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Novel blooms of the diatom *Asteroplanus karianus* deplete nutrients from Ariake Sea coastal waters

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ABSTRACT: In the Ariake Sea, Japan, the pennate diatom *Asteroplanus karianus* has formed massive blooms each winter since 2008. During the peak of the bloom, seaweeds of the genus *Pyropia*, which are cultivated to make nori products, are discolored due to nutrient deficiency. We investigated the nutrient dynamics associated with *A. karianus* blooms in the Ariake Sea and measured the uptake of nitrate and phosphate by an axenic strain of *A. karianus*. Dissolved inorganic nitrogen and dissolved inorganic phosphorus in the coastal waters were significantly lower in areas where *A. karianus* cells were proliferating ($r \leq -0.955$, $p < 0.01$), and these nutrients were severely depleted during the period of blooming. In the laboratory, the maximum uptake rate and half-saturation constant of *A. karianus* cultures were 27.3 fmol N cell^{-1} h^{-1} and 7.44 µmol N l^{-1}, respectively, for nitrate and 22.6 fmol P cell^{-1} h^{-1} and 3.61 µmol P l^{-1}, respectively, for phosphate. This study demonstrates that *A. karianus* blooms significantly deplete nutrients in the water column. The winter blooms presumably deplete dissolved inorganic nitrogen and dissolved inorganic phosphorus from the water column and may indirectly cause *Pyropia* nutrient deficiency.

KEY WORDS: Ariake Sea · *Asteroplanus karianus* · Nutrient depletion · *Pyropia* · Winter bloom

INTRODUCTION

Seaweeds of the genus *Pyropia* (formerly *Porphyra*), such as *Pyropia yezoensis* (Ueda) Hwang et Choi, have been consumed in Japan since the 700s (Nisizawa et al. 1987). Known as nori (Fig. 1), they have been cultivated around Japanese coasts since the 1600s (Shimbo 2000). Nori products are utilized in various Japanese foods, including sushi and onigiri, and are exported widely to various countries. Nori is one of the top fisheries products in Japan (Nisizawa et al. 1987). Cultivation of *Pyropia* in Japan is therefore a significant form of aquaculture, and its consumption is part of a traditional seafood culture. The major production regions in Japan are the Ariake Sea (see Fig. 2) (Shimazu 2003, Watanabe et al. 2004) and the Seto Inland Sea (Nishikawa et al. 2007, Oyama et al. 2008); however, cultivation of *Pyropia* is at risk due to blooms of a marine diatom species (Imai et al. 2006).

Several centric diatoms, such as *Coscinodiscus wailesii* Gran (Nishikawa et al. 2010), *Eucampia zodiacus* Ehrenberg (Uno & Sasaki 1989, Nishikawa & Yamaguchi 2006), and *Rhizosolenia imbricata* Brightwell (Sasaki & Kito 2003), form blooms during the winter season. They are believed to cause nutrient deficiency in cultivated *Pyropia* through competitive utilization of nutrients (Uno & Sasaki 1989, Nishikawa et al. 2007). Nishikawa et al. (2007) reported that these diatom blooms have sometimes caused serious underdevelopment of *Pyropia*, causing economic losses on the order of 1

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billion yen (over US$10 million) in the Seto Inland Sea.

In February 2008, the pennate diatom *Asteroplanus karianus* (Grunow) Gardner et Crawford (formerly *Asterionella kariana* or *Asterionellopsis kariana*) (Crawford & Gardner 1997) suddenly formed massive blooms of 10^4 cells ml^{-1} in the *Pyropia* cultivation farms in the Ariake Sea (Matsubara et al. 2014). This region produces approximately 40% of Japanese nori products (Shimazu 2003). Since that year, winter blooms of *A. karianus* have occurred annually in the Ariake Sea. In December 2011, *A. karianus* appeared in the cold waters and proliferated to yield 10^4 cells ml^{-1} by early January 2012 (Matsubara et al. 2014). During the winter blooms, large amounts of cultivated *Pyropia* were discolored, probably due to nutrient deficiency (Fig. 1A), resulting in a dramatic decrease in nori production and/or quality (Fig. 1B). Indeed, a trial calculation by Saga Prefectural Ariake Fisheries Research and Development Center showed that the unit price and total fishery output of nori in early 2012 were putatively reduced to approximately two-thirds of the price and yield in January 2008 — a time at which blooms of *A. karianus* had yet to develop.

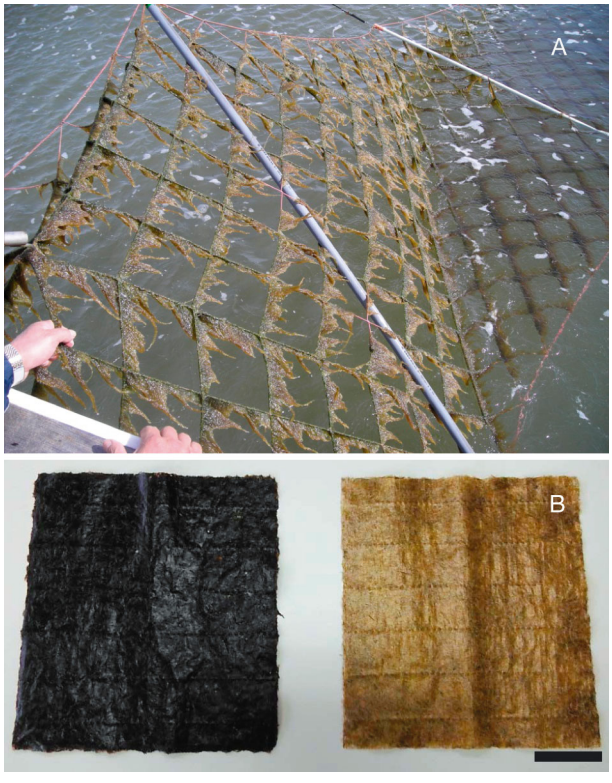

Fig. 1. (A) Cultivated seaweeds of *Pyropia*, which were discolored during the bloom development of a marine diatom *Asteroplanus karianus* in the Ariake Sea, Japan. (B) Japanese nori products made from discolored (right) and typical (left) *Pyropia*. The scale bar indicates 50 mm

