Communications

Compensatory growth of the bloom-forming dinoflagellate *Prorocentrum donghaiense* induced by nitrogen stress*

doi:10.5697/oc.55-1.269
OCEANOLOGIA, 55 (1), 2013.
pp. 269–276.

KEYWORDS
Compensatory growth
Prorocentrum donghaiense
Nitrogen

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Received 21 November 2012, revised 13 February 2013, accepted 18 February 2013.

* This study was supported by the Natural Science Foundation of China-Guangdong Province Joint Key Project (U1133003), the Natural Science Foundation of China (41176104, 31070103), and the Natural Science Foundation of Guangdong Province Key Project (10251007002000001).

Abstract

Although the phenomenon of compensatory growth has been documented in some animals and higher plants, little information is available on its manifestation in marine microalgae. We have conducted the first study on the compensatory growth of the red tide causative dinoflagellate *Prorocentrum donghaiense* after its recovery from different nitrogen concentrations. The results showed that $NaNO_3$ concentrations of 0 and 7.5 mg l^{-1} significantly reduced the growth of *P. donghaiense*, as compared to 37.5 and 75 mg l^{-1}. When the microalgal cells were returned to 75 mg l^{-1}, they exhibited subsequent compensatory growth. The most significant compensatory growth was found in those cells previously experiencing 0 mg l^{-1}, followed by 7.5 mg l^{-1}, indicating that compensatory growth depended on the extent of nitrogen stress they had been subjected to. Our results suggest that compensatory growth can be induced in the marine microalga *P. donghaiense* after its recovery from nitrogen fluctuation, and that this should be taken into consideration in the prevalence of *P. donghaiense* blooms in coastal waters.

Compensatory growth (CG), an interesting physiological phenomenon characterized by the unusually fast growth of organisms after a period of reduced growth following stressful conditions, has been commonly observed in a variety of animals and terrestrial higher plants (Lennartsson et al. 1998, Oba et al. 2000). For instance, CG can be elicited in animals, e.g. fish, by various methods such as food restriction and subsequent refeeding for a period, as a result of which they achieve the same size as or even a larger size than their non-restricted counterparts (Sevgili et al. 2012). To take an example from the plant kingdom, it has been well documented that grassland productivity can be promoted by herbivory, whereby the photosynthetic rates and relative growth rates of the plants are stimulated after grazing or clipping, that is to say, the plants exhibit compensatory growth (Watt et al. 2007, Zhao et al. 2008).

However, the compensatory responses of marine microalgae, a type of lower plant with no differentiation into stem, root or leaf, are poorly understood. They are the primary producers in marine environments driving ocean life, but under certain ecological conditions, some marine microalgae may proliferate rapidly in a short time and eventually give rise to red tides. During these events, biological responses, such as a compensatory growth response resulting from environmental changes, may be a major causative factor, together with other complex ecological, meteorological and oceanographic processes. Red tide causative microalgae may be exposed to a variety of fluctuating conditions; understanding compensatory growth in microalgae during their recovery from adverse environments is therefore

not merely of theoretical interest, but may also have profound ecological implications (Cai et al. 2009).

Prorocentrum donghaiense is an ecosystem-disruptive algal bloom dinoflagellate species that frequently occurs during late spring and early summer in the Changjiang River estuary and the adjacent East China Sea (Sunda et al. 2006), where the nitrogen input into the water system is very complex (Hu et al. 2012). It is becoming increasingly evident that the input of nitrogen from industrial or agricultural activities is linked with red tide scenarios, yet it is not clear whether compensatory growth in this dominant species is elicited as a result of fluctuations in the nitrogen concentration. Here we report on the first study specifically assessing the compensatory growth of *P. donghaiense* recovering from nitrogen deprivation. We measured the cell proliferation of this species at different nitrogen concentrations to evaluate the restricted growth of cells due to nitrogen limitation. After the original nitrogen levels had been restored, the subsequent compensatory response of *P. donghaiense* was elucidated by comparing the growth of cells recovering from nitrogen deprivation and of those in the control group.

The marine microalga *P. donghaiense* was obtained from the Institute of Hydrobiology, Jinan University, Guangzhou, China. It was grown in f/2 medium at constant irradiance (70 μmol m^{-2} s^{-1}) and temperature (23°C) in a 12 h/12 h (light/dark) photoperiod cycle in a plant growth chamber. Artificial seawater was passed through a 0.45 μm filter prior to the experiment (Harrison & Berges 2005). Algal cells in the exponential growth phase were used, and one mL of microalgal culture was inoculated into 250 mL Erlenmeyer flasks containing 99 mL culture medium. All the glassware and media in the experiments were previously sterilized. The salinity of the artificial seawater was about 30 PSU and the initial pH of the culture was 7.0.

