Vol. 77: 155–166, 2016
doi: 10.3354/ame01793

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online September 16

Effects of temperature, irradiance and pCO_2 on the growth and nitrogen utilization of *Prorocentrum donghaiense*

Zhangxi Hu[1,2], Margaret R. Mulholland[3,*], Ning Xu[4], Shunshan Duan[4]

[1]Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, PR China

[2]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, PR China

[3]Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, 4600 Elkhorn Avenue, Norfolk, VA 23507-0276, USA

[4]Research Center of Hydrobiology, Jinan University, 601 W Huangpu Blvd, Guangzhou 510632, PR China

ABSTRACT: Environmental factors such as temperature, irradiance, and nitrogen (N) supply affect the growth of *Prorocentrum donghaiense*, but the interactive effects of these physical factors and the effects of atmospheric CO_2 (pCO_2) on growth and N uptake have not been examined. We compared growth kinetics of *P. donghaiense* grown on 4 different N substrates (nitrate [NO_3^-], ammonium [NH_4^+], urea, and glutamic acid [glu]) with respect to temperature, irradiance, and pCO_2. Temperature (15 to 30°C) had a positive effect on growth (max. growth rates: 0.17 to 0.65 d^{-1}; optimal temperature: 25 to 30°C); maximum specific growth rates (μ_{max}) declined when cultures were grown at 30°C. *P. donghaiense* grew well on all 4 N sources, under irradiances ranging from 10 to 180 µmol quanta m^{-2} s^{-1}. μ_{max} (2.0 ± 0.1 d^{-1}) was observed in cultures growing with NH_4^+ as the sole N source in the highest irradiance treatment (180 µmol quanta m^{-2} s^{-1}). These rates were significantly higher than those measured in cultures grown on NO_3^-, urea, and glu (all ~1.4 d^{-1}). Half-saturation constants (K_s) ranged from 66.5 ± 4.6 to 99.4 ± 6.7 µmol quanta m^{-2} s^{-1} for cultures grown with glu or NH_4^+, respectively, as the sole source of N. Both growth and N uptake rates were higher in cultures grown under elevated pCO_2. Our results suggest that *P. donghaiense* exhibits flexible adaptation for N utilization under broad environmental conditions (temperature, irradiance, pCO_2), which may play an important role in the formation and duration of *P. donghaiense* blooms.

KEY WORDS: *Prorocentrum donghaiense* · Temperature · Irradiance · CO_2 · Nitrogen · Growth rate · Nitrogen uptake

INTRODUCTION

Harmful algal blooms (HABs) are a worldwide ecological problem that have become more frequent, extensive, and severe in recent years (Wang & Wu 2009, Anderson et al. 2012, Dia et al. 2014, Glibert 2014, Song et al. 2016). HABs most often occur in coastal waters, where they exert serious economic impacts on marine fisheries and aquaculture, and threaten public health and aquatic ecosystems (Heisler et al. 2008). HABs commonly occur in the coastal areas of China, including the South China, East China, and Bohai seas, and their frequency, intensity, and duration have increased dramatically in the last 2 decades (Wang & Wu 2009, Zhou 2010, Lu et al. 2014, Song et al. 2016). Since 2000, blooms

*Corresponding author: mmulholl@odu.edu

of *Prorocentrum donghaiense*, a photosynthetic eukaryote, have occurred every year in the Changjiang River Estuary and coastal waters adjacent to Zhejiang province, causing serious economic impacts (Li et al. 2009, Zhou 2010, Lin et al. 2014). While blooms of *P. donghaiense* have been studied since they were first recorded in early 2000s (Lu & Goebel 2001, Lu et al. 2005, Wang & Wu 2009, Zhou 2010, Lin et al. 2014), the identity of factors promoting the initiation and persistence of these blooms remains elusive.

Growth of marine phytoplankton is inherently complex, and there are generally predictable but nonlinear relationships between their growth and environmental parameters such as temperature, irradiance, and nutrient concentrations (Harrison & Platt 1986, Litchman & Klausmeier 2008, Boyd et al. 2013, Edwards et al. 2015a,b). HABs have most often been linked to nutrient over-enrichment and consequent eutrophication of coastal waters (Heisler et al. 2008). Both the form of dissolved nitrogen (N) and its concentration in coastal waters are thought to be important in the formation and development of HABs and phytoplankton blooms (Dugdale et al. 2007). Increased nutrient loads (i.e. N) from the Changjiang (Yangtze) River have led to increased HAB events, including *P. donghaiense* blooms (Li et al. 2014). Over the progression of a *P. donghaiense* bloom, concentrations of dissolved inorganic and organic N can vary from several µM to more than 50 µmol N l^{-1} (Li et al. 2010). Previous studies have examined the growth kinetics of *P. donghaiense* on different N sources and determined that *P. donghaiense* has high affinities for and exhibits maximum growth rates under a diverse suite of N compounds (Hu et al. 2012, 2014).

Responses of marine phytoplankton to temperature are species-specific and occasionally strain-specific, depending on the geographic region in which they are isolated (Thomas et al. 2012, Boyd et al. 2013). Temperature is an important regulator of morphology, growth rate, resource allocation, and metabolism in phytoplankton (Lomas & Glibert 1999, Persson et al. 2013, Toseland et al. 2013), and can also affect toxin production by some harmful algae (Band-Schmidt et al. 2014, Thorel et al. 2014). Blooms of *P. donghaiense* typically occur in late April or early May at temperatures of 15 to 26°C (Li 2009, Li et al. 2010, Lu et al. 2005, 2014), and monitoring data has confirmed that temperature is a key factor affecting the initiation and development of *P. donghaiense* blooms (Li 2009, Zhu et al. 2009). Blooms of this species usually last several weeks to more than a month, over which time surface water temperatures generally increase. Even though *P. donghaiense* can efficiently utilize different N compounds (Hu et al. 2012), even when present at low concentrations (Hu et al. 2014), the activity of enzymes involved in nutrient utilization varies with temperature. Water temperatures in the Changjiang River Estuary and adjacent waters varies from 15 to 26°C during the period when blooms commonly occur (Li 2009, Li et al. 2010, Lu et al. 2005, 2014). Previous culture studies have been conducted at temperatures between 20 and 25°C (Hu et al. 2012, Wang et al. 2013), but the ability of *P. donghaiense* to grow on different N sources at the range of temperatures experienced in the environment has not yet been fully investigated.

Irradiance is another major factor controlling growth of autotrophic phytoplankton because it is essential for photosynthesis. In highly turbid areas such as East China Sea (Sun et al. 2008), light availability can limit photosynthesis. The intensity of photosynthetically active radiation (PAR) can be estimated from the depth of the mixed layer and turbidity (Diehl et al. 2002). In spring, the surface PAR of the East China Sea has been estimated to be ~200 W m^{-2} (Sun et al. 2008), with light intensity attenuating rapidly with depth. *P. donghaiense* blooms mainly appear near the surface, but in some cases the bloom depth can extend to 10 m. The maximum recorded cell density during a bloom was 36×10^7 cells l^{-1}, which gave the water a brownish discoloration (Lu et al. 2005). Because algal cells are also particles that contribute to turbidity, during dense surface blooms light can rapidly attenuate with depth and limit the ability of photosynthetic cells to grow and take up N.

