Aquatic Botany 170 (2021) 103343

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Responses of the germination and growth of *Ulva prolifera* parthenogametes, the causative species of green tides, to gradients of temperature and light

Yuanzi Huo [a,b,*], Jang Kyun Kim [a,c], Charles Yarish [a], Simona Augyte [a], Peimin He [b]

[a] Department of Ecology and Evolutionary Biology, University of Connecticut, 1 University Place, Stamford, CT 06901, USA
[b] College of Marine Ecology and Environment, Shanghai Ocean University, Shanghai 201306, PR China
[c] Department of Marine Science, School of Natural Sciences, Incheon National University, 119 Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea

ARTICLE INFO

Keywords:
Green tides
Green macroalgae bloom
Ulva
Parthenogametes
Neopyropia aquaculture
Yellow Sea

ABSTRACT

Responses of the germination and growth of *Ulva prolifera* parthenogametes to gradients of temperature and light were evaluated. Results showed that *U. prolifera* parthenogametes could not germinate at 5 °C and 35 °C, and at all temperatures combined with dark conditions, but had high germination rates at the temperature of 15–25 °C and photosynthetically active radiation (PAR) of 80–160 μmol m^{-2} s^{-1}. There was a significant interaction between temperature and PAR on the growth rate of *U. prolifera* germlings germinated from parthenogametes ($P < 0.001$), which indicated that *U. prolifera* germlings achieved the highest growth rate at specific combinations of temperature and light. Growth rate of *U. prolifera* germlings germinated from parthenogametes was as high as 93.5–99.2 % d^{-1} at combined conditions of 22 °C and 26 °C with 100 μmol m^{-2}·s^{-1} and 200 μmol m^{-2}·s^{-1}, respectively. *Ulva prolifera* parthenogametes survived over two months at the temperature of 3 °C, and germinated and grew when the temperature increased from 3 °C to 13 °C. *Ulva prolifera* thalli germinated from parthenogametes maintained a relatively better state under the condition of 30 °C and 10 μmol m^{-2}·s^{-1} compared with thalli cultured at 30 °C combined with PAR of 100 μmol m^{-2}·s^{-1} and 200 μmol m^{-2}·s^{-1}, respectively. These results suggest that *U. prolifera* parthenogametes may largely contribute to green tides due to their high germination and growth rates, and their ability to survive over stressful environments in the southern Yellow Sea.

1. Introduction

Over the past four decades, green macroalgal blooms, referred to as green tides, have been occurring with an increasing frequency in coastal areas worldwide (Liu et al., 2013; Huo et al., 2016). These blooms are formed due to excessive growth of some green macroalgal species, driven mainly by eutrophication in the coastal waters (Hernández et al., 1997; Valiela et al., 1997; Raffaelli et al., 1998; Samanta et al., 2019). These blooms negatively affect the ecology and economy of coastal environments (Fletcher, 1996; Morand and Merceron, 2005; Hiraoka et al., 2011).

The world's largest green macroalgal bloom, which was caused mainly by *Ulva prolifera* subsp. qingdaoensis J. Cui, W. Zhu & M. Hiraoka (Cui et al., 2018a), occurred during the summer of 2008 along the coast of the southern Yellow Sea of China. Since then, green macroalgal blooms have been an annual summer occurrence in the Yellow Sea (Liu et al., 2009 and 2013; Huo et al., 2016; Wu et al., 2018). It is now widely accepted that the green macroalgal blooms in the Yellow Sea arise from *Ulva* fouling the rafts used for *Neopyropia* aquaculture along the coast of Jiangsu Province, China (Liu et al., 2010; Zhang et al., 2014; Huo et al., 2015; Wang et al., 2015; Wu et al., 2017).

Ulva prolifera has a complex life history with multiple reproduction modes (Arasaki and Shihira, 1959; Bliding, 1963; Kapraun, 1970; Kim et al., 1991; Burrows, 1991; Hiraoka et al., 2003). These reproduction modes are considered important strategies for *U. prolifera* to form green tides (Wang et al., 2007; Lin et al., 2008; Ye et al., 2008; Zhang et al., 2011a,b; Zhang et al., 2016; Cui et al., 2018a,b). Among the different reproduction modes, parthenogenesis is considered an important strategy in the Yellow Sea because biflagellate parthenogametes of *U. prolifera* can germinate into thalli directly without fusion (Hoxmark, 1975; Philips, 1990; Wang et al., 2007; Ma et al., 2009). Parthenogametes are also considered to play an important role in the proliferation

* Corresponding author at: College of Marine Ecology and Environment, Shanghai Ocean University, Shanghai 201306, PR China.
E-mail address: yuanzi1979-11@163.com (Y. Huo).

https://doi.org/10.1016/j.aquabot.2020.103343
Received 7 January 2020; Received in revised form 7 December 2020; Accepted 11 December 2020
Available online 17 December 2020

of attached populations of *U. prolifera* and the subsequent formation of green tides (Liu et al., 2015).

To our knowledge, few studies have been conducted to determine the effects of environmental factors on the germination of *Ulva prolifera* parthenogametes and the subsequent growth of their germlings. Sousa et al. (2007) reported the influence of salinity, nutrients and light on the germination and growth of *Ulva* spores. Geng et al. (2015) conducted experiments on the germination of *U. prolifera* gametes on various substrates. Cui et al. (2015) reported the growth rate of *U. prolifera* germlings germinated from spores at different photosynthetically active radiation (PAR) and temperatures. Some researchers have reported the germination and growth of *Ulva* spores at different temperatures and PAR, however, the germination and subsequent growth of *U. prolifera* parthenogametes at variable temperatures and PAR are still unknown.

It is hypothesized that *Ulva prolifera* parthenogametes exhibit high germination and growth rates at suitable combinations of light and temperature, which make them contribute more to green tides in the southern Yellow Sea. In this study, we present the results on the germination and growth of *U. prolifera* parthenogametes isolated from the Yellow Sea. The aims of this study were to evaluate the responses of the germination and growth of *U. prolifera* parthenogametes to gradients of light and temperature, and its survival mechanism under stressful environments in the Yellow Sea.

