La mer 47 : 75-88, 2009
Société franco-japonaise d'océanographie, Tokyo

High tolerance of phytoplankton for extremely high ammonium concentrations in the eutrophic coastal water of Dokai Bay (Japan)

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Abstract: The tolerance of phytoplankton in Dokai Bay for an extremely high ammonium concentration in culture media has been studied. Six species of phytoplankton, three diatoms (two clones of *Skeletonema* sp. and *Chaetoceros* sp.) and three flagellates (*Heterosigma akashiwo*, *Chattonella antiqua* and *Karenia mikimotoi*) were grown in various concentrations of NH_4Cl. The results suggested that high ammonium concentrations had negative effects on phytoplankton growth. Non-indigenous species in Dokai Bay, Japan, *C. antiqua* and *K. mikimotoi*, were unable to grow at 200 and 150 μM, respectively. Growth rates of *Skeletonema* sp. isolated from Harima Nada (Seto Inland Sea, Japan), *Chaetoceros* sp. and *H. akashiwo* were reduced significantly at higher ammonium concentrations compared to the control treatment. However, such a high ammonium concentration of even 1,500 μM could not produce a significant adverse effect on the growth rate of *Skeletonema* sp. isolated from Dokai Bay. Furthermore, the maximum chlorophyll fluorescence of tested species was also gradually decreased with an increase in ammonium concentration. The influence of a high ammonium level on phytoplankton growth observed in this study confirmed the phytoplankton species composition observed in Dokai Bay. Our results suggested that such a high ammonium concentration was an important factor in determining the species composition of the phytoplankton assemblage in that bay.

Keywords: *Ammonium toxicity, growth inhibition, Dokai Bay*

1. Introduction

Since the last century, the role of ammonium in phytoplankton growth has attracted the attention of scientists, and several studies have been published underlining its importance. Among dominant nitrogen sources, ammonium is known as an excellent nitrogen source for phytoplankton growth. Phytoplankton are believed to prefer ammonium to nitrate even when both substrates are available due to the higher energy cost of nitrate utilization. Phytoplankton resume the uptake of nitrate when ammonium concentration declines (e.g. SYRETT, 1981 and reference therein; FLYNN, 1991; FLYNN *et al.*, 1997; LEVASSEUR, *et al.*, 1993; WASER *et al.*, 1998). Although ammonium is an important factor related to primary productivity in aquatic environments (NATARAJAN, 1970; DORTCH and CONWAY, 1984), it is generally found in lower concentrations than nitrate in natural environments. The availability of nitrate determined a new primary productivity in aquatic environments (DUGDALE and GOERING, 1967).

However, both negative and positive effects

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of ammonium on the growth of phytoplankton have been reported. For example, the inhibitory effects of ammonium on the phytoplankton growth rate and photosynthesis have been variously reported (e.g. NATARAJAN, 1970; THOMAS *et al.*, 1980; AZOV and GOLDMAN, 1982; LIVINGSTON *et al.*, 2002). Moreover, several studies have reported in both culture and field experiments that the presence of ammonium significantly reduced the nitrate uptake rate of several phytoplankton species (e.g. DORTCH and CONWAY, 1984; DORTCH *et al.*, 1991; HORRIGAN and MCCARTHY, 1982; LOMOS and GLIBERT, 1999). SYRETT (1981) summarized that active nitrate reductase (NR), an important enzyme for nitrate uptake, is not formed in the presence of ammonium. A decrease in NR activity by ammonium reflects the inhibition of nitrate incorporation (BERGES *et al.*, 1995). Changes in NR activity are most likely mediated by changes in enzyme protein at longer time scales, but perhaps by an inactivation mechanism on a scale of only minutes.

Generally, though the amounts of ammonium in unpolluted coastal waters rarely exceed 5 μM, a high ammonium concentration could be observed in several coastal areas due to the discharge of high-loading or untreated wastewater from human activity (e.g. LIVINGSTON *et al.*, 2002; TADA *et al.*, 2001). In Dokai Bay, one of the eutrophic embayments in Japan, a high ammonium concentration (e.g. >200 μM) that was toxic to organisms (e.g. RANDALL and TSUI, 2002) has often been observed, and due to its negative effect on growth rates, ammonium might be one of the factors that have determined the species composition of the phytoplankton community in that bay. Species tolerant of such a high ammonium concentration should produce a bloom more frequently observed than in species with a lower tolerance. These hypotheses were derived from field observations of dominant phytoplankton species during phytoplankton blooms in Dokai Bay. YAMADA and KAJIWARA (2004) reported that *Skeletonema* spp. usually formed the major component of the phytoplankton assemblage in Dokai Bay, while only a few blooms of other species were observed. Blooms of flagellates seldom occurred even though sufficient nutrients were available. In addition, the phytoplankton assemblage in the bay was dominated all year around by *Skeletonema* sp.

Although TADA *et al.* (2001, 2004) had already shown that the growth rates of phytoplankton were an important factor in species composition because of the rapid advection of the surface water mass by a strong estuarine circulation without vertical mixing, indicating that the influence of an extremely high ammonium concentration in the bay should also be discussed. The goal of this study was to clarify the influence of a high concentration of ammonium on phytoplankton growth and its consequences for species determination under eutrophic conditions such as those in Dokai Bay. The effects of ammonium were examined at various concentrations (including those similar to that of Dokai Bay) on the growth of indigenous and non-indigenous phytoplankton species.

