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Atomic ratio of N to P influences the impact of UV irradiance on photosynthesis and growth in a marine dinoflagellate, *Alexandrium tamarense*

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Abstract

The effects of the atomic ratio of N to P (N:P) on the response of *Alexandrium tamarense* to UV radiation (UVR) were investigated in this study. Artificial sea water of 5 different N:P ratios for indoor culture and with 3 different N:P ratios for outdoor culture were used for a period of 14 and 9 d, respectively. The short-term response of cells to UVR was analyzed using a fluorometer. Cells that acclimated to nutrient conditions at the Redfield value (16:1) showed the fastest growth rate and highest pigment concentrations in both indoor and outdoor conditions, compared to those acclimated to the non-Redfield conditions. Moreover, these physiological parameters were functions of the N:P ratio according to a two-order equation ($y = a + bx + cx^2$, R^2>0.95). The fluorescence data of indoor cultures showed that *A. tamarense* grown at 16:1 (N:P) exhibited the greatest ratio of repair rate/damage rate (r/k) and minimum level of UVR-induced inhibition. among those grown at all of the N:P ratios following UVR exposure. Outdoor cultures had the same patterns of fluorescence as indoor cultures, but the less UVR-induced inhibitions were detected compared the former with the latter. The following three parameters, the r/k, level of inhibition caused by the two radiation treatments following 60 min of exposure (PAR and PAB, respectively), and level of UVR-induced inhibition, were also functions of the N:P ratio according to the two-order equation (R^2>0.96). Further, there was a negative correlation between UVR-induced inhibition and the r/k ratio. In summary, the Redfield value (16:1) was the optimal nutrient stoichiometry for the protection of *A. tamarense* against the deleterious effects of UVR. Results were not impacted by previous light history experienced by cells.

Additional key words: algal bloom; effective photochemical efficiency; nutrient stoichiometry; solar ultraviolet radiation.

Introduction

Over the past decade, large-scale dinoflagellate blooms have occurred in the Pear River Estuary and Yangtze River Estuary and adjacent areas of both South and East China Sea from late spring to early summer. *Alexandrium* species blooms with those of *Prorocentrum donghaiense* together in China coastal water (Tang *et al.* 2006). The more frequent and persistent algal blooms of dinoflagellates have been determined to be caused by high concentrations of nutrients coming from rivers and from human activities (Anderson *et al.* 2002, Glibert and Burkholder 2006). Areas associated with the highly frequent formations of blooms, including the Pear River Estuary and Changjiang River Estuary, are impacted by both dilution by large rivers and the Kuroshio Current (*e.g.*, the Taiwan Warm Current). Seasonal variation in nutrients, such as the total N concentration, ranges from 70 to 100 μM (Shen *et al.* 2003, Zhang *et al.* 2007) and the amount of total phosphorus (P) is approximately 2−25 μM (Yan and Zhang 2003). Thus, it results in the ratio (N:P) of dissolved inorganic N (DIN) to the dissolved inorganic P (DIP) being typically over 50 at river mouths (Chai *et al.* 2006, Zhang *et al.* 2007). Thus, the growth of phytoplankton in coastal sea waters very often fluctuates between states of N and P limitation due to

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Abbreviations: ASW – artificial sea water; Car – carotenoids; Chl − chlorophyll; DIN – the dissolved inorganic N; DIP - the dissolved inorganic P; k – damage rate constant; OD – optical density; P – phosphorus; PAB − photosynthetically active radiation plus UV-A and UV-B radiation; PMA – personal measurement assistant; PSIN – photon system instruments; r – repair rate constant; UV_{abc} − ultraviolet absorbing compounds; UVR – ultraviolet radiation; Y' − effective photochemical efficiency of PSII.
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discharge from rivers, currents, waste waters, or rainfall.

Although natural phytoplankton possesses N and P at an atomic ratio (N:P) of approximately 16 (Redfield 1958, Ryther and Dunstan 1971), the optimal ratio may vary from species to species due to species specificity (Lagus *et al.* 2004). The factors, which control the growth and responses of harmful bloom-forming marine algal species, such as dinoflagellates, have been intensively studied because of the impacts of these blooms on the oceanic environment and human health. In addition, the combined effects of nutrient limitation and UVR have been widely studied (Beardall *et al.* 2009, Villafañe *et al.* 2003). Studies evaluating the effects of acute UVR exposure on photosynthesis have reported enhanced susceptibility due to nutrient limitation (Lesser *et al.* 1994, Litchman *et al.* 2002; Shelly *et al.* 2002, 2005) and changes in competition among different species at the phytoplankton community level (Marcoval *et al.* 2007, 2008, Cabrerizo *et al.* 2014). Other studies have reported no change in sensitivity of photosynthesis to UVR under nutrient limitation caused by chronic UVR exposure (Behrenfeld *et al.* 1994, Veen *et al.* 1997).

