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Relationship between phytoplankton community succession and environmental parameters in Qinhuangdao coastal areas, China: A region with recurrent brown tide outbreaks

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ABSTRACT

The picoplanktonic pelagophyte *Aureococcus anophagefferens* could trigger harmful algal blooms (HABs) to discolor water in brown, known as brown tide. Since 2009, large-scale brown tides, caused by *A. anophagefferens*, had been occurred in early summer for three consecutive years in the coastal waters of Qinhuangdao, China and resulted considerable deleterious effects on the scallop mariculture industry. The causes for the occurrence of brown tides were not fully understood. Therefore, we conducted a one-year survey from June 2013 to May 2014 to study the seasonal succession of the phytoplankton community, including *A. anophagefferens* and its relationship with environmental variables in the area. The results revealed that the population dynamics of the phytoplankton community were significant variation with seasonal succession, in which *A. anophagefferens* played an important role during the entire year. The trend of the whole diversity index indicated that the community structure became more stable in winter. The results of principle component analysis (PCA) applied to the environmental factors indicated four major seasonal groups in the environmental variables. The water temperature, silicate and total nitrogen were contributed to the environment in summer, autumn and spring, respectively. In addition, a few another environmental factors commonly contributed to the winter waterbody, indicated that the aquatic environment is more complex in the cold season. The result revealed that the phytoplankton community structure and its variation were mainly affected by the hydrological factors, by using the redundancy analysis (RDA) for the relationship between dominant species and the environment. Furthermore, we inferred *Chaetoceros decipiens* as a potential species for the breakout of harmful algae blooms (HABs) by RDA ordination. We concluded that the key factor for the seasonal variations in the dynamics of phytoplankton community could be the hydrological parameters in Qinghuangdao coastal area. This research may provide more insight into the occurrence mechanism of brown tide.

1. Introduction

The Bohai Sea is a semi-enclosed sea, and it is linked together with the Yellow Sea by the Bohai Strait. Therefore, the water exchange capacity is relatively weak (Tao, 2006). Qinhuangdao, China is located in the northwest of the Bohai Sea. Along the coastal area of Qinhuangdao, there are many rivers, such as the Xinkai River, Yanghe River, Xinhe River and Daihe River. The river flows are much less than those of the Yellow River and Liao River within the Bohai Sea. However, there is a remarkable seasonality of water flow in these rivers. When there are rain storms in the summer, large amounts of nutrients are discharged with runoff into the seawater. The area also has the largest bay scallop mariculture industry in China.

In the last decade, there were frequently observed HABs events in the area. In 2009, a brown tide caused by *Aureococcus anophagefferens* was first observed in China, which was the third country following the USA and South Africa to suffer from brown tides. Since then, the brown tide has reoccurred every year (Probyn et al., 2001; Sieburth et al., 2010). A large-scale brown tide occurred in the Qinhuangdao coastal area from June to August 2012, covering 3400 km^2 and lasted for 73 days. It resulted in the massive death of cultured bay scallops (Hebei Provinvial Department of Land and ResourcesOceanic Administration, 2010; Zhang et al., 2013).

The phytoplankton community is closely related to the aquatic environment. The phytoplankton community is unavoidably influenced by environmental elements (such as temperature, pH and nutrients), that induce HABs (Buschmann et al., 2006; David et al., 2009). In contrast, as a primary producer of marine organic matter,

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phytoplankton plays a key role in energy transformation to grazers (e.g., zooplankton, and shellfish) and material circulation of the marine ecosystem (Olsen et al., 2007; Danielm et al., 2009; Pitta et al., 2009; Silva et al., 2012). In addition to the role of phytoplankton, human activity has similarly altered the marine environment and led to nutrient enrichment and eutrophication (Smith et al., 1999). Eutrophication has become the most widespread water quality problem in China, as well as in many other countries (Feng et al., 2004; Xiao et al., 2007). There are few previous studies about brown tide outbreaks along the Qinhuangdao coast; therefore, it is important to research the phytoplankton community, including *A. anophagefferens*, its environment and role in the ecosystems in the area.

In this study, samples of phytoplankton and water column were collected monthly in the Qinhuangdao coastal area from June 2013 to May 2014 and analysed to research physical-chemical variables and phytoplankton succession. A multivariate statistical analysis was performed to detect the diversity and the relationships between environmental parameters and the phytoplankton community. The objectives of this study were to: a. clarify the diversity structure of the phytoplankton community; b. characterize the succession of the phytoplankton community; c. detect the key factor for the seasonal variations in the dynamics of phytoplankton community; and d. investigate potential linkages between environmental parameters and brown tide outbreaks.

2. Materials and methods

2.1. Site description and sample collection

The Qinhuangdao coastal area is located in the coastal area of the Northwestern Bohai Sea, China. This is a well-known summer resort area in China, which is famous for its beautiful beaches and pleasant climate. The area is characterized by a typical continental monsoon climate, dry and rainless springs, tepidity without intense heat in the summers, cool autumns and dry winters. The study area (119°26′11–119°44′18″ E, 39°42′48–39°54′07″ N, Fig. 1) was chosen along the coast of Qinhuangdao, representing the 20 sampling stations that experienced brown tides from 2009 to 2011.