While not generally thought to limit algal growth, during dense blooms dissolved inorganic carbon (DIC) can be rapidly drawn down, thereby raising the pH and limiting photosynthesis (Boyd & Hutchins 2012). Atmospheric CO_2 concentrations are expected to double by the end of this century (IPCC 2013), and elevated pCO_2 increases growth rates of cyanobacteria and some other phytoplankton (Wu et al. 2014), perhaps by alleviating carbon (C) limitation of photosynthesis (Hutchins et al. 2007). Elevated pCO_2 also results in higher DIC concentrations in seawater, and this alters the inorganic carbon buffer system and decreases pH in oligotrophic waters where photosynthetic drawdown of DIC is lower. In nutrient-enriched eutrophic coastal systems, however, increasing the supply of DIC may alleviate C limitation of algal growth, provided light or some other element does not limit their growth. Higher supplies of DIC could also impact marine phytoplankton growth, element ratios, photosynthetic rates, nutrient utilization,

toxin production, and community structure (Burkhardt et al. 1999, Hutchins et al. 2013, Li & Campbell 2013, Tatters et al. 2013, Errera et al. 2014, Celis-Plá et al. 2015, Johnson et al. 2015).

Environmental factors such as temperature and irradiance affect the growth of *P. donghaiense* in culture experiments and in the field (Deng et al. 2009, Zhu et al. 2009, Xu et al. 2010), but the interactive effects of these physical factors and N availability have not been examined. A diverse suite of N compounds are available to phytoplankton in natural seawater that can potentially support their growth. These include inorganic N compounds (nitrate [NO_3^-], nitrite [NO_2^-], and ammonium [NH_4^+]) and organic N compounds (including urea and amino acids) (Bronk 2002, Mulholland & Lomas 2008, Mulholland & Lee 2009). Many enzymes facilitating the uptake of these compounds are sensitive to environmental factors such as temperature and irradiance (Gao et al. 2000). In previous studies, we examined the growth kinetics of *P. donghaiense* on different N sources and determined that *P. donghaiense* had high affinities to, and experienced maximum growth rates under a diverse suite of N compounds (Hu et al. 2012, 2014). Here, we examined the growth and N uptake responses of cultured populations of *P. donghaiense* to changes in temperature, irradiance, and pCO_2 concentration, in order to better understand how physical factors affect growth and N (NO_3^-, NH_4^+, urea, and amino acid) uptake.

MATERIALS AND METHODS

Alga and culture conditions

Prorocentrum donghaiense was isolated from the coastal waters of Zhoushan, Zhejiang province, China, and obtained from the Research Center of Hydrobiology, Jinan University, Guangzhou, China. Cultures were grown in artificial seawater enriched with sterile, silicate-free medium supplied with f/2 trace metals and vitamins (Guillard 1975). Concentrations of N in the culture media were 50 µmol N l^{-1} (supplied as NO_3^-, NH_4^+, urea, or glutamic acid [glu]), and phosphate concentrations were 3.12 µmol P l^{-1} (as phosphate, PO_4^{3-}). A series of experiments were conducted wherein temperature, irradiance, or pCO_2 were varied to examine their individual effects on *P. donghaiense* growth rates. Growth responses of *P. donghaiense* with respect to temperature and irradiance were examined in cultures grown on 4 different N sources (NO_3^-, NH_4^+, urea, and glu), while growth responses of *P. donghaiense* with respect to pCO_2 were measured only in cultures containing NO_3^- as the sole N source. Experiments in which temperature and irradiance were the experimental variables were conducted in an incubator maintained at present day pCO_2 and either temperature or irradiance was varied; temperature treatments were 15, 20, 23, 25, or 30°C and irradiance treatments were 10, 20, 50, 80, 120, and 180 µmol quanta m^{-2} s^{-1}. In all experiments, light was supplied on a 12 h light:12 h dark cycle. For temperature treatments, cultures were maintained at a constant irradiance (60 µmol quanta m^{-2} s^{-1}) and for irradiance treatments, cultures were maintained at a constant temperature (23°C). For pCO_2 experiments, cultures were maintained in an environmental room at constant temperature (23°C) and irradiance (60 µmol quanta m^{-2} s^{-1}), and N was supplied as NO_3^- (50 µmol N l^{-1}). To estimate growth rates, *in vivo* fluorescence was monitored at the same time each day using a Turner Designs AU-10 fluorometer.

Experimental design

Growth response to temperature. For each nutrient treatment (NO_3^- [data from Boyd et al. 2013], NH_4^+, urea or glu), growth curves were constructed from batch cultures grown at 15, 20, 23, 25, and 30°C. For each nutrient and temperature treatment, cultures were grown in triplicate, capped 50 ml Pyrex test tubes containing 35 ml of medium. Cultures were maintained under the treatment growth conditions for more than 5 generations prior to the start of growth experiments to ensure cells were acclimated to treatment conditions (temperature and N source combination). On the first (the day of culture inoculation) and last days (the day of culture just going to stationary stage) of the experiments, samples were collected to measure cell abundance and chlorophyll *a* (chl *a*).

Growth response to irradiance. Using neutral density screening and by adjusting the distance between cool white fluorescent lamps, cultures growing on 50 µmol l^{-1} NO_3^-, NH_4^+, urea or glu as the sole N source were grown at irradiances of 10, 20, 50, 80, 120 or 180 µmol quanta m^{-2} s^{-1}, supplied on a 12 h light:12 h dark cycle. Cultures were maintained under each treatment condition (irradiance and N source combination) for more than 5 generations prior to the start of experiments in order to ensure cells were acclimated prior to the start of the experiments. For each treatment, cultures were grown in

triplicate, capped, 50 ml Pyrex test tubes containing 35 ml of medium. On the first (the day of culture inoculation) and last days (the day of culture just going to stationary stage) of the experiments, samples were collected to measure cell abundance, and chl *a*.

Growth response to pCO_2. For experiments examining the effects of pCO_2 on growth of *P. donghaiense*, triplicate cultures were equilibrated to 190 (glacial maximum pCO_2), ~380 (present day), 500 (predicted 2050 pCO_2), or 750 (predicted 2100 pCO_2) ppm pCO_2. Present day pCO_2 was maintained with filtered air from the room (0.2 µm filtered) using an air pump. The other 3 CO_2 concentrations were commercially prepared certified standard CO_2 gas mixtures (Gilmore Liquid Air Company). As with previously described experiments, cultures were acclimated to different pCO_2 levels for more than 5 generations prior to the start of experiments. *In vivo* fluorescence, cell density, chl *a*, particulate nitrogen and carbon (PN and PC), and pH were monitored daily, and nutrient concentrations (NO_3^-, NH_4^+, urea, dissolved free amino acids [DFAAs], and PO_4^{3-}) were measured every other day over the course of experiments.

On the 9th day of each pCO_2 experimental treatment, N uptake rates were measured using stable isotopes as tracers. Uptake experiments were initiated by adding 100 nmol N l^{-1} of highly enriched (96 to 99%) ^{15}N-labeled NO_3^- or NH_4^+, or dually labeled (^{15}N and ^{13}C) urea or algal amino acid mixture to culture aliquots placed in 30 ml acid-washed bottles. Incubations were terminated after 30 to 60 min by gentle filtration through pre-combusted (450°C for 2 h) GF/F (nominal pore size ~0.7 µm) filters. Filters were then frozen in sterile polypropylene cryovials until analysis.

Analytical methods

Measurement of chl *a*, cell density, PN/PC and nutrient concentrations. Chl *a* concentrations were measured fluorometrically after extraction in 90% acetone (Welschmeyer 1994). Cells were enumerated using a Sedgwick-Rafter counting chamber and a Zeiss inverted microscope or a FACSCalibur flow cytometer (Becton Dickinson Instruments). PC and PN concentrations and isotopic ratios were analyzed on a Europa Scientific 20-20 isotope ratio mass spectrometer equipped with an automated N and C analyzer. Dissolved NO_3^- and urea concentrations were measured on an Astoria Pacific autoanalyzer using standard colorimetric methods according to the manufacturer's specifications (Parsons et al. 1984). NH_4^+ was determined using the manual phenol hypochlorite method (Solorzano 1969). Concentrations of DFAAs were measured by high performance liquid chromatography (HPLC) (Cowie & Hedges 1992).

