

## The Yellow Sea green tide: A risk of macroalgae invasion

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### ABSTRACT

Large scale green tides have bloomed successively in the Yellow Sea since 2007. The floating ecotype of *Ulva prolifera*, which is responsible for the environmental disaster, drifted a long distance during the blooming time and was exotic to the coastal area. The Yellow Sea green tide can be a potential source to incur bio-invasion. In this study, the distribution pattern and propagule pressure of the floating ecotype was investigated along the Qingdao coastline, which was seriously impacted by the green tide. Two out of 661 attached *Ulva* specimens collected in different seasons were identified as the floating ecotype by molecular markers, indicating that a few individuals of the floating ecotype had settled down, and their attached population could have spontaneously established. In seawater and sediments, the proportion of the floating ecotype in *Ulva* propagules reached up to 32% and 69% respectively when the floating algae was accumulating on seashore, which was a great propagule pressure to the local ecosystem. Results of the field test indicated that the available resources and the competition between the floating ecotype and the local *Ulva* species might be the main restrictions for settlement. Though the current scale of the established population is still small, the risk of biological invasion by the floating ecotype exists and it deserves more attention.

### 1. Introduction

With climate change and intensive anthropogenic activities, biological invasion is an increasingly serious problem globally with substantial economic and ecological costs (Mack et al., 2000; Pimental et al., 2002). Macroalgae are considered to be especially anxious non-indigenous marine species because they may bring dramatic effects on ecosystem by monopolizing space, changing foodwebs, and spreading beyond the initial introduction site through efficient dispersal capacities (Schaffelke et al., 2006). On the other hand, “Green tides”, which are vast accumulations of green macroalgae biomass associated with intense eutrophication, have been frequently reported in North America (Vadas and Beal, 1987), Europe (Lappalainen and Ponni, 2000), and the Asia-Pacific region (Morand and Briand, 1996; Ye et al., 2011). In addition to various ecological impacts on indigenous biodiversity (Berger et al., 2003) and biogeochemical cycles (Sandjensen and Borum, 1991), these algal blooms affect aquaculture and tourism, causing great economic losses (Sun et al., 2008). The majority of the green tide-forming algae belonging to one genus, *Ulva* (Fletcher, 1996), which includes the

genus formerly known as *Enteromorpha* (Hayden et al., 2003). *Ulva* has the typical characteristics of an invasive species, such as eurythermal, euryhaline, resistance to environmental adversity and high reproductivity. However, most of the green tides occurred in closed or semi-closed bays, and the blooming algae were local species, which became floating in the eutrophic condition (Blomster et al., 2002). Therefore, biological invasion was not involved in these green tide cases.

For the Yellow Sea green tides, the scenario is different. From 2007 to 2017, green tides have continuously appeared in the Yellow Sea every summer for eleven years, which were documented as the largest green tides that ever occurred worldwide (Liu et al., 2013a). Besides the astonishing scale, the most significant feature of the Yellow Sea green tide is the long-distance drifting (> 500 km) of the floating seaweed. The blooms originated in the coastal area of Jiangsu province in late April, spread north-east with the wind and surface current, arrived at Shandong Peninsula in June and July, and disappeared by precipitation and decomposition in late July (Keesing et al., 2011). Qingdao is one of the most seriously impacted cities of the green tides (Fig. 1). Genetic

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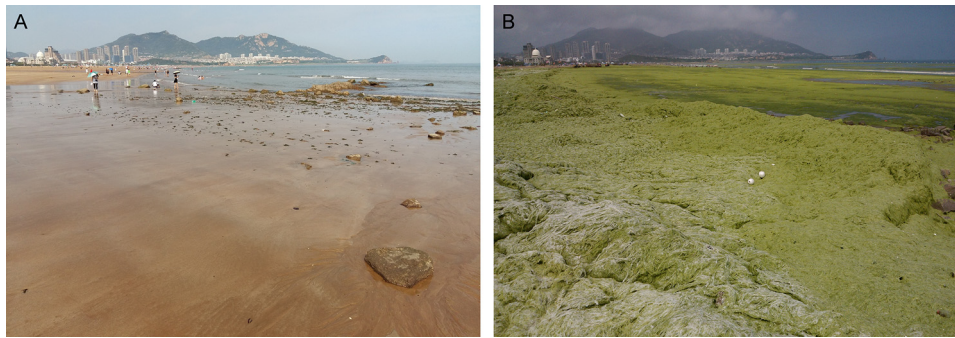


Fig. 1. Impacts of the Yellow Sea green tide on Qingdao coastal area. A) The coastline when there is no floating *U. prolifera*. B) The same coastline having the great amount of floating *U. prolifera* accumulating in summer.

analysis of the bloom forming algae proved that the Yellow Sea green tides were dominated by only one species *Ulva prolifera* (Zhao et al., 2013), which is exotic to Qingdao coastal area (Jiang et al., 2008). Further study at the intraspecific level showed that the floating *U. prolifera* was highly homogeneous and was genetically different from the attached populations in the Yellow Sea intertidal zone (Zhao et al., 2011). In combination with the morphology and physiological features, the genetic analysis results suggested it was a unique ecotype of *U. prolifera* that was responsible for the Yellow Sea green tides (Zhao et al., 2015). Former studies indicated that the floating ecotype was foreign to the north Yellow Sea coastal area, and the Yellow Sea green tides might be an ongoing macroalgal invasion.

