

## The distribution of picophytoplankton across Kuroshio Current off the Western Pacific Coast of Japan

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**Abstract :** The distribution of picophytoplankton ( $<3\ \mu\text{m}$ ) across the Kuroshio Current off the Pacific coast of Japan was studied using epifluorescence microscopy. Three groups of picophytoplankton were delineated: *Synechococcus*, *Prochlorococcus* and eukaryotic picophytoplankton. The two prokaryotic picoplankton, *Synechococcus* and *Prochlorococcus*, had a differential pattern as their distribution. The former was dominant in the main body of the Kuroshio Current, while the latter was dominant on the south side of the Kuroshio seaward boundary. The factors that contributed most to their distribution patterns were suggested to be temperature and nutrient levels within the mixed layer. Maximum concentrations of eukaryotic picophytoplankton were found at the depths of the subsurface chlorophyll maximum. *Synechococcus* occurred abundantly in the surface mixed layer of Kuroshio water when nutrients were supplied by frontal eddy pumping or autumn deepening of mixed layer. *Prochlorococcus* (low-light adapted type) distribution was limited in the subsurface depths with higher temperature than  $20^{\circ}\text{C}$ .

### 1. Introduction

The discovery of the dominance of pico-sized ( $0.2-2\ \mu\text{m}$ ) oxygenic photosynthetic phytoplankton in oligotrophic regions led to a general reconsideration of the structure of marine ecosystems (e.g. Li *et al.*, 1983). The picophytoplankton that tend to dominate these oligotrophic regions are generally known to be prokaryotic genera belonging to *Synechococcus* (JOHNSON and SIEBURTH, 1979; WATERBURY *et al.*, 1979) and *Prochlorococcus* (CHISHOLM *et al.*, 1988). In contrast, the eukaryotic picophytoplankton is much more diverse and evidently composed of organisms that belong to several algal divisions (e.g. SMITH and HOBSON, 1994), including Bolidophyceae (GUILLOU *et al.*, 1999) and Pelagophyceae (ANDERSON *et al.*, 1993) in Chromophyta, and Pedinophyceae (MOESTRUP, 1991) and Prasinophyceae (e.g. EIKREM and THRONDSSEN, 1990; CHERÉTIENNOT-DINET *et al.*, 1995) in Chlorophyta.

Since the study of FURUYA and MARUMO

(1983), there have been many studies aimed at assessing the relative abundance and species composition of the smaller phytoplankton size classes off Japan in the North Pacific. TAKAHASHI *et al.* (1985) found that picophytoplankton distributed anywhere from 20 to 100% of the total chlorophyll *a* (Chl *a*) biomass, off the Sanriku coast of Japan. ODATE *et al.* (1990) compared the distribution of picocyanobacteria and other picophytoplankton along  $155^{\circ}\text{E}$  meridian by epifluorescence microscopy. Shimada *et al.* (1995a) found that picophytoplankton accounted for about 25% of the total Chl *a* biomass in Suruga Bay and measured *Prochlorococcus* abundance of  $2.5 \times 10^4$  cells  $\text{ml}^{-1}$  by flow cytometry.

The Pacific coast of Japan is highly influenced by the northward flowing Kuroshio Current. Because the Kuroshio Current is the border between two fairly diverse water masses: eutrophic coastal and oligotrophic offshore waters, it is expected that the phytoplankton composition should also change across the current. This change in the phytoplankton community composition should be most notable in the picophytoplankton size