The pennate diatom *A. karianus* (*A. kariana*) is reported to be distributed in the coastal regions of Australia, New Zealand (Harper et al. 2012), and the UK (Sato et al. 2008). In the Ariake Sea, *A. karianus* has been observed putatively since at least the 1980s, but did not form massive blooms (Matsubara et al. 2011) or cause economic damage until 2008. To date, centric diatoms, such as *E. zodiacus* (Sasaki & Kito 2003) and *R. imbricata* (Sasaki & Kito 2003, Shimazu 2003), have been considered to be the causative agents of the underdevelopment of cultivated *Pyropia* in Japan. The pennate diatom *A. karianus*, like most diatom species, was therefore considered to be an 'inconspicuous' primary producer rather than a 'nuisance'.

As the discoloration phenomenon of nori is thought to be caused by nutrient depletion in the environment, it is essential to understand the relationship between *A. karianus* blooms and depletion of nutrients. Recent research by Matsubara et al. (2014) reported the distribution and seasonal variation of *A. karianus* in the Ariake Sea; however, it is also important to clarify the nutrient dynamics during the blooming of *A. karianus* and to understand the nutrient uptake kinetics of this species.

This study investigated the dynamics of dissolved inorganic nutrients associated with the bloom development of *A. karianus* in coastal waters of the Ariake Sea during winter 2011 to 2012. The data were compared with data from 2006 to 2007, when *A. karianus* infrequently appeared. We established an axenic strain of *A. karianus* isolated from the Ariake Sea and clarified its uptake kinetics regarding nitrate and phosphate. The results obtained from the field and our laboratory work will help clarify the effects of *A. karianus* blooms on the depletion of nutrients in this region and improve our understanding of nutrient deficiency in cultivated *Pyropia*.

MATERIALS AND METHODS

Field investigation

The Ariake Sea is approximately 90 km long, 20 km wide, and 20 m deep, on average, and has a total area of almost 1700 km^2 (Shimazu 2003). Sampling was conducted at a station (33° 4.72′ N, 130° 10.89′ E; depth 6 m) in the Ariake Sea (Fig. 2) from 6 December 2006 to 25 January 2007, and also

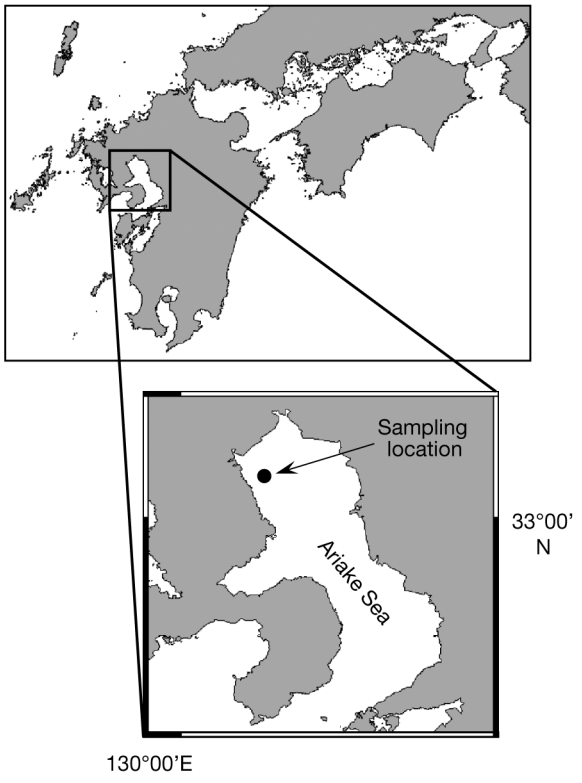

Fig. 2. Sampling location in the Ariake Sea, Japan

from 2 December 2011 to 30 January 2012. Throughout the former period, *Asteroplanus karianus* cells were observed only once (on 18 January; see 'Results'). Seawater from the surface and from the layer 1 m above the bottom was collected using a RIGO-B transparent water bottle sampler (RIGO) and transferred into a 500 ml polypropylene bottle (SANPLATEC). The water temperature of each sample was immediately measured using a mercury thermometer (SANSYO). These samples were transported to the laboratory. The salinity of each sample was measured using a digital salinometer (E-202; Tsurumi-seiki).

