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Note

Surface distribution and abundance of small-sized phytoplankton in the western and central subarctic North Pacific and the Bering Sea in winter

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Recent studies have reported that small-sized phytoplankton significantly contribute to phytoplankton biomass and productivity in the subarctic North Pacific (Booth et al. 1993; Welschmeyer et al. 1993; Boyd et al. 1995a, b; Odate 1996; Shiomoto et al. 1997, 1999c) and the Bering Sea (Odate 1996; Shiomoto et al. 1999b; Shiomoto 1999; Sukhanova et al. 1999). Small-sized phytoplankton, that is, pico- and nanophytoplankton, are divided into *Synechococcus*, *Prochlorococcus* and eukaryotic phytoplankton. In the oceanic region of the eastern subarctic North Pacific, the Alaskan Gyre, cell densities of *Synechococcus* and autotrophic nanoflagellates in summer (magnitudes of 10^6–10^7 cells l^{-1}) were similar to those in winter (magnitude of 10^6 cells l^{-1}) (Boyd et al. 1995a). Similar cell densities were also observed in summer for *Synechococcus* and eukaryotic small-sized phytoplankton (magnitudes of 10^6–10^7 cells l^{-1}) in the oceanic region of the western subarctic North Pacific (Odate et al. 1990) and in the central subarctic North Pacific (Ishizaka et al. 1994). In the oceanic region of the Bering Sea, cell densities of nano- and picophytoplankton were reported to be of a magnitude of 10^5–10^6 cells l^{-1} in summer (Sukhanova et al. 1999). Cell densities of small-sized phytoplankton seem to be higher in the subarctic North Pacific than in the Bering Sea in summer. However, cell densities of the small-sized phytoplankton have not yet been reported in the western and central subarctic North Pacific and the oceanic Bering Sea in winter. Thus, observation of the cell densities of small-sized phytoplankton in these subarctic regions in winter will reveal their seasonal variations in the subarctic North Pacific and the Bering Sea, and their regional variations throughout the subarctic regions in winter.

Hence, we observed the distribution and abundance of cell densities of small-sized phytoplankton in the subarctic North Pacific and the Bering Sea in winter. In this paper, combining the results from previous studies, we elucidate the seasonal and regional variations in the cell density of small-sized phytoplankton in the subarctic North Pacific and the Bering Sea.

Water sampling was conducted during cruises of the R/V *Kaiyo Maru*, which belongs to the Fisheries Agency of Japan, in February 1998 (Fig. 1). Surface seawater samples were collected using an acid-cleaned plastic bucket. Flow cytometry was used for analysing small-sized phytoplankton, because this technique provides varied information about the plankton quickly and precisely (e.g. cell density, phytoplankton groups and cell size) (e.g. Olson et al. 1993). Seawater samples (5 ml) were pooled in polyethylene bottles, and fixed with 1.2% (final concentration) formaldehyde neutralized by potassium hydroxide. After storage for about 20 min in a refrigerator, the samples were frozen and kept at −80°C on the ship for about one month and at −30°C in the laboratory for about one year until analysis. Chemical fixation (Partensky et al. 1996), and short- and long-term storage (5–260 d) in liquid N_2 (Vaulot et al. 1989) caused a decrease of picophytoplankton cell density. The cell densities reported in our study should be considered conservative estimates.

The samples were analysed with an EPICS-Elite-ESP flow cytometer (Beckman Coulter) equipped with a 15-mW argon laser exciting at 488 nm. According to Olson et al. (1993), we measured forward light scatter (FLS, an indicator of size), orange fluorescence from phycoerythrin (560–590 nm) and red fluorescence from chlorophyll (>660 nm) after excitation by 488-nm light. Data were collected in list mode, then analyzed on a personal computer using the WinMDI, version 2.7, free software (Joseph Trotter). We recognized populations of various kinds of small-sized phytoplankton and estimated their equivalent spherical diameters, by comparing with 0.5, 1, 2, 3 and 6-μm sized fluorescent beads ("Fluoresbrite", Polysciences, Inc., Warrington, PA). *Synechococcus* cells are easily recognized by the orange fluorescence of their phycoerythrin, whereas *Prochlorococcus* have smaller scatter signals than *Synechococcus* and have only red fluorescence (e.g. Olson et al. 1990). The eukaryotic phytoplankton also have red fluores-

