

Dynamics of picocyanobacteria in the Seto Inland Sea (Japan) during summer

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ABSTRACT: Temporal and vertical changes of picocyanobacteria (PC) were monitored daily together with environmental variables during the summer stratification period (16 July to 9 August 1992) in the Seto Inland Sea, Japan. Coupled with wind mixing, N- and P-nutrients were introduced to the surface layer twice (19 July and after 30 July). A PC bloom ($5 \times 10^4 \text{ ml}^{-1}$) occurred following the first mixing event, and a rapid decrease of this bloom seemed to be attributed to high abundance ($> 2 \times 10^3 \text{ ml}^{-1}$) of heterotrophic nanoflagellates (HNAN). In contrast, the second mixing event did not induce a PC bloom in spite of the high nutrient concentration, low abundance of HNAN ($< 2 \times 10^3 \text{ ml}^{-1}$) and optimum temperature for growth. Field experiments on PC growth in the $< 2 \mu\text{m}$ seawater fraction conducted during the second mixing period revealed that addition of metal buffer solution enhanced the PC growth rates significantly. Although trace metal concentrations were not monitored and clean techniques were not used in the experiments, we infer that growth stimulation was not an experimental artifact, but due to Fe-deficiency of the PC populations. This is based on results obtained in our previous studies concerning trace metal contamination. It is suggested that Fe-deficiency was responsible for the lack of a PC bloom during the second mixing period.

INTRODUCTION

The ubiquitous and abundant distribution of picocyanobacteria (PC) in the size range of 0.2 to $2 \mu\text{m}$ (Sieburth et al. 1978) is now well documented, and the ecological importance of PC as primary producers has been highlighted in open ocean ecosystems (Johnson & Sieburth 1979, Waterbury et al. 1979, reviewed by Waterbury et al. 1986 and Stockner 1988). Recent research has also revealed that *in situ* growth rates of PC are high ($> 1 \text{ d}^{-1}$) and at the same time, PC are grazed vigorously by heterotrophic nanoflagellates (HNAN) and ciliates (e.g. Landry et al. 1984, Campbell & Carpenter 1986a, b, Iturriaga & Mitchell 1986, Iturriaga & Marra 1988, Weisse 1988, Kudoh et al. 1990, Caron et al. 1991, Kuosa 1991, Sherr et al. 1991, Pick & Bérubé 1992). Furthermore, using a sensitive chemiluminescence method for NO_3^- analysis, Glover et al. (1988a, b) found that introduction of ca 10 nM NO_3^- (below the detection limit of the conventional colorimetric method) to surface waters in the stratified Sargasso Sea was followed by a *Synechococcus* spp.

(= typical PC) bloom. However, in spite of their pioneering efforts, there are still very few studies dealing with the relationship between the changes in chemical variables (nutrients and trace metals) and PC population dynamics. This is probably due to the high growth rate of PC even in 'nutrient-depleted' (i.e. below the detection limit of conventional methods) waters (e.g. Campbell & Carpenter 1986a, Iturriaga & Mitchell 1986, Iturriaga & Marra 1988) and/or to infrequent (weekly or monthly) monitoring of PC populations, which makes it difficult to relate changes of PC populations with chemical variables.

We have monitored the marine environment around the Ie-shima Islands (Seto Inland Sea, Japan) each summer since 1985 (Fig. 1). This area is usually stratified and nutrient concentrations are very low (e.g. $\leq 0.1 \mu\text{M}$ NO_3^-) above the thermocline (Nakamura et al. 1988). However, sporadic introduction of nutrients to the surface waters coupled with wind mixing causes diatom 'summer blooms' or red tides due to the raphidophycean flagellate *Chattonella antiqua* (Nakamura et al. 1989). Furthermore, the PC concentration increased rapidly to

