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Succession of phytoplankton communities from macro-scale to micro-scale in coastal waters of Qinhuangdao, China

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The coastal area of Qinhuangdao, particularly the Changli Gold Coast Nature Reserve, is experiencing ecological degradation and frequent Harmful Algal Blooms (HABs). This study focuses on the changing phytoplankton communities in these coastal waters, examining them from both a macroscopic and microscopic perspective. Utilizing microscopy, molecular techniques, and pigment analysis, seasonal shifts were observed, with diatoms predominating in June and July, and dinoflagellates in August. Our morphological examination enabled the classification of 89 species into four distinct groups. The species *Paralia sulcata* and *Pseudo-nitzschia pungens* were most abundant in early summer, while *Tripos furca*, a dinoflagellate, dominated in August. This indicates a shift in phytoplankton communities due to environmental factors such as phosphate deficiency and high nitrogen/phosphorus ratios. Additionally, the study notes the impact of reduced river runoff and reintroduction of scallop farming contributing to nitrogen-rich eutrophication in August. Molecular analysis revealed a disparity between microscopic observations and the prevalence of *Teleaulax* blooms during early summer. Elevated concentrations of TN and DOC, coupled with limited water exchange, emerged as primary factors contributing to their occurrence. Sediment analysis revealed a high diversity but low abundance of dinoflagellates in August, with a significant presence of harmful species. The study highlights the shift from diatoms to harmful dinoflagellate populations, exacerbated by eutrophication and pollution, leading to HABs. These findings provide a theoretical basis for understanding toxic algal blooms and are crucial for environmental agencies in developing strategies to protect and sustainably develop offshore environments.

KEYWORDS

scallop farming, phytoplankton community, cryptophytes, dinoflagellate cysts, 18S rRNA

Introduction

Qinhuangdao city, located in the northeast of Hebei province, China, is an important harbor city within the Bohai Rim economic zone. This port city is situated near the Bohai Sea, a semi-enclosed sea characterized by poor circulation (Tao, 2006; Qiao et al., 2017). In recent years, human activities have significantly affected the coastal waters of Qinhuangdao, making it one of the most polluted sea areas (Cao et al., 2016). Consequently, the occurrence of harmful algal blooms (HABs) in these coastal waters has become increasingly severe (Zhang et al., 2012; Cao et al., 2016; Ou et al., 2018; Wang et al., 2021).

HABs in the coastal waters of the Qinhuangdao region typically occur from May to August, mainly in summer (Oceanic Administration of Hebei Province, 2005–2021). However, the dominant species have varied considerably. Before 2000, the dominant species were primarily microphytoplankton, such as *Skeletonema costatum* and *Pseudo-nitzschia delicatissima* (Su and Zhou, 2001; Yu et al., 2020). Since 2000, the causative species of HABs have tended to become more diversified, harmful, and miniaturized, including *Alexandrium catenella* and *Aureococcus anophagefferens* (Kong et al., 2012; Zhang et al., 2012; Yu et al., 2019; Wang et al., 2021). Earlier studies predominantly focused on diatoms (usually > 20μm), identified and quantified primarily through light and electron microscopy. However, recent research has increasingly focused on harmful dinoflagellates and other nanophytoplankton (2μm - 20μm), which are not only being gathered from water samples but also from sediment samples (Xu et al., 2017; Yu et al., 2020; Wang et al., 2021). The presence of harmful dinoflagellates within communities can lead to harmful algal blooms (HABs), making it crucial to study the cysts deposited in sediment as a preventive measure. Obtaining samples of dinoflagellate cysts from sediment is thus imperative for this purpose. Additionally, novel methodologies, such as the pigment method (Kong et al., 2012) and molecular techniques (Metfies et al., 2010; Yu et al., 2020), have been developed to explore various aspects of HABs. However, there remains a lack of studies comparing the outcomes derived from these distinct methodologies.

The sampling site was located near the Changli coast of Qinhuangdao, positioned between the heavily polluted Yang River Estuary and Dai River Estuary. These regions are typical areas influenced by riverine discharge into the marine environment (Li, 2012). Additionally, this site is proximal to tourist and aquaculture areas, significantly impacted by human activities. It is also located in the Changli ecology-monitoring area, between the Gold Coast National Nature Reserve and the Mariculture zone, where HABs have recently occurred frequently, posing threats to the ecological environment and resources such as amphioxus and lagoons (Li, 2012). In our study, we combined traditional classification techniques with the pigment method and molecular techniques to investigate the abundance and diversity of phytoplankton. Our main objectives were: (1) to explore their response to environmental dynamics, and (2) to evaluate the marine ecological system through the utilization of indicator species and the analysis of alterations in community structure. Such investigations can provide valuable insights to relevant authorities for the formulation of effective conservation policies.