2. Materials and methods

2.1 Influence of ammonium on phytoplankton growth

To examine and compare the influence of ammonium on phytoplankton growth under eutrophic conditions such as those in Dokai Bay, cultures of indigenous and non-indigenous species in the bay i.e. *Skeletonema* sp. and *Chaetoceros* sp. (Diatoms), *Heterosigma akashiwo* and *Chattonella antiqua* (Raphidophyceae) and *Karenia mikimotoi* (Dinoflagellates) were chosen for this study. *Skeletonema* sp. was isolated from both Dokai Bay and Harima Nada. Moreover, other phytoplankton species (*Chaetoceros* sp., *H. akashiwo*, *C. antiqua* and *K. mikimotoi*) were isolated from Harima Nada and maintained in the culture collection of the Kagawa University Laboratory.

Seawater (filtrated through Whatman GF/F filters) from Harima Nada was used to prepare an ESM culture medium (OKAICHI *et al.*, 1983) without Tris-hydroxymethyl-aminomethane. The pH of each culture medium was initially adjusted to 8.0. Culture media were then transferred to a 50-ml borosilicate glass test tube with a Teflon-lined cap, and autoclaved at 65℃ for 60 minutes. The resulting medium contain-

ing 1,400 μM of nitrate as the sole N source was used for the following experiments together with various concentrations of ammonium.

Each test species was inoculated into the culture media with a different level of ammonium concentrations by the addition of NH_4Cl and incubated at 21℃ in 100 μmol photons m^{-2} s^{-1} (14:10 hours Light-Dark cycle). Based on the ammonium concentration of the surface water in Dokai Bay, eleven different final ammonium concentrations (0 (no ammonium addition and nitrate as the sole N source), i.e. 10, 50, 100, 150, 200, 500, 700, 1000, 1250 and 1500 μM (with 1,400 μM of nitrate)) were chosen for *Skeletonema* sp., *Chaetoceros* sp. and *H. akashiwo*, while lower levels (0, 5, 10, 25, 50, 100, 150, 200, 300 and 500 μM) were used for *C. antiqua* and *K. mikimotoi*. All treatments were conducted independently in triplicate. Sterile technique was used throughout the study to prevent bacterial contamination. Prior to the experiment, all test species were acclimated by being grown in the desired ammonium concentration for at least six generations. The acclimated exponential phase of test species was inoculated into the new culture medium. Following inoculation, *in vivo* chlorophyll fluorescence was determined at 24-h intervals using a fluorometer (Turner Design 10-AU-005) (BRAND *et al.*, 1981). The experiment was terminated when most replicates began to decline. The growth rate (μ_2, divisions day^{-1}) was estimated from *in vivo* chlorophyll fluorescence data during the exponential phase of growth using the following formula:

$$\mu_2=\frac{(\log_2 F_2-\log_2 F_1)}{(t_2-t_1)},$$

where F_2 and F_1 are the *in vivo* chlorophyll fluorescences in the exponential phase at times 2 (t_2) and 1 (t_1) after incubation, respectively. A minimum of 3 sampling points was included in each calculation. The growth rate and maximum chlorophyll fluorescence of test species in a concentration series of ammonium were compared to the no-ammonium addition treatment (control treatment) using one-way analysis of variance (SPSS® version 10.0 software) to evaluate the influence of ammonium on phytoplankton growth. A post-hoc comparison of means was conducted when a significant difference ($p<0.05$) was observed.

2.2 Relationship between *in vivo* chlorophyll fluorescence and cell density

Another experiment, using the protocol described above, was conducted to assess the correlation between *in vivo* chlorophyll fluorescence and cell density. That correlation was tested on *Skeletonema* sp. and *C. antiqua* at two ammonium concentrations (i.e. no ammonium addition with nitrate (1,400 μM) as the sole N source, and high ammonium concentration with 1,400 μM NO_3^-). That correlation was determined at high ammonium concentrations (500 and 150 μM, respectively) with no ammonium addition. *In vivo* chlorophyll fluorescence was determined using a fluorometer (Turner Design 10-AU-005), and cell density by using a cell and particle counter (Beckman Z2™ Coulter Counter®).

2.3 Influence of pH on growth of *Skeletonema* sp.

An additional experiment using the protocol described above was carried out to assess the influence of pH. The variations of pH during the growth of *Skeletonema* sp. isolated from Dokai Bay under a number of ammonium concentrations (0, 10, 50, 100, 150, 200 and 500 μM) were determined daily using a Shidengen ISFET pH-meter (KS 701).

2.4 Phytoplankton growth and ammonium concentration of Dokai Bay water

The influence of high ammonium on the species composition of the phytoplankton assemblage in Dokai Bay was assessed. To evaluate that influence, the previous data set of ammonium concentrations of surface water obtained from an intensive monitoring program in the bay (SUKSOMJIT *et al.*, 2005) were used. In addition, ammonium concentrations of surface water at 7 stations in the bay from 1996 to 1997 as well as the tolerance data obtained in this study were examined.

