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Characteristics of eukaryotic microalgal community and its abiotic influencing factors during brown tide blooms near Qinhuangdao, China

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ABSTRACT

The brown tides caused by the picoplanktonic pelagophyte *Aureococcus anophagefferens* in the coastal waters of Qinhuangdao, China have occurred periodically since 2009 and exerted deleterious effects on scallop cultivation in the area. In this study, clone libraries were constructed to determine the characteristics of the local eukaryotic microalgae community, and a real-time fluorescent quantitative PCR assay was performed to analyze the temporal and spatial variations in the 18S rDNA copies of *A. anophagefferens* samples collected in 2012. The results showed that *A. anophagefferens* was the dominant species in the local eukaryotic microalgae community during the brown tide in June 2012 and accounted for a large fraction of the community. A redundancy analysis (RDA) showed that the decreasing concentration of dissolved inorganic nitrogen (DIN), increasing amount of human aquaculture activities and suitability of spring/summer temperatures for the growth of *A. anophagefferens* may be the primary causes of the brown tide outbreaks in the Qinghuangdao scallop culture area.

1. Introduction

The coastal water near Qinhuangdao in the Bohai Sea is one of the most productive ecosystems in China in terms of primary productivity and scallop harvests. In recent years, outbreaks of brown tides have had a deleterious effect on the local shellfish mariculture industry. Brown tides generally persist from late May to late June or even August and have caused the stunted growth and even death of scallops and led to a dramatic collapse of the local scallop population and large economic losses (Zhang et al., 2012). The brown tide bloom in 2009 affected approximately two-thirds of the mariculture zone (~260 km^2) in the area. In 2010, the affected sea area reached 3350 km^2 (Bulletin of Marine Disaster of China, 2010), and in 2012, a bloom covering 3400 km^2 lasted for 73 days from June 8 to August 20 (Bulletin of Marine Disaster of China, 2012). Therefore, determining the species that cause blooms and the factors that contribute to brown tide outbreaks is vital for local scallop cultivation.

The brown tide species that causes blooms in the coastal waters of Qinhuangdao was recently identified as the picoplanktonic pelagophyte *Aureococcus anophagefferens* Hargraves et Sieburth (Kong et al., 2012; Zhang et al., 2012). The outbreak in the Qinhuangdao coastal waters is not the first reported case of a brown tide caused by *A. anophagefferens*. The first observed *A. anophagefferens* brown tide occurred in the inland bays and estuaries of Long Island, NY, and Narragansett Bay, RI, in 1985 (Cosper et al., 1987; Sieburth et al., 1988), and since then, numerous and widespread ecological and economic impacts in ecosystems afflicted by these blooms, such as Chesapeake Bay (Glibert et al., 2001) and Chincoteague Bay in the USA (Trice et al., 2004) and Saldanha Bay in South Africa (Probyn et al., 2001, 2010), have been recorded.

Considerable research efforts have been undertaken to identify suitable detection approaches (Anderson et al., 1993;

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Caron et al., 2003; Popels et al., 2003; Stauffer et al., 2008) and characterize the physiology (Popels et al., 2007; Alami et al., 2012), biochemistry (Frischkorn et al., 2014), nutrient utilization (Berg et al., 2008) and competitive ecology of *Aureococcus anophagefferens* (Gobler et al., 2011) with the aim of better understanding the initiation and termination of blooms and developing more effective monitoring measures and remediation and prevention proposals. The brown tides caused by *A. anophagefferens* in recent years off the coast of Qinhuangdao are the first recorded in Chinese coastal waters, and Chinese scientists have paid increasing attention to these tides. For example, Dong et al. (2014) performed a system-level analysis using RNA-seq technology to determine the expression of genes regulated by urea or nitrate in a Chinese strain of *A. anophagefferens* grown on urea, nitrate, or a mixture of urea and nitrate under N-repletion, N-limitation and N-recovery conditions, and the results suggest that *A. anophagefferens* preferentially utilizes urea rather than nitrate. Gong et al. (2015) conducted a toxicity experiment with *A. anophagefferens* and showed that the brown tide affected the survival of *Artemia salina* and inhibited the ingestion of *A. salina* and *Brachionus plicatilis* by downstream consumers; however, the study of brown tides is in the preliminary stages, and many issues must be investigated. In particular, to identify the mechanisms underlying outbreaks of *A. anophagefferens*, it is necessary to determine the distribution and variation of nutrients and phytoplankton communities in the coastal waters of Qinhuangdao as well as the interactions between these factors and the local environment.