Materials and methods

Study area and sampling stations

The sampling cruise (119.418°E; 39.743°N) was conducted in the vicinity of the Changli coast of the Qinhuangdao (Figure 1). During the summer months of 2017 (June, July, and August), a total of nine samples were collected, consisting of six water samples and three sediment samples. Detailed information can be found in Figure 1.

Sampling and analysis

A CTD profiler (Seabird SBE 911 CTD) installed onto a rosette sampler was used to measure the *in-situ* temperature (T), pH, salinity, dissolved oxygen (DO) and total nitrogen (TN). Samples for measurement of inorganic nutrients and size-fractionated chlorophyll *a* (Chl-*a*) concentrations were collected from the two layers (0 m and 6 m) in the water column and passed through a 200 μm mesh filter to remove large zooplankton and debris. For nutrient analysis, water samples were collected into 100 mL HCl-rinsed plastic bottles and stored at 4°C. For Chl-*a* measurements, 500 mL of seawater from each layer was filtered through GF/F filter paper and preserved in liquid nitrogen until analysis. Under negative pressure of < 100mm Hg, 5 l of seawater was filtered through a 25 mm GF/F filter membrane. The filter membrane obtained was then wrapped in tin foil and stored in a -80°C refrigerator (or in liquid nitrogen). In the laboratory, the filter membrane was thawed and extracted with 100% methanol and analyzed by High-Performance Liquid Chromatography (HPLC) (Mackey et al., 1996; Schlüter et al., 2011). To collect DNA, 800 mL of seawater was filtered through 0.22 μm GTTP filters (Millipore, Eschbonn, Germany) under a low-pressure vacuum. Sediment samples for further DNA analysis were collected using a bottom sampler, placed into 2 mL microtubes, and immediately frozen in liquid nitrogen. For microphytoplankton estimation, water samples were placed into 250 mL polyethylene (PE) bottles, fixed with a 1% final concentration formaldehyde solution, and stored in the dark until further identification in the laboratory. Surface sediments were collected using a grab sampler (June sediment (JUNsedi); July sediment (JULsedi); August sediment (AUGsedi)), following the methods of Gu et al. (2010). Dinoflagellate cyst abundance was measured as the number of cysts per gram of wet weight sediment.

Nutrients and Chl-*a* estimation

In the laboratory, inorganic nutrient concentrations including ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), silicate (SiO_3-Si), and phosphate (PO_4-P) were determined using a

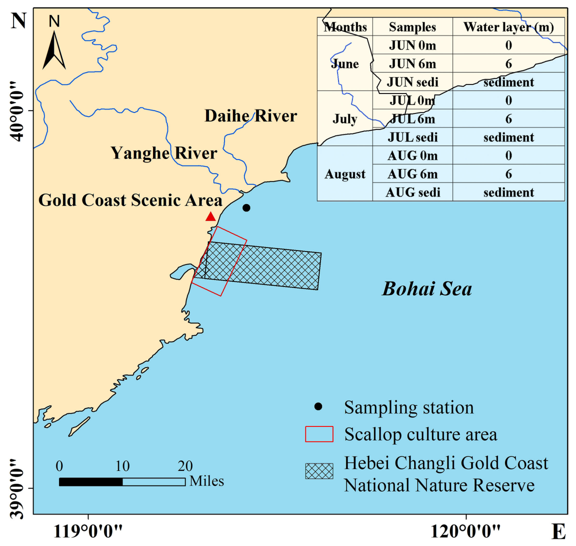

FIGURE 1
Map of study area and sampling station in Bohai Sea showing the Hebei Changli Gold Coast National Nature Reserve and scallop culture area.

Technicon AA3 Auto-Analyzer (Bran+Luebbe, Norderstedt, Germany) with standard procedures (Guo et al., 2016). Dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) concentrations were measured using high-temperature combustion in a Shimadzu TOC-V analyzer equipped with an inline chemiluminescence nitrogen detector (Davis and Benner, 2005). For the determination of Chl-*a* concentrations, 500 mL water samples were filtered sequentially through 20 µm, 2 µm and 0.72 µm Waterman GF/F filter (each 25 mm in diameter). This process facilitated the classification of Chl-*a* into micro-, nano-, and pico-sized fractions. For total Chl-*a* determination, another sample was filtered through a 20µm Waterman GF/F filter, followed by 2 µm GF/F filter, specifically targeting the nano-sized Chl-*a* fraction. These filters were then placed into aluminum-foil bags and kept in the dark at -20°C. For pigment extraction, filters were submerged in 20 mL vials containing 90% acetone for 24 h at 4°C. Subsequently, the concentration of Chl-*a* was determined using a Trilogy (CHL NA, Model #046) fluorometer following the method described by Parsons et al. (1984).