3. Results

3.1 Relationship between *in vivo* chlorophyll

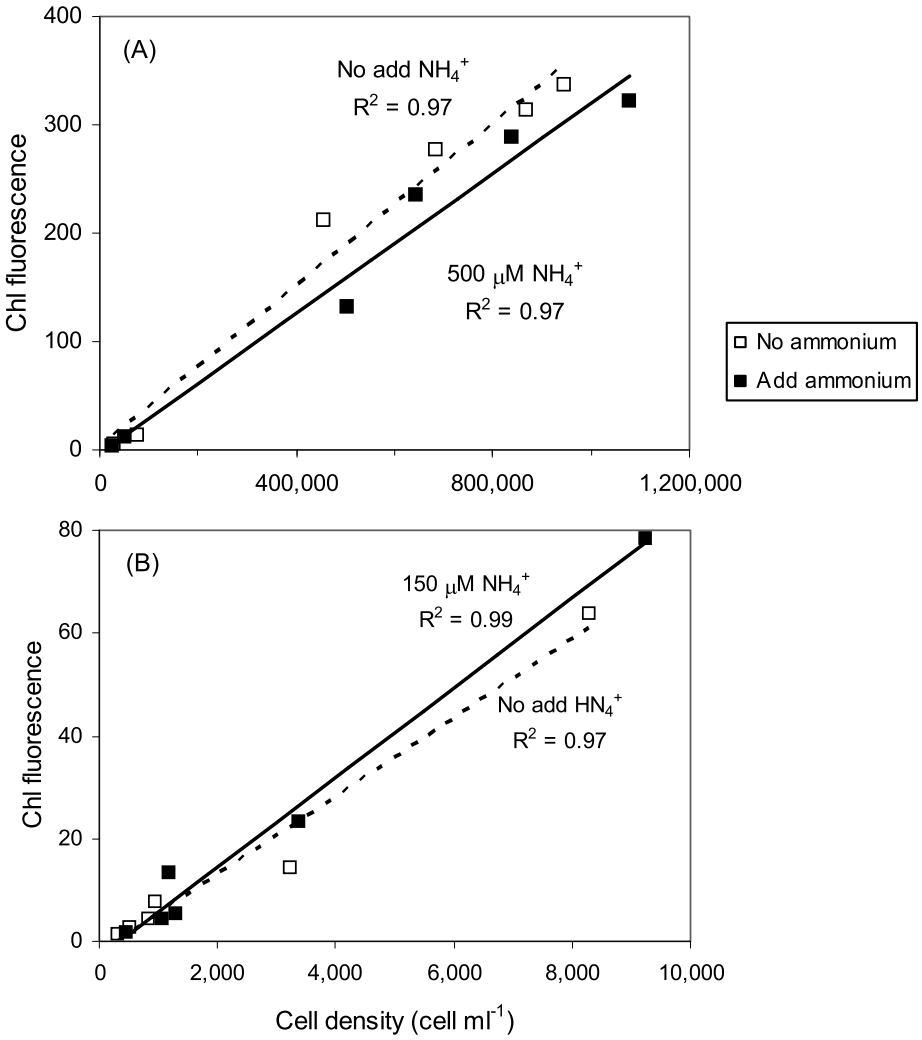

Fig. 1. Correlation between chlorophyll fluorescence and phytoplankton cell density of *Skeletonema* sp. (A) and *Chattonella antiqua* (B) under different ammonium concentrations.

fluorescence and cell density

Figure 1 shows the relationship between the *in vivo* chlorophyll fluorescence and phytoplankton cell density of *Skeletonema* sp. and *C. antiqua* under different ammonium concentrations. Linear regressions of *in vivo* chlorophyll fluorescence versus cell density observed at all ammonium concentrations of both test species and correlation coefficients (R^2) were > 0.97. Moreover, there was no difference (p>0.05) in the correlation between *in vivo* chlorophyll fluorescence and cell density at high ammonium concentration vs. no ammonium addition. This showed that the cells responding to severe conditions of high ammonium concentration based on their cellular chlorophyll contents were not different from those under normal conditions. This result indicated that we could calculate the growth rate using fluorescence instead of cell density, and could discuss our results using data calculated from *in vivo* chlorophyll fluorescence for cell yields.

