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Ammonium accelerates the growth rate of *Skeletonema* spp. in the phytoplankton assemblage in a heavily eutrophic embayment, Dokai Bay, Japan

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Abstract: High ammonium loading in Dokai Bay produces large diatom blooms and therefore laboratory experiments was conducted to determine if ammonium stimulated the occurrence of the observed massive phytoplankton blooms. The presence of ammonium as a nitrogen source significantly increased the growth rate of *Skeletonema* spp. compared to nitrate. Growth rate (μ_{chl}) on ammonium was significantly higher (~13–15%) than on nitrate. However, the effect of ammonium on growth rate acceleration was species specific, because the effect was not observed when the field assemblage was a mixture of *Skeletonema* spp. and other diatoms. In addition, the magnitude of the growth acceleration effect varied, depending on the irradiance level. The largest significant increase in growth rate on ammonium compared to nitrate occurred when irradiance was at irradiance around 200 μmol photons m^{-2} s^{-1}. Our results suggested that the growth of *Skeletonema* spp. which was dominant species in Dokai Bay was accelerated by ammonium and particularly under an irradiance which occurs in the mixed surface layer of this bay in summer. This bay may act like a selective growth incubator for certain diatoms such as *Skeletonema* that are subsequently exported to nearby coastal waters.

Keywords: *High ammonium, growth rate stimulation, irradiance, Dokai Bay, Skeletonema, nitrogen.*

1. Introduction

In most coastal environments, nitrogen is the important macronutrient for the growth of phytoplankton. A deficit of nitrogen reduces primary productivity and an excess of nitrogen can stimulate excessive algal blooms. Among the various forms of nitrogen, ammonium and nitrate are traditionally considered to be the most important nitrogen sources for a natural phytoplankton assemblage (e.g. SYRETT, 1981; LEVASSEUR *et al.*, 1993; HERNDON and COCHLAN, 2007).

Anthropogenic nutrient pollution is considered to cause one of the most pervasive changes in lower trophic levels in the coastal environment. The input of inorganic nitrogen, particularly ammonium from sewage, agricultural runoff, or untreated wastewater from industry, is steadily increasing and causes the deterioration of water quality. In Japan, for example, untreated wastewater from

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industries along the coast of Dokai Bay, Kyushu Island, caused the deterioration of water quality since 1960. High ammonium concentrations were observed throughout the year particularly in the inner bay (TADA *et al.*, 2001). High concentrations of ammonium are increasingly found in several discharge areas (e.g. LIVINGSTON *et al.*, 2002).

The responses of phytoplankton to an increase of ammonium concentration have been reviewed previously (e.g. SYRETT, 1981; FLYNN, 1991; FLYNN *et al.*, 1997). The adverse effect of high (toxic) ammonium on phytoplankton growth has been reported in several studies (e.g. NATARAJAN (1970); AZOV and GOLDMAN (1982); LIVINGSTON *et al.* (2002)). On the other hand, the stimulation of growth rate of phytoplankton by ammonium has also been reported for laboratory cultures (THOMPSON *et al.,* 1989; LEVASSEUR *et al.*, 1993; HERNDON and COCHLAN, 2007).

Early studies in Dokai Bay, suggested that strong estuarine circulation was the explanation for the occurrence of phytoplankton blooms during only summer (YANAGI and YAMADA, 2000; TADA *et al.*, 2001 and 2004). YAMADA and KAJIWARA (2004) reported that 51 phytoplankton blooms were observed in this bay between 1980 and 1995, and 20 phytoplankton blooms were dominated by *Skeletonema costatum* and *S. tropicum* and it was also a co-dominant 16 times in blooms with other species. However, *S. costatum* has long been considered one of the most conspicuous and widespread members of the coastal marine phytoplankton. Recently, it was discovered that a number of distinct species were included under this name (SARNO *et al.*, 2005) and they are difficult or impossible to distinguish based on morphological characters. So, we expressed the species *Skeletonema* spp. in this paper. In contrast, *Chaetoceros* sp. and *Heterosigma akashiwo* blooms were rarely observed. TADA *et al.* (2001) reported that the phytoplankton assemblage in Dokai Bay was dominated by *S. tropicum* in summer season and *S. costatum* in other seasons. However, the occurrences of phytoplankton blooms in this bay appear to be associated with the high ammonium concentrations. This hypothesis is derived from the observation of *Skeletonema* spp. as a dominant species throughout the year and *Skeletonema* spp. usually formed the major component of these phytoplankton blooms. Recently, it was determined that the high ammonium level that was found in the inner bay was an important factor regulating phytoplankton growth and dominant species through differences in ammonium tolerance efficiency (SUKSOMJIT *et al.*, in press).

It was not clear whether the presence of high ammonium enhanced the occurrence of very frequent *Skeletonema* spp. blooms in this bay with strong estuarine circulation, although the growth stimulation of phytoplankton by ammonium has been previously reported in the laboratory (THOMPSON *et al.*, 1989; LEVASSEUR *et al.*, 1993). In addition, there is a need to clarify the effect of irradiance on the growth rate of *Skeletonema* spp. under different nitrogen sources, because the previous studies revealed that the influence of ammonium acceleration of growth rate depends on light irradiance (e.g. HERNDON and COCHLAN, 2007; WOOD and FLYNN, 1995). The goal of this study was to clarify the effect of ammonium on the acceleration of phytoplankton growth rate in Dokai Bay, with a focus on *Skeletonema* since it was always the dominant bloom forming species.

2. Materials and methods

2.1 Effect of ammonium on phytoplankton growth

Natural phytoplankton assemblages and natural seawater were collected from the surface of the middle and outer part of Dokai Bay (station DK1 and DK2, Fig. 1) on June 20 and July 31, 2007. The samples were pre-filtered through a 300 μm mesh net in order to remove large zooplankton and brought back to the laboratory.

The natural phytoplankton assemblage collected from station DK1 was diluted 50-fold with filtered (pre-combusted GF/F) natural seawater from station DK2 and transferred into six 1-L Erlenmeyer flasks (initial chlorophyll fluorescence ca. 2). Either 100 μM ammonium (1^{st} treatment) or nitrate (2^{nd} treatment) was added along with phosphate (29 μM) and silicic acid (88 μM) in triplicate.

These flasks were incubated at 20℃ under 100 μmol photons m^{-2} s^{-1} (14 L:10 D cycle) using cool white fluorescence lamps with gentle stirring. After nitrogen in the culture medium of each treatment was depleted, both the NH_4^+ and NO_3^- grown cultures were transferred into new medium, but the nitrogen source was switched (i.e. from NH_4^+ to NO_3^- and vise versa). According to this incubation experiment, we tested the effect of either ammonium or nitrate individually, because of the following reasons. In Dokai Bay, the DIN concentration often exceeded 100 μM of which 40–70% was ammonium. Namely, both ammonium and nitrate concentration were high in this bay. TADA *et al.* (in press) conducted the incubation experiment of surface water and they showed that natural phytoplankton assemblage of mainly diatoms took up ammonia instead of nitrate. Moreover, nitrate was only taken up after ammonium was almost depleted, although ammonium was never depleted in the natural seawater in this bay.

