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Effects of Different Concentrations of Nitrogen and Phosphate on Growth of *Sargassum thunbergii* Germlings (Phaephyta)

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Abstract

In the present study, the germlings shortly released from fertile thalli of *S. thunbergii* were collected and cultured in the laboratory. The stress resistances of germlings to adverse conditions of different nutrient concentrations and N:P ratios on growth of germingling sporophyte and rizoids were analyzed. Results showed that different concentrations of nitrogen and phosphate could significanly affect the relative grwoth rate and the most obvious growth inhibition was observed in group I which stood for the heavily eutrophicated nutrient condition. Moreover, the negative nutrient condition exerted more serious inhibition on germling sporophyte compared to that on the rizoid, and this might be a suvival strategy for the sustainability of *S. thunbergii* population.

Keywords: Nitrogen, Phosphate, Growth, *Sargassum thunbergii* germlings

1. Introduction

Macroalgal beds in the intertidal zone have been recognized to play important ecological roles, such as primary producer, spawning, nursery and feeding ground for marine organisms, and nutrient cycling controller (Zhang and Sun, 2007). However, the worldwide coastal area has been affected by multiple anthropogenic stressors which drive the seagrass and macroalga habitat change, and pollution, nutrient over-enrichment, over-exploitation and toxic algal blooms (HABs) are suggested to be the most notable factors impacting seagrass beds (Kennish et al., 2010). Therefore, effective measures helpful to the seagrass restoration are of great concern now. The common brown alga, *Sargassum thunbergii*, which forms extensive beds in the intertidal zones, is widely distributed in the coastal areas of China, Japan and Korea (Zhao et al., 2007; Zhang et al., 2009). *I*t is especially important as a feed source of sea cucumber and abalone. Moreover, it is usually suggested to be used as a candidate for the restoration of intertidal seaweed beds (Chu et al., 2012a,b; Yu et al., 2012). However, the natural populations of *S. thunbergii* along the northern coast of China have been depleted (Zhao et al., 2007, 2008). Developing strategies to restore *S. thunbergii* using its germlings is drawn much attention now. . Sexual production of *S. thunbergii* has been fully understood now. During this period, *S. thunbergii* produces numerous receptacles along the branches of thalli (Zhang et al., 2009), and eggs then derive from receptacles and adhere to the surface of the receptacle. After eggs' fertilization, young germlings are formed (Zhao et al., 2008). Period of germling is more sensitive to the ambient stress, and the *S. thunbergii* germling usuallyexperiences highly stressful challenges as compard to other growth periods. Eutrophicationis one of the most fearful stressors leading to not only the water pollution but also the occurrence of harmful algal blooms (HABs), which is suggested to have obviously negative effects on *S. thunbergii bed.* However, little is paid attention to the effects of nutrient conditions on *S. thunbergii* in its early life stages. We thus performed the present study, aiming at elucidating adverse conditions of different nutrient concentrations and N:P ratios on the germling growth. Results in the present study will provide useful information to develop strategies to restore intertidal seaweed beds.

2. Materials and Methods

2.1 Macroalgal Collection and Cultivation

The male and female *S. thunbergii* at reproductive phase with well-developed receptacles were collected from Dongtou Couty, Zhejiang Province from late May to early July, 2012. Samples were collected and immediately transported to the laboratory. The fresh thalli were brushed using a toothbrush and rinsed with autoclaved seawater to remove the attached microorganisms and temporarily cultured under controlled lab conditions. The male and female were cultivated separately and were maintained at 20±0.5 °C, illuminated with 80 μmol photon m^{-2} s^{-1} under a 12h: 12h light-dark cycle in illuminating incubator. The initial salinity was adjusted to 30. The culture medium was changed every 3 days for the half. The development of receptacle was observed under light microscope every 3 days till eggs were found on it. Thereafter, the observation was performed everyday and the numbers of receptacle with eggs were recorded as well as the egg's quality. During this period, the culture medium was still changed every 3 days but the volume was decreased to 1/3 of the total and the aeration intensity was lowered to decrease the effect on the matured receptacle.

2.2 The Collection of Fertilized Eggs

The well-developed fertile receptacles were taken out and dried in the shade for about 30min, and then transferred in the sterilized seawater. The system was shaken so that the eggs and

sperms released into the culture medium. The sperms and eggs were collected and mixed at a ratio of 6:1. The mixture was filtered using 60-mash sieve to remove the macro-impurities. The filtered mixture, was filtered again using the 200-mash sieve, and rinsed repetitively three-time cycle disposal.