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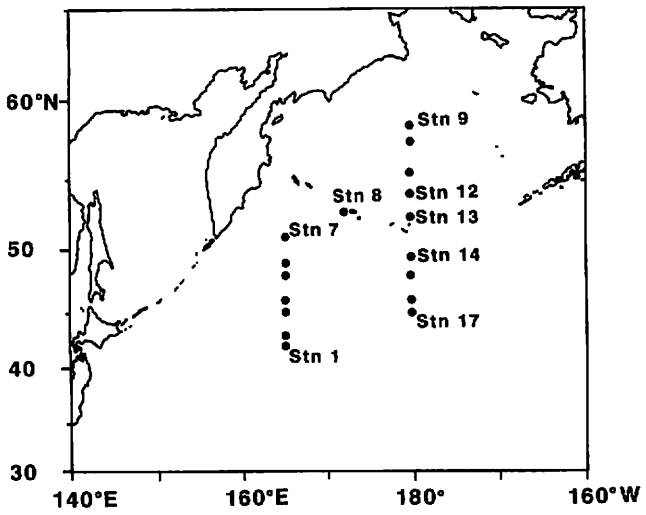

Fig. 1. Location of sampling stations in the subarctic North Pacific and the Bering Sea, February 1998.

cence but have larger scatter signals than *Synechococcus*, so they are easily distinguished from *Prochlorococcus*. We divided the phytoplankton into two groups on the basis of their flow cytometric signatures: *Synechococcus* and eukaryotic phytoplankton. *Prochlorococcus* was not observed, possibly because of growth limitation due to the low temperature (<17°C) (Olson et al. 1990). Cell numbers were counted at a calibrated flow rate of 60 μl min^{-1} for 5 min. Five samples were weighed daily before and after analysis at selected times, and the flow rate was corrected based on the difference in weight reduction between the time intervals. For *Synechococcus* and eukaryotic phytoplankton, precision of the counting of cell density was estimated at better than 4% as the coefficient of variation across three replications, using five coastal samples.

The water temperature, nitrite+nitrate concentration and chlorophyll-*a* (Chl-*a*) concentrations at the surface, and salinity at 10-m depth were cited from a cruise report (Anonymous 1999). The temperature and salinity were measured with a Sea Bird CTD (SBE 9plus) and a Guild Line salinometer (Auto Sal Model 8400A), respectively. The nutrient concentrations were determined with a Bran and Luebbe Auto Analyser II immediately after sample collection. Seawater samples (0.5 liter) for determining the Chl-*a* concentrations were filtered on Whatman GF/F filters. Chl-*a* concentrations were measured on board with a Shimadzu RF-5000 flourophotometer calibrated with commercial Chl-*a* (Sigma Chemical) after extraction with 90% acetone (Parsons et al. 1984).

The subarctic North Pacific is defined as the area north of the Subarctic Boundary, denoted by a vertical 34.0 isohaline in the surface layers (Dodimead et al. 1963). Stations with salinity less than 34.0 were chosen for our analysis (Fig. 1). The stations were divided into the western subarctic North Pacific (WSNP, Stns 1–7), vicinity of the Aleutian Islands (AI, Stns 8 and 13), the Bering Sea (BS, Stns 9–12) and the central subarctic North Pacific (CSNP, Stns 14–17).