The experiment comprised two stages: a nitrogen stress stage consisting of four different $NaNO_3$ concentrations (0, 7.5, 37.5 and 75 mg l^{-1}), and a recovery stage, during which the microalgal cells were restored to the standardized condition (75 mg l^{-1}). The nitrogen stress trial lasted for six days, the recovery trial for eight days. The flasks containing microalgal cells were gently shaken by hand three times a day, and all the experiments were carried out in triplicate. Every morning during the experiment, a 0.5-mL algal sample was taken from each flask. The numbers of algal cells were counted immediately using a haemocytometer under an optical microscope in the laboratory. The growth rate (μ, divisions d^{-1}) was calculated using the following equation:

$$\mu = (\mathrm{Ln}\, X_1 - \mathrm{Ln}\, X_0)/(t_1 - t_0), \tag{1}$$

where X_1 and X_0 (cells/mL) are the cell densities at times t_1 and t_0 (d) (Piedras & Odebrecht 2012). Statistical tests were conducted using Microsoft Excel 2003 (Microsoft Company, USA) and SAS (SAS Institute Inc., Cary, NC, USA). The statistical significance of the specific growth rate within each treatment was determined by one-way ANOVA, and the probability level of 0.05 was used as the threshold for statistical significance. All the data were expressed as mean $\pm$ SE.

During the first three days, no significant differences were observed in the growth of *P. donghaiense* in the 0, 7.5, 37.5 and 75 mg dm^{-1} $NaNO_3$ concentrations; the cell density of *P. donghaiense* was 20×10^4 cells mL^{-1} in each treatment. Thereafter, the cells at 7.5, 37.5 and 75 mg l^{-1} grew rapidly, and significant differences were recorded among the different nitrogen concentrations. Low nitrogen restricted the growth of *P. donghaiense* (Figure 1a). As can be seen from Figure 1b, there were considerable differences in the specific growth rates of *P. donghaiense*, which increased with rising nitrogen concentrations: 0.32, 0.68, 0.77 and 0.78 d^{-1} at respective $NaNO_3$ concentrations of 0, 7.5, 37.5 and 75 mg l^{-1}. Our results extend the observation that low nitrogen restricts the growth of *P. donghaiense*. This can be explained by the fact that nitrogen is an essential nutrient for plant growth, and a shortage of nitrogen restricts the biosynthesis of the nucleic acids, hormones and enzymes involved in microalgal cell proliferation (Shskara et al. 2011, Pirastru et al. 2012). It needs to be pointed out that even at 0 mg dm^{-1}, the cells were still able to survive, suggesting that *P. donghaiense* could adapt to nitrogen

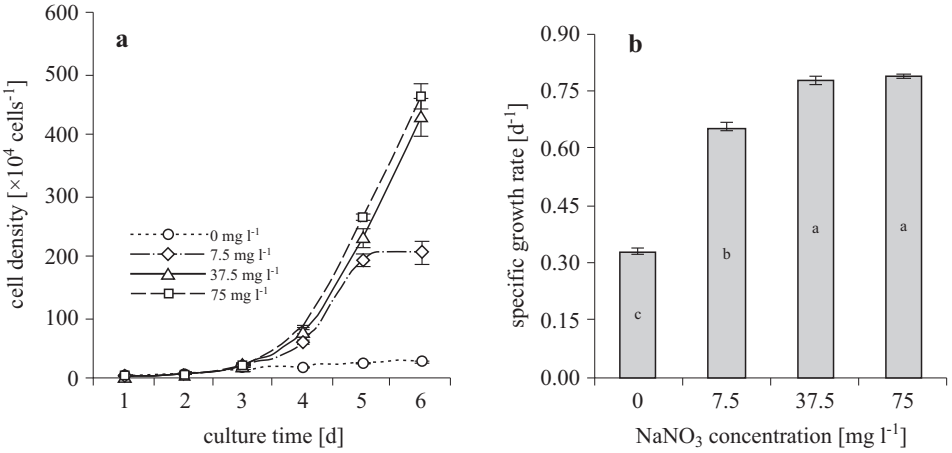

Figure 1. Cell growth of *Prorocentrum donghaiense* at various $NaNO_3$ concentrations (cell density (a); specific growth rate (b); each bar represents mean $\pm$ SE; the bars with different letters are significantly different at $P < 0.05$)

deprivation for a certain time. It has been reported that microalgae may possess a capacity for storing nitrogen for later use, or that they may alter the expression of nitrogen-related enzymes and genes, which enables them to survive in conditions of nitrogen depletion (Lomas & Glibert 2000, Hockin et al. 2012).

