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# Effects of UV-B radiation on the growth interaction of *Ulva pertusa* and *Alexandrium tamarense*

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Abstract: Enhanced UV-B(280 – 320 nm) radiation resulting from ozone depletion is one of global environmental problems. Not only marine organisms but also marine ecosystems can be affected by enhanced UV-B radiation. The effects of UV-B radiation on interaction of macro-algae and micro-algae were investigated using *Ulva pertusa* Kjellman and *Alexandrium tamarense* as the materials in this study. The results demonstrated that UV-B radiation could inhibit the growth of *Ulva pertusa* and *Alexandrium tamarense* when they were both mono-cultured, and the growth inhibition of algae was more significant with increasing doses of UV-B radiation. *Alexandrium tamarense* could inhibit the growth of *Ulva pertusa* in mixed culture, and the growth inhibition was more significant when increasing the initial cell density. However, *Ulva pertusa* could inhibit the growth of *Alexandrium tamarense* in early phase and stimulate the growth in latter phase when they were grown in mixed culture. Lower initial cell density(10<sup>2</sup> cell/ml) of *Alexandrium tamarense* could inhibit the growth of *Ulva pertusa* under UV-B radiation treatment, however, with the initial cell density increasing(10<sup>3</sup> and 10<sup>4</sup> cell/ml), the growth of *Ulva pertusa* was stimulated under lower dose of UV-B radiation and inhibited under higher dose of UV-B radiation by *Alexandrium tamarense*. Compared with that in mixed culture, *Ulva pertusa* showed more positive inhibition to the growth of *Alexandrium tamarense* under UV-B radiation treatment.

Keywords: UV-B radiation; mono-culture; mixed culture; Ulva pertusa; Alexandrium tamarense; growth

### Introduction

UV-B radiation levels have increased due to ozone depletion through anthropogenic emissions of chlorofluorocarbons (CFCs) and other gases which adversely affect the ozone layer. Caldwell estimated that a 1% decrease in the stratospheric ozone concentration would result in an increase of approximately 2% in biologically effective UV-B radiation at temperate latitudes (Caldwell, 1977). UV-B radiation has been shown to reach ecologically significant depths in many freshwater and marine ecosystems (Hader, 1998; Caldwell, 1998; Premkumar, 2001). Submerged aquatic plants are subject to the influences of UV-B radiation due to the penetration of harmful UV-B wavelengths to depths of 10 m (Larkum, 1993), with maximum UV-B penetration reaching to depths of 70 m in the clear Antarctic Ocean (Smith, 1992). The effects of UV-B radiation on marine plants are expected to be the greatest in shallow intertidal environments because the plants are often at or above the water during low tide. The reaction of these plants to UV-B radiation could range from inhibition of photosynthetic activity as seen in marine algae to the increased metabolic cost of producing UV-B blocking compounds within plant tissue (Raven, 1991). Not only marine organisms but also marine ecosystems can be affected by enhanced UV-B radiation. Clearly, enhanced exposure of marine ecosystems to UV-B radiation may have world-wide significance.

Red tide is a ubiquitous and natural phenomenon caused by excessive growth of phytoplankton. It has caused serious social problems and heavy damage to fisheries throughout the world (Hallegraeff, 1993). From March to April 1998, a massive "red tide" occurred in coastal waters of south China. The "red tide" rapidly killed various species of caged fish and affected coral fishes, killing a few of them, and caused great economic loss and ecological damage (Yang, 2004). The inshore eutrophication and red tide have become an

important marine environmental problem which has been paid close attention by international social and need to be solved urgently because of its many characteristics, such as globality, repetitiousness, complexity, endangered severity and so on. However, the process of occurring to subsidizing, physical, chemical and ecological interaction is complex and still little understood, especially in the process of sporangium bourgeon, upper floatage, biologic competition, feeding and so on. Therefore, the red tide process and causes went short of evaluation data(Li, 1999).