Calculation of specific growth rates. Specific growth rates (µ; d^{-1}) were calculated using a least squares fit to a straight line after logarithmic transformation of *in vivo* fluorescence data, as described by Guillard (1973):

$$\mu = \frac{\ln N_1 - \ln N_0}{T_1 - T_0} \quad (1)$$

where N_1 and N_0 are the *in vivo* fluorescence at time T_1 and T_0, respectively, during the linear portion of exponential phase growth.

The relationship between growth rate and irradiance was described using the equation modified from Lederman & Tett (1981):

$$\mu = \frac{\mu_{max}(I - I_0)}{I + K_s - 2I_0} \quad (2)$$

where μ_{max} is the maximum specific growth rate (d^{-1}), I is the irradiance (µmol quanta m^{-2} s^{-1}), I_0 is the compensation irradiance (µmol quanta m^{-2} s^{-1}) and K_s is the irradiance at $\mu_{max}/2$ (i.e. half-saturation light intensity).

Calculation of N uptake rates. N uptake rates were calculated using the following equation (Mulholland & Lee 2009):

$$^{15}\text{N uptake}\,(V) = \frac{(\text{atom\% PN})_{final} - (\text{atom\% PN})_{initial}}{(\text{atom\% N source pool} - \text{atom\% PN})_{initial} \times \text{time}} \quad (3)$$

where the source pool was the dissolved N pool that was enriched.

Statistical analysis. All statistical tests were conducted using Microsoft® Excel 2007 and SPSS 13.0 with the level of significance set at $\alpha = 0.05$. Differences in maximum *in vivo* fluorescence, specific growth rates and N uptake rates were compared using a 1-way ANOVA.

RESULTS

Growth response of *P. donghaiense* to temperature

Temperature had a positive effect on the growth of *Prorocentrum donghaiense* when NO_3^-, NH_4^+, urea or glu were supplied as the sole N source. NO_3^- results were obtained from Boyd et al. (2013), who

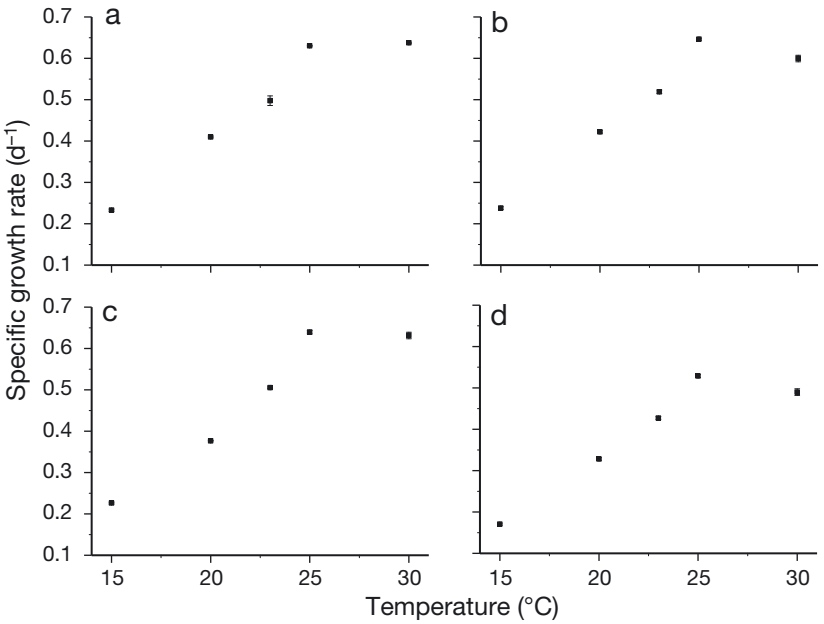

Fig. 1. Specific growth rates of *Prorocentrum donghaiense* grown on (a) NO_3^- (redrawn from Boyd et al. 2013), (b) NH_4^+, (c) urea, and (d) glutamic acid as a function of temperature. Values are means ± SD of triplicate samples

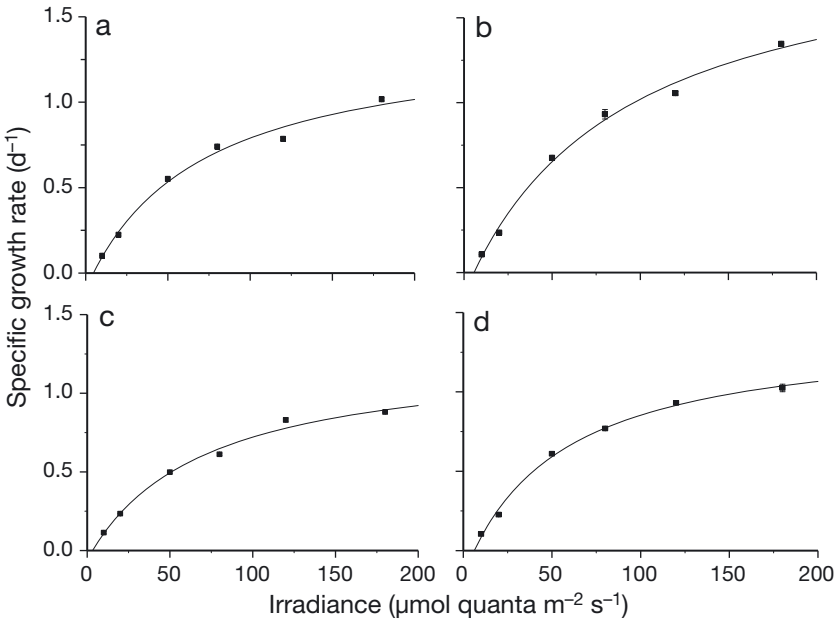

Fig. 2. Specific growth rates of *Prorocentrum donghaiense* grown on (a) NO_3^-, (b) NH_4^+, (c) urea, and (d) glutamic acid as a function of irradiance. Solid lines are fitted iteratively to the data according to the Monod equation. Values are means ± SD of triplicate samples

addressed the temperature versus growth responses of phytoplankton from polar to tropical waters. Here, we redrew the NO_3^- data to compare with the growth response of *P. donghaiense* grown on NH_4^+, urea, and glu. Specific growth rates ranged from 0.17 to 0.65 d^{-1} (Fig. 1); maximum specific growth rates were observed in cultures of *P. donghaiense* grown at 25°C (Fig. 1). The specific growth rates for cultures grown on NO_3^- at 25 and 30°C were not significantly different from each other (Fig. 1). Specific growth rates of cultures supplied with NO_3^-, NH_4^+ and urea as the sole source of N were significantly higher than those supplied with glu as an N source at all temperatures tested (15 to 30°C) ($p < 0.01$; Fig. 1).

Growth response of *P. donghaiense* to irradiance

Growth curves for *P. donghaiense* with respect to irradiance were fit using maximum specific growth rates measured in cultures growing at irradiances between 10 and 180 µmol quanta m^{-2} s^{-1} for each of the 4 N compounds (NO_3^-, NH_4^+, urea and glu) as a sole source of N (Fig. 2). The average specific growth rates of *P. donghaiense* cultured using the 4 different N compounds were not significantly different from each other, and averaged ~0.1 d^{-1} in cultures maintained at the lowest irradiance (10 µmol quanta m^{-2} s^{-1}) (Fig. 2). However, at the highest irradiance tested (180 µmol quanta m^{-2} s^{-1}), the average specific growth rates ranged from 0.9 d^{-1} in cultures grown on urea to 1.4 d^{-1} in cultures growing with NH_4^+ as the sole N source (Fig. 2). We calculated μ_{max} for *P. donghaiense* grown on the 4 different N sources to range from 1.3 ± 0.0 to 2.0 ± 0.1 d^{-1}. The highest μ_{max} were found in cultures grown with NH_4^+ as the sole source of N (2.0 ± 0.1 d^{-1}) and these were significantly higher than those calculated for cultures grown on NO_3^- (1.4 ± 0.0 d^{-1}), glu (1.4 ± 0.1 d^{-1}) or urea (1.3± 0.0 d^{-1}) (Table 1). The calculated I_0 ranged from 2.8 ± 0.3 to 6.2 ± 0.1 µmol quanta m^{-2} s^{-1} (Table 1). The K_s for cultures grown on NO_3^-, NH_4^+, urea, and glu were 78.0 ± 3.5, 99.4 ± 6.7, 79.7 ± 1.4 and 66.5 ± 4.6 µmol quanta m^{-2} s^{-1}, respectively (Table 1).