In this study, we first investigated the growth and photosynthetic response of *A. tamarense* to visible and UV irradiances under different atomic N:P ratios in indoor and outdoor cultures. Second, the short-term response of *A. tamarense* to UVR was assessed under artificial UVR by examining photochemical efficiency. Emphasis was focused on question how changes in the N:P atomic ratio could impact the response of this organism to UVR. The rationale behind this work is that nutrient stoichiometry (*e.g.*, N:P ratio) is the major factor affecting the composition of blooms and phytoplankton communities, and this ratio is undergoing gradual and significant changes worldwide due to human activities (Hodgkiss and Ho 1997, Glibert and Burkholder 2006). Thus, this ratio should influence the responses of harmful algal species to UV stress in coastal waters. Two different cultures (indoor and outdoor) were used for testing if the light history can impact on the response of *A. tamarense* to UVR in the media with the various N:P ratios.

Materials and methods

Species and culture conditions: *Alexandrium tamarense* was isolated from the Pearl River estuary, Guangdong Province, China (22°30'N, 114°20'E) in May, 2012. It was maintained at 20°C in F/2 medium (Guillard and Ryther 1962) in a growth chamber (*MGC-100P, Shanghai Yiheng Co. Ltd.,* China) under white light (LED lamp) (12L:12D) at 100 μmol(photon) m^{-2} s^{-1}. Two different cultures were set in our experiments: (*1*) the indoor culture, which was grown in artificial sea water (ASW) (Berges *et al.* 2001) with five different N:P ratios for 14 d. The *Nalgene* bottles (500 ml, *Thermo Scientific*, NY, USA) were used as the culture vessels. (*2*) The outdoor culture with three different N:P ratios, which was grown in big quartz tubes (6 cm in diameter, 50 cm in length) and incubated with ASW in a running tap-water bath at 20 ± 1°C under full spectrum of solar radiation without or with UVR (PAR and PAB treatments) and lasted 9 d from 30 May to 7 June, 2015. Then, both indoor and outdoor cultures were diluted to 5×10^3 cells ml^{-1} in fresh ASW medium and put in small (2 cm in diameter, 9 cm in length) quartz tubes kept in a water bath containing circulating tap water (20 ± 0.5°C) in an air-conditioned room to examine the short-term responses of cells to artificial UVR by measuring the photochemical efficiency. The whole experiment was performrd at Wenzhou Medical University, China (28.0°N,120.7°E).

Nutrient stoichiometry and treatments: *Alexandrium tamarense* was grown in a defined inorganic ASW medium. Nitrate ($NaNO_3$) and phosphate (Na_2HPO_4) were the sole sources of N and P, respectively. For the growth studies at various N:P ratios, the phosphate concentration was kept constant at 3.6 μM, while the nitrate concentration was adjusted between 3.6 and 720 μM to obtain five different N:P ratios (1:1, 16:1, 50:1, 100:1, and 200:1) in the indoor culture. The pH values in the media were not significantly different between various N:P ratio conditions. Three different N:P ratios (1:1, 16:1, and 200:1) were used in the outdoor culture. Cell density was determined in a phytochamber by a microscope (*CH30RF200, Olympus Optical Co. Ltd.*, Japan).

UVR irradiance and treatments: A 150-W xenon lamp (*HMI, OSRAM,* Germany) was used as the source of UVR. The lamp was adjusted to provide 213.3 W m^{-2} of PAR and 21.0 W m^{-2} of UVR, which were measured by *PMA* (*PMA2100, Solar Light,* USA); the distance from the samples to the front of the lamp was 50 cm. The solar radiation received by outdoor cultures was also monitored by *PMA* recording the solar PAR and UVR per 5 min; the radiation data were downloaded into computer every night.

Cells were exposed to the following treatments: (*1*) PAB (PAR+UVR), where quartz tubes were covered with 295-nm cut-off filters (*Ultraphan, Digefra,* Munich, Germany), transmitting irradiance of above 295 nm; (*2*) PAR, where quartz tubes were covered with 395-nm cut-off filters (*Ultraphan UV Opak, Digefra,* Munich, Germany). The filters were provided by Marine Biology Institute, Shantou University. During 60 min of exposure to an artificial UV, photochemical efficiency (yield) was measured, and exposure-response curves (ERCs) were created. For outdoor cultures, the diurnal variation of effective photochemical efficiency (Y') was measured three times every day in the morning (07:00 h), noon

(12:00 h), and afternoon (18:00 h) (local time), respectively.

UV-absorbing compounds (UV_{abc}) and pigments: The spectral characteristics of the cultures were determined by filtering 50 ml of culture (the volume filtered varied for the different cell concentrations) with a *Whatman GF/F filter* (25 cm in diameter). Extraction of pigments was carried out in absolute methanol (5 ml) overnight at 4˚C, followed by centrifugation (10 min at 1,500 × *g*) (*TGL-16GR, Anting,* China). Absorbance measurements of the supernatant were performed from 280 to 750 nm using a scanning spectrophotometer (*A590 UV/VIS Spectrophotometer, Xiangyi,* China). The concentrations of chlorophyll (Chl) *a* and carotenoids (Car) were calculated using the following equations of Porra (2002) and Strickland and Parsons (1972), respectively:

$$\text{Chl } a \text{ [}\mu\text{g ml}^{-1}\text{]} = 16.29 \times A_{665.2} - 8.54 \times A_{652} \quad (1)$$

$$\text{Car [}\mu\text{g ml}^{-1}\text{]} = 4\ A_{480} \quad (2)$$

where A is the optical density (ODs) of the supernatant at 665.2, 652, and 480 nm.

