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Maintenance mechanism of *Enteromorpha prolifera* green tide: From perspective of nutrients utilization

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Green tide caused by macroalgae is one of the global ocean ecological disasters and nutrients with high concentration are considered as materials base for the outbreak of green tide. Nevertheless, there is no continuous nutrients supply for macroalgae during their floating in sea areas. Thus, there must be special nutrients utilization strategy for the macroalgae to maintain growth and proliferation even if the nutrients in seawater can not supply enough nutrients for them. To verify the hypothesis, *Enteromorpha prolifera* responsible for green tide was exposed to nutrients with different concentrations. *E. prolifera* absorbed and stored excrescent nutrients when it encountered nutrient eutrophication, then released and reutilized those stored nutrients for growth and proliferation in the nutrient-shortage seawaters. Thus, the green tide can be maintained by the nutrients regulation ability of *E. prolifera*. Results of the present work may be helpful to provide enlightenment on prediction and controlling of macroalgae green tide.

[Keywords: *Enteromorpha prolifera;* Green tide; Nutrient enrichment; Nutrient shortage; Nutrient starvation]

Introduction

Green tide caused by the multiplication of macroalgae is one of the global ocean ecological disasters[1], and it occurs frequently in the lagoon, estuary and coastal zones which are heavily influenced by intensive anthropogenic activities such as urbanization, aquaculture and agriculture. Because of its huge negative ecological effects including decreasing biodiversity[2], potential dissolved oxygen concentration reduction, massive nutrient regeneration from decayed macroalgae[3] and reduction of aesthatic value of seawater landscape[4], green tides get more and more attention from governments, researchers and ordinary public[5,6], e.g., the yearly eruptive *Enteromorpha prolifera* green tide in the Yellow Sea of China from 2007 is listed as one of the serious ocean ecological disasters that should be curbed by establishing early warning system[7,8]. Moreover, lots of environmental factors such as water temperature, wind and current speed, topography and livestock breeding along the coastal zones may lead to outbreak of green tide[9], and the nutrients with high concentration are considered as material bases for the outbreak of green tide[8]. The correlation analysis of field investigation on green tide seems to support the conclusion that nutrient eutrophication leads to the outbreak of green tide[9]. In general, the macroalgae multiply and develop into the green tide when they drift across different sea areas under wind and current. Nevertheless, because of the heterogeneity of nutrients distribution, there is no continuous nutrients supply for the macroalgae responsible for green tide during their floating in seawaters[10]. Moreover, the outbreak of the green tide depends not only on the fast growth, but also on the quick proliferation of macroalgae, both of which are independent of nutrients[11]. Thus, there must be special nutrients utilization strategy for the macroalgae to maintain growth and proliferation even if the nutrients in seawaters can not supply enough nutrients for them during the floating process. The hypothesis is related to the outbreak and the maintenance mechanism of macroalgae green tide. Nevertheless, there are few data about the nutrient utilization strategy of the macroalgae responsible for the green tide, which implies that it is difficult to establish an early warning system to prevent and control the green tide.

E. prolifera is one typical species of macroalgae responsible for green tide[9], hence, it was used in the present work to evaluate the hypothesis, during which the macroalgae was exposed to nutrients with different concentrations. The result of the present work may be helpful to provide enlightenment on prediction and to control macroalgal green tide.

Materials and methods

Enteromorpha prolifera responsible for green tide

In July of 2014, *E. prolifera* was sampled from the First Bathing Beach of Qingdao, Shandong Province, China (36.06° N,120.34° E), where *E. prolifera* green tide outbroke yearly since 2007. After washing by degreasing cotton filtered seawater, the healthy *E. prolifera* was temporary reared for one week to make the species nutrient starved[12], during which the water temperature, the illumination intensity and the illumination period was 20 ± 1 °C, 4000 - 4500 Lux and 12 L : 12 D, respectively. Moreover, some algae washed by de-ionized water were dried to get a constant weight at 60 °C to determine the water content in the species of macroalga.