Table 1. Mean (±SD; n = 3) growth parameters as a function of irradiance for *Prorocentrum donghaiense* growing with NO_3^-, NH_4^+, urea, or glutamic acid (glu) as the sole source of nitrogen in the culture medium. The maximum specific growth rate (μ_{max}; d^{-1}), compensation irradiance (I_0; µmol quanta m^{-2} s^{-1}) and half-saturation light intensity (K_s; µmol quanta m^{-2} s^{-1}) for each nitrogen compound was calculated according to the equation modified from Lederman & Tett (1981)

	μ_{max}	I_0	K_s	R^2
NH_4^+	2.0 ± 0.1	5.7 ± 0.4	99.4 ± 6.7	0.99
NO_3^-	1.4 ± 0.0	4.7 ± 0.1	78.0 ± 3.5	0.98
Glu	1.4 ± 0.1	6.2 ± 0.1	66.5 ± 4.6	0.99
Urea	1.3 ± 0.0	2.8 ± 0.3	79.7 ± 1.4	0.99

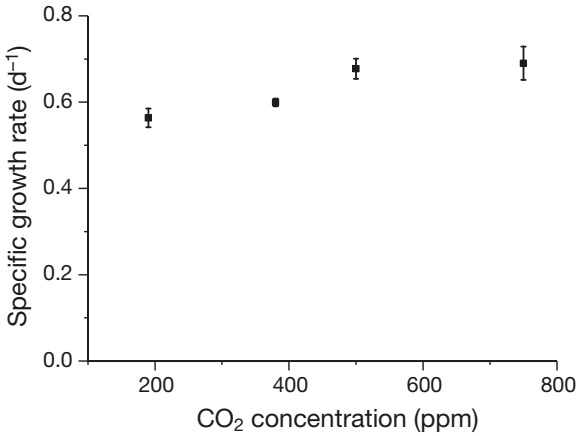

Fig. 3. Specific growth rates of *Prorocentrum donghaiense* as a function of CO_2. Values are means ± SD of triplicate samples

Growth response of *P. donghaiense* to pCO_2

P. donghaiense growth rates positively responded to elevated pCO_2 (Fig. 3) but data were insufficient to perform a kinetic analysis. Cultures of *P. donghaiense* maintained at 500 and 750 ppm pCO_2, had significantly higher specific growth rates than those grown using air mixtures containing 190 or 380 ppm pCO_2 (Fig. 3; $p < 0.05$). There was no significant difference in growth rates between cultures grown at 190 and 380 or 500 and 750 ppm pCO_2 (Fig. 3).

Although all N compounds tested were taken up, NH_4^+ and DFAA were taken up at higher rates than urea and NO_3^- in all cultures regardless of the pCO_2 treatment (Fig. 4). Furthermore, rates of NO_3^- and urea uptake were not significantly different in the 4 pCO_2 treatments (Fig. 4; $p > 0.05$). In contrast, NH_4^+ uptake rates were significantly higher in cultures grown at 750 ppm pCO_2 relative to those grown under the other pCO_2 treatments, although rates were also high in the 190 ppm pCO_2 treatment (Fig. 4; $p < 0.05$). DFAA uptake rates were significantly higher in cultures grown under 750 ppm pCO_2 than in those grown at 190 or 380 ppm pCO_2 (Fig. 4; $p < 0.05$).

DISCUSSION

Environmental factors including temperature, irradiance, N availability, and pCO_2 are important controls for the growth of phytoplankton (Thomas et al. 2012, Boyd & Hutchins 2012). In previous studies, we examined the role of N availability and uptake kinetics on the growth of *Prorocentrum donghaiense* (Hu et al. 2012, 2014). In this study, we found that temperature, irradiance, and pCO_2 all had significant impacts on *P. donghaiense* growth, and that growth kinetics varied depending on the N source.

Temperature

Temperature affects growth, morphology, and rates of enzyme activity, and thereby affect rates of nutrient utilization and the seasonal and geographical distribution of phytoplankton, as well as toxins production by some harmful algae (Lomas & Glibert 1999, Levitan et al. 2010, Thomas et al. 2012, Boyd et al. 2013, Mousing et al. 2014). Until recently, most

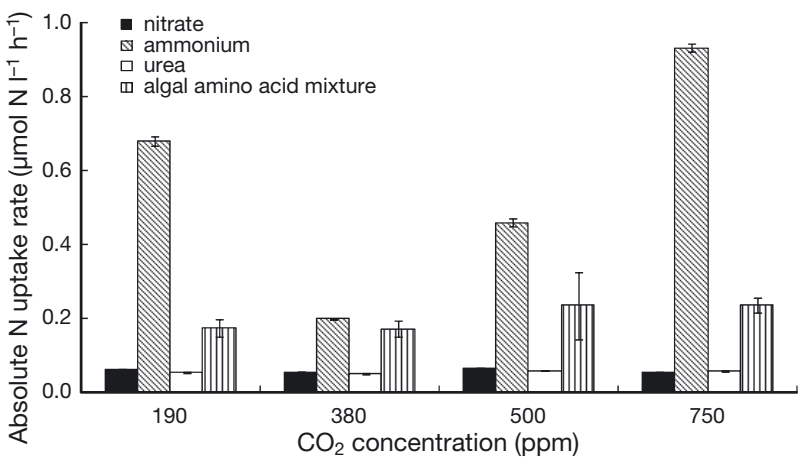

Fig. 4. Nitrogen (NO_3^-, NH_4^+, urea, and algal amino acid mixture) uptake rates of *Prorocentrum donghaiense* grown on different concentrations of CO_2. Values are means ± SD of triplicate samples

studies examining the effects of temperature on the growth rates of phytoplankton have used cultures wherein NO_3^- was supplied as the sole N source (e.g. Xu et al. 2010, Laabir et al. 2011). However, there are many other forms of N available in natural seawater, including NO_2^-, NH_4^+, urea, and amino acids (Bronk 2002). In this study, we examined growth of *P. donghaiense* under a wider range of temperatures (15 to 30°C), representative of those during which blooms occur. The maximum specific growth rates were obtained at 25°C in cultures growing on all N sources tested (NO_3^-, NH_4^+, urea and glu), however, the maximum specific growth rate was lower for cultures grown on glu relative to those observed in cultures growing on the other 3 N substrates (Boyd et al. 2013) (Fig. 1).

Previous culture studies suggested that *P. donghaiense* grows optimally at ~22°C (Chen et al. 2005), however, Xu et al. (2010) found that *P. donghaiense* grew fastest at higher temperatures (27°C). In the field, blooms of *P. donghaiense* typically occur in late April or early May in the Changjiang River Estuary, when water temperatures are generally between 15 and 26°C (Li 2009, Li et al. 2010, Lu et al. 2005, 2014). Monitoring data suggests that temperature is a key factor affecting the initiation and development of *P. donghaiense* blooms (Li 2009, Zhu et al. 2009). In our culture experiments, *P. donghaiense* exhibited the maximum specific growth rates at 25°C when supplied with NO_3^-, NH_4^+, urea, and glu (Boyd et al. 2013) (Fig. 1), which is comparable with the other culture and field studies.

