JARE-33第一レグ(東京〜フリマントル,オーストラリア)に沿ったピコ植物プランクトンの表面分布

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Surface Distribution of Picophytoplankton along the First Leg of the JARE-33 Cruise, from Tokyo to Fremantle, Australia

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Abstract
The abundance of picophytoplankton (2 - 0.2 μm) was investigated by chlorophyll a determinations and cell counts with an epifluorescence microscope during the first leg of the 33rd Japanese Antarctic Research Expedition (JARE-33) cruise in the coastal waters of Southeast Asia and the open waters of the western North Pacific Ocean and the eastern Indian Ocean in November of 1991. Contribution of picophytoplankton to the total phytoplankton chlorophyll was lower in the coastal waters (mean ± 1SD, 58 ± 15%) than in the open waters (73 ± 3.2 % and 73 ± 2.9%, respectively), although the concentration was higher in the former (0.14 ± 0.043 μg l⁻¹) than in the latter (0.052 ± 0.012 μg l⁻¹ and 0.057 ± 0.008 μg l⁻¹, respectively). Cell densities of cyanobacteria and other picophytoplankton were also more abundant in the coastal (74 ± 56 × 10³ cells ml⁻¹ and 2.0 ± 0.64 × 10³ cells ml⁻¹, respectively) than in the western North Pacific (1.4 ± 0.51 × 10³ and 0.63 ± 0.20 × 10³ cells ml⁻¹, respectively) and in the eastern Indian Ocean (3.6 ± 2.0 × 10³ and 0.84 ± 0.20 × 10³ cells ml⁻¹, respectively). A significant relationship was obtained based on the multiple regression analysis for the data on chlorophyll a concentration (y, fg ml⁻¹), cell densities (cells ml⁻¹) of cyanobacteria (x₁) and other picophytoplankton (x₂) of the 2 - 0.2 μm size fraction (y=0.48 x₁ + 34 x₂ + 32000) (p<0.001, r=0.95, n=30). Coefficient for cyanobacteria was two orders of magnitude lower than that for other picophytoplankton. Since the coefficient is considered as mean chlorophyll a content per cell, the present results imply that chlorophyll a content per cell is largely different between the two groups. The relatively large intercept seems to result from contribution of prochlorophytes, which could not be exactly evaluate by the epifluorescence microscope. The averaged contribution of prochlorophytes to the picoplankton chlorophyll a is estimated as 30 ± 14% in the coastal waters of Southeast Asia, 58 ± 7.3 and 48 ± 6.2% in the western North Pacific and the eastern Indian Ocean, respectively.

Keywords: cyanobacteria, picophytoplankton, chlorophyll a, cell density, Southeast Asian sea

The structure of the pelagic ecosystem has been reconsidered after the discovery of the widespread occurrence of picophytoplankton (WATERBURY et al. 1979),
which are smaller than 2 µm. Primary production in subtropical and tropical open waters is largely attributed to picophytoplankton. Picophytoplankton community is composed of prokaryotic cyanobacteria (Synechococcus spp.) and eukaryotic microalgae (Murphy & Haugen 1985, Takahashi et al. 1985, Odate et al. 1990, Blanchot et al. 1992, Campbell & Vaulot 1993, Morel et al. 1993). Novel free-living prochlorophyte was revealed by Chisholm et al. (1988). Li & Wood (1988) also found very small red-fluorescing bodies by flow cytometry in the North Atlantic Ocean. They considered that the very small red-fluorescing bodies corresponded to prochlorophyte described by Chisholm et al. (1988). More recently, the prokaryotic alga was isolated and named Prochlorococcus marinus (Chisholm et al. 1992).

The Japanese Antarctic Research Expedition (JARE) has determined concentration of chlorophyll a to assess phytoplankton abundance along cruise tracks to and from the Antarctica since 1965 (Hoshiai 1968). The first leg of the JARE cruise is conducted every year across the subtropical open waters of the western North Pacific and the eastern Indian Ocean and the coastal waters of Southeast Asia. In the previous JAREs concentration of chlorophyll a was routinely determined using a single filter, which can retain all phytoplankters in a sample, regardless of phytoplankton size and taxon. Although a bulk measurement of chlorophyll a provides a good estimate of pigment biomass of primary producers, it can mask many important differences that affect ecosystem structure and function (Li et al. 1993). It is necessary to recognize details of constituents of marine ecosystems. The present study aims to reveal abundance and community structure of picophytoplankton along the first leg of the JARE-33 cruise.