Nutrient dynamics of *E. prolifera* exposed to nutrients eutrophication and shortage

After nutrient starvation, the healthy *E. prolifera* washed by the filtered seawater was put into a conical flask with 500 ml seawater. These macroalgae were exposed to nutrients with different concentrations (Table 1), during which the nitrogen (N) and the phosphorus (P) was presented by NH_4Cl and NaH_2PO_4, respectively. Moreover, the biomass of experimental macroalgae was 0.50 ± 0.01 g/L (calculated by dry weight). Furthermore, the seawater without nutrients addition was regarded as the normal seawater with low-nutrient concentration. The culture solution without macroalgae was considered as the corresponding blank control. These flasks covered by para-film were put into illumination incubator (SPX-250I-G), and the water temperature, the illumination intensity and the illumination period was 20 ± 1 °C, 4000 - 4500 Lux and 12 L : 12 D, respectively. Moreover, there were 9 repeats in every treatment. And the experiment lasted 2 days, after which the concentration of phosphate, ammonium, nitrate and nitrite of experimental seawater was measured.

Table 1 — Nutrients concentrations of different nutrient-enrichme (μmol/L)

Treatments	N	P
N+	160	2
N+P+	160	20
P+	20	20

Note: N-enrichment, N and P-enrichment and P-enrichment represented by N+, N+P+ and P+, respectively.

E. prolifera treated by the same level of nutrient eutrophication was mixed and rinsed by the filtered seawater. Then, *E. prolifera* with biomass 0.50 ± 0.06 g/L (calculated the dry weight of *E. prolifera*) was put into conical flasks with 500 ml seawater with low nutrients concentration. The culture solution without macroalgae was considered as the corresponding blank control, too. There were 3 repeats at each treatment. The 150 ml seawater was sampled every day to measure the concentrations of phosphate, ammonium, nitrate and nitrite, and equal volume of seawater with design nutrients concentration was supplied in to. Moreover, the experiment lasted for 4 days.

The phosphate, ammonium, nitrate and nitrite concentration was determined by the phate-molybdenum blue spectrophotometric method, indophenol blue method, Zinc-Cadmium reduction method and diazo-azo method, respectively.

Calculation method

The nutrient absorption or release rate of *E. prolifera* was calculated as following, $D_i = \frac{(C_i - C_{i-1} - \Delta C_0)V}{WT}$

Where, D_i, C_i, C_{i-1}, ΔC_0, V, W and T was the nutrient absorption or release rate of *E. prolifera* at the i^{th} day (μmol/g/h) (calculated by the dry weight of *E. prolifera*), the final nutrient concentration (μmol/L) and the initial nutrient concentration (μmol/L), the concentration variation of the corresponding blank control (μmol/L), the experimental water volume (L), the dry weight of *E. prolifera* (g), and the time span of water sampling (h), respectively.

Statistical analysis

The statistical analysis was conducted by SPSS 13.0. If the homogeneity of variances was satisfied, the One-way ANOVA was used to analyze the treatment effects on nutrients dynamics of *E. prolifera*, during which multiple comparisons were conducted by the Bonferroni-test. Otherwise, the possible difference among these effects caused by different treatments was analyzed by the Dunnet's-T3 test. Moreover, the correlation analysis was conducted through the Pearson test at significant 5 % significance.

Results

Nutrient dynamics of *E. prolifera* exposed to nutrients eutrophication

After the nutrient starvation treatment, *E. prolifera* exposed to nutrients eutrophication with different concentration levels stored N and P fast (Fig. 1-2). Under the N+ and the N+P+ treatment, the absorption rate of DIN (dissolved inorganic nitrogen) including ammonium, nitrate and nitrite was similar, which was 17.49 ± 0.30 (mean ± standard error) and 16.38 ± 2.71 µmol/g/h, respectively. Moreover, they were significantly higher than that of *E. prolifera* under the P+ treatment and that of *E. prolifera* cultured in seawater without nutrient addition ($p < 5$ %) (Fig. 1).

E. prolifera had the highest phosphate absorption rate under the N+P+ treatment, and the phosphate absorption rate reached to 0.88 µmol/g/h, which was higher than that of *E. prolifera* treated by P+, N+ and seawater without nutrient addition ($p < 5$ %) (Fig. 2).

