Variation of photosynthetic activity during the process of green tides induced by nutrient additions in the coastal sea of Qingdao, China

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Abstract. Three nutrient addition experiments were conducted to induce green tides of Ulva prolifera from April to August in 2016 in the coastal waters of Qingdao, China. Photosynthetic activity (Fv/Fm), biomass, nutrients and temperature were measured. We found that Fv/Fm of U. prolifera increased to the maximum about 0.7 ~ 0.75 under favorable environment, and declined significantly when the growth of U. prolifera was limited by nutrient and temperature. In addition, the results showed that Fv/Fm increased to the maximum prior to occurrence of green tide and fell significantly before green tide dissipated. Fv/Fm can be used to predict the outbreak and dissipation of green tides.

1. Introduction
Since 2007, large-scale green tides caused mainly by Ulva prolifera have occurred in the Yellow Sea for 11 consecutive years [1,2]. During 2016, the affected area of the green tide in the Yellow Sea about 57500 km², is the largest in the last five years. Green tides have many negative effects, such as severely impacting the local ecosystem structures, inducing hypoxic zone, jeopardizing fisheries and marine aquaculture industry, and leading to tourism losses [3-5]. Laboratory experiments, shipboard surveys and remote-sensing have conventionally been used to detect the developmental process of the green tides in Yellow Sea. Wet weight, Chlorophyll a contents and covered area of algae have been used as indicators of U. prolifera biomass. However, less research has focused on early warning of green tides. It is wise to detect the growth potential and green tide forming probability before any green tide break out.

Chlorophyll a fluorescence can determine the physiological state of algae rapidly, sensitively, and non-invasively [6,7]. The most commonly used parameter is the potential maximum PS II quantum yield (Fv/Fm), an index of photosynthetic activity of algae. Laboratory culture experiment and field investigation have suggested that Fv/Fm rose to the maximum and remained constant under suitable environment conditions, and decreased significantly when phytoplankton experience physiological stress such as nutrient limitation, unfavourable light and temperature [8,9]. Wang et al. found that in
the adjacent area of Changjiang River estuary, $F_v/F_m$ rose to higher value before outbreak of *Skeletonema* sp. and *Prorocentrum donghaiense* blooms and $F_v/F_m$ may be used to predict onset of *Skeletonema* sp. and *P. donghaiense* blooms [10].

Here we carried out 3 ship-based mesocosm experiments with nitrogen (N) and phosphorus (P) additions to induce *U. prolifera* blooms in the coastal sea of Qingdao, China, from March to August in 2016. $F_v/F_m$, biomass, temperature and nutrient concentrations were observed during the process of green tides to test whether $F_v/F_m$ could be used as an indicator of early warning of green tides.

2. Material and methods

2.1. Enclosure experimental design

Qingdao locates in the western part of the Yellow Sea, where green tides occurred frequently. Three ship-based nutrient enrichment experiments were conducted at station E (35°58′30″N 120°24′20″E) in the coastal sea of Qingdao on April 25, June 25 and August 20 in 2016, respectively (figure 1).

![Figure 1. The experimental station in the coastal sea of Qingdao.](image)

In each experiment, surface water was prescreened through 100 μm mesh to remove larger algae, and then injected into the 40-liter transparent polyethylene bag, where healthy *U. prolifera* was put in with initial biomass about 10g. 40 μmol·L⁻¹ NO₃⁻ and 5 μmol·L⁻¹ PO₄³⁻ were added to the bag at about 8 o’clock every day to keep nutrients sufficient. Experiments were performed on deck, in temperature-controlled water baths to approximate *in situ* light and temperature levels. $F_v/F_m$ and biomass of *U. prolifera*, dissolved inorganic nutrients and temperature were measured at the same time every few days.