Phytoplankton and dinocyst quantification

The water-column samples of phytoplankton were placed in Utermöhl counting chambers (100 mL), allowed to settle for 24 h, and then examined and enumerated using an inverted microscope (Motic BA300) at ×200 or ×400 magnification. Sample volume was optimized to ensure that settled cells did not overlap. Phytoplankton morphotypes were identified following the standard identification keys (Sun et al., 2002).

For dinocyst community analysis, surface sediment samples were processed using the previously published protocol (Gu et al., 2010) with modifications. Approximately 3 g of wet sediment was mixed with filtered seawater (20 mL) and sonicated for 2 min to dislodge detritus particles. The obtained slurry was then passed through two-tier (100 and 20 µm) sieves. The fraction accumulated on the 20 µm sieve was washed, suspended in 1 mL filtered seawater, and used for quantification of dinocysts under an inverted microscope (Motic BA300) at ×200 magnification. Dinocyst morphotypes were identified using published literature (Accoroni et al., 2016). Dinocyst abundance was measured as the number of cysts per gram of wet weight sediment (cysts g^{-1} wet weight).

DNA extraction, amplification, and analysis

Total genomic DNA samples were extracted using a DNA Extraction Kit, following the established protocols (Bio-Rad, Hercules, CA, USA). The purity and concentration of DNA were assessed with NanoDrop (ThermoFisher, Waltham, MA, USA) and agarose gel electrophoresis. These validated DNA samples were diluted to 1 ng/µl and stored at -20°C until they were used as templates for PCR amplification of eukaryotic 18S rRNA genes using barcoded primers and HiFi Hot Start Ready Mix (KAPA Biosystems, Roche, Basel, Switzerland). To analyze eukaryote diversity, variable regions of the 18S rRNA genes were amplified using universal primers for the V9 region, namely 817F (5'-TTAGCATGGAATAATRRAATAGGA-3') and 1196R (5'-TCTGGACCTGGTGAAGTTTCC-3') (Rousk et al., 2010). Amplicon quality was visualized via gel electrophoresis. The PCR products were then purified using AMPure XP beads (Agencourt, Beckman Coulter, Brea, CA) and subjected to another round of PCR amplification. The final amplicon concentration was quantified using the Qubit dsDNA assay kit (ThermoFisher). Equal amounts of the purified amplicons were pooled for subsequent sequencing.

MiSeq sequencing (Illumina, San Diego, CA, USA) was used to generate raw reads. to the initial step in analyzing microbial diversity sequencing involved stitching the original double-end sequencing data and removing irrelevant materials. Subsequently, low-quality and fuzzy base sequences were excised, and chimera sequences were identified and removed using RStudio. After the preliminary filtration, the resulting high-quality sequences were considered valid tags and used for further analysis. The remaining effective tags were clustered using VSEARCH (v2.4.2) (Torbjørn et al., 2016), with sequences exhibiting ≥97% similarity deemed operational taxonomic units (OTUs) (Edgar, 2010). Subsequently, the OTU classification table were generated through comparison with the Silva database (v. 123). Species comparison annotations were performed using the RDP classifier software, retaining annotation results with confidence intervals greater than 0.7.

Raw sequences obtained from Miseq were deposited in theNCBI Sequence Read Archive under accession number PRJNA503792. For each sample, the relative abundance of phytoplankton OTUs was calculated by dividing the number of sequences from the same taxa by the total number of phytoplankton sequences. Richness was calculated as the number of OTUs from the same taxa over total number of algal OTUs.

Statistical analysis

Redundancy analysis (RDA) is a constrained principal component analysis, which combines correspondence analysis with multiple regression analysis. In this study, nutrient paraments were used as environmental factors, and various indicators were used to elucidate the relationship between phytoplankton abundance. The RDA analysis was performed using CANOCO 5.0 software. Significant differences between phytoplankton abundance and environmental factors were determined using SPSS one-way ANOVA. The Shannon-Wiener Biodiversity (H') and Pielou Evenness (J) indices were calculated using the "DIVERSITY" function of the "vegan" package in R v3.6.1 software (R Development Core Team, 2008). The results of HPLC pigment analysis were evaluated using the CHEMTAX program developed by Mackey et al. (1996). CHEMTAX calculated the contributions from different phytoplankton groups to Chl-*a* based on ratios between accessory pigments and Chl-*a*, which were input into the program together with the field measurements of pigment concentrations.