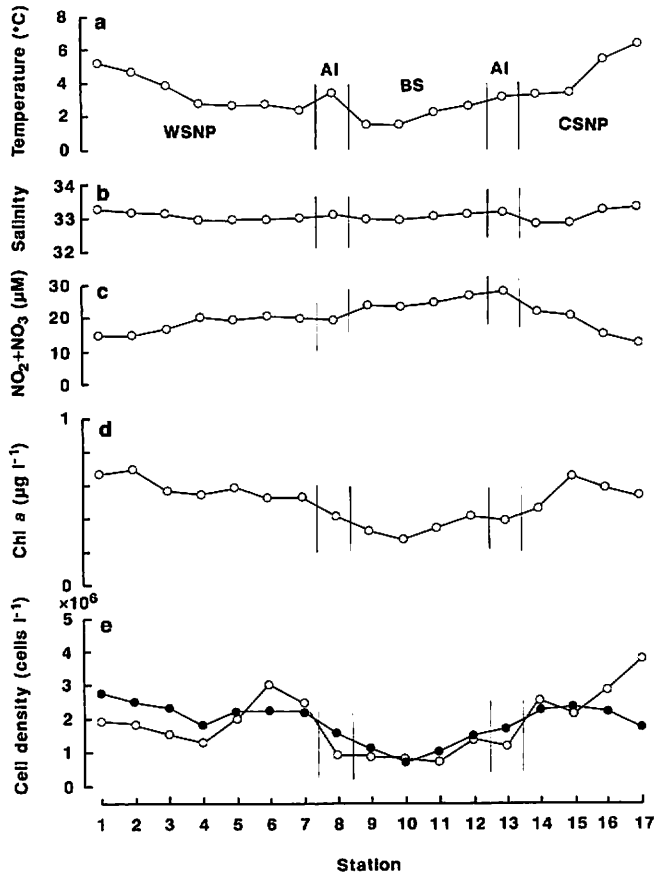

Fig. 2. Variations in surface water temperature (**a**), salinity at 10 m depth (**b**), surface nitrite+nitrate concentration (**c**), surface chlorophyll-*a* concentration (**d**) and surface cell densities of *Synechococcus* (open circles) and eukaryotic phytoplankton (solid circles) (**e**) in the subarctic North Pacific and the Bering Sea, February 1998. WSNP: the western subarctic North Pacific (Stns 1–7); AI: vicinity of the Aleutian Islands (Stns 8 and 13); BS: the Bering Sea (Stns 9–12); CSNP: the central subarctic North Pacific (Stns 14–17).

Water temperatures tended to be lower in the BS than in the other regions (Fig. 2a). Salinity was nearly uniform, around 33, at all stations (Fig. 2b). Nitrite+nitrate concentrations tended to be higher in the BS and AI than in the WSNP and CSNP (Fig. 2c). Chl-*a* concentrations ranged from 0.28 to 0.70 μg l^{-1} (Fig. 2d). Concentrations tended to be higher in the WSNP and CSNP than in the AI and BS. Mean±SD of Chl-*a* concentration was 0.59±0.07 μg l^{-1} (n=7) in the WSNP, 0.57±0.08 μg l^{-1} (n=4) in the CSNP, 0.41 μg l^{-1} (n=2) in the AI and 0.35±0.06 μg l^{-1} (n=4) in the BS. The mean values in the WSNP and CSNP were almost equal, and were about 1.5 times higher than those in the AI and BS.

On the basis of a comparison of flow cytometric signatures of various sized beads and samples, the equivalent spherical diameters of *Synechococcus* and eukaryotic phytoplankton were between 0.5 and 1 μm and ca. 1 and 3 μm, respectively. Cell densities of *Synechococcus* and eukaryotic phytoplankton ranged from 1.31 to 3.07×10^6 cells l^{-1} and from 1.90 to 2.83×

Table 1. Mean±standard deviation (σ_{n-1}) of cell densities ($\times 10^3$ cells l^{-1}) of *Synechococcus* and small-sized eukaryotic phytoplankton at the surface in the western North Pacific (WSNP), the central subarctic North Pacific (CSNP), vicinity of the Aleutian Islands (AI) and the Bering Sea (BS), February 1998. The number of data point is given in parentheses.