The effects of UV-B radiation on the growth, physiology and biochemistry of algae have been extensively studied (Helbing, 1996; Krizek, 1998; Brian, 1999). UV-B radiation enhance could reduce the biomass of red tide algae (Lesser, 1996). However, few studies have focused on the effects on the interspecific growth interaction of algae. The effects of UV-B radiation on interaction of macro-algae and micro-algae were studied using *Ulva pertusa* Kjellman and Alexandrium tamarense as the materials in this paper. It could provide the fundamental for us to clarify the effects of UV-B radiation on marine ecosystems and the biological mechanisms of red tide occurrence.

### 1 Materials and methods

### 1.1 Plant materials and treatment

Ulva pertusa Kjellman was collected from Taiping Angle of Qingdao, China. The material was washed with natural seawater immediately after sampling, and pierced to circle slices of 1.3 cm diameter by bore machine, then prepared to culture for 7 d in indoor temperature with illumination of 3000 lx.

Alexandrium tamarense was offered by Marine Microalgae Research Center in Ocean University of China.

Triangle bottles (300 ml) were used as culture containers. They were washed with 1 mol/L HCl and 90% ethanol, and then used f/2 medium (Guillard, 1975)

preparing equilibrium.

#### 1.2 Culture conditions

The initial cell densities of Alexandrium tamarense were 0 (mono-culture), 100 (mixed culture A), 1000 (mixed culture B) and 10000 (mixed culture C) cell/ml, respectively. Ulva pertusa  $(0.05~\mathrm{g})$  was inoculated in 150 ml f/2 culture medium, and aged sea water of the Qingdao Current(salinity of 31) was used as the culture medium. The culture was maintained at  $20~\mathrm{C}$  under 3000 lx cool-white fluorescent illumination on a 12 h light 12 h dark cycle.

### 1.3 System of UV-B radiation

UV-B radiation was provided by two UV-B tubes(Philips TL 40 W/12  $\mu V)$  covered by a film of cellulose acetate(0.12 mm) to remove all radiation below 280 nm. In order to minimize the change of the filter properties of the film, the cellulose acetate was preburnt for 48 h at a distance of 1 m from two UV-B lamps. The spectral irradiance was measured with UV spectroradiometer (Beijing Normal University, Beijing). Only a thin layer of the algal suspension was used to ensure adequate UV-B penetration. Cellulose acetate filters were changed weekly to avoid aging effects on the UV-B spectral transmission through the filters.

#### 1.4 UV-B radiation treatment

The intensity of radiation was controlled in 1.25  $\mu$ W/cm². The dose of radiation was controlled by adjusting radiation time. The trial designed the dose of 0 (control experiment with the normal fluorescent lamp tube irradiation), 0.72(U-1), 1.44(U-2), 2.16(U-3) and 2.88 (U-4) J/m² radiation treatment.

### 1.5 Ulva pertusa weighs and Alexandrium tamarense numbers

Ulva pertusa was weighed after absorbed the superficial water on it using filter papers. Alexandrium tamarense was fixed by Lugol iodine solution and then counted using optical microscopy.

#### 1.6 Statistics

All results were represented as means  $\pm$  SD from at least five independent series of experiments (3 measurements each). For all data collected SPSS 11.0 J for Windows (SPSS, Chicago, IL, USA): Two-factor analysis was used. p < 0.05 was considered as significant, p < 0.01 was considered as extraordinary significant.

### 2 Results

### 2.1 The growth interaction of *Ulva pertusa* and *Alexandrium tamarense*

# 2.1.1 Effects of Alexandrium tamarense on the growth of Ulva pertusa

Alexandrium tamarense could inhibit the growth of *Ulva* pertusa in mixed culture (Fig. 1). The inhibition of *Ulva* pertusa was extraordinary significant (p < 0.01) between mono-culture and mixed culture A, B and C on the day 12. The growth inhibition was more obvious with increased initial cell density of *Alexandrium tamarense*.