3.2 Effect of ammonium on growth rates

The growth rates of all tested species and the

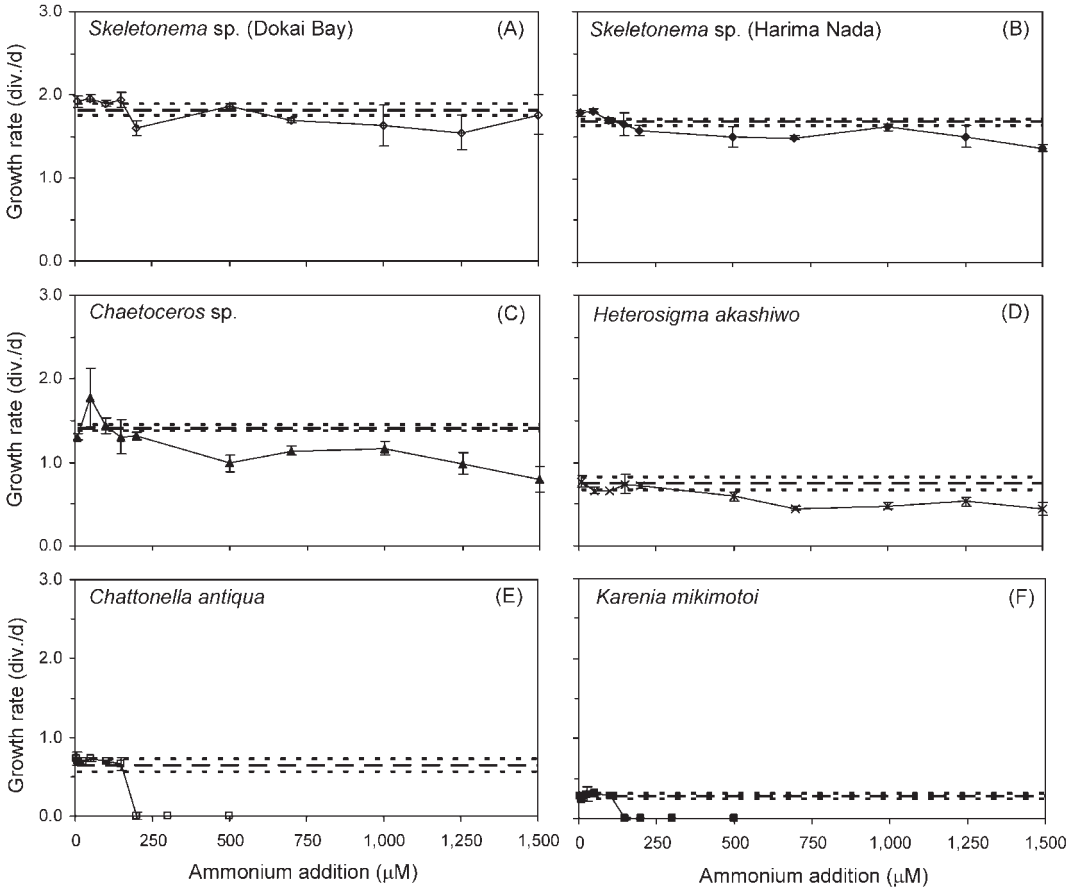

Fig. 2. Growth rates (μ_2, divisions day^{-1}) of *Skeletonema* sp. isolated from Dokai Bay (A) and Harima Nada, Seto Inland Sea (B), *Chaetoceros* sp. (C), *Heterosigma akashiwo* (D), *Chattonella antiqua* (E) and *Karenia mikimotoi* (F) exposed to various ammonium addition. Vertical bars are standard deviations (S.D.) of replicate samples. Longer dashed lines indicate the growth rate of the control treatment (no NH_4^+ addition) and shorter dashed lines indicate ± S.D.

growth rate variations in a series of ammonium concentrations were shown in Fig. 2. Among test species, the growth rates of *Skeletonema* sp. isolated from both Dokai Bay and Harima Nada were slightly higher than those of *Chaetoceros* sp. and markedly higher than those of other test species. The presence of high ammonium exerted a significant impact on the growth rate of indigenous test species, i.e. *Skeletonema* sp., *Chaetoceros* sp. and *H. akashiwo*. Moreover, the lethal effect of such high ammonium concentrations as those found in Dokai Bay became evident in two non-indigenous flagellates, i.e. *C. antiqua* and *K. mikimotoi*.

Since no lethal effect at even the highest ammonium concentrations was found in all indigenous species, the effect of ammonium on the growth rate varied among each species tested. Among the indigenous species, ammonium at a high concentration of even 1,500 μM had no significant effect on the growth rate of *Skeletonema* sp. isolated from Dokai Bay compared to the control, which had no added ammonium and in which nitrate was the sole N source (Fig. 2A). The growth rate of *Skeletonema* sp. varied from 1.60 to 1.96 divisions day^{-1}, and the rates at high ammonium concentrations (700, 1,000, 1,250 and 1,500 μM) were similar to those in with the control

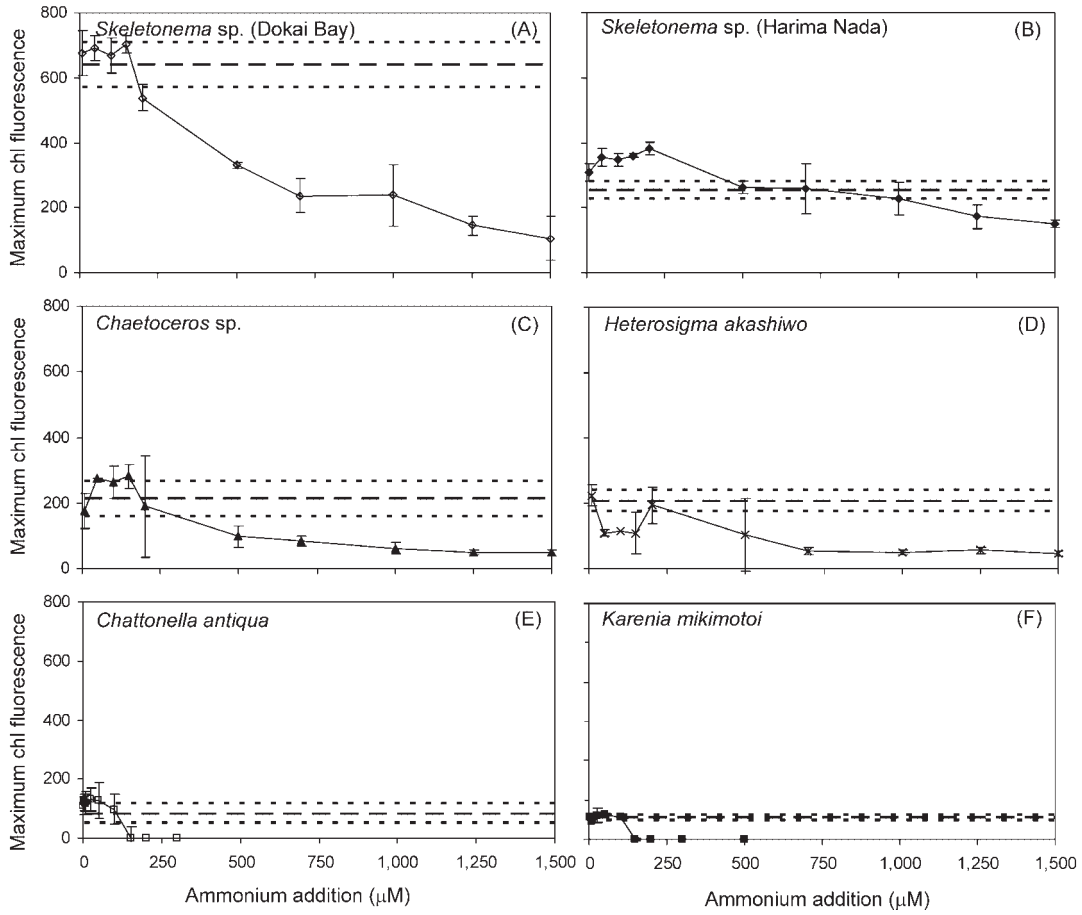

Fig. 3. Maximum chlorophyll fluorescence of *Skeletonema* sp. isolated from Dokai Bay (A) and Harima Nada, Seto Inland Sea (B), *Chaetoceros* sp. (C), *Heterosigma akashiwo* (D), *Chattonella antiqua* (E) and *Karenia mikimotoi* (F) exposed to various ammonium addition. Vertical bars are standard deviations of replicate samples. Longer dashed lines indicate the maximum chlorophyll fluorescence of the control treatment (no NH_4^+ addition) and shorter dashed lines indicate ± S.D.