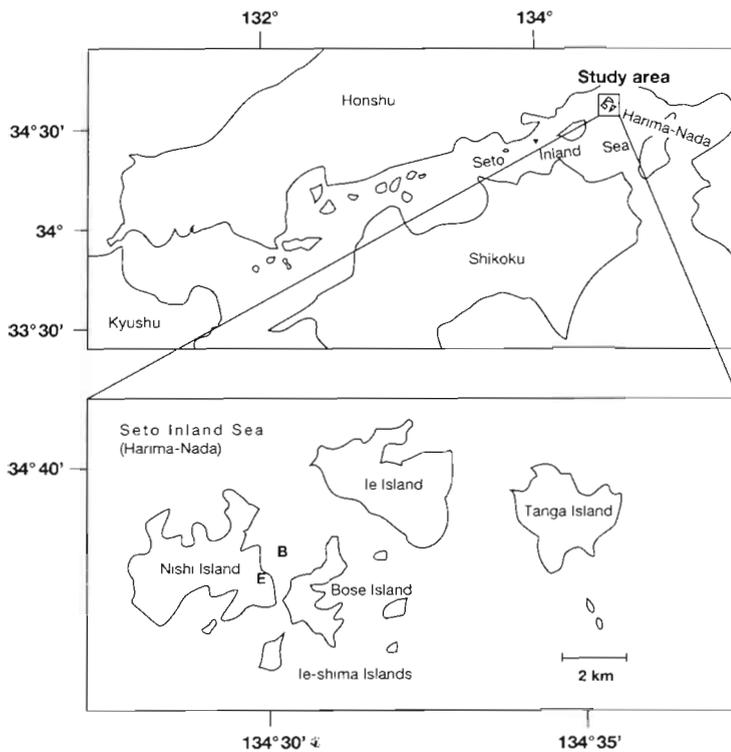


Fig. 1 Sampling station locations around the Ie-shima Islands, Seto Inland Sea. (B) Sampling station; (E) field lab

reach $> 1 \times 10^5 \text{ ml}^{-1}$ following strong wind mixing in 1991 (K. Fukami & Y. Nakamura unpubl.). Based on the above observations, we hypothesized that the introduction of N- and/or P-nutrients liberated PC from N- and/or P-limitation and that the growth rate of PC in this coastal area does not reach maximum even at nutrient levels (e.g. 0.1 to $1 \mu\text{M NO}_3^-$) higher than the detection limit for conventional colorimetric methods. In order to verify this, and to assess the roles of chemical variables on population dynamics of PC, we monitored temporal and vertical changes in PC populations together with environmental variables on a daily basis during summer 1992. We also conducted field experiments on PC growth in $< 2 \mu\text{m}$ fractions of the seawater enriched with NO_3^- , PO_4^{3-} , and/or metal buffer solution (composed mainly of Fe^{III} and EDTA). In the present paper, results from field observations and experiments are described and the importance of trace metals (rather than N and P) for PC blooming is discussed.

METHODS

Monitoring of marine environment. In summer 1992 (16 July to 9 August), field surveys were conducted at Stn B (depth = 21 m) around the Ie-shima Islands

(Fig. 1). Water temperature, salinity, and dissolved oxygen were monitored at 2.5 m intervals using a Surveyor II (Hydrolab Co.). Transparency of the seawater was measured using a Secchi disk. Water samples for chemical analysis (NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} , $\text{Si}(\text{OH})_4$, chlorophyll *a*) and for enumeration of PC and HNAN were taken from depths of 0, 5, 10, 15 and 19 m (= for chemical analysis only) using a 10 l Van Dorn-type bottle. Monitoring and sampling were conducted daily (except 4 and 8 August due to typhoons) in the morning (06:20 to 07:00 h), and water samples were treated (filtration, fixation, etc.) immediately in our field laboratory (Fig. 1). Analytical procedures for each chemical variable have been summarized in a previous paper (Nakamura et al. 1988).

Field experiments. PC growth experiments in $< 2 \mu\text{m}$ fractions were conducted 3 times (Run 1: 22 to 23 July; Run 2: 1 to 3 August; Run 3: 6 to 8 August). Seawater samples were obtained from 5 m depth in the morning together with routine samples (see above) and poured into a 2 l polypropylene (PP) bottle. Samples were stored in an incubator, where the temperature was set at that of 5 m ($\pm 1^\circ\text{C}$) and irradiance was ca $25 \mu\text{E m}^{-2} \text{ s}^{-1}$, until routine treatments were finished (ca 09:30 h). Samples were then filtered through Nuclepore $2 \mu\text{m}$ filters mounted on a polysulfone filtration apparatus (1 l, Nalgene Co.) with gentle negative pressure ($< 50 \text{ mm Hg}$). About 400 ml of seawater was filtered through 1 filter (47 mm diameter). Filtrates were poured into a 2 l PP bottle, then dispensed into glass bottles (250 ml in volume with 200 ml of sample solution) and received enrichments. Modes of enrichment were: 'N+P' ($10 \mu\text{M NO}_3^-$ and $1 \mu\text{M PO}_4^{3-}$, Runs 1, 2 & 3); 'Metals' [$0.3 \text{ ml N-1 metal solution}$ (Nakamura & Umemori 1991) per bottle, Run 3]; 'Full' (NO_3^- , PO_4^{3-} and N-1 metals were added at the same levels of 'N+P' and 'Metals', Runs 2 & 3); and a control (no addition, Runs 1, 2 & 3). Added concentrations of trace metals and EDTA through N-1 metal solution were $350 \text{ nM Fe}^{\text{III}}$, $5 \text{ nM Co}^{\text{II}}$, $63 \text{ nM Zn}^{\text{II}}$, $240 \text{ nM Mn}^{\text{II}}$, $1 \text{ nM Cu}^{\text{II}}$, 1 nM MoO_4^{2-} and $4 \mu\text{M EDTA}$. In parallel with enrichment, a sample (100 ml) for enumeration of PC and HNAN was also taken from the $< 2 \mu\text{m}$ filtrates. Filtration apparatus, filters and glass bottles (pre-sterilized by autoclaving) were rinsed thoroughly by GF/F filtered seawater (from 5 m) before use. The bottles which had received enrichment were then suspended at 5 m depth (around 11:00 h) and the growth of PC was monitored for 1 (Run 1) or 2 d (Runs 2 & 3). The bottles were recovered, subsamples (15 ml)