However, in most environments, recycled N compounds, not NO_3^-, are the dominant N source satisfying the N demand of resident microbes (Mulholland & Lomas 2008), particularly at high water temperatures. We found that temperature and N sources had interactive effects on *P. donghaiense*. The highest specific growth rates occurred when *P. donghaiense* was grown on NH_4^+ at temperatures ranging from 15 to 25°C, while cultures grown on glu always had lower specific growth rates than cultures grown on other N sources at the temperatures tested. The difference could be N transporters and enzyme activity. Many enzymes are involved in the assimilation of different N species (Lomas 2004, Berges & Mulholland 2008). Temperature affects enzymes associated with N metabolism differently, and the relative responses of these enzymes to changes in temperature may also contribute to differences in N utilization by phytoplankton with respect to temperature (Gao et al. 2000). For example, temperature affects urease activity in *P. donghaiense*, with the highest urease activity observed at 25°C (Cai et al. 2016). In addition, growth phase, temperature, irradiance, and nitrogen source all impact the amino acid oxidase (AO) activity of *P. donghaiense*, which plays an important role in N utilization (Liu et al. 2013).

Once initiated, blooms of this species usually last several weeks to more than a month in the estuary and adjacent waters (Li 2009, Li et al. 2010, Lu et al. 2005, 2014). *P. donghaiense* can efficiently utilize many N compounds (Hu et al. 2012), even when present at low concentrations (Hu et al. 2014). The ability to grow under a wide range of temperatures and use a broad spectrum of N compounds is likely an important factor affecting the success of *P. donghaiense* in an environment where temperatures warm seasonally and concentrations of N species (NO_3^-, NH_4^+, urea and DFAA) vary (Wang & Huang 2003, Li 2009, Li et al. 2009). This metabolic flexibility may offer *P. donghaiense* an advantage in a highly variable estuarine environment.

Irradiance

In addition to temperature, irradiance is one of the most important physical factors impacting the growth and photosynthetic efficiency of phytoplankton. Both irradiance and duration affect the efficiency of photosynthesis and the overall photosynthetic rate. In previous culture studies examining the effects of irradiance on *P. donghaiense* growth rates, NO_3^- was used as the sole N source (Chen et al. 2005, Xu et al. 2010). However, in the Changjiang River Estuary, there are many forms of bioavailable N, including NO_3^-, NH_4^+, urea, and DFAAs, and their relative abundance changes over the course of blooms (Li et al. 2009, 2010). Therefore, to better understand the interaction between irradiance and N sources on the growth of *P. donghaiense*, we examined growth responses of this organism to irradiances ranging from 10 to 180 µmol quanta m^{-2} s^{-1}, a realistic range of irradiance during dense blooms, in cultures grown on 4 different N substrates (NO_3^-, NH_4^+, urea and glu). We found that *P. donghaiense* grew on all 4 N sources at each irradiance tested, and that growth rates increased as irradiance increased (Fig. 2). The μ_{max} and K_s values for irradiance for *P. donghaiense* grown on NH_4^+ were significantly higher ($p < 0.05$) than those measured in cultures grown on NO_3^-, urea or glu as the sole source of N (Table 1, Fig. 2).

Most autotrophic phytoplankton cannot survive, or grow very slowly at low irradiances, but growth may

Table 2. Summary of compensation irradiance (I_0; µmol quanta m^{-2} s^{-1}) and K_s (µmol quanta m^{-2} s^{-1}) for different dinoflagellate species

Species	I_0	K_s	Reference
Akashiwo sanguinea	14.4	114	Matsubara et al. (2007)
Karenia mikimotoi	0.7	110	Yamaguchi & Honjo (1989)
Prorocentrum donghaiense (grown on NH_4^+)	5.7 ± 0.4	99.4 ± 6.7	This study
Alexandrium tamarense	76	90	Yamamoto & Tarutani (1997)
Cochlodinium polykrikoides	10.4	90	Kim et al. (2004)
P. donghaiense (grown on urea)	2.8 ± 0.3	79.7 ± 1.4	This study
P. donghaiense (grown on NO_3^-)	4.7 ± 0.1	78.0 ± 3.5	This study
Gyrodinium instriatum	10.6	70	Nagasoe et al. (2006)
P. donghaiense (grown on glutamic acid)	6.2 ± 0.1	66.5 ± 4.6	This study
P. donghaiense	0.1	30	Xu et al. (2010)
Gymnodinium catenatum	10	16.8	Yamamoto et al. (2002)
Alexandrium fundyense	15.0	–	Etheridge & Roesler (2005)
Alexandrium minutum (grown at 20°C)	10.6 ± 5.6	–	Lim et al. (2006)
A. minutum (grown at 25°C)	10.0 ± 1.1	–	Lim et al. (2006)
Alexandrium tamiyavanichii (grown at 20°C)	10.3 ± 3.3	–	Lim et al. (2006)
A. tamiyavanichii (grown at 25°C)	9.9 ± 1.6	–	Lim et al. (2006)

also be inhibited at high irradiances (MacIntyre et al. 2002, Dubinsky & Stambler 2009). We observed low growth rates for *P. donghaiense* under low light conditions but did not observe growth inhibition at the highest irradiance tested, which was about 20% of the average spring surface PAR estimated for the East China Sea (Sun et al. 2008) (Fig. 2). The values of I_0 and K_s determined for *P. donghaiense* in this study were within the ranges of those observed for other dinoflagellate species (Table 2). For example, *Karenia mikimotoi* had lower I_0 than *P. donghaiense*, but higher K_s (Yamaguchi & Honjo 1989), while *Alexandrium tamarense* had higher I_0 but similar values of K_s (Yamamoto & Tarutani 1997). Growth temperatures (20 versus 25°C) were shown to affect I_0 values for *A. minutum* and *A. tamiyavanichii* (Lim et al. 2006).

Sun et al. (2008) found that the optimal growth irradiance for *P. donghaiense* was 174 µmol quanta m^{-2} s^{-1} , which is similar to the highest irradiance used in the present study. In contrast, Xu et al. (2010) showed that *P. donghaiense* grew at high rates at light intensities as low as 2 µmol quanta m^{-2} s^{-1} and that irradiance was saturating at just 30 but not yet inhibited at 230 µmol quanta m^{-2} s^{-1}. Liu et al. (2011) observed maximum specific growth rates for *P. donghaiense* at a light intensity of 70 µmol quanta m^{-2} s^{-1}, but reported that growth rates were inhibited at irradiances higher than 150 µmol quanta m^{-2} s^{-1}. In highly turbid areas such as the East China Sea (Sun et al. 2008), light availability can limit photosynthesis. The intensity of PAR can be estimated from the depth of mixed layer and turbidity (Diehl et al. 2002). In spring, the surface PAR in the East China Sea was estimated to be ~914 µmol quanta m^{-2} s^{-1} (Sun et al. 2008), with light intensity attenuating rapidly with depth. *P. donghaiense* blooms are observed mainly near the surface (upper 10 m) and the maximum recorded cell density was 36×10^7 cells l^{-1} (Lu et al. 2005). Because algal cells are also particles that contribute to turbidity and light scattering and attenuation, during dense surface blooms the euphotic depth can become shallow, and the light reaching cells is likely to be substantially less than that measured at the surface. While light may be abundant as blooms initiate and cell densities are still low, as *P. donghaiense* abundances increase (reaching densities as high as 36×10^7 cells l^{-1}; Lu et al. 2005) light may become rapidly attenuated, causing self-shading. The ability of *P. donghaiense* to grow at low irradiances (10 to 20 µmol quanta m^{-2} s^{-1}), is likely important, because dense blooms often last more than a month. All the results from field and laboratory are comparable with ours, which suggests that *P. donghaiense* can easily adjust to widely fluctuating irradiance levels.