2. Materials and methods

2.1. Gametophytes of cultured Ulva prolifera

Floating thalli of *Ulva prolifera* were collected from the coast of the Rudong sea area in the southern Yellow Sea, China on April 8, 2012 (Zhang et al., 2013). After debris and epiphytes were removed from the surface of *Ulva* using a soft brush and rinsing the thalli with autoclaved seawater, the thalli were transferred to a 1000 mL flask containing 800 mL Von Stosch's Seawater Enrichment medium (Ott, 1965). To establish a single genetic strain, a single thallus of *U. prolifera* was separated based on its specific morphological features (Huo et al., 2013). *Ulva prolifera* was then cultured at a temperature range of 20–25 °C and photosynthetically active radiation (PAR) of 130–160 μmol m^{-2} s^{-1} with a photoperiod of 12 L:12D. Molecular confirmation of *U. prolifera* was established using a TiangenDNAsecure Plant Kit (Zhang et al., 2011a,b). Internal transcribed spacer (ITS) sequences and 5S ribosomal deoxyribonucleic acid (5S rDNA) spacer sequences were chosen for species identification (Huo et al., 2013). Phylogenetic analysis using ITS sequences indicated that the strain used in the present study was clustered into the so-called "LPP complex" clade (including morphological species *Ulva linza*, *U. prolifera* and *Ulva procera*; Huo et al., 2016). Based on the sequence of the 5S rDNA spacer region, this strain was clustered together with known strains of *U. prolifera*, which can be clearly distinguished from *U. linza*.

The gametophytes of *Ulva prolifera* were determined based on the type of zoids, which were examined by their size, number of flagella and phototactic characteristics (Hiraoka and Yoshida, 2010). *Ulva prolifera* parthenogametes, had two flagella. These zoids were significantly smaller than zoospores, which were quadriflagellate and exhibited positive phototaxis. Fig. 1 shows the biflagellate parthenogametes of *U. prolifera,* which were produced by gametophytes which germinated from unfertilized parthenogametes.

2.2. Parthenogametes release

Some of the *Ulva prolifera* gametophyte biomass was chopped into 1.0–2.5 mm long pieces using a blender (Osterizer, Galaxie, USA). These small pieces were then rinsed with autoclaved seawater 2–3 times, transferred to a 1000 mL flask containing 800 mL VSE medium, and cultured at the same conditions described above for *U. prolifera* gametophyte cultures. After 2–3 days, gametangia were formed from almost all of the cells of the thallus or from cut edges of large disks (Gao et al., 2010). These small pieces of *U. prolifera* were filtered through a sieve (80 μm mesh size). After rinsing with sterile seawater, small pieces of *U. prolifera* were transferred to 250 mL flasks containing 150 mL of VSE medium and cultured under the same environmental conditions mentioned above. Within half an hour to one hour, a large number of parthenogametes were released simultaneously from *U. prolifera* gametangia. The abundance of *U. prolifera* parthenogametes was counted using a hemocytometer and the parthenogametes were diluted into a series of densities depending on the needs of the experiments.

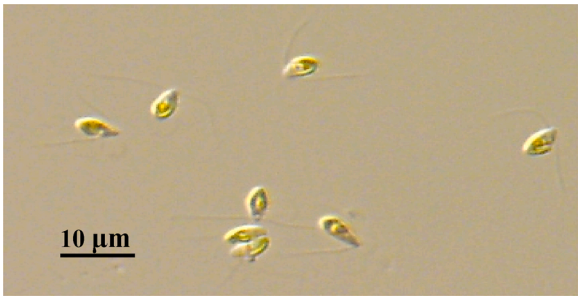

Fig. 1. Biflagellate parthenogametes of *Ulva prolifera*, which were produced by gametophytes germinated from unfertilized parthenogametes.

2.3. Germination of Ulva prolifera parthenogametes

The density of the *Ulva prolifera* parthenogametes was diluted to 1000 cells mL^{-1} using autoclaved seawater, and a 1 mL suspension was pipetted into a dish containing 20 mL VSE medium to the final density of 50 cells per mL. All the experiments in this part had at least three replicates. The dishes were gently shook and cultured at different temperatures and photosynthetically active radiation (PAR). For the temperature experiments, the gradients of temperature were set at 5, 10, 15, 20, 25, 30 and 35 °C under a PAR of 100 μmol m^{-2} s^{-1} with a 12 L:12D. For the PAR experiments, the gradients of PAR were set at 0, 40, 80, 120, 160 and 200 μmol m^{-2} s^{-1} under a temperature of 20 °C with a 12 L:12 D. After 2–3 weeks, the germlings of *U. prolifera*, which were attached to the walls and bottoms of the glass beakers and had grown to a length of 1–5 cm, were counted. The germination rate was calculated using the following equation:

$$G = Pn/Gn \times 100\ \%$$

where *G* represents the germination rate of parthenogametes, *Pn* represents the quantity of young germlings, and *Gn* represents the quantity of initial *Ulva prolifera* parthenogametes, which was equal to 1000 cells.

2.4. Growth of Ulva prolifera germlings germinated from parthenogametes

The density of the *Ulva prolifera* parthenogametes was adjusted to 2×10^6 cells mL^{-1} using sterile seawater, and a 0.2 mL suspension containing about 4×10^5cells of *U. prolifera* parthenogametes was pipetted onto glass coverslips (22 × 22 mm). All coverslips were placed into moisture chambers for over 24 h for the parthenogametes to settle down on coverslips, and then these coverslips were gently rinsed using autoclaved seawater to remove any parthenogametes, which had not attached to the coverslips (Yarish and Edwards, 1982). These coverslips were cultured in 100 × 80 mm Pyrex deep storage Petri dishes. Each deep well dish contained seven coverslips with 200 mL VSE medium.

A total of 63 deep welled Pyrex dishes were placed on a crossed-gradient culture apparatus described by Yarish et al. (1979). A total of 21 combinations of temperature (3, 8, 13, 18, 22, 26 and 30 °C; ±1 °C) and PAR (10, 100 and 200 μmol m^{-2}·s^{-1}) were used for each gradient plate experiment. Irradiance was supplied by high output deluxe

cool-white fluorescent bulbs with a 12 L: 12 D photoperiod. On the 1 st, 2nd, 3rd, 4th and 7th day after inoculation, one coverslip with settled parthenogametes was removed from each deep well dish. The coverslips from each experimental condition were randomly selected and 100 randomly selected *Ulva prolifera* germlings were measured for length using a compound microscope. All the experiments in this part were done separately with at least three replicates. The growth rate was calculated according to the following equation:

$$SGR = 100\times(\ln L_i - \ln L_{i\text{-}1})/t$$

where *SGR* represents specific growth rate (% days^{-1}), L_i represents the length of *Ulva prolifera* thallus measured at time *i*, $L_{i\text{-}1}$ represents the length of *U. prolifera* thallus measured at time *i*-1, and *t* represents days (d) between *i* and *i*-1.