Samples were taken daily and chlorophyll fluorescence was determined using a fluorometer (Turner Design 10-AU-005) according to BRAND *et al.* (1981). Samples were preserved in formaldehyde (final concentration 4%) and phytoplankton species and cell density was determined using an inverted microscope and a Sedgewick-Rafter counting chamber. Part of the daily sample was filtered through a 25 mm pre-combusted GF/F filter (450℃, 2 h) and frozen at −30℃ for measuring NH_4^+, NO_3^- $+NO_2^-$, PO_4^{3-} and Si $(OH)_4$ using an autoanalyzer (Bran+Luebbe, TRACCS 2000) according to STRICKLAND and PARSONS (1972). To determine the approximate time when nitrogen was depleted, NH_4^+ and NO_3^- were determined daily using fluorometric and optical methods according to HOLMES *et al.* (1999) and COLLOS *et al.* (1999), respectively.

Specific growth rate (μ, d^{-1}) was determined during exponential growth phase and was calculated by a least-squares linear fit to the logarithmically transformed chlorophyll fluorescence (μ_{Chl}) or cell density ($\mu_{Cell\ density}$) using the following formula:

$$\mu = \frac{\ln C_2 - \ln C_1}{d_2 - d_1}$$

where C_2 and C_1 are the chlorophyll fluorescence, or cell density (cell ml^{-1}) in the exponential phase at time 2 (d_2) and 1 (d_1), respectively. For each treatment, a paired t-test was used to determine the difference between the growth rates on ammonium and nitrate using Microsoft® Excel software.

2.2 Influence of irradiance on Skeletonema japonicum growth

Additional laboratory experiments were carried out to assess the influence of irradiance on the growth of the dominant phytoplankton of Dokai Bay, *S. japonicum* under two nitrogen sources. *S. japonicum* was isolated from a germination cyst from Dokai Bay sediment which collected in 2005, and maintained under 20℃ in the culture collection at Kagawa University. The culture was grown in 30 ml of enriched artificial seawater (modified from HARRISON *et al.,* 1980) containing either 100 μM ammonium or nitrate in 50 ml borosilicate glass test tubes. Cultures were incubated under cool white fluorescence lamps with four different irradiances of 58, 197, 260 and 450 μmol photons m^{-2} s^{-1} using black color nylon screen. The irradiance levels were measured inside the tube of each irradiance condition using a QSL-2101 irradiance meter (Biospherical Instruments Inc.). Cultures were grown at 20℃ under a 14 L:10 D cycle and each treatment was also conducted independently in three replicates. Prior to the experiment, all cultures were acclimatized to each irradiance for at least six generations. Acclimated exponential phase cultures were inoculated into the new medium and determined *in vivo* chlorophyll fluorescence at 24 h intervals using a fluorometer (Turner Design 10-AU-005). Specific growth rate (μ, d^{-1}) was determined using the protocol described above. For each irradiance condition, a paired t-test was used to determine the difference between the growth rates on ammonium and nitrate.

2.3 Role of ammonium and irradiance in Dokai Bay

The influence of high ammonium and

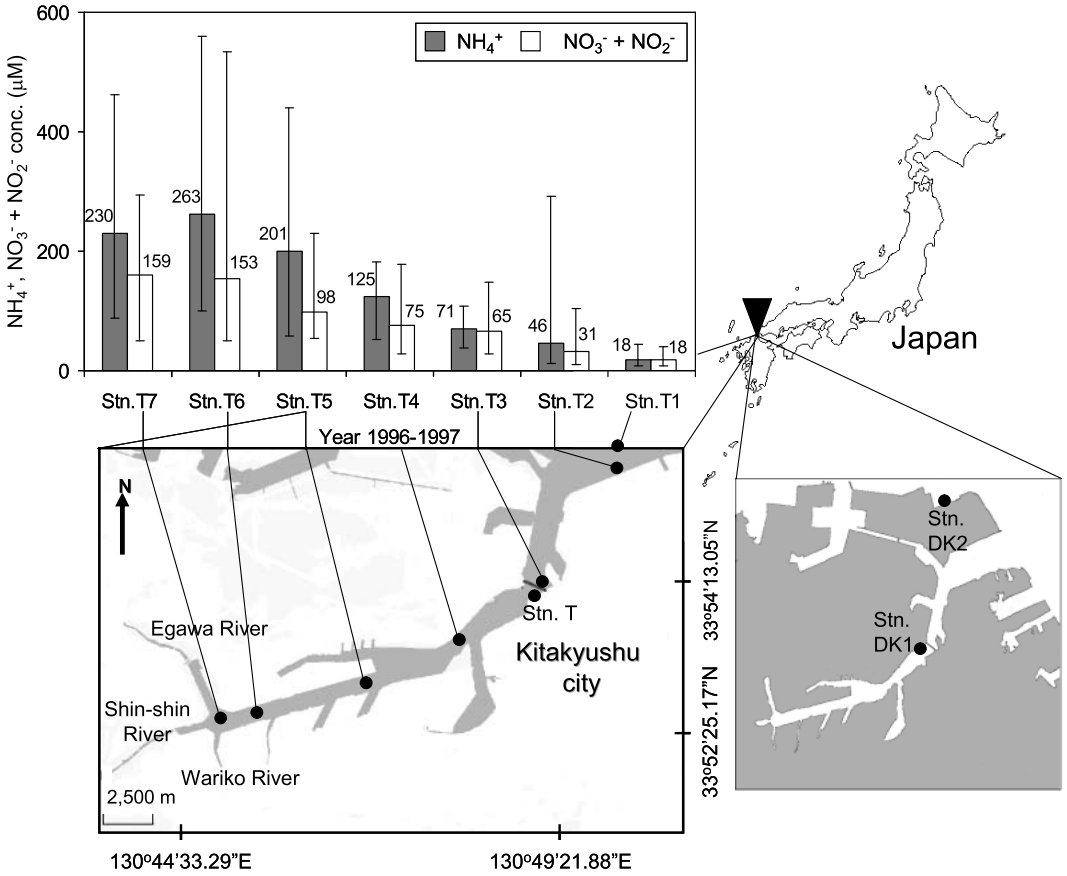

Fig. 1. Map of Dokai Bay in northern Kyushu Island, Japan and the 8 sampling stations. DK1 and DK2 represent sampling stations for the collection of the natural phytoplankton assemblages and natural seawater. The average ammonium and nitrate + nitrite concentration (μM) of surface water during 1996-1997 for station T1 (bay mouth) to T7 (inner bay) is given above the bar. Vertical bars show range (minimum to maximum) at each sampling station.

irradiance on the determination of species composition of phytoplankton assemblage in Dokai Bay was assessed. The previous data set of ammonium concentrations of surface water obtained from an intensive monitoring program in this bay (SUKSOMJIT *et al.*, 2005) was used. A vertical profile of irradiance in Dokai Bay from the inner part to mouth of the bay (Station T1 to T7 and Station T of central part in the bay, Fig. 1) determined in mid-summer of 2000, using a quantum irradiance meter (LI-205 Light meter, LI-COR) was used. Moreover, species composition of phytoplankton in Doaki Bay was determined in August, 1995 and 1998. Phytoplankton cell density and species along a transect from station T1 to T7 were counted and identified under an inverted microscope.