Materials and Methods
Seawater sample was collected with three to four times a day during the first leg of the JARE-33 cruise of the icebreaker Shirase (JARE-33) (Figure 1) from the outlet of the Surface Water Monitoring System (Fukuchi & Hattori 1987). The present study covered the open water of the western North Pacific Ocean (Stations 1-10), the coastal waters of Southeast Asia (Stations 11-24), and the open water of the eastern Indian Ocean (Stations 25-30).

One aliquot of the seawater (200 ml) was directly filtered with Whatman GF/F glass fiber filter (25 mm diameter) (less than 100 mm Hg vacuum pressure) for measurement of chlorophyll a concentration as bulk. The other (200 ml) was screened using a Nuclepore filter (47 mm diameter) with 2.0 µm pore size (<50 mm Hg) to remove micro- and nanophytoplankton. And then, picophytoplankton in the filtrate (100 ml) was retained onto a Nuclepore filter (47 mm diameter) with 0.2 µm pore size (<100 mm Hg) for evaluation of picophytoplankton. These filters were put into glass vials contained 6 ml of N, N-dimethylformamide (Suzuki & Ishimaru 1990). Extraction of pigments was conducted for 24 h in dark at −20°C. Concentrations of chlorophyll a was determined by the fluorometry (Parsons et al. 1984). Calibration of the Turner Design Fluorometer Model 10R was performed with pure chlorophyll a (Sigma Chemical Co.).

On the other hand, the rest of the filtrate from the 2.0 µm pore sized filter (<100 ml) was also filtered using a Nuclepore filter (25 mm diameter) with 0.2 µm pore size (<100 mmHg). This filter was prepared for direct counts of algal cells
Fig. 1. Cruising track of the icebreaker Shirase during the JARE-33, from November 14 to 28, 1991. The first leg was from Tokyo to Fremantle, Australia. Surface water samples were collected at thirty points. Open circles, the western North Pacific Ocean; closed circles, the coastal waters of Southeast Asia; open squares, the eastern Indian Ocean.

with an epifluorescence microscope. Under blue excitation (excitor, 420-490 nm), yellow and red fluorescing cells are usually recognized (MURPHY & HAUGEN, 1985). The former is considered as cyanobacteria while the latter contains small
eukaryotes and prokaryotes (e.g. CAMPBELL & VAULT 1993). In the present study, discrimination between picoeukaryotes and prokaryotes could not be conducted. Then, the red fluorescing cells are referred as other picophytoplankton.

Results
Surface distribution of chlorophyll $a$ concentration is shown in Figure 2a. The highest bulk concentration occurred at Station 24, which was in Lombok Strait. The relatively high concentrations were observed at Station 11, which was located at the entrance of the Celebes Sea, and Stations 16-18, which were in Makassar Strait. However, the concentration in the central part of the Celebes Sea (Station...
Table 1. Summary of chlorophyll $a$ concentrations and cell densities (mean ± 1SD) in surface water along the first leg of the JARE-33 cruise by the icebreaker Shirase.

<table>
<thead>
<tr>
<th>Sea area</th>
<th>Chlorophyll $a$ concentration ($\mu g, l^{-1}$)</th>
<th>Contribution of 2-0.2 $\mu m$ fraction (%)</th>
<th>Picophytoplankton cell density ($\times 10^3$ cells ml$^{-1}$)</th>
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<tr>
<td>Open water of the western North Pacific Ocean (n=10)</td>
<td>0.076 ± 0.017</td>
<td>73 ± 3.2**</td>
<td>1.4 ± 0.63***</td>
</tr>
<tr>
<td>Open water of the eastern Indian Ocean (n=6)</td>
<td>0.087 ± 0.018</td>
<td>73 ± 2.9**</td>
<td>3.6 ± 0.84***</td>
</tr>
<tr>
<td>Coastal waters of Southeast Asia (n=14)</td>
<td>0.30 ± 0.20</td>
<td>58 ± 15</td>
<td>2.0 ± 0.64***</td>
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Mean value marked with *, **, and *** is significantly different from mean value of the coastal waters of Southeast Asia at significant level of 0.05, 0.01, and 0.001, respectively.

Concentration of chlorophyll $a$ in the 2-0.2 $\mu m$ size fraction was also more abundant in the coastal waters than in the open waters (Figure 2a). The mean concentration in Southeast Asia (0.14 ± 0.043 $\mu g\, l^{-1}$) was significantly higher than that in the western North Pacific (0.052 ± 0.012 $\mu g\, l^{-1}$) and that in the eastern Indian Ocean (0.057 ± 0.008 $\mu g\, l^{-1}$) (Table 1). The concentration of this fraction was not highest in Lombok Strait (see also Figure 3b), where the highest concentration of bulk chlorophyll $a$ was recorded. On the other hand, concentration in picoplankton fraction was less in the central part of the Celebes Sea (Station 13) as observed in bulk chlorophyll $a$.