Ulva prolifera parthenogametes did not germinate and stayed as single cells at the temperature of 3 °C under all light conditions tested for up to 60 days. After 60 days, the temperature was increased from 3 °C to 13 °C, and the number of thalli germinated from the parthenogametes were counted. At the temperature of 30 °C combined with all experimental light conditions, *Ulva prolifera* parthenogametes germinated and grew very fast. In order to evaluate the effects of high temperature on the *U. prolifera* thallus, the experimental time of all 30 °C groups was also designed to extend up to 60 days. Then, *Ulva prolifera* thalli were checked for thallus color, cell morphology, chloroplast structure and pyrenoids. The medium in these experiments was changed weekly.

2.5. Data analysis

All data concerning thallus length, germination rate of parthenogametes and growth rate of germlings were displayed as average ± standard deviation. Tests of homogeneity of variance were conducted, and a one-way analysis of variance (ANOVA) was performed to test for differences in the germination rate of *Ulva prolifera* parthenogametes between different experimental groups. The growth rate (% d^{-1}) data were transformed (log10x) before the analysis in order to obtain consecutive distributions, and a two-way ANOVA was performed to test the effects of temperature and PAR on the growth rate of the *U. prolifera* germlings germinated from parthenogametes at the end of experiment. Least significant difference (LSD) was used to make post hoc comparisons between different groups. Differences were considered significant at $P < 0.05$. Statistical analyses were conducted using SPSS 19.0.

3. Results

3.1. Germination rate of Ulva prolifera parthenogametes

Germination rate of the *Ulva prolifera* parthenogametes was greatly affected by temperature and PAR (Tables 1 and 2 in the supplemental material; Fig. 2). *Ulva prolifera* parthenogametes did not germinate at 5 °C or 35 °C. The germination rate of the *U. prolifera* parthenogametes was 78.1 ± 1.6 % at 20 °C, which was not significantly different from that at 15 °C (76.3 ± 1.1 %, $P = 0.066$). However, the germination rate at 20 °C and 15 °C was significantly higher than those at the other temperatures tested ($P < 0.001$). The germination rate at 25 °C was 69.7±1.6 %, which was higher than those at 10 °C and 30 °C ($P < 0.001$ and $P < 0.001$, respectively). These results indicate that the suitable temperature range for the germination of *U. prolifera* parthenogametes is from 15 °C to 25 °C.

The germination rate of *Ulva prolifera* parthenogametes was significantly different between all experimental light conditions ($P < 0.001$, Fig. 2B). *Ulva prolifera* parthenogametes did not germinate in dark conditions. The germination rate was only 11.6 ± 1.1 % at 200 μmol m^{-2}·s^{-1}, which was significantly lower than those at other PAR conditions ($P < 0.001$). The germination rate increased from 59.3 ± 0.9 % to 88.1 ± 0.8 % when PAR increased from 40 to 120 μmol·m^{-2}·s^{-1}. However, germination rate decreased to 64.5 ± 1.2 % at PAR of 160 μmol m^{-2}·s^{-1}.

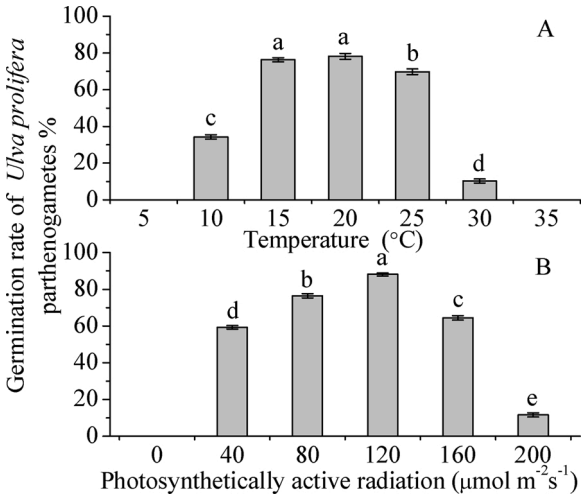

Fig. 2. Germination rate of *Ulva prolifera* parthenogametes at different temperature (A, °C) and photosynthetically active radiation (PAR) (B, μmol·m^{-2}·s^{-1}) conditions (n = 3).

3.2. Growth rate of Ulva prolifera parthenogametes

Ulva prolifera parthenogametes remained as single cells at 3 °C regardless of the PAR conditions (Figs. 3 and 4). When the temperature was at 8 °C–26 °C, the average thallus length of *U. prolifera* germlings increased sharply, especially at PAR of 100 μmol m^{-2}·s^{-1} and 200 μmol m^{-2}·s^{-1} (Fig. 3). The growth rate of *U. prolifera* germlings germinated from parthenogametes was not significantly different between 100 μmol m^{-2}·s^{-1}and 200 μmol m^{-2}·s^{-1} within the temperature range of 8–30 °C ($P > 0.05$, Fig. 4). The growth rate of *U. prolifera* germlings was in the range of 93.5 %d^{-1} to 99.2 %d^{-1} at the combined conditions of 22 or 26 °C with 100 or 200 μmol m^{-2}·s^{-1}, respectively, which was significantly higher than those at other experimental conditions ($P \leq 0.023$, Fig. 4). When the PAR was 10 μmol m^{-2}·s^{-1}, the growth rate of *U. prolifera* germlings was in the range of 41.8 % d^{-1} to 51.2 % d^{-1} at the temperature treatments from 13 °C to 30 °C, which was significantly lower than those at the PAR conditions of 100 μmol m^{-2}·s^{-1}and 200 μmol m^{-2}·s^{-1} ($P < 0.001$, Fig. 4). Two-way ANOVA analysis indicated that there was a significant interaction between temperature and PAR on the growth rate of *U. prolifera* germlings at the end of experiment (Table 3 in the supplemental materials, $P < 0.001$).

3.3. Ulva prolifera parthenogametes and thalli after 60 days

Results of the present study showed that the parthenogametes can remain as a single cell for over two months (Fig. 5). At 10 μmol m^{-2}·s^{-1}, the remaining amount of *Ulva prolifera* parthenogametes was higher than that at the other two PAR conditions after 60 days. The diameter of parthenogametes at 10 μmol m^{-2}·s^{-1} was also bigger than those at other two PAR conditions (Fig. 5). After 60 days, when the parthenogametes cultured at 3 °C were moved to 13 °C, they started to germinate and grow into germlings within 3–4 days (Fig. 6). The average number of germlings was up to 6.8 ± 0.9 thalli mm^{-2} at 10 μmol m^{-2}·s^{-1}, which was significantly higher than those at 100 μmol m^{-2}·s^{-1} (0.2 ± 0.2 thalli mm^{-2}) and 200 μmol m^{-2}·s^{-1} (0.02 ± 0.01 thalli mm^{-2}), respectively ($P < 0.001$, Fig. 6).