3. Results

3.1 Effect of ammonium on the growth of natural phytoplankton assemblages

In the first experiment using the sample collected on June 20, the dominant species in the natural phytoplankton assemblage was *Skeletonema* spp. All additional nutrients i.e. ammonium, nitrate, phosphate and silicic acid were assimilated for phytoplankton growth in the both treatments. In Fig. 2A and 3A, the concentration of all nitrogen sources decreased rapidly during the growth of phytoplankton.

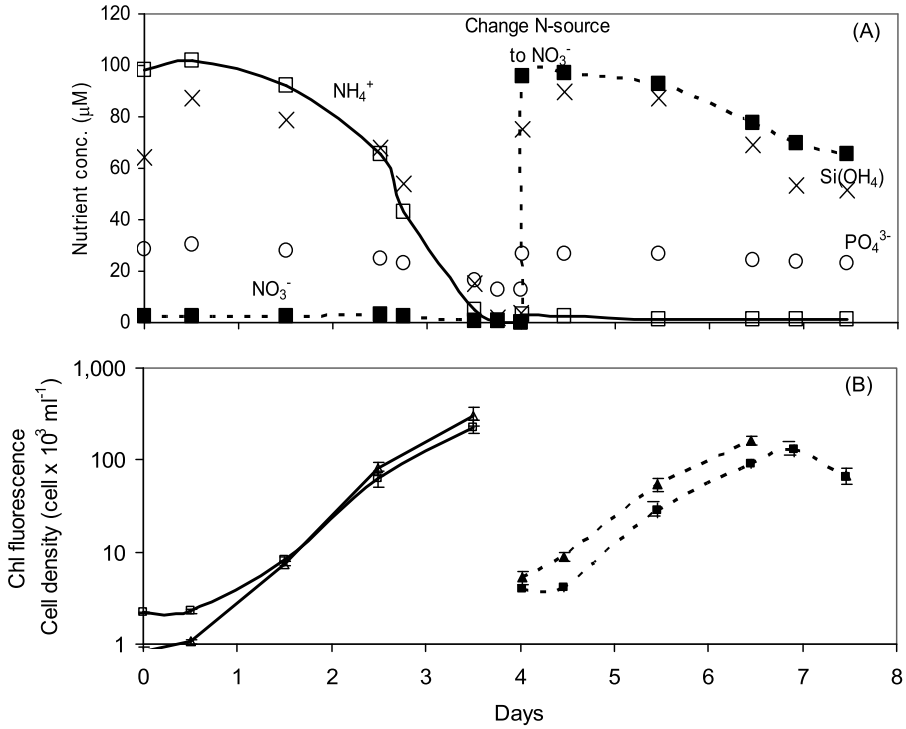

Fig. 2. Time series for the natural assemblage grown in the laboratory: (A) nutrients, NH_4^+ = □, NO_3^- = ■, Si $(OH)_4$ = X and O = PO_4^{3-} and (B) cell density (□) and chlorophyll fluorescence (Δ) of an NH_4^+-grown culture (solid lines and open symbols, n = 3) and then switched to growth on NO_3^- on day 4 (dashed lines and fill symbols, n = 2). Error bars show +/- 1 standard deviation (S.D.) of replicate samples.

Although the depletion of silicic acid (<1.8 μM for NH_4^+ and <8.0 μM for NO_3^-grown culture) occurred at the end of the incubation period on day 4 of the both treatments (Fig. 2A and 3A), it did not affect to the calculation because specific growth rate was determined during the exponential growth phase from day 1 to day 3, when silicic acid was >50 μM. In this experiment, *Skeletonema* spp. were always dominated and its contribution varied from 82 to 100% with the mean value 96% in both treatments at before and after nitrogen source was changed.

In the first treatment, the cell density of NH_4^+-grown culture reached a maximum (3.0×10^5 cells ml^{-1}) and the chlorophyll fluorescence increased to 229 after 4 days (Fig. 2B). When the nitrogen source was switched to nitrate, the highest cell density was 1.6×10^5 cells ml^{-1} and the fluorescence was only 128 on day 7.

For the second treatment, the phytoplankton cell density and chlorophyll fluorescence of the NO_3^-grown culture reached a maximum of 2.1×10^5 cells ml^{-1} and 182, respectively after 4 days. After NO_3^- was depleted and the nitrogen source was switched to ammonium, the highest cell density was 1.8×10^5 cells ml^{-1} and chlorophyll fluorescence reached 160 on the day 7 (Fig. 3B).

In this experiment, the specific growth rates (μ_{Chl} and $\mu_{Cell\ density}$) on ammonium were significantly higher ($p < 0.05$) compared to those on nitrate (Table 1). In the first treatment, the growth rate (μ_{Chl}) on ammonium was significantly higher ($1.59 \pm 0.05\ d^{-1}$, $p = 0.015$) than the growth rate ($1.40 \pm 0.01\ d^{-1}$) after the culture was switched to nitrate. Similarly, the growth rate (μ_{Chl}) of the natural phytoplankton assemblage of the NO_3^-grown culture in the second treatment was only $1.47 \pm 0.01\ day^{-1}$ and increased significantly to $1.69 \pm 0.01\ d^{-1}$ ($p < 0.01$) after the nitrogen source was switched to ammonium. For $\mu_{Cell\ density}$, a

