTAX analysis) is widely distributed in Qinzhou Bay. However, most previous investigations of phytoplankton community based on microscopic method that undetected small-sized cell (< 3 μm), and limited by scarce studies on CHEMTAX analysis, the long-term dynamic data of small-size phytoplankton are lacking in Qinzhou Bay. It is recognized that combining microscopic examination with CHEMTAX analysis could provide a good taxonomic reliability for large cells and valuable information about small-size groups. In this study, microscopic examination and High Performance Liquid Chromatography (HPLC)-CHEMTAX analysis were employed to characterize the spatiotemporal variability of the phytoplankton community structure in Qinzhou Bay in winter and summer of 2021. The results of microscopic observations showed that the phytoplankton community was characterized by diatoms and dinoflagellates mainly. Diatoms dominated in both seasons, of which *Skeletonema costatum* bloom occurred in the summer. CHEMTAX analysis not only agreed well with microscopy data for diatoms and dinoflagellates, but also helped identification of other small-sized flagellates and cyanobacteria that hard to observe by microscope. The results of CHEMTAX analysis found that diatoms, prasinophytes and cryptophytes co-dominated the total chlorophyll *a* in winter while diatoms became the dominant group in summer. In addition, our results indicate that the proportion of small-sized flagellates has increased in the past decade in Qinzhou Bay, especially for cryptophytes. Temperature, nutrient availability, and selective grazing of oyster affected the succession of phytoplankton community from co-dominance of diatoms and flagellates in winter to absolute diatoms dominance in summer. The distribution of

prasinophytes and cryptophytes on a spatial scale were related to the location of shellfish culture area and estuary, respectively, rather than by nutrients. Eutrophication, selective grazing of oyster and warming were the driving factors of long-term changes in phytoplankton composition in Qinzhou Bay. This study enhanced our understanding of entire phytoplankton community dynamics and its relationship with environmental factors in Qinzhou Bay.

KEYWORDS

phytoplankton, pigments, environmental factor, HPLC-CHEMTAX, microscopy, Qinzhou Bay

1 Introduction

Phytoplankton acts as a primary producer in marine ecosystems and maintains the balance of the global ecosystem by supplying organic matter to higher trophic levels (Paerl et al., 2003). Phytoplankton community reflects environmental changes sensitively and rapidly, and variation in the structure of phytoplankton community could affect the global climate in terms of carbon flux (Sarmiento et al., 1988), cloud albedo (Charlson et al., 1987), light flux, and heat flux (Sathyendranath et al., 1991). Therefore, quantifying the variations in phytoplankton biomass and taxonomic composition and analyzing the relationship between phytoplankton and the environment are important for the evaluation of ecosystem function and status, as well as for the study of global biogeochemical cycles (Eker-Develi et al., 2008).

The widespread techniques regarding phytoplankton monitoring are morphological observation, pigment analysis, and high throughput sequencing analysis (Egge et al., 2015; Pan et al., 2020). The traditional identification and quantification of phytoplankton *via* microscopy techniques can provide accurate taxonomical information (at species or genus) of phytoplankton (Brito et al., 2015b). However, phytoplankton cells in small size (<3 μm) and unobvious morphological characteristics are difficult to identify accurately, which restricts the application to the species of small size group, such as prasinophytes and cyanobacteria (Chen et al., 2016). In addition to morphological characteristics, some photosynthetic pigments (such as fucoxanthin, peridinin, zeaxanthin and alloxanthin), determined by high-performance liquid chromatography (HPLC), can also be used as taxonomic indicators for phytoplankton classification (Zapata et al., 2000). The contribution of different phytoplankton groups to the total chlorophyll *a* can be estimated by combining the photosynthetic pigment data with CHEMTAX, a mathematical program, based on the ratios of mark pigments to Chl *a* (Mackey et al., 1996). This technology overcomes the drawback of the microscopy method. However, the CHEMTAX estimates can only classify phytoplankton into classes or phyla. Many studies have proposed a combination of the two approaches for better understanding of phytoplankton community structures (Agirbas et al., 2015; Brito et al., 2015b; Pan et al., 2020).

Qinzhou Bay (QZB) is a semi-enclosed subtropical bay located in Qinzhou, China. It receives a considerable amount of chemicals, organic matter, and nutrients from Maoling River, Qinjiang River, and Jingu River (Luo et al., 2019). Nutrients usually increased in gradient from south to north and exhibit significant seasonal change in QZB, which reflects the dominant control role of land runoff (Lai et al., 2013). QZB is an important oyster aquaculture area in Guangxi Zhuang Autonomous Region, in-bay oyster farming area has expanded from inner bay (Maowei Sea) to the Qinzhou Port coastal area in recent decades. With accelerated expansion of port transportation, aquaculture industry, and coastal industry in the local rim region, the QZB has undergone a shift from oligotrophic to mild, moderate and even heavy (Maowei Sea) eutrophication status from 1980s to the 2010s (Wei et al., 2002; Lan, 2012; Yang et al., 2012; Lai et al., 2013), inorganic nitrogen showed an obvious increasing trend (Wei et al., 2002; Wei and He, 2008). In addition, the Guangxi Fangchenggang Nuclear Power Plant located in the west coast of QZB (Figure 1) began operating in January 2016, the thermal discharge causes sea surface temperature increase near the water outlet (Pan et al., 2022).

Phytoplankton in terms of biomass, abundance and species information, have been measured in QZB over many decades, many studies have shown that the phytoplankton dynamics is closely related to environmental physical and chemical factors (Wei and He, 2008; Wang et al., 2013; Liu et al., 2020). Under the background of environmental changes over the past decades, phytoplankton responded to qualitative and quantitative changes in community structure; for example, intensified blooms (Qin et al., 2016; Shen et al., 2018; Liu et al., 2020; Su et al., 2022), small-sized diatoms become dominant group and the contribution of non-diatom (especially dinoflagellates) to total species number increased significantly (Jiang et al., 2012; Wang et al., 2013; Luo et al., 2019; Liu et al., 2020). Furthermore, several studies used HPLC-CHEMTAX revealed that smaller phytoplankton groups (pico- or nano-sized), including prasinophytes, cryptophytes, and cyanobacteria, contributed greatly to the total Chl *a* in QZB (Lan et al., 2011; Lan et al., 2013; Lan et al., 2014; Pan et al., 2022). The feeding pressure on oysters is an important factor in the prevalence of nano- and pico-phytoplankton in QZB (Lan et al., 2011; Lan et al., 2013; Lan et al., 2014; Pan et al., 2022), as shellfish mainly

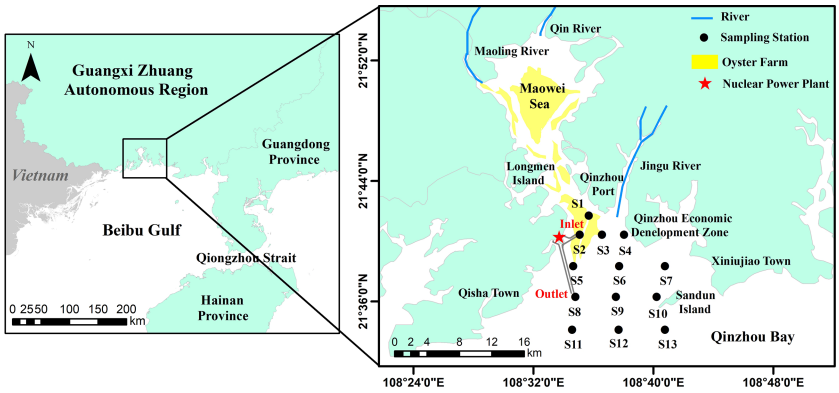

FIGURE 1
Deployment of sampling stations in QZB, the Beibu Gulf, China.

filter larger phytoplankton group (>3 μm) (Ward and Shumway, 2004). We hypothesized that seasonal and spatial changes in phytoplankton biomass and structure in QZB are largely regulated by size-selective grazing of shellfish, nutrients, and temperature, and the long-term size-selective filtration of shellfish may increase the contribution of small-sized phytoplankton groups.