### 2.1.2 Effects of *Ulva pertusa* on the growth of *Alexandrium tamarense*

The effects of *Ulva pertusa* on the growth of *Alexandrium tamarense* were shown in Fig. 2, Fig. 3 and Fig. 4. *Ulva pertusa* could inhibit the growth of *Alexandrium tamarense* in early phase (0—9 d) and stimulate the growth in

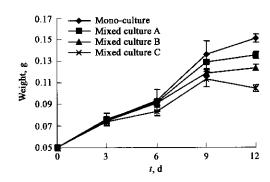


Fig. 1 The growth curves for *Ulva pertusa* with different initial *Alexandrium tamarense* density

later phase (9—12 d). On the day 6, the growth inhibition to Alexandrium tamarense was significant (p < 0.05) in the initial cell density B and C, while it was not significant (p > 0.05) in the initial cell density A. The growth of stimulation was significant (p < 0.05) in the initial cell density B, and extraordinary significant (p < 0.01) in the initial cell density A and C on the day 12.

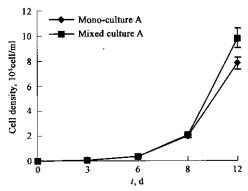


Fig. 2 The growth curves for Alexandrium tamarense in density A with Ulva pertusa

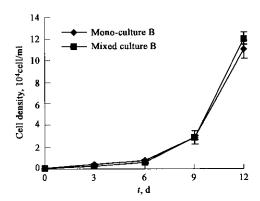


Fig. 3 The growth curves for Alexandrium tamarense in density B with Ulva pertusa

# 2.2 Effects of UV-B radiation on the growth of *Ulva pertusa*

The effects of UV-B radiation on the growth of Ulva pertusa are shown in Fig.5. UV-B radiation could inhibit the growth of Ulva pertusa, and the growth inhibition was more significant with increased doses of UV-B radiation. On the day 6, the growth inhibition to Ulva pertusa was significant (p < 0.05) except the U-1 group. The growth inhibition to Ulva pertusa was more notable, including the U-1 group, and

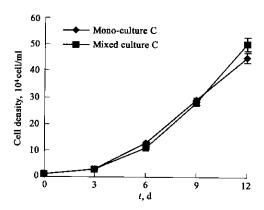


Fig. 4 The growth curves Alexandrium tamarense in density C with  $Ulva\ pertusa$ 

significant (p < 0.05) on the day 9.

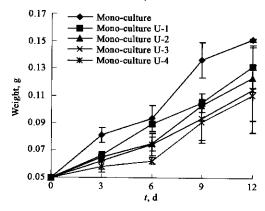


Fig. 5 Effects of UV-B radiation treatment on growth of Ulva pertusa

# 2.3 Effects of UV-B radiation on the growth of Alexandrium tamarense

The effects of UV-B radiation on the growth of Alexandrium tamarense are shown in Fig.6, Fig.7 and Fig. 8. The UV-B radiation could inhibit the growth of Alexandrium tamarense and the growth inhibition was more significant with increased doses of UV-B radiation.

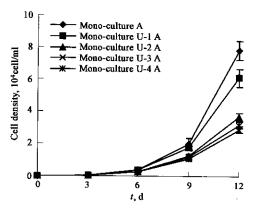


Fig. 6 Effects of UV-B radiation treatment on growth of Alexandrium tamarense in density A

With regard to the initial cell density A, the growth inhibition of Alexandrium tamarense was significant (p < 0.05) except the U-1 group on the day 9 and it was extraordinary significant (p < 0.01) on the day 12. As for the initial cell density B, each of the four groups in the growth of Alexandrium tamarense was inhibited extraordinary

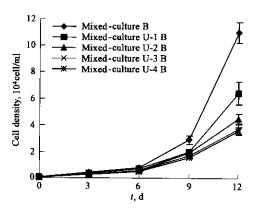


Fig. 7 Effects of UV-B radiation treatment on growth of Alexandrium tamarense in density B