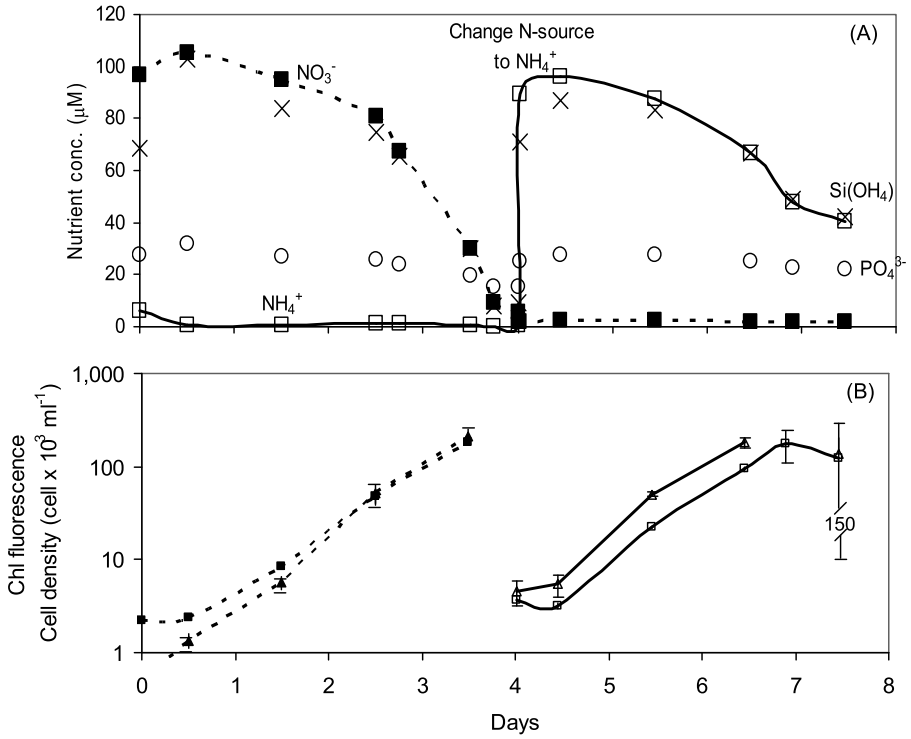

Fig. 3. Time series for the natural assemblage grown in the laboratory: (A) nutrients, NH_4^+ = □, NO_3^- = ■, Si $(OH)_4$ = X and O = PO_4^{3-} and (B) cell density (□) and chlorophyll fluorescence (Δ) of an NO_3^-grown culture (dashed lines and fill symbols, n = 3) and then switched to growth on NH_4^+ on day 4 (solid lines and open symbols, n = 2). Error bars show +/- 1 S.D. of replicate samples.

Table 1. Summary of average growth rates of the natural phytoplankton assemblage grown on ammonium and nitrate during the first experiment. The nitrogen source of each treatment is shown in parentheses. The difference between the two nitrogen sources is significant at $p < 0.05$ level (*) ; $p < 0.01$ level (**) ; n.s. = non-significant difference.

Growth rate (μ_{Chl}, d^{-1})	NH_4^+-sufficient treatment		p
	(NH_4^+) 1.59±0.05 (n=3)	(NO_3^-) 1.40±0.01 (n=2)	0.0152*
	NO_3^-sufficient treatment		
	(NO_3^-) 1.47±0.01 (n=3)	(NH_4^+) 1.69±0.01 (n=2)	0.0008**
Growth rate ($\mu_{Cell\ density}$, d^{-1})	NH_4^+-sufficient treatment		p
	(NH_4^+) 1.94±0.06 (n=3)	(NO_3^-) 1.46±0.01 (n=2)	0.046*
	NO_3^-sufficient treatment		
	(NO_3^-) 1.74±0.09 (n=3)	(NH_4^+) 1.80±0.01 (n=2)	n.s.

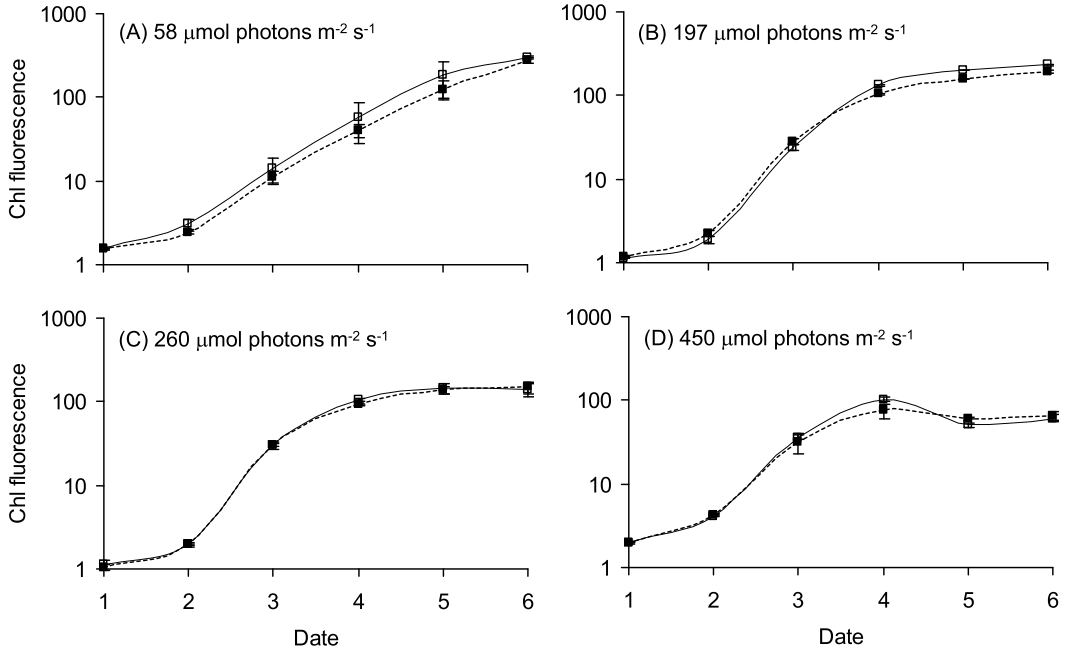

Fig. 4. Growth of *Skeletonema japonicum* at 58 (A), 197 (B), 260 (C) and 450 (D) μmol photons m^{-2} s^{-1} using NH_4^+ (solid line and open block) or NO_3^- (dash line and fill block). Error bars show +/- 1 S.D. of triplicate samples.

similar response of an increase in growth rate on ammonium compared to nitrate was also observed. The growth rate ($\mu_{Cell\ density}$) on ammonium was significantly higher ($1.94 \pm 0.06\ d^{-1}$, $p < 0.05$) than the growth rate ($1.46 \pm 0.01\ d^{-1}$) after the culture was switched to nitrate. μ_{Chl} of the NO_3^-grown culture in the second treatment was $1.74 \pm 0.09\ day^{-1}$ and increased to $1.80 \pm 0.08\ d^{-1}$ after the nitrogen source was switched.