Growth rate: Samples were taken daily for cell counts and the specific growth rate (μ) was calculated as follows:

$$\mu = \ln (C_n/C_{n-1})/(t_n - t_{n-1}) \quad (3)$$

where C_n and C_{n-1} are the cell concentrations [cells ml^{-1}] over the $(t_n - t_{n-1})$ period.

Photochemical efficiency, repair rate (r) and damage rate (k): Samples were exposed to PAR and PAB (UVR+PAR) for 60 min at the end of both indoor and outdoor experiments. The effective photochemical efficiency (Y') was measured every 5−30 min during the exposure period. Y' was detected by a *PSI* fluorometer (*AquaPen-C, Photon System Instruments*, Czech Republic) and calculated according to Genty *et al*. (1989) as follows:

$$Y' = \Delta F/F_m' = (F_m' - F_t)/F_m' \quad (4)$$

where Y' was determined by measuring the instant maximal fluorescence (F_m') and the steady-state fluorescence (F_t) of light-adapted cells. The saturating-light pulse was 3,000 μmol(photon) m^{-2} s^{-1}, with duration of 0.6 s. The measuring light was approximately 0.3 μmol(photon) m^{-2} s^{-1}, and actinic irradiance was 100 μmol(photon) m^{-2} s^{-1}.

The rate constant of UVR-induced damage to the photosynthetic apparatus (k, in min^{-1}) and the corresponding repair rate constant (r, in min^{-1}) were estimated according to previous studies (Lesser *et al.* 1994, Heraud and Beardall 2000) as follows:

$$Y_n'/Y_0' = r/(r + k) + k/(k + r) \times e^{-(k + r) \times t} \quad (5)$$

where Y_n' and Y_0', respectively, are Y' values at time t_n (after UVR treatment) or t_0 (before UVR treatment). The ratio of r to k (r/k) indicates the whole response of cells to UVR. The higher r/k means the lower UVR-induced inhibition because of faster repair rate constant and lesser damage rate constant of D1 protein in PSII.

UVR-induced inhibition of effective photochemical efficiency was calculated as follows:

$$Inh_{UVR}\ (\%) = (Y_P' - Y_x') \times Y_P^{-1} \times 100 \quad (6)$$

where Y_P' is Y' under PAR treatment, and Y_x' is Y' under PAB treatment.

Two radiation-induced inhibition values (*i.e.*, PAR and PAB) at 60 min were calculated as follows:

$$Inh_Y\ (\%) = (Y_0 - Y_{60}) \times Y_0^{-1} \times 100 \quad (7)$$

where Y_0 indicates the initial value, and Y_{60} is Y' after 60 min of exposure to the PAR or PAB treatment, respectively.

Data analysis: All experiments were performed in three replicates for each radiation and each nutrient treatment; for each determination of the effective photochemical efficiency and cell density, at least 2 measurements were performed. Thus, a total of 6 measurements were used to calculate the mean and standard deviation of the data presented. One-way or two-way analysis of variance (*ANOVA*) followed by *post-hoc Tukey*'s test was used to determine significant differences between the radiation treatments. The significant level was set at 0.05.

Results

Growth and Y': Outdoor culture: the doses during the experimental period were shown in Fig. 1*A*. The highest and the lowest doses were observed on the third and fourth day, respectively. During the 9-d acclimation period under solar radiation, the cell densities increased from 3.16 to 4.19 × 10^3 cells per ml (PAR) and 3.87 (PAB) for the Redfield ratio (16:1), and UVR caused 27.6% inhibtion in a specific growth rate during the first phase (from day 0 to day 4) (Fig. 1*C*). After 5 d of acclimation, there was no significant inhibition by UVR during the second phase (Fig. 1*E*); the specific growth reached 0.15 d^{-1}. However, the non-Redfield ratio led to the different variation in growth. In 1:1 (N:P) condition, both PAR and PAB inhibited the growth of *A. tamarense* totally during the first 4 d (Fig.1*B*). After 5 d of acclimation, the cells began growing and the specific growth rates were 0.13 (PAR) and 0.11 d^{-1} (PAB), respectively, but there was still 10.3% inhibition caused by UVR (Fig. 1*B*). In 200:1 (N:P) condition, the UVR-induced inhibition was 60.2%, which was higher than that in 16:1