Ulva prolifera parthenogametes quickly germinated and grew into germlings within 7 days at 30 °C regardless of PAR (Figs. 3 and 4). When the *U. prolifera* germlings were continuously cultivated for 60 days, the

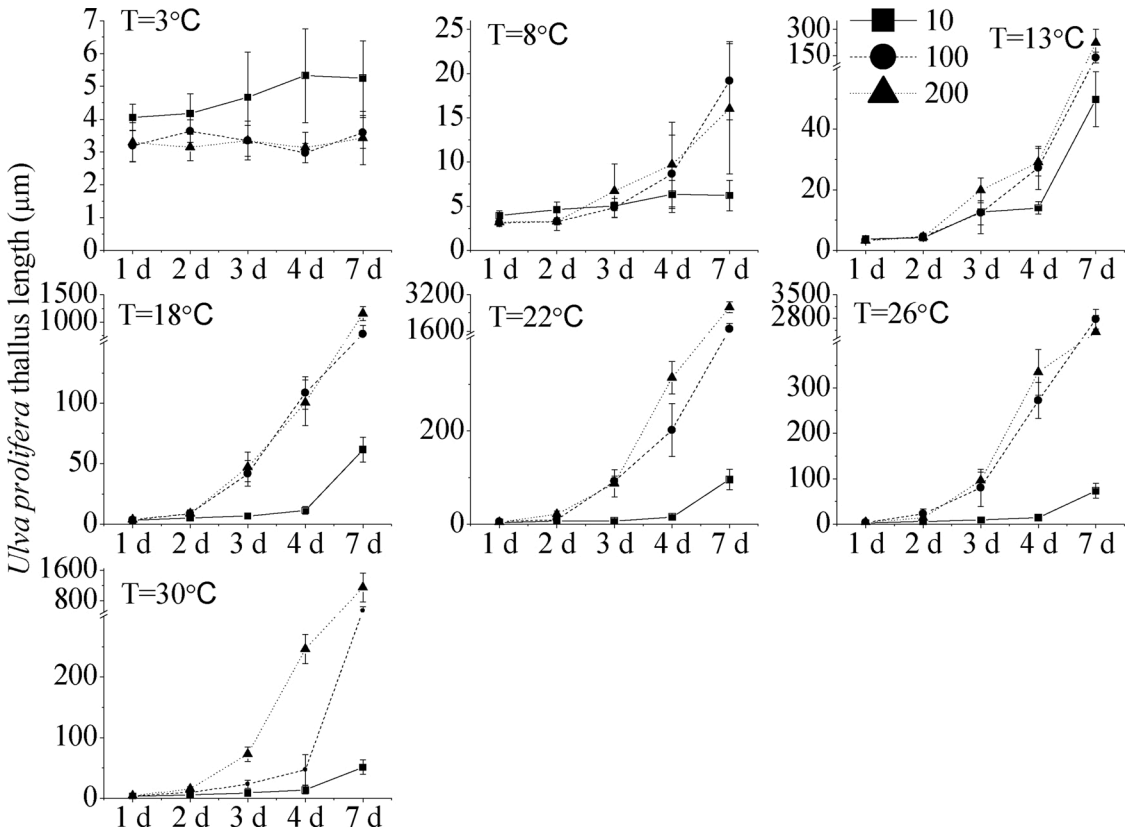

Fig. 3. Daily thallus length measurements of *Ulva prolifera* germlings germinated from parthenogametes at designated temperatures (°C) and photosynthetically active radiation (PAR) (μmol·m^{-2}·s^{-1}) (n = 3).

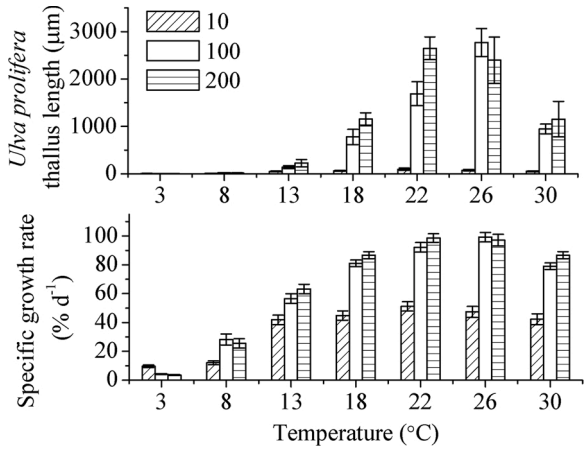

Fig. 4. Final thallus length (μm) and specific growth rate (% d^{-1}) of *Ulva prolifera* germlings germinated from parthenogametes at designed temperatures (°C) and photosynthetically active radiation (PAR) (μmol·m^{-2}·s^{-1}) at the end of the experiment (n = 3).

thallus at 10 μmol m^{-2}·s^{-1} and 30 °C was dark green in color, with cells well arranged in a regular longitudinal pattern, and each cell had several chloroplasts and one pyrenoid (Fig. 7A). However, the thalli grown at other PAR conditions appeared to be seriously impacted by high PAR combined with high temperature (Fig. 7B and C).

4. Discussion

4.1. Contributions of Ulva prolifera parthenogametes to blooms

The results of present study showed that *Ulva prolifera* parthenogametes could not germinate at 5 °C, but can survive over two months at 3 °C as single cells regardless of PAR (10–200 μmol m^{-2}·s^{-1}). It also indicated that the germination rate of *U. prolifera* parthenogametes was higher at 10 μmol m^{-2}·s^{-1} compared with those at other PARs when these parthenogametes were transferred from 3 °C to 13 °C. Parthenogenesis is considered an important strategy to *U. prolifera* blooms in the southern Yellow Sea (Ma et al., 2009; Zhang et al., 2011a,b). Therefore, the capacity of *U. prolifera* parthenogametes to survive under low temperatures and low PAR, and to germinate quickly when temperature becomes preferable significantly contribute to the initial *Ulva* blooms in the southern Yellow Sea. During the winter months, the seawater temperature is lower than 5 °C in the southern Yellow Sea (Huo et al., 2015; Song et al., 2015a,b). The irradiance on the sea surface in the mid intertidal areas in *Neopyropia* J. Brodie & L.-E.Yang, aquaculture regions of the southern Yellow Sea was relatively high (Huo et al., 2013; Keesing et al., 2016). However, the high turbidity of seawater, which was mainly caused by large amounts of different-sized suspended particles, can reduce the radiation reaching parthenogametes. It was reported that *U. prolifera* parthenogametes appear to be mainly attached on the *Neopyropia* rafts in the original area of green tides in the southern Yellow Sea (Huo et al., 2013, 2016). The *U. prolifera* parthenogametes which were attached to the rafts can be protected from relatively high irradiance, which is supported by the results of the present study. Therefore, *U. prolifera* parthenogametes can successfully survive over the cold winter period at the origin of the green tides in the southern Yellow Sea.