were withdrawn and fixed at intervals of 21 to 24 h. These experiments were conducted in duplicate.

Enumeration. Samples for PC (and HNAN) counts were fixed with glutaraldehyde (final concentration 1%), stored in a refrigerator (5°C), and enumerated within 2 d after sampling. Subsamples (3 ml) were concentrated on black 0.2 µm Nuclepore filters under low vacuum (ca 100 mm Hg). The filters were examined by epifluorescence microscopy using a Nikon Optiphot-2 equipped with a Hg 100 W lamp and B2 filter cassette (exciter 450 to 490 nm, dichroic mirror 510 nm, barrier 520 nm). PC were characterized by bright yellow to orange fluorescence. It should be noted that the PC detected in the present study were cells containing phycoerythrin, since we used 'blue excitation' only (cf. Pick 1991). PC cells were counted at 1250× using a fluor 100/1.30 oil objective by observing at least 50 and 80 fields (80 × 80 µm) in natural and experimental samples, respectively. Using 30 ml of subsamples, we also enumerated HNAN by 4',6-diamidino-2-phenylindole (DAPI) staining, details of which will be described in another article.

RESULTS

Temporal changes in environmental variables

Vertical profiles of environmental variables throughout the survey period are summarized in Table 1 and Fig. 2A to D. Water was weakly stratified between 16 and 22 July and strong stratification developed between 23 and 28 July due to calm and sunny weather. After 29 July, weather was still sunny, but a continuous westward-blowing wind weakened the stratification.

The typhoon (No. 9) on 4 August destroyed the stratification completely (Fig. 2A).

A significant increase of NO₃⁻ (and PO₄³⁻) concentration in the surface layer (0 to 5 m) was observed on 19 July (Fig. 2B, C) due to the strong winds on 18 July. This increase caused a bloom of diatoms (see below) and NO₃⁻ in the surface layer then decreased rapidly to below the detection limit (0.1 µM). After 30 July, NO₃⁻ concentrations in the surface layer increased again, coupled with the weakening of thermal stratification. The concentration of NH₄⁺ in the surface layer was usually below 0.2 µM before 3 August, but after that it increased to a maximum of 3 µM. Changes in PO₄³⁻ concentration were similar to those of NO₃⁻, but PO₄³⁻ was not depleted completely (detection limit = 0.01 µM) except for 25 and 27 July (0 to 5 m).

Following the introduction of nutrients to the surface layer on 19 July, chlorophyll *a* concentrations (0 to 5 m) increased rapidly to reach 4 µg l⁻¹ (20 to 22 July, Fig. 2D) due to a bloom of diatoms (*Chaetoceros* spp.). The dense layer of chlorophyll *a* was then shifted downward (23 to 29 July), apparently due to the sinking of diatoms. After 30 July, nutrients were introduced again to the surface layer, but chlorophyll *a* did not increase significantly.

Temporal changes in PC cell concentrations

PC cells in the study area were dominated by spherical cells (ca 70% of total PC) ranging in size from 0.8 to 1.4 µm diameter, and the rest were rod-shaped *Synechococcus*-type cells (0.8 to 1.0 × 1.0 to 1.6 µm). Irregular clumps of PC were rare throughout the survey period.