Many previous studies on phytoplankton were based on microscopy examination and small-sized cells were undetected in QZB. However, the studies using HPLC-CHEMTAX to analyze phytoplankton biomass and community in QZB are few (Lan et al., 2011; Lan et al., 2013; Lan et al., 2014), and lack of information on the long-term dynamics of small-sized phytoplankton in this area. To test our hypothesis, we combined microscopic counting (provides information on species and abundance of large cells) and HPLC-CHEMTAX analysis (provides information on classes or phyla and biomass of full-size cells) to investigate the whole phytoplankton community in winter and summer in QZB, and subsequently compared with historical data. Our objectives were: (i) to add to the knowledge on the distribution and structure of phytoplankton community in QZB; (ii) to analyze the leading factors affecting the succession of various phytoplankton groups from summer to winter based on the environmental data; (iii) to explore driving factors of the long-term changes in phytoplankton community structure. Our work is useful to understand the seasonal succession and long-term changes in whole phytoplankton community structure and its controlling factors in this semi-enclosed culture bay of the South China Sea.

2 Materials and methods

2.1 Study area and sampling strategy

QZB (108.45°–18.95°E, 21.50°–21.90°N), a semi-enclosed bay located in the north of the Beibu Gulf, covers an area of 380 km^2 and characterized by a southern subtropical oceanic monsoon climate (Jiang et al., 2012). Recent studies had found that the circulation in north of the Beibu Gulf is mainly counterclockwise all year round (Xia et al., 2001; Cao Z et al., 2019; Li et al., 2022), and the water masses were classified into three types, i.e., the bottom mixed shelf water with low temperature and high salinity, the surface mixed shelf water, and the coastal water with low salinity (Cao Z et al., 2019). QZB is affected by the westward coastal current (Chen and Shi, 2019) and the coastal water masses (Cao Z et al., 2019) in the Beibu Gulf both in winter and summer. The tide in QZB belongs to diurnal tide type, and the tidal current is dominated by reciprocating flow. The flood current enters QZB from south to north, and the velocity of flood current is generally smaller than that of ebb current (Dong et al., 2014; Lyu et al., 2021). Tidal current are stronger in summer than in winter in QZB (Dong et al., 2014).The inflow rivers include Maoling River, Qinjiang River, and Jingu River, with average annual runoff of 2.61×10^9 m^3, 2.47×10^9 m^3 (Lyu et al., 2021), and 1.50×10^8 m^3 (Chen et al., 2016), respectively, which bring in large nutrient loads into QZB. Influenced by runoff, tide and wave (Cao X et al., 2019), sea water exchange capacity in the estuary area of Maowei Sea and offshore area is stronger than that in coastal area. There is a large area of shellfish culture (mainly *Crassostrea rivularis*) in QZB (Lan et al., 2014) (Figure 1).

Two in-bay cruises were conducted at 13 stations in the winter (January 27) and summer (June 23) in 2021. At each sampling station, 2.0-L seawater was collected from near surface (0.5 m in depth) with Niskin bottle, from which 1.0-L seawater was fixed in acidic Lugol's solution for microscopic identification, and the rest 1.0-L seawater was filtered through GF/F filters (0.7 μm, Whatman). The filters were immediately deep-frozen in liquid nitrogen and maintained at −80°C for HPLC analyses, and 100 mL filtrate was stored at −20°C for nutrient analysis. Hydrographic (water temperature and salinity) measurements were performed using AAQ-RINKO (JFE, Japanese).

2.2 Nutrient analysis

Dissolved reactive phosphate (DRP), dissolved inorganic nitrogen (DIN), and dissolved silicate (DSi) were determined with a SKALAR flow analyzer in methodological standard (Strickland and Parsons, 1972). DIN is to the sum of nitrite (NO_2^-), nitrate (NO_3^-), and ammonium (NH_4^+) contents.

2.3 Microscopical detection of phytoplankton

The samples fixed with Lugol's solution were concentrated to 5–15 mL *via* sedimentation, after which the supernatants were discarded. Identification and quantification were conducted under an inverted microscope (Nikon ECLIPSE Ti-S) according to the method of Utermöhl (1958).

2.4 HPLC-pigment analysis

The frozen filters were cut into pieces and then placed in a screw-cap centrifuge tube with 3-5 mL of 95% cold-buffered methanol. The filters were sonicated for 5 min in ice-water bath, and the extracted solution were filtered through PTFE membrane filters (0.2 mm pore size) to remove filter and cell debris. Prior to injection, 200 µL of sample was mixed in 67-µL Milli-Q water in 2.0-mL amber glass sample vials. The procedures of HPLC analyses using an Agilent series 1200 HPLC system fitted with Waters C8 column fully followed the work of Zapata et al. (2000). Standard curves were established using standard pigments from DHI (Institute for Water and Environment, Denmark). The standard pigments included Chl *a*, chlorophyll *b* (Chl *b*), chlorophyll *c*2 (Chl *c*2), chlorophyll *c*3 (Chl *c*3), divinyl chlorophyll *a* (divinyl Chl *a*), fucoxanthin (Fuco), 19′-hex-fucoxanthin (Hex-Fuco), 19′-butfucoxanthin (But-Fuco), 19'-Hexanoyloxy-4-ketofucoxanthin (Hex-kfuco), peridinin (Peri), prasinoxanthin (Pras), alloxanthin (Allo), zeaxanthin (Zea), neoxanthin (Neo), lutein (Lut), diatoxanthin (Diato), diadinoxanthin (Diadino), violaxanthin (Viol), Mg-2,4-divinylpheoporphyrin (MgDVP), pheophorbide *a* (Pheide *a*), β,ε-Carotene (βε-Car) and β, β-Carotene (ββ-Car).

2.5 CHEMTAX analysis

The relative contribution of different phytoplankton groups to the total Chl *a* biomass was estimated from pigment data using CHEMTAX (version 1.95) (Mackey et al., 1996; Wright et al., 1996; Wright et al., 2009). The input pigment ratios were obtained from Wang et al. (2015) and Mackey et al. (1996). To optimize the input ratios for our data set, a series of 60 derivative pigment ratio matrices were built by multiplying each ratio of the initial matrix using a random function. The best 10% of results were considered as the optimized results. Data from the winter and summer were run separately into 2 bins to consider the potential variations with irradiance and/or nutrient availability in the optimization of CHEMTAX procedures. The initial and final ratios are presented in Table,32] ?> S1. We present the output data as absolute concentrations (µg/L) of Chl *a* and relative proportions attributed to each phytoplankton group.

2.6 Statistical analysis

To test the significant difference of physical, chemical, and biological variables between the two seasons, Student's t-test was conducted using the SPSS Statistics (version 23). A redundancy analysis (RDA) was performed using software CANOCO 5 (Lai, 2013) to explore the relationships between the environmental parameters and phytoplankton data. Correlation between phytoplankton cell abundances and CHEMTAX results was evaluated by regression analyses using the Origin 2017 software.

3 Results

3.1 Oceanographic conditions

In winter, the sea surface temperature ranged from 15.41 to 17.69°C, with an average of 16.15°C, and it showed a trend of high in the west and low in the east, with the maximum in the southwest stations (Table 1; Figure 2A). In summer, the surface temperature significantly increased (with an average of 28.07°C), and it decreased in gradient from south to north in the sampling area (Table 1; Figure 2B). Due to the influence of the fresh water input from Qinjiang River, Maoling River, and Jingu River (Figure 1), sea surface salinity decreased from south to north in the study area in the two cruises (Figures 2C, D). The sea surface salinity in summer (with an average of 28.07) was significantly lower than that in winter (with an average of 30.55) (P<0.01; Table 1) because the influence of the runoff was relatively large in summer.