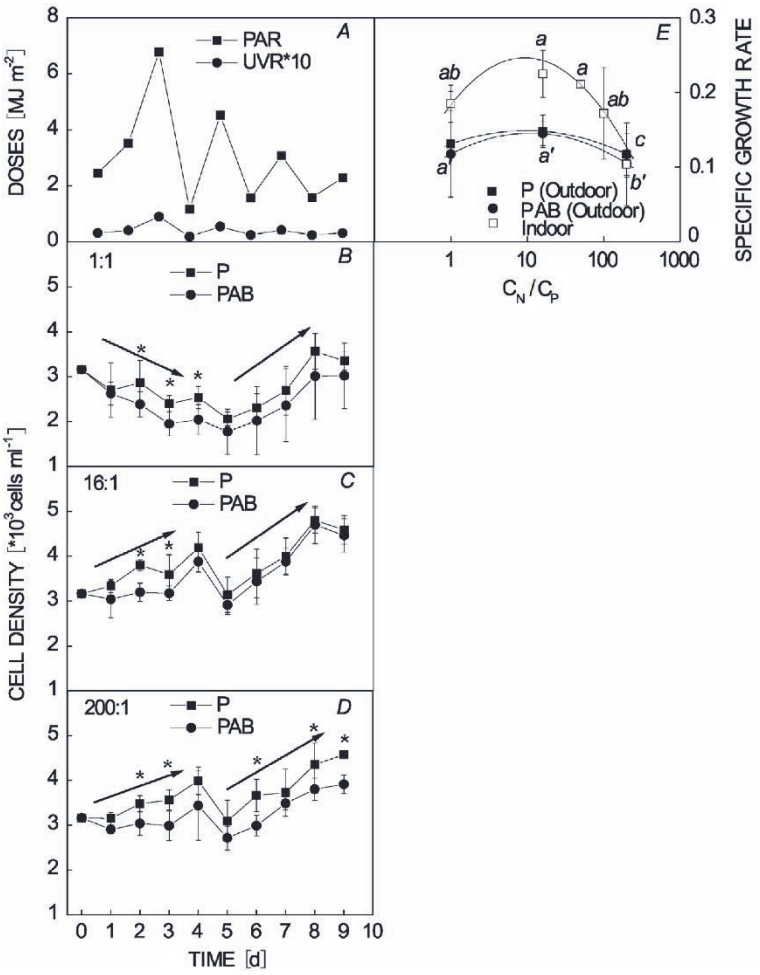

Fig. 1. (*A*) Doses of solar radiation during the experiments. Cell densities throughout the outdoor experiments in 1:1 (*B*), 16:1 (*C*), and 200:1 (*D*). (*E*) The specific growth rates of both indoor and outdoor cultures as a function of N:P ratio in the medium, respectively. Specific growth rate for outdoor culture was calculated by the data from day 5 to day 8. *Solid and open symbols* indicate the outdoor and indoor cultures, respectively. The vertical bars mean the SD (n = 6). * – significant differences between PAR and PAB treatment. The *different letters* indicate significant differences between the N:P ratio treatments and different radiations. There is no significant difference between those with *the same letters*.

during the first 4 d. Then the specific growth rates increased from 0.05 (PAR) and 0.02 d^{-1} (PAB) to 0.10 d^{-1} (PAR) and 0.09 d^{-1} (PAB), respecitively. The UVR-induced inhibition decreased to 10.0% (Fig. 1*D,E*). The specific growth rates and UVR-induced inhibition indicated that the non-Redfield ratio slowed down the growth of *A. tamarense* and enhanced the deleterious effect of UVR (Fig. 1*E*).

Indoor culture: during the 14-d of acclimation to five different N:P ratios, the cells growing at the Redfield ratio (16:1) exhibited the highest growth rate (0.22 d^{-1}) (Fig. 1*E*). The higher and lower N:P ratios inhibited cell growth significantly (Fig. 1*E*). The lowest growth rate was *ca*. 0.10 d^{-1}, which was observed at a ratio of 200:1. The growth rates corresponding to the other N:P ratios were intermediate. The specific growth rates associated with the different N:P ratios were fitted using the two-order equation: $y = a + bx + cx^2$ (R^2>0.95) (Fig. 1*E*). The statistical data indicated that the growth rates of cells exposed to the N:P ratios of 16:1 and 50:1 were significantly higher compared to cells exposed to other ratios (*e.g.*, 1:1, 100:1, and 200:1) (Fig. 1*E*), while there was no pronounced difference in cell growth between the 16:1 and 50:1 ratios. Comparing the two different cultures methods, the growth was slower in the outdoor than that in indoor culture, except for that at 200:1 (Fig. 1*E*).

The effective photochemical efficiency (Y') showed that Y' varied according to the diurnal change of solar radiation; the lowest Y' was observed at noon (12:00 h local time) and recovered in the evening (18:00 h local time) (Fig. 2*A*−*C*). The inhibition of solar UVR at noon proved that the non-Redfield ratio enhanced the UVR-induced inhibition during the 9-d outdoor acclimation compared to the Redfield ratio (16:1) (Fig. 2*D*).

UV-absorbing compounds (UV_{abc}) and pigments: The absorption spectra showed solar UVR increased the UV_{abc} content in *A. tamarense* for all outdoor cultures (Fig. 3*A*−*C*), and higher PAR also induced UV_{abc} synthesis in 1:1 (N:P) compared to those in 16:1 (Fig. 3*A,B*). The peak in optical density was found between 310 and 360 nm. However, the N:P ratio did not impact the synthesis of UV_{abc} in the indoor culture.