Table 1. Vertical profiles of environmental variables at Stn B from 16 July to 9 August 1992. Secchi depth during this period changed from 3 to 10.5 m (avg. = 7.2 m). Values in parentheses are averages of each parameter throughout the survey period. DO: dissolved oxygen

Depth (m)	Temp. (°C)	S (‰)	DO (ppm)	NO ₃ ⁻ (µM)	NO ₂ ⁻ (µM)	NH ₄ ⁺ (µM)	PO ₄ ³⁻ (µM)	Si (OH) ₄ (µM)	Chl <i>a</i> (µg l ⁻¹)
0	22.0–25.7 (24.0)	29.0–32.4 (31.4)	6.0–8.6 (7.3)	<0.1–1.7 (0.4)	<0.1–0.4 (0.1)	<0.2–2.9 (0.3)	<0.01–0.55 (0.13)	1.5–17.1 (7.1)	0.6–4.1 (1.6)
5	21.8–25.1 (23.6)	31.6–32.4 (32.0)	5.8–8.6 (6.9)	<0.1–1.8 (0.5)	<0.1–0.5 (0.2)	<0.2–2.9 (0.3)	<0.01–0.55 (0.16)	2.0–16.2 (7.6)	0.8–3.8 (1.8)
10	21.5–24.3 (22.6)	31.9–32.6 (32.3)	5.2–8.0 (6.2)	<0.1–3.4 (1.5)	<0.1–1.1 (0.4)	<0.2–2.5 (0.3)	<0.01–0.50 (0.24)	2.2–18.2 (10.8)	1.3–4.1 (2.4)
15	20.3–23.9 (21.8)	32.1–32.7 (32.4)	4.0–6.0 (5.2)	1.2–5.8 (3.3)	0.3–1.8 (0.9)	<0.2–2.1 (0.6)	0.20–0.71 (0.50)	9.5–26.8 (17.9)	1.5–6.6 (2.5)
19	20.1–23.8 (21.3)	32.3–32.7 (32.5)	3.5–5.8 (4.6)	1.6–7.8 (4.9)	0.5–1.8 (1.2)	<0.2–2.1 (0.8)	0.34–0.89 (0.67)	13.1–30.3 (23.5)	1.4–6.3 (2.5)
21 (bottom)	20.0–23.8 (21.2)	32.4–32.9 (32.6)	3.3–5.7 (4.3)						

