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Responses of *Ulva prolifera* to short-term nutrient enrichment under light and dark conditions

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ABSTRACT

To define responses of short-term nutrient uptake in *Ulva prolifera*, we measured uptake rates, enzyme activity, and tissue nutrient content in lab experiments where we manipulated nutrient supply and irradiation. Nitrate uptake of *U. prolifera* was significantly impacted by the external nitrate concentrations, and ammonium uptake was mainly determined by the light availability. The measured nitrogen contents in tissues were higher than the calculated values from the uptake of dissolve inorganic nitrogen, indicating that *U. prolifera* might use multiple nitrogen sources. High external phosphate concentrations and sufficient light can accelerate the phosphate uptake of *U. prolifera*, while the measured phosphorus contents in tissues were lower than the calculated values from the uptake of phosphate, suggesting a possibility of internal phosphorus release. The enzymatic activities of nitrate reductase (NR), acid phosphatase (AcP) and alkaline phosphatase (AP) showed little changes, indicating that enzymatic activity might not a direct factor determining the short-term nutrient uptake of *U. prolifera*.

1. Introduction

Macroalgal blooms of the genus *Ulva* driven by nutrient enrichment were well documented worldwide over the last century (Valiela et al., 1997; Dailer et al., 2012). To understand the mechanism between nutrient enrichment and macroalgal blooms, nutrient-enriched experiments were extensively carried out in field and laboratory, and the results demonstrated a series of important physiological characteristics of *Ulva* species in nutrient uptake (e.g., Fong et al., 2004; Teichberg et al., 2008, 2010; Dailer et al., 2012). *Ulva* species can regulate nutrient uptake rates, tissue nutrient contents and enzymatic activities in response to the variable environmental conditions (e.g., light, salinity, and nutrient increase) (Cohen and Fong, 2004; Choi et al., 2010; Chow, 2012). The prior storage of nutrient in algal tissue can affect the capability to nutrient uptake (Fong et al., 2003; Teichberg et al., 2007; Kennison et al., 2011). High nitrogen (N) storage can decrease the uptake rates of dissolved inorganic nitrogen (DIN) and increase nitrate reductase (NR) activity. Kennison (2008) even found that *Ulva* spp. in bloom conditions, across all seasons, may not assimilate nitrogen effectively due to the storage of nitrogen during the eutrophic period. In comparison, little was known about the physiological response of *Ulva* species to short-term nutrient variations. The enzymatic activity determining the uptake of phosphorus (P) was generally measured in a few days. For example, in *U. lactuca* under P-deficient conditions, acid phosphatase (AcP) and alkaline phosphatase (AP) activities were detected after a culture of 1–4 days (Weich and Granéli, 1989; Lee, 2000). NR activities were measured in hours (Lartigue and Sherman, 2005) and in days (Teichberg et al., 2007). However, few differences were found among nitrate-enriched treatments in hours (Lartigue and Sherman, 2005).

Ulva prolifera blooms in the Yellow Sea were mainly developed and accumulated during the drifting in coastal and offshore waters (Liu et al., 2013), where the environmental conditions (e.g., nutrients, light, salinity and temperature) changed continuously. It indicated a physiological character of *U. prolifera* rapidly adapting to environmental change. Therefore, it is important to understand the short-term physiological response of *U. prolifera* to the variable environment, and this can help to explain the formation of drifting bloom. In this study, the manipulated experiment was designed in laboratory, with four treatments (2 light levels × 2 nutrient levels) for the nutrient uptake of *U. prolifera*. The nutrient uptake rates,

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enzyme activities, and nutrient contents in algal tissue were measured within the three hours after experimental treatment, with an aim of knowing whether the short-term responses of *U. prolifera* to the light and nutrient variations are beneficial to the formation of *U. prolifera* bloom during early period.

2. Materials and methods

2.1. Algae collection

Fresh thalli of *Ulva prolifera* were collected from *Porphyra* rafts in Rudong, Jiangsu province, China (32°40′53″N, 121°05′24″E) on April 30, 2013. The algal thalli were immediately transported to the laboratory in a cool beaker. The intact healthy thalli were carefully chosen, and they were rinsed gently using filtered sterilized seawater to remove debris and epiphytes.

2.2. Experimental design

Approximately 0.4 ± 0.1 g of thalli were cultured in a transparent plastic bowl containing 250 mL of filtered seawater (salinity 31.7) at 20 °C (Table 1). To inhibit the growth of diatoms, 0.2 mg/L of germanium oxide (GeO_2) was added to the culture medium. The sterilized natural seawater was collected from Qingdao coast, and modified f/8-enriched natural seawater was used for the NP-enriched treatments (Guillard, 1975). The nutrient concentrations (C_0) are listed in Table 1. Three samples were examined for the initial nutrient concentrations (*% tissue* $N_{measured}$, *% tissue* $P_{measured}$) and enzymatic activities (NR, AcP and AP) of the algal tissue at Time 0 through the procedures described below. These samples were not acclimated in lab conditions and represented the origin status of *Ulva prolifera* from the *Porphyra* rafts in Rudong coast.