In the second experiment using the sample collected from Dokai Bay on July 31, 2007, there was no difference in the growth rate of natural phytoplankton assemblage on either ammonium or nitrate The specific growth rate (μ_{Chl} and $\mu_{Cell\ density}$) of NH_4^+-growing cell was not significantly different ($p > 0.05$) from nitrate (data not shown). In this second experiment, the dominant species of natural phytoplankton assemblages collected on July 31 were mixed diatoms, i.e. *Skeletonema* spp., *Chaetoceros* spp., *Nitzschia* spp. and *Pseudo-nitzschia* spp. The proportion of *Skeletonema* spp. at the initial was 56.8%. However, the proportion of *Chaetoceros* spp. was 27.5%, while *Nitzschia* spp. and *Pseudo-nitzschia* spp. was 16%. Moreover, the proportion of *Skeletonema* spp. also decreased gradually and showed the lowest at the end of this experiment. Average proportion of *Skeletonema* spp. and *Chaetoceros* spp. in this experiment was 30.1% and 12.8%, respectively, while *Nitzschia* spp. and *Pseudo-nitzschia* spp. was 57.1%. This evidence was differed from the first experiment where *Skeletonema* spp. was almost completely dominant.

3.2 Growth of Skeletonema under different four irradiances

Variations of chlorophyll fluorescence of *S. japonicum* at various irradiances under two nitrogen sources were shown in Fig. 4. The photoadapted response allowed *S. japonicum* to grow well in a wide range of irradiances. At any irradiance (i.e. 58, 197, 260 and 450 μmol photons m^{-2} s^{-1}), all cultures grew by using either ammonium or nitrate. In Fig. 5, the growth rate of *S. japonicum* on either ammonium or nitrate increased with the irradiance up to 197 and 260 μmol photons m^{-2} s^{-1},

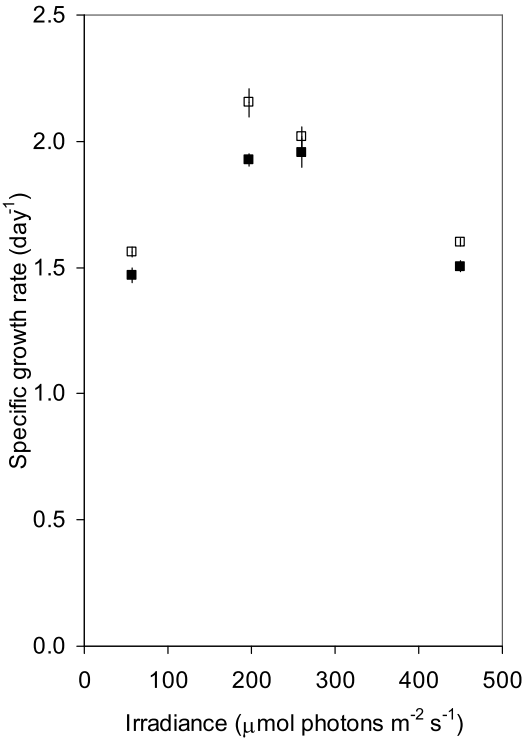

Fig. 5. Relationship between growth rate and irradiance for *Skeletonema japonicum* grown on either ammonium (□) or nitrate (■).The error bars show +/- 1 S.D. of triplicate samples.

respectively. Higher irradiance resulted in the inhibition of growth rate of the both nitrogen sources. However, the growth rates of *S. japonicum* on ammonium at all irradiances particularly at 197, 260 and 450 μmol photons m^{-2} s^{-1} were significant (p <0.05) higher than on nitrate. Individual growth rates on ammonium and nitrate at the above three irradiances were 2.15 $\pm$ 0.06 and 1.93 $\pm$ 0.02, 2.02 $\pm$ 0.04 and 1.95 $\pm$ 0.06, 1.60 $\pm$ 0.01 and 1.51 $\pm$ 0.02 d^{-1}, respectively. Moreover, the largest difference on the growth rate between the two nitrogen sources occurred at 197 μmol photons m^{-2} s^{-1} (p = 0.01). At 58 μmol photons m^{-2} s^{-1}, the growth rate of ammonium-grown cells was 1.56 $\pm$ 0.02 d-1 and not different from nitrate (1.47 $\pm$ 0.03 d^{-1}).

3.3 Irradiance and species composition of phytoplankton in Dokai Bay

Vertical profiles of irradiance on a sunny day in mid-summer (August 23, 2000) as an example of the light condition in the Dokai Bay at 8 stations when diatom blooms could form are shown in Fig. 6. The irradiance levels in the surface water (1 m depth) ranged between 183 to 959 μmol photons m^{-2} s^{-1} (average irradiance was 577 μmol photons m^{-2} s^{-1}). However, the irradiance showed a rapid decrease with water depth to about 200 μmol photons m^{-2} s^{-1} at 2 to 3 m depth) through the bay. Actually, the irradiance level at 2 to 3 m ranged from 71 to 675 (mean 267 $\pm$ 181) and from 32 to 284 (mean 102 $\pm$ 79) μmol photons m^{-2} s^{-1}, respectively.

The species composition of phytoplankton in Doaki Bay in mid-summer on August 31, 1995 and August 20, 1998 are showed in Fig. 7. In the both years, *Skeletonema* spp. was clearly dominant over other species from the inner to the bay mouth. The proportion of *Skeletonema* spp. ranged from 61–92% of the other species (except station T1 in 1995).

4. Discussion

4.1 Accelerated growth rate of natural phytoplankton assemblages

The growth of the natural phytoplankton assemblage collected from Dokai Bay was increased due to a 100 μM ammonium addition under laboratory conditions. The significantly higher phytoplankton growth rate on ammonium (~13 to 15%) compared to nitrate agreed with the previous studies summarized in Table 2. LEVASSEUR *et al.* (1993) found that the growth rate of *Thalassiosira pseudonana* on ammonium was 12% higher compared to nitrate. HERNDON and COCHLAN (2007) reported that the addition of ammonium at 50 μM increased the growth rate of *Heterosigma akashiwo* and growth rates on ammonium were 9 to 24% faster than those on urea and nitrate respectively. WOOD and FLYNN (1995) reported that during exponential growth of *H. carterae*, ammonium-grown cells attained higher growth rates by at least 20%.

The higher growth rates of ammonium-grown cells in this study might be caused by the extra energy cost for growth on nitrate. A higher energy cost of nitrate utilization over ammonium was reported in several previous studies (e.g. SYRETT, 1981 and reference