Results

Morphological examination

A total of 89 phytoplankton species belonging to 43 taxa were identified from the water samples through conventional microscopic analysis. Diatoms (Bacillariophyceae) were predominant, comprising 56 species (65.2% of the total phytoplankton), while dinoflagellates, with 30 species, contributed 33.7%. Additionally, a small number of Dictyochophyceae were observed (Figure 2A). Within the diatoms, the genus *Chaetoceros* was notably abundant, with 9 species representing 15.5% of the total diatom assemblage. In contrast, the genus *Protoperidinium* predominated among the dinoflagellates, constituting 33.3% of its total. During June and July, *Paralia sulcata* and *Pseudo-nitzschia pungens* were the most abundant phytoplankton species, comprising approximately 40% of the total phytoplankton. Conversely, in August, *Tripos furca* (a dinoflagellates) emerged as the dominant species, constituting up to 70% of the total. The number of *Tripos furca* reached to 4.68×10^4 cells/L in AUG0m layer, accounting for over 80% of the total phytoplankton population. Phytoplankton diversity index, measured by Shannon-Wiener diversity index, varied from 1.433 to 3.899 (average 2.647), with the highest diversity observed in June and the lowest in August. The Pielou evenness index ranged from 0.039 to 0.186, with an average of 0.089.

Regarding trophic modes, mixotrophic and heterotrophic species predominantly characterized the dinoflagellates assemblage in the region. Nineteen dinocyst species from 8 genera were identified in the surface sediments. Notably, *Alexandrium* species (mainly *A. minutum*) dominated the cyst assemblage in June, with a concentration of 75 cysts g^{-1} wet weight, but their prevalence decreased over time (Figure 3). These 19 dinocyst species were categorized as autotrophs (8 species), heterotrophs (9 species), and mixotrophs (2 species). The abundance of heterotrophs and mixotrophs decreased monthly towards August. The dinocyst diversity index (Shannon-Wiener diversity index) ranged from 2.654 to 3.039 (average 2.810), with the highest diversity observed in August and the lowest in June. The Pielou evenness index ranged from 0.649 to 0.798, with an average of 0.757.

18S rRNA

In total, 305,018 effective tags were included in our study after quality control. The sequencing coverages (C) exceeded 99.9% for all, suggesting adequate depth for 18S rRNA gene diversity analysis. Clustering the unique sequences at a 97% similarity threshold resulted in 292 OTUs, with individual sample OTUs ranging from 94 to 186. To accentuate the abundance and richness of eukaryotic phytoplankton, non-algal and unclassified OTUs were excluded, yielding a total of 118,325 sequences with ≥97% similarity to the eukaryotic algae reference sequence from 9 samples. These accounted for 39% of the total sequences across 53 OTUs.

Phylogenetic analysis indicated that these 53 OTUs spanned five major types of marine algae: Cryptophyceae, Bacillariophyceae, Dinophyceae, Prymnesiophyceae and Dictyochophyceae (Figure 2B). Notably, diatoms and dinoflagellates each had 15 species, representing 57% of the total OTUs. A minor disparity in OTU richness between samples suggested a relative stable composition of the eukaryotic phytoplankton community at higher taxonomic levels. However, the relative abundance of different eukaryotic phytoplankton groups varied considerably between samples (Figure 2B). Most sequences in the water samples were attributed to Cryptophyceae, which accounted for 71.65–94.40% of phytoplankton community, except in August. The genus *Teleaulax* was the dominant species within Cryptophyceae. Notably, the abundance of dinoflagellates in water samples increased rapidly from 0.89% (JUL0m) to 68.09% (AUG0m), with the genera *Amoebophrya* and *Ceratium* being the most prevalence. In the sediments, dinoflagellates were the most abundant, though their prevalence significantly diminished in August, similar to the diatoms. The sediment diversity index (Shannon-Wiener diversity index) ranged from 2.23 to 3.53 (average 2.98), with the highest diversity observed in August and the lowest in July. Dinoflagellates dominated in July, accounting for approximately 74.50% of the total sequences, but their dominance rapidly decreased from July (74.50%) to August (46.15%).