An aliquot of each sample was filtered through a pre-combusted glass fiber filter (GB-100R; ADVANTEC) and the filtrates were stored at −30°C prior to nutrient analysis. Dissolved inorganic nitrogen (DIN: sum of NO_3^-, NO_2^-, and NH_4^+), dissolved inorganic phosphate (DIP: PO_4^{3-}), and silicate (SiO_2) concentrations in the filtrate were analyzed with an autoanalyzer (QuAAtro 2-HR, BL-TEC), following a standard method (Strickland & Parsons 1972). A 10 to 500 µl sample of the water from the surface was dropped on a plankton-counter glass plate (MPC-200 and/or S617, Matsunami). The microalgal cells, which included *A. karianus* and were ≥2 µm in size, were directly counted under an optical microscope (E600, Nikon). The cell shape and size (e.g. length: 20 to 49 µm; width: <3.5 µm) of *A. karianus* cells in the samples were similar to those originally described by Crawford & Gardner (1997). The net exponential growth rate for *A. karianus* in the coastal waters was determined using a least-squares method (Guillard 1979). The proportion of *A. karianus* in the planktonic microalgae assemblage was determined by dividing the number of *A. karianus* cells by the total number of microalgal cells. The numbers and variability of *A. karianus* from 2011 to 2012 have been reported by Matsubara et al. (2014), and these data were used in the present study.

Using the data set obtained from 19 December 2011 to 8 January 2012, during the blooming of *A. karianus* (see 'Results'), we conducted Pearson correlation and regression analysis to estimate the relationships among nutrient concentrations, the number of *A. karianus* cells, and the abundance of other diatoms. DIN, DIP, and SiO_2 concentrations and the abundance of other diatoms obtained from December 2011 to January 2012 (n = 15) were compared with those obtained in 2006 to 2007 (n = 8), when *A. karianus* infrequently appeared. The significance of the comparison was analyzed at $\alpha = 0.05$ using Welch's *t*-test for the data set, because heteroscedasticity ($p < 0.05$) of the data set was confirmed using Levene's test (Brown & Forsythe 1974).

Uptake kinetics for nutrients

A clonal strain of *A. karianus* (Ak5) was isolated from the Ariake Sea in February 2008 and maintained in SWM-3 medium by Dr. Mineo Yamaguchi (National Research Institute of Fisheries and Environment of Inland Sea), who provided the strain for this research. We cultivated *A. karianus* Ak5 in SWM-3 medium that was maintained at pH 7.8 and contained 2 mmol l^{-1} $NaNO_3$, 65 µmol l^{-1} NaH_2PO_4, 0.2 mmol l^{-1} Na_2SiO_3, 30 µmol l^{-1} Na_2EDTA, 2 µmol l^{-1} Fe-EDTA, 2 nmol l^{-1} Na_2SeO_3, 1% (v v^{-1}) PI metals, 0.2% (v v^{-1}) S3 vitamins, and 4.1 mmol l^{-1} Tris (Imai et al. 1996). The axenic strain was established by washing with the antibiotic reagent AM9 (Provasoli & Shiraishi 1959). The absence of bacteria in the cultures was confirmed by visualizing DAPI (4′,6-diamidino-2-phenylindole)-stained microflora under an epifluorescence microscope (Porter & Feig 1980). The axenic cultures were maintained in

50 ml glass Erlenmeyer flasks containing 25 ml of modified SWM-3 medium at 15°C under ~110 µmol photons m^{-2} s^{-1} of cool-white fluorescent illumination on a 12:12 h light:dark cycle (light period 06:00 to 18:00 h).

We prepared nitrogen (N)-limited or phosphorus (P)-limited SWM-3 medium, as well as non-limited SWM-3 medium, in 3 l glass flasks (VIDREX). For the N-limited medium, nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations were adjusted to 2.5 µmol l^{-1} and 65 µmol l^{-1}, respectively. For the P-limited medium, nitrate and phosphate concentrations were adjusted to 2000 µmol l^{-1} and 0.65 µmol l^{-1}, respectively. The salinity of the medium was adjusted by dilution with distilled water to 25, which was optimal for growth of the *A. karianus* strain Ak5 (M. Yamaguchi pers. comm.).

Using these media, we conducted culture experiments to measure the nutrient uptake of the Ak5 strain. The stock culture was inoculated into N-limited, P-limited, or non-limited SWM-3 medium, and these cultures were incubated under 110 µmol photons m^{-2} s^{-1} of cool-white fluorescent illumination on a 12:12 h light:dark cycle (light period 06:00 to 18:00 h). As winter blooms of *A. karianus* are found in the temperature range of 4.9 to 15.4°C (Matsubara et al. 2014), we carried out the culture experiments at a temperature (10.8°C) similar to the median (10.2°C) of the range. A 5 ml sample of the cultures was removed and placed in a polypropylene-capped glass test tube (12 mm × 17.5 mm; Eiken) at 3 d intervals. The chlorophyll *a* fluorescence in the suspension samples was measured with a Turner fluorometer (Model 10AU, Turner Design). The cultures were considered to be N-limited or P-limited when the chlorophyll *a* fluorescence of the cultures dropped below that of the non-limited culture. The N-limited and P-limited cultures, in which cell sizes of *A. karianus* (length: ~8 to 13 µm; width: ~3 to 5 µm) were smaller than those originally described for the species (Crawford & Gardner 1997), were used as samples in subsequent experiments.

After the incubation period was complete, 150 g samples from the cultures were transferred into autoclaved 300 ml Erlenmeyer flasks, in duplicate. The cell densities of N-limited (1.67×10^5 cells ml^{-1}) and P-limited (4.19×10^4 cells ml^{-1}) samples were determined as described above. Nitrate and phosphate were added to the N-limited and P-limited cultures at final concentrations of 0, 5, 10, 20, 40, 60, and 100 µmol l^{-1} for nitrate and 0, 0.5, 1, 2, 4, and 6 µmol l^{-1} for phosphate.