Relative abundance of picophytoplankton to the total phytoplankton abundance was less in the coastal waters of Southeast Asia than in the open waters of the western North Pacific and the eastern Indian Ocean (Figure 2b). At the central part of the Celebes Sea (Station 13), contribution of picophytoplankton was high as observed in the open waters of the western Pacific and the eastern Indian Ocean. The contribution was the lowest in Lombok Strait. On the average the contribution was significantly lower in the coastal waters of Southeast Asia (58 ± 15%) than in the open waters of the western North Pacific Ocean (73 ± 3.2%) (p<0.01) and in the eastern Indian Ocean (73 ± 2.9%) (p<0.01) (Table 1).

Cell densities of picophytoplankton communities are shown in Figure 3. Throughout the observations of the present study, cell density of cyanobacteria was more than that of the other picophytoplankton (Figure 3a). As observed in
chlorophyll $a$ concentration of picophytoplankton, the cell densities of cyanobacteria and other picophytoplankton populations were more abundant in the coastal waters of Southeast Asia than in the open water of the western North Pacific Ocean and the eastern Indian Ocean. The mean cell density of cyanobacteria in the coastal waters of Southeast Asia ($74 \pm 56 \times 10^3$ cells ml$^{-1}$) was significantly higher than that in the western North Pacific Ocean ($1.4 \pm 0.51 \times 10^3$ cells ml$^{-1}$) ($p<0.001$) and in the eastern Indian Ocean ($3.6 \pm 2.0 \times 10^3$ cells ml$^{-1}$) ($p<0.001$), respectively (Table 1). The mean cell density of other picophytoplankton was also significantly higher in the coastal waters ($2.0 \pm 0.64 \times 10^3$ cells ml$^{-1}$) than in the western Pacific ($0.63 \pm 0.20 \times 10^3$ cells ml$^{-1}$) ($p<0.001$) and the Indian Ocean ($0.84 \pm 0.20 \times 10^3$ cells ml$^{-1}$) ($p<0.001$), respectively (Table 1).

Regional variation in the total cell density of cyanobacteria plus other picophytoplankton was well consistent with that in chlorophyll $a$ concentration of the 2-0.2 $\mu$m size fraction (Figure 3b). Consequently, cyanobacteria and other picophytoplankton are considered to contribute chlorophyll $a$ concentration in the

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**Fig. 3.** Surface distributions of cyanobacteria and other picophytoplankton along the first leg of the icebreaker Shirase (a). Regional variations in the total cell density of two groups and chlorophyll $a$ concentration in the 2-0.2 $\mu$m size fraction are illustrated in lower panel (b). Date is expressed with GMT.
this fraction (Li et al. 1993). The multiple regression analysis was conducted for
the data on chlorophyll $a$ concentration and cell densities of two groups. Chlorophyll $a$ concentration in this fraction was represented by the following equation,

$$y = 0.48 x_1 + 34 x_2 + 32000 \quad (p<0.001, r=0.95, n=30)$$

where $y$ is chlorophyll $a$ concentration (fg ml$^{-1}$), $x_1$ and $x_2$ are cell densities (cells ml$^{-1}$) of cyanobacteria and other picophytoplankton, respectively. Coefficient for cyanobacteria cell density ($0.48 \pm 0.10$) was about two orders of magnitude lower than that for other picophytoplankton ($34 \pm 6.6$). Intercept of the equation ($0.032 \pm 0.007 \mu g l^{-1}$) was equivalent to 62 and 56% of the mean concentration of chlorophyll $a$ in this size fraction observed in the open waters of the western North Pacific Ocean ($0.052 \mu g l^{-1}$) and the eastern Indian Ocean ($0.057 \mu g l^{-1}$).