DIN, DRP, and DSi exhibited a similar distribution pattern that increased northward in the study area in both cruises due to influence of terrigenous input (Figures 2E–J). The highest nutrients concentrations were recorded at the northwest stations during both cruises. Although the nutrients concentrations in summer were higher than those of winter, only the differences of DSi between the two seasons was statistically significant (P<0.05; Table 1).

3.2 Phytoplankton assemblages

3.2.1 HPLC pigments

The spatio-temporal variability of Chl a and generally-used accessory pigment proxies of biomass for diatoms (Fuco), dinoflagellates (Peri), prasinophytes (Pras), cyanophytes (Zea), and cryptophytes (Allo) are showed in Figure 3. Other pigments (e.g. Lut, Hex-Fuco, But-Fuco, etc.) detected *in situ* at stations were minor and ignorable, and thus were not presented here.

A high-Chl *a* region was noted at the eastern part of the sampling region in a clear gradient of decrease southwestward in the two cruises (Figures 3A, B). Fuco was the dominant accessory pigment at all stations, showing a similar distribution pattern to Chl *a* (Figures 3C, D). The mean concentrations of Chl *a* and Fuco in the summer were significantly higher than those in the winter (P<0.01; Table 1). The third accessory pigment was Peri showing no

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TABLE 1 Mean values of physical, chemical, and biological variables during winter and summer.

Parameters	Mean values ± Standard deviation (ranges)	
	Winter	Summer
Temperature (°C)	16.15 ± 0.55 (15.41-17.69)	28.07 ± 2.49** (23.57-31.78)
Salinity	30.55 ± 1.22 (27.34-31.78)	27.80 ± 1.86** (24.50-30.50)
DIN (μmol/L)	3.34 ± 3.05 (0.94-11.56)	4.60 ± 4.73 (0.97-18.59)
DRP (μmol/L)	0.48 ± 0.20 (0.25-0.94)	0.59 ± 0.56 (0.28-2.21)
DSi (μmol/L)	5.28 ± 2.92 (2.12-12.86)	12.44 ± 10.53* (3.63-42.31)
Chl *a* (μg/L)	1.11 ± 0.42 (0.46-1.81)	3.24 ± 2.45 ** (0.90-10.21)
Fuco (μg/L)	0.72 ± 0.44 (0.10-1.35)	3.23 ± 2.63** (0.68-10.64)
Peri (μg/L)	0.15 ± 0.10 (0.01-0.30)	0.27 ± 0.3 (0.01-0.99)
Pras (μg/L)	0.07 ± 0.05 (0.02-0.16)	0.02 ± 0.02** (0.00-0.07)
Allo (μg/L)	0.05 ± 0.02 (0.02-0.09)	0.06 ± 0.04 (0.01-0.14)
Zea (μg/L)	0.01 ± 0.005 (0.01-0.02)	0.16 ± 0.06** (0.07-0.27)
Chl *b* (μg/L)	0.13 ± 0.08 (0.04-0.31)	0.07 ± 0.04* (0.03-0.17)
Diatoms (μg Chl *a*/L)	0.52 ± 0.34 (0.07-1.03)	2.53 ± 2.29** (0.44-9.18)
Dinoflagellates (μg Chl *a*/L)	0.11 ± 0.07 (0.01-0.22)	0.23 ± 0.24 (0.01-0.76)
Prasinophytes (μg Chl *a*/L)	0.23 ± 0.14 (0.08-0.54)	0.10 ± 0.06** (0.00-0.20)
Cryptophytes (μg Chl *a*/L)	0.20 ± 0.08 (0.07-0.34)	0.20 ± 0.14 (0.05-0.47)
Haptophytes (μg Chl *a*/L)	0.02 ± 0.01 (0.00-0.05)	0.01 ± 0.01 (0.00-0.04)
Chrysophytes (μg Chl *a*/L)	0	0.02 ± 0.02** (0.00-0.06)
Chlorophytes (μg Chl *a*/L)	0.02 ± 0.02 (0.00-0.07)	0.03 ± 0.03 (0.01-0.10)
Cyanobacteria (μg Chl *a*/L)	0.01 ± 0.004 (0.00-0.02)	0.12 ± 0.05** (0.05-0.22)
Diatoms (10^4 cells/L)	31.33 ± 25.47 (0.51-71.32)	188.13 ± 144.19** (23.80-569.25)
Dinoflagellates (10^4 cells/L)	2.92 ± 3.24 (0.08-10.00)	3.68 ± 4.10 (0.86-14.63)

Asterisks denote the difference between the two seasons. *: P<0.05; **: P<0.01 by t-test.

significant seasonal variation (P<0.01; Table 1). The peak Pras level was recorded in the northwest part of QZB, and high-Zea region was noted in the northwestern and southeastern parts of QZB in the two seasons (Figures 3G–J). The mean Pras and Zea concentrations varied significantly between the two seasons (P<0.01; Table 1). Allo presented a peak in the estuaries zone (Figures 3K, L), which was not significantly different between the two cruises (P>0.05; Table 1).

3.2.2 CHEMTAX results

CHEMTAX analysis identified eight major phytoplankton groups (Table 1). Although the relative contribution of the different phytoplankton groups to the total Chl *a* biomass was variable, diatoms were generally the dominant group (Figure 4). This dominance was accentuated in the summer (Figure 4B). In the winter, on average, the diatoms contributed 43.12% to the total phytoplankton biomass, followed by the prasinophytes (24.18%), cryptophytes (18.54%), and dinoflagellates (9.17%) (Figure 5A). Prasinophytes dominated in the northwest part of the study area (Stations S1, S2, and S5) (Figure 4A). In the summer, a sharp increase was observed in diatom biomass (P<0.01; Table 1), which accounted for up to 72.12% of total biomass (Figure 5A). In contrast, the contributions of prasinophytes and cryptophytes decreased significantly from winter to summer (P<0.01). The dinoflagellates showed similar relative contributions during both cruises (accounting for 9.17% and 8.03% in the winter and summer respectively). The contribution of cyanobacteria increased significantly from 1.21% in the winter to 5.80% in the summer (P<0.01; Figure 5A).

3.2.3 Phytoplankton community: microscope observation

A total of 99 species were identified in both cruises (Table S2), of which 73.7% were diatom species, 22.2% were dinoflagellate species, and 4% were other flagellates. In terms of cell number, diatoms contributed over 90% of total phytoplankton abundance in both the seasons (Figure 5B). Diatoms and dinoflagellates exhibited a similar distribution pattern to those of Fuco and Peri (or to

