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Effect of the large-scale green tide on the species succession of green macroalgal micro-propagules in the coastal waters of Qingdao, China

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ABSTRACT

In order to evaluate the effect of the large-scale green tide on micro-propagule community in the coastal waters of Qingdao, a year-round survey was conducted to investigate the abundance and species succession of the green macroalgal micro-propagules. Five *Ulva* and one *Blidingia* species were detected and an evident shift on species composition was observed in summer when the large-scale floating biomass of *Ulva prolifera* approached the coasts of Qingdao. Propagules of *U. prolifera* were only dominant in summer. Detection of the 'floating' type of *U. prolifera* in summer, a unique strain responsible for the green tide in Yellow Sea, supported that large-scale floating *U. prolifera* biomass could affect local micro-propagule community. There were, however, no 'floating' *U. prolifera* propagules were detected in the following winter, indicating that influence from the large-scale green tide was transient, and it has not leave prolonged seeds in Qingdao coastal waters.

1. Introduction

Since 2007, large-scale green tide, dominated by a single green seaweed species *Ulva prolifera*, recurred annually in the western Yellow Sea of China (Leliaert et al., 2009; Liu et al., 2009; D. Liu et al., 2010; Wang et al., 2010). The maximum distribution of the floating mats ranged from 2.0×10^4 to 5.8×10^4 km^2, covering 267–2100 km^2 during 2008–2016, which was recognized to be the world's largest green tide (BCMES, 2008–2016; Liu et al., 2009; D. Liu et al., 2013). Based on the remote sensing data, the drifting green algae could be traced back to the southwestern coastal water of Yellow Sea, near Subei Shoal (Ciappa et al., 2010; Hu, 2009; Hu et al., 2010; Keesing et al., 2011; Lee et al., 2011). Subei Shoal is a large intertidal muddy flat of about 1.8×10^4 km^2 with over 70 sand ridges (10–60 km long) extending from coastline to the offshore water (Wang et al., 2011). The seaweed *Pyropia yezoensis* (formerly known as *Porphyra yezoensis*; Sutherland et al., 2011) has been intensively cultured on these sand ridges since 1980s, which is one of the most important industries in that region. Years of comprehensive field surveys directly pointed to the green macroalgal wastes from the *Pyropia* aquaculture rafts as the primary seed source for the initial floating biomass of the large-scale green tide (Liu et al., 2009; D. Liu et al., 2010; D. Liu et al., 2013; Zhang et al., 2014; Wang et al., 2015; Zhou et al., 2015).

In contrast to the 'visible' process that floating biomass expanded and developed into the large-scale green tide depicted by multiple research (Zhou et al., 2015), there are still quite a lot of unknowns about the micro-propagules (an 'invisible' part of the green tides). Micro-propagules are gametes, spores, zygotes, micro-germlings and vegetative fragments of macroalgae (Hoffmann and Santelices, 1991; Liu et al., 2012). They could survive in harsh environment and develop into visible thalli forming a large amount biomass under the optimal environmental conditions (Fletcher and Callow, 1992; Santelices et al., 1995; Lotze et al., 1999, 2001; Worm et al., 2001). Some research showed that green macroalgal micro-propagules are distributed widely in Yellow Sea with a high density in and around Subei Shoal (Huo et al., 2014, 2016; Liu et al., 2012). The large tempo-spatial fluctuations of the micro-propagule density suggested a close correlation between the floating green tide biomass and the abundance of environmental micro-propagules (Zhang et al., 2011; Y. Li et al., 2014; Song et al., 2014; Zhang et al., 2014). It was further implied that environmental micro-propagules in Subei Shoal were the important seed source for the attached green algae on the *Pyropia* aquaculture rafts which grew rapidly in the following spring (Song et al., 2015; Huo et al., 2016). Some other research, based on the laboratory experiment, proposed a 'floating

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germination' hypothesis suggesting the environmental micro-propagule might directly develop into and contribute substantially to the floating biomass (Liu et al., 2012), while it was not supported by the field observations (Wang et al., 2015; Huo et al., 2016). At present, detection and quantification of the environmental micro-propagules were mostly based on a laboratory culture method, by which the 'invisible' micropropagles in the environmental water or sediment samples were cultured under the laboratory controlled conditions and developed into 'visible' germlings, and subsequent quantification and species identification were performed on these germlings (Liu et al., 2010b, 2012; Huo et al., 2014; Song et al., 2014, 2015). Little, however, was known about the existing form of the microscopic propagules in nature, their strategy to survive the advent environmental conditions and maintain the population. Even less was known about whether the re-emergent large-scale green tides could leave prolonged propagules in the non-indigenous regions causing population expansion.

Significant economic losses were resulted from the large-scale green tides in Yellow Sea due to its destructive impacts on the coastal aquaculture, substantial costs on clearing macroalgal biomass on the beaches and coastal waters and indirect detrimental effects on tourisms etc. (Sun et al., 2008; Ye et al., 2011; Wang et al., 2015). More seriously, great concerns have been proposed about the long-term environmental and ecological impacts caused by the large-scale green tides in the blooming region, especially along the downstream cities, such as Qingdao. Since 2008, huge amount of floating algal biomass was piled up on the beaches and coastal waters of Qingdao during late spring to summer every year. The practical mitigation strategies, such as clearing algae mass from beaches and coastal waters manually, blocking drifting mats by arresting nets, tend to remove and block the large amount of visible biomass. Little, however, has been done on the micro-propagules (e.g. broken fragments, somatic cells etc.), which are often associated with the floating biomass (Zhang et al., 2011; Y. Li et al., 2014; Song et al., 2015; Huo et al., 2016). Hence, concerns are existing whether the non-indigenous green tide could affect the local green macroalgae assemblage and leave prolonged micro-propagules after consecutive re-emergence in the coastal water of Qingdao for 9 years (2008–2016). Compared to the multiple large-scale surveys in the bloom region of Yellow Sea, especially in and around Subei Shoal (Y. Li et al., 2014; Song et al., 2014, 2015; Huo et al., 2014, 2016), few studies have been conducted on the micro-propagules along Qingdao coasts. During the winter of 2008 (December 2008 – April 2009), Liu and his colleagues screened the micro-propagules after the large-scale green tide hit Qingdao coastline for the first time, and found no *U. prolifera* propagules suggesting non-indigeneity of the green tide *U. prolifera* in the coasts of Qingdao (Liu et al., 2010a). Since then, no further research has been performed to characterize the micro-propagule assemblage in Qingdao coastal water, especially after years of large-scale green tides.