The results of present study showed that *Ulva prolifera* parthenogametes have high germination rates and growth rates at suitable

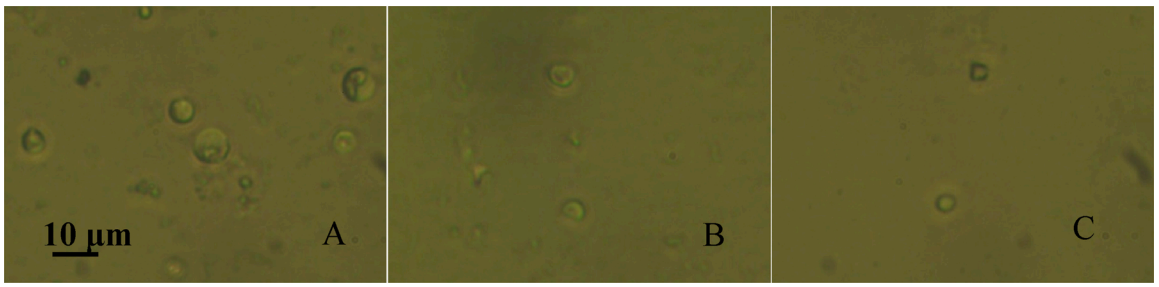

Fig. 5. Parthenogametes of *Ulva prolifera* at 3 °C and 10 μmol $m^{-2}·s^{-1}$ (A), 100 μmol $m^{-2}·s^{-1}$ (B) and 200 μmol $m^{-2}·s^{-1}$ (C) after 60 days of cultivation.

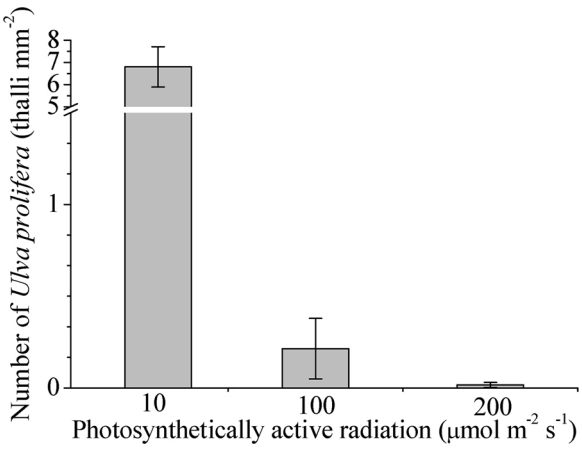

Fig. 6. Number of *Ulva prolifera* germlings (thalli mm^{-2}) germinated from parthenogametes when temperature increased from 3 °C to 13 °C at different photosynthetically active radiation (PAR) conditions (μmol·$m^{-2}·s^{-1}$) after 60 days (n = 3).

temperatures combined with adequate PAR (Figs. 2,3). The surface seawater temperature gradually increased starting in March in the southern Yellow Sea (Huo et al., 2013, 2016), and from late May to August, ranged of 18 °C–28 °C during the period when green macroalgal blooms occurred (Huo et al., 2014). When temperatures became preferable, *Ulva prolifera* parthenogametes, which survived the cold winter season, can quickly germinate, grow rapidly and mature into gametophytes (Zhang et al., 2015). These mature *U. prolifera* vegetative thalli can contribute significantly to the initial stages of green tides during the *Neopyropia* harvest period in the southern Yellow Sea (Liu et al., 2010; Zhang et al., 2014; Wang et al., 2015). When annual *U. prolifera* blooms largely occurred from late May to August, the free-floating *Ulva* vegetative thalli may have become the substrate for *U. prolifera* micropropagules that were in the water column (Zhang et al., 2014).

The results of present study showed that the mature gametophytes of *U. prolifera* germinated from parthenogametes could survive over two months at the temperature of 30 °C combined with low irradiance. When the seawater temperature increased to more than 28 °C during the period from July to August in the southern Yellow Sea, *Ulva* blooms generally disappeared (Huo et al., 2014). Even though most of *Ulva* biomass disappeared from the southern Yellow Sea, some attached *Ulva* biomass which resided in low PAR areas could have survived this period, such as in *Neopyropia* aquaculture regions. When the seawater temperature decreased to 20−25 °C during the period from September to October in the southern Yellow Sea, the surviving mature *U. prolifera* gametophytes can recover and again contribute to the *Ulva* biomass in the in the following year.

4.2. Germination and growth of Ulva prolifera parthenogametes

It is certain that the germination of *Ulva prolifera* parthenogametes could be influenced by different environmental factors (Lotze et al., 1999; Sousa et al., 2007; Agrawal, 2009). The present results clearly suggest that the germination of *U. prolifera* parthenogametes is affected by temperature and PAR. The highest germination rate of *U. prolifera* parthenogametes was obtained at 20 °C. Our results are in contrast to the germination rates of *U. prolifera* microscopic propagules reported by Song et al. (2015a,b) who reported that the germination rate of *U. prolifera* microscopic propagules maximized at 15 °C. *Ulva prolifera* parthenogametes did not germinate at 5 °C, which was in accordance with the results reported by Liu et al. (2012) and Song et al. (2015a,b). Germination of *U. prolifera* parthenogametes was not observed at 35 °C, which indicates that high temperatures are lethal to *U. prolifera* parthenogametes. Highest germination rate of the parthenogametes was 88.10 ± 0.80 % at 120 μmol $m^{-2}·s^{-1}$, which is similar to the results reported by Zhang et al. (2013). *Ulva prolifera* parthenogametes did not germinate in the dark environment, which was in accordance with the results reported by Liu et al. (2012) and Song et al. (2015a,b), however, Kolwalkar et al. (2007) reported that *Ulva flexuosa* spores could germinate in complete darkness. These results indicated that the demand for light for germination in various *Ulva* species is species-specific. In the southern Yellow Sea, salinity and pH varies from 27.5–30.9 and 7.24–8.10, respectively (Huo et al., 2014, 2016), and nutrients are continuously supplied through the Yangtze River and other nearby rivers (Liu et al., 2013). These ranges of salinity, pH, and nutrient concentrations are all suitable for *U. prolifera* parthenogametes to