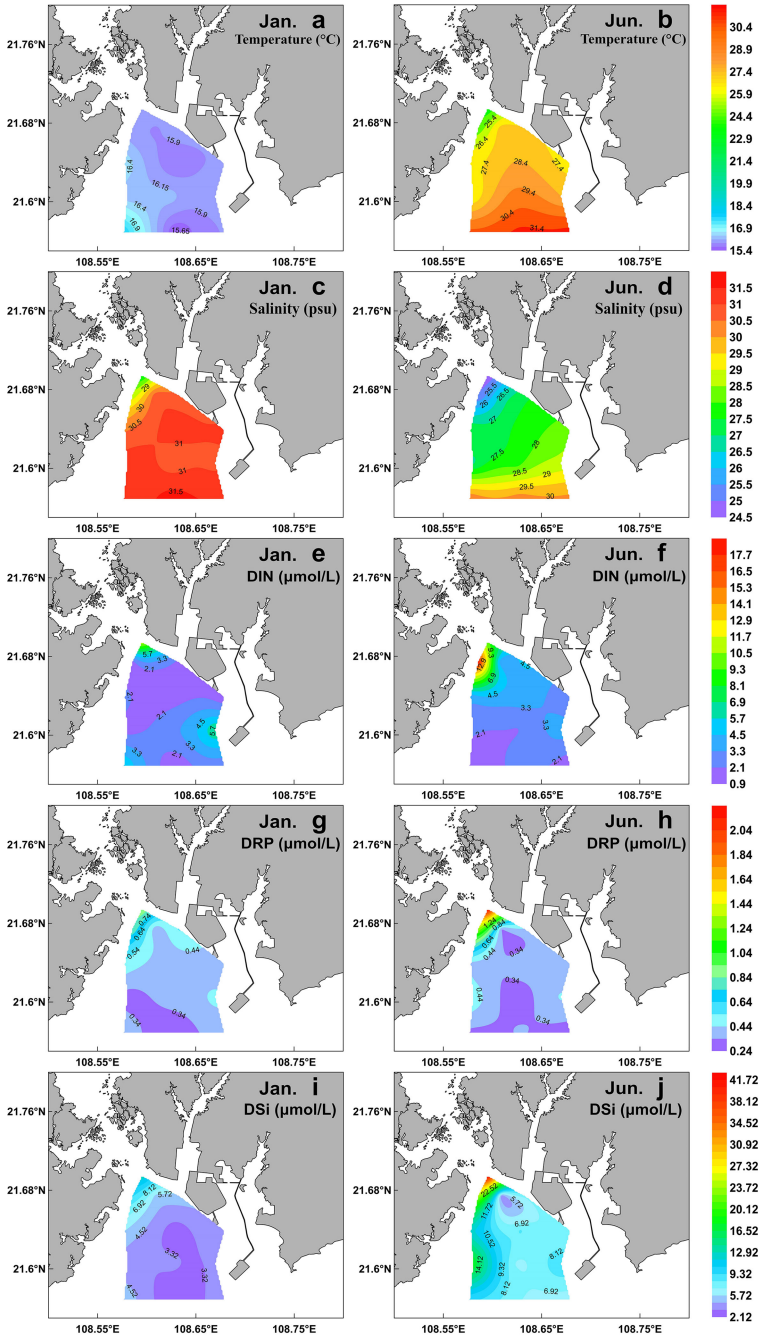

FIGURE 2
Spatial distribution of surface temperature **(A, B)**, salinity **(C, D)**, DIN **(E, F)**, DRP **(G, H)**, and DSi **(I, J)** during winter (left) and summer (right) in the QZB.

diatoms and dinoflagellates biomass using CHEMTAX) in the two seasons, respectively (Figures 6A–D). In the winter, diatoms were composed mostly of *Asterionella glacialis*, *Rhizosolenia sinensis*, *Synedra* sp., *Guinardia delicatula*, and *Skeletonema costatum* (Figure 7A), of which *Asterionella glacialis* was the most dominant species in the winter and concentrated in the eastern part (Figure 6A). Dinoflagellates were composed mainly of *Prorocentrum minimum* (Figure 7A), and was abundant in the eastern part of the study area (Figure 6C). In the summer, the first diatoms dominant species of *Skeletonema costatum* (Figure 7B) concentrated in the eastern part of the study area (Figure 6B), followed by other *Chaetoceros minutissimus*, *Chaetoceros curvisetus*, *Guinardia delicatula*, and *Leptocylindrus danicus* (Figure 7B). The dinoflagellates peaked in the northern part of the study area (Figure 6D).

The abundance of diatoms increased significantly from the winter to summer ($P<0.01$; Table 1). The abundance of dinoflagellates was not significantly different between the two seasons ($P>0.05$; Table 1). A high abundant of diatom was observed in the summer, during which the mean phytoplankton

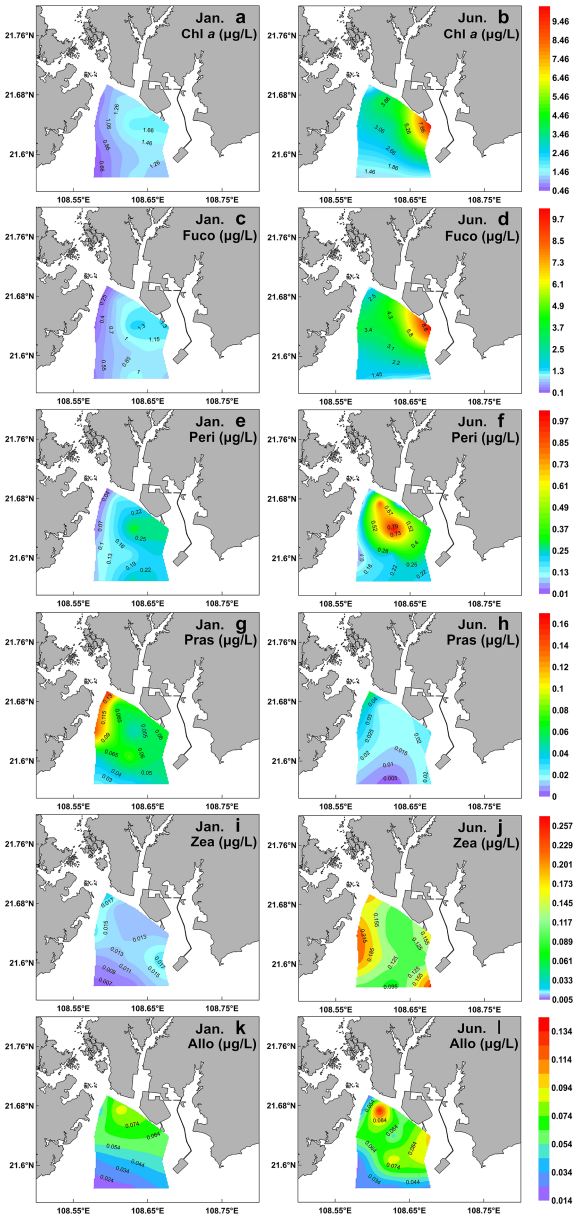

FIGURE 3
Spatial distribution of main surface pigments during winter (left) and summer (right) in the QZB. Chl a **(A, B)**; Fuco **(C, D)**; Peri **(E, F)**; Pras **(G, H)**; Zea **(I, J)**; Allo **(K, L)**.

abundance was 191.81×10^4 cell/L, which was 5.6 times higher than in the winter (Table 1).

3.3 Cell counting *vs.* CHEMTAX estimates

Good agreement was shown between cell counting (abundance) with microscope and CHEMTAX-derived Chl *a* for both diatoms and dinoflagellates ($P<0.01$; Figure 8).

All the coefficients of correlation were above 0.55 (e.g. $R^2 = 0.58$ for dinoflagellates in the summer). The highest coefficients were from diatoms in the winter ($R^2 = 0.87$). The coefficients for diatoms were better than dinoflagellates in both seasons.

3.4 Relationships between phytoplankton composition and environmental factors

A redundancy analysis (RDA) was used to investigate the response of the phytoplankton community (the CHEMTAX) to the environmental variables observed in this study (Figure 9). Results of the Monte Carlo test show that five environmental variables explained significantly the variability of phytoplankton data ($P<0.01$).

For both the cruises together, the RDA explained 52.9% of the variance associated with the phytoplankton environment relationship. The first canonical axis alone explained 33.71% of the variance. Cyanobacteria, chrysophytes and diatoms correlated positively with temperature. Prasinophytes correlated negatively with temperature. Cryptophytes, haptophytes, and dinoflagellates were strongly associated with lower concentrations of nutrients, and were positively associated with salinity. Chlorophytes biomass increased with nutrients concentrations but decreased with salinity (Figure 9).