Irradiance can affect the cell size of phytoplankton, its nutrient quota, and also nutrient preference and uptake (Thompson et al. 1989, Chang & Page 1995, Fan & Glibert 2005, Herndon & Cochlan 2007). In this study, we found that irradiance affected the N preference of *P. donghaiense*. At lower irradiance (<20 µmol quanta m^{-2} s^{-1}), *P. donghaiense* had similar growth rates when grown on the 4 N species tested, but when the irradiance

increased, the growth rates varied in different N sources. At the highest irradiance (180 µmol quanta m^{-2} s^{-1}), the average specific growth rates ranged from 0.9 d^{-1} in cultures grown on urea, to 1.4 d^{-1} in cultures growing with NH_4^+ as the sole N source (Fig. 2); μ_{max} for *P. donghaiense* grown on the 4 different N sources ranged from 1.3 ± 0.0 to 2.0 ± 0.1 d^{-1} (Table 1). Our results are comparable with other phytoplankton species. Chang & Page (1995) found that light affected cell size of *Heterosigma carterae*, and also had more influence on cultures grown with NO_3^- than cultures grown with NH_4^+ or urea. In *Thalassiosira pseudonana* culture experiments at lower irradiance (≤29 µE m^{-2} s^{-1}), NO_3^--grown cells had equal growth rates, N quotas, chl *a*, and equal or greater carbon quotas compared to NH_4^+-grown cells, but at higher irradiance, growth rates were lower for cells growing on NO_3^- compared to cells growing on NH_4^+ (Thompson et al. 1989) — results similar to ours. At lower irradiance, growth rates of *T. pseudonana* grown on NO_3^- and NH_4^+ were not significantly different, which suggests that NO_3^--grown cells may make other physiological adjustments to compensate for their greater need for reductant. Conversely, at higher irradiance, culture of this organism grown on NO_3^- had lower carbon and N quotas, implying that the higher energy requirements of NO_3^--grown cells (for N reduction) are compensated for by a reduction of C quota, N quota, chl *a*, and growth rate (Thompson et al. 1989), and suggesting that phytoplankton can adapt by changing different parameters at specific irradiance levels in order to maintain a high growth rate. We did not measure the same parameters as Thompson et al. (1989), but from our *in vivo* fluorescence data, we found cultures grown on NH_4^+ had higher fluorescence than cultures grown on NO_3^- at higher irradiance (>80 µmol quanta m^{-2} s^{-1}), hence, our result might be partially explained in a similar way as for *T. pseudonana*. In another similar work, the growth rate of *Heterosigma akashiwo* was found to be slightly higher in cultures grown on NH_4^+ (0.89 d^{-1}) compared to those grown on NO_3^- or urea at saturating irradiance (110 µE m^{-2} s^{-1}), but at sub-saturating irradiance (40 µE m^{-2} s^{-1}), both cultures supplied with urea and NH_4^+ grew faster than cultures supplied with NO_3^- (Herndon & Cochlan 2007). Besides the culture experiments, field studies also demonstrate that irradiance has an important role in N uptake and preference (Hu & Smith 1998, Kudela & Cochlan 2000, Fan & Glibert 2005). Previous studies and our results clearly demonstrated that irradiance and N source have interactive effects on N preference and uptake. Adaptation to varying irradiance and an ability to utilize an array of N species may contribute to the ability of *P. donghaiense* to form dense blooms in highly turbid waters.

CO_2

CO_2 concentrations in the atmosphere have risen from 280 ppm during the Industrial Revolution to 400 ppm in 2015, and are still rising at an accelerating rate. It is expected that pCO_2 will double by the end of this century (IPCC 2013). The increased CO_2 concentration in the atmosphere has led to a series of environmental problems, including ocean acidification. Increasing pCO_2 may also influence phytoplankton growth rates, element ratios, photosynthetic rates, nutrient utilization, toxin production, and community structure (Burkhardt et al. 1999, Fu et al. 2007, Hutchins et al. 2013, Li & Campbell 2013, Tatters et al. 2013, Errera et al. 2014). While an increase in HABs has long been linked with nutrient overenrichment (Heisler et al. 2008), the effects of climate warming and increased pCO_2 are not as well understood for most taxa (Moore et al. 2008, Paerl & Huisman 2009, Hallegraeff 2010, O'Neil et al. 2012, Paerl & Paul 2012).

We found that *P. donghaiense* growth rates increased by >10%, and NH_4^+ uptake rates increased by about 57 to 79% in cultures maintained at elevated pCO_2 (500 to 750 ppm) relative to those grown at present-day pCO_2 (Figs. 3 & 4). This suggests that increases in pCO_2 may positively affect the growth of *P. donghaiense*, and this could affect the magnitude and duration of blooms. Higher atmospheric CO_2 concentrations and elevated pCO_2 may offset some of the DIC drawdown. Elevated pCO_2 increases growth rates of many cyanobacteria and some other phytoplankton (Fu et al. 2007, 2012, Wu et al. 2014), likely by alleviating C limitation of photosynthesis (Hutchins et al. 2007). Elevated pCO_2 also results in higher DIC concentrations in seawater, and this alters the inorganic carbon buffer system and decreases pH in oligotrophic waters where photosynthetic drawdown of DIC is relatively low. In contrast, in nutrient-enriched coastal systems, increasing the supply of DIC may alleviate C limitation of algal growth, provided light or some other element does not limit their growth.

Acknowledgements. We acknowledge 3 anonymous reviewers and the editor Prof. Paul del Giorgio for their helpful comments and suggestions regarding an earlier version of the manuscript. This work was supported by the Natural Science Foundation of China (NSFC) (Grant Nos. 41506143, 61533011, 41576159), China Postdoctoral Science Foundation (Grant No. 2014M561974), the Open Fund of Key Laboratory of Marine Ecology and Environmental Science, Institute of Oceanology, Chinese Academy of Sciences (Grant No. KLMEES201309) and NOAA and NSF grants to MRM.

LITERATURE CITED

Anderson DM, Cembella AD, Hallegraeff GM (2012) Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. Annu Rev Mar Sci 4:143–176

Band-Schmidt CJ, Bustillos-Guzmán JJ, Hernández-Sandoval FE, Núñez-Vázquez EJ, López-Cortés DJ (2014) Effect of temperature on growth and paralytic toxin profiles in isolates of *Gymnodinium catenatum* (Dinophyceae) from the Pacific coast of Mexico. Toxicon 90: 199–212

Berges JA, Mulholland MR (2008) Enzymes and nitrogen cycling. In: Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (eds) Nitrogen in the marine environment, 2nd edn. Academic Press, Burlington, MA, p 1385–1444

Boyd PW, Hutchins DA (2012) Understanding the responses of ocean biota to a complex matrix of cumulative anthropogenic change. Mar Ecol Prog Ser 470:125–135

Boyd PW, Rynearson TA, Armstrong EA, Fu FX and others (2013) Marine phytoplankton temperature versus growth responses from polar to tropical waters — outcome of a scientific community-wide study. PLoS ONE 8:e63091

Bronk DA (2002) Dynamics of DON. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Academic Press, San Diego, CA, p 153–249

Burkhardt S, Zondervan I, Riebesell U (1999) Effect of CO_2 concentration on C:N:P ratio in marine phytoplankton: a species comparison. Limnol Oceanogr 44:683–690

Cai Y, Liu JY, Qin JL, Sun SG, Duan SS, Xu N (2016) Effects of environmental factors on the growth and urease activity of the harmful dinoflagellate *Prorocentrum donghaiense.* Acta Ecol Sin 36:1711–1718 (in Chinese with English Abstract)

Celis-Plá PSM, Hall-Spencer JM, Horta PA, Milazzo M, Korbee N, Cornwall CE, Figueroa FL (2015) Macroalgal responses to ocean acidification depend on nutrient and light levels. Front Mar Sci 2:26

Chang FH, Page M (1995) Influence of light and three nitrogen sources on growth of *Heterosigma carterae* (Raphidophyceae). NZ J Mar Freshw Res 29:299–304