The batch cultures were immediately incubated under the same conditions for 8 d as described above. During the incubation, a portion of approximately 30 ml of each cell suspension was sampled hourly and immediately filtered through a pre-combusted glass fiber filter (GF/F; Whatman). The filtrates were maintained frozen at −30°C for subsequent nutrient analysis. Nitrate and phosphate concentrations in the filtrates were sampled during incubation at 0, 60, 120, and 180 min and were analyzed in duplicate by using an AutoAnalyzer II (BL-TEC), according to the method of Strickland & Parsons (1972).

The nutrient concentrations and incubation times were plotted and the significance of their correlation was tested at $\alpha = 0.05$. Using a least-squares method, the constant time-course of nutrient concentration was determined as an uptake rate (ρ; mol $cell^{-1}$ h^{-1}) at an initial nutrient concentration (the concentration at 0 time). The ρ values that fell outside the 95% confidence interval were excluded from subsequent analysis. The significant ρ values within the 95% confidence interval and the initial nutrient concentrations were plotted and fitted to a Michaelis-Menten-like equation (Goldman & Glibert 1983):

$$\rho = \rho_{max} \frac{S}{S + K_s} \quad (1)$$

where ρ_{max} is the unattainable maximum uptake rate at infinite S, S is the ambient nutrient concentration, and K_s is the constant when $\rho = 0.5\rho_{max}$. The equation was fitted by 6 or 7 repeated calculations. The significance of the multiple correlation coefficient (R) between the observed and theoretical values was tested at $\alpha = 0.05$ by using the F-based method. The upper and lower 95% confidence intervals for the regression equations were also determined by using the statistical software OriginPro 8.5.0J (OriginLab).

RESULTS

Field investigation

Within the bloom development period of 26 December 2011 to 8 January 2012, *Asteroplanus karianus* at the sampling station in the Ariake Sea appeared when the temperature was 7.6 to 9.8°C and salinity was 28.5 to 30.0 (Fig. 3A,B), and grew exponentially, at a maximum growth rate of 0.684 divisions d^{-1}, from 27 December 2011 to 3 January 2012 (Fig. 3A,B). The dominant algae were diatoms, and flagellates were rarely found. Among the planktonic diatoms, *A. karianus* was the dominant species during the bloom development period, accounting for

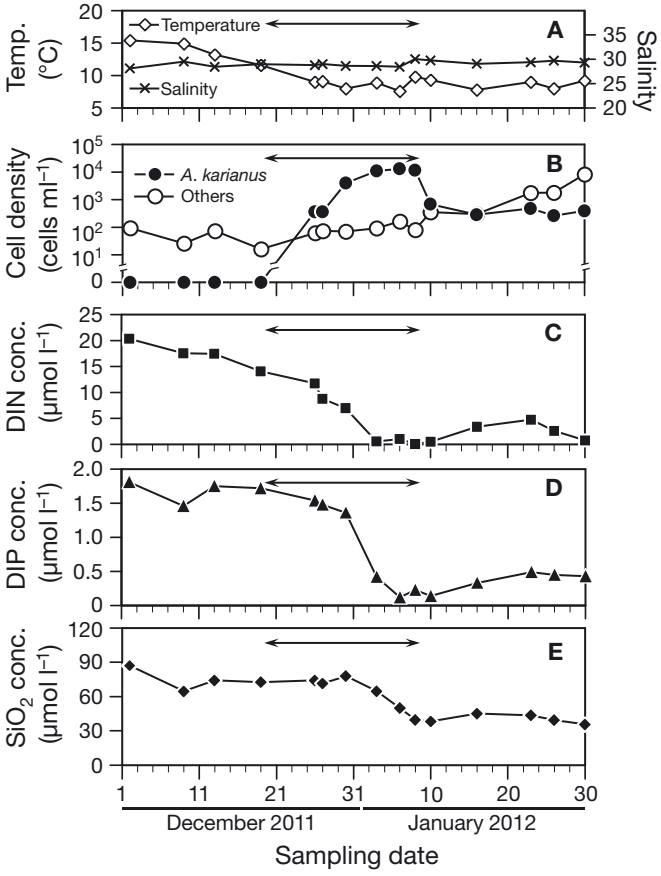

Fig. 3. Changes in (A) water temperature and salinity, (B) the abundance of *Asteroplanus karianus* and other diatoms, and the concentrations of (C) dissolved inorganic nitrogen (DIN: sum of NO_3^-, NO_2^-, and NH_4^+), (D) dissolved inorganic phosphorus (DIP: PO_4^{3-}), and (E) silicate (SiO_2) in surface waters at the sampling station in the Ariake Sea. Arrows indicate the period of bloom development of *A. karianus*. The cell numbers of *A. karianus* reported in Matsubara et al. (2014) were used