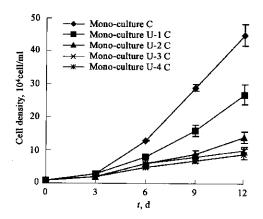


Fig. 8 Effects of UV-B radiation treatment on growth of Alexandrium tamarense in density C

significantly (p < 0.01) by UV-B radiation from the day 9 to 12. As for the initial cell density C, the growth of Alexandrium tamarense was inhibited more obviously than the initial cell density A and B by UV-B radiation. Each of the four groups in the growth of Alexandrium tamarense was inhibited extraordinary significantly (p < 0.01) by UV-B radiation from the day 6 to 12.

# 2.4 Effects of UV-B radiation on the growth interaction of *Ulva pertusa* and *Alexandrium tamarense*

# 2.4.1 Effects of Alexandrium tamarense on the growth of Ulva pertusa under UV-B radiation treatment

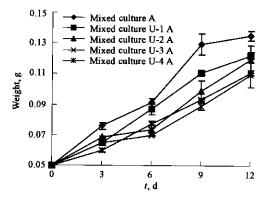


Fig. 9 Effects of UV-B radiation on growth of *Ulva pertusa* with *Alexandrium tamarense* in density A

The effects of Alexandrium tamarense on the growth of Ulva pertusa under UV-B radiation treatment are shown in Fig. 9, Fig. 10 and Fig. 11.

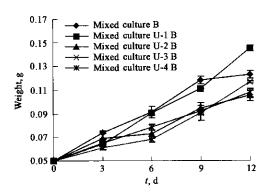


Fig. 10 Effects of UV-B radiation on growth of  $Ulva\ pertusa$  with  $Alexandrium\ tamarense$  in density B

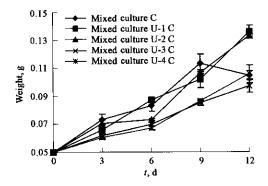


Fig. 11 Effects of UV-B radiation on growth of *Ulva pertusa* with Alexandrium tamarense in density C

The growth inhibition of Alexandrium tamarense with the initial cell density A on Ulva pertusa was significant (p < 0.05) under UV-B radiation on the day 3. It was extraordinary significant (p < 0.01) under UV-B radiation from the day 6 to 12 except that the U-1 group was from the day 9 to 12.

The growth effect of Alexandrium tamarense with the initial cell density B on Ulva pertusa was different under different doses of UV-B radiation. The growth of Ulva pertusa was inhibited by Alexandrium tamarense before the day 9 under UV-B radiation, while it was stimulated from the day 9 to 12 in the U-1 group. This inhibition was extraordinary significant(p < 0.01) under UV-B radiation on the day 3, and the growth stimulation of Ulva pertusa by Alexandrium tamarense was extraordinary significant(p < 0.01) under UV-B radiation on the day 12 in the U-1 group. The growth of Ulva pertusa was inhibited extraordinary significantly (p < 0.01) by Alexandrium tamarense under UV-B radiation before the day 9, significantly (p < 0.05) from the day 9 to 12 in the groups of U-2, U-3 and U-4.

The growth effect of Alexandrium tamarense with the initial cell density C on Ulva pertusa was also different under different doses of UV-B radiation. The growth of Ulva pertusa was inhibited insignificantly (p > 0.05) by Alexandrium tamarense under UV-B radiation before the day 9, while it was stimulated extraordinary significantly (p < 0.01) from the day 9 to 12 in the groups of U-1 and U-2. The growth of Ulva pertusa was inhibited significantly (p < 0.05) by Alexandrium tamarense under UV-B radiation before the day 9, insignificantly (p > 0.05) on the day 12 in the groups of U-3 and U-4.