4 Discussion

4.1 Phytoplankton community structure as revealed by microscopic methods and CHEMTAX analysis in QZB

Previous investigations on phytoplankton community in QZB using microscopic method showed that diatoms were always the dominant group accounting for over 90% of the total abundance, except during *Phaeocystis globosa* bloom (Jiang et al., 2012; Wang et al., 2013; Luo et al., 2019; Liu et al., 2020). In our study, based on microscopic examination, similar high contribution rate of diatoms for abundances was observed in both the seasons, followed by dinoflagellates (Figure 5B). However, CHEMTAX estimates found that diatom was the most advantageous group during the investigation, but its importance decreased in winter (Figures 4, 5A). Compared to microscopic method, CHEMTAX estimates recognized the important contribution of small-sized flagellates and cyanobacteria to the total phytoplankton biomass, especially during winter period (Figure 5). In fact, due to the limitation of microscopic method, pico- and nano-phytoplankton could not be completely identified for a long time, and it was not until the application of CHEMTAX estimates found that prasinophytes and cyanobacteria had ubiquitous distribution in QZB (Lan et al., 2011; Lan et al., 2013; Lan et al., 2014). A study conducted on January 13, 2021 showed that cryptophytes had the secondly higher contribution (20.25%) to the total biomass in QZB (Pan et al., 2022). Given that the small-sized phytoplankton is responsible for the recycling of the organic matter within the euphotic layer (Seoane et al., 2011) and the partitioning between pico-phytoplankton and larger cells affects the pathway of material transfer to higher trophic levels (Vaquer et al., 1996), we support the use of CHEMTAX as a suitable tool to assess the long-term changes of these small-sized groups in QZB.

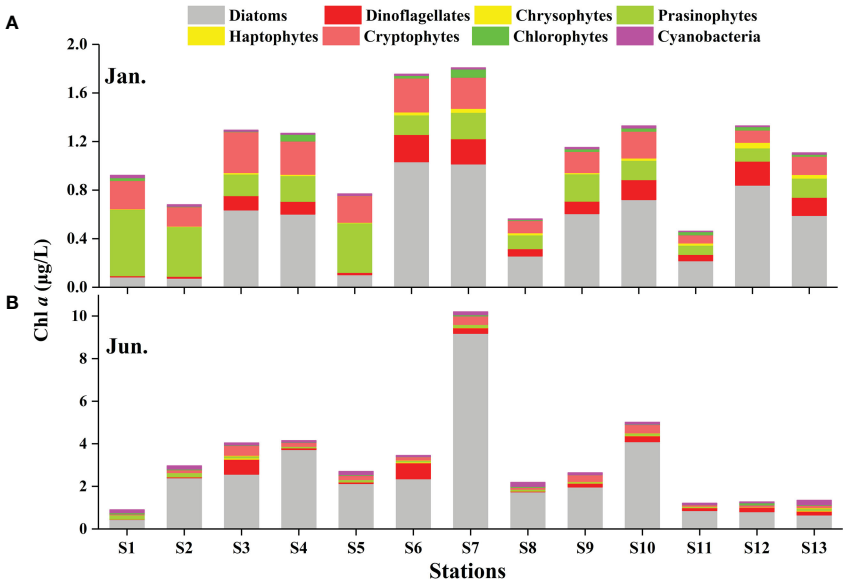

FIGURE 4
The absolute contributions of eight taxonomic groups to Chl *a* (obtained by HPLC-CHEMTAX) in the winter **(A)** and summer **(B)** in the QZB.

Former investigations performed in different seas suggested that the Chl *a* allocation to certain algal groups (e.g., diatoms and dinoflagellates) by CHEMTAX are consistent with those of microscopy (Agirbas et al., 2015; Brito et al., 2015b; Wang et al., 2018). In this study, the correlation between algal cell abundance and CHEMTAX biomass was significant for diatoms and dinoflagellates in both cruises (Figure 8). CHEMTAX estimates is an essential method to identify fragile or small cells (such as prasinophytes, cryptophytes, and cyanobacteria) that would be neglected by microscopic method as shown in Figure 5. Nevertheless, CHEMTAX estimates blind analyses may produce a distorted picture of phytoplankton communities. Irigoien et al. (2004) reported that the dinoflagellates bloom in summer and the haptophytes bloom in spring were interpreted as being composed of diatoms due to the high concentration of fucoxanthin. Furthermore, dinoflagellates might not contain Peri because they are often heterotrophic in summer (Xu et al., 2017). Therefore, it is necessary to understand the species level by microscopic analysis before applying CHEMTAX estimates. In this study, CHEMTAX estimates provided a clear picture of the whole phytoplankton community, especially that of small-sized organisms, whereas microscopy presented accurate taxonomical information (of species or genus) of large-sized phytoplankton, such as diatoms and dinoflagellates.

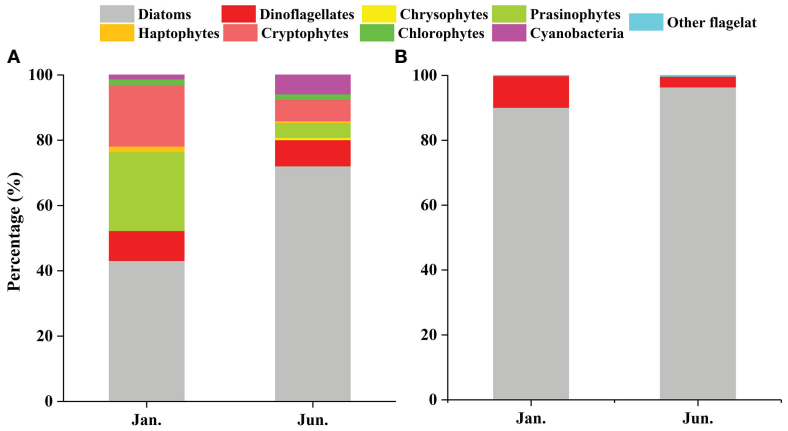

FIGURE 5
The relative contributions of various taxonomic groups in the QZB: CHEMTAX-derived Chl *a* **(A)** and cell abundances **(B)**.