The cultures were treated at two nutrient levels (N, P-enriched and control) crossed with two light levels (light and dark, Table 1), resulting in four treatments: nitrogen (nitrate and ammonium) and phosphorus (phosphate)-enriched with light *(NPL)*; NP-enriched under dark conditions (*NPD*); control under light conditions (*CL*); and control under dark conditions (*CD*). There were 9 cultures for each treatment, and 36 cultures in total. For each treatment, algae from three out of the 9 cultures were harvested at 1-hr intervals (Time 1, 2 and 3 h). The nutrient concentrations (*% tissue* $N_{measured}$, *% tissue* $P_{measured}$) and the enzymatic activities of the algal tissues were measured individually using the methods described below. The nutrient concentrations (C_t) of the culture media were examined simultaneously to calculate how much nutrient was absorbed by the algae.

2.3. Measurements of medium nutrient concentrations, enzymatic activities and tissue nutrient concentrations

The water samples were filtered through 0.22 μm cellulose acetate membranes and stored at −20 °C. A segmented continuous flow analyzer (SAN^{++} SYSTEM , Skalar Analytical B.V., Breda, Netherlands) was used to measure the nutrient concentrations (C_t) of the water samples, including the concentrations of nitrate, ammonium and phosphate.

The nutrient uptake rate (*Ur*, $\mu mol \bullet g_{FW}^{-1} \bullet h^{-1}$) of *Ulva prolifera* at each sampling was then calculated as:

$$Ur = \frac{(C_0 - C_t) \times V}{m \times t}$$

where C_0 and C_t are the nutrient concentrations ($\mu mol \bullet L^{-1}$) of water at Time 0 and *t* h, respectively; *t* is the culture period (h); *V* is the volume of the culture medium (L); and *m* is the fresh weight of the testing algae (g_{FW}).

The gain or loss of ammonium (mg, NH_4—N) and nitrate (mg, NO_3—N) was calculated as:

$$M = \frac{(C_0 - C_t) \times Mr \times V}{1000}$$

where C_0 and C_t are the ammonium or nitrate concentrations (μM) of water at Time 0 and *t* h, respectively; *V* is the volume of the culture medium (L); *Mr* is the relative molecular mass of N.

The tissue nutrient concentrations (*% tissue* $N_{measured}$, *% tissue* $P_{measured}$) were determined according to the method modified from Sjöö and Mörk (2009). In general, 0.20 ± 0.05 g_{FW} of the algal tissues were dried at 60 °C for 12 h and ground into a fine powder. The powder was dried for another 12 h at 60 °C and weighed. Then, approximately 5.0 ± 2.0 mg of dry powder was used for measuring tissue N (mg) using a Vario ELIII Elemental analyzer (Elementar Analysen systeme GmbH, Hanau, Germany). To determine the tissue P, approximately 5.0 ± 2.0 mg of dry powder was combusted for 3 h at 450 ~ 480 °C in a muffle furnace and was then digested in a persulfate solution (50 g $K_2S_2O_8$ + 30 ml H_2SO_4 L^{-1}) for 1 h at 120 °C. The total amount of P (mg) in the dried powder was determined colorimetrically using a phosphomolybdenum blue reagent solution (Strickland and Parsons, 1972). The tissue nutrient concentrations (*% tissue* $N_{measured}$ and *% tissue* $P_{measured}$) were expressed as the percentages of the elemental masses (N and P, mg) per unit of dry weight (DW, mg).

Previous studies reported that *Ulva* spp. utilized multiple sources of nutrients other than DIN and phosphate (Tyler et al., 2001; Fong et al., 2004; Runcie et al., 2004; Tyler et al., 2005; Shi et al., 2015). To test whether other forms of nutrients (N, P) were used during the experiments, we estimated the total amount of nutrients (*% tissue* $N_{predicted}$, *% tissue* $P_{predicted}$) derived from the DIN (DW_{DIN}) and phosphate ($DW_{phosphate}$) absorbed by *Ulva prolifera* from the culture medium. We compared the estimated amount of nutrients with the real measurements of the nutrients (*% tissue* $N_{measured}$, *% tissue* $P_{measured}$) in tissues after a period of culturing (*t*). The equations calculating DW_{DIN}, $DW_{phosphate}$, *% tissue* $N_{predicted}$, *% tissue* $P_{predicted}$, and *% tissue* $N_{measured\text{-}predicted}$, *% tissue* $P_{measured\text{-}predicted}$ at *t* h are listed below:

Table 1
Experimental design and culture conditions.

Irradiation (μmol photon•m^{-2}•s^{-1})		Initial nutrient concentration (μM)		Salinity	Temperature (°C)	pH
Light (L)	Dark (D)	NP-enriched (NP)[a]	Control (C)[b]			
80	0	Phosphate: 13.81 Nitrate : 255.27 Ammonium : 12.33	Phosphate: 5.40 Nitrate: 15.35 Ammonium: 7.82	31.7	20 ± 1	8.00 ± 0.02

[a] Modified f/8-enriched natural seawater according to Guillard (1975).
[b] Natural seawater collected from Shazikou, Qingdao.