# 2.4.2 Effects of *Ulva pertusa* on the growth of *Alexandrium tamarense* under UV-B radiation treatment

The effects of *Ulva pertusa* on the growth of *Alexandrium tamarense* under UV-B radiation treatment are shown in Fig. 12, Fig. 13 and Fig. 14. With regard to the initial cell density A, the growth inhibition of *Alexandrium tamarense* was extraordinary significant (p < 0.01) except the U-1 group by *Ulva pertusa* under UV-B radiation from the day 9 to 12. As for the initial cell density B, each of the four groups in the growth of *Alexandrium tamarense* was inhibited extraordinary significant (p < 0.01) by *Ulva pertusa* under UV-B radiation from the day 9 to 12. As for the initial cell density C, each of the four groups in the growth of *Alexandrium tamarense* was inhibited extraordinary significant (p < 0.01) by *Ulva pertusa* under UV-B radiation from the day 6 to 12.

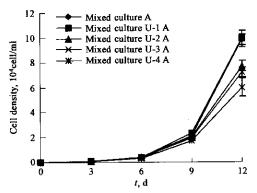


Fig. 12 Effects of UV-B radiation treatment on growth of Alexandrium tamarense in density A with Ulva pertusa

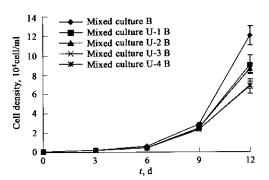


Fig. 13 Effects of UV-B radiation treatment on growth of Alexandrium tamarense in density B with Ulva pertusa

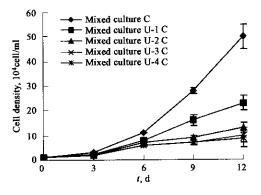


Fig. 14 Effects of UV-B radiation treatment on growth of Alexandrium tamarense in density C with Ulva pertusa

### 3 Discussion

### 3.1 Growth interaction of *Ulva pertusa* and *Alexandrium tamarense*

The r- and K-selection played a key role in stimulating empirical and theoretical work on life-history evolution. The theory as presented by MacArthur (MacArthur, 1967) and extended by Pianka (Pianka, 1970) was sufficiently compelling to draw biologists to the study of life histories. Alexandrium tamarense belongs to r-selection, while Ulva pertusa belongs to K-selection in this experiment.

The growth of *Ulva pertusa* was inhibited by Alexandrium tamarense in the experiment all the while and the growth inhibition was more significant with the increasing initial cell density of Alexandrium tamarense. This growth inhibition could be due to the following: (1) The niche between Ulva pertusa and Alexandrium tamarense was overlapped, and they consumed nutrition and space together, so that the growth of Ulva pertusa was necessarily inhibited by Alexandrium tamarense. (2)With propagating Alexandrium tamarense, the density would increase gradually. The efficiency of photosynthesis in Ulva pertusa will decrease for the effects of shading light by Alexandrium tamarense. Therefore, the growth of Ulva pertusa must be inhibited by Alexandrium tamarense. (3) Alexandrium tamarense inhibited the growth of Ulva pertusa for allelopathic effect in different algae (Keating, 1978; VanDonk, 2002; Nan, 2004).

Ulva pertusa could inhibit the growth of Alexandrium tamarense in the early phase and stimulate the growth in the latter phase. The effects of macro-algae on the growth of micro-algae have been extensively studied. Marshall and Orr applied fertilizer in a neritic gulf of England as early as 1949. They found that the red tide would take place for micro-algae only under the condition that macro-algae lack (Marshall, 1949). Fong reported that the emergence of macro-algae and micro-algae presented the negative relativity, the observation to the gulf in Tijuana of the South California in 1989, both of the abundance with the variety of the space and season (Fong, 1993a; 1993b; 1994). Nan and Dong studied the effects of initial algal density and phosphorus concentration on the competition between macro-algae and micro-algae. They found that macro-algae could express inhibition on the growth of micro-algae in large mass (Nan, 2003; 2004). The growth of micro-algae was inhibited by macro-algae in latter phase according to the Sfriso's result (Sfriso, 1989), Sfriso studied the salt lake of Wearnes and found that red tide would appear only after macro-algae putrefied, harvest or in the extreme rich nourishment area (Sfriso, 1989). The reasons can be inferred as follows: (1) the allelopathic effect took place in different algae. The growth of micro-algae was stimulated by macro-algae and propagating quickly because they must adapt to environments. (2) Ulva pertusa was placed in the passive position gradually in the competition on the latter period of experiment. Ulva pertusa putrefied for the depravation living conditions. Some nourishment materials, which produced by the Ulva pertusa putrefied, promoted the growth of micro-algae.