The pigments (both Chl *a* and Car) (Fig. 4*A,B*) were dependent on the N:P ratio in both indoor and outdoor cultures. They varied as a function of the ratios according

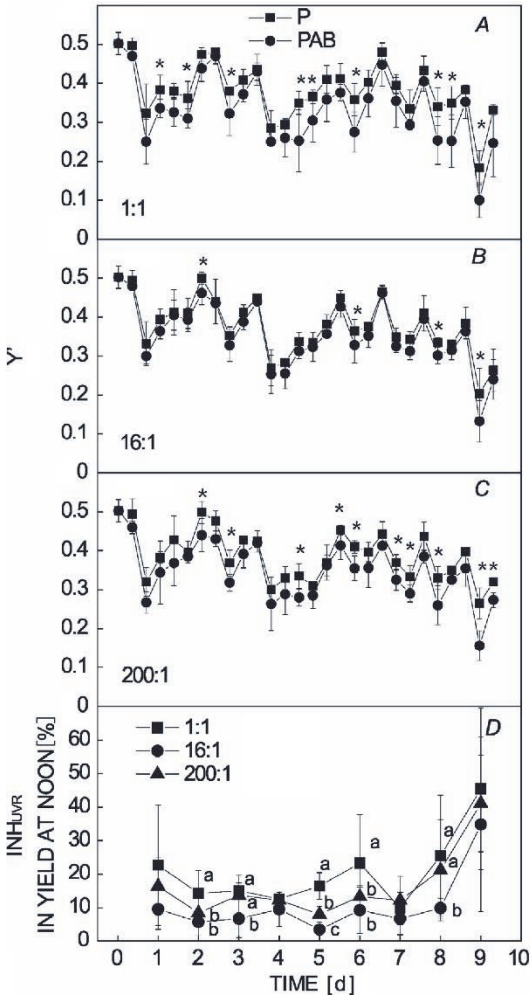

Fig. 2. The variation of photochemical efficiency (Y') during a 9-day acclimation of *Alexandrium tamarense* cells to solar radiation with UVR (PAB) or without UVR (PAR). Y' was measured at 07:00, 12:00 and 18:00 h (local time), respectively, during the experiment. *A, B*, and *C* mean the N:P ratio in the medium, 1:1, 16:1, and 200:1, respectively. (*D*) UVR-induced inhibition in Y' at noon. The vertical bars assign SD (n = 6). * and *the different letters* indicate significant differences among two radiation treatments (PAR and PAB) and three N:P ratio treatments, respectively. There is no significant difference between those with *the same letters*.

to the two-order equation $y = a + bx + cx^2$ (R^2>0.99). The highest pigment concentration (Chl *a* and Car) was observed at 16:1 (N:P) in both cultures. However, both a decrease and an increase in this ratio caused declines in the concentrations of these pigments. The outdoor higher solar radiation (both PAR and PAB) declined the contents of both Chl *a* and Car (Fig. 4*A,B*). Concerning the ratio of Car to Chl *a*, solar radiation (both PAR and PAB) increased the ratio in all N:P ratios compared to that of indoor conditions, furthermore, solar UVR (PAB) led to the higher ratio than solar PAR treatments (Fig. 4*C*).

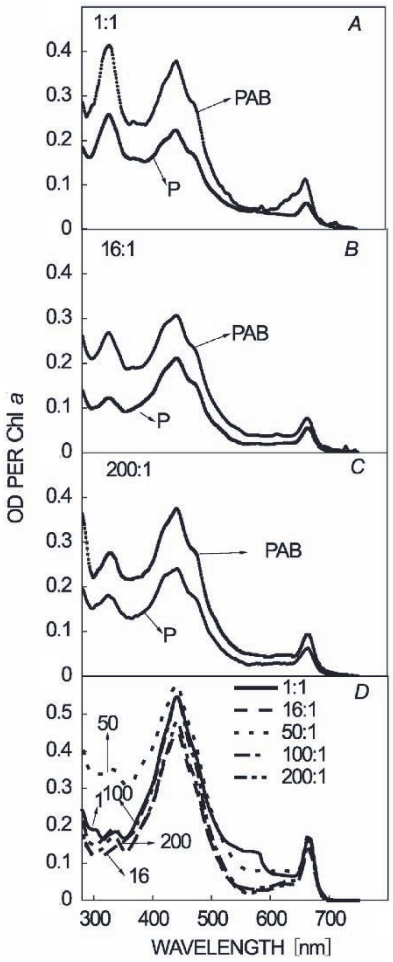

Fig. 3. Spectral absorption characteristics of *Alexandrium tamarense* (methanol extraction) in outdoor (*A, B*, and *C*) and indoor (*D*) cultures, respectively. PAR and PAB indicate two different radiation treatments. The different N:P ratios in Fig. *D* are indicated by 1:1, 16:1, 50:1, and 100:1, and 200:1.

Exposure-response curves: In order to ensure that conditions were comparable over the course of the experiments and in both indoor and outdoor cultures, we used an artificial UV lamp that provided constant UVR and artificial sea water containing specific concentrations of substrates to analyze the short-term response of *A. tamarense* to UVR. Artificial UVR was provided by an Xe lamp, and it closely resembled natural solar radiation up to a wavelength of approximately 700 nm. The intensities of radiation were 213.3 W m^{-2} of PAR, 21.0 W m^{-2} of UVR, and the exposure time was 60 min.