Nutrient dynamics of *E. prolifera* in seawater with low-nutrient concentration

At the 1st day of exposition to seawater with low concentration of nutrients, there were nutrients released from *E. prolifera* treated by nutrients eutrophication (Figs. 3 - 6), and the maximum release rate of phosphate, ammonium, nitrate and nitrite from *E. prolifera* was 1.29 ± 0.19, 9.19 ± 1.38, 31.66 ± 9.77 and 0.52 ± 0.09 µmol/g/h, respectively.

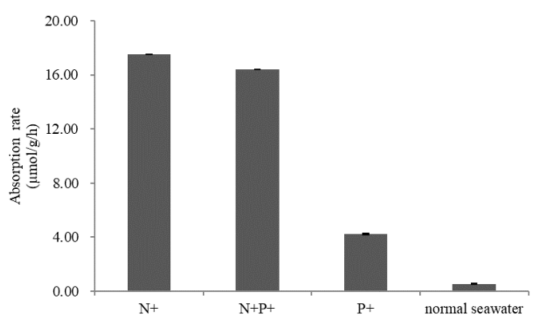

Fig. 1 — DIN absorption rate of *E. prolifera* exposed to nutrients eutrophication.

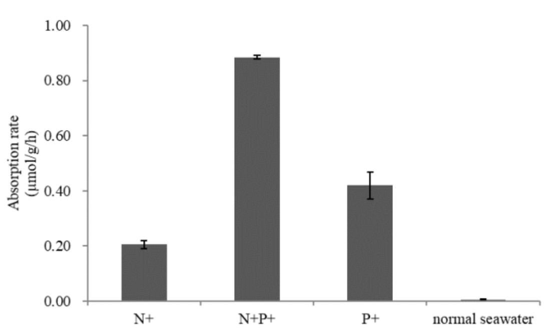

Fig. 2 — Phosphate absorption rate of *E. prolifera* exposed to nutrients eutrophication.

Moreover, there was similar nutrients dynamic characteristics of *E. prolifera* under different treatments of nutrients eutrophication, and the nutrient release and absorption occurred alternatively.

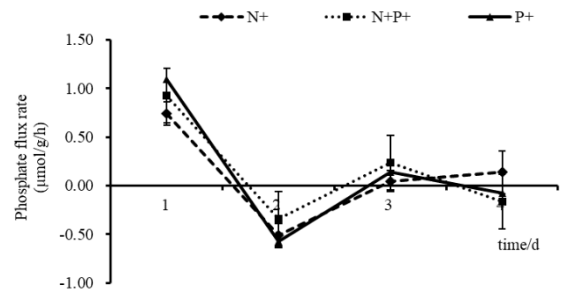

Fig. 3 — Phosphate dynamic of *E. prolifera* in seawater with low-nutrient concentration.

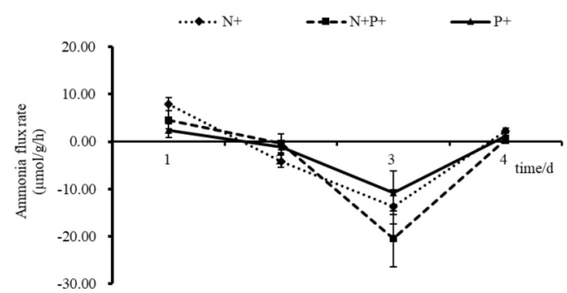

Fig. 4 — Ammonia dynamic of *E. prolifera* in seawater with low-nutrient concentration.

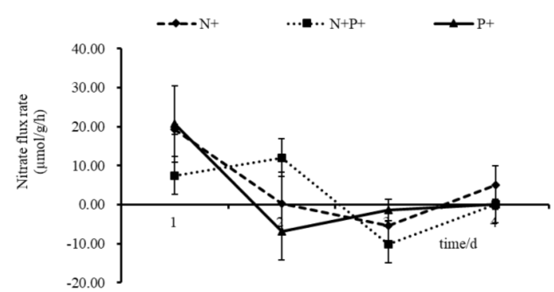

Fig. 5 — Nitrate dynamic of *E. prolifera* in seawater with low-nutrient concentration.

