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# Variations of dominant free-floating *Ulva* species in the source area for the world's largest macroalgal blooms, China: Differences of ecological tolerance

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ABSTRACT

Species composition and seasonal variations of free-floating *Ulva* species were investigated in the source area of the world's largest macroalgal blooms during 2009–2015. Based on a combination of a morphological analysis and sequences of nuclear-encoded ITS and 5S rDNA spacer regions, the dominant species in the free-floating *Ulva* community at the early stage of green tides were *Ulva compressa*, *Ulva flexuosa*, and *Ulva linza*. The first appearance of *Ulva prolifera* on the sea surface was in mid-May and it dominated the floating *Ulva* community in June from 2009 to 2011. From 2012–2015, *U. prolifera* was not only the first species to appear on the sea surface but also the dominant species during the whole early stage of green tides. To explain the successional mechanism, the effects of environmental factors on the growth of four *Ulva* species were examined in the laboratory under different combinations of light intensity and temperature. It was found that the highest growth rate of *U. prolifera* was 44.9%/d, which was much higher than the other three *Ulva* species. The strong tolerance of *U. prolifera* to extreme conditions also helps it survive and bloom in the Yellow Sea.



## 1. Introduction

Green tides, which occur widely in many coastal areas, are recognized as a special type of harmful algal bloom (HAB) (Nelson et al., 2003). Green macroalgal blooms have negative effects on marine ecosystems, including the alteration of ecosystem structure and a decrease in biodiversity (Hernández et al., 1997; McGlathery, 2001; Nelson and Lee, 2001; Franz and Friedman, 2002; Zhang et al., 2013a,b). Green tides are caused primarily by flotation, accumulation, and the excessive proliferation of green macroalgae, which are typically members of the genus *Ulva* (Blomster et al., 2002; Merceron et al., 2007; Nelson et al., 2008). In recent decades, green tides have increased in severity, frequency, and geographic range, and have become a growing concern globally, especially in the coastal areas of France, Italy, Holland, China, America, and England (Fletcher, 1996; Taylor et al., 2001; Blomster et al., 2002; Ye et al., 2011; Huo et al., 2016).

In the Yellow Sea, large-scale green tides have broken out annually from 2007 to 2017. In late June 2008, a massive green algae bloom in the coastal region of Qingdao was brought the world's attention because the algal bloom covered an area of approximately 13,000–30,000 $km^2$ and was thought to be one of the largest in recorded history (Leliaert et al., 2009; Liu et al., 2009). Various methods were used to maintain an algae-free water area near Qingdao for the sailing events in the Olympic games, with a total cost exceeding U.S. $100 million (Wang et al., 2009). More than 16,000 people using over 1000 transportation vehicles and 1600 fishing and transportation vessels volunteered to clean up the massive bloom. By 15 July 2008, more than 700,000 tons of algae had been removed from beaches and nearby coastal waters (Hu and He, 2008).

Through the combination of satellite remote sensing, shipboard monitoring, and numerical analysis, the radial sand ridge sea area in the southern Yellow Sea has been identified as the source area of

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the *Ulva* macroalgal blooms (Huo et al., 2014; Zhang et al., 2014; Zhang et al., 2017). The significant amount of *Ulva* species attached to *Pyropia* aquaculture rafts in the bloom's source area contribute to the initial occurrence and formation of the blooms (Liu et al., 2009; Liu et al., 2010; Hu et al., 2010; Tian et al., 2011; Liu et al., 2013; Zhang et al., 2013a,b; Huo et al., 2015; Liu et al., 2015; Geng et al., 2015; Liu et al., 2016).

The dominant species of the blooms has been identified as *Ulva prolifera* (Leliaert et al., 2009; Zhao et al., 2013); however, the *Ulva* species on the *Pyropia* rafts consist of many green algae, such as *Ulva compressa*, *Ulva linza*, *Ulva flexuosa*, and *U. prolifera* (Han et al., 2013; Huo et al., 2013). It is unclear why only the *U. prolifera* survive and drifted into the Qingdao sea area, while the other three species gradually disappeared. The first objective of the present study was to investigate the seasonal variation of the floating *Ulva* species in the bloom's source area.

Many macroalgae species show different growth responses to environmental factors (Dawes et al., 1978). Light intensity, temperature, and salinity are major environmental factors associated with algal growth in the marine environment. Some algae are tolerant to low irradiance, while others require high irradiance (Taylor et al., 2001; Cui et al., 2015). Temperature and salinity can also determine algal distribution on the shore (Wiencke and Lauchli, 1980; Round, 1981); however, according to a field survey, obvious variations in salinity are not observed in the Yellow Sea. Thus, the second objective of this study was to determine the effects of irradiance and temperature on the growth of four *Ulva* species and further explain the seasonal variation of the floating *Ulva* species.