The effective photochemical efficiency (Y') data were fitted using a 1st-order exponential equation ($y = a + b \times e^{c \times t}$) (Fig. 5*A*–*E*) for both indoor and outdoor cultures. After a 14-d acclimation in the indoor culture to 16:1 (N:P), Y' decreased from 0.65 to 0.43 in 15 min during the PAR treatment and thereafter remained constant (Fig. 5*B*). Addition of UVR resulted in increased PSII damage, and

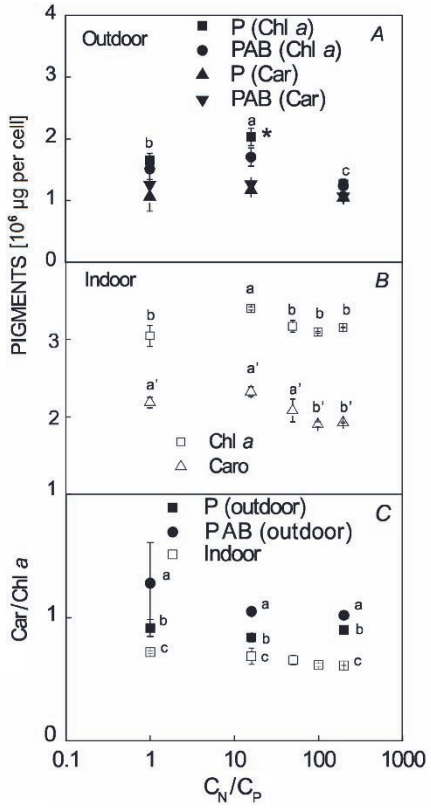

Fig. 4. Pigment concentrations for outdoor (*A*) and indoor (*B*) cultures and the ratio of carotenoids (Car) to chlorophyll (Chl) *a* (*C*) at the end of experiments. *Solid and open symbols* indicated the outdoor and indoor cultures, respectively. The vertical bars assign SD (n = 6). *– significant difference of Chl *a* between PAR and PAB treatments. *The different letters* in *A* and *B* indicate significant differences of Chl *a* (*e.g.*, a,b,c) or Car (*e.g.*, a',b',c') among different N:P ratio treatments, respectively. There is no significant difference in Car between three N:P ratio treatments for outdoor cells (*A*). *The different letters* in *C* indicate significant differences between outdoor and indoor treatments.

a decrease in Y' to 0.35 (PAB) (Fig. 5*B*). UVR led to 15.7% greater decrease compared with the PAR treatment after 60 min of exposure. After acclimation of cells to other N:P ratios (*e.g.*, 1:1, 50:1, 100:1, and 200:1), the patterns of Y' were the same as that observed at 16:1 (N:P) (Fig. 5*A,C–E*). Inhibition of Y' at 60 min caused by solar PAR or PAB varied as a function of these ratios according to the two-order equation $y = a + bx + cx^2$ (R^2>0.99) (Fig. 5*F*). Our results indicated that an enhancement or reduction in the media N:P ratio increased the sensitivity of cells to both PAR and UVR and that cells were more intolerant to UVR than to PAR (Fig. 5*F*).

After 9-d of outdoor acclimation, the same patterns were found as those in the indoor culture (Fig. 5*A,B,E*). But the decrease of Y' after a 60-min exposure under artificial solar lamp, was lesser than that in indoor cultures.

UVR-induced inhibition, repair and damage rate: The rate of UVR-induced damage to the photosynthetic apparatus (k, in min^{-1}) and the corresponding repair rate (r, in min^{-1}) were estimated according to the exposure-response curves (ERCs) above. There was no significant pattern for both r and k (Fig. 6*A,B*). However, the r/k ratios for both indoor and outdoor (Fig. 6*C*) and levels of UVR-induced inhibition (Fig. 6*D*) were functions of the N:P ratio according to the two-order equation $y = a + bx + cx^2$. The highest r/k and the lowest UVR-induced inhibition level were observed at 16:1, while the former decreased and the latter increased when the N:P ratio decreased to 1:1 or increased to 50:1, 100:1, and 200:1 (Figs. 5*F*, 6*D*). Thus, there was an obvious negative linear correlation between r/k and inhibition.

According to the outdoor data, the same phenomenon was observed; though the UVR-induced inhibitions were lesser than that of the indoor culture (Fig. 6*D*). The N:P ratio influcenced the photosynthetic performance of *A. tamarense* in response to solar UVR and this was not related to the history of light acclimation in cells.

Discussion

The response of *A. tamarense* to the N:P ratio: Redfield (1958) has reported that the C:N:P ratio in normal sea water is 106:16:1 (Redfield ratio); therefore, the optimum N:P ratio is approximately 16:1, and other ratios, such as N:P > 22 or < 10, have been proposed to indicate P limitation and N limitation, respectively (Dortch and Whitledge 1992, Justic *et al.* 1995). Nutrient limitation influences the growth (John and Flynn 2000, Lippemeier *et al.* 2001) and contents of pigments, proteins (Geider *et al.* 1993), and enzymes in microalgae (Brooks 1986, Geider *et al.* 1998). For instance, N limitation inhibits or even stops the growth of *Dunaliella salina* (Lippemeier *et al.* 2001), and P limitation decreases the growth of *A. fundyense* (John and Flynn 2000). These effects occur primarily due to a reduction in Chl *a* caused by N limitation, in addition to the blocking of the synthesis of central proteins in PSI and PSII, a decrease in the energy conversion efficiency (Geider *et al.* 1993), and decreases in the activity and concentration of Rubisco (Geider *et al.* 1998). In addition, the phosphorus content impacts the Calvin cycle by influencing the ATP/ADP ratio and reducing the activity of Rubisco (Brooks 1986). Nutrient limitation can also affect protein, carbohydrate, fat, and polysaccharide syntheses. For example, N and P limitation has been reported to influence carbon, nitrogen, phosphorus, and protein concentraitons in *Scenedesmus* sp. (Rhee 1978) and fatty acid contents in *Chlamydomonas reinhardtii*