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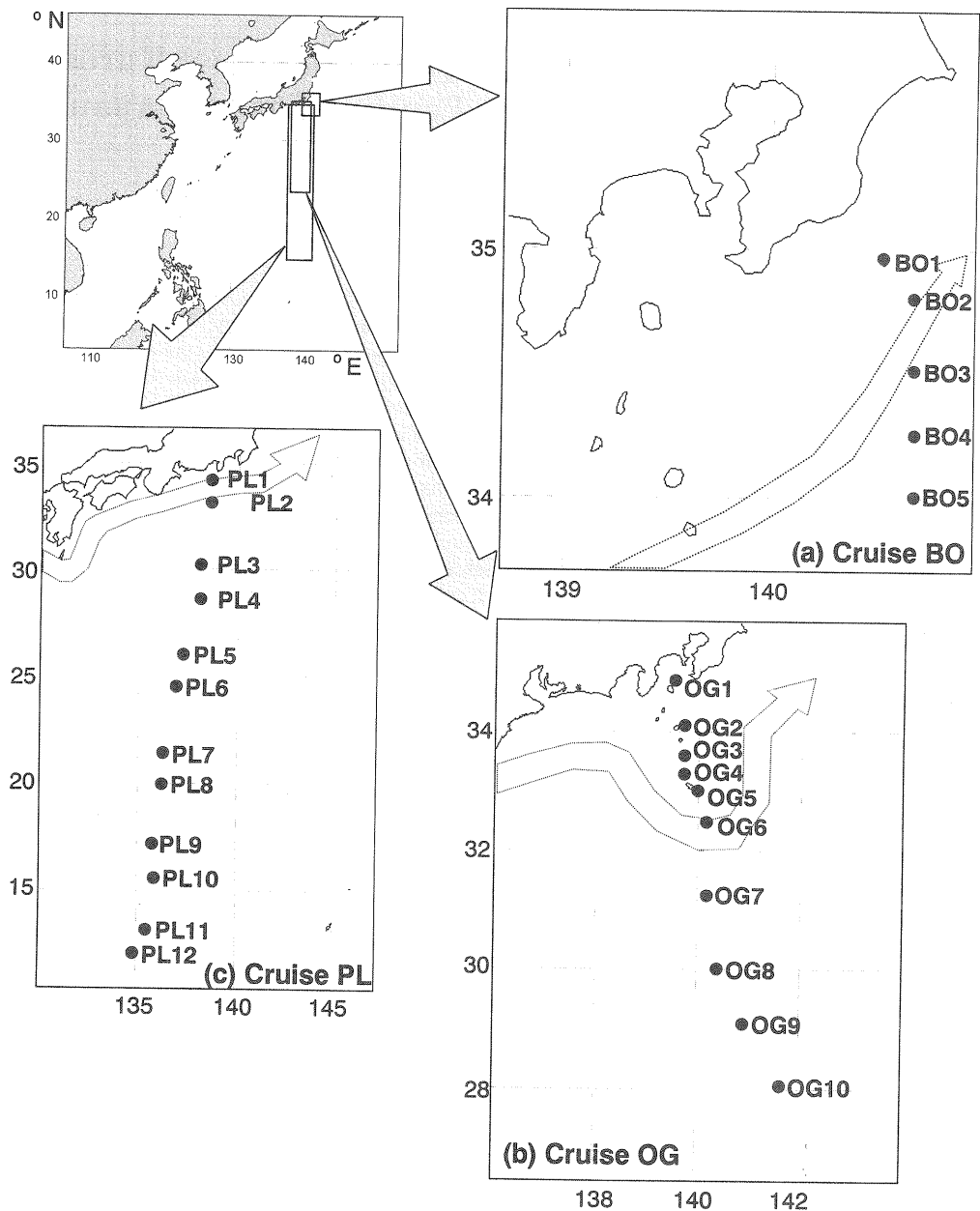


Fig. 1. The stations sampled in the western Pacific Ocean in (a) cruise BO: October 1994, (b) cruise OG: June 1995, and (c) cruise PL: March 1998. A broken line corresponds to Kuroshio Paths measured by the Hydrographic Department of the Japanese Coast Guard.

range, because of their high relative dominance in oligotrophic regions compare to coastal regions. However, there have been no studies conducted to ascertain the biomass and composition of picophytoplankton across the

Kuroshio Current, systematically.

In the present study, we examined the distribution of the two prokaryotic picophytoplankton groups, *Synechococcus* and *Prochlorococcus*, and also the eukaryotic picophyto-

plankton across the Kuroshio Current. Recently, some evidences for niche partitioning has been observed between the prokaryotes, *Synechococcus* and *Prochlorococcus* (OLSON *et al.*, 1990; CAMPBELL *et al.*, 1994; LIU *et al.*, 1998), however, it is still not clear what environmental parameters separate these two groups. Here, we discuss the relationship between the distribution of these groups and the water properties—temperature, salinity, strength of the stratification, and nutrients levels—throughout the Kuroshio Current region.