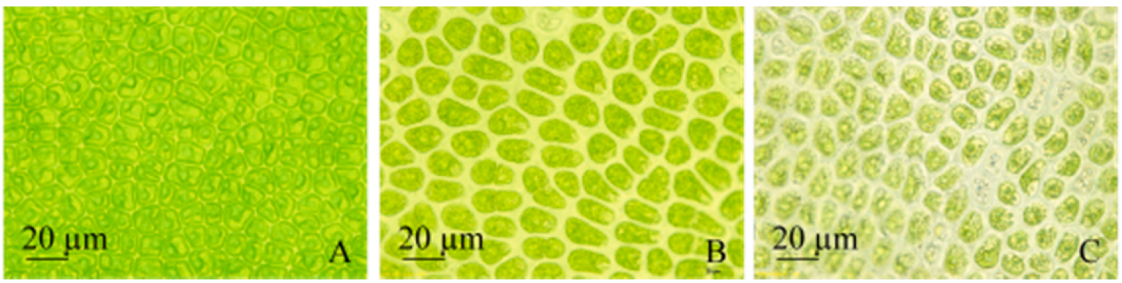

Fig. 7. *Ulva prolifera* thallus after 60 days of cultivation at 30 °C combined with photosynthetically active radiation (PAR) of 10 μmol $m^{-2}·s^{-1}$ (A), 100 μmol $m^{-2}·s^{-1}$ (B) and 200 μmol $m^{-2}·s^{-1}$ (C).

germinate.

The fate of *Ulva* microscopic propagules in darkness has been of interest to some researchers. It was found that only 35 % of *Ulva flexuosa* zoospores survived after 51 days in darkness (Kolwalkar et al., 2007). Santelices et al. (2002) report that zoospores of *U. intestinalis* and *U. compressa* were able to germinate in the dark at 15 °C. In the present study, *U. prolifera* parthenogametes survived for 60 days at 3 °C regardless of PAR. They were able to germinate and grow quickly when the temperature increased to 13 °C. These results suggest that low temperature is the key environmental factor that restricts the germination of *U. prolifera* in this alga (Schories, 1995). It is interesting to note that the germination rate of *U. prolifera* parthenogametes was significantly higher at 10 μmol m^{-2}·s^{-1} than those at other higher PAR conditions when the temperature increased to 13 °C. This result suggests that high PAR may negatively affect the survival of *U. prolifera* parthenogametes at 3 °C. Song et al. (2015a,b) reported that *Ulva* microscopic propagules did not germinate at 5 °C, but they did not report the effects of PAR on the survival of *Ulva* microscopic propagules under low temperatures. To our knowledge, there has not been any study on the effects of PAR on the survival of *Ulva zooids* (zoospores and gametes).

After attachment on substrates, *Ulva prolifera* parthenogametes can rapidly germinate and grow under suitable environments. Our results showed that the maximum growth rate of *U. prolifera* germlings was 99.2 %d^{-1}, which is higher than 78.9 % d^{-1} and 74.5 % d^{-1} reported by Tian et al. (2010) and Cui et al. (2015). The growth rate of *U. prolifera* germlings was 79.0 ± 2.3 %$d^{-1}$and 83.1 ± 2.4 %d^{-1} at the temperature of 30 °C combined with the PAR of 100 and 200 μmol m^{-2}·s^{-1}, respectively, which indicated that these *U. prolifera* germlings have a high tolerance to high temperature.

In summary, we report the responses of the germination and growth of *Ulva prolifera* parthenogametes to different combinations of temperature and PAR. The results of present study indicate that *U. prolifera* parthenogametes may largely contribute to green macroalgal blooms in the southern Yellow Sea due to their high germination and growth rates. The parthenogametes will make significant contributions to future green tide events since these micropropagules can survive winter conditions.

Author statement

The authors thank all the staff and particularly, Yuanzi Huo, Jang Kyun Kim and Charles Yarish conceived and designed the experiments; Yuanzi Huo performed the experiments and wrote the paper; Yuanzi Huo, Simona Augyte and Peimin He analyzed the data.

No any potential financial or other interests could be perceived to influence the outcomes of the research.

No conflicts, informed consent, human or animal rights applicable exit in the submission of this manuscript.

This manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

Y. Huo acknowledges the support by the Public Science and Technology Research Funds Projects of the Ocean (201205010), the National Science & Technology Pillar Program (2012BAC07B03), the National Natural Science Foundation of China (41206111), the Shanghai Universities First-class Disciplines Project (Discipline name: Marine Science (0707)), the Plateau Peak Disciplines Project of Shanghai Universities (Marine Science 0707). C. Yarish acknowledges support from Perkin-Elmer research funds to the Seaweed Marine Biotechnology Labs. We thank Dr. Cui Jianjun from Shanghai Ocean University for supplying the parthenogamete pictures. Thanks is also due to the anonymous reviewers for their valuable comments and suggestions.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aquabot.2020.103343.