# 3.2 Effects of UV-B radiation on the growth of *Ulva* pertusa and Alexandrium tamarense and their responses

The effects of UV-B radiation on the growth of *Ulva* pertusa and Alexandrium tamarense showed inhibition in the experiment all the while. The effects of UV-B radiation on the growth of plants were also reported. A great deal of research expressed that PS II was not only the most sensitive part on thylakoid membrane in photosynthesis, but also the main part involved in photosynthesis. The growth of plants must be influenced if their photosynthesis is affected by UV-B radiation. Moreover, UV-B radiation could also induce DNA to produce the reactive oxygen species which could injury plants and subsequently affect the growth of plants.

The pigment in *Ulva pertusa* leaf appeared asymmetric phenomenon and thicken through UV-B radiation. In plants, a certain amount of UV-absorbing compounds (usually phenolic compounds) could be synthesized when plants were exposed to increased levels of UV-B radiation. This was naturally important in reducing the penetration of UV-B radiation to underlying tissues (Li, 1993; Reuber, 1996). Other adjustments in plant leaves after exposure to increased UV-B radiation may also contribute to a heightened UV defense. At the structural level, increased leaf thickness was often induced by UV-B radiation and reduces UV-B penetration to internal leaf tissues. An increased UV-B radiation resulted in enhanced synthesis of UV-screening pigment due to the expression of particular genes. It appeared that the effects of UV-B radiation on photosynthesis, growth, and development of plants were caused by altered gene action. However, the mechanisms of how the organism perceives UV-B radiation and how signals are transmitted are not yet well understood (Jenkins, 1997).

The cubage of micro-algae is so small that the exterior phenomenon is not obvious. However, micro-algae could get together after UV-B radiation. This is also a kind of protection behavior on micro-algae.

# 3.3 Effects of UV-B radiation on the growth interaction of *Ulva pertusa* and *Alexandrium tamarense*

The results demonstrated that both tamarense and UV-B radiation showed inhibition on the growth of Ulva pertusa. However, the growth of Ulva pertusa was not inhibited completely by Alexandrium tamarense under UV-B radiation. Lower initial cell density of Alexandrium tamarense could inhibit the growth of Ulva pertusa under the UV-B radiation treatment, however, with increasing initial cell density, the growth of *Ulva pertusa* was stimulated under lower dose of UV-B radiation and inhibited under higher dose of UV-B radiation by Alexandrium tamarense. The possible explanation for this phenomenon is that UV-B radiation inhibited the growth of both Ulva pertusa and Alexandrium tamarense, Alexandrium tamarense strongly inhibited the growth of Ulva pertusa, so that low density of Alexandrium tamarense decreased the growth inhibition of Ulva pertusa. The inhibition of UV-B radiation on the growth of Ulva pertusa is smaller than that of Alexandrium tamarense. Consequently, the growth of *Ulva pertusa* was stimulated under lower dose of UV-B radiation in mixed culture. With increasing initial density of Alexandrium tamarense, the range of UV-B radiation dose, which could stimulate the growth of Ulva pertusa would enhance.

Compared with that in mixed culture, our results showed that the inhibition of *Ulva pertusa* was more positive to the

growth of Alexandrium tamarense under UV-B radiation treatment. Both Ulva pertusa and UV-B radiation could inhibit the growth of Alexandrium tamarense in the early phase. Therefore, the growth of Alexandrium tamarense was inhibited by Ulva pertusa more obviously under UV-B radiation treatment.

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