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Comparative study of the germination of *Ulva prolifera* gametes on various substrates

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ABSTRACT

Since 2007, massive green tides have occurred every summer in the southern Yellow Sea (YS), China. They have caused severe ecological consequences and huge economic losses. *Ulva prolifera* originated from Subei Shoal of the YS was confirmed as causative species of the green tides. The *Porphyra yezoensis* aquaculture rafts in the Subei Shoal have been highly suspected to be the "seed bed" of the green tides, because *U. prolifera* abundantly fouled the *Porphyra yezoensis* aquaculture facilities. Besides, various habitats of aquaculture ponds along the Jiangsu coastline and mudflat in the Subei Shoal were proposed to be possible sources of green tides. To understand the "seed" of the green tides in the southern YS and mitigate the original biomass of the green tide, various materials used as substrates for the germination of *U. prolifera* gametes were tested in this study. Culture experiments showed the following: 1) materials used in the *P. yezoensis* rafts (plastic, bamboo, jute rope, plastic rope, nylon netting, and plastic netting) displayed a significantly higher germination rate than those associated with mudflats and aquaculture ponds (mud, sand and rock); 2) plastics were the best substrates for the germination of *U. prolifera* gametes; 3) poor germination was found on old fronds of *U. prolifera*,, and rubber showed inhibitory effect on germination. The success in germination on *P. yezoensis* rafts related materials supports the notion that these mariculture structures may be involved in acting as a seed bed for green tide macroalgae. The lack of germination on rubber surfaces may suggest one way to limit the proliferation of early stages of *U. prolifera*.

1. Introduction

Since 2007, large-scale green tides have occurred between May and July annually in the southern Yellow Sea (YS), China (Liang et al., 2008; Liu et al., 2009, 2013a). They have caused severe ecological problems and huge economic losses (Ye et al., 2011). *Ulva prolifera*, which formed the green tides along the southern coastline of Shandong province, is not a local species (Jiang et al., 2008; Duan et al., 2011). The origin of green tides was traced back to the Jiangsu coast of the southern YS based on remote-sensing and numerical simulation models (Hu et al., 2010; Qiao et al., 2009, 2011; Bao et al., 2015). It has been reported widely that *Porphyra yezoensis* aquaculture rafts are the main source of green tides (Liu et al., 2009, 2013a; Keesing et al., 2011). *U. prolifera* abundantly fouled the *Porphyra yezoensis* aquaculture facilities. They were scraped off the rafts after harvesting *P. yezoensis*, thereby generating floating algae and forming a large-scale green tide (Liu et al., 2010, 2013a). But there are still debates about the origin of green tides. Various habitats of aquaculture ponds along the coastline (Pang et al., 2010) and mudflats in Subei Shoal (Liu et al., 2012, 2013b) have been proposed to be possible sources of green tides as well. Therefore, further studies are needed for clarifying why *P. yezoensis* aquaculture rafts, but not other habitats, served as the main source of *U. prolifera*.

The massive proliferation of *Ulva prolifera* is closely related to its complex modes of reproduction (Lin et al., 2008; Q. Liu et al., 2015). Microscopic propagules, which play an important role in its life cycle, were observed at various sites along the Jiangsu coast (Liu et al., 2012, 2013b; Song et al., 2015; Zhang et al., 2015); these propagules are able to overwinter successfully and provide large reserves for green tide outbreaks the following year (Schories, 1995; Pang et al., 2010). When environmental conditions are

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favorable and substrates are suitable for settlement and growth, the microscopic propagules can grow rapidly and form huge biomass. A green tide is then likely to occur (Hernandez et al., 1997; Lotze and Worm, 2001; Morand and Merceron, 2005; Song et al., 2015; Zhang et al., 2015). The settlement and germination of its gametes and spores are key stages in this process (Fletcher and Callow, 1992; Fletcher, 1996). The spores swim with flagella and identify an appropriate substrate; they then shed flagella and release alginate to complete attachment (Callow and Callow, 1997; Stanley et al., 1999). Settlement and germination of microscopic propagules on a suitable substrate is a prerequisite for rooting, growth and colonization of *U. prolifera*.

The settlement and germination of microscopic *Ulva prolifera* propagules is very important to further clarify the role played by various habitats in the green tides in the southern YS. Besides, to mitigate the original biomass of the green tide, searching for potential anti-fouling materials is important. But the knowledge on the settlement and germination of microscopic *U. prolifera* propagules on various substrates linked to above habitats is poorly understood. Previous studies mainly focused on the effects of the physical and chemical factors (Dan et al., 2002; Sousa et al., 2007; Wang et al., 2007) but not the effects of various substrates on the settlement and germination. *Porphyra yezoensis* aquaculture rafts are mainly constructed by bamboo sticks, plastic or jute ropes and plastic or nylon nettings. Macroalgal substrates in mudflats and aquaculture ponds include mud, sand and rocks. Previous studies have shown that wood was a preferred substrate for macroalgal attachment, followed by blue nylon netting, white ABS (Acrylonitrile Butadiene Styrene) plastic and rock, but sand, mud were not suitable (Wu et al., 2000), but more concerted study of settlement and germination on diverse substrates is essential.