2.2. $F_v/F_m$

$F_v/F_m$, which is calculated as $(F_m - F_0) / F_m$, was obtained by a water-PAM Fluorometer(Heinz Walz GMBH, Effeltrich, Germany). $F_0$ is the minimal fluorescence measured at a low light intensity about 5 μmol photons·m⁻²·s⁻¹. $F_m$ is the maximum fluorescence analysed by saturating light pulses at approximately 6000 μmol photons·m⁻²·s⁻¹ with 800ms duration. Samples of *U. prolifera* were dark adapted for 20 min before measurement.

2.3. Nutrients

Water samples were filtered through acid-washed 0.45 μm cellulose ester filters. Filtrates were frozen at −20°C and later measured for dissolved inorganic nitrogen (DIN = NO₃⁻+ NO₂⁻ + NH₄⁺) and phosphate (PO₄³⁻) by an automated segmented flow analyzer (QuAAtor, Bran+ Luebbe GmbH, Norderstedt, Germany). Detection limits were 0.01 μmol·L⁻¹ for NO₃⁻ and NO₂⁻, 0.034 μmol·L⁻¹ for
NH$_4^+$ and 0.02 μmolL$^{-1}$ for PO$_4^{3-}$.

2.4. Biomass and temperature

U. prolifera was sampled and drained to remove extra water. Then its biomass (expressed as wet weight) was obtained by electronic balance. Salinity and water temperature was determined by a portable CTD (AAQ1183, Alec Electronics Co., Japan).

3. Results and discussions

The initial conditions of the three experiments are shown in table 1. Some laboratory and field experiments have found that F$_v$/F$_m$ of U. prolifera increased to the maximum around 0.7~0.75 under favorable environment conditions, and declined observably when U. prolifera is limited by nutrients, temperature, light and salinity[2-4].

| Table 1. Initial conditions for the three mesocosm experiments. |
|---|---|---|---|---|
| Experiment | Date | Temperature (°C) | Salinity | DIN (μmolL$^{-1}$) | PO$_4^{3-}$ (μmolL$^{-1}$) |
| E1 | 4/25/2016 | 13.2 | 30.5 | 7.26 | 0.24 |
| E2 | 6/25/2016 | 21.0 | 30.8 | 4.84 | 0.13 |
| E3 | 8/20/2016 | 26.1 | 30.7 | 5.31 | 0.16 |

Figure 2. Variations in parameters during nutrient addition in experiment E1.

In experiment E1, on April 25, the growth of U. prolifera was limited by low water temperature and F$_v$/F$_m$ decreased gradually, although nitrogen and phosphorus were replete. With the water
temperature rising to more than 17 °C, suitable for the growth of *U. prolifera*, \( F_v/F_m \) enhanced to more than 0.7 promptly on May 28, indicating that *U. prolifera* was in a healthy state. Subsequently, biomass of *U. prolifera* rose sharply and green tide broke out on May 31 (figure 2).

In experiment E2, because of favourable temperature and nutrients, \( F_v/F_m \) maintained higher than 0.7, biomass of *U. prolifera* rocketed and green tide formed. With the addition of nutrients stopped on July 1 and nitrogen run out, \( F_v/F_m \) fell to below 0.7 on July 5. Then the biomass of *U. prolifera* began to decrease on July 11, and the green tide gradually died out (figure 3).

**Figure 3.** Variations in parameters during nutrient addition in experiment E2.

In experiment E3, the growth of *U. prolifera* was stressed by excessive temperature, although nutrients were sufficient. \( F_v/F_m \) fell before the decrease of biomass and decreased to 0.29 on August 30 (figure 4).

Similar to the research of Wang et al. on red tides [10], our experiments showed that \( F_v/F_m \) rising to the maximum happened a few days prior to marked increases in biomass of *U. prolifera* and occurrence of green tide. Furthermore, we also found \( F_v/F_m \) declined significantly before the green tide dissipated. The mentioned above indicated that \( F_v/F_m \) can be used to predict the outbreak and dissipation of green tides and can provide more rapid results than biomass in early warning of green tides.
Figure 4. Variations in parameters during nutrient addition in experiment E3.

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