treatment. Unlike the clone from Dokai Bay, the *Skeletonema* sp. isolated from Harima Nada, *Chaetoceros* sp. and *H. akashiwo*, evidenced a significant reduction in their growth rate at higher ammonium concentrations. The growth rates of *Skeletonema* sp. from Harima Nada and *Chaetoceros* sp. were significantly (p <0.05) reduced at ammonium concentrations of 500, 700, 1,250 and 1,500 μM but not at 1,000 μM compared to the control treatment (Fig. 2B and 2C). Those rates also varied from 1.37 to 1.51, and from 0.80 to 1.14 divisions $d-a y^{-1}$, whereas the growth rate of the control treatment was 1.68 and 1.41 divisions day^{-1}. In addition, *H. akashiwo* growth rates were significantly lower than the control treatment when ammonium concentrations reached 500 μM or higher (Fig. 2D). The growth rate at those concentrations varied from 0.44 to 0.59 divisions day^{-1}, whereas that in the control treatment was 0.75 divisions day^{-1}.

The lethal effect of high ammonium concentrations was shown in both non-indigenous flagellates, *C. antiqua* and *K. mikimotoi*. These test species were unable to grow when the ammonium concentrations reached 200 and 150 μM, respectively (Figs. 2E and 2F), whereas below those concentrations, they could have survived and grown at the same rate as the control treatment.

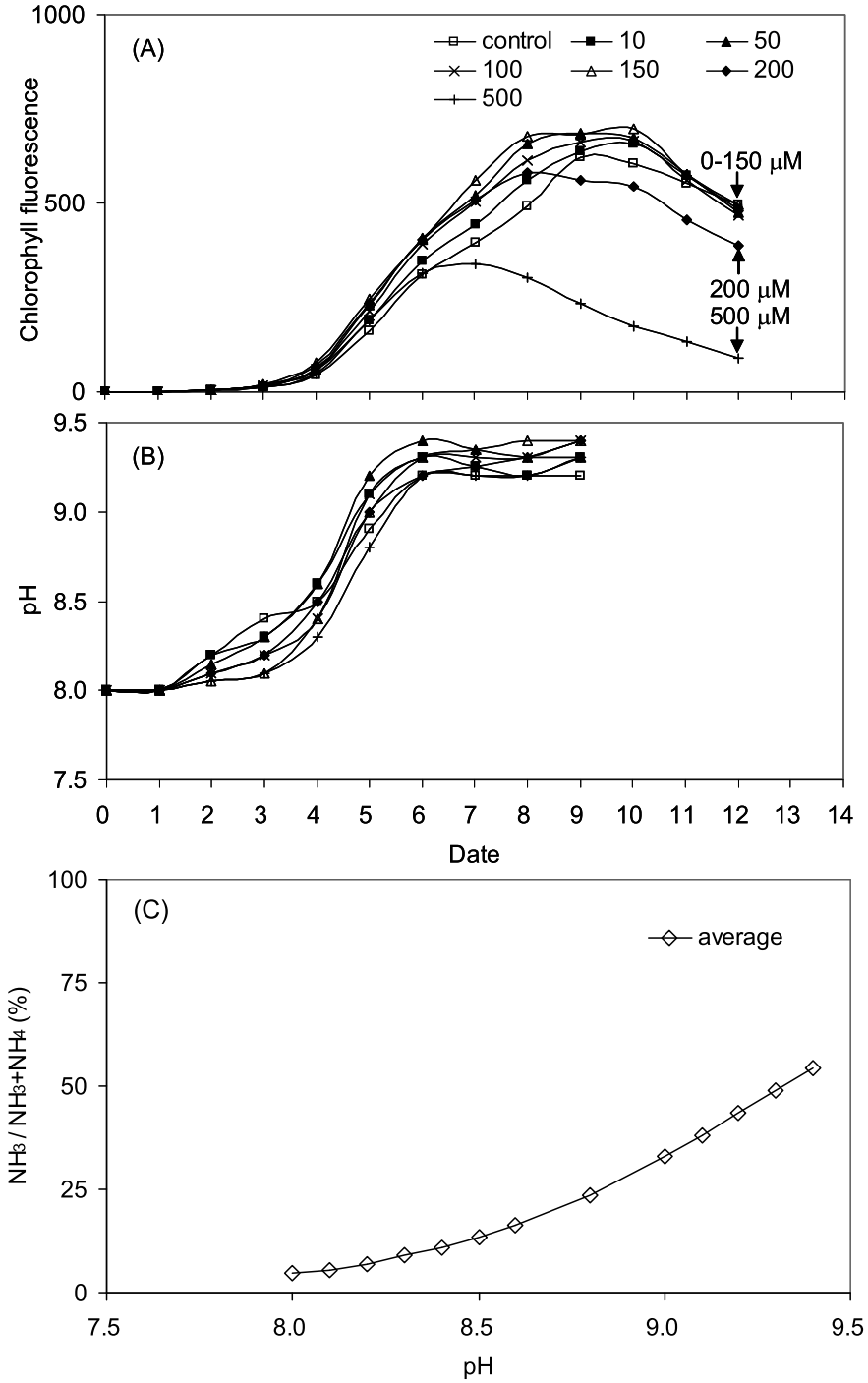

Fig. 4. Influence of pH on toxicity of ammonium. (A) indicates the variation of chlorophyll fluorescence of *Skeletonema* sp. (Dokai Bay) and (B) indicates pH variation at various ammonium concentrations. Average relative percentage of NH_3 in NH_3 NH_4 due to increase of pH at various ammonium concentrations is shown in (C).

