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Rapid expansion of *Ulva* blooms in the Yellow Sea, China through sexual reproduction and vegetative growth

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ABSTRACT

Green algal blooms have occurred in the Yellow Sea for 11 consecutive years since 2007. A "seed bank" comprising micro-propagules including gametes, meiospores, and zygotes, played an important role in the rapid formation of a green tide. In the present study, germination differences among zygotes, meiospores, and gametes were examined. The growth ability and maturation period of alternating generations of sexual *Ulva prolifera* strains were also assessed. The zygote and meiospore germination rate was 91.67% and 80.29%, respectively, approximately three times greater than that of gametes (30%). In addition, the highest daily growth rate of sporophytes and gametophytes was 266.7% and 288.1%, respectively, and the maturation period of sporophytes and gametophytes was 35.7 and 31.3 days, respectively. These results indicate that sexual reproduction and vegetative growth are mainly responsible for the rapid expansion of macroalgal blooms in the Yellow Sea.

1. Introduction

Green macroalgae are globally ubiquitous in marine and estuarine habitats, where they show a great ability to acclimate to adverse conditions and grow rapidly in eutrophic waters (Tan et al., 1999). Vast accumulations and rapid growth of unattached green macroalgae, known as "green tides", and are closely associated with eutrophicated marine environments (Raffaelli et al., 1998; Shimada et al., 2003; Charlier et al., 2007; Nelson et al., 2008). Green macroalgal blooms have been reported throughout the oceans globally (Fletcher, 1996; Blomster et al., 2002; Nelson et al., 2003; Merceron et al., 2007). Over the last few decades, green tides in particular have been increasing in severity, frequency, and geographic range, becoming a growing global concern (Largo et al., 2004).

In the Yellow Sea, large-scale green tides have occurred consecutively between 2007 and 2017. Particularly, in late June 2008, a massive green algae bloom in the coastal region of Qingdao garnered global attention because it covered an area of 13,000–30,000 km^2, thought to be one of the largest in recorded history. *Ulva prolifera*, a dominant bloom species, exhibits unique morphological and reproductive features. The extremely filamentous growth form, diversified reproductive mode, and high nutrient absorption activity of pelagic *U. prolifera* are considered physiological adaptations to the floating environment, leading to significant biomass accumulation (Lin et al., 2008; Gao et al., 2010; Wang et al., 2012). Although asexual and sexual reproduction (Bliding, 1963; Koeman and van den Hoek, 1982; Hiraoka et al., 2003) and the heterogeneous life history of *U. prolifera* (parthenogenetic reproduction with biflagellate gametes), have been reported recently (Liu et al., 2015), it is still unclear which form of reproduction will be advantageous in the field. In addition, *Ulva* species are generally characterized by the quick release of zoids and short maturation time; however, it is still unknown how floating *Ulva* species maintain growth and large biomass. Therefore, in the present study, germination and growth experiments using different generations of sexual *U. prolifera* were designed. The aims of this study were to reveal: (1) the physiological differences between sporophytes and gametophytes, and (2) the contribution of sexual history and vegetative growth to green tide bloom-forming *U. prolifera*.

2. Materials and methods

2.1. Specimen collection

Ulva prolifera strains in the present study were collected from a green tide blooming area in the Qingdao region of China in July 2011. Live materials were brought back to the laboratory, and the periphyton

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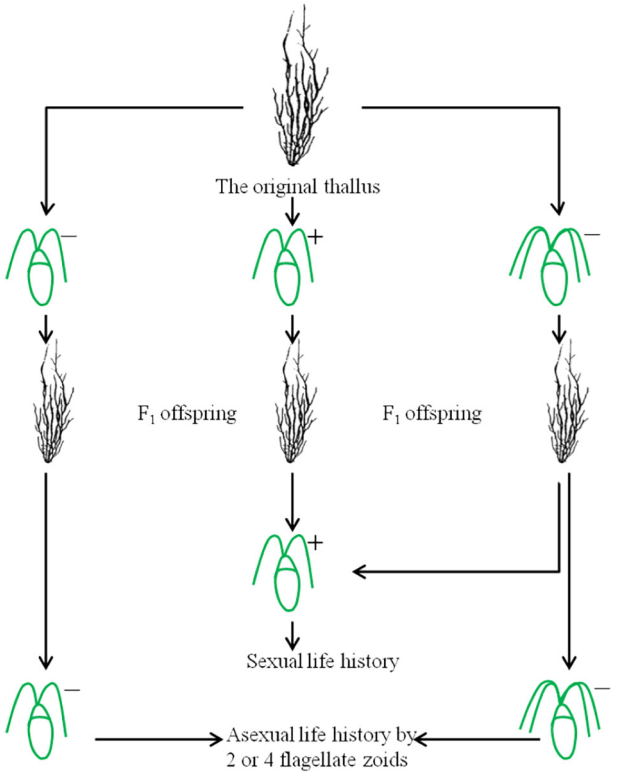

Fig. 1. Diagrammatic map in the determination of the sexual and asexual life history for the targeted *Ulva* strain. : biflagellate zoids; : quadriflagellate zoids; −: negative phototaxis; +: positive phototaxis.

and surface impurities were removed using sterilized seawater and a soft brush. Collected samples were identified by molecular and morphological characteristics. The strains were then kept as unialgal cultures in an incubator under the condition of 15 °C and 20–30 μmol·m^{-2}·s^{-1}, under a 12 h:12 h light:dark cycle.