References

Agrawal, S.C., 2009. Factors affecting spore germination in algae-review. Folia Microbiol. 54, 273–302.
Arasaki, S., Shihira, I., 1959. Variability of morphological structure and mode of reproduction in *Enteromorpha linza*. Jpn. J. Bot. 17, 92–100.
Bliding, C.V., 1963. A critical survey of European taxa in Ulvales. Part I:*Capsosiphon, Percursaria, Blidingia, Enteromorpha*. Opera Bot. 8, 1–160.
Burrows, E.M., 1991. Seaweeds of the British Isles. In: Chlorophyta, vol. 2. National History Museum Publications, London, p. 238.
Cui, J.J., Zhang, J.H., Huo, Y.Z., Zhou, L.J., Wu, Q., Chen, L.P., Yu, K.F., He, P.M., 2015. Adaptability of free-floating green tide algae in the Yellow Sea to variable temperature and light intensity. Mar. Pollut. Bull. 101, 660–666.
Cui, J., Monotilla, A.P., Zhu, W., Takano, Y., Shimada, S., Ichihara, K., Matsui, T., He, P., Hiraoka, M., 2018a. Taxonomic reassessment of *Ulva prolifera* (Ulvophyceae, Chlorophyta) based on specimens from the type locality and Yellow Sea green tides. Phycologia 57 (6), 692–704.
Cui, J.J., Shi, J.T., Zhang, J.H., Wang, L.T., Fan, S.Y., Xu, Z.Y., Huo, Y.Z., Zhou, Q.Y., Lu, Y.W., He, P.M., 2018b. Rapid expansion of *Ulva* blooms in the Yellow Sea, China through sexual reproduction and vegetative growth. Mar. Pollut. Bull. 130, 223–228.
Fletcher, R.T., 1996. The occurrence of "green-tide''. In: Schramm, W., Nienhuis, P.H. (Eds.), Marine Benthic Vegetation: Recent Changes and the Effects of Eutrophication. Springer Verlag, pp. 7–43.
Gao, S., Chen, X.Y., Yi, Q.Q., Wang, G.C., Pan, G.H., Lin, A.P., Peng, G., 2010. A strategy for the proliferation of *Ulva prolifera*, main causative species of green tides, with formation of sporangia by fragmentation. PLoS One 5 (1), e8571.
Geng, H.X., Yan, T., Zhou, M.J., Liu, Q., 2015. Comparative study of the germination of *Ulva prolifera* gametes on various substrates. Estuar. Coast. Shelf Sci. 163, 89–95.
Hernández, I.G., Peralta, G., Pérez-lloréns, J.L., Vergara, J.J., Niell, F.X., 1997. Biomass and dynamics of growth of *Ulva* species in Palmones River Estuary. J. Phycol. 33, 764–772.
Hiraoka, M., Yoshida, G., 2010. Temporal variation in isomorphic phase and sex rations of a natural population of *Ulva pertusa* (Chlorophyta). J. Phycol. 46, 882–888.
Hiraoka, M., Dan, A., Shimada, S., Hagihira, M., Migita, M., Ohno, M., 2003. Different life histories of *Enteromorpha prolifera* (Ulvales, Chlorophyta) from four rivers on Shikoku Island, Japan. Phycologia 42, 275–284.
Hiraoka, M., Ichihara, K., Zhu, W.L., Ma, J.H., Shimada, S., 2011. Culture and hybridization experiments on an *Ulva* clade including the Qingdao strain blooming in the Yellow Sea. PLoS One 6 e19371.
Hoxmark, R.C., 1975. Experimental analysis of the life cycle of *Ulva mutabilis*. Bot. Mar. 18, 123–129.
Huo, Y.Z., Zhang, J.H., Chen, L.P., Yu, K.F., Chen, Q.F., He, Q., He, P.M., 2013. Green algae blooms caused by *Ulva prolifera* in the southern Yellow Sea: Identification of the original bloom location and evaluation of biological processes occurring during the early northward floating period. Limnol. Oceanogr. 58 (6), 2206–2218.
Huo, Y.Z., Hua, L., Zhang, J.H., Cui, J.J., Huang, X.W., Yu, K.F., Shi, H.H., He, P.M., Ding, D.W., 2014. Abundance and distribution of *Ulva* microscopic propagules associated with a green tide in the southern coast of the Yellow Sea. Harmful Algae 39, 357–364.
Huo, Y.Z., Han, H.B., Shi, H.H., Wu, H.L., Zhang, J.H., Yu, K.F., Xu, R., Liu, C.C., Zhang, Z.Z., Liu, K.F., He, P.M., Ding, D.W., 2015. Changes to the biomass and species composition of *Ulva* sp. on *Porphyra* aquaculture rafts, along the coastal radial sandbank of the southern Yellow Sea. Mar. Pollut. Bull. 93, 210–216.
Huo, Y.Z., Han, H.B., Hua, L., Wei, Z.L., Yu, K.F., Shi, H.H., Kim, J.K., Yarish, C., He, P. M., 2016. Tracing the origin of green macroalgal blooms based on the large-scale spatio-temporal distribution of *Ulva* microscopic propagules and settled mature *Ulva* vegetative thalli in coastal regions of the Yellow Sea, China. Harmful Algae 59, 91–99.
Kapraun, D.F., 1970. Field and cultural studies of *Ulva* and *Enteromorpha* in the vicinity of Port Aransas, Texas. Contrib. Mar. Sci. 15, 205–285.
Keesing, J.K., Liu, D.Y., Shi, Y.J., Wang, Y.J., 2016. Abiotic factors influencing biomass accumulation of green tide causing *Ulva* spp. on *Pyropia*culture rafts in the Yellow Sea, China. Mar. Pollut. Bull. 105, 88–97.
Kim, K.Y., Ahn, Y.S., Lee, I.K., 1991. Growth and morphology of *Enteromorpha linza* (L.) J. Ag. and *E. prolifera*(Müller) J. Ag. (Ulvales, Chlorophyceae). Korean J. Phycol. 6, 31–45.
Kolwalkar, J.P., Sawant, S.S., Dhargalkar, V.K., 2007. Fate of *Enteromorpha flexuosa* (WULFEN) J. AGARDH and its spores in darkness: implications for ballast water management. Aquat. Botany 86, 86–88.
Lin, A.P., Shen, S.D., Wang, J.W., Yan, B.L., 2008. Reproduction diversity of *Enteromorpha prolifera*. J. Integr. Plant Biol. 50, 622–629.
Liu, D.Y., Keesing, J.K., Xing, Q.G., Shi, P., 2009. World's largest macroalgal bloom caused by expansion of seaweed aquaculture in China. Mar. Pollut. Bull. 58, 888–895.

Liu, D.Y., Keeding, J.K., Dong, Z.J., Zhen, Y., Di, B.P., Shi, Y.J., Ferans, P., Shi, P., 2010. Recurrence of the world's largest green-tide in 2009 in Yellow Sea, China: *Porphyra yezoensis* aquaculture rafts confirmed as nursery for macroalgal blooms. Mar. Pollut. Bull. 60, 1423–1432.

Liu, F., Pang, S.J., Zhao, X.B., Hu, C.M., 2012. Quantitative, molecular and growth analyses of *Ulva* microscopic propagules in the coastal sediment of Jiangsu province where green tides initially occurred. Mar. Environ. Res. 74, 56–63.

Liu, D.Y., Keesing, J.K., He, P.M., Wang, Z.L., Shi, Y.J., Wang, Y.J., 2013. The world's largest macroalgal bloom in the Yellow Sea, China: Formation and implications. Estuar. Coast. Shelf Sci. 129, 2–10.

Liu, Q., Yu, R.C., Yan, T., Zhang, Q.C., Zhou, M.J., 2015. Laboratory study on the life history of bloom-forming *Ulva prolifera* in the Yellow Sea. Estuar. Coast. Shelf Sci. 163, 82–88.

Lotze, H.K., Schramm, W., Schories, D., Worm, B., 1999. Control of macroalgal blooms at early developmental stages: *Pilayella littoralis* versus *Enteromorpha* spp. Oecologia 119, 46–54.