Region	*Synechococcus*	Eukaryotic phytoplankton
WSNP	2.03±0.58 (7)	2.34±0.29 (7)
CSNP	2.85±0.71 (4)	2.16±0.29 (4)
AI	1.10 (2)	1.66 (2)
BS	0.95±0.30 (4)	1.13±0.31 (4)

10^6 cells l^{-1}, respectively, in the WSNP; from 2.17 to 3.82× 10^6 cells l^{-1} and from 1.73 to 2.38×10^6 cells l^{-1}, respectively, in the CSNP; 0.96×10^6 cells l^{-1} and 1.56×10^6 cells l^{-1}, respectively, at Stn 8 and 1.24×10^6 cells l^{-1} and 1.76×10^6 cells l^{-1}, respectively, at Stn 13 in the AI; from 0.73 to 1.38× 10^6 cells l^{-1} and from 0.77 to 1.53×10^6 cells l^{-1}, respectively, in the BS (Fig. 2e). There were not many differences in cell densities of both phytoplankton groups between the WSNP and CSNP, and between the AI and BS, whereas there were significant differences between the former two regions and the latter two regions (Mann–Whitney *U*-test, $p<0.01$, two-tailed test). The mean values of both phytoplankton groups in the WSNP and CSNP were 1.3–3.0 times higher than those in the AI and BS (Table 1).

A positive linear relationship was observed for *Synechococcus* between cell density and Chl-*a* concentration when the cell densities were less than 2.20×10^6 cells l^{-1}, whereas Chl-*a* concentrations were nearly constant when the cell densities were more than 2.40×10^6 cells l^{-1} (Fig. 3a). For eukaryotic phytoplankton, a significant positive linear relationship was observed between both parameters throughout the full range of cell densities (Fig. 3b). The ratio of contribution, r^2, for a positive linear relationship between cell densities of eukaryotic phytoplankton and Chl-*a* concentrations was high (0.83), indicating that cell densities were significantly correlated to and probably affected Chl-*a* concentrations. Odate et al. (1990) showed that the cell density of small-sized eukaryotic phytoplankton significantly contributed to the variation in Chl-*a* concentration in the western subtropical and subarctic North Pacific in summer. The same result was obtained in this study for the winter period.

The slope of the straight line in Fig. 3b is 2.0×10^{-7} μg Chl *a* cell^{-1}, indicating the mean Chl-*a* content per cell of the eukaryotic phytoplankton. Furuya (1990) established the relationship between cell volume and Chl-*a* per unit cell volume for eukaryotes as follows:

$$\text{Log (pg Chl } a\ \mu\text{m}^{-3}) = -0.27\ \text{Log}\ (\mu\text{m}^3) - 1.49.$$

Using this equation, the equivalent spherical diameter of phytoplankton that contain 2.0×10^{-7} μg Chl *a* cell^{-1} is calculated as 2.9 μm. This is consistent with the cell sizes obtained

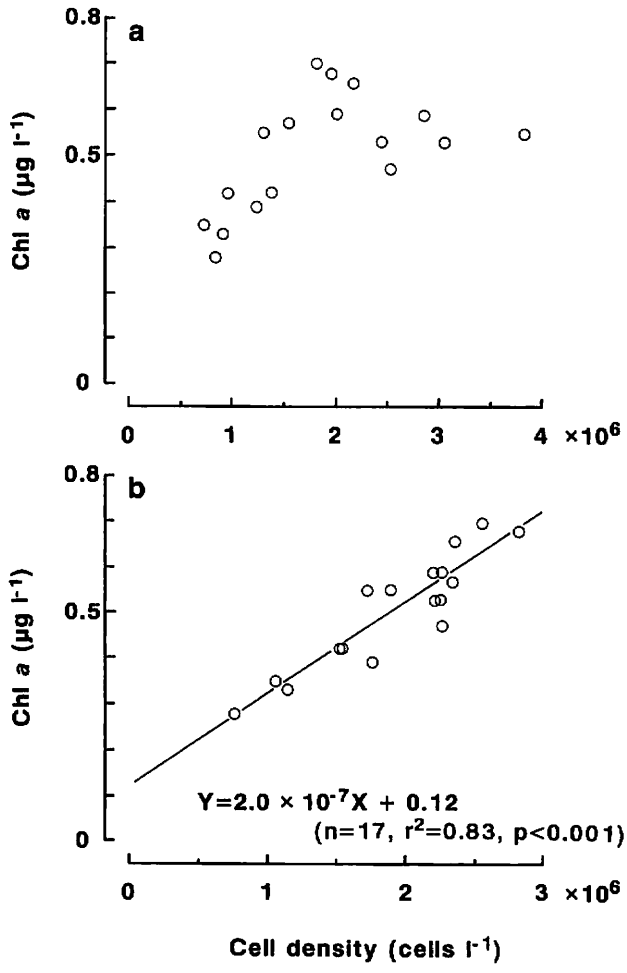

Fig. 3. Relationships between cell densities (cells l^{-1}) of *Synechococcus* (**a**) and eukaryotic phytoplankton (**b**) and chlorophyll-*a* concentration (μg l^{-1}) at the surface. The straight lines were obtained by the least squares method.