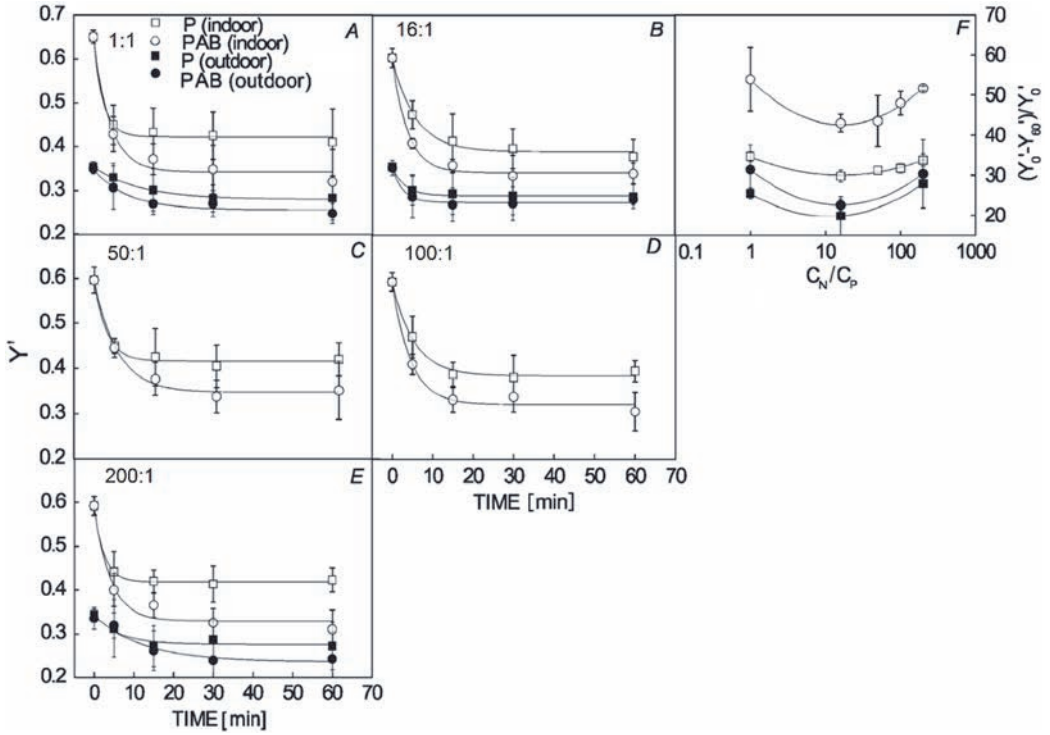

Fig. 5. Changes in the effective photochemical efficiency (Y') in *Alexandrium tamarense* during a 60-min exposure to PAR or PAB under UV lamp at the end of experiments in both outdoor and indoor cultures. (*F*) The two radiations induced inhibition at 60 min $[(Y_0' - Y_{60}')/Y_0']$ as a function of N:P ratio in the medium. *Solid and open symbols* indicate the outdoor and indoor cultures, respectively. The vertical bars indicate SD (n = 6). The "P" in Figure *A* indicates PAR treatment.

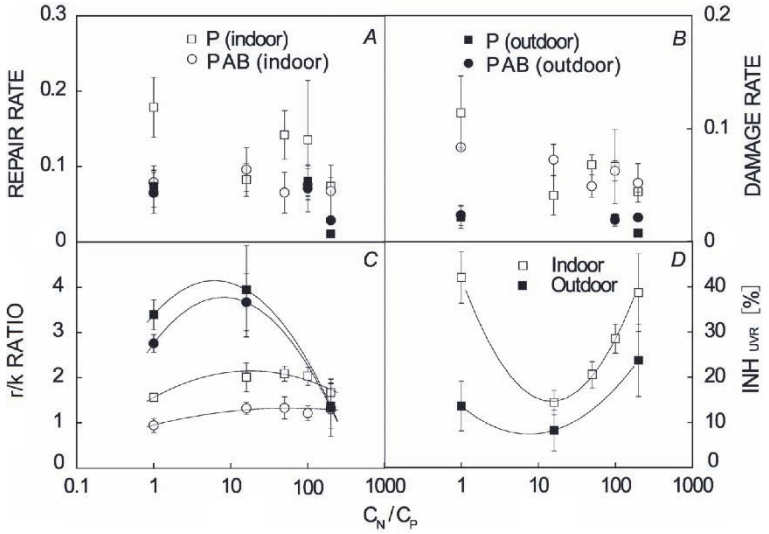

Fig. 6. (*A*) The repair rate (r) and (*B*) damage rate (k) under PAR and PAB treatments as a function of N:P ratio in the medium. (*C*) The ratio of r to k under PAR and PAB treatments as a function of N:P ratio in the medium. (*D*) The UVR-induced inhibition as a function of N:P ratio in the medium. *Solid and open symbols* indicate the outdoor and indoor cultures, respectively. The vertical bars indicate SD (n = 6). The "P" in Figure *A* and *B* indicates PAR treatment.

