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Tempo-spatial distribution and species diversity of green algae micro-propagules in the Yellow Sea during the large-scale green tide development

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ABSTRACT

From 2008 to 2013, vast green tides mainly composed of *Ulva prolifera* consecutively invaded the coast of Qingdao (36°06′N, 120°25′E, PR China) in June and July. Previous studies have shown that the early green tides initially formed in the *Porphyra yezoensis* aquaculture area of the Subei Shoal, southern Yellow Sea. To date, multiple studies have demonstrated that green algae micro-propagules play an important role in the formation of green tides. In this study, we aimed to assess the temporal and spatial distribution of green algae micro-propagules in an extensive area of the Yellow Sea and to determine the species diversity of propagules during the development of the large-scale green tide. We found that the quantity of micro-propagules increased with the free-floating biomass from the initial generation to the development phase of the green tide in mid May. From late May to mid June, the micro-propagule density decreased sharply despite a continuous increase of the floating macroalgae biomass. In addition, our data indicate that the coastal area of the Subei Shoal has always been the distribution center of the micro-propagules, even prior to the large-scale green tide formation. Furthermore, diverse green algae species, including *Ulva prolifera*, *Ulva linza*, *Ulva flexuosa*, *Ulva compressa*, *Ulva pertusa* and *Blidingia* sp., were identified among the micro-propagules in the survey sea area. Finally, we determined that the distribution of *U. prolifera* micro-propagules is closely related to the floating algal mats and attached macroalgae on *Porphyra* aquaculture rafts.

1. Introduction

'Green tides' are mainly caused by the bloom and accumulation of *Ulva* species (Ohno, 1999). The first green-tide event was recorded in 1905 at Belfast Lough, Ireland (Lettes and Richards, 1911). Public attention was aroused when these phenomena began to occur more frequently, negatively affecting ecosystems and deteriorating the coastal economy in the 1970s (Fletcher, 1974; Buttermore, 1977). Blooms of *Ulva* species have been reported in the southwestern Yellow Sea in the past a few years (Liang et al., 2008; Liu et al., 2009, 2012; Ye et al., 2011); the first small bloom was observed in 2007. In late June of 2008, before the opening of the Olympic Sailing Regatta, a massive free-floating *Ulva* bloom (400 km^2) occurred in the coastal area of Qingdao (36°06′N, 120°25′E, PR China), constituting the world's largest recorded green tide. From 2008 to 2013, large-scale green tides, mainly consisting of *Ulva prolifera* (Liu et al., 2010a,b,c,d), have consecutively invaded the coastal cities of the Shandong peninsula, resulting in drastic impacts on local economies and environmental conditions.

Usually, green-tide events are associated with poor seawater quality, *e.g.*, eutrophication caused by domestic and industrial sewage (Fletcher, 1996; Valiela et al., 1997; Raffaelli et al., 1998; Morand and Merceron, 2005). However, unlike previous macroalgal blooms, the large-scale Yellow Sea green tides had two distinct characteristics: they were formed in remote sea areas (Keesing et al., 2011) and were transported a great distance (Liu et al., 2009, 2010a). Previous studies showed that earlier green tides initially formed in the coastal area of the Subei Shoal, especially at 32°N and 33°N (Liu et al., 2009). In that area, affected by the riverine input and seawater animal aquaculture activities

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(Pang et al., 2010; Liu et al., 2013a,b), nutrient levels have increased over the past 10 years. Moreover, frequent nutrient enrichment may have provided the material base for the development of early green tides. In the green-tide movement from south to north, the dominant species, *Ulva prolifera*, characterized by high nutrient uptake rates as well as extensive temperature and salinity adaptability, rapidly increased the biomass in seawaters (Li et al., 2009; Tian et al., 2010). Indeed, early green-tide patches have generally emerged in late April and developed into vast green macroalgal blooms in just 6 weeks, affecting the coastal cities of the Shandong Province by middle or late June.

Micro-propagules including gametes, spores, zygotes, micro-germlings and vegetative fragments (Hoffmann and Santelices, 1991; Liu et al., 2012) constitute important factors in the opportunistic propagation strategy of *Ulva* spp. (Worm and Lotze, 2006; Fang et al., 2012). Indeed, micro-propagules help the species survive in harsh environmental conditions (Santelices et al., 1995), and under suitable conditions they develop into visible thalli (Lotze and Worm, 2001). Schories (1995) showed that *Ulva* micro-propagules could persist in the water column for at least 8 days and that several propagules could survive in unfavorable conditions for 10 months. Song et al. (unpublished data) found that green algae micro-propagules are present year-round in the coastal area of the Subei Shoal and serve as the "seed stock" in the Yellow Sea large-scale green tides. Liu et al. (2010d) showed that micro-propagules play an important role during the outbreak of green tides by enlarging the floating biomass of *Ulva prolifera*. Gao et al. (2010) discovered that micro-propagules can attach to floating thalli; thus, reproduction *via* propagules can take place completely within the bloom. Liu et al. (2012) argued that the green algae micro-propagules in the sediments along the coastal areas of Jiangsu Province are the source of the large-scale green tides. However, in the sediment samples collected during the major cruises of this study, few micro-propagules were detected. Thus, the micro-propagules in the water column were the focus of this study.