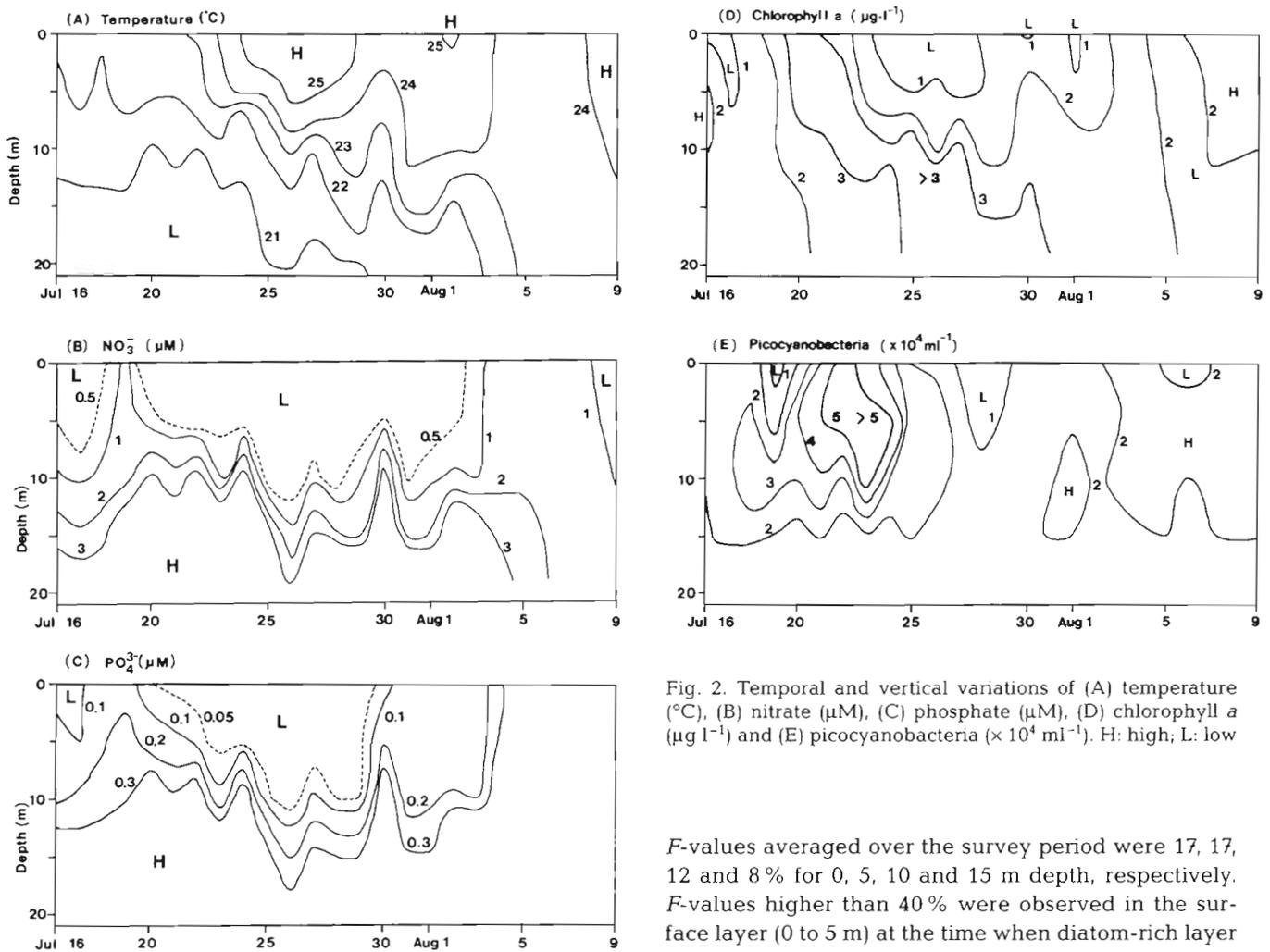


Fig. 2. Temporal and vertical variations of (A) temperature ($^{\circ}\text{C}$), (B) nitrate (μM), (C) phosphate (μM), (D) chlorophyll *a* ($\mu\text{g l}^{-1}$) and (E) picocyanobacteria ($\times 10^4 \text{ ml}^{-1}$). H: high; L: low

F-values averaged over the survey period were 17, 17, 12 and 8% for 0, 5, 10 and 15 m depth, respectively. *F*-values higher than 40% were observed in the surface layer (0 to 5 m) at the time when diatom-rich layer shifted downward and PC were still blooming (i.e. 23 to 24 July).

Concentrations of eukaryotic picoalgae (N_{EPA}) were also monitored at depths of 5 and 15 m, and $N_{\text{EPA}}:N_{\text{PC}}$

Temporal changes in PC concentration (N_{PC}) are shown in Fig. 2E and N_{PC} were in the range of 0.7 to $5.7 \times 10^4 \text{ ml}^{-1}$ during the survey period. Following the introduction of nutrients to the surface layer (0 to 5 m) on 19 July (Figs. 2B & 3), N_{PC} increased rapidly, especially in the surface layer, and N_{PC} averaged over 0 to 15 m depth reached a maximum ($4.8 \times 10^4 \text{ ml}^{-1}$) on 23 July (Fig. 3). N_{PC} then decreased during a strong thermal stratification period (23 to 28 July). In contrast to the chlorophyll *a* change (Fig. 2D), the dense layer of PC did not shift downward (Fig. 2E) apparently due to the slow sinking rate of PC. Although N_{PC} increased again coupled with weakening of thermal stratification (after 29 July), its rate of increase was rather slow (Fig. 3).

Cell concentrations of PC were converted to chlorophyll *a* assuming a PC cell diameter of $1.2 \mu\text{m}$ and using $250 \text{ fg cell C } \mu\text{m}^{-3}$ (Kana & Glibert 1987, Booth 1988) and a C:chl *a* ratio of 22.5 (Takahashi et al. 1985, Geider 1987, Ray et al. 1989). The contribution of PC to total chlorophyll *a* (*F*) was in the range of 3 to 63%.

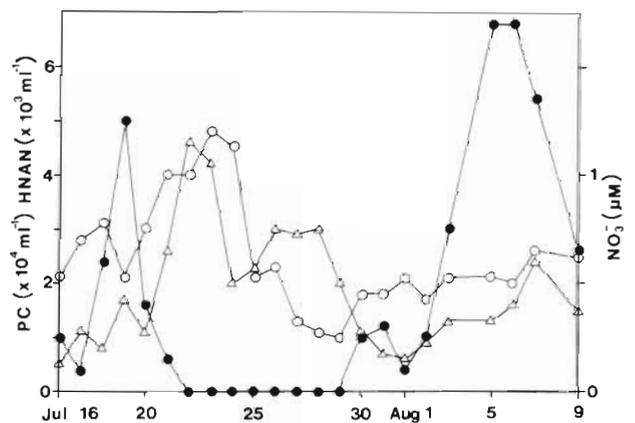


Fig. 3. Temporal variations of nitrate (●; averaged over 0 to 5 m), picocyanobacteria (○; averaged over 0 to 15 m) and heterotrophic nanoflagellates (Δ; averaged over 0 to 15 m)

ratios averaged over the survey period were 14 and 15 % for 5 and 15 m depth, respectively.