Chen B, Wang Z, Zhu M, Li R (2005) Effects of temperature and salinity on growth of *Prorocentrum dentatum* and comparisons between growths of *Prorocentrum dentatum* and *Skeletonema costatum.* Adv Mar Sci 23:60–64 (in Chinese, with English Abstract)

Cowie GL, Hedges JI (1992) Improved amino acid quantification in environmental samples: charge-matched recovery standards and reduced analysis time. Mar Chem 37:223–238

Deng G, Geng YH, Hu HJ, Qi YZ, Lu SH, Li ZK, Li YG (2009) Effects of environmental factors on photosynthesis of a high biomass bloom forming species *Prorocentrum donghaiense.* Mark Sci 33:34–39 (in Chinese with English Abstract)

Dia A, Guillou L, Mauger S, Bigeard E, Marie D, Valero M, Destombe C (2014) Spatiotemporal changes in the genetic diversity of harmful algal blooms caused by the toxic dinoflagellate *Alexandrium minutum.* Mol Ecol 23: 549–560

Diehl S, Berger S, Ptacnik R, Wild A (2002) Phytoplankton, light, and nutrients in a gradient of mixing depths: field experiments. Ecology 83:399–411

Dubinsky Z, Stambler N (2009) Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications. Aquat Microb Ecol 56:163–176

Dugdale RC, Wilkerson FP, Hogue VE, Marchi A (2007) The role of ammonium and nitrate in spring bloom development in San Francisco Bay. Estuar Coast Shelf Sci 73: 17–29

Edwards KF, Klausmeier CA, Litchman E (2015a) Nutrient utilization traits of phytoplankton. Ecology 96:2311

Edwards KF, Thomas MK, Klausmeier CA, Litchman E (2015b) Light and growth in marine phytoplankton: allometric, taxonomic, and environmental variation. Limnol Oceanogr 60:540–552

Errera RM, Yvon-Lewis S, Kessler JD, Campbell L (2014) Reponses of the dinoflagellate *Karenia brevis* to climate change: pCO_2 and sea surface temperatures. Harmful Algae 37:110–116

Etheridge SM, Roesler CS (2005) Effects of temperature, irradiance, and salinity on photosynthesis, growth rates, total toxicity, and toxin composition for *Alexandrium fundyense* isolates from the Gulf of Maine and Bay of Fundy. Deep-Sea Res II 52:2491–2500

Fan C, Glibert PM (2005) Effects of light on nitrogen and carbon uptake during a *Prorocentrum minimum* bloom. Harmful Algae 4:629–641

Fu FX, Warner ME, Zhang Y, Feng Y, Hutchins DA (2007) Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (*Cyanobacteria*). J Phycol 43:485–496

Fu FX, Tatters AO, Hutchins DA (2012) Global change and the future of harmful algal blooms in the ocean. Mar Ecol Prog Ser 470:207–233

Gao Y, Smith GJ, Alberte RS (2000) Temperature dependence of nitrate reductase activity in marine phytoplankton: biochemical analysis and ecological implications. J Phycol 36:304–313

Glibert PM (2014) Harmful algal blooms in Asia: an insidious and escalating water pollution phenomenon with effects on ecological and human health. ASIANetwork Exchange 21:52–68

Guillard RRL (1973) Division rates. In: Stein JR (ed) Handbook of phycological methods: culture methods and growth measurements. Cambridge University Press, Cambridge, p 289–311

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum Press, New York, NY, p 26–60

Hallegraeff GM (2010) Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. J Phycol 46:220–235

Harrison WG, Platt T (1986) Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. Polar Biol 5:153–164

Heisler J, Glibert PM, Burkholder JM, Anderson DM and others (2008) Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8:3–13

Herndon J, Cochlan WP (2007) Nitrogen utilization by the raphidophyte *Heterosigma akashiwo*: growth and uptake kinetics in laboratory cultures. Harmful Algae 6: 260–270

Hu S, Smith WO (1998) The effects of irradiance on nitrate uptake and dissolved organic nitrogen release by phytoplankton in the Ross Sea. Cont Shelf Res 18:971–990

Hu ZX, Mulholland MR, Duan SS, Xu N (2012) Effects of nitrogen supply and its composition on the growth of *Prorocentrum donghaiense.* Harmful Algae 13:72–82

Hu ZX, Duan SS, Xu N, Mulholland MR (2014) Growth and nitrogen uptake kinetics in cultured *Prorocentrum donghaiense.* PLoS ONE 9:e94030

Hutchins DA, Fu FX, Zhang Y, Warner ME and others (2007) CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry. Limnol Oceanogr 52:1293–1304

Hutchins DA, Fu FX, Webb EA, Walworth N, Tagliabue A (2013) Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations. Nat Geosci 6: 790–795

IPCC (2013) Climate change 2013: the physical science basis. In: Stocker TF, Qin D, Plattner GK, Tignor M and others (eds) Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate. Cambridge University Press, Cambridge

Johnson VR, Brownlee C, Milazzo M, Hall-Spencer JM (2015) Microalgal assemblage shift along a marine CO_2 gradient subjected to multiple environmental stressors. J Mar Sci Eng 3:1425–1447

Kim D, Matsuyama Y, Nagasoe S, Yamaguchi M and others (2004) Effect of temperature, salinity and irradiance on the growth of the harmful red tide dinoflagellate *Cochlodinium polykridoides* Margalef (Dinophyceae). J Plankton Res 26:61–66

Kudela RM, Cochlan WP (2000) Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California. Aquat Microb Ecol 21: 31–47

Laabir M, Jauzein C, Genovesi B, Masseret E and others (2011) Influence of temperature, salinity and irradiance on the growth and cell yield of the harmful red tide dinoflagellate *Alexandrium catenella* colonizing Mediterranean waters. J Plankton Res 33:1550–1563

Lederman TC, Tett P (1981) Problems in modelling the photosynthesis-light relationship for phytoplankton. Bot Mar 24:125–134

Levitan O, Brown CM, Sudhaus S, Campbell D, LaRoche J, Berman-Frank I (2010) Regulation of nitrogen metabolism in the marine diazotroph *Trichodesmium* IMS101 under varying temperatures and atmospheric CO_2 concentrations. Environ Microbiol 12:1899–1912

Li R (2009) Study on the ecology of red tide *Prorocentrum donghaiense* in the north of Fujian coast. Mar Environ Sci 28(Suppl 1):65–69 (in Chinese with English Abstract)

Li G, Campbell DA (2013) Rising CO_2 interacts with growth light and growth rate to alter photosystem II photoinactivation of the coastal diatom *Thalassiosira pseudonana.* PLoS ONE 8:e55562

Li J, Glibert PM, Zhou M, Lu S, Lu D (2009) Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. Mar Ecol Prog Ser 383:11–26

Li J, Glibert PM, Zhou M (2010) Temporal and spatial variability in nitrogen uptake kinetics during harmful dinoflagellate blooms in the East China Sea. Harmful Algae 9:531–539

Li HM, Tang HJ, Shi XY, Zhang CS, Wang XL (2014) Increased nutrient loads from the Changjiang (Yangtze) River have led to increased harmful algal blooms. Harmful Algae 39:92–101

Lim PT, Leaw CP, Usup G, Kobiyama A, Koike K, Ogata T (2006) Effects of light and temperature on growth, nitrate uptake, and toxin production of two tropical dinoflagellates: *Alexandrium tamiyavanichii* and *Alexandrium minutum* (Dinophyceae). J Phycol 42:786–799

Lin JN, Yan T, Zhang QC, Wang YF, Liu Q, Zhou MJ (2014) *In situ* detrimental impacts of *Prorocentrum donghaiense* blooms on zooplankton in the East China Sea. Mar Pollut Bull 88:302–310