Here in this study, a year-round field survey was conducted to investigate the abundance and species assemblage of environmental micro-propagules in the Qingdao coastal waters. The remote sensing data were also analyzed to illustrate the association between the large-scale floating *U. prolifera* biomass and the fluctuation of micro-propagule community. The major objectives of this research are to characterize the tempo-spatial distribution and species composition of green macroalgal micro-propagules, and to evaluate the influence of the large-scale floating *U. prolifera* biomass on the micro-propagule assemblage along Qingdao coast.

2. Materials and methods

2.1. Sample collection

The water samples were collected from four locations off the coast of Qingdao (Zhanqiao: 36°03′40″ N, 120°18′50″ E, Yiyu: 36°03′24″ N, 120°20′15″ E, Olympic: 36°03′27″ N, 120°23′21″ E and Shilaoren: 36°05′27″ N, 120°27′53″ E, Fig. 1b), where high floating macroalgal biomass piled up every year (Fig. 1c). At each station, about 4 L of sea surface water were sampled using a clean beaker at the last week of every month from March 2016 to March 2017. Water samples were then transported to the laboratory in a cooler at 4 °C within 2 h. Temperature and salinity of sea surface water were measured and recorded in situ using the Multi-Parameter Water Quality Detector (YSI, USA).

2.2. Cultivation and quantification of green algae micro-propagules

In the laboratory, water samples were filtered through a 200 μm mesh net to remove the major zooplanktons. One liter of filtered seawater was then cultured in a glass beaker with addition of 20 mL Provasoli-enriched seawater medium (PES, Berges et al., 2001). The saturated GeO_2 was also added at a final concentration of 0.5 mg mL^{-1} to inhibit the growth of diatoms. Three replicates of each water sample were cultured as described above in an Artificial Climatic Chamber (202728–380, Jiangnan Inc., Ningbo, China) at 16 °C with 80–100 μmol photons m^{-2} s^{-1} and 12:12-h light:dark light cycle. The medium was renewed every 5 days to maintain sufficient nutrients for the growth of micro-propagules.

During the culturing, the green micro-propagules continuously attached to the wall and bottom of the glass beakers. After about 20 days, they developed into approximately 1–3 cm germlings, which could be counted with the naked eyes. The number of germlings in each beaker was then counted under a magnifier and considered as the total number of micro-propagules in the water sample. The abundance of micro-propagules (A, inds L^{-1}) was then calculated as A = N/V (N: total number of germlings, V: volume of water sample cultured).

After counting, the germlings were randomly sampled and identified to species individually. For the beaker with < 30 germlings grew, all the germlings were harvested for species identification. For the others (≥ 30 germlings in each beaker), we randomly sampled 30–76, which accounted for 10%–80% of the total amount of germlings in each beaker. The individual germling was removed from glass beaker by a sterilized forceps, rinsed with distilled water for 2–3 times and then frozen in a 1.5 mL Eppendorf tube for subsequent molecular identification.

2.3. DNA extraction, PCR amplification and molecular identification

An efficient deoxyribonucleic acid (DNA) extraction method modified from Hwangbo et al. (2010) was used to minimize the time and procedures for molecular identification. In general, the unialgal thallus was homogenized with a pestle in 100–150 μL of 5% Chelex 100 (Bio—Rad, USA). The homogenate was then vortexed for 10 s, incubated in boiling water for 5 min and centrifuged at 13000 rpm for 1 min to remove the tissue debris. The supernatant was readily used as the template for the following polymerase chain reaction (PCR) amplification.

Molecular identification of the green algal species was conducted based on the method described in Xiao et al. (2013). In brief, the internal transcribed spacer (ITS) fragment, including ITS1, 5.8S and ITS2 regions, was amplified using the primer pair: forward 5′-TCGTAACAAGGTTTCCGTAGG-3′, reverse 5′-GCTTATTGATATGCTTAAGTTCAGCGGGT-3′ (Leskinen and Pamilo, 1997; Hayden et al., 2003). The amplicons were then digested with series of restriction enzymes (BspT107 I and EcoO109 I, TaKaRa Bio, Inc., Dalian, China), and the restriction fragment length polymorphism (RFLP) pattern of each amplified sample was compared with the standard molecular keys of common ulvoid species (Xiao et al., 2013). To further distinguish *U. linza* and *U. prolifera*, 5S spacer regions were amplified with the primer pair: forward 5′-GGTTGGGCAGGATTAGTA-3′ and reverse 5′-AGGCTTAAGTTGCGAGTT-3′ (Yotsukura et al., 2002; Shimada et al., 2003). The two species were differentiated based on the polymorphism of amplified 5S spacer fragments. This method has proven useful to distinguish the common *Ulva* spp. and one *Blidingia* species along the west coasts of