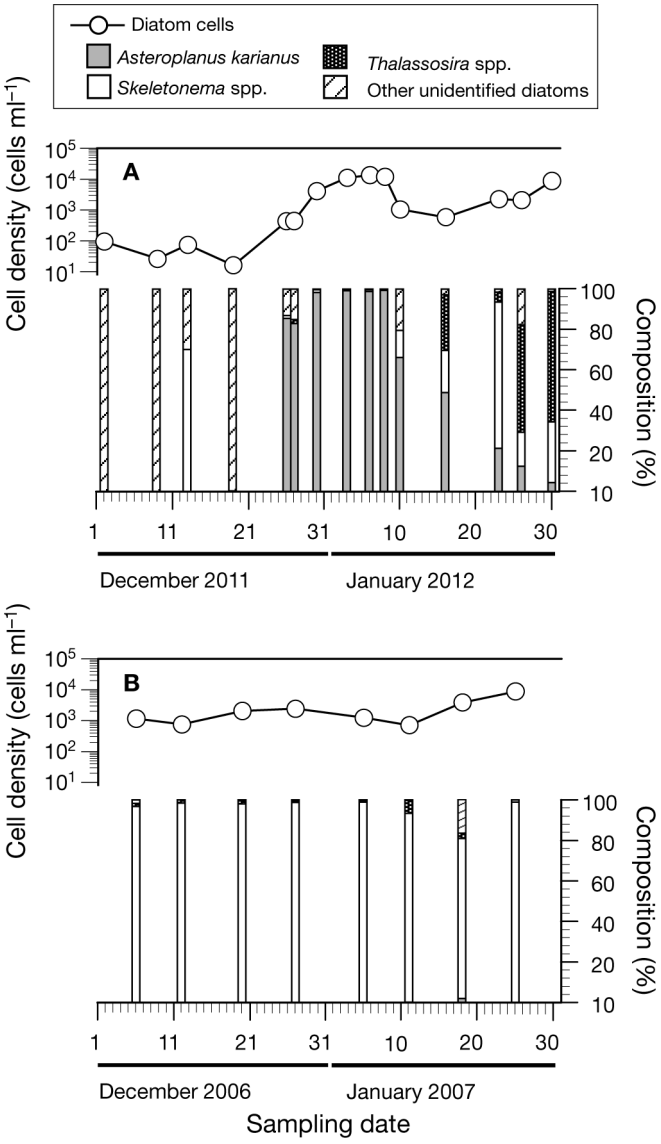

Fig. 4. Variations in the abundance and species composition of diatoms observed from (A) December 2011 to January 2012, compared with those obtained in (B) 2006 to 2007

83.1 to 93% of the total cells of the diatom assemblage, which also included, *Skeletonema* spp. and *Thalassosira* spp. (Fig. 4A). DIN and DIP concentrations in the surface waters during the bloom development period decreased from 11.7 to 0.50 µmol l^{-1} and from 1.54 to 0.14 µmol l^{-1}, respectively (Fig. 3C,D). The DIN concentration, in particular, was at the starvation level (<0.5 µmol l^{-1}) at the time that *A. karianus* was forming massive blooms, its population at ≥10^4 cells ml^{-1}, from 3 to 8 January (Fig. 3C). Meanwhile, the SiO_2 concentration exceeded 30 µmol l^{-1} (Fig. 3E). The nutrient concentrations in the surface water and the bottom waters were equivalent ($r \geq 0.982$, $p < 0.001$, $n = 12$). The DIN:DIP ratios in the surface waters, <1.0 to 8.6, were less than the Redfield ratio of 16 throughout the period of the bloom development.

The results of the Pearson correlation analysis showed significant negative correlations ($r \leq -0.816$, $p < 0.05$, $n = 7$) between *A. karianus* cells and each nutrient concentration (Fig. 5). There was no significant correlation ($p > 0.05$) between the populations of other diatoms and the DIN concentration (data not shown). Slopes of the regression between *A. karianus* cells and nutrients were 0.917 pmol N cell^{-1} for DIN and 0.115 pmol P cell^{-1} for DIP (Fig. 5).

The DIN and DIP concentrations from December 2011 to January 2012 were <0.5 to 20.4 (average 7.36 ± 7.15) µmol N l^{-1} and 0.12 to 1.81 (average 0.92 ± 0.669) µmol P l^{-1}, respectively (Fig. 6). Com-

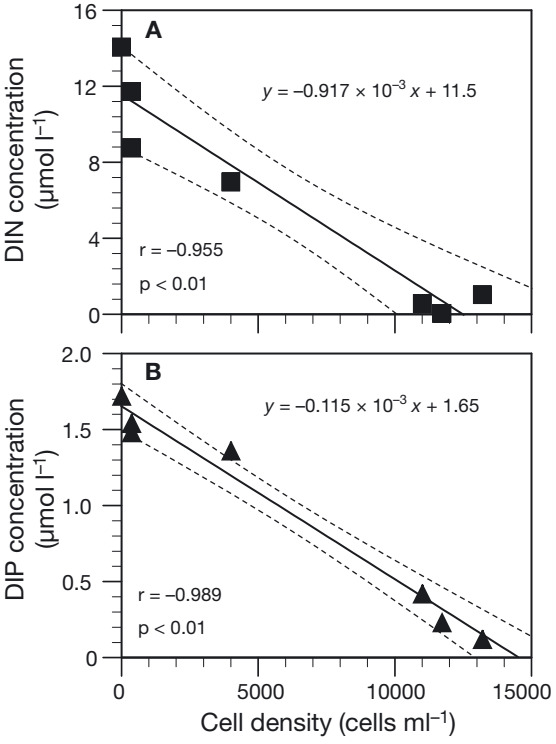

Fig. 5. Correlations between *Asteroplanus karianus* cell density and (A) dissolved inorganic nitrogen (DIN: sum of NO_3^-, NO_2^-, and NH_4^+) and (B) dissolved inorganic phosphorus (DIP: PO_4^{3-}). The dashed lines indicate the 95% confidence interval for the regression lines