2.3 Experimental Design

The prepared fertilized eggs were then exposed to different concentrations of nutrient conditions, and the responses of early development to different nutrient changes were analyzed. We divided the growth of *S. thunbergii* into two phases: phase Ⅰ was from fertilized eggs to the appearance of rhizoid, and this phase would last for 2h. Phase Ⅱ was the period from the appearance of rhizoid till the complete development of the germling. Samples at these two phases were collected and were exposed to different nitrogen concentrations as that in Tab.1. The temperature was kept at (22±0.5) °C and the other experimental conditions were the same as described in 2.1.

Table 1. Experimental design on different nitrogen concentrations and ratios

groups	Nitrogen (N) (mg/L)	Phosphate (P) (mg/L)	N:P ratio
Ⅰ	22.8	10.8	2:1
Ⅱ	14.4	0.45	32:1
Ⅲ	4.5	0.45	10:1
Ⅳ	0.45	0.45	1:1
Ⅴ	4.5	4.5	1:1
Ⅵ	4.5	0.14	32:1

During the experiment on phase I, the observation was performed every 3h and the development of the fertilized eggs was recorded till rhizoid appeared. The time when 90% of the samples were found to have rhizoid was designated as rhizoid phase. The culture medium was supplemented every 24h and the nitrogen and phosphate concentrations were adjust to the set concentrations. The adhesive rate of rhizoid was recorded and the length of rhizoid and the length and width of the thallus were recorded every 12h during the experiment on phase I. As to phase Ⅱ, the growth of germlings at phase Ⅱ was measured in terms of changes in adhesive rate and the length and width of rhizoid every 24h. A total of 20 germlings in each dish was used to estimate of relative growth rate (RGR, % day^{-1}). RGR was calculated as 100 ($\ln(L_1) - \ln(L_0)$)/t, from initial versus final values, where L_0 and L_1 are germlings lengths at the start and end of treatment, respectively, and *t* is the length of treatment period in days (Hunt, 1978 ; Navarro et al., 2008).

2.4 Statistics

All data were analyzed using SPSS for Windows 17.0. Prior to all statistical analyses, the homogeneity of variances was verified with Levene's test. Tukey's tests were used for the post hoc comparisons. The differences were considered to be statistically significant if the probability value was less than 5% ($p < 0.05$).

3. Results

3.1 Different Concentrations of Nitrogen and Phosphate on Growth of S. thunbergii in Phase I

Different concentrations of nitrogen and phosphate had obviously effects on the growth of samples at phase I, which referred to fertilized eggs developing into germlings. RGR at 48h after exposure showed that it decreased steadily with a time and concentration manner in the treated groups, and great difference was observed among the treated groups by paired *t*-test ($P<0.05$). Among the treated groups, RGR in group I which was represented the heavy eutrophicated condition decreased to the lowest as compared to the other groups, while that in the groups of Ⅲ and Ⅶ were relatively higher at 24h after exposure. Differently, RGR presented the highest value at 48h, but little difference was observed among groups of Ⅱ、Ⅳ and Ⅵ (paired t-test, $P>0.05$).

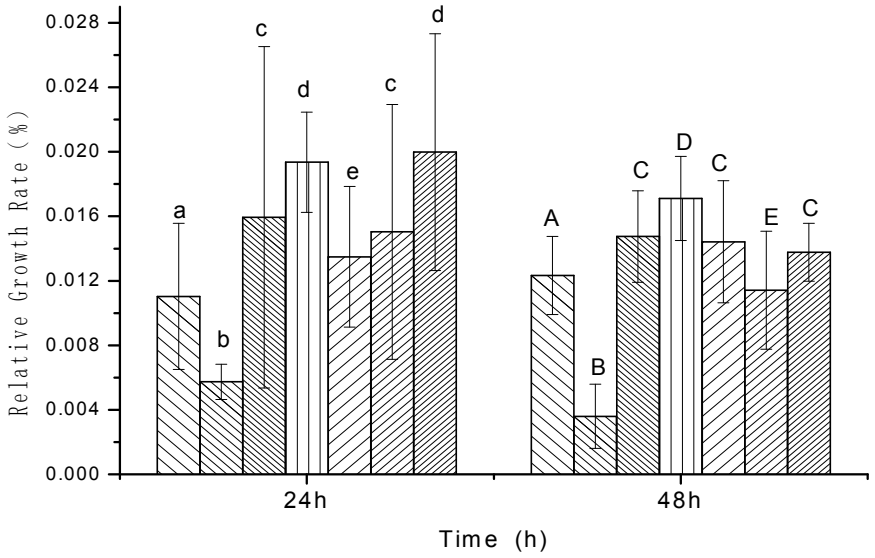

Figure 1. Effects of different concentrations of Nitrogen and Phosphorus on growth of *S.thunbergii* sporophytes during Phase I