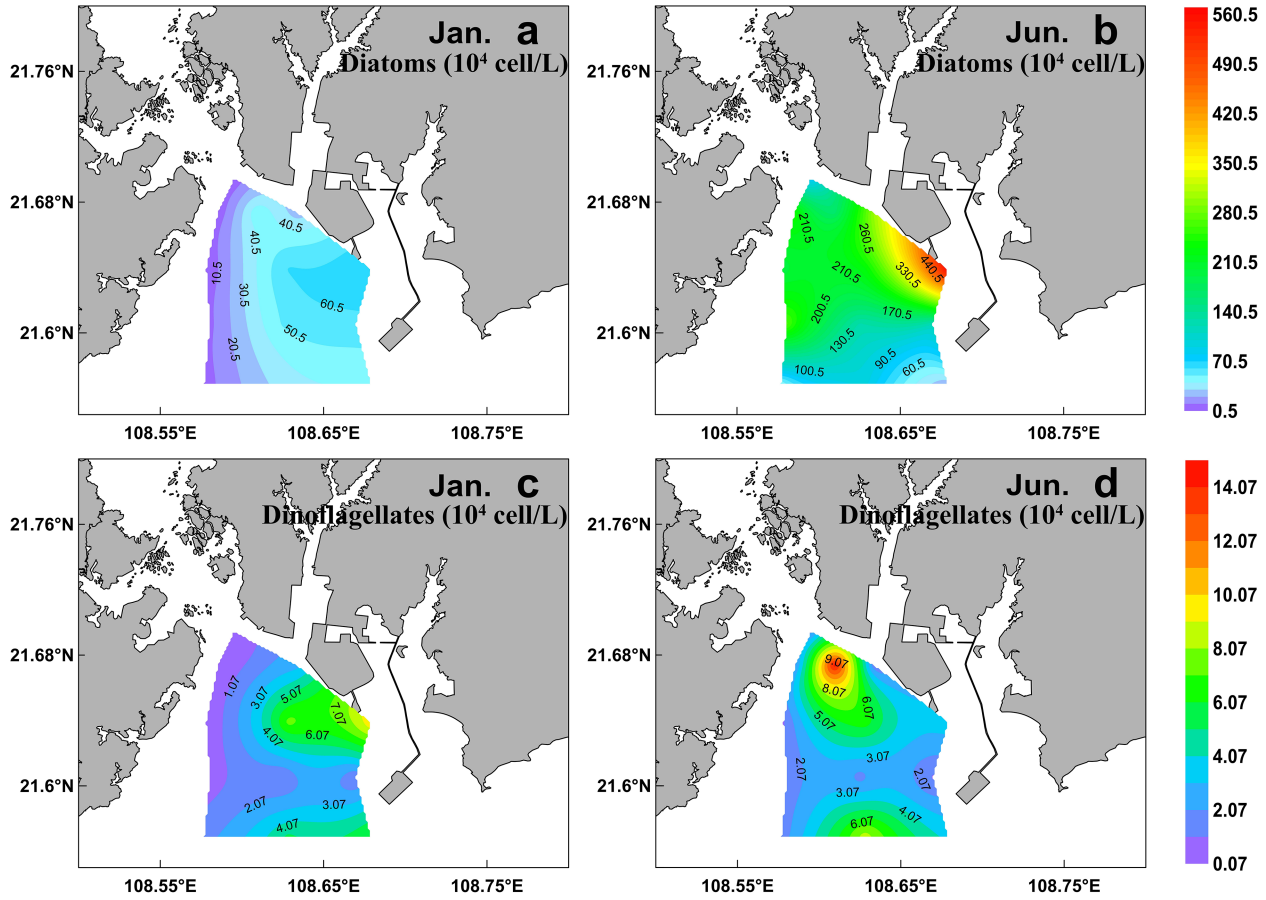

FIGURE 6
Spatial distribution of cell abundance of diatom **(A, B)** and dinoflagellates **(C, D)** (determined by microscope) during winter (right) and summer (left) in the QZB.

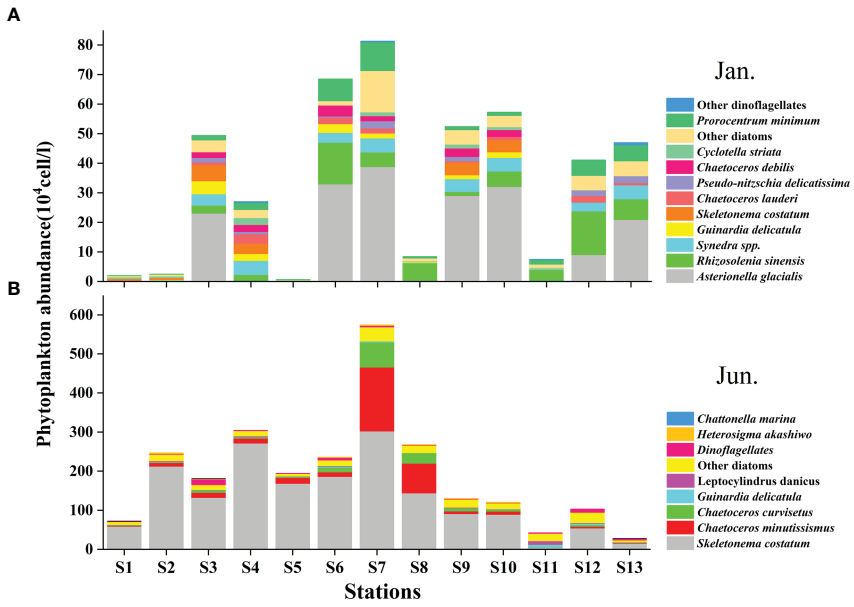

FIGURE 7
Distribution of main phytoplankton species (determined by microscope) during the winter **(A)** and summer **(B)** in the QZB.

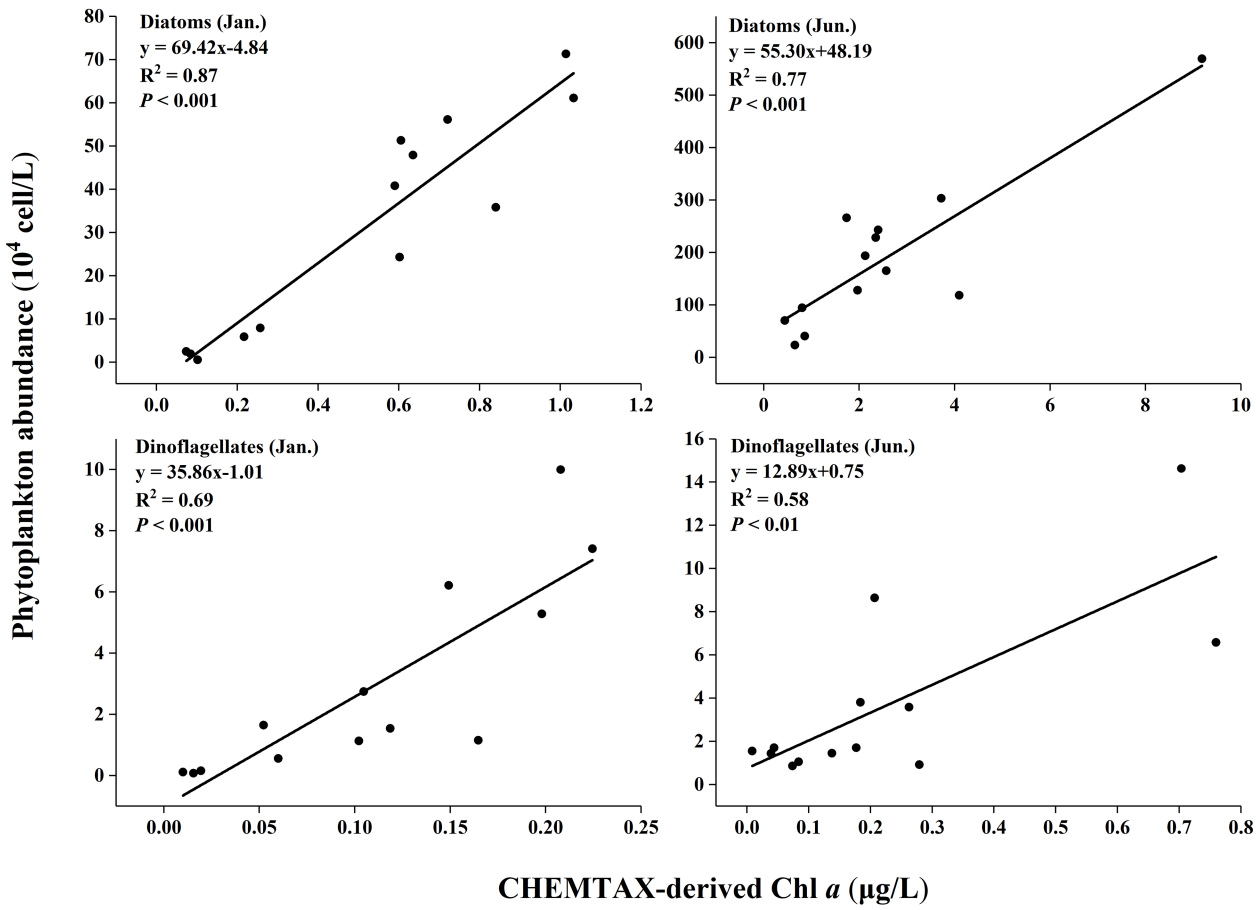

FIGURE 8
Phytoplankton abundance (determined by microscopic counting) against CHEMTAX-derived Chl *a*.