**Discussion**

Surface distribution of bulk chlorophyll $a$ along the first leg of the JARE-33 cruise has been demonstrated based on analysis of *in vivo* fluorescence intensity by ODATE & FUKUCHI (1994). They showed the relatively high concentration in the coastal waters of Southeast Asia and the highest concentration in Lombok Strait, as observed in the present study. The mean concentrations of bulk chlorophyll $a$ determined by the present study in the open waters of the western North Pacific and the eastern Indian Ocean are ca. 30% and 40% lower than those determined by ODATE & FUKUCHI (1994), respectively. This results from that less samplings were conducted from 12:00 November 17 to 00:00 November 18 (GMT) and 12:00 November 24 to 00:00 November 25 (GMT), during which relatively high *in vivo* fluorescence occurred (ODATE & FUKUCHI 1994). On the other hand, the highest concentration of the present study is about a half of that of ODATE & FUKUCHI (1994). This discrepancy seems to be attributed to the difference of sampling resolution of both studies. That is, ODATE & FUKUCHI (1994) collected data every five minutes, while samples were taken with three to four times a day in the present study as the previous JARE cruises. The present results are comparable to the observations conducted by the previous JAREs during the same season (see ODATE & FUKUCHI 1994).

Chlorophyll $a$ concentration in the picoplankton fraction was also more abundant in the coastal waters of Southeast Asia than in the open waters as same as bulk chlorophyll $a$. Percent contribution of picophytoplankton was, however, less in the former than in the latter. In particular, the percent contribution of picoplankton fraction was lowest in Lombok Strait, where bulk chlorophyll $a$ was most abundant. These results are due to that increases of micro- and nano­phytoplankton were larger than that of picophytoplankton, being consistent with the general trend that an increase of total phytoplankton abundance results from that of larger cell sized phytoplankton (CHISHOLM 1992).

Surface cell density of cyanobacteria has been reported as $10^4$-$10^6$ cells ml$^{-1}$ in the open waters of the Pacific and the Atlantic Oceans (BLANCHOT et al. 1992, CAMPBELL & VAULOT 1993, MOREL et al. 1993, SHIMADA et al. 1993), while as $10^2$-$10^6$ cells ml$^{-1}$ in the coastal waters of Southeast Asia (ZEVENBOOM 1990) and as $10^4$-$10^6$ cells ml$^{-1}$ in the coastal waters of the Mediterranean Sea (Li et al. 1993). Our results are consistent with these findings. Moreover, the present cell counts
of other picophytoplankton in the open waters are similar to those of picoeukaryotes in the oligotrophic open waters \((6 \times 10^7 \text{ cells ml}^{-1})\) (BLANCHOT et al. 1992, CAMPBELL & VÁLOUT 1993, MOREL et al. 1993). However, they are one to three orders of magnitude lower than prochlorophytes abundance in the open waters of the Pacific Ocean \((10^8-10^9 \text{ cells ml}^{-1})\) (CAMPBELL & VÁLOUT 1993, SHIMADA et al. 1993) and the coastal water of the Mediterranean Sea \((10^3-10^4 \text{ cells ml}^{-1})\) (Li et al. 1993). Consequently, other picophytoplankton represent abundance of not prochlorophytes but picoeukaryotes. This may result from that prochlorophytes are difficult to be recognized by an ordinary epifluorescence microscope because of their small size and weak fluorescence (Li & Wood 1988).

In the present study multiple regression analysis was conducted based on chlorophyll \(a\) concentration in the picoplankton fraction and cell densities of cyanobacteria and other picophytoplankton to predict contribution of the two groups to the total picophytoplankton abundance. Coefficient of cyanobacteria was two orders of magnitude lower than that of other picophytoplankton, although cell density of the former was higher than that of the latter. Since the coefficient is considered as mean chlorophyll \(a\) content per cell, the present analysis implies that chlorophyll \(a\) content per cell is largely different between the two groups as suggested by ODATE et al. (1990).

Chlorophyll \(a\) content per cell of cyanobacterium \((Synechococcus)\) is estimated between 0.5 and 7.5 fg cell\(^{-1}\) (GLOVER et al. 1988, KANA & GLIBERT 1987, MOREL et al. 1993). Our result for cyanobacteria \((0.48 \pm 0.10 \text{ fg cell}^{-1})\), which was conducted with the surface water samples collected in the subtropical and tropical waters, is consistent with the lower estimates. The low chlorophyll content per cell occurs in near-surface water (PRÉZELIN et al. 1986) since the chlorophyll content becomes low at high incident irradiance (KANA & GLIBERT 1987, MOREL et al. 1993). Theoretical solar radiation received at the sea surface is calculated as about \(2,000 \mu \text{E m}^{-2} \text{s}^{-1}\) between \(10^\circ\) N and \(10^\circ\) S in November and December (LALLI & PARSONS 1993). Under such the high irradiance, the chlorophyll content becomes less than 2 fg cell\(^{-1}\) (KANA & GLIBERT 1987). Moreover, nitrate-depletion also makes it smaller in the surface water (ca. 0.5 fg cell\(^{-1}\)) (GLOVER et al. 1988). These environmental factors may result in the relatively small chlorophyll content of the present study.