After the microalgal cells previously subjected to nitrogen limitation were transferred to the nitrogen repletion condition (75 mg l^{-1}), they showed, to some extent, a compensatory growth response, which was dependent on the level of nitrogen stress that the cells had been subjected to before (Figure 2a). The cells exposed to lower nitrogen concentrations (0 and 7.5 mg l^{-1}) appeared to exhibit faster growth when they were recultured at 75 mg l^{-1}. The cells previously kept at 0 mg l^{-1} exhibited the most obvious compensatory growth when they were recultured at 75 mg l^{-1}, followed by those previously kept at 7.5 mg l^{-1}. This indicates that the level of stress was an important factor affecting the subsequent compensatory growth of microalgal cells (Figure 2b). These results were consistent with our earlier ones, which indicated that compensatory growth in the marine phytoplankton *Phaeodactylum tricornutum* triggered by darkness and UV radiation took place in response to a stress condition (Guo et al. 2005, Cai et al. 2009). However, the biological mechanisms possibly involving the alteration of enzyme and gene expression, underlying such compensatory growth, are not yet fully understood. After the growth of cells in relatively severe conditions, a protection system like anti-oxidative enzymes can be

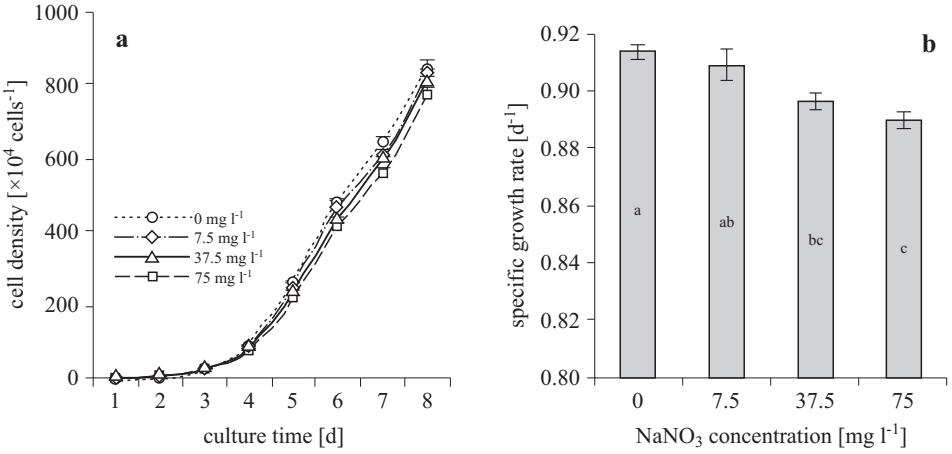

Figure 2. Compensatory response of *Prorocentrum donghaiense* after recovery from nitrogen depletion conditions (cell density (a); specific growth rate (b); each bar represents mean ± SE; the bars with different letters are significantly different at $P < 0.05$)

induced, and ultimately overcorrection of physiological function substances of POD, SOD, and CAT is elicited, which results in the subsequent rapid growth of cells (Metcalfe & Monaghan 2001).

Few studies of the compensatory growth of red tide causative microalgae have yet been carried out. The properties of microalgae, the severity of eutrophication, and concerns about the increasingly frequent outbreaks of red tides have triggered great interest in the question whether microalgae exhibit compensatory growth after environmental fluctuations. To our knowledge, this is the first attempt to evaluate such growth phenomena in the dinoflagellate *P. donghaiense*, an ecosystem-disruptive algal bloom species frequently proliferating in Chinese coastal waters. Our preliminary results provide strong evidence that compensatory growth does take place in the recovery stage, and that such a response is closely related to the level of stress that the algal cells were previously subjected to. It is worth pointing out that compensatory growth has been considered an adaptive response of organisms to wide fluctuations in environmental factors (Marshall et al. 2006, Ruiz-R et al. 2008); hence, the results presented here could have profound ecological implications. In recent years, red tides have increased in frequency, intensity and geographic distribution, and have led to many negative environmental and economic consequences. Based on the findings of our study, therefore, it is reasonable to hypothesize that *P. donghaiense* may proliferate exceptionally fast once a normal climate is restored following an inclement one. Moreover, this phenomenon is in accordance with field observations: red tides usually occur after a rainy spell.

In brief, our results extend the observation that compensatory growth also takes place in the dinoflagellate *P. donghaiense* once nitrogen stress has been relieved, and that such a compensation response is closely related to the extent of the stress that the algal cells were previously subjected to. We propose that such an unusual growth response should be taken into account when analysing the occurrences of this microalga in coastal waters. However, further physiological, biochemical and molecular analytical studies are urgently required to unravel the mechanisms underlying this compensatory growth in *P. donghaiense*.

Acknowledgements

The authors would like to thank Dr. and Prof. Yuanjiao Feng of the Institute of Tropical and Subtropical Ecology, South China Agricultural University, for her kind assistance in revising our manuscript. The authors also wish to thank the reviewer for the insightful comments which helped to improve the quality of our manuscript.

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