## 2. Material and methods

### 2.1. Study area and collection of free-floating Ulva samples

In the source area of the blooms, the water depth was less than 10 m, which created ideal conditions for *Pyropia* aquaculture (Fig. 1). Semi-floating raft cultivation techniques are widely used in these *Pyropia* farms, with a combination of the pillar and floating methods in use, especially for intertidal cultivation. At high tide, the net floats on the water, maximizing the light available to the seaweed, while at low tide, the net settles on the ground.

In the *Pyropia* harvesting season, which runs from March-June every year, thousands of local farmers will work every day in the bloom's source area to collect *Pyropia*. At the same time, they will help to monitor the process of formation and distribution of green tide. Once floating *Ulva* species were discovered in the *Pyropia* aquaculture area by farmers, they recorded the GPS coordinates, take pictures of the blooms, and sent them to us by cellphone. They also collected samples and kept them in an ice-cold box. These samples would be transported to the lab under cool conditions as soon as possible for further analysis. The details of the collection sites are listed in Table 1 in the Supplemental Material.

### 2.2. Species identification in the free-floating Ulva macroalgae

Before morphological identification, debris and epiphytes were removed from the thalli, which were then rinsed gently using sterile seawater and examined with a light microscope (E200; Nikon, Tokyo, Japan). The identification of morphotypes was based on the morphology of the blade following the criteria of Koeman and van den Hoek (1981), Tanner (1986), Dion et al. (1998), and Loughnane et al. (2009).

A phylogenetic analysis was performed for unidentified green algae. Before DNA extraction, the samples were classified roughly according to their morphology, with four morphotypes identified. Three single thalli (three replicates) for each morphotype were sampled and cleaned several times with sterile seawater. The DNA extraction and polymerase chain reaction (PCR) amplification techniques used were previously described by Han et al. (2013). The nuclear encoded internal transcribed spacer (ITS) DNA region was amplified using the primers reported by Leskinen and Pamilo (1997). The *U. prolifera* and *U. linza* belonged to one group, referred to as the LPP cluster (*Ulva linza-procera-prolifera*). Therefore, the 5S rDNA spacer region was amplified using the primers to identify the LPP samples according to Shimada et al. (2008). Sequences of the ITS and 5S rDNA regions were aligned using Clustal X (Thompson et al., 1997). Neighbor Joining (NJ) analyses of the aligned sequences were performed using Mega 4.0 (Tamura et al.,

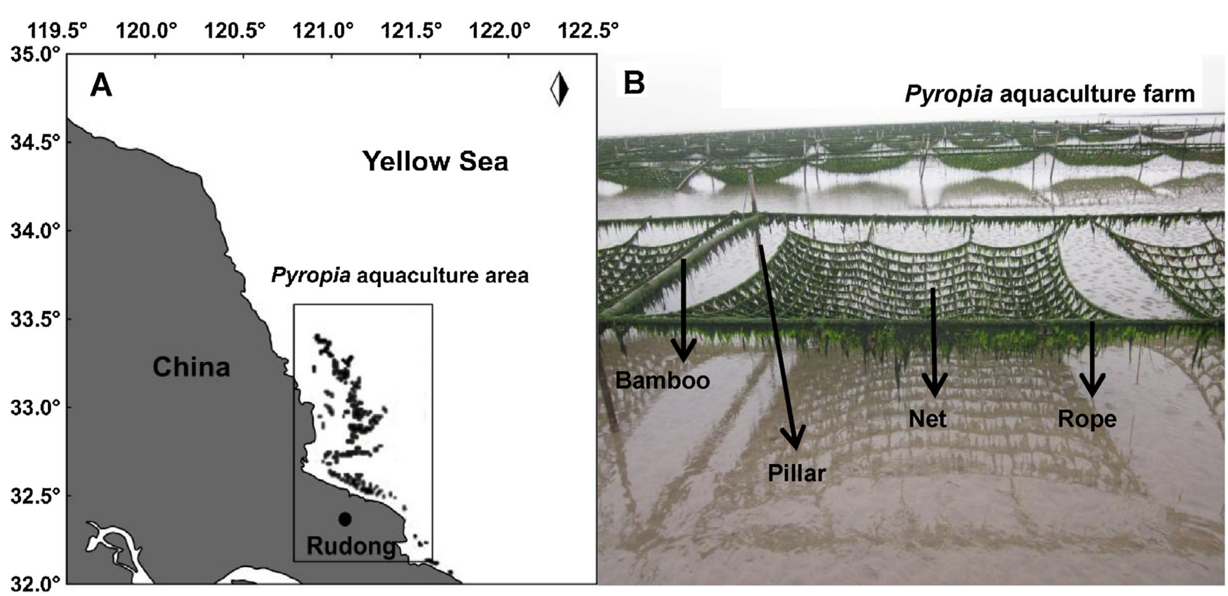


**Fig. 1.** A, Large-scale *Pyropia* aquaculture in the source area of the *Ulva* macroalgal blooms. B, An example of a *Pyropia* aquaculture farm showing components including ropes, bamboos, nets, and pillars. *Pyropia* grows on the net and *Ulva* can easily be attached on the bamboo and rope. The pillar legs can support the net at the low tide.