In this study, monthly investigations were conducted from June 2013 to May 2014 in this area. Triplicate water samples of surface water (1.0 m) were collected in a 5 L sample bottle at each sampling site. Phytoplankton samples of each station were collected and preserved with 1.5% final concentration of Lugol's solution.

2.2. Environmental parameters and phytoplankton

Water temperature (WT), salinity (Sal), dissolved oxygen (DO), and pH value were recorded with a YSI meter (YSI-6600; YSI Incorporated, Ohio, USA) at the sampling sites. The concentrations of ammonia nitrogen (NH3-N), nitrate (NO3-N), nitrite (NO2-N), phosphate (PO4-P), metasilicate (SiO_3-Si), total nitrogen (TN) and total phosphate (TP) were measured using spectrophotometry (Bendschneider, 1952; Mullin and Riley, 1955; Murphy and Riley, 1962; Sagi, 1966; Ebina et al., 1983).

A sedimentation method was used for algae count and species identification. A 0.1-mL Lugol subsample was taken for taxonomy and enumeration (cells L^{-1}) with an OLYMPUS CX31 microscope (to ensure statistical significance, at least 200 cells per sample were counted). Phytoplankton species were identified based on morphology. In addition, we enumerated the brown tide alga, *A. anophagefferens*, by a flow cytometry technique (Flow cytometer: BD FacsCalibur).

2.3. Statistical analysis

The phytoplankton species diversity indices of Shannon-Wiener (Shannon, 1948), Simpson (Simpson, 1949), Pielou's evenness (Pielou and Levandowsky, 1975), Margalef (Margalef, 1968) and dominance index (*Y*) were calculated with Primer 5 software. The annual variations of the diversity indices were obtained by Gaussian fitting (Gauch and Chase, 1974). The environmental data analyses were performed using SPSS 19.0. A Two-way ANOVA (LSD test) was conducted to evaluate the differences of the environmental parameters of the seawater among the twenty sites. The statistical significance was set at $p < 0.05$.

A principle component analysis (PCA) was used to explore the influences of different variables on environmental parameters. The relationship between the environmental parameters and phytoplankton community were determined by a redundancy analysis (RDA). Dominant species and environmental parameters entered into the PCA and RDA were normalized through a logarithmic transformation (log10 (n + 1)). A large number of species only occurred occasionally and did not offer much useful ecological information; therefore, only those with an occurrence frequency more than 10% were selected for the ordination analysis. All canonical axes were applied to evaluate the significant variables under analysis with Monte Carlo permutation tests (499 permutations). PCA and RDA were performed using the CANOCO 4.5 software (Braak and Smilauer, 2002).

3. Results

3.1. Environmental parameters

Seasonal variations of physical–chemical parameters at the twenty sampling sites are shown in Fig. 2. WT in Qinhuangdao coastal areas varied significantly with time (ranged from −1.05 °C in January 2014–25.33 °C in August 2013) (Fig. 2A). The DO concentration ranged from 2.2 mg/L (August 2013) to 11.42 mg/L (January 2014) (Fig. 2B). The salinity of samples varied from 28.06 Psu to 32.3 Psu, and the highest value was obtained in July 2014(Fig. 2C). The water samples had a wide range of pH values varying from 6.00 to 9.80 (Fig. 2D). No significant differences were found between the twenty stations for WT, DO, and pH ($P > 0.05$, two-way ANOVA testing, Table S1), but a significant difference was found for salinity ($P < 0.05$, two-way ANOVA testing, Table S1).

With regards to the trophic conditions of the Qinhuangdao coastal area, total nitrogen (TN) and total phosphorus (TP) covered a wide range of concentrations, from 0.87 mg/L to 4.75 mg/L and 0.01 mg/L to 0.4 mg/L, respectively (Fig. 2E-F). Other nutrients (NH3-N, NO3-N, NO2-N, PO4-P, SiO_3-Si) (Fig. 2G-K) also covered a wide range of concentrations, with 0.01 mg/L to 0.34 mg/L, 0.01 mg/L to 1.13 mg/L, 0.001 mg/L to 0.059 mg/L, 0.0005 mg/L to 0.029 mg/L and 0.03 mg/L to 2.11 mg/L, respectively. The twenty sampling sites have no significant differences for these nutrient parameters ($P > 0.05$, two-way ANOVA testing, Table S1), except for SiO3-Si ($P < 0.05$, two-way ANOVA testing, Table S1).

3.2. Distribution of environmental parameters

We analysed the variation in the aquatic environment factors to accurately detect the seasonal change of the environment during the entire year in order to research the spatial and temporal distributions of environmental parameters. It is an effective method to determine the relationship between the phytoplankton and environmental factors better while also exploring the impact on the annual succession of phytoplankton by the environment.

PCA is an effective method to determine the main environmental parameters on a spatial scale via dimensionality reduction (Facchinelli et al., 2001). Many surveys have used this approach to obtain an ordination analysis in aquatic ecology research (Gu et al., 2012). Most of the values determined at different regions had similar importance in PCA and were strongly intercorrelated. According to the results of PCA (Table S2), the first axis of PCA explained 88.6% of the variance of