Field experiments

Initial conditions for the 3 experiments are summarized in Table 2, and growth curves of PC in each run are shown in Fig. 4A to C. In Run 1 (Fig. 4A) where NO_3^- and NH_4^+ were below the detection limit, N_{PC} in the control and 'N+P'-enrichment decreased after 24 h incubation. However, the rate of decrease in 'N+P' was lower than in the control, and PC cells in 'N+P' showed much brighter fluorescence than in the control after incubation. In Run 2 (Fig. 4B), where NO_3^- (and PO_4^{3-}) were not completely depleted, N_{PC} in the control and 'N+P'-enrichment increased significantly during 48 h incubation. Although a slight lag was observed (0 to 24 h), the growth rates of PC in the control and 'N+P'-enrichment between 24 and 48 h reached 0.87 and 0.80 d^{-1} , respectively. The growth of PC in 'Full'-enrichment was more rapid than in the control and 'N+P'-enrichment, and the growth rate between 24 and 48 h was 1.26 d^{-1} . In Run 3 (Fig. 4C), where N- and P-

Table 2. Initial conditions for PC growth experiments

	Run 1 (22 to 23 July)	Run 2 (1 to 3 August)	Run 3 (6 to 8 August)
Temp. ($^{\circ}\text{C}$)	22.7	24.6	23.5
Salinity (‰)	32.1	32.0	32.4
NO_3^- (μM)	<0.1	0.1	1.8
NO_2^- (μM)	<0.1	0.1	0.5
NH_4^+ (μM)	<0.2	<0.2	0.8
PO_4^{3-} (μM)	0.06	0.12	0.41
HNAN (ml^{-1}) ^a	1.2×10^3	$<0.1 \times 10^3$	0.2×10^3

^aConcentration in $<2 \mu\text{m}$ filtrates

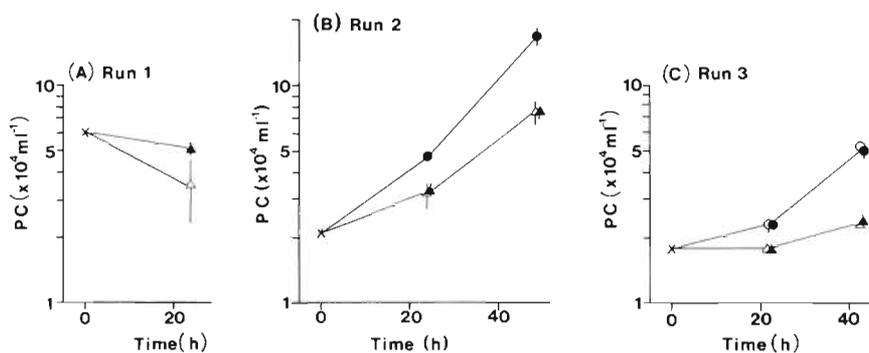


Fig. 4. Growth curve of picocyanobacteria in $<2 \mu\text{m}$ seawater fraction for (A) Run 1: 22 to 23 July; (B) Run 2: 1 to 3 August; (C) Run 3: 6 to 8 August. (Δ) Control; (\blacktriangle) N+P, NO_3^- and PO_4^{3-} added; (\circ) Metals, N-1 metal solution added; (\bullet) Full, NO_3^- , PO_4^{3-} and N-1 metals added. Vertical error bars show the range of duplicate counts

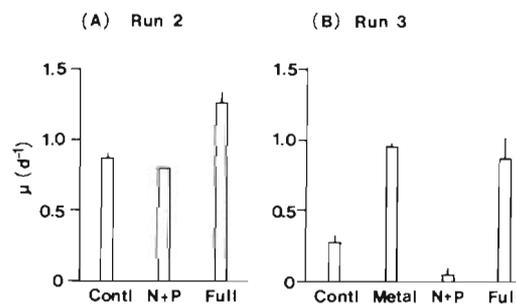


Fig. 5. Effects of N-, P- and metal buffer-enrichment on the growth rates (μ) of picocyanobacteria. (A) Run 2: 1 to 3 August; (B) Run 3: 6 to 8 August. Vertical error bars show the range of duplicate experiments

nutrients were at a sufficient level, N_{PC} in the control and 'N+P'-enrichment did not increase significantly between 0 and 22 h, and the growth rates in these modes between 22 and 43 h were below 0.3 d^{-1} . Although growth of PC in 'Metals'- and 'Full'-enrichment showed a lag between 0 and 22 h, it was rapid between 22 and 43 h. The growth rates during this period were 0.95 and 0.88 d^{-1} for 'Metals'- and 'Full'-enrichment, respectively. The growth rates obtained in Run 2 (24 to 48 h) and Run 3 (22 to 43 h) are summarized in Fig. 5.

