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Physiological acclimation of the green tidal alga *Ulva prolifera* to a fast-changing environment

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ABSTRACT

To aid early warning and prevent the outbreak of green tides in the Yellow Sea, both the growth and photosynthetic performance of *Ulva prolifera* were studied after culture in different temperatures (18, 22, and 26 °C) and light intensities (44, 160, and 280 μmol $m^{-2}·s^{-1}$). Furthermore, their instantaneous net photosynthetic performance (INPP) was studied to determine the resulting environmental acclimation. The relative growth rates of *U. prolifera* significantly decreased in response to increasing temperature, while they increased with increasing light intensity. Culture at higher light intensities significantly increased INPP, while higher temperatures decreased the INPP. Culture at lower temperatures lowered INPP, while increased growth temperature increased the effect. These results suggest that high temperatures during the cold season inhibited *U. prolifera* growth. However, low temperatures during the warm season increase biomass and may cause a large-scale green tide. These results help to understand the correlation between *U. prolifera* blooms and extreme weather.

1. Introduction

Green tides as a consequence of the proliferation of green algae such as *Ulva* and *Chaetomorpha* have been reported worldwide. Considerable blooms of green macroalgae have already happened in Europe (Denmark, Netherlands, France, and England), Asia (China, Japan, and Korea), North America, and Australia (Choi et al., 2010; Kim et al., 2011). Since 2007, *Ulva* blooms have consecutively occurred along the coastal areas of the Yellow Sea. In June 2008, the world's largest green-tide with an affected area of about 600 km^2 occurred along the coast of the Yellow Sea near Qingdao in China, severely threatening the 2008 Olympic Games sailing regatta (China Ocean News, 2008; Liu et al., 2010), causing considerable economic loss for the local government (Liu et al., 2009). The enormous biomass of green algae was suggested to destruct marine ecosystems and destroy ecological service functions, since it promotes oxygen depletion of both the water column and the benthic environment (Lomstein et al., 2006). Furthermore, green algae can negatively impact coastal ecosystems via rapid expansion and via interfering with coastal nitrogen and carbon cycles (Lomstein et al., 2006; Nelson et al., 2008). Therefore, increasing focus has been directed to green tide research with particular attention on its blooming mechanisms. According to previous records, no signs were observed prior to the sudden bloom (Liu et al., 2010; Zhang et al., 2014).

Numerous researchers have tried to explain the *Ulva* bloom. According to previous studies, *Ulva* microscopic propagules were widespread throughout the southern Yellow Sea, and *Porphyra* aquaculture rafts contributed to the attachment of *Ulva* spores (Huo et al., 2014; Zhang et al., 2016). Moreover, different species of *Ulva* showed varied competitive advantages, such as *Ulva prolifera* showed higher nutrient uptake (eg. with an N uptake rate of 33.9 μmol g^{-1} DW·h^{-1} and P uptake rate of 11.1 μmol g^{-1} DW·h^{-1}), growth (eg. *U. prolifera* with a growth rate of 37%·d^{-1}) and diverse reproductive system than non-bloom forming *Ulva* species (Huo et al., 2013; Liu et al., 2013; Fan et al., 2014; Gao et al., 2017a,b). Especially the distinctive growth and reproductive strategies of *Ulva* spp., including enlarging tubular diameter, formation of new branches, release of zoids, and polarized growth, result in a high growth rate during green-tide formation (Ye et al., 2008; Zhang et al., 2016). These findings indicated *Ulva* as bloom-forming genus.

The influence of ecological factors on the growth and proliferation of *U. prolifera* have also been studied (Dan et al., 2002; Luo et al., 2012; Zhang et al., 2013; Gao et al., 2017a). Seaweeds enhance biomass via photosynthesis; however, excessive light intensity affects both photosynthesis and growth (Copertino et al., 2006; Gao et al., 2016). The level of nutrients including combined nitrogen, phosphorus, and dissolved inorganic carbon influences the photosynthesis, growth, and nutrient contents of macroalgae (Xu et al., 2014; Sjøtun et al., 2015; Ueno et al., 2017). Damaging nitrogen could increase the

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Table 1
Treatments of different light intensity and temperature.

Treatment	44 μmol $m^{-2}·s^{-1}$ (LL)	160 μmol $m^{-2}·s^{-1}$ (ML)	280 μmol $m^{-2}·s^{-1}$ (HL)
18 °C (LL)	LL-LT	ML-LT	HL-LT
22 °C (ML)	LL-MT	ML-MT	HL-MT
26 °C (HL)	LL-HT	ML-HT	HL-HT

photoprotective capacity of *Ulva* and sufficient nutrients dissolved in the Yellow Sea ensured the rapid growth of *Ulva* species (Zhang et al., 2013). In addition, Dan et al. (2002) reported the suitable salinity range for the release of reproductive cells of *U. prolifera* of about 13.2–45.3, with a light intensity above 16 μmol $m^{-2}·s^{-1}$. Furthermore, salinity levels of 20 and 35 have been suggested as helpful for the growth of *Ulva* spores (Sousa et al., 2007). Moreover, the photosynthesis and growth showed differences in response to different temperatures, CO_2 and pH levels (Xu and Gao, 2012; Li et al., 2016; Gao et al., 2017b).