by flow cytometry (mostly 1–3 μm). Moreover, if the cell densities in our study were underestimated by more than one order of magnitude compared with actual cell density, due to long-term sample storage at −30°C, the equivalent spherical diameters of phytoplankton calculated using Furuya's equation become less than 1.0 μm. This is inconsistent with the cell sizes obtained by flow cytometry. Accordingly, cell densities of the eukaryotic phytoplankton were unlikely to be seriously underestimated in our study.

The cell densities of *Synechococcus* and eukaryotic picophytoplankton in the surface waters were reported to be of the magnitude of 10^6–10^7 cells l^{-1} during summer in the oceanic region of the western (Odate et al. 1990) and central (Ishizaka et al. 1994) subarctic North Pacific. Their cell densities in the WSNP and CSNP in our study (magnitude of 10^6 cells l^{-1}, Fig. 2e) are similar to the summertime values. In the oceanic region of the Bering Sea, cell densities of nano- and picophytoplankton were of the magnitude of 10^5–10^6 cells l^{-1} in summer (Sukhanova et al. 1999). Cell densities of small-sized phytoplankton in the BS in our study (magnitudes of 10^5–10^6

cells l^{-1}, Fig. 2e) are also similar to the summertime values. Cell densities of small-sized phytoplankton were noticed to be similar in summer and winter in both these subarctic regions, a pattern which is similar to that observed in the eastern subarctic North Pacific (Boyd et al. 1995a). Considering that summer and winter are at the extremes of the four seasons, it is unlikely that the cell density of small-sized phytoplankton varies greatly with season in the subarctic North Pacific and the Bering Sea.

In contrast, combining data from the present and previous studies, we noticed that cell density of small-sized phytoplankton tended to be higher in the subarctic North Pacific (magnitudes of 10^6–10^7 cells l^{-1}) than in the Bering Sea (magnitudes of 10^5–10^6 cells l^{-1}) in both summer and winter. It is well known that water temperature plays an important role in the variation of cell densities in small-sized phytoplankton (Murphy & Haugen 1985; Waterbury et al. 1986; Odate 1989; Booth et al. 1993). By using all of the data, significant positive linear relationships were observed between water temperature and cell densities of *Synechococcus* and the eukaryotic phytoplankton ($p<0.05$, Fig. 4). The surface temperature is higher in the subarctic North Pacific than in the Bering Sea in summer and winter (Anonymous 1993, Fig. 2a). Is water temperature a primary factor leading to the regional difference in their cell densities between the subarctic North Pacific and the Bering Sea? Significant seasonal variations were not noticed in the cell density of small-sized phytoplankton in the subarctic North Pacific and the Bering Sea (see above). Moreover, examining the relationship between water temperature and cell densities of small-sized phytoplankton in the subarctic North Pacific (Stns 1–7 and 14–17) and the Bering Sea (Stns 9–12), significant relationships were not found in either region ($p>0.2$) (Fig. 4). The significant linear relationship between the two parameters obtained using all the data in our study is considered to be artifact. Temperature is thus unlikely to be related to the observed differences in cell densities between the two regions.

What are the factors leading to the regional differences in cell densities of small-sized phytoplankton between the subarctic North Pacific and the Bering Sea? In summer, Taniguchi (1984) showed that the biomass of microzooplankton in the surface layers was higher in the basin waters of the Bering Sea than in the subarctic North Pacific. Microzooplankton graze on small-sized phytoplankton (e.g. Stockner 1988), implying that such grazing pressure may greatly influence the cell densities of small-sized phytoplankton. In winter, Shiomoto et al. (1999a) suggested that daily primary production was lower in the Bering Sea than in the subarctic North Pacific, and that respirative loss by the phytoplankton throughout the water column was larger in the former region than in the latter region. Thus, we can submit more intense microzooplankton grazing in summer, and lower daily primary production with higher respirative loss in winter as factors leading to the regional difference in cell densities of small-sized phytoplankton between the subartcic North Pacific and the Bering Sea.