Pigment analysis

The HPLC analysis identified a total of 18 pigment components in water samples, with the content incrementally increasing monthly at the station. CHEMTAX analysis revealed seven phytoplankton groups present, including diatoms, Cyanophyceae,

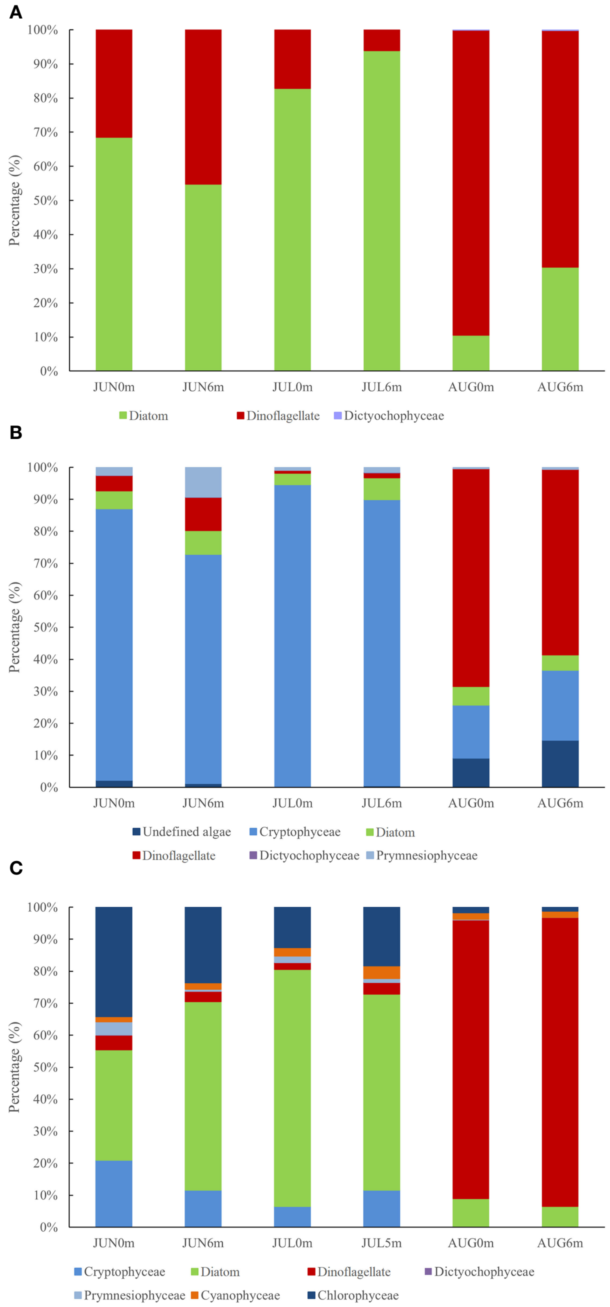

FIGURE 2
Phytoplankton structure based on **(A)** Morphological examination; **(B)** 18S rRNA; **(C)** Pigment analysis.

Dinophyceae, Cryptophyceae, Chrysophyceae, Prymnesiophyceae, and Chlorophyceae (Figure 2C). In June, pigment distribution at JUN0m was predominantly composed of diatoms (fucoxanthin 0.825 ug/L), Chlorophyceae (peridinin 0.820 ug/L), and Cryptophyceae (alloanthin 0.503 ug/L), with pigment/Chl-*a* ratios of 35%, 34% and 21% respectively. Conversely, at JUN6m, diatoms dominated, exhibiting a fucoxanthin/Chl-*a* ratio of 58.8%. In July and August, surface and bottom waters displayed similar trends. Specifically, in July, diatoms were prevalent in water samples with an average concentration of 2.637 ug/L and an average fucoxanthin/Chl-*a* ratio of 68%. In August, dinoflagellates predominated with an average concentration of 5.884 ug/L, and the peridinin/Chl-*a* ratio averaged 89%.

Influence of environmental factors on phytoplankton community

The RDA analysis of both conventional microscopic and molecular data identified SiO_3^{2-}, TN (total nitrogen), PO_4^{3-}, NO_2^- and NO_3^- as the most influential factors shaping the composition of phytoplankton assemblages, accounting for up to 97% of the total variation. In the