Although large-scale green tides have impacted a vast area in the Yellow Sea, previous studies have only attempted to determine the changes in quantity and distribution of green algae micro-propagules in a limited sea area along the Jiangsu coast (Liu et al., 2010c, 2012, 2013a; Song et al., unpublished data); this research did not sufficiently illustrate the relationship between floating green algae and micro-propagules. Thus, a more extensive survey sea area in the Yellow Sea is imperative. From late April to early June 2012, four small and four major expeditions were conducted in the coastal area of the Subei Shoal and the central part of the Yellow Sea (120–123°E, 32–36°N). This work aimed (1) to determine the temporal and spatial distribution of green algae micro-propagules in the large area of the Yellow Sea during the development of the large-scale green tide, (2) to analyze species diversity of green algae micro-propagules before the green tide invasion of the coastal area of the Shandong peninsula, and (3) to ascertain the relationship between micro-propagules and large-scale green tides.

2. Materials and methods

2.1. Selection of sampling locations

The main study areas included two regions of the Yellow Sea: the inshore waters near the coastal area of the Subei Shoal and the offshore waters within the Yellow Sea (Fig. 1). The Subei Shoal lies on the northern coast of the Jiangsu Province from the Yangtze River delta up to the Sheyang River. It is a colossal radial sand body that stretches for 200 latitudinally and 90 km longitudinally. This area is characterized by unconsolidated sediments, intense tidal activity, complex hydrodynamic forces, and continual water turbidity (Wang et al., 2011). In most parts of the Subei Shoal, water depth is less than 15 m, with a depth of approximately 20–30 m at the periphery; the shoal is submerged in high tide and exposed during ebb tide (Zhou and Sun, 1981). On these radial sand ridges, greater than 65% of the Chinese commercial seaweed *Porphyra yezoensis* is cultured. After significant expansion of *Porphyra* aquaculture from 2006 to 2008, the total aquaculture area has reached approximately 20,000 ha.

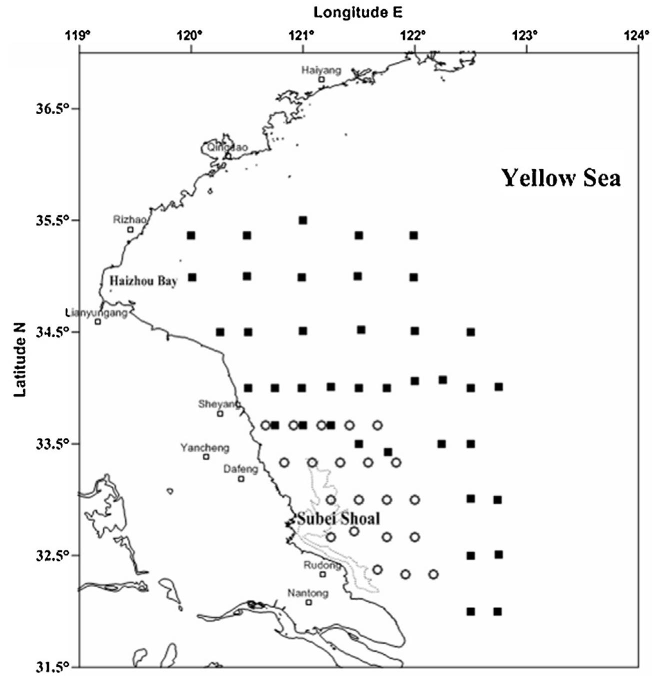

Fig. 1. Survey area and sampling stations in the Yellow Sea, 2012 (○, sample stations in small cruises; ■, sample stations in major cruises).

In this study, four small survey cruises (SCs) were conducted (SCI, April 25 to May 06; SCII, May 11 to May 21; SCIII, May 27 to May 29; and SCIV, June 03 to June 09) to identify the distribution of green algae micro-propagules as well as species diversity. In addition, four major cruises (MC) were conducted (MCI, April 22 to May 06; MCII, May 14 to May 21; MCIII, May 25 to May 29; and MCIV, May 31 to June 05) to investigate the influence of green algae micro-propagules on the large-scale green tide in the central part of the Yellow Sea as well as their temporal and spatial distribution. All eight cruises were conducted during the initial formation and development of the green tide, and the surveyed sea area included nearly all affected regions (Fig. 1).

2.2. Sample collection and quantification of green algae micro-propagules in sea water

During the eight cruises, temperature and salinity were determined *in situ* using a KNDXR-620CTD + Tu (RBR, Inc., Ottawa, Canada). Surface seawater samples (2 L) were collected using a HQM-1 water sampler at each station and were transported to the laboratory after filtration through a 200-μm mesh net. Seawater sample aliquots (1 L) were poured into glass beakers followed by the addition of 20 mL polyethersulfone (PES) culture medium to maintain its nutrient-enriched status (Provasoli, 1963; modified by Bold and Wynne, 1978). Then, 1 mL of saturated GeO_2 was added to the mixture for benthic diatom growth inhibition. The beakers were stored in an artificial climate chamber (202728-380, Jiangnan, Inc., Ningbo, China). The culture conditions were 20 °C, 100 μmol photons m^{-2} s^{-1}, and 12 h:12 h light–dark cycle. The green algae microscopic stages in seawater were assessed using