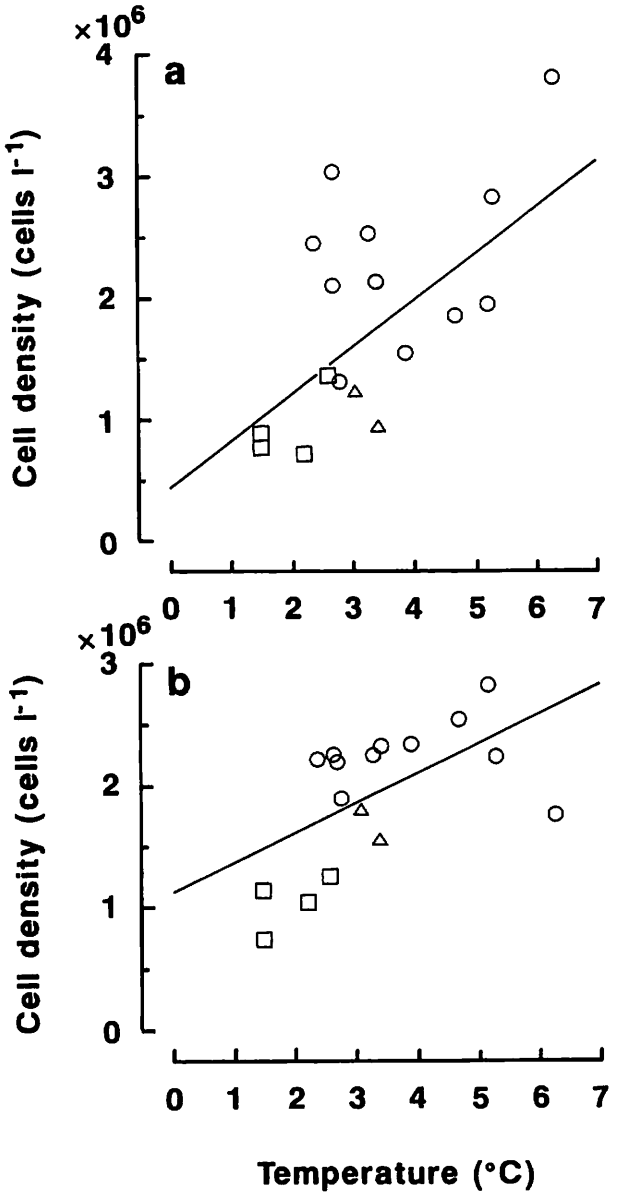

Fig. 4. Relationships between the water temperature (°C) and cell densities (cells l^{-1}) of *Synechococcus* (**a**) and eukaryotic phytoplankton (**b**) at the surface. The straight lines were obtained by the least squares method, using all of the data. (a): $Y=4.2\times10^5X+4.6\times10^5$ ($n=17$, $r^2=0.41$, $p<0.01$); (b): $Y=2.4\times10^5X+11.2\times10^5$ ($n=17$, $r^2=0.33$, $p<0.05$). ○: the western and central subarctic North Pacific (Stns 1–7 and 14–17); △: vicinity of the Aleutian Islands (Stns 8 and 13); □: the Bering Sea (Stns 9–12). The Spearman rank correlation coefficient, r_s, was calculated between the temperature and cell densities. (a): $r_s=0.12$, $n=11$ and $p>0.69$ (two-tailed test) in the western and central subarctic North Pacific, and $r_s=0.32$, $n=4$ and $p>0.58$ in the Bering Sea; (b): $r_s=0.28$, $n=11$ and $p>0.37$ in the western and central subarctic North Pacific, and $r_s=0.63$, $n=4$ and $p>0.27$ in the Bering Sea.

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