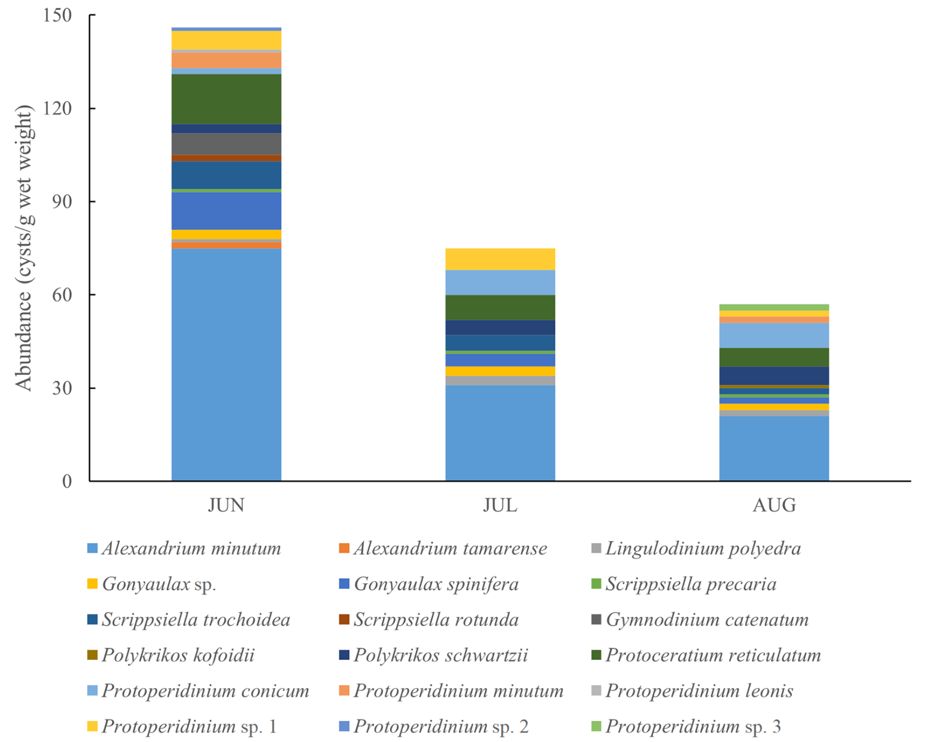

FIGURE 3
Abundances of dinoflagellate cyst by morphological examination.

conventional microscopic analysis, all these factors exhibited a significant positive correlation with diatoms. Conversely, dinoflagellates, demonstrated a positive correlation with SiO_3^{2-}, TN and NO_3^-, but a negative correlation with PO_4^{3-} and NO_2^-. Please refer to Table 1 for specific environmental parameters.

Discussion

Shift from diatoms to dinoflagellates as the dominant phytoplankton group

During June and July, diatoms were the predominant species, whereas dinoflagellates became more prevalent in August. Specifically, *P. sulcata* and *P. pungens* were the most abundant phytoplankton species during the earlier months, constituting approximately 40% of the total. In contrast, by August, *T. furca* (a dinoflagellate) accounted for up to 70% of the total phytoplankton. This transition was validated through pigment and molecular analyses, indicating that the shift was largely attributed to phosphate deficiency. Nutrients serve as the foundational basis for phytoplankton growth, and terrestrial rivers inputs significantly influence the content and structure of marine nutrients (Strokal et al., 2017; Kubo et al., 2018). Notably, the average precipitation during the flood season in Hebei Province in 2017 decreased by 12% compared to the previous year, indicating a drier year (https://mip.ys137.com/zhishi/fanwen/15581844.html). Additionally, runoff monitoring at the Yang hydrological control station revealed that the annual runoff of the Yang River in 2017 was 0.2823 million cubic meters, a substantial 52% decrease from

TABLE 1 Variations in environmental parameters at the sampling station in the coastal waters of Qinhuangdao.

Samples	T/ °C	Salinity/ psu	Total Chl-*a*/ µg/L	DO/ mg/ L	pH	DOC/ umol/ L	DIC/ mmol/ L	PO_4^{3-}/ µmol/ L	NO_3^-/ µmol/ L	NH_4^+/ µmol/ L	SiO_3^{2-}/ µmol/ L	NO_2^-/ µmol/ L	TN/ umol/ L
JUN0m	24.74	31.73	4.022	7.41	7.94	162.1	2.20	0.106	1.205	0.650	11.368	0.129	21.15
JUN6m	23.88	31.89	4.729	7.85	7.97	164.7	2.22	0.035	1.233	0.350	10.500	0.079	18.77
JUL0m	27.14	30.96	9.734	6.64	7.81	167.28	2.12	0.403	2.291	5.607	26.364	0.364	27.70
JUL6m	26.67	31.38	9.384	6.68	7.82	161.67	2.20	0.203	1.913	3.336	21.371	0.236	20.26
AUG0m	28.44	30.56	17.326	9.68	8.11	298.28	1.96	0.010	1.909	0.179	38.364	0.086	34.34
AUG6m	28.42	30.57	14.839	9.41	8.10	179.12	1.97	0.010	1.912	0.071	38.411	0.086	25.40