4.2 Phytoplankton groups response to environmental factors

The distributions of physical indicators and nutrients demonstrate that, as a typical estuarine bay, the QZB is vulnerable to terrigenous input and anthropogenic activity whether in dry or wet season (Figure 2). The nutrient concentration in summer is usually much higher than that in winter as terrigenous input spikes in summer. Therefore, phytoplankton biomass is generally greater in summer than in winter in the QZB (Wei and He, 2008; Lai et al., 2013; Wang et al., 2013), the similar seasonal changes in phytoplankton biomass were also revealed by this study (Figure 4, 7). The high biomass of diatoms (Figure 6B) was associated with the bloom of *Skeletonema costatum* in summer (Figure 7B). As a common bloom species in QZB, *Skeletonema costatum* prefer to a warm environment (Chin et al., 1965; Brito et al., 2015b), and usually prevails in summer (Jiang et al., 2012; Wang et al., 2013; Luo et al., 2019; Liu et al., 2020). Temperature played an important role in the variation of diatoms from winter to summer as revealed by RDA (Figure 9). Moreover, diatoms are opportunistic organisms and often show a quick response to nutrient enrichments (Barlow et al., 2004), the DSi in surface seawater replenished by summer runoff may be one of the reasons for the abundant growth of *Skeletonema costatum* (Table 1; Figure 7B) (Brito et al., 2015b). Although the differences of DIN and DRP between the two seasons were not statistically significant (P>0.05; Table 1), it is important to keep in mind that phytoplankton response may be delayed for certain days, when a high abundance of phytoplankton was observed, the nutrients had already been depleted by cells (Davidson et al., 1992; Davidson et al., 1993; Brito et al., 2015b).

Prasinophytes, a group common in temperate and cold waters (Not et al., 2004), were highly abundant during winter in this study (Figures 4A, 5A). The RDA analysis presenting a negative correlation between prasinophytes and temperature, indicateing temperature is one of the main factors affecting the seasonal distribution of prasinophytes (Figure 9). In spatial distribution, prasinophytes concentrated in the oyster culture area in both the seasons (Figures 3G, H). Shellfish preferentially filter larger cells (>3μm) (Ward and Shumway, 2004), thus encouraging the development of the small-size phytoplankton (such as prasinophytes) in shellfish culture area (Dupuy et al., 2000; Jiang et al., 2019; Jiang et al., 2019a). Pico-phytoplankton constitutes an important abundant component of the phytoplankton community in other shellfish aquaculture areas in the world (Safi and Gibbs, 2003; Cranford et al., 2008), which is also mainly ascribed to size-

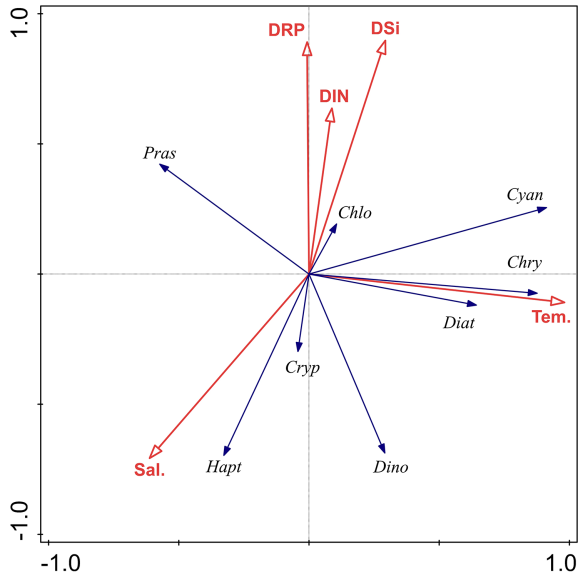

FIGURE 9
Ordination diagram of redundancy analysis for major environmental parameters and phytoplankton groups in the QZB. Red arrows refer to environmental variables. Sal, salinity; Tem, temperature; DIN: DRP and DSi. Variables used: Diat, diatoms; Cryp, cryptophytes; Pras, prasinophytes; Dino, dinoflagellates; Cyan, cyanobacteria; Chlo, chlorophytes; Hapt, haptophytes; Chry, chrysophytes.

selective grazing of shellfish. Lan et al. (2013); Lan et al. (2014) reported that prasinophytes were mainly distributed in oyster culture area during cold seasons. In this study, prasinophytes dominated in oyster culture area (Stations S1, S2, and S5) during winter, where the biomass of larger-size diatoms was very low under the feeding pressure on oyster (Figure 4A). To summarize, oyster aquaculture could stimulate prasinophyte growth, and the seasonal variations are mainly controlled by the water temperature in QZB as shown in the two cruises, which consistent with the results of Jiang et al. (2016).

As a mixotrophic organism, cryptophytes can obtain nutrition through photosynthesis and phagocytosis, especially during the periods of low nutrient concentrations (Lee, 2008). In general, cryptophytes respond to the input of freshwater (Mendes et al., 2013; Brito et al., 2015a) rather than to nutrient enrichment (Balode et al., 1998; Arhonditsis et al., 2007; Mendes et al., 2013). High abundances of cryptophytes were revealed in the Jingu River estuary in both seasons (Figures 3K, L, 4A). Moreover, cryptophytes are an important phytoplankton group in China's coastal waters, such as Jiaozhou Bay (Peng et al., 2010) and Daya Bay (Wang et al., 2018).

The previous study shows that *Synechococcus* and *Prochlorococcus* were common groups of cyanobacteria in the Beibu Gulf (Zhao et al., 2019). In this study, the abundance of cyanobacteria was related to the abundance of *Synechococcus* because divinyl Chl *a*, the exclusive marker pigment of *Prochlorococcus*, was not detected. Although cyanobacteria (mainly *Synechococcus*) were a minority group in terms of average contribution to total Chl *a*, they exhibited strong seasonal fluctuation that thriving in summer and declining in winter (Figures 3I, J, 4). It is known that cyanobacteria are thermophilic (Seoane et al., 2011), and the optimum water temperature range for *Synechococcus* is 27–30°C (Rajaneesh and Mitbavkar, 2013), which is close to the summer water temperature in QZB (Figure 2B). This finding gains support from the RDA analysis presenting a positive correlation between temperature and cyanobacteria (Figure 9).

Nuclear power plant located in the west coast of QZB (Figure 1). According to the horizontal distribution of temperature, the southwestern part of the study area was more affected by the thermal effluent of the nuclear power plant in winter than in summer (Figures 2A, B), which may be related to the strong water exchange capacity during summer in QZB (Dong et al., 2014). Jiang et al. (2019) reported that the thermal discharge from power plants stimulated phytoplankton growth in Xiangshan Bay, China, particularly in cold seasons. However, Pan et al. (2022) found that Chl *a* concentrations were lower in the areas affected by thermal discharge, similar to the results of this study. It is noteworthy that, in addition to thermal pollution, the residual chlorine of the thermal effluent also affects phytoplankton growth (Jiang et al., 2008). Therefore, further attention should be paid to research the response of phytoplankton community structure and biomass to thermal discharge from nuclear power plant in QZB and its mechanism.