From the multiple regression analysis, cellular chlorophyll \(a\) of other picophytoplankton is estimated as \(34 \pm 6.6 \text{ fg cell}^{-1}\). FURUYA (1990) established relationships between cell volume and chlorophyll \(a\) per unit cell volume for eukaryotes as follows,

\[
\log (\text{pg chlorophyll} \ a \ \mu \text{m}^{-3}) = 0.27 \log (\mu \text{m}^3) - 1.49.
\]

Using this equation, equivalent spherical diameter of phytoplankton, which contain 34 fg chlorophyll \(a\) per cell, is calculated as ca. 1.3 \(\mu\)m. This is consistent with the present size-fractionation, but is about two times larger than diameter of \(Prochlorococcus\) \((0.59 \mu \text{m})\) determined by MOREL et al. (1993), further suggesting that other picophytoplankton in the present study does not represent prochlorophytes.

The relatively large intercept predicted from the multiple regression analysis \((=0.032 \mu \text{g} \text{l}^{-1})\) may be explained by abundance of prochlorophytes. Chlorophyll
Fig. 4. Estimated picophytoplankton community structure in the surface waters along the first leg of the icebreaker Shirase in terms of chlorophyll $a$ concentration (a) and cell density (b). Picoeukaryotes represent other picophytoplankton in the present study. In this estimation one data collected from Station 15 is eliminated, since the sum of chlorophyll $a$ due to cyanobacteria and picoeukaryotes exceed picophytoplankton chlorophyll $a$. Date is expressed with GMT.

$\alpha$ concentration in picoplankton fraction can be expressed as follows,

\[
\text{Chlorophyll } a = n_1 \cdot c_1 + n_2 \cdot c_2 + n_3 \cdot c_3
\]

where $n_1$ to $n_3$ and $c_1$ to $c_3$ are cell densities and mean cellular chlorophyll $a$ contents of cyanobacteria, picoeukaryotes (= other picophytoplankton in the present study), and prochlorophytes, respectively. Although the major photosynthetic pigment of prochlorophytes is divinyl-chlorophyll $a$ and chlorophyll $a$ is absent, the ordinal fluorometry for determination of chlorophyll $a$ can not distinguish them (GOERICKE & WELLSCHMeyer 1993). The cell densities of the former two groups have been shown and the mean cellular chlorophyll $a$ contents of them are
predicted based on the multiple regression analysis. Then, chlorophyll $a$ abundance due to prochlorophytes can be estimated, subtracting chlorophyll $a$ due to cyanobacteria and picoeukaryotes from chlorophyll $a$ in the picoplankton fraction (Figure 4a). In this estimation, chlorophyll $a$ due to cyanobacteria and picoeukaryotes exceeds the picoplankton chlorophyll $a$ in this fraction with only one sample in the coastal waters in Southeast Asia (Station 15). This data is eliminated from the following calculation. The averaged contribution of prochlorophytes to the picoplankton chlorophyll $a$ is 58 ± 7.3 and 48 ± 6.2% in the western North Pacific and the eastern Indian Ocean, respectively, while relatively small contribution (30 ± 14%) is noted in the coastal waters of Southeast Asia. Since 73% of the total chlorophyll $a$ was due to picophytoplankton in the western North Pacific and the eastern Indian Ocean (Table 1), prochlorophytes contribute 42 and 35% of the whole chlorophyll $a$ in the respective waters. The similar percentage has been established by GOERICKE & WELSCHMEYER (1993) in the Sargasso Sea.

Assuming a mean cellular chlorophyll $a$ content of prochlorophytes of 1.4 fg cell$^{-1}$ (MOREL et al. 1993), their cell density is estimated as 23 ± 5.0 and 19 ± 1.5 \times 10^3$ cells ml$^{-1}$ in the open waters of the western Pacific and the eastern Indian Ocean, respectively. The similar cell density was founded in the surface water of the western North Pacific Ocean by SHIMADA et al. (1993). Prochlorophytes are likely to contribute 91 ± 1.9 and 82 ± 6.3% of picophytoplankton cell density, whereas the less contribution (35 ± 23%) is noted in the coastal waters of Southeast Asia (Figure 4b). Since prochlorophytes seem to be an important component in primary producer, it is necessary to directly evaluate their abundance and growth rates for further understanding of marine ecosystems.

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