Ma, J.H., Ji, J.M., Xu, R., He, P.M., Zhang, T.F., Wang, X.K., Li, Y.H., Ren, S., Xu, P., Lu, Q.Q., 2009. Preliminary study on life history of *Ulva linza* Linnaeus (*Enteromorpha linza* (L.) J. Ag.). J. Fisheries China 33, 45–52 (In Chinese with English Abstract).

Morand, P., Merceron, M., 2005. Macroalgal population and sustainability. J. Coast. Res. 21, 1009–1020.

Ott, F.D., 1965. Synthetic media and techniques for the xenic cultivation of marine algae and flagellate. Va. J. Sci. 16, 205–218.

Raffaelli, D.G., Raven, J.A., Poole, L.J., 1998. Ecological impact of green macroalgal blooms. Oceanogr. Mar. Biol. 36, 97–125.

Samanta, P., Shin, S.K., Jang, S.J., Song, Y.-C., Oh, S.S., Kim, J.K., 2019. Stable carbon and nitrogen isotopic characterization and tracing nutrient sources of Ulva blooms around Jeju coastal areas. Environ. Pollut. 254 (Part A), 113033.

Santelices, B., Aedo, D., Hoffmann, A., 2002. Banks of microscopic forms and survival to darkness of propagules and microscopic stages of macroalgae. Rev. Chil. Hist. Nat. 75, 547–555.

Schories, D., 1995. Sporulation of *Enteromorpha* spp. (Chlorophyta) and overwintering of spores in sediments of the Wadden Sea, island Sylt, North Sea. Netherlands J. Aquat. Ecol. 29, 341–347.

Song, W., Peng, K.Q., Xiao, J., Li, Y., Wang, Z.L., Liu, X.Q., Fu, M.Z., Fan, S.L., Zhu, M.Y., Li, R.X., 2015a. Effects of temperature on the germination of green algae micro-propagules in coastal waters of the Subei Shoal. China. Estuar. Coast. Shelf Sci. 163, 63–68.

Song, W., Li, Y., Fang, S., Wang, Z.L., Xiao, J., Li, R.X., Fu, M.Z., Zhu, M.Y., Zhang, X.L., 2015b. Temporal and spatial distributions of green algae micro-propagules in the coastal waters of the Subei Shoal, China. Estuar. Coast. Shelf Sci. 163, 29–35.

Sousa, A.I., Martins, I., Lillebø, A.I., Flindt, M.R., Pardal, M.A., 2007. Influence of salinity, nutrients and light on the germination and growth of *Enteromorpha* sp. spores. J. Exp. Mar. Biol. Ecol. 341, 42–150.

Tian, Q.T., Huo, Y.Z., Zhang, H.Y., Li, X.S., Feng, Z.H., Wang, Y.Y., Zhang, Y.J., He, P.M., 2010. Preliminary study on growth and NH_4- N uptake kinetics of *Enteromorpha prolifera* and *Enteromorpha clathrata*. J. Shanghai Ocean U. 19, 252–258 (In Chinese with English Abstract).

Valiela, I., Mcclelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K., 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. 42, 1105–1118.

Wang, X.K., Ma, J.H., Ye, D.C., Chen, X.D., 2007. Preliminary study on the life history of *Enteromorpha prolifera*. Mar. Sci. Bull. 26, 112–116 (In Chinese with English Abstract).

Wang, Z.L., Xiao, J., Fan, S.L., Li, Y., Liu, X.Q., Liu, D.Y., 2015. Who made the world's largest green tide in China?—an integrated study on the initiation and early development of the green tide in Yellow Sea. Limnol. Oceanogr. 60, 1105–1117.

Wu, H.L., Kim, J.K., Huo, Y.Z., Zhang, J.H., He, P.M., 2017. Nutrient removal ability of seaweeds on *Pyropia yezoensis* aquaculture rafts in China's radial sandbanks. Aquat. Bot. 137, 72–79.

Wu, H.L., Huo, Y.Z., Yarish, C., Kim, J.K., He, P.M., 2018. Bioremediation and nutrients migration of the *Ulva* blooms in the Yellow Sea. Phycologia 57, 223–231.

Yarish, C., Edwards, P., 1982. Field and cultural studies on the seasonal and horizontal distribution of estuarine red algae of New Jersey. Phycologia 21, 112–124.

Yarish, C., Lee, K.W., Edwards, P., 1979. An improved apparatus for the culture of algae under varying regimes of temperature and light intensity. Bot. Mar. 22, 395–397.

Ye, N.H., Zhang, X., Mao, Y.Z., Zhuang, Z.M., Wang, Q.Y., 2008. Life history of *Enteromorpha prolifera* under laboratory conditions. J. Fish. Sci. China 15, 853–859 (In Chinese with English Abstract).

Zhang, H.W., Ma, J.H., Hu, X., Yang, J.Q., Zhang, T.F., Chen, B.B., Xu, R., Ye, S.F., 2011a. Reproductive characteristics of the floating algae in green tide. J. Shanghai Ocean. Univ. 20, 600–606 (In Chinese with English Abstract).

Zhang, X.W., Xu, D., Mao, Y.Z., Li, Y.X., Xue, S.Y., Zou, J., Lian, W., Liang, C.W., Zhuang, Z.M., Wang, Q.Y., Ye, N.H., 2011b. Settlement of vegetative fragments of *Ulva prolifera* confirmed as an important seed source for succession of a large-scale green tide bloom. Limnol. Oceanogr. 56, 233–242.

Zhang, J.H., Huo, Y., Yu, K.F., Chen, Q.F., He, Q., Han, W., Chen, L.P., Cao, J.C., Shi, D.J., He, P.M., 2013. Growth characteristics and reproductive capability of green tide algae in Rudong coast, China. J. Appl. Phycol. 25, 795–803.

Zhang, J.H., Huo, Y.Z., Yu, K.F., Kim, J.K., Yarish, C., Qin, Y.T., Liu, C.C., Xu, R., He, P. M., 2014. The origin of the *Ulva* macroalgae blooms in the Yellow Sea. Mar. Pollut. Bull. 89, 276–283.

Zhang, Q.C., Liu, Q., Kang, Z.J., Yu, R.C., Yan, T., Zhou, M.J., 2015. Development of a fluorescence in situ hybridization (FISH) method for rapid detection of *Ulva prolifera*. Estuar. Coast. Shelf Sci. 163, 103–111.

Zhang, J.H., Kim, J.K., Yarish, C., He, P.M., 2016. The expansion of *Ulva prolifera* O.F. Müller macroalgal blooms in the Yellow Sea, PR China, through asexual reproduction. Mar. Pollut. Bull. 104, 101–106.