In this study, an acceleration of growth rates at lower ammonium concentrations was observed. The growth rate of *Chaetoceros* sp. at 50 μM was significantly higher ($p<0.05$) than that of the control treatment as well as other ammonium concentrations. However, there was no acceleration effect on growth rates of other indigenous and non-indigenous species at lower ammonium concentrations.

3.3 Effect of ammonium on maximum chlorophyll fluorescence

The maximum chlorophyll fluorescence of all species tested in a series of ammonium concentrations was shown in Fig. 3. Maximum chlorophyll fluorescence of all test species gradually decreased with the increase in ammonium concentration. However, the level of ammonium that adversely affected the maximum chlorophyll fluorescence varied from species to species.

In Fig. 3A, the maximum chlorophyll fluorescence of *Skeletonema* sp. isolated from Dokai Bay did not differ from that in the control treatment at low ammonium concentrations from 10 to 150 μM. That maximum level rapidly decreased when the ammonium concentration reached 200 μM. From 200 to 1,500 μM, the maximum chlorophyll fluorescence decreased gradually as a function of ammonium concentration, reaching a significantly lower level ($p<0.05$) compared to that in the control treatment. In the case of the clone from Harima Nada (Fig. 3B), the maximum chlorophyll fluorescence was significantly higher ($p<0.05$) than that in the control treatment at low ammonium concentrations from 10 to 200 μM. That maximum decreased when the ammonium reached over 500 μM. However, the maximum chlorophyll fluorescences at 1,250 and 1,500 μM were significantly lower ($p<0.05$) than those in the control treatment. As for *Chaetoceros* sp., its maximum chlorophyll fluorescences did not differ from those of the control treatment at low ammonium concentrations and then decreased when ammonium exceeded 200 μM (Fig. 3C). The *Chaetoceros* sp. maximum was significantly lower ($p<0.05$) than that of the control treatment when ammonium concentrations ranged between 500 and 1,500 μM. Similar to the other indigenous species, the adverse effect on the maximum chlorophyll fluorescence of *H. akashiwo* emerged from an ammonium concentration of 25 μM up to the highest concentration (Fig. 3D). The maximum chlorophyll fluorescence of this test species at 500 to 1,500 μM was significantly lower ($p<0.05$) than the one in the control-treatment maximum.

Although those two non-indigenous species, *C. antiqua* and *K. mikimotoi*, were unable to grow at ammonium concentrations of 200 and 150 μM, respectively, no adverse effect on their maximum chlorophyll fluorescence at lower ammonium concentrations was observed. In Figs. 3E and 3F, the maximum chlorophyll fluorescence of *C. antiqua* and *K. mikimotoi* varied over a small range and did not differ from the control-treatment maximum.

3.4 Variation of pH

During the growth of *Skeletonema* sp. isolated from Dokai Bay under various ammonium concentrations (Fig. 4A), the pH in the culture medium of all treatments increased slightly between days 1 and day 3, but then increased rapidly, reaching 9.0 on day 5. This phenomenon was also observed in the control treatment. On day 6, the pH of all treatments varied over 9.0 (Fig. 4B).

4. Discussion

4.1 Inhibition effect of high ammonium on phytoplankton growth

In this study, the presence of high ammonium caused significant effects on the growth of phytoplankton. At a high ammonium concentration, suppression of the growth rates and maximum chlorophyll fluorescence of all indigenous species or a total growth inhibition in the case of *C. antiqua* and *K. mikimotoi* were observed. Suppressions of the growth rates found in the present study also corresponded with those in previous studies (Admiraal, 1977; Abeliovich and Azov, 1976; Bates *et al.*, 1993; Källqvist and Svenson, 2003; Livingston *et al.*, 2002). Admiraal (1977) reported that ammonia at >500 μM reduced the growth of benthic diatoms. Källqvist and Svenson (2003) found that ammonium at 224

Table. 1. Summary of negative ammonium effects and ranges on several phytoplankton species

Species	Effect	NH_4^+ conc. (μM)	Remark	Sources
Thalassiosira pseudonana	reduced NO_3^- uptake	3	6-min incubation	Yin *et al.* (1998)
Scenedesmus obliquus, Phaeodactylum tricornutum				
Dunaliella tertidecta	reduced photosynthesis	>2,000	90-min incubation	Azov and Goldman (1982)
D. tertidecta				
	reduced photosynthesis	15	15-min incubation	Turpin (1983)
Alexandrium tamarense	increased growth	6-50	−	Leong *et al.* (2004)
	reduced growth	100		
Navicular arenaria, N. c.f. dissipata				
N. dubiformis,Amphiprora c.f. paludosa	reduced growth	>500	−	Admiraal (1977)
Stauroneis constricta, Gyrosigma spencerii, N. sigma				
		95-166	(pH 7-8)	
Nephroselmis pyriformis	inhibited growth rate	21-70.7	(pH>8)	Källqvist and Svenson (2003)
			24-h exposure time	
Nitzschia pungens	reduced cell yield	>110		
	prevented growth	>880	−	Bates *et al.* (1993)
Skeletonema costatum	no effect	>880		
Pseudonitxchia pungens f. multiseries Hasle	reduced growth rate	>20		
			−	Hillbrand and Sommer (1996)
S. costatum	increased growth	4.2	−	
	reduced chlorophyll *a*	32.8		Livingston *et al.* (2002)

μM reduced the growth rate of *Nephroselmis pyriformis* (Chlorophyta) within 24h. LIVINGSTON *et al.* (2002) showed that the chlorophyll *a* concentration was significantly decreased at ammonium concentrations from 0.11 to 0.24 mg l^{-1} and even much lower at over 0.46 mg l^{-1}. HILLEBRAND and SOMMER (1996) reported that ammonium inhibited or at least slowed down the nitrate uptake at a high ammonium/nitrate ratio, leading to a lower growth rate of *Pseudo-nitzschia pungen* f. *multiseries* Hasle. A summary of the negative ammonium effects and ranges on several phytoplankton species was shown in Table 1. Moreover, the concentration of ammonium that caused the inhibition of growth rates in test species was about the same across several species. BATES *et al.* (1993) reported little suppression of the *Skeletonema costatum* cell yield at 880 μM (the highest test concentration). FUKAZAWA (1980) found that the growth rate of *Gymnodinium* sp. decreased at 50 μM of NH_3. NAKAMURA and WATANABE (1983) indicated that ammonium concentrations higher than 150 μM caused a severe inhibition of *C. antiqua* growth, and that the species could not survive at 300 μM.