The microalga *Aureococcus anophagefferens* is a coccoid, non-motile, golden-colored alga (DeYoe and Stockwell, 1997), which is difficult to monitor due to its miniscule size (approximately 2–3 μm in diameter) and lack of distinctive taxonomic characteristics (DeYoe and Stockwell, 1997). Therefore, direct microscopic cell counts from environmental samples are virtually impossible because their cells are not easily identified as *A. anophagefferens*. The rapid development of modern molecular biological technologies offers new approaches for the identification of harmful microalgae and may aid diversity and quantitative analyses of nano- or pico-phytoplankton (Moon-van der Staay et al., 2001; Lee et al., 2010). Clone libraries and high-throughput sequencing based on rDNA sequences obtained from natural assemblages have provided new insights into the diversity of marine microbial phytoplankton (Amann et al., 1990; Pace, 1997; Palenik et al., 2009; Cheung et al., 2010), and quantitative PCR (qPCR) has played an increasing role in the identification and enumeration of microalgae. Popels et al. (2003) used qPCR to detect and enumerate *A. anophagefferens* in environmental samples collected along the East Coast of the United States and validated the feasibility of using this method for the detection of *A. anophagefferens*. Guo et al. (2015) designed 18S rDNA-based TaqMan® probes for qPCR assays to enumerate *A. anophagefferens* and determine the distribution and diffusion of brown tides in the coastal waters of Qinhuangdao. In this study, samples were collected from the Qinhuangdao coast in 2012 and analyzed to explore the relationships between physical/chemical complexity and brown tides. In addition, 18S rDNA clone libraries were used to assess the diversity of the phytoplankton community assembly in terms of correlations with the large environmental gradients in the ecosystem (e.g., salinity, temperature, inorganic nutrients, etc.), and qPCR analyses were performed to detect the temporal and spatial variations in *A. anophagefferens* abundance in this area. The primary aims were to (i) clarify the diversity structure of the phytoplankton community during brown tides, (ii) investigate the distribution of *A. anophagefferens* in this area, and (iii) explore potential linkages between environmental parameters and brown tide outbreaks.

2. Materials and methods

2.1. Sample collection

Environmental samples were collected from six sampling sites in the scallop culture area on the Qinhuangdao coast in April (spring), May/June (summer) and December (winter) 2012 (Fig. 1). At each site, the surface seawater was sampled, and 1 L of seawater was filtered through a 200-μm mesh net to remove large suspended solids and zooplankton as well as other large phytoplankton cells. The filtrate was filtered again using a 0.45-μm Millipore membrane (Millipore, USA) to collect the microalgal cells, and the membranes were then maintained at −80 °C until DNA extraction.

2.2. Analysis of environmental parameters

At each site, in situ measurements of the physico-chemical parameters of the surface seawater (temperature, salinity and dissolved oxygen) were recorded with a Model HQ30d multi-parameter meter (HACH, China), and other parameters were measured in the laboratory. A QuAAtro nutrient auto analyzer (Seal Analytical Ltd., UK) was used to measure the concentrations of dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP) and dissolved silicate (DSi) in the surface seawater from all of the sites.

2.3. Genomic DNA extraction

The total genomic DNA of the samples was extracted with the modified method described by Gou et al. (2003). Briefly, the membranes were cut using sterile scissors and resuspended in 250 μL of TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0). Five hundred microliters of extraction buffer (3% CTAB, 1% sarkocyl, and 20 mM EDTA, pH 8.0) was added, and the cells were incubated at 55 °C for 1 h. The mixture was gently inverted every 10 min, and the suspension was allowed to cool down at 4 °C for 3–5 min. A total of 1 mL of chloroform/isoamyl alcohol (24:1, v/v) was added, and the mixture was mixed by gentle inversion (approximately 25–30 times) until an emulsion formed. After centrifugation (10,000 × *g* and 4 °C for 10 min), the supernatant was transferred

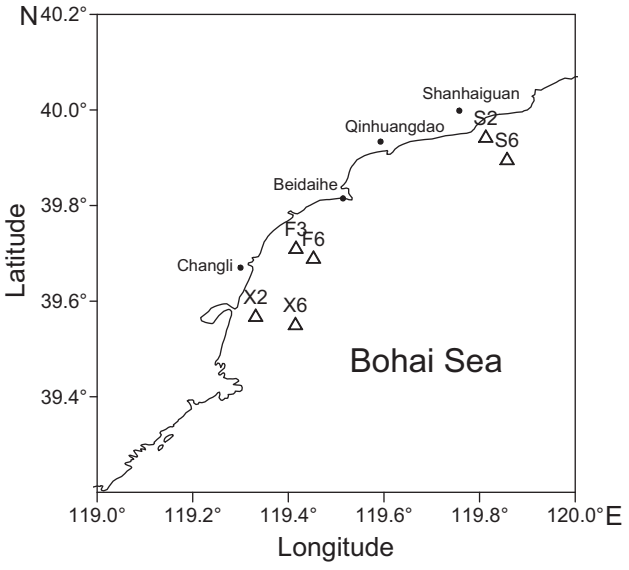

Fig. 1. Sampling sites in the coastal waters of Qinhuangdao. Sampling was performed in April, May/June and December 2012.