Seaweed invasion, a complex multi-stage process, can be divided into separate phases of arrival, establishment, spread and persistence (Mollison, 1986). The arrival of the floating ecotype of *U. prolifera* had been observed every summer since 2007, and a great amount of its biomass accumulated on seashore. Establishment, which is defined as the development of a macroscopic thalli population in an area that did not previously support the species, is a crucial phase for the macroalgae invasion (Valentine and Johnson, 2003). It is also the crucial period to prevent the invasion of the exotic seaweed (Valentine et al., 2007). The process of establishment is affected by many factors. In addition to the biological characteristics of the exotic organism, such as the adaptability and resistance to adversity, it is also closely related to the properties of the recipient place, including the environmental conditions, resources availability and interspecies competition (Dunstan and Johnson, 2007; Britton-Simmons and Abbott, 2008; Hu and Juan, 2014). Making sure whether the floating ecotype of *U. prolifera* has settled down and established along the coast is essential to assess the bio-invasion risk and the ecological effects of the Yellow Sea green tide.

Most of former studies on the Yellow Sea green tides focused on tracking the origin of the floating algae (Liu et al., 2009; Pang and Liu, 2010; Zhang et al., 2011), explaining the mechanism behind the green tide blooming (Gao et al., 2011; Lin et al., 2011a; Lin et al., 2011b) and utilizing the floating biomass (Zhao et al., 2016). Few reports concerned the settlement of the floating ecotype. Liang et al. (2010) studied the species diversity of 12 *Ulva* strains collected from Qingdao coastline in April 2009, following the bloom in 2008, and no *U. prolifera* was found. Liu et al. (2010b) collected 48 seawater samples in the intertidal zone at six sites along Qingdao coast from December 2008 to April 2009, and the dominant *U. prolifera* strain of the 2008 bloom was absent in all the water-derived cultures. However, only a small sample set collected during one season was not enough for finishing the concerns. A systematic study based on the representative sampling with widely temporal and spatial distribution is earnestly required to confirm whether the floating *U. prolifera* have settled down in the non-native area.

In this study, we collected the attached *Ulva* specimens from ten sites along the Qingdao coastline in different seasons and classified all the specimens based on molecular markers. To evaluate the propagule

pressure of the exotic algae, we also collected seawater and sediment samples around the year to analyze the species diversity of the *Ulva* propagules and investigated the distribution of the floating ecotype propagules in Qingdao coastal area. Furthermore, taking the solid substrates as representative, we attempt to explain the restriction for the establishment of the floating *U. prolifera* by field experiments.

## 2. Materials and methods

### 2.1. Sample collection

From ten sites distributing along the 30 km of Qingdao coastline, attached green seaweed samples were collected for eight times during 2014 (February, April, May, June, August, October, December) and 2015 (February; Fig. 2, Table S1). All specimens were collected randomly from intertidal zones. After removing epiphytes, apical branches (10–20 mg) of each individual specimen were placed into a 1.5 mL Eppendorf tube and stored at  $-20^{\circ}\text{C}$  for DNA extraction. Seawater and surface sediment samples were collected from nine sites on the Qingdao intertidal zone eight times during 2014 (July, August, October, December) and 2015 (February, April, June, July; Fig. 2).

### 2.2. Quantification of *Ulva* propagules in seawater and sediment samples

Seawater and sediment samples were transported to laboratory in cool boxes within 5 h after collection. The abundance of *Ulva* propagules in each sample was evaluated using the method described by Liu et al. (2013b) with some modification. For seawater, 500 mL of each sample was enriched with 500  $\mu\text{L}$  of 1000  $\times$  VSE (Von Stosch's Enriched) solution in 500 mL glass beaker. For sediments, a wet weight of 300 g of each sample was put into a 1000 mL glass beaker filled with 500 mL sterile VSE medium. The sediment suspension was stirred vigorously and then the liquid was filtered out with a Nylon mesh (with the bore size of 75  $\mu\text{m}$ ) into a 500 mL glass beaker. Saturated  $\text{GeO}_2$  solution of 250  $\mu\text{L}$  was added in each beaker to inhibit the growth of diatoms (Shea and Chopin, 2007). All beakers were placed in an incubator (Jiangnan, Ningbo, China) at  $20^{\circ}\text{C}$  with 100  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  and 12:12-h light:dark light cycle. After three-week culture, the number of green algae germlings was determined. After counting, the algae were continuously cultured for about 2 weeks until the individual thallus grew up to 10 cm for further DNA extraction.

### 2.3. Molecular identification

Genomic DNA was extracted from individual thallus using a modified CTAB method (Zhao et al., 2010). Firstly, all of the specimens were identified by their ITS (internal transcribed spacer) sequences (Leskinen and Pamilo, 1997). Then, for the LPP (*U. linza-procera-prolifera*) clade, morphological features of the samples were analyzed to separate *U. linza* and *U. prolifera* on the basis of the criterion proposed by Tseng

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