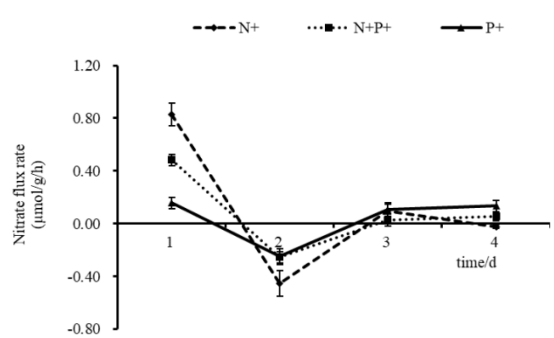

Fig. 6 — Nitrite dynamic of *E. prolifera* in seawater with low-nutrient concentration.

Discussions

Nutrient dynamics of *E. prolifera*

The opportunistic macroalgae can absorb and store excessive nutrients fast when they encounter nutrient eutrophication[13-16], and the luxury consumption of nutrient can indeed make the algae sustain in nutrient-scarce seawater[12,17]. When *E. prolifera* treated by nutrients eutrophication was taken into the seawater with low-nutrient concentration, the species of algae firstly released phosphate and DIN, which could be reused subsequently. Furthermore, nutrients release and uptake of *E. prolifera* occurred alternately in the present work, which indicates *E. prolifera* can repeat this process continuously to regulate the nutrients content to adapt different condition. Thus, *E. prolifera* has similar nutrient utilization characteristics.

E. prolifera prefers to ammonium because ammonium can be absorbed by passive diffusion which will not cost any energy[18]. By releasing ammonium first, the osmotic pressure balance between inside and outside of the species of macroalgae can be maintained when *E. prolifera* treated by nutrients eutrophication are taken into nutrient-scarce seawater. Moreover, there was correlativity between the absorption or release rate of ammonium and that of other nutrients. Other nutrients might be released or absorbed later to keep the osmotic pressure balance in cell due to the ammonium release.

Ecological implications of the nutrients dynamics of *E. prolifera*

Only when there is enough quantity of macroalgae such as *E. prolifera*, there might be the possibility of green tide outbreak. The quantity of *E. prolifera* depends not only on the growth but also on the proliferation that include both asexual and sexual reproduction.

The growth of macroalgae is independent of real-time surrounding nutrients. Moreover, most opportunistic macroalgae uptake nutrients as much as possible at the expense of growth when they encounter nutrient eutrophication[13,19], and accelerate growth rate with stored nutrients as the nutrients are not rich enough[13,20]. *E. prolifera* absorbs more nitrogen in nutrient-enrichment seawater regardless of phosphate limitation in N+ treatment (high ratio of N/P). Ren et al.[21] finds that growth speed of algae can reach to the maximum if the ratio of N/P is 10, which means that growth might need special nutrient proportion[22]. Thus, *E. prolifera* releases stored nutrients to make nutrients within cell approximate to special proportion to stimulate growth when it encounters nutrient-scarce seawater.

Most green algae belong to the opportunistic r-strategist, which will release lots of gametophyte during the life history. The development of gametophyte into sporophore and the growth of sporophore are independent of nutrients. The sporophore of *E. prolifera* has divaricate structure, which implies that there are sporophore fragment and the active fragment can grow up through utilization of nutrients. The process can be called as clonal reproduction. The *E. prolifera* green tide does not outbreaks in the origin seawaters generally. Driven by the wind and current, macroalgae responsible for green tide such as *E. prolifera* can go through seawaters with different nutrients concentration. Nevertheless, the distribution of nutrients is not homogeneous among different sea areas, e.g., some sea areas may be rich in nutrients, however, there may be nutrients shortage in some sea areas. How can these macroalgae keep growth and population multiplication as they go through these sea areas with low nutrients concentration? And how they get enough nutrients which are necessary for them? According to the results of the present work, the luxury uptake and release subsequently of nutrient will be the effective pathway to support the gametophyte and active fragment of sporophore.

Hu et al.[7] estimated that biomass of *E. prolifera* can could reach to 2 kg/m^2 at maximum during green tides in the Yellow Sea. Thus, the biomass of macroalgae responsible for green tide is limited by carrying capacity which is determined by nutrients supply generally. Up to present, green tide is mainly controlled by salvage which implies the fade away depends on not more community succession but on anthropogenic activity. If the nutrients in one given seawaters cannot meet the need of primary production, the green tide cannot last longer even though appropriate temperature and illumination for green algae responsible for green tide are present.

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