DISCUSSION

Coupled with the first mixing event on 19 July, N_{PC} increased rapidly to reach $\text{ca } 5 \times 10^4 \text{ ml}^{-1}$ (Fig. 3). However, the next mixing after 30 July did not cause a PC bloom. Enrichment experiments conducted in the second mixing period showed enhancement of PC growth by addition of metal buffer solution. These results suggest that chemical variable(s) can control the population of PC even in a eutrophic inland sea (cf. Glover et al. 1988a, b).

Field observations

It has often been pointed out that the abundance of PC is positively correlated with temperature (e.g. Caron et al. 1985, Murphy & Haugan 1985, El Hag & Fogg 1986, Kuosa 1991). However, this was not the case in the present study, since PC was abundant (3.9 to $5.0 \times 10^4 \text{ ml}^{-1}$; 0 and 5 m) on 21 July when the corresponding temperature was still in the range 22.1 to 22.9 $^{\circ}\text{C}$ and the lowest abun-

dance of PC (27 to 29 July; Fig. 3) coincided with the highest temperature period (24.2 to 25.5°C; 0 and 5 m). Furthermore, PC bloomed when the surface temperature was 24.0 to 26.3°C in summer 1991 (K. Fukami & Y. Nakamura unpubl.). Thus we conclude that the abundance of PC was not significantly affected by temperature in the range (22 to 26°C) encountered in both years.

Among the processes of PC loss, grazing by HNAN and/or ciliates is considered to be most important (reviewed by Stockner 1988). During the period of decreasing PC (23 to 27 July), cell concentrations of HNAN (N_{HNAN}) remained relatively high in comparison with those of the pre-bloom period (16 to 19 July) or the second mixing period (after 30 July; Fig. 3). HNAN cells containing PC cells inside them were also observed (>10% of the total by impression, not quantitative). These observations suggest that the high abundance of HNAN contributed, to some extent, to the disappearance of the PC bloom. However, it should be noted that grazing by ciliates or viral lysis of PC cells (Porter & Fuhrman 1990, Suttle et al. 1990) might also have played significant roles in the mortality of PC.

The second mixing period (after 30 July) was characterized by high concentrations of N- and P-nutrients in the surface layer, low abundance of HNAN (Fig. 3) and high transparency of the seawater (7.5 to 11 m from 30 July to 3 August). N_{HNAN} in this period was comparable to that observed during the PC bloom period in summer 1991 (K. Fukami & Y. Nakamura unpubl.). These conditions seem to be favorable for PC blooming. However, the increase of N_{PC} during this period was not rapid in comparison with the bloom period (Fig. 3). These observations suggest that the population of PC was controlled by factors such as trace metals (see below) and/or ciliates (not monitored in both years), and not by N- and P-nutrients, HNAN, light or temperature.

Chemical variables affecting PC growth

Before the field survey of 1992, we hypothesized that addition of N- and P-nutrients would enhance the growth of PC (see 'Introduction'). In this context the field experiment of Run 1 (22 to 23 July) was conducted only in the control and 'N+P'-enrichment modes. However, N_{PC} in both modes decreased during 24 h incubation (Fig. 4A) and this suggested to us the addition of a metal buffer solution in Run 2 (1 to 3 August) and Run 3 (6 to 8 August). The reason for the decrease of N_{PC} in Run 1 is still uncertain. HNAN slightly contaminated the <2 μm fraction ($1.2 \times 10^3 \text{ ml}^{-1}$; 30% of original seawater) and may have

suppressed the increase of N_{PC} to some extent. However, N_{PC} of the natural population increased under N_{HNAN} of $2.5 \times 10^3 \text{ ml}^{-1}$ (21 July, Fig. 3) and the decrease of N_{PC} in Run 1 cannot be explained by the contamination of HNAN alone. N_{HNAN} in Runs 2 & 3 were $\leq 0.2 \times 10^3 \text{ ml}^{-1}$ and probably had a minor effect on PC growth.

Effects of metal buffer solution

The most striking feature of the results obtained in the present study was the enhancement of PC growth by addition of the metal buffer solution (Fig. 5). Since we did not expect this effect before the survey, a clean technique (Fitzwater et al. 1982) was not used, and thus it might be considered that the growth stimulation was due to an experimental artifact. However, based on our previous results obtained from field observations and experiments (Nakamura et al. 1989, Nakamura 1990), we infer that the growth stimulation was due to Fe deficiency of natural PC populations, as discussed below.