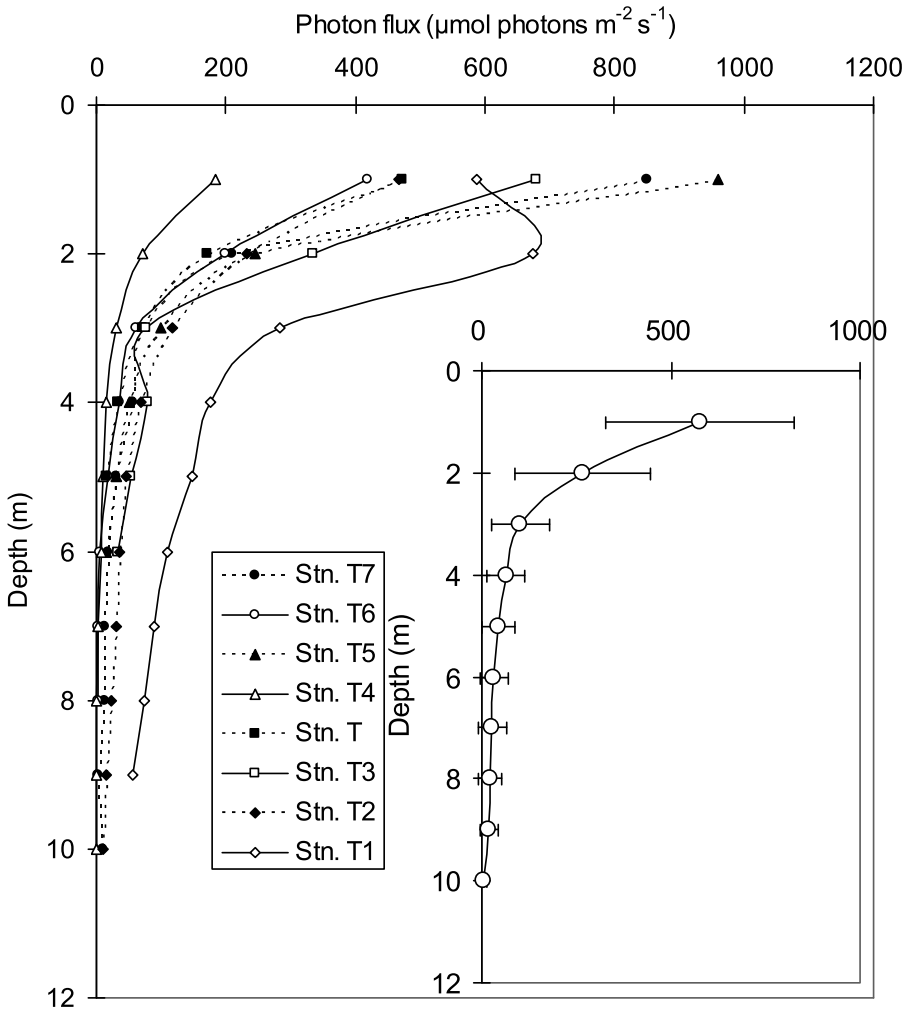

Fig. 6. Vertical profiles of photon flux in Dokai Bay from Station T1 (bay mouth) to Station T7 (inner bay), including Station T (middle of the bay), in mid-summer, 2000. See map in Fig. 1 for station locations. Small vertical profiles show the average photon flux of all stations and error bars show +/- 1 S.D. of replicate samples.

therein; FLYNN, 1991; FLYNN *et al.*, 1997; LEVASSEUR *et al.*, 1993; WASER *et al.*, 1998). LEVASSEUR *et al.* (1993) and THOMPSON *et al.* (1989) concluded that the higher energy requirement for nitrate reduction might be the reason of the lower growth rate of nitrate-growing cell, although there are several strategies that may be used to compensate for the higher energy requirement. WOOD and FLYNN (1995) demonstrated that nitrate-grown *H. carterae* decreased its growth rate possibly to compensate for the higher energy requirement of nitrate reduction.

Although ammonium had the potential to accelerate the growth rate of the natural phytoplankton assemblage in the first experiment when the dominant species was *Skeletonema* spp., this effect was not observed for other dominant species. In the second experiment, there was no significant difference for the growth of natural phytoplankton assemblages on ammonium and nitrate when the dominant species were a mixture of diatoms (i.e. *Skeletonema* spp., *Chaetoceros* spp.,

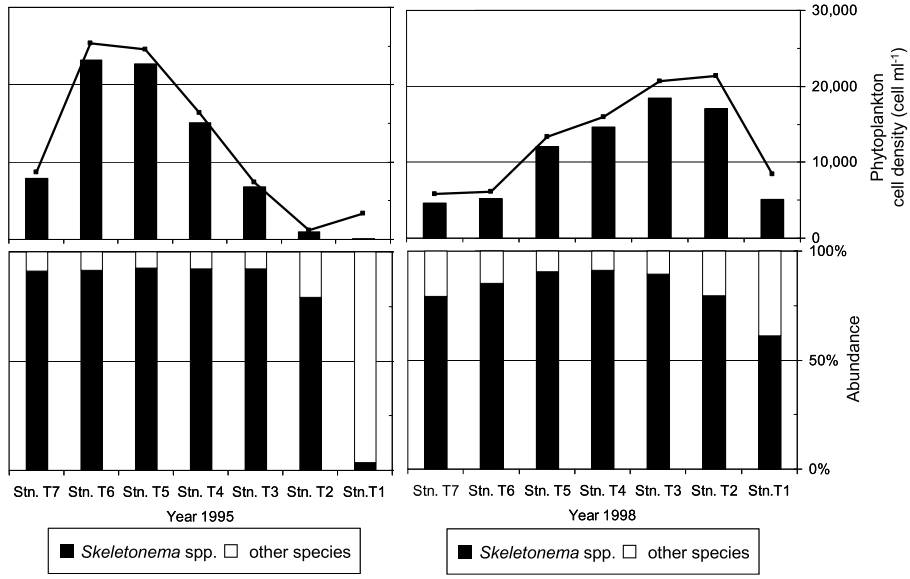

Fig. 7. Distribution of total phytoplankton abundance and proportion (%) between *Skeletonema* spp. and other species along a transect from station T1 (bay mouth) to station T7 (inner part) in mid-summer, 1995 (left) and 1998 (right). Solid lines indicate total phytoplankton cell density in the surface water and fill bars indicate the cell density of *Skeletonema* spp.

Nitzschia spp. and *Pseudo-nitzschia* spp). These results indicated that the effect of ammonium on growth acceleration was species specific and this agreed with previous studies. LEVASSEUR *et al.* (1993) reported that growth rate of *Thalassiosira pseudonana* on ammonium was higher compared to nitrate. In contrast, *Chaetoceros gracilis* grew significantly faster on nitrate than ammonium at similar irradiances. However, *Dunaliella tertiolecta* and *Gymnodinium sanguineum* showed very little influence of nitrogen source on growth rate. Moreover, species specificity on the growth acceleration effect of high ammonium had also been observed in higher plants. TYLOVA-MUNZAROVA *et al.* (2005) found that the growth rate of *Glyceria maxima* was 16% higher on ammonium than on nitrate, but the growth rate of *Phragmites australis* was not affected by the different forms of nitrogen.