Fig. 4C showed the relative percentage of NH_3 in NH_3 plus NH_4^+, which was calculated by the equation presented in KÖRNER *et al.* (2001). In the present study, a lower yield of maximum chlorophyll fluorescence at higher ammonium concentrations with almost a similar growth rate was observed. The onset of premature senescence (Fig. 4A) seemed to be triggered by the increase in ammonia (NH_3) due to the pH increase initially from 8.0 to over 9.0 on day 6 (Fig. 4B). We considered that the lower yield of maximum chlorophyll fluorescence of phytoplankton test species at the higher ammonium concentration shown in Fig. 3 was due to the increase in ammonia concentration following the elevation of pH. This suggested that the inhibition effect on phytoplankton growth occurred when the proportion of NH_3 increased due to pH elevation. KÄLLQVIST and SVENSON (2003) also suggested that the toxicity of total ammonia (the sum of NH_4^+ and NH_3) strongly depended on pH and NH_3 is the main toxic form of total ammonia.

4.2 Tolerances among phytoplankton species

The tolerances of test phytoplankton species to ammonium varied with each phytoplankton species showing different growth rates under various ammonium concentrations. Comparing the growth rates of all test species, we found that *Skeletonema* sp. isolated from Dokai Bay showed similar growth rates even at 1,500 μM, whereas the growth rates of *Skeletonema* sp. isolated from Harima Nada, *Chaetoceros* sp. and *H. akashiwo*, gradually diminished with the increase in ammonium concentrations over 500 μM. Moreover, *C. antiqua* and *K. mikimotoi* were unable to grow with ammonium concentrations of 200 and 150 μM, respectively. An unchanged growth rate despite high ammonium concentrations indicated that *Skeletonema* sp. isolated from Dokai Bay was more tolerant than *Chaetoceros* sp. and *H. akashiwo*. Moreover, the lethal effect on *C. antiqua* and *K. mikimotoi* also suggested that, compared to indigenous species, non-indigenous species in Dokai Bay were less tolerant. Superior tolerance for the ammonium of diatoms, particularly *Skeletonema* sp., than that shown by other phytoplankton species was reported in several previous studies. FUKAZAWA (1980) found that 50 μM of NH_3 failed to inhibit the growth rate of *S. costatum,* whereas those of *H. akashiwo* and *Gymnodinium* sp. were inhibited at that concentration. BATES *et al.* (1993) concluded that *Skeletonema* was the species most tolerant of high ammonium. Levels higher than 110 μM were found to significantly reduce the photosynthetic rate of *Nitzschia pungens*, while having no effect on that of *S. costatum*. In addition, we could assume that *Skeletonema* sp. isolated from Dokai Bay was more tolerant of ammonium than *Skeletonema* sp. isolated from Harima Nada. The differences in ammonium tolerance among phytoplankton species isolated from different locations have also been reported in a previous study. HILLEBRAND and SOMMER (1996) discovered major differences in ammonium tolerances among *Pseudo-nitzschia* clones isolated from locations under a variety of original conditions.

Therefore, the result from this study simulated conditions of high ammonium

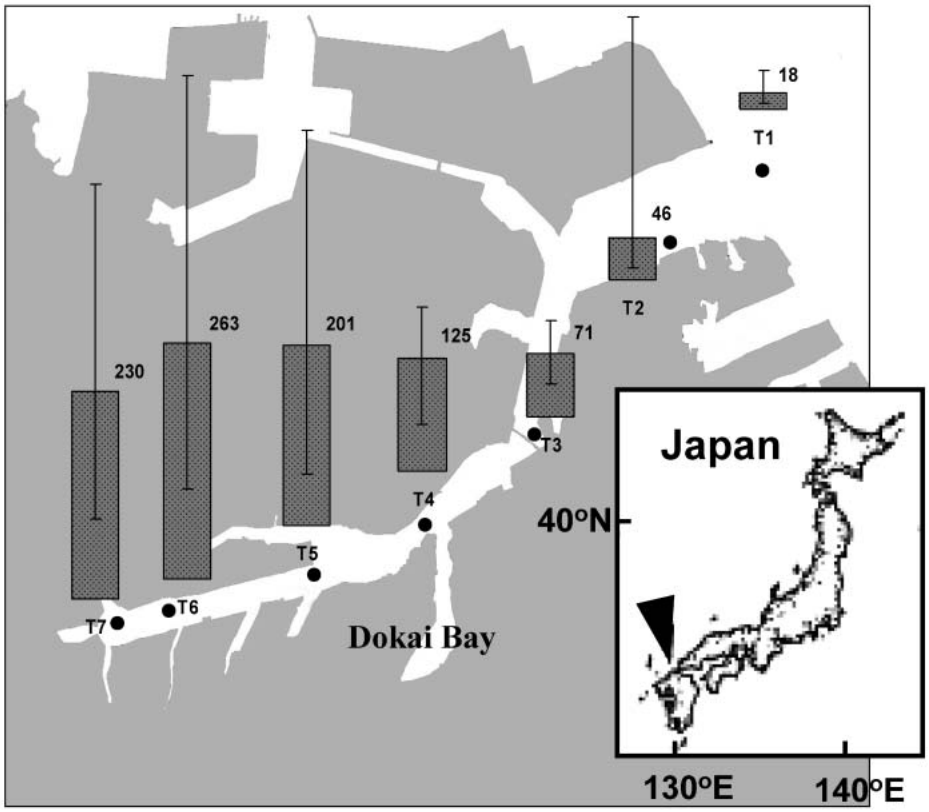

Fig. 5. Map of Dokai Bay, in northern Kyushu Island, Japan. Average ammonium concentration (μM) of surface water since 1996–1997 from Station T1 (bay mouth) to T7 (inner part). Bar graphs represent maximum and minimum ammonium concentrations and black dot (●) indicates sampling stations. Vertical bars show range (minimum to maximum) of ammonium concentration at each sampling station.

