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Effects of the decomposing green macroalga *Ulva (Enteromorpha) prolifera* on the growth of four red-tide species

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ABSTRACT

The green macroalga *Ulva (Enteromorpha) prolifera* formed large-scale blooms (the so-called "green tide") from 2007 to 2010 in the Yellow Sea, China. In June 2008, huge amounts of floating *U. prolifera* accumulated along the coast of Qingdao. At the late stage of the green tide, a large amount of green algae sank to the bottom and decomposed, which led to concerns about derivative ecological and environmental problems, such as red tides. The effects of nutrient recycling from decomposing green tides on the growth of four selected red-tide causative species, i.e. *Heterosigma akashiwo*, *Alexandrium tamarense*, *Prorocentrum donghaiense* and *Skeletonema costatum*, were studied in the laboratory. It was found that the decomposing green algae released considerable amounts of ammonium and phosphate into the surrounding seawater. The addition of effluent from the decomposing green algae promoted the rapid proliferation of the raphidophyte *H. akashiwo*, but inhibited the growth of the diatom *S. costatum*. The growth of two dinoflagellate species *A. tamarense* and *P. donghaiense* was triggered by the addition of low-concentration decomposing algal effluent, but inhibited by the high-concentration decomposing algal effluent, probably due to toxicity from high concentrations of ammonium or water-soluble allelochemicals present in the decomposing effluent. We conclude that the decomposition of green algae releases inorganic nutrients, particularly ammonium, into seawater where the nutrients could support red tides once they were taken up by the opportunistic microalgae.

1. Introduction

Large-scale blooms of green macroalgae, such as *Ulva*, *Chaetomorpha*, *Cladophora*, *Codium* and *Caulerpa*, etc. (the so-called "green tides"), occurred worldwide with increasing frequency during the last few decades (Fletcher, 1996; Blomster et al., 2002; Hiraoka et al., 2004; Lapointe et al., 2005a,b). In summer 2007, a bloom of green algae first occurred along the coast of Qingdao, China. In 2008, just weeks before the start of the Olympic sailing games in Qingdao, green algae appeared on an unprecedented scale in the near shore area of Qingdao, and caused serious concerns for the local administration. The over-growing green algae species was identified as Chlorophycophyta, Chlorophyceae, Ulvales, Ulvacea, *Ulva* (*Enteromorpha*) *prolifera* (Mueller) J. Agardh (Hayden et al., 2003; Ding and Luan, 2009; Ding et al., 2009).

Intensive studies have been performed on green tides in the Yellow Sea and several hypotheses were advanced to explain the occurrence of green tides in this area. It was found that the green algae were floating from the southern area of the Yellow Sea to Qingdao, driven by the prevailing monsoon in summer (Sun et al., 2008). Molecular biological data supported the unialgal composition of the floating algae in the Yellow Sea during the bloom (Jiang et al., 2008; Ye et al., 2008), which was different from the local attached Ulvaceae species in Qingdao (Jiang et al., 2008). The original source of the green algae was suggested to be the rafts intensively used by the *Porphyra yezoensis* culture industry in the intertidal zone along Jiangsu province (Liu et al., 2009), but a detailed study found that land-based animal aquaculture ponds were more likely to be the origin of the green algae in 2008 (Pang et al., 2009). The same bloom occurred again in 2009 (Xinhua news) and 2010, and the largest affected sea area reached 51,000 km^2 in the Yellow Sea, suggesting that green tides of *U. prolifera* have become a recurrent phenomenon in this area.

Green tides normally lack significant acute toxicity, but they have a broad range of ecological impacts and are considered to be harmful algal blooms (ECOHAB, 1995). Many studies have addressed the potential ecological and environmental consequences of green tides (Fletcher, 1996; Valiela et al., 1997), which include uncoupled biogeochemical cycles, decreased water transparency, degraded intertidal environments, reduced biodiversity,

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hypoxia or anoxia, destruction of coastal marine habitats (e.g., sea grass) and economic losses to marine industries (e.g., fisheries and tourism) etc. (Sand-Jensen and Borum, 1991; Tarmanowska, 1995; Pieczyńska and Tarmanowska, 1996; Hernandez et al., 1997; McGlathery, 2001; Nelson and Lee, 2001; Franz and Friedman, 2002). The large-scale green tides in the Yellow Sea led to tremendous economic loss in Shandong and Jiangsu Provinces (~0.2 billion US $ in 2008) (State Ocean Administration, 2008), but the potential ecological and environmental consequences of the green tides were not well understood. At the late stage of the green tide in 2008, large amounts of *U. prolifera* sank to the bottom and decomposed, which led to concerns of derivative ecological and environmental problems, such as red tides or hypoxia. In the southwestern coast of Florida, the findings of Lapointe and Bedford (2007) and Yentsch et al. (2008) suggested that macroalgal blooms of red algae in this region appeared to have nutrient recycling linkages with red tides in Florida. Shortly after the collapse of the green tide in August, 2008, small red tides of the raphidophyte *Chattonela* sp. and the dinoflagellate *Noctiluca scintillans* were observed along the coast of Qingdao (Zhang et al., 2009). However, there is still little data available on the relationship between the decomposing green algae and the occurrence of red tides.