4.3 The long-term change in phytoplankton composition and driving factors

The contribution of diatoms to total species number decreased from 90.2% in 1984-1985 (Chen, 1989) to 67.1% in 2014 (annual average), and the proportion of dinoflagellates increased to 28.8% in the same period, so did other groups including haptophytes, chrysophytes, and cyanobacteria etc. (Jiang et al., 2012; Wang et al., 2013; Luo et al., 2019; Liu et al., 2020). On the other hand, the dominant species of phytoplankton in QZB were relatively monotonous, and large-size *Rhizosolenia* spp. were the absolutely dominant species during 1984-1985 (Liu et al., 2020). However, small-sized diatoms, dinoflagellates, and haptophytes gradually were added to the list of dominant species over the last decade (Jiang et al., 2012; Wang et al., 2013; Luo et al., 2019) (Table 2). Our research shows a similar phenomenon that the contribution of dinoflagellates to total species number were 22.2%, small-sized diatoms (*Skeletonema costatum*) and dinoflagellates (*Prorocentrum minimum*) became dominant species (Figure 7). Furthermore, we found that prasinophytes and cryptophytes acted alternatively as the second dominant group during cold season in QZB, and the proportion of small-sized flagellates has been increased since 2010 (excluding data from the Maowei Sea), especially cryptophytes (Table 3). In addition, a study on size-fractionated Chl *a* showed that pico- and nano-phytoplankton were major contributors to Chl *a* in QZB (Mo et al., 2017). These studies suggest that the phytoplankton community of the QZB was developing towards diversification and miniaturization.

The eutrophication of QZB (Wei et al., 2002; Lan, 2012; Yang et al., 2012; Lai et al., 2013) played an important role in the changes of phytoplankton class structure. Generally, increased nitrogen and

TABLE 2 Long-term variation in dominant species and various taxonomic groups contribution (%) to total species number in QZB.

Investigation time	Diatoms	Dinoflagellate	Other groups	Main dominant species	References
1984-1985	90.2%	9.8%	0%	*Rhizosolenia alata f. gracillima Rhizosolenia imbricate Thalassionema nitzschioides*	Chen, 1989
2008*	77.1%	17.6%	5.3%	*Chaetoceros curvisetus Skeletonema costatum Rhizosolenia alata*	Wang et al., 2013
2010-2011*	77.2%	14.5%	8.3%	*Chaetoceros curvisetus Skeletonema costatum Thalassionema nitzschioides*	Jiang et al., 2012
2013-03,07*	86.9%	12.2%	0.9%	*Coscinodiscus radiates Ditylum brightwelii Skeletonema costatum Thalassiosira nordenskioldii*	Luo et al., 2019
2013-2014	67.1%	28.8%	4.1%	*Skeletonema costatum Chaetoceros curvisetus Phaeocystis globosa Thalassiosira nordenskioldii*	Liu et al., 2020
2021-01,06	73.7%	22.2%	4.1%	*Skeletonema costatum Asterionella glacialis Chaetoceros minutissismus Rhizosolenia sinensis*	This study

*The stations data in Maowei Sea are included.

phosphorus in the areas of eutrophication often leads to excessive phytoplankton growth, however, diatoms require silica for their shells in addition to these nutrients. For this reason, non-diatom phytoplankton groups increase in species number and biomass in eutrophic waters; for example, the species number of dinoflagellates were obviously dominant compared to diatoms in the south-eastern Black Sea (Agirbas et al., 2015) and the phytoplankton community changed from the diatoms dominance to co-dominance of diatoms and dinoflagellates in the Bohai Sea (Pan et al., 2020). According to the statistics, several bloom of haptophytes (*Phaeocystis globosa*) and dinoflagellates (e.g. *Noctiluca scintillans*, *Gymnodinium sanguineum*, and *Gymnodinium catenatum* and so on) occurred in QZB over the past decade (Qin et al., 2016; Shen et al., 2018; Liu et al., 2020; Su et al., 2022).

Variation in phytoplankton size scaling usually related to temperature and nutrients (Jiang et al., 2019b). It is widely recognized that small-sized phytoplankton have been considered an indicator to stressful environments, they tend to dominate in oligotrophic sea area because of its large relative surface area and high utilization rate of nutrients (Sabetta et al., 2008), whereas large-celled phytoplankton benefit from a eutrophic environment (González-García et al., 2018). However, in this study, prasinophytes were concentrated in the northwest of the study area where nutrient was enriched the most in both the seasons (Figures 2E–J, 3G, H). As described in section 4.2, in addition to nutrients, phytoplankton community structure also depends on biological factors (grazing of shellfish) in high-density shellfish culture area. Cloern (1982) suggested that phytoplankton is much more controlled by shellfish feeding than by nutrients concentration, indicating that the phytoplankton community structure in oyster culture area of QZB was mainly affected by oyster feeding rather than by high nutrient concentration. Therefore, the long-term size-selective filtration of shellfish stimulated the small-size phytoplankton growth in QZB.

TABLE 3 Long-term variation in various taxonomic groups contribution (%) to phytoplankton biomass in the QZB.

Investigation time	Contribution of various taxonomic groups to phytoplankton biomass obtained by HPLC-CHEMTAX (%)					References
	Diatoms	Prasinophytes	Cyanobacteria	Cryptophytes	Dinoflagellates	
2010-06*	84.61	4.16	4.81	1.00	2.20	Lan et al., 2011
2010-10*	76.67	11.40	5.28	1.18	1.27	Lan et al., 2013
2011-03*	71.89	19.06	1.73	0.36	4.57	Lan et al., 2014
2021-1-13	62.29	9.77	0.96	20.25	3.43	Pan et al., 2022
2021-1-27	43.12	24.18	1.29	18.54	9.17	This study
2021-6-23	72.11	4.53	5.80	6.54	8.03	This study

*The stations data in Maowei Sea are excluded.

Mesocosm experiments (Yvon-Durocher et al., 2011; Peter and Sommer, 2015) and field investigations (Morán et al., 2010) have confirmed a tendency toward smaller size at higher temperatures for different phytoplankton groups, include diatoms, picophytoplankton (*Prochlorococcus*, *Synechococcus*, and picoeukaryotes), and chlorophytes. The water temperature in QZB was up from 1983 to 2003 (He and Wei, 2010), possibly indicating warming in QZB as well under climate change. Moreover, thermal discharge may potentially accelerated the warming trend in QZB. We inferred that increased dominance of small-sized diatoms in QZB is closely related to size-selective grazing of oyster and increased warming. It is noteworthy that the biomass and proportion of cyanobacteria may also increase in the future driven by these two factors. Overall, size-selective grazing of oyster farming and warming might the main reasons causing phytoplankton miniaturization in QZB, especially in oyster culture area.

5 Conclusions

The microscopy and HPLC-CHEMTAX was combined to obtain complete information about species composition and spatial distribution of entire phytoplankton community in QZB. Microscopy analysis revealed that diatoms were absolute dominant group in summer and winter, 2021, followed by dinoflagellates. As a complementary method, HPLC-CHEMTAX detected other small-size flagellates and cyanobacteria, of which prasinophytes and cryptophytes were important groups in winter. This study confirms our hypothesis that the spatiotemporal distributions of various phytoplankton groups were affected by physicochemical factor and size-selective grazing of shellfish. High temperature and adequate nutrients in summer could promote the algal blooms of *Skeletonema costatum*. A high grazing pressure of shellfish and low temperature were the driving force to the prevalence of prasinophytes in the winter. The distribution of cryptophytes was related to the location of estuary. Driven by high temperature, cyanobacteria (mainly *Synechococcus*) thrived in summer while declined in winter. In addition, the negative impact of thermal discharge on phytoplankton occurred mainly in winter. Compared with previous reports, the proportion of small-sized flagellates has been increased over the past decade. The change of phytoplankton taxonomic structure may be related to the eutrophication of QZB, phytoplankton miniaturization was largely regulated by grazing of oyster and climate warming. The microscopy derived cell abundances and CHEMTAX analysis information for diatoms and dinoflagellates were in good agreement. Microscopy provided accurate species information for large cells, while HPLC-CHEMTAX provided detailed data of small-sized phytoplankton groups. However, no single technique is ideal for the investigation of the structure and dynamics of phytoplankton community. We suggest that in future, research should combine CHEMTAX estimates with microscopic counting to improve the long-term understanding of phytoplankton community dynamic in QZB.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

HP, MX and JLai wrote the original manuscript. CL, JM, JLi and JLu attended the experiments and took part in the data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1186106/full#supplementary-material

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