In response to an increasing frequency of climatic fluctuations and extreme weather events, tolerance and adaptive performance of seaweeds to variable environmental factors have received more attention. Over the past 40 years, the temperature of the oceans increased with a rate of 0.1 °C per decade (IPCC, 2013). Since seaweeds showed a different tolerance to temperature, both abundance and distribution of seaweeds changed apparently after acclimation to environmental changes (Gallon et al., 2014; Sjøtun et al., 2015; Piñeiro-Corbeira et al., 2016). More recently, the correlation between the occurrence and distribution of seaweeds with temperature and varying salinity were reported (Sjøtun et al., 2015). Although the oceanic temperature follows an increasing trend in the long-term, temperature fluctuations have always been observed. A further study suggested that global warming enhances the frequency of extreme weather (Sun et al., 2016). When extreme weather occurs, environmental factors such as light intensity, transparency, and temperature change considerably during a short time, which raises new challenges for seaweed survival, even leading to succession and bloom of several species.

Previous studies have demonstrated the advantageous physiological characteristic of *Ulva*, particularly in response to climate changes (Xu and Gao, 2012; Cui et al., 2015; Sun et al., 2016; Gao et al., 2017b). However, no clear evidence was presented to date to explain the blooming mechanisms and the relationship between *Ulva* bloom and extreme weather. We therefore hypothesized that photosynthetic performances of *Ulva* serve to address the environmental variation. To test this hypothesis, we chose the dominate bloom species *U. prolifera* for this study and investigated its growth and photosynthetic performance when cultured at different light intensities and temperatures. For the first time, we exposed the experimental algae under instantaneous light intensities and temperature conditions to study their instantaneous photosynthetic performance. The results of this study will be helpful to understand the *Ulva* blooming mechanisms with relation to physiological acclimation. Particularly, the relationship between algae bloom and a fast-changing environment has been investigated, which will benefit the early warning and thus, the prevention of green tides.

2. Materials and methods

2.1. Sample collection and preparation of the Ulva materials

In May 2011, about 50 g thalli of *Ulva* at the vegetative state and at a length of about 7 cm were collected from the Lianyungang sea area (119.38 E, 34.58 N), Jiangsu province, China. The temperature and salinity at the sampling site were 22 °C and 28, respectively. Algae were cleaned of debris and epiphytes, gently rinsed using sterile seawater, and transported to the laboratory in a cool box at about 5 °C. Molecular methods were adopted to identify *Ulva prolifera* (Zhang et al., 2016). The healthy thalli of *U. prolifera* were cultured in tanks at 20 °C, 12:12 L:D photoperiod, and 100 μmol m^{-2} s^{-1} light intensity for two days prior to the experiments (Jiangnan, Ningbo, China). The natural seawater enrichment medium was obtained from sterile natural seawater under addition of 60 μM $NaNO_3$ and 8 μM KH_2PO_4 and gentle bubbling with filtered air.

2.2. Experimental design

Three light intensities (44, 160, and 280 μmol m^{-2} s^{-1}) and three temperatures (18, 22 and 26 °C) were set via GXZ-300C intelligent llumination incubators (Jiangnan, Ningbo, China). Therefore, a total of nine growing/instantaneous conditions were used (see Table 1). Healthy thalli were cultured in 500 ml conical flasks in filtered natural seawater (salinity 30, enriched with 60 μM NO_3^- and 8 μM PO_4^{3-}) at a stocking density of 0.02 g L^{-1}, and three triplicates were conducted per treatment. The photoperiod was set to 12:12 light:dark. The seawater medium was vigorously aerated and exchanged every two days; fresh weight was measured to evaluate the growth rate. After one week, the net photosynthetic rates (NPR) of *U. prolifera* were measured in the acclimation conditions and also after a sudden transfer to all conditions (i.e., 81 treatments were performed).

2.3. Measurement of growth

The fresh weight was obtained during the experiment and was used to calculate the growth rate of seaweeds according to the following formula: $RGR = 100*(lnW_2-lnW_1)/(T_2-T1)$, where *RGR* represents the relative growth rate (%·day^{-1}), W_1 and W_2 represent the fresh weight at days T_1 and T_2, respectively.

2.4. Measurement of net photosynthetic rate

A Clark-type oxygen electrode (YSI Model 5300, USA) was used to measure the net photosynthetic rates of *U. prolifera* in response to different conditions. The thalli were cut into segments with a length of about 1 cm, and then restored under growth conditions for 1 h (Zhou et al., 2016). After that, about 0.05 g of fresh weight thalli of every condition was introduced into a photosynthetic chamber containing 8 mL seawater. The photosynthetic rate was measured using normal seawater, the time for measurement was less than 6 min, and the O_2 concentration in seawater medium exceeded less than 10% at the end of measurement. The treatments under different light and temperature conditions were randomly distributed among the O_2 measurements.

2.5. Statistical analysis

All data analyses were conducted using the software Origin 9.0 and are displayed as mean ± standard deviation. A normal distribution (Shapiro-Wilk, $p > 0.05$) of the data under every treatment was conformed and the variances were equal (Levene's test, $p > 0.05$). Two-way or three-way ANOVA (Tukey's post-hoc test) were used to test for differences using the SPSS 17.0. $P < 0.05$ was considered to be significant.

3. Results

The effects of light and temperature on the growth of *U. prolifera* are