In the current study, a simulation experiment was carried out in the laboratory to study the effects of decomposing green algae on four selected red-tide causative species, i.e. *Heterosigma akashiwo*, *Alexandrium tamarense*, *Prorocentrum donghaiense* and *Skeletonema costatum*. The raphidophyte *H. akashiwo*, distributed widely in coastal seas of temperate and semitropical embayments, is one of the dominant red-tide causative species along the coast of China (Honjo, 1993; Tseng et al., 1993). The dinoflagellate *A. tamarense* species complex (including three morphospecies *A. tamarense*, *A. catenella* and *A. fundyense*) forms recurrent blooms in many coastal waters around the world, including sea areas adjacent to the Changjiang River estuary in the East China Sea (Anderson, 1997; Zhou et al., 2001; Zhang and Lian, 1999; Lin, 1996; Lilly et al., 2007). It is known for producing potent neurotoxins, which cause paralytic shellfish poisoning (PSP) in many coastal regions (Hallegraeff, 1995; Anderson et al., 1994). *P. donghaiense*, which forms large-scale red tides in the Changjiang River estuary and Zhejiang coastal waters almost every year, has become the major causative species of the large-scale red tides in the East China Sea since the late 1990s. *S. costatum* is a cosmopolitan diatom species (Tomas, 1997) and often dominates the spring bloom in coastal waters (Han et al., 1992; Thornton and Thake, 1998). In the Jiaozhou Bay of Qingdao, *S. costatum* is the dominant species of the phytoplankton community in spring and summer (Li et al., 2005; Liu et al., 2003). The potential effects of the decomposing *U. prolifera* on the growth of four selected red-tide species were studied to probe the relationship between green tides and red tides.

2. Materials and methods

2.1. Sample collection and preparation

The macroalga *U. prolifera* used in the present study was collected from the Center of the Olympic Sailing Game in Fushan Bay, Qingdao (36°03′N, 120°23′E) in July, 2008. The algal mud (mainly decomposed algae and sand) formed after the decomposition of accumulated green algae was collected from the sixth swimming beach (36°03′N, 120°18′E). The algae collected were flushed several times with sterilized seawater to remove epiphytes and slime, and frozen in darkness at −20 °C together with the algal mud until use.

Prior to the experiment, the frozen algae were thawed and flushed with fresh water to remove salt. Algae were then squeezed gently with filter paper to remove free water. The decomposition process of the green algae was simulated in the laboratory by adding 70 g algae (wet weight) into a 3 L flask with 1 L membrane-filtered (0.45 μm) seawater, and keeping the samples at 20 ± 0.5 °C for 15 days in darkness. The supernatant, filtered with 1 mm mesh and a 0.45 μm membrane filter successively, was used in the experiment as the effluent of decomposing green algae. The decomposing algal effluent was diluted with membrane-filtered (0.45 μm) seawater to two different concentrations (the high-concentration solution, 7×, nominal concentration of 10 g algae/L; the low-concentration solution, 70×, nominal concentration of 1 g algae/L) for the following growth experiment. To examine the effects of the algal mud, 70 g (wet weight) was also added into a 3 L flask with 1 L membrane-filtered (0.45 μm) seawater, and the mixture was shaken thoroughly on a rotary shaker at 150 rpm for 24 h at room temperature (20 °C). The supernatant was filtered with 0.45 μm membrane filter and used in the experiment as algal mud extract. The algal mud extract was also diluted with membrane-filtered (0.45 μm) seawater to two different concentrations (the high-concentration solution, 7×, nominal concentration of 10 g mud/L; the low-concentration solution, 70×, nominal concentration of 1 g mud/L) for the following growth experiment. Both the decomposing algal effluent and the algal mud extract were prepared just prior to the growth experiment and protected against light during the preparation process. Nutrient concentrations in the decomposing algal effluent and algal mud extract were determined as described in Section 2.4.

2.2. Algal cultures

Unialgal cultures of four red-tide causative species, *H. akashiwo*, *A. tamarense*, *P. donghaiense* and *S. costatum*, were selected to study the effects of decomposing green algae on their growth. *H. akashiwo* (Strain HA-1) was isolated from the Jiaozhou Bay in Qingdao. *A. tamarense* (Strain ATHK) was isolated from the South China Sea and provided by Jinan University. *P. donghaiense* (Strain PDDH) was isolated from the East China Sea and provided by the National Basic Research Priority Program (973). *S. costatum* (Strain SC-1) was obtained from the Algal Culture Center of the Institute of Oceanology, Chinese Academy of Sciences.

All the microalgal cultures were grown in f/2-Si medium in 1 L flasks, except for *S. costatum*, which was cultured in f/2 medium supplemented with silicate. Natural seawater used for algal cultures was pumped from Taipingjiao (no known pollution history), Qingdao, and sand-filtered prior to use in the laboratory. The pH and salinity were 8.0 ± 0.1 and 30 ± 1, respectively. Seawater was filtered with 0.45 μm membrane and autoclaved before nutrient enrichment.

Algae were cultured at 20 ± 0.5 °C, with an irradiance of 56 $\mu E\ m^{-2}\ S^{-1}$ and a photoperiod of 12:12 (L:D). The flasks of *S. costatum* were shaken once a day. Microalgae were collected at the exponential phase and used for inoculation in the following experiments.

2.3. Experiment design

For each species of microalgae, 18 flasks were used to establish six treatments in triplicate. Two treatments were used to test the effects of decomposing algal effluent (at two different concentrations) on the growth of microalgae. Another two treatments were used to test the effects of algal mud extract at two different concentrations. A nutrient-simulation treatment was used to test the effects of major nutrients in the decomposing algal effluent, and the final treatment was used as the control. For each treatment, microalgae were inoculated into 500 mL flasks containing 300 mL sterilized seawater. No nutrients were added except in