: Blank group ; : Group Ⅰ; : Group Ⅱ ; : Group Ⅲ; : Group Ⅳ; : Group Ⅴ; :Group Ⅵ

3.2 Different Concentrations of Nitrogen and Phosphate on Growth of Rhizoid of S. thunbergii

Regarding to rhizoids, their growth exposed to different concentrations of nitrogen and phosphate were greatly affected, and the treatment in group I had the most obvious growth inhibiton on RGR while treatment in group Ⅲ presented little effect on it as compared to

that in the control. Significance was observed among the treated groups at 48h after exposure by paired t-test, suggesting that nutrient concentrations do affect the early development of *S. thunbergii* ($P<0.05$). Not only the growth of rhizoids were affected by the different nutrient concentrations, but also the time entering rhizoid phase varied at different exposure.

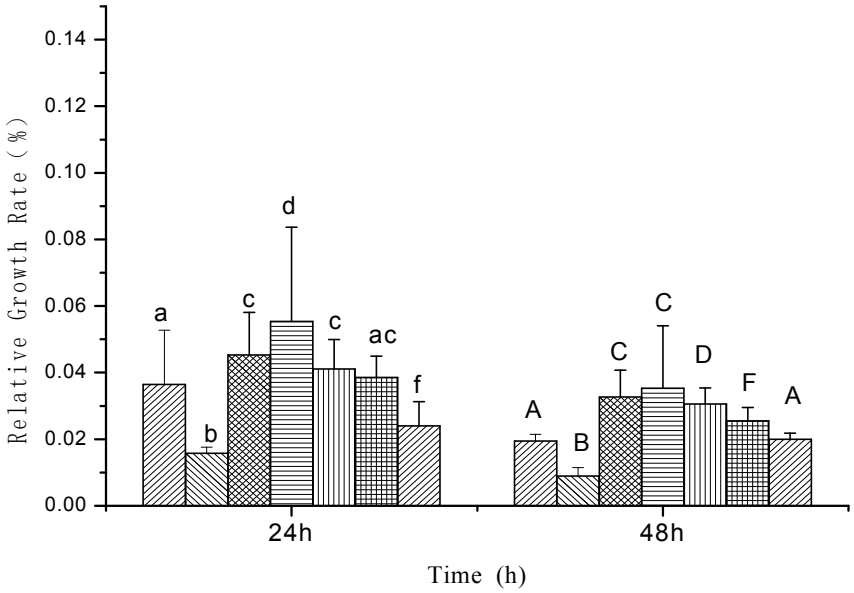

Figure 2. Effects of different concentrations of Nitrogen and Phosphorus on growth of *S. thunbergii* rhizoid during Phase I

: Blank group ; : Group Ⅰ; : Group Ⅱ ; : Group Ⅲ; : Group Ⅳ; : Group Ⅴ; :Group Ⅵ

3.3 Different Concentrations of Nitrogen and Phosphate on Growth of S. thunbergii in Phase Ⅱ

The RGR of germling of *S. thunbergii* in phase Ⅱ decreased fast during 72h after exposure and kept stable thereafter, but RGR in group I which was in heavily eutrophicated condions kept lowest throughout the whold experiment. Differently, RGR in groups of Ⅱ、Ⅲ and Ⅵ kept relatively higher value as compared to other groups. Great difference was observed on the 12h after exposure（$P<0.05$）, but it presented fluctuation according with the stressing time(Fig.3)

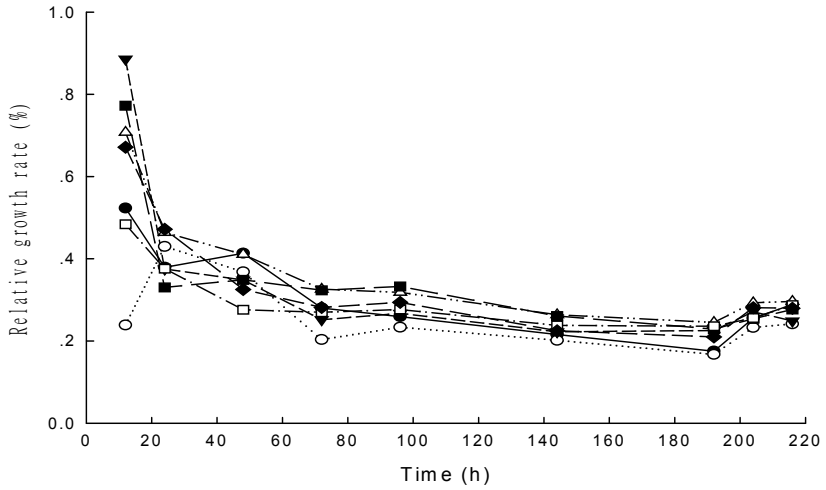

Figure 3. Effects of different concentrations of Nitrogen and phosphorus on growth sporophytes of *S. thunbergii* in phase Ⅱ