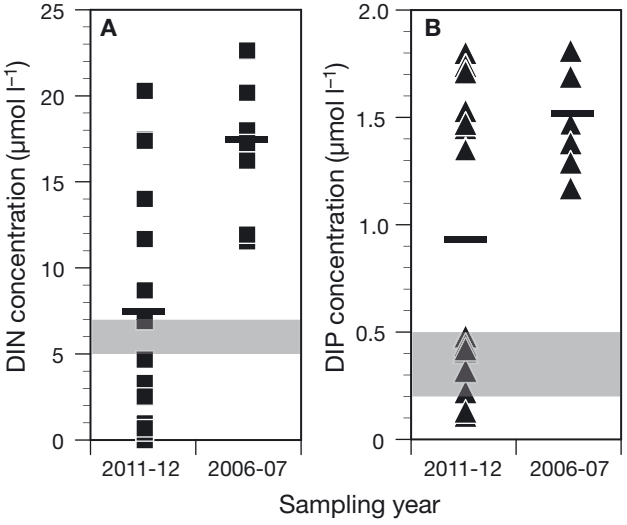

Fig. 6. Comparison of (A) dissolved inorganic nitrogen (DIN: sum of NO_3^-, NO_2^-, and NH_4^+) and (B) dissolved inorganic phosphorus (DIP: PO_4^{3-}) concentrations obtained from December 2011 to January 2012 (n = 15), with those obtained in 2006 to 2007 (n = 8). Bars indicate average values. The ranges of the lower limits of DIN and DIP for cultivation of the seaweed *Pyropia*, according to Japanese quality standards for fishery waters, are shown as shaded areas

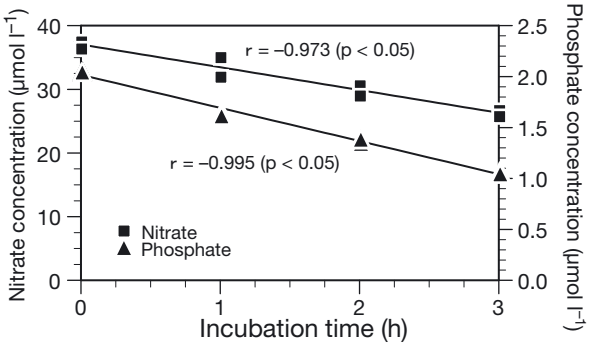

Fig. 7. Decrease in nitrate and phosphate concentrations in the N-limited or P-limited axenic cultures of *Asteroplanus karianus* strain Ak5 as the incubation period progressed. Two symbols indicate duplicate measurements

pared with the DIN and DIP concentrations from the same months in 2006 to 2007, the concentrations in 2011 to 2012 were significantly lower ($p < 0.05$) (Fig. 6). The DIN:DIP ratios of <1.0 to 11.2 (average 6.74 ± 3.75) from 2011 to 2012 were also significantly lower ($p < 0.05$) than those (average 11.1 ± 1.34) from 2006 to 2007. The difference between the SiO_2 concentrations from 2011 to 2012 (average 58.6 ± 17.4 µmol l^{-1}) and 2006 to 2007 (average 61.5 ± 3.82 µmol l^{-1}) was not significant ($p > 0.05$).

During the period from December 2006 to January 2007, *A. karianus* cells were observed once, at 78 cells ml^{-1}, on 18 January, when water temperature and salinity were 9.4°C and 29.9, respectively (data not shown). The population of other diatoms, mainly *Skeletonema* spp. and *Thalassosira* spp., ranged from 0.756 to 8.81 × 10^3 cells ml^{-1} during the period from December to January (Fig. 4B). The former species was dominant, accounting for 79 to 99% of the total cells of the diatoms (Fig. 4B). There was no significant difference ($p > 0.05$) between the average cell densities of other diatoms during the period from December to January in 2011 to 2012 and 2006 to 2007 (data not shown).

Uptake kinetics for nutrients

Nitrate and phosphate concentrations in the N-limited or P-limited *A. karianus* cultures decreased linearly and significantly ($p < 0.05$) as the incubation period progressed (Fig. 7); there was no significant correlation in any plot (data not shown). The significant uptake rates (unit: fmol cell^{-1} h^{-1}) for nitrate (ρ_N) and phosphate (ρ_P) varied with the nitrate (S_N) and phosphate (S_P) concentrations (unit: µmol l^{-1}), respectively (Fig. 8). Their relationships respectively fitted Michaelis-Menten-like equations (2) and (3) as follows:

$$\frac{S_N}{S_N + 7.44} \quad (2)$$

$$\frac{S_P}{S_P + 3.61} \quad (3)$$

where R values were >0.879 ($p < 0.01$, $N \geq 9$) (Fig. 8). The upper and lower 95% confidence intervals of the parameters — maximal uptake rate (ρ_{max}) and half-saturation constant (K_s) — for both nutrients (Table 1) were close to each parameter value. Nutrient uptake rates of the *A. karianus* 'assemblage' forming a massive bloom (10^4 cells ml^{-1}) were calculated as 32.3 to 157 nmol NO_3^- l^{-1} h^{-1} and 6.09 to 49.0 nmol PO_4^{3-} l^{-1} h^{-1} when nitrate and phosphate concentrations reached 1.0 to 10 µmol NO_3^- l^{-1} and 0.1 to 1.0 µmol PO_4^{3-} l^{-1}, respectively.

DISCUSSION

In recent years, winter blooms of the pennate diatom species *Asteroplanus karianus* have occurred annually in the Ariake Sea. The present study provides the first evidence that the winter blooms cause significant depletion of nutrients — specifically, DIN and DIP — in coastal waters.