4.2 The influence of irradiance on the growth rate of Skeletonema japonicum

The growth rates of *S. japonicum* on ammonium at all irradiances of 58, 197, 260 and 450 μmol photons m^{-2} s^{-1} were significantly higher than on nitrate. However, the largest difference in growth rate between the two nitrogen sources was observed at 197 μmol photons m^{-2} s^{-1}. This effect of irradiance level on the growth of *S. japonicum* between the two nitrogen sources in our study agreed with previous reports (HERNDON and COCHLAN, 2007; WOOD and FLYNN, 1995). WOOD and FLYNN (1995) reported that a significant difference between cell specific growth rates of *H. carterae* on ammonium and nitrate was observed at mid irradiances (200 μmol photons m^{-2} s^{-1}). In contrast, at low and high irradiances (50 or 350 μmol photons m^{-2} s^{-1}), they found that there was no significant difference between ammonium and nitrate.

In our study, the largest significant difference in growth rate at 197 μmol photons m^{-2} s^{-1} and smaller at high irradiances (260 and 450 μmol photons m^{-2} s^{-1}) between ammonium and nitrate might be explained by the following reason. At 197 μmol photons m^{-2} s^{-1}, the irradiance was optimal for growth. However, at 260 and 450 μmol photons m^{-2} s^{-1}, the growth rate was photoinhibited on either nitrogen sources. In contrast, light was limited

Table 2. Summary of the acceleration of growth rate of ammonium for several phytoplankton species.

Species	Concentration (μM)	Irradiance (μmol photons m^{-2} s^{-1})	Growth rate (d^{-1})	Reference
Thalassiosira pseudonana	100	170	1.48 (NH_4^+) 1.30 (NO_3^-)	LEVASSEUR *et al.* (1998)
Chaetoceros gracilis			1.56 (NH_4^+) 1.84 (NO_3^-)	
Dunaliella tertiolecta			1.42 (NH_4^+) 1.41 (NO_3^-)	
Gymnodinium sanguineum			0.43 (NH_4^+) 0.41 (NO_3^-)	
Heterosigma akashiwo	50	40	0.57 (NH_4^+) 0.46 (NO_3^-)	HERNDON and COCHLAN (2007)
		110	0.89 (NH_4^+) 0.82 (NO_3^-)	
Thalassiosira pseudonana	75	$\leq$29 $\geq$61	$NH_4^+ = NO_3^-$ $NH_4^+ > NO_3^-$	THOMPSON *et al.* (1989)
Heterosigma carterae	100	50 200 350	$NH_4^+ > NO_3^-$ $\sim$ 20%	WOOD and FLYNN (1995)
Skeletonema sp.	100	58	1.56 (NH_4^+) 1.47 (NO_3^-)	This study
		197	2.15 (NH_4^+) 1.93 (NO_3^-)	
		260	2.02 (NH_4^+) 1.95 (NO_3^-)	
		450	1.60 (NH_4^+) 1.51 (NO_3^-)	

at 58 μmol photons m^{-2} s^{-1} for cells using either nitrogen source. The equal growth rates of ammonium and nitrate-grown cells at 58 μmol photons m^{-2} s^{-1} agreed with the previous studies (THOMPSON *et al.*, 1989; WOOD and FLYNN, 1995). Our study clearly showed that the growth acceleration of *Skeletonema* spp. in this bay was particularly significant at an irradiance level of about 200 μmol photons m^{-2} s^{-1}.

4.3 Field implications

Generally, it is well known that phytoplankton growth in the natural environment is controlled by physical (e.g. water circulation, irradiance, water temperature, etc.), chemical (e.g. nutrient, trace element, etc.) and biological (e.g. taxonomic variation, origin of phytoplankton, zooplankton grazing, etc.) factors. YANAGI and YAMADA (2000) and TADA *et al.* (2001 and 2004) concluded that phytoplankton blooms in Dokai Bay were mainly controlled by the fast flushing time of the bay because nutrient concentrations in this bay were sufficient for phytoplankton growth during the entire year. They suggested that phytoplankton species that have a higher growth rate than their loss rate due to the high flushing rate of the surface water in the bay, could bloom and become dominant. However, evidence from two laboratory experiments helps to explain why *Skeletonema* spp. is the dominant species in the phytoplankton assemblage throughout the year in Dokai Bay.

Due to the strong estuarine circulation in Dokai Bay, phytoplankton in the inner bay would be flushed out of the bay within 2.5 days. Only phytoplankton species that are able to obtain a high density due to their high growth rate, could become dominant and produce a bloom in this bay (YANAGI and YAMADA, 2000; TADA *et al.*, 2001 and 2004). Data from the intensive monitoring program in 1996 to 1997 (SUKSOMJIT *et al.*, 2005) indicated that an

ammonium concentration of 100 μM occurred in the middle part of Dokai Bay (station T4 and T3, Fig. 1.), while a higher ammonium concentration >200 μM occurred at the inner bay. In addition, the variation of nitrate + nitrite in this bay also showed a similar pattern as ammonium. The highest concentration was observed at the inner bay and decreased gradually to the bay mouth. The ammonium concentration of 100 μM that was found in the middle part of this bay, was in the range of ammonium that caused the acceleration effect of phytoplankton growth. This suggests that the growth of natural phytoplankton assemblages in Dokai Bay would be accelerated by this ammonium concentration during the approximate 2.5 days flushing time out of the bay. This conclusion agreed with the observation that *Skeletonema* spp. was the dominant species in Dokai Bay in 1995 and 1998 (Fig. 7). The proportion of *Skeletonema* spp. of the total phytoplankton assemblage was 90% in both years throughout the bay. We therefore concluded that high ammonium was one reason to regulate the phytoplankton composition in Dokai Bay through the acceleration on growth rate of *Skeletonema* spp., although there were many factors regulating the phytoplankton composition, such as phytoplankton ability of adaptation to eutrophication, sinking rate, cyst formation characteristic and the dependences of temperature on growth and this might also explain the dominance of *Skeletonema* spp. in Dokai Bay over other species.

However, the effect of ammonium on the acceleration of growth rate was influenced by the irradiance level. The growth of *S. japonicum* was significantly accelerated by ammonium at an irradiance level of about 200 μmol photons m^{-2} s^{-1}. Although irradiance at the surface water on a sunny day in summer was higher and might inhibit the growth of phytoplankton, the acceleration in growth rate could occur in the surface (2 or 3 m) mixed layer, or on cloudy days. In Dokai Bay, an irradiance level around 200 μmol photons m^{-2} s^{-1} was observed in sub-surface waters (2 and 3 m depth) through the bay as showed in Fig. 6. This observation was consistent with a previous report in this bay. TADA *et al.* (2001) also reported that the transparency in Dokai Bay was low and irradiance was strongly limited in the water column. In the field, high irradiance would be observed only for a few hours around noon, while the irradiance of 200 μmol photons m^{-2} s^{-1} in laboratory was maintained over a 14 h period. Hence, we conclude that the present results showed the possibility of growth acceleration of phytoplankton community in Dokai Bay and the importance of the irradiance level on the growth of phytoplankton in this bay.