●: Blank group ;○: Group Ⅰ;▼: Group Ⅱ ;△: Group Ⅲ;■: Group Ⅳ;□: Group Ⅴ; ◆ :Group Ⅵ

The growth of rhizoid at phase Ⅱ presented similar changes as that at phase Ⅰ. RGR in all groups decreased significantly within 72h after exposure and slowed down thereafter. RGR in group Ⅰ still presented the lowest value at the end of the experiment compared to other groups, and groups Ⅲ showed ralatively higher RGR during the treatment. Moreover, great significance was observed among groups of Ⅰ、Ⅲ、Ⅴ during 96h after treatment（paired t-test, $P<0.05$）, but little was obtained thereafter till the end of the experiment(Fig.4).

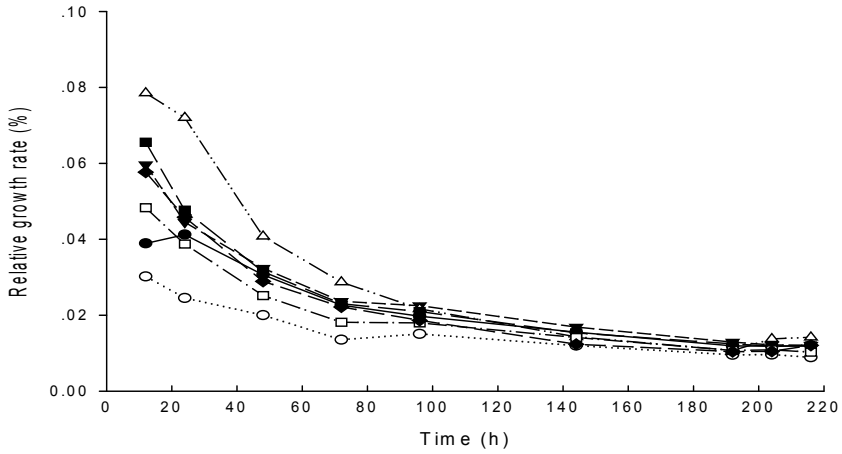

Figure 4. Effects of different concentrations of Nitrogen and phosphorus on growth rhizoid of *S. thunbergii* in phase Ⅱ

●: Blank group ;○: Group Ⅰ;▼: Group Ⅱ ;△: Group Ⅲ;■: Group Ⅳ;□: Group Ⅴ; ◆ :Group Ⅵ

4. Discussion

Environmental stress in the upper distributional limit is driven by many environmental factors (Petes et al., 2007). Nutrient pollution is one of the most serious pollution in coastal area. However nutrient conditions play a decisive role in algal species growth and distribution in the intertidal region. At different intertidal levels, seaweeds are exposed to various frequencies and durations of emergence (Ji and Tanaka, 2002), but overlaid of nutrient would damage the macroalgal growth. When combined considered the results, we found that high concentrations of nitrogen and phosphate presented obviously negative effects on growth and development of germling sporophyted during the early stage, and the N:P ratio also played an essential role in the growth inhibition. For instance, groups of Ⅲ and Ⅶ presented higher relative growth rate in phase I with 24h, which was much similar as that in the control grown in favaroble nutrient condition. However, RGR in treated group of Ⅶ decreased a lot at 48h. Since the concentrations of nitrogen were the same in these groups, result inferred that germling sporophyte was inhibited in Phase I. As to the growth of rizoid, it could grow even in heavily entrophicated conditions, but the growth rate was relatively lower. This was very important to the sustaining of *S. thunbergii* population. Results also showed that the recovery of the negative condition would benefit to the rizoid growth. Another interesting result in the present study was that rizoid had higher growth rate compard to the germling sporophyte, inferring that it be a suvival stragegy since the fast growth of rizoid is good for its adherence on the rocky basement. This stragegy would prevent the germling sporophytes from being washed away and prove the refreshing rate of the germlings.

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