2016 (China Water Resources Bulletin). This reduction in river runoff led to lower levels of DIN (Dissolved Inorganic Nitrogen, DIN) and DIP (Dissolved Inorganic Phosphorus), especially DIP, in 2017 compared to preceding years. In August, the area showed a stoichiometric limitation of phosphate. However, nutrient concentration analyses indicated no nitrogen limitation near the scallop farming waters during the sampling period. The high levels of nitrogen concentration might be attributed to the regeneration from scallop farming through direct excretion of ammonium (Prins and Smaal, 1994; Kong et al., 2022). Phosphorus, a fundamental component of nucleic acids, cell membranes, and high-energy compounds like adenosine triphosphate, plays a critical role in cellular functions. Notably, dinoflagellates exhibit a higher tolerance to low phosphate levels compared to diatoms (Egge, 1998; Liang et al., 2019). Our findings corroborate previous research indicating that the peak cell densities of Tripos consistently occur subsequent to a diatom bloom, as nutrients are provided through diatom decomposition (Holligan and Harbour, 1977). Another reason was that the higher N/P ratio significantly contributes to the displacement of diatoms by dinoflagellates (Wei et al., 2004; Xu et al., 2010; Kong et al., 2022). Particularly in aquaculture areas of the Bohai Sea, the elevated N/P ratio and phosphate deficiency are emerging concerns, adversely affecting phytoplankton growth and shellfish farming (Xu et al., 2010; Kong et al., 2022).

Occurrence of *Teleaulax*-dominated blooms in early summer

High-throughput sequencing revealed that the dominant species in the water samples belonged to the phylum Cryptophyta, with the highest quantity reaching 10^8 DNA copies. Pigment analysis corroborated this finding, showing that the concentration of alloxanthin, the characteristic pigment of cryptophytes, was 0.503 μg/L. This constituted a ratio of 21% to Chl-*a* in June, slightly lower than that of diatoms (35%) and dinoflagellates (34%). However, microscopic examination presented a contrasting result from the high-throughput sequencing. The discrepancy primarily arises because cryptophytes are nano-sized phytoplankton, challenging to be observed under a light microscope (Robinson et al., 1999; Novarino, 2005). Additionally, the cells of *Teleaulax* lack a cellulose-based cell wall and tare morphologically variable, complicating their qualitatively and quantitatively identification using conventional microscopy (Cerino and Zingone, 2007; Metfies et al., 2010).

The proliferation of *Teleaulax* during this period can be ascribed to several factors. Firstly, nutrient conditions were sufficient to the growth of cryptophyte in early summer (Sommer, 1989). High concentrations of TN, PO_4-P, and elevated temperature all supported growth in June (Šupraha et al., 2014). The proximity of the survey site to a scallop culture area led to an accumulation of excrement and food residue during the early summer growing season. On one hand, the direct excretion by shellfish and release of substantial amounts of NH_4-N from bottom sediments significantly increased NH_4-N concentrations. These high levels of NH_4-N, often exceeding 4 μmol/L, inhibited the uptake and utilization of NO_3-N, thus limiting the growth of micro-sized phytoplankton like dinoflagellates and promoting the proliferation of smaller primary producers (Dugdale et al., 2012). On the other hand, limited exchange and higher DOC were also conducive to the growth of bacteria, providing sufficient food for the proliferation of *Teleaulax* (Jeong et al., 2013; Yoo et al., 2017). The dominance of cryptophytes has been documented in various studies, including pigmentation measurements in the central North Sea and the Weddell-Scotia confluence area in the 1980s and 1990s, as well as in Masan Bay, Korea in 2004–2005 (Gieskes and Kraay, 1983; Buma et al., 1992; Jeong et al., 2013; Yoo et al., 2017). Cryptophytes have been identified as the primary cause of red tides in Masan Bay, with densities reaching 10^8 cells/L. Along the coast of British Columbia, marine photosynthetic cryptophytes were observed as the dominant species in summer surface water. Molecular technology has further confirmed that *Teleaulax acuta* and *Plagioselmis prolonga*, both belonging to the phylum Cryptophyta, are the causative species of algal blooms in various coastal waters, including those in Zhejiang Province, Xiamen Port, Hong Kong's Tolo Harbour, and the Mediterranean Sea (Cerino and Zingone, 2007; Environmental Protection Department, Hong Kong, 2005–2006; Šupraha et al., 2014). Consequently, it is plausible that *Teleaulax*-dominated blooms occurred in our survey area during early summer.