The high dominance of *Skeletonema* spp. over other species at most stations in the bay and the high flushing rate of ～2.5 days, indicates that this bay exports large amount of *Skeletonema* spp. to coastal waters. Hence, Dokai Bay acts like a selective growth incubator for certain diatoms such as *Skeletonema* spp. whose growth rate is greater that the loss rate as a result of flushing. During the 2.5 day flushing period, 7.8 doublings would occur (assuming growth rate = 2.15 d^{-1}) on ammonium, while 7.0 doublings would occur (assuming a growth rate of 1.95 d^{-1}) if *Skeletonema* spp. used only nitrate. This nearly one extra doubling during the 2.5 day transient out of the bay for growth on ammonium at optimum light, may only partially explain Skeletonema's dominance and other factors such as grazing should be explored.

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References

AZOV, Y. and J. C. GOLDMAN (1982): Free ammonia inhibition of algal photosynthesis in intensive cultures. *Appl. Environ. Microbiol.*, **43**, 735–739.

BRAND, L.E., R. R. L. GUILLARD and L. S. MURPHY (1981): A method for the rapid and precise determination of acclimated phytoplankton. *J. Plankton Res.*, **3**, 193–201.

COLLOS, Y., F. MORNET, A. SCIANDRA, N. WASER, A. LARSON and P. J. HARRISON (1999): An optical method for the rapid measurement of micromolar concentration of nitrate in marine phytoplankton cultures. *J. Appi. Phycol.*, **11**, 179

–184.

FLYNN, K. J. (1991): Algal carbon–nitrogen metabolism: a biochemical basis for modelling the interactions between nitrate and ammonium uptake. *J. Plank. Res.*, **13**, 373–387.

FLYNN, K. J., M. J. R. FASHAM and C. HIPKIN (1997): Modelling the interactions between ammonium and nitrate uptake in marine phytoplankton. *Phil. Trans. R. Soc. Lond. B.*, **352**, 1625–1645.

HARRISON, P. J., R. E. WATER and F. J. R. TYLOR (1980): A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *J. Phycol.*, **19**, 28–35.

HERNDON, J. and W. P. COCHLAN (2007): Nitrogen utilization by the raphidophyte *Heterosigma akashiwo*: Growth and uptake kinetics in laboratory cultures. *Harmful Algae*, **6**, 260–270.

HOLMES, R. M., A. AMINOT, R. KÉROUEL, B. A. HOOKER and B. J. PETERSON (1999): A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish Aquat. Sci.*, **56**, 1801–1808.

LEVASSEUR, M., P. A. THOMPSON and P. J. HARRISON (1993): Physiological acclimation of marine phytoplankton to different nitrogen sources. *J. Phycol.*, **29**, 587–595.

LIVINGSTON, R. J., A. K. PRASAD, X. NUI and S. E. MCGLYNN (2002): Effects of ammonia in pulp mill effluents on estuarine phytoplankton assemblages: field descriptive and experimental results. *Aquat. Bot.*, **74**, 343–367.

NATARAJAN, K. V. (1970): Toxicity of ammonia to marine diatoms. *J. WPCF*, 42, 184–190.

SARNO, D., W.H.C.F. KOOISTRA, L.K. MEDLIN, I. PERCOPO and A. ZINGONE (2005): Diversity in the genus *Skeletonema* (Bacillariophyceae). II. an assessment of the taxonomy of *S. costatum*–like species with the description of four new species. *J. Phycol.*, **41**, 151–176

STRICKLAND, J. D. H. and T. R. PARSONS (1972): *A practical hand book of seawater analysis* (*2nd Ed'n*). Fisheries Research Board Canada Bulletin, **167**, 311 pp.

SUKSOMJIT, M., K. TADA, K. ICHIMI and S. MONTANI: High tolerance of phytoplankton for extremely high ammonium concentrations in the eutrophic coastal water of Dokai Bay (Japan), *La mer*, (in Press).

SUKSOMJIT, M., K. TADA, K. ICHIMI, M. YAMADA and S. MONTANI (2005): The effect of high ammonium concentration on phytoplankton growth in coastal water. p. 209. In *The Fourth Asian–Pacific Phycological Forum, Advances in Phycological Research: Biology, Chemistry and Biotechnology* (Abstract), The Asian–Pacific Phycological Association, Thailand.

SYRETT, P. J. (1981): Nitrogen metabolism of microalgae. *Can. Bull. Fish. Aquat. Sci.*, **210**, 182–210.

TADA, K., M. SUKSOMJIT, K. ICHIMI, Y. FUNAKI, S. MONTANI, M. YAMADA and P. J. HARRISON :Ammonium Increases Diatom Growth Rates in Rapidly Flushed Eutrophic Dokai Bay, Japan. *J. Oceanogr.* (in press)

TADA, K., K. ICHIMI, H. T. YOKOTA, M. YAMADA and S. MONTANI (2004): Why flagellates do not produce bloom in Dokai Bay, Japan. *Oceanogr. Japan*, **13**, 271–279 (in Japanese, with English abstract).

TADA, K., M. MORISHITA, K. I. HAMADA, S. MONTANI and M. YAMADA (2001): Standing stock and production rate of phytoplankton and a red tide outbreak in heavily eutrophic embayment, Dokai Bay, Japan. *Mar. Poll. Bull.*, **42**, 1177–1186.

THOMPSON, P. A., M. E. LEVASSEUR and P. J. HARRISON (1989): Light–limited growth on ammonium vs. nitrate: What is the advantage for marine phytoplankton?. *Limnol. Oceanogr.*, **34**, 1014–1024.

TYLOVA–MUNZAROVA, E., B. LORENZEN, H. BRIX and O. VOTRUBOVA (2005): The effects of NH_4^+ and NO_3^- on growth, resource allocation and nitrogen uptake kinetics of *Phragmites australis* and *Glyceria maxima*. *Aquat. Bot.*, **81**, 326–342.

WASER, N. A., K. YIN, Z. YU, K. TADA, P. J. HARRISON, D. H. TURPIN and S. E. CALVERT (1998): Nitrogen isotope fractionation during nitrate, ammonium and urea uptake by marine diatoms and coccolithophores under various conditions of N availability. *Mar. Ecol. Prog. Ser.*, **169**, 29–41.

WOOD, G. J. and K. J. FLYNN (1995): Growth of *Heterosigma carterae* (Raphidophyceae) on nitrate and ammonium at three photon flux densities: evidence for N stress in nitrate–growing cells. *J. Phycol.*, **31**, 859–867.

YAMADA, M. and Y. KAJIWARA (2004): Characteristics of phytoplankton occurrence in the hyper–eutrophic environment, Dokai Bay, Japan. *Oceanogr. Japan*, **13**, 281–293. (in Japanese, with English abstract).

YANAGI, T. and M. YAMADA (2000): Reason why red tides do not occur during winter in Dokai Bay, Japan. *Umi no Kenkyu*, **9**, 125–132. (in Japanese).

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