2.2. Determination of the life history of the targeted Ulva prolifera strain

Six thalli of *U. prolifera* were chosen randomly to confirm the life history. Life history was determined by the size, phototactic response, and zooid flagella number of at least two successive generations (Fig. 1). Flagella numbers and phototactic response of zoids were observed following the methods of Hiraoka et al. (2003). Asexual life history characteristics comprised zoids of two successive generations of the same size and with negative phototaxis. In contrast, sexual life history presented either two sizes of zoids with opposite phototactic responses or zoids of the same size with positive phototaxis in two successive generations. The size of quadriflagellate meiospores with negative phototaxis was larger than biflagellate gametes with positive phototaxis. The morphological characteristics of zoids were examined under a microscope (BX51, Olympus, Tokyo, Japan). The sporophytes and gametophytes of *U. prolifera* were selected for germination and growth experiments.

2.3. Germination experiments using alternating generations of the targeted Ulva prolifera strain

The selected sporophytes and gametophytes were cultivated at 20 °C and 100 μmol·m^{-2}·s^{-1}, under a 12 h:12 h light:dark cycle. VSE medium (Ott, 1965) was refreshed every 2 days until thalli matured. Mature thalli were induced to release zoids in a Petri dish under a unilateral light source (50–80 μmol·m^{-2}·s^{-1}) from a white fluorescent tube. The meiospores and gametes were purified at least twice according to their respective phototaxis for a further germination experiment. The other isolated anisogametes were induced to zygote-forming and the zygotes were isolated from anisogametes by negative phototaxis. The zygotes also were purified at least twice using the same method as mentioned above for a further germination experiment.

For the germination experiment, four marked sterilized coverslips were placed into one sterilized Petri dish containing 40 mL of VSE medium (Ott, 1965). Suspensions comprising several hundred purified meiospores, zygotes, and gametes were added to the different Petri dishes, which were then placed in the dark at 20 °C for zoid settlement. The next day, two marks on each coverslip were selected and microphotographs of settled zoids were taken. Following this, the Petri dishes were transferred for cultivation in an incubator at 20 °C and 70 μmol·m^{-2}·s^{-1}, under a 12 h:12 h light:dark cycle. The germination process of each type of zoid was observed at the same time of day for 1 week and the microphotographs were taken at the exactly same marks as mentioned above. The development of the settled zoids was individually traced on the photomicrographs. Finally, only zoids that developed into multicellular germlings after 1 week were regarded as having successfully germinated. Two hundred viable settled zoids of each type were analyzed; three replicates of each type of zoid were examined. The germination rate was calculated based on the formula: germination rate (%) = $(G_t / S_0) \times 100\%$. Where, S_0 was the number of settled zoids after 1 day in the dark, G_t was the number of germlings after t days of culture, and t was the culture period in days.

2.4. Growth characteristics of alternating generations of the targeted Ulva prolifera strain

Intact sporophyte and gametophyte germlings in good condition were selected for the growth experiment. For each generation, one individual (~2 cm in length) was cultivated in a spherical glass flask (500 mL) under continuous aeration at 20 °C and 100 μmol·m^{-2}·s^{-1}, under a 12 h:12 h light:dark cycle. VSE medium was refreshed every 2 days for 1 week (Ott, 1965). Daily growth rate was calculated based on the formula: daily growth rate (%/d) = $[(W_t / W_0)^{1/t} - 1] \times 100\%$. The initial wet weight (W_0) and final wet weight (W_t) were measured at the same time after removing the surface moisture of thalli with paper towels, t was the culture period in days. Three parallel samples from each generation were analyzed.

2.5. Maturation period of alternating generations of the targeted Ulva prolifera strain

After the growth experiment, the cultivation of each generation occurred under the same condition as mentioned earlier until the thalli matured, and the thalli maturation period was recorded. Three parallel samples in each generation were analyzed.

2.6. Data analysis

The germination rate, growth rate, and maturation period was expressed as mean ± standard deviation. One-way analysis of variance was used for the variances analysis of germination and growth rates. For post hoc analysis, Tukey's test was used for multiple mean comparisons. SPSS software (v.13.0 SPSS Inc., Stanford, CA, USA) was used to conduct statistical analyses. $P < 0.05$ indicated statistical significance.

3. Results

3.1. Reproductive pattern of the targeted Ulva prolifera strain

In the six thalli of the targeted *U. prolifera* strain, four mother thalli released 4-flagellate zoids with negative phototaxis (Fig. 2B), while F_1 generation thalli produced two types of 2-flagellate zoids with intensive