Gametophytes are the dominant survival forms of floating *Ulva prolifera* in the southern YS (Chen et al., 2011). Therefore, the germination of *U. prolifera* gametes is important in the studies on the "seed" of green tides. We conducted a comparative study to investigate the germination of *U. prolifera* gametes on a variety of substrates, such as materials used in *Porphyra yezoensis* aquaculture rafts and materials associated with mudflats and aquaculture ponds; and materials aids in the quest for potential anti-fouling substances. We aim to provide evidence for clarifying why *P. yezoensis* rafts most likely serves as the "seed" of the large-scale green tides and what kind of materials could be used as potential "anti-fouling" substrates in the mitigation of green tides in the southern YS.

2. Materials and methods

2.1. Culture methods and collection of gametes

Ulva prolifera samples were collected from Qingdao Huiquan Bay in July 2011. They were confirmed as *U. prolifera* through morphological and molecular identification. The thalli were washed with sterile seawater several times to remove attachments and were then cultured in an Erlenmeyer flask with f/2-Si medium in the laboratory at a salinity of 30 ± 1, pH of 8.2–8.3, temperature of 20 ± 1 °C and light intensity of 50 μmol m^{-2} s^{-1} with a light: dark circle of 14: 10. To obtain gametes, a mature frond was cultured in a Petri dish containing sterile seawater after being dried out for 12 h in the dark. The released microscopic propagules collected were confirmed to be gametes by phototaxis and morphology assessment (Q. Liu et al., 2015) with a biological microscope (Nikon YS100).

2.2. Substrate materials tested

The substrate materials used in the experiments were classified into three groups (Fig. 1). Materials in group 1 were those used in the *Porphyra yezoensis* rafts, including sheet-type materials of plastic (Polypropylene, PP) and bamboo, plastic (Polyethylene, PE) and jute ropes, and plastic (PE) and nylon (Polyamide, PA) nettings. Materials in group 2 were that associated with mudflats and aquaculture ponds, including mud, sand, and rock. Materials in group 3 were others which could be found in the green tides area or could be used as potential anti-fouling substances, which includes wood, glass, rubber, fishmeal bag and old fronds of *Ulva prolifera*. Glass was selected because some experiments were conducted in Petri dishes made of glass.

Sheet-type substrates in group 1 and some materials (i.e., wood, glass, rubber) in group 3 were cut into circles with a diameter of 9 cm, so they could be fixed to the bottom of the Petri dishes. Plastic ropes and jute ropes were cut to lengths of 1 cm with a nominal diameter of 5 mm. Nettings were measured to be 1 cm in length and 1 mm in diameter. Sand, mud and rocks were spread evenly over the bottom of Petri dishes. Old fronds and fishmeal bags were tied with a string to a plastic sheet and fixed to the bottom of Petri dishes. In addition, in order to avoid the release of microscopic propagules and facilitate germling count, the old fronds were decolorized by being soaked in a 95% alcohol solution for 24 h prior to the experiments.

2.3. Timing of gamete settlement after release

Gametes were collected in time after release. Different substrates tested were sterilized via boiling and placed into Petri dishes with 30 ml of sterile seawater. Gametes were added into Petri dishes and reached a final density of 1×10^5 cells ml^{-1} before being cultured in the dark for 1 d. The gamete densities in each dish were assessed every 2 h with a biological microscope (Nikon YS100). The gamete settlement rates on materials tested were all calculated by the formula:

$$\text{Settlement rate}(t) = (D_0 - D_t)/D_0 \times 100\%$$

where D_0 represents the gamete density in the seawater at the start; D_t represents the gamete density at time t.

2.4. Germination of Ulva prolifera gametes on various materials

2.4.1. Group 1: materials used in the Porphyra yezoensis rafts

2.4.1.1. Sheet-type materials. After sterilization by boiling, substrates (i.e., plastic, bamboo) were placed into Petri dishes with 30 ml of sterile water. A specific number of gametes were added to Petri dishes and reached a desired density of 2×10^4 cells ml^{-1}, and then cultured under the same conditions in the dark for 48 h. When gamete settlement was completed, the substrates were cultured in the light in beakers with adequate nutrients. After 10 days, the germlings were counted under a stereomicroscope (Nikon SMZ800). If a high density of germination on the substrates were observed, which occurred for plastic and bamboo, 10 views with areas of 5 mm × 5 mm were selected randomly to obtain an appropriate average number. When the germlings had grown to 3–5 cm in length, they were scraped off and their wet weights and lengths were measured. Fifty germlings were selected randomly to be measured, and the average length was determined.

2.4.1.2. Nettings. The methods used for material treatments, gamete collection and density determination were the same as those described in Section 2.4.1.1. The nettings were separated before counting, and the total numbers of germlings on the substrates were calculated. The total surface area of nettings was also calculated to determine the germling amount per unit area.