concentrations and sufficient nutrients in Dokai Bay, demonstrated that the tolerance of ammonium was also one factor that determined the species composition of the phytoplankton assemblage in that bay.

4.3 Field implications

It is widely acknowledged that phytoplankton growth in natural environments is controlled by three key factors, i.e. physical (e.g. water circulation, light intensity, water temperature, etc.), chemical (e.g. nutrients, trace elements, etc.) and biological (e.g. taxonomic variations, phytoplankton origins, zooplankton grazing, etc.). Under suitable conditions, phytoplankton grow actively and often produce blooms. TADA *et al.* (2001 and 2004) concluded that the nutrient concentration in Dokai Bay was sufficient for phytoplankton growth during the entire year, and that phytoplankton blooms were controlled by strong water circulation. They suggested that only phytoplankton species having a higher growth rate than the flash-out speed of the surface water mass could be dominant and would subsequently produce a bloom. However, the results from this study indicated that high ammonium had the potential to function as a selective factor in determining the species composition and the dominant species of phytoplankton blooms in Dokai Bay.

YAMADA and KAJIWARA (2004) reported that the frequency of *Skeletonema* red tides was highest in Dokai Bay. They reported 51 red tides occurring during the observation period, 36 of which were attributed to *Skeletonema*

species, which 20 times were sole and 16 were a mixture with other diatoms or flagellates; however, a few *Chaetoceros* sp. and *H. akashiwo* blooms were also observed. Furthermore, there were no reports on blooms of *C. antiqua* or *K. mikimotoi*. Data from an intensive monitoring program from 1996 to 1997 (SUKSOMJIT *et al.*, 2005) indicated that a high ammonium concentration was always found in this bay throughout the year. The average ammonium concentrations of surface water between 1996 and 1997 plotted from the bay mouth (Station T1) to the inner part (Station T7) were shown in Fig. 5. In this bay, phytoplankton blooms were usually observed at the inner part, with phytoplankton biomass decreasing gradually from there to the bay mouth (YANAGI *et al.*, 1997). Moreover, at Stations T5, T6 and T7, important areas for bloom development (YANAGI *et al.*, 1997), average ammonium concentrations were at their highest, often exceeding 200 μM. As a rule, concentrations decreased gradually from the inner part to the bay mouth (Station T1). Thus, blooms of *Skeletonema* spp., *Chaetoceros* sp. and *H. akashiwo* but none of *C. antiqua* or *K. mikimotoi* were compatible with each species tolerance of the existing level of ammonium concentration. Although a level of 200 μM had a lethal effect on *C. antiqua* and *K. mikimotoi* growth, no inhibitory effect on *Skeletonema* sp., *Chaetoceros* sp. or *H. akashiwo* could be observed at the existing level of ammonium concentration. This would explain why these highly tolerant species (i.e. *Skeletonema* sp., *Chaetoceros* sp. and *H. akashiwo*) could be readily observed in this area, whereas *C. antiqua* and *K. mikimotoi* could not. Moreover, superior tolerance for the ammonium of the *Skeletonema* sp. isolated from Dokai Bay should be observed more frequently than species with lower tolerance. This finding coincided with the observations of *Skeletonema* sp. all year long (TADA *et al.*, 2004). This influence of high ammonium on the determination of species composition was reported in several previous studies. ADMIRAAL (1977), for example, revealed that the distribution of benthic diatoms in an estuary would be affected by the occurrence of ammonia, and that *Navicula salinarum*, with its extreme tolerance of high ammonium, was the dominant species in that polluted mudflat. LIVINGSTON *et al.* (2002) found that phytoplankton abundance and species richness were significantly lower in the Amelia River-Estuary, which received wastewater from a nearby pulp mill. HÜRLIMANN and SCHANZ (1993) reported that the addition of 364 μM or more resulted in a decrease in the biomass and triggered drastic changes in species composition; after a 42-day enrichment period, diatoms (which are known to be tolerant of organic pollution) were found.

5. Conclusion

In summary, we have concluded that ammonium at high concentrations functions as an important factor in regulating phytoplankton growth and dominant phytoplankton species via the different ammonium tolerance levels of each species. TADA *et al.* (2001, 2004) had already shown that the growth rates of phytoplankton were an important factor in determining species composition because of the sudden advections of the surface water mass by a strong estuarine circulation without vertical mixing. However, we considered that the results of this current study provide additional evidence for why *Skeletonema* sp. was such a dominant species in Dokai Bay, producing blooms more frequently than *Chaetoceros* sp. or *H. akashiwo*. These results also provided convincing evidence why some flagellates, (i.e. *C. antiqua* and *K. mikimotoi*) were not observed in this bay.

Acknowledgements

We would like to thank Professor Dr. Machiko YAMADA of Fukuoka Women's University for her critical comments and encouragement during preparation of the manuscript. We also thank Dr. Hideki KAERIYAMA, Kagawa University for his suggestion in statistical analyses.

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Received: December 27, 2008
Accepted: July 13, 2009