Diversity and Dynamics of dinoflagellate cysts, including toxic varieties

In China, a latitudinal gradient was observed, with a decrease in the abundance of dinoflagellate cysts from north to south, as noted by Chen et al. (2011) and Shao et al. (2012). Molecular and microscopic analyses revealed that the number of dinoflagellate cysts in sediment diminished with rising temperatures, reaching its lowest point in August. This aligns with previous studies suggesting that elevated temperatures promote the germination of dinoflagellate cysts (Estrada and Berdalet, 1997; Smayda, 1997; Rose and Caron, 2007). Concurrently, TN reached its highest level in August, corroborating extensive research that confirms nitrogen as a primary factor influencing cyst germination (Qi et al., 1997; Wang et al., 2011; Figueroa et al., 2015). Significantly, the trophic mode of specific dinoflagellate critically influences the dynamics and impacts of resultant harmful algal blooms, which can inflict economic, ecological, and health repercussions (Park et al., 2013). Microscopic analysis indicated that heterotrophic dinoflagellate cysts were prevalent, constituting over 40% of the species during summer. Heterotrophic dinoflagellates can obtain adequate nutrients through feeding in nutrient-deficient waters, potentially leading to the formation of red tides under favorable environmental conditions (Jeong et al., 2010). Moreover, they have the capacity to foster the successive emergence of multiple dominant species within a single red tide through their feeding activities (Jeong et al., 2005). Furthermore, the proportion of toxic dinoflagellate cysts exceeded 85%, displaying a gradual decreasing trend from June to August. An analysis of species symbiosis revealed that parasitic marine dinoflagellate Syndiniales comprised half of the total OTUs of dinoflagellate algae in the water sample. These parasites terminate their hosts and release copious independent planktonic spores (Coats and Park, 2002; Guillou et al., 2010). In the sediment, *Alexandrium* cysts, prevalent in Bohai Bay, China, predominated. Notably, species like *Gonyaulax spinifera*, *Lingulodinium polyedra*, and *Protoceratium reticulatum* were present, known for producing Yessotoxins (YTXs). These toxins, not typical shellfish toxins, inflict damage on the heart, liver, nervous system, and other organs (Paz et al., 2008; Alvarez et al., 2016). The environmental concentration and form of phosphorus notably affect the growth and toxicity of dinoflagellates capable of producing paralytic shellfish poison. Low levels of DIP create favorable conditions for the accumulation of toxins (Touzet et al., 2007). Based on 18S rRNA and light microscope analysis, Polykrikos was identified as highly abundant among dinoflagellates in the sediment samples from August. Previous research also recognized Polygonum cysts, such as *P. schwartzio* and *Polykrikos kofoidii*, as common in Bohai Bay, China (Wang et al., 2004). In our study, *P. schwartzio* cysts were detected in all sediment samples, with the highest abundance occurring in August. Additionally, the prevalence of Polykrikos cysts can serve as indicators of eutrophication or high primary productivity (D'Silva et al., 2013; Narale and Anil, 2017). The presence, absence, and abundance of heterotrophic algae cysts also serve as markers for these conditions (D'Silva et al., 2013; Narale and Anil, 2017). The predominant species in the sediment samples of the survey area, all belonging to the same type, suggest a high level of eutrophication, significant pollution, and poor water quality in the survey area.

Conclusions

During the summer survey period, we observed a notable decline in phytoplankton diversity in the Qinhuangdao Changli coastal area. The dominant phytoplankton populations underwent a significant shift from diatom predominance to a dinoflagellate-dominated ecosystem, with cryptophytes emerging as a dominant species in early summer. They thrived in an environment rich in organic matter. The sediments were characterized by a wide variety of harmful dinoflagellate cysts exhibiting high richness. Specifically, Polykrikos, an indicator of water eutrophication, was notably abundant in August sediment samples. Additionally, various dinoflagellate cysts capable of producing YTXs were present in all sediment samples. These findings suggest that the marine ecosystem has suffered serious degradation. Scallop farming may be under threat, and the region faces an increased risk of harmful algal blooms in the future. Cryptophyte blooms are likely to occur in the most polluted stations. Furthermore, the area serves as a habitat for amphioxus, and changes in the marine environment could jeopardize this species, leading to a reduction in its density and biomass. This potential impact warrants attention in future research endeavors.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Author contributions

XS: Writing – review & editing, Writing – original draft, Investigation, Formal Analysis, Data curation. SY: Data curation, Writing – review & editing. JS: Visualization, Supervision, Resources, Project administration, Methodology, Conceptualization, Writing – review & editing.

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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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Publisher's note

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