A possible explanation of the growth enhancement by metal buffer solution is that toxic metals (such as Cu) already present in natural seawater or as contaminants from experimental procedures suppressed the growth of PC in the control and 'N+P'-enrichment, and that the enrichment of EDTA through the metal buffer solution liberated PC from growth suppression. However, in the study area in the summers of 1986 to 1988 (Nakamura 1990), Cu (ca 10 nM in 0.8 μm filtrates) was highly chelated by natural organic ligand(s), pCu (-log of cupric ion activity) was in the range 11.5 to 11.7 (= non-toxic range for all phytoplankton species examined; Gavis et al. 1981), Cu-complexing capacity was ca 100 nM, and addition of inorganic Cu (60 nM) to natural seawater did not have any toxic effects on the growth of *Chattonella antiqua* (= Raphidophyceae, a species sensitive to cupric ion activity; Nakamura et al. 1986). Furthermore, in our experiments to determine the *in situ* growth rate of *C. antiqua* (Nakamura et al. 1989), the experimental procedures (sampling, filtration apparatus, incubation bottles) were essentially the same as those in the present study, and the obtained growth rates could be explained satisfactorily by the ambient concentrations of N- and P-nutrients alone (cf. Nakamura et al. 1988) without any deleterious effects of contaminants. Thus it is unlikely that the growth enhancement of PC was due to the detoxification of toxic metals by EDTA.

Another explanation of the growth enhancement mechanism is the adsorption of essential metals (such as Fe) to incubation bottles (Fitzwater et al. 1982). Metals might have been scavenged by adsorp-

tion in the control and 'N+P'-enrichment, and the growth of PC might have been suppressed. However, based on our experiments (Nakamura 1990), adsorption of Fe in filtered seawater (initial concentration = 10 nM) to borosilicate flasks was ca 10% during 4 d incubation (25°C, 150 $\mu\text{E m}^{-2} \text{s}^{-1}$, 12 h light:12 h dark cycle). The insignificance of Fe adsorption to glass bottles was also indicated by Takeda & Kamatani (1989).

We monitored the Fe concentration (in 0.8 μm filtrates) and conducted growth bioassay experiments in the present study area taking care to avoid trace metal contamination during the summers of 1986 to 1988 (Nakamura 1990). The results indicated that Fe in the seawater was usually insufficient to support the maximum growth rate of *Chattonella antiqua* in spite of the relatively high concentration of Fe (4 to 10 nM). Furthermore, Brand (1991) indicated that Fe demand by PC (assessed by Fe/P ratios inside the cell) was much higher ($>10^{1.3}$) than for oceanic species of eukaryotic phytoplankton and comparable to a raphidophycean flagellate *Heterosigma akasiwo* (closely related to *C. antiqua* in taxonomic and physiological aspects; Watanabe et al. 1982, Nakamura & Watanabe 1983). Based on the above arguments, it seems reasonable to infer that growth enhancement of PC by addition of the metal buffer solution was due to insufficient Fe in the original seawater and not to an experimental artifact, although growth stimulation by other metals (such as Mn) cannot be ruled out.

Nitrogen and phosphorus

In order to assess the roles of N- and P-nutrients on the growth of PC, growth rates between 'Metals'- and 'Full'-enrichment (i.e. both modes are set at 'optimum' for trace metals) should (ideally) be compared, since trace metals enhanced the growth significantly (Fig. 5). Unfortunately, the growth in 'Metals' was examined only in Run 3. In this case, the initial ambient nutrient concentrations were very high (Table 2) and it is not surprising that the growth rates in 'Metals' were comparable with those in 'Full'. On the other hand, Run 2 was conducted under the conditions that initial concentration of NO_3^- was at the detection limit (0.1 μM) and the growth rate in the control was 70% of that in 'Full'. This indicates that 0.1 μM NO_3^- can support >70% of the maximum growth rate of PC and the half-saturation constant for growth is well below 0.1 μM NO_3^- . Thus, although PC populations in 'nutrient-depleted' waters (e.g. 22 to 29 July, 0 to 5 m; Fig. 2B) might have been controlled by NO_3^- (and/or PO_4^{3-}), our initial hypothesis that introduction of nutrients (e.g. 0.1 to 1 μM NO_3^-) to the surface layer liberates the PC

populations from N- (and/or P-) limitations (see 'Introduction') is not realistic.

In summary, the results obtained in the present study strongly suggest that trace metal(s) play important roles in the wax and wane of PC populations. However, questions still remain: what is the difference between the 2 mixing events? Or in other words, were sufficient trace metals introduced in the first event, but not in the second event? Since we did not monitor trace metal concentrations (especially available Fe; see Wells et al. 1991), we cannot answer the above questions at present. Finally, we stress here the importance of trace metal monitoring for full understanding of PC population dynamics.

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