(Weers and Gulati 1997). Our results showed that the optimum N:P ratio was 16:1 and that growth was limited by either the increase or decrease in the N:P ratio (*e.g.*, 1:1, 50:1, 100:1, and 200:1, Fig. 1). The ratio of these nutrients significantly limited algal growth, except for that at 16:1. The growth rate of *A. tamarense* determined in this study was similar to that of this previously reported in species as long as the N:P ratio was higher or lower than the Redfield ratio (Fig. 1*B,D*), and the concentrations of Chl *a*, and Car were reduced when the N:P ratio was higher or lower than this ratio (Fig. 4*A,B*). Our results indicated that the growth and metabolic rates were reduced, thus the alga could acclimate to the new nutrient conditions by re-allocating intercellular energy, which was proven by pigments, UV_{abc}, and growth (Figs. 1–4). These processes are the main mechanisms of the response to N or P limitation in microalgae (Davies and Grossman 1998, Giordano and Hell 2001). Physiological activity decreases in cells due to declines in the Chl *a* and Car concentrations, which is a common

phenomenon in algae exposed to nutrient-limited environments (Geider *et al.* 1993). This might be related to N, which is the raw source for synthesis of both UV_{abc} and Chl *a* synthesis, while P limitation leads to intracellular energy redistribution, thereby also reducing the Chl *a* and UV_{abc} contents. Under 1:1 condition, the cell densities decreased during the first 4 d because of the accumulation of UV_{abc}, which cost a lot of energy, in both PAR and PAB treatments (Figs. 1*B*, 3*A*). Thus, UV_{abc} enhanced the tolerance of cells to both solar PAR and UVR to accelerate the growth after 5 d of acclimation.

The requirements of phytoplankton for potassium (K) are important because K in cytoplasm impacts ionic strength and affects ionic osmosis across the cell membrane (Reynolds 2006). The previous research indicated the potassium concentration (different N:K ratio) affected the net photosynthetic rate, Chl *a* content, chloroplast ultrastructure *etc*. in cotton (Zhao 2001). In our work, N:K was also changed following the variation of N:P in the media (refer to nutrient stoichiometry and treatments in Materials and methods). However, there is no regulated effect of potassium on the phytoplankton growth found by previous research (Jaworshi *et al.* 2003, Reynolds 2006). The variations of growth in *A. tamarense* were influenced by the N:P ratio only.

N:P ratio dependency of UVR-induced inhibition in *A. tamarense*: *A. tamarense* cells grown indoor under four different N:P conditions for 14 d were found to be more sensitive to UV radiation, with the lower r/k ratio, compared to those cultures with the nutrients at the Redfield ratio (16:1) (Figs. 1*E*, 5*F*). These differences in cells cultured with N and P at Redfield and non-Redfield ratios reflected the effects of the N:P ratio on cellular defense strategies against UVR. The N:P ratio influenced the response of *A. tamarense* to UVR by regulating the r/k (Fig. 5*E*) and pigments (Figs. 3, 4). In addition, the cells acclimated to outdoor showed the same patterns as those grown indoor. The Redfield ratio represented the most optimal nutrient conditions for adaptation of *A. tamarense* to UVR stress.

The lower r/k led to the reductions in the effective photochemical efficiencies under the non-Redfield nutrient conditions under both two radiation treatments (PAR and PAB). Although there is no significant patterns in the repair and damage rate, the sensitivity of cells to UVR is normally decided by the ratio of r/k, such as, the results in *Emiliania hulexyi* (Guan and Gao 2010), *Scrippsiella trochoidea* (Guan and Lu 2010), and *Chaetoceros curvisetus* (Guan *et al.* 2011). Then UVR resulted in the lowest inhibition of the effective photochemical efficiency at the Redfield value (16:1). Thus, the N:P ratio regulated the deleterious effects of UVR in *A. tamarense* by modifying the ratio of r/k. Accumulation of UV_{abc}, such as mycosporine-like amino acids (Garcia-Pichel 1994), is known to be protective against UVR. However, this effective protection mechanism was observed under the outdoor condition only. It indicated that the synthesis of UV_{abc} in *A. tamarense* is induced under higher solar PAR and UVR conditions.

Therefore, we concluded that the different N:P ratios resulted in varying photosynthetic and growth responses to UVR. The N:P ratio affected UVR-induced inhibition in *A. tamarense* by regulating the ratio of r/k and UV_{abc} and the ratios of Car to Chl *a*. However, light history of cell acclimation did not influence the effect of N:P on the response of *A. tamarense* to UVR.

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