Litchman E, Klausmeier CA (2008) Trait-based community ecology of phytoplankton. Annu Rev Ecol Evol Syst 39: 615–639

Liu S, Yu Z, Yao P, Zheng Y, Li D (2011) Effects of irradiance on pigment signatures of harmful algae during growth process. Acta Oceanol Sin 30:46–57

Liu JY, Lai HY, Xu N, Duan SS (2013) Effects of growth phase and environmental factors on amino acid oxidase activity of *Prorocentrum donghaiense*. Ecol Sci 32:27–33 (in Chinese with English Abstract)

Lomas MW (2004) Nitrate reductase and urease enzyme activity in the marine diatom *Thalassiosira weissflogii* (Bacillariophyceae): interactions among nitrogen substrates. Mar Biol 144:37–44

Lomas MW, Glibert PM (1999) Temperature regulation of nitrate uptake: a novel hypothesis about nitrate uptake and reduction in cool-water diatoms. Limnol Oceanogr 44:556–572

Lu DD, Goebel J (2001) Five red tide species in genus *Prorocentrum* including the description of *Prorocentrum donghaiense* Lu sp. nov. from the East China Sea. Chin J Oceanology Limnol 19:337–344

Lu DD, Goebel J, Qi YZ, Zou JZ, Han XT, Gao YH, Li RX (2005) Morphological and genetic study of *Prorocentrum donghaiense* Lu from the East China Sea, and comparison with some related *Prorocentrum* species. Harmful Algae 4:493–505

Lu DD, Qi YZ, Gu HF, Dai XF and others (2014) Causative species of harmful algal blooms in Chinese coastal waters. Algol Stud 145:145–168

MacIntyre HL, Kana TM, Anning T, Geider RJ (2002) Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. J Phycol 38:17–38

Matsubara T, Nagasoe S, Yamasaki Y, Shikata T, Shimasaki Y, Oshima Y, Honjo T (2007) Effects of temperature, salinity, and irradiance on the growth of the dinoflagellate *Akashiwo sanguinea.* J Exp Mar Biol Ecol 342:226–230

Moore SK, Trainer VL, Mantua NJ, Parker MS, Laws EA, Backer LC, Fleming LE (2008) Impacts of climate variability and future climate change on harmful algal blooms and human health. Environ Health 7(Suppl 2):S4

Mousing EA, Ellegaard M, Richardson K (2014) Global patterns in phytoplankton community size structure — evidence for a direct temperature effect. Mar Ecol Prog Ser 497:25–38

Mulholland MR, Lee C (2009) Peptide hydrolysis and the uptake of dipeptides by phytoplankton. Limnol Oceanogr 54:856–868

Mulholland MR, Lomas MW (2008) N uptake and assimilation. In: Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (eds) Nitrogen in the marine environment. Academic Press, Burlington, MA, p 303–338

Nagasoe S, Kim DI, Shimasaki Y, Oshima Y, Yamaguchi M, Honjo T (2006) Effects of temperature, salinity and irradiance on the growth of the red tide dinoflagellate *Gyrodinium instriatum* Freudenthal et Lee. Harmful Algae 5: 20–25

O'Neil JM, Davis TW, Burford MA, Gobler CJ (2012) The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14: 313–334

Paerl HW, Huisman J (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ Microbiol Rep 1:27–37

Paerl HW, Paul VJ (2012) Climate change: links to global expansion of harmful cyanobacteria. Water Res 46: 1349–1363

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, NY

Persson A, Smith BC, Morton S, Shuler A, Wikfors GH (2013) Sexual life stages and temperature dependent morphological changes allow cryptic occurrence of the Florida red tide dinoflagellate *Karenia brevis.* Harmful Algae 30:1–9

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr 14:799–801

Song NQ, Wang N, Lu Y, Zhang JR (2016) Temporal and spatial characteristics of harmful algal blooms in the Bohai Sea during 1952-2014. Cont Shelf Res 122:77–84

Sun BY, Wang XL, Li YB, Wang CY, Wang AJ, Liang SK, Zhang CS (2008) Effects of irradiance on blooms of the dinoflagellate *Prorocentrum donghaiense* Lu in the coastal area in East China Sea. J Environ Sci (China) 29: 362–367 (in Chinese with English Abstract)

Tatters AO, Schnetzer A, Fu F, Lie AY, Caron DA, Hutchins DA (2013) Short- versus long-term responses to changing CO_2 in a coastal dinoflagellate bloom: implications for interspecific competitive interactions and community structure. Evolution 67:1879–1891

Thomas MK, Kremer CT, Klausmeier CA, Litchman E (2012) A global pattern of thermal adaptation in marine phytoplankton. Science 338:1085–1088

Thompson PA, Levasseur ME, Harrison PJ (1989) Light-limited growth on ammonium vs. nitrate: What is the advantage for marine phytoplankton? Limnol Oceanogr 34: 1014–1024

Thorel M, Fauchot J, Morelle J, Raimbault V and others (2014) Interactive effects of irradiance and temperature on growth and domoic acid production of the toxic diatom *Pseudo-nitzschia australis* (Bacillariophyceae). Harmful Algae 39:232–241

Toseland AD, Daines SJ, Clark JR, Kirkham A and others (2013) The impact of temperature on marine phytoplankton resource allocation and metabolism. Nat Clim Change 3:979–984

Wang J, Huang X (2003) Ecological characteristics of *Prorocentrum dentatum* and the cause of harmful algal bloom formation in China Sea. Chin J Appl Ecol 14:1065–1069 (in Chinese with English Abstract)

Wang J, Wu J (2009) Occurrence and potential risks of harmful algal blooms in the East China Sea. Sci Total Environ 407:4012–4021

Wang J, Zhang Y, Li H, Cao J (2013) Competitive interaction between diatom *Skeletonema costatum* and dinoflagellate *Prorocentrum donghaiense* in laboratory culture. J Plankton Res 35:367–378

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol Oceanogr 39:1985–1992

Wu Y, Campbell DA, Irwin AJ, Suggett DJ, Finkel ZV (2014) Ocean acidification enhances the growth rate of larger diatoms. Limnol Oceanogr 59:1027–1034

Xu N, Duan SS, Li AF, Zhang CW, Cai ZP, Hu ZX (2010) Effects of temperature, salinity and irradiance on the growth of the harmful dinoflagellate *Prorocentrum donghaiense* Lu. Harmful Algae 9:13–17

Yamaguchi M, Honjo T (1989) Effect of temperature, salinity and irradiance on the growth of the noxious red tide flagellate *Gymnodinium nagasakiense* (Dinophyceae). Bull Jpn Soc Sci Fish 55:2029–2036 (in Japanese with English Abstract)

Yamamoto T, Tarutani K (1997) Effect of temperature, salinity and irradiance on the growth of toxic dinoflagellate *Alexandrium tamarense* isolated from Hiroshima Bay, Japan. Jpn J Phycol 45:95–101 (in Japanese with English Abstract)

Yamamoto T, Oh SJ, Kataoka Y (2002) Effects of temperature, salinity and irradiance on the growth of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae) isolated from Hiroshima Bay, Japan. Fish Sci 68:356–363

Zhou M (2010) Environmental settings and harmful algal blooms in the sea area adjacent to the Changjiang River Estuary. In: Ishimatsu A, Lie HJ (eds) Coastal environmental and ecosystem issues of the East China Sea. TERRAPUB, Tokyo, p 133–149

Zhu DD, Lu DD, Wang YF, Su JL (2009) The low temperature characteristics in Zhejiang coastal region in the early spring of 2005 and its influence on harmful algae bloom occurrence of *Prorocentrum donghaiense.* Acta Oceanol Sin 31:31–39 (in Chinese with English Abstract)

Editorial responsibility: Paul del Giorgio, Montreal, Quebec, Canada

Submitted: October 16, 2015; Accepted: July 11, 2016
Proofs received from author(s): August 23, 2016