According to the Japanese quality standards for fishery waters, the lower limits of DIN and DIP for cultivation of the seaweed *Pyropia* are 5 to 7 µmol N l^{-1} and 0.2 to 0.5 µmol P l^{-1}, respectively. DIN and DIP concentrations in the water column of the Ariake Sea during the winter bloom development of *A. karianus* in 2011 to 2012 were below these lower limits. The observed ratios of DIN:DIP (<1 to 8.6) were lower

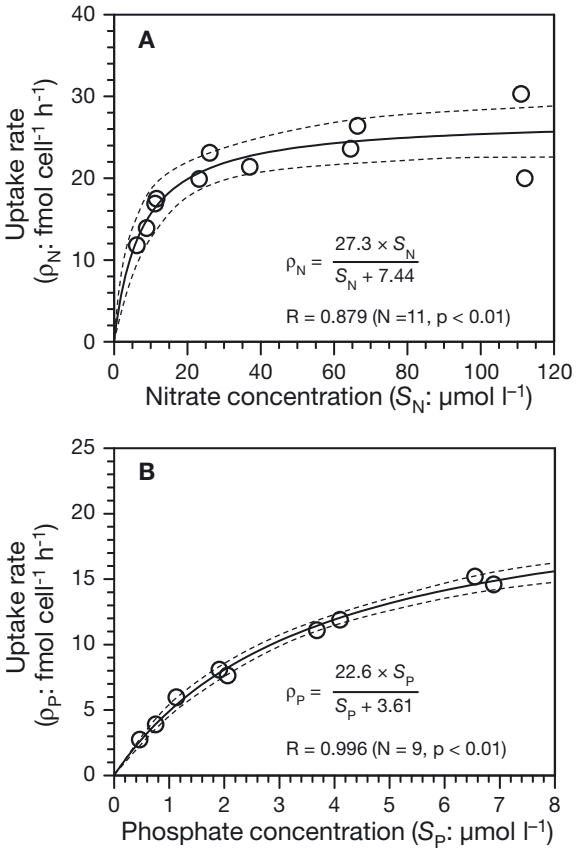

Fig. 8. (A) Nitrate (N) and (B) phosphate (P) uptake rates (ρ) of N-limited and P-limited axenic cultures of *Asteroplanus karianus* strain Ak5 as each single function of the ambient substrate concentration (*S*). The solid curve of the Michaelis-Menten equation was fitted to the observed value using the maximum-likelihood method. The dashed lines indicate the 95% confidence interval for the regression lines

Table 1. Uptake kinetic parameters — half-saturation constant (K_s), and maximum uptake rate (ρ_{max}) — for nitrate and phosphate in *Asteroplanus karianus* Ak5 and other marine diatoms. Standard errors are given for the *A. karianus* data

Substrate	Order Species	K_s (µmol l^{-1})	ρ_{max} (pmol cell^{-1} h^{-1})	Reference
Nitrate	Pennales			
	Asteroplanus karianus	7.44 ± 2.2	0.0273 ± 0.0017	Present study
	Centrales			
	Chaetoceros spp.	3.10	0.0240	Lomas & Glibert (2000)
	Coscinodiscus wailesii[a]	2.91 – 5.08	58.3 – 95.5	Nishikawa et al. (2010)
	Eucampia zodiacus[a]	2.59 – 2.92	0.777 – 0.916	Nishikawa et al. (2009)
	Skeletonema costatum	0.40	0.063	Lomas & Glibert (2000)
	Thalassiosira weissflogii	2.80	0.310	Lomas & Glibert (2000)
Phosphate	Pennales			
	Asteroplanus karianus	3.61 ± 0.40	0.0226 ± 0.0012	Present study
	Centrales			
	Coscinodiscus wailesii[a]	5.62 – 6.67	41.9 – 59.1	Nishikawa et al. (2010)
	Eucampia zodiacus[a]	1.83 – 4.85	0.224 – 0.550	Nishikawa et al. (2009)
	Skeletonema costatum	0.68	0.0384	Tarutani & Yamamoto (1994)

[a]These data were obtained at 9 and 20°C incubations

than the Redfield ratio, indicating that nitrogen was likely to be limited rather than phosphorus. In contrast, the DIN and DIP concentrations in 2006 to 2007, when *A. karianus* blooms did not appear, exceeded the lower limits of the quality standards throughout the winter (Fig. 4), indicating good growing conditions for *Pyropia*. These data suggest that winter blooms of *A. karianus* cause DIN- and DIP-depleted environments in the Ariake Sea, and therefore may indirectly cause the nutrient deficiency, particularly the nitrogen-deficient state, of cultivated *Pyropia*.

The present study is the first to clarify the uptake kinetics of nitrate and phosphate by a pennate diatom forming winter blooms (Table 1), although the patterns of centric diatoms have been well studied. The maximum uptake rates (ρ_{max}) of nitrogen and phosphate by a 'single cell' of *A. karianus* are considerably lower than those of the representative nuisance species *Coscinodiscus wailesii* (Nishikawa et al. 2010) and *Eucampia zodiacus* (Nishikawa et al. 2009) (Table 1). In contrast, the maximum cell yields of *A. karianus* in coastal waters often reach 10^4 cells ml^{-1} (Matsubara et al. 2014) and they easily exceed the ~10 cells ml^{-1} of *C. wailesii* (Ono et al. 2009), the ~10^2 cells ml^{-1} of *E. zodiacus* observed in the Seto Inland Sea (Nishikawa et al. 2007), as well as the ~10^3 cells ml^{-1} of *E. zodiacus* in Ariake Sea (Uno & Sasaki 1989, Ito et al. 2013).

The nutrient uptake ability of *A. karianus* when proliferating at 10^4 cells ml^{-1} appears to be close (a similar order of magnitude) to the nutrient uptake abilities of the *C. wailesii* and *E. zodiacus* assemblages. By substituting each uptake parameter of diatoms (Table 1) into Eq. (1), nutrient uptakes of *C. wailesii*, present with a population of 10 cells l^{-1} in cold waters (9°C), are calculated to be 149 to 452 nmol NO_3^- l^{-1} h^{-1} and 7.33 to 63.3 nmol PO_4^{3-} l^{-1} h^{-1} when nitrate and phosphate concentrations are 1.0 to 10 µmol NO_3^- l^{-1} and 0.1 to 1.0 µmol PO_4^{3-} l^{-1}, respectively. Under the same conditions, nutrient uptake rates of *E. zodiacus* growing at 10^2 cells ml^{-1} are 21.6 to 61.7 nmol NO_3^- l^{-1} h^{-1} and 1.26 to 8.62 nmol PO_4^{3-} l^{-1} h^{-1}. The uptake rates are 10-fold larger at 10^3 cells ml^{-1}. The results of our field and culture experiments suggest that *A. karianus*, as well as *C. wailesii* and *E. zodiacus*, has the ability to remove DIN and DIP from the water column when it forms blooms.

Differences among the nutrient uptake rates of *A. karianus* and other diatoms (Table 1) might be explained partially by the algal cell sizes, as indicated by Nakamura & Watanabe (1983). For example, *A. karianus* cells show lower uptake rates than *C. wailesii* and *E. zodiacus* cells (Table 1), as *A. karianus* has smaller cells than the other two. Furthermore, the cells of *A. karianus* cultures are smaller in size than those of the field populations. Hence, it is possible that the nutrient uptake by *A. karianus* cells is an underestimate. In general, small microalgae take up nutrients more efficiently than large microalgae, because small species have a relatively larger cell surface area per cell volume (Friebele et al. 1978, Grover 1989). Meanwhile the index of affinity to nitrogen for *A. karianus* — also called its half-saturation constant (K_s) — is higher than those of *C. wailesii* and *E. zodiacus* (Table 1) and apparently does not depend on cell size. We infer that the small diatom species *A. karianus* may not necessarily have an ecological advantage over relatively large diatoms such as *C. wailesii* and *E. zodiacus* in competition for nutrients. Also, we speculate that the nutrient uptake rates of diatoms probably differ between the diatom orders of Centrales and Pennales, and differ among the species in the latter order. Further studies are needed to compare nutrient uptake kinetics (e.g. minimum cell quota) among bloom-forming pennate diatoms such as toxic species of *Pseudo-nitzschia* (Bates 1998, Cochlan et al. 2008) to improve our understanding of the ecological strategy of competitive utilization of nutrients shown by *A. karianus*.

As shown by the present study and Matsubara et al. (2014), *A. karianus* in the Ariake Sea seem to grow preferentially in cold waters. To date, however, winter blooms of *A. karianus* can not be explained by the diatom's growth characteristics: growth rates of an Ariake Sea strain of *A. karianus* in laboratory experiments were 0.8 divisions d^{-1} at a temperature of 10°C, and clearly lower than growth rates (2 divisions d^{-1}) at 25°C (Matsubara et al. 2014). Therefore, this pennate diatom does not have a great potential to grow in cold waters. After comparing the dynamics of diatoms between 2006–2007 and 2011–2012, we suggest that *A. karianus* may be able to grow and to be predominant only when other diatoms such as *Skeletonema* spp. appear at low abundance of $<10^2$ cells ml^{-1} (Fig. 4).

To understand the DIN and DIP dynamics associated with the winter blooms of *A. karianus* in the Ariake Sea, it is essential to understand the sources of the nutrients. The muddy sediments of the Ariake Sea play an important role as a nutrient reservoir (Koriyama et al. 2013) and putatively supply nutrients to the water column when the water mass is mixed vertically during the winter season. Our results clearly showed that DIN and DIP were depleted from the waters during the time of the winter blooms

of *A. karianus*, suggesting that the supplied amounts of nutrients might have been insufficient to support a high abundance of both *A. karianus* and *Pyropia*.

Riverine waters are also important sources of nutrients; many rivers along the coast of the Ariake Sea discharge a large amount of nutrients into the sea during the warm season (Watanabe et al. 2004). In contrast, the amounts of nutrients during the winter season putatively decrease as the riverine water discharge diminishes (Watanabe et al. 2004); these riverine waters are stored in dams that control the discharge. We suggest that when *A. karianus* forms massive blooms during the winter season, their competitive consumption of DIN and DIP is accelerated. Therefore, it may be essential to manage the water reservoirs in upstream dams and the discharge of the reservoirs of the rivers along the coast of the Ariake Sea to sustain the growth of cultivated *Pyropia*.

CONCLUSIONS

Recent severe depletion of dissolved nutrients in the Ariake Sea caused by nuisance blooms of *A. karianus* may be responsible for the discoloration of economically important seaweeds of the genus *Pyropia*, which are cultivated to produce nori products. Therefore, careful monitoring of *A. karianus* is essential to forecast nutrient dynamics in the waters of the Ariake Sea. Future studies should clarify the growth kinetics, nutrient uptake kinetics, genetic features, and distribution and population structures of *A. karianus* to improve our understanding of bloom dynamics. Future work should also examine the bloom dynamics of *A. karianus* in relation to coexisting diatom species and to environmental factors, such as temperature, salinity, and light.

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