## 2. Methods

### *Study area*

Three cruises were carried out on the research and training vessels of the Tokyo University of Fisheries: off the Bouso Peninsula in October 1994 (Cruise BO) on the *Sinyo-maru*, over the Izu Ridge down the south to the Ogasawara Islands during June 1995 (Cruise OG) on the *Seiyo-maru*, and the south of Izu Ridge down the south to Palau Island during March 1998 (Cruise PL) on the *Umitaka-maru*. Station locations for each survey line and the position of the Kuroshio (Hydrographic Department of Japan Coast Guard, 1994, 1995 and 1998) are shown in Fig. 1.

### *Hydrographic observation*

Hydrographic data were taken by OCTOPUS system (OCTO-Parameter Underwater Sensors; ISHIMARU *et al.*, 1984). In order to estimate the water column stability in the surface layer, the Brunt-Väisälä frequency ( $N$ ), in radians  $s^{-1}$ , one of the important descriptors of the oceanic vertical structure (MILLARD *et al.*, 1990), was calculated from vertical profiles of temperature and salinity:

$$N = \sqrt{\left(\frac{g}{\rho}\right) \frac{\Delta\rho}{\Delta z}},$$

where  $g$  is the acceleration due to gravity,  $\rho$  is density and  $z$  is depth. Values of  $N$  larger than  $0.01 s^{-1}$  roughly corresponded to the position of seasonal thermocline (see Fig. 1 in MILLARD *et al.*, 1990).

Water samples for the analysis of phytoplankton abundance and species composition were taken with 1.7L Niskin bottles attached

on a Rosette Multi-Sampler, which were mounted on the OCTOPUS system. Inorganic nitrate was analyzed using a segmented flow colorimetric auto analyzer (after STRICKLAND and PARSONS, 1972). 200 ml of seawater from each Niskin bottle was filtered onto a 25 mm diameter glass fiber filter (Watmann Co., GF/F) at  $<150$  mm-Hg pressure for later chlorophyll  $a$  (Chl  $a$ ) measurement. Chl  $a$  was extracted from the filters by  $N,N$ -dimethylformamide for one day at  $-20^{\circ}C$  (SUZUKI and ISHIMARU, 1990), and then stored at  $-20^{\circ}C$  until measurement. Chl  $a$  was determined using a Turner Designs 10R fluorometer following the procedures of STRICKLAND and PARSONS (1972). Relative irradiance within the water column was calculated from an attenuation coefficient using mean water column Chl  $a$  concentration after RILEY (1975).

### *Plankton sampling*

For picophytoplankton analysis, 5–25 ml of water was taken from each Niskin bottles. The sample was immediately fixed in glutaraldehyde, with a final concentration of 1.0 %, and stored under cool ( $<4^{\circ}C$ ) and dark conditions for at least 1 day, then filtered onto a 7 mm diameter,  $0.2 \mu m$  membrane filter (Millipore Co., JG type). The filter was then embedded between glass cover plates with glycerin-water (3 : 2, v/v) with agar (2% w/v) for preventing the filter from drying out. Reagent grade glycerin (Wako Pure Chem. Ind. Ltd.) and non-fluorescent, low-melting-point-temperature agar (Sigma Chemical Co., type IV) were used to make this agent. All of these processes were conducted under extremely dim light in order to avoid decay of the fluorescence of phytoplankton. Once fully embedded, the samples were stored in a freezer ( $<-20^{\circ}C$ ) for later counting and identification.

### *Enumeration of picoplankton*

Picophytoplankton were enumerated using an epifluorescence microscope (Olympus BH2-RFCA) with an IB (interference blue excitation) cube, that consisted of excitation, dichroic, and absorbance filters: EY495, DM505 and O515, respectively. All cell counting was completed within one month of

collection. Because fluorescence intensity depends on excitation intensity, the mercury lamps of the microscope (Ushio-102D, 100W) were replaced every 100 hours of use. This method was sensitive enough to detect a 0.2  $\mu\text{m}$  fluorescent bead (Polyscience, Inc., cat #09834). All picoplankton within 140 fields per slide, near the centerline of the filter, were counted and identified at a magnification of 1000.

*Synechococcus* was identified by its vivid yellow to orange fluorescence, a coccid to bacillus shape, and a size between 0.5 and 1.5  $\mu\text{m}$ . We also detected bright yellow cells, individually about 2–3  $\mu\text{m}$  in diameter, that were found both solitary and sometimes in aggregates of up to 20 cells, at 16 to 18°N, 135°W (Stns. PL 9 and PL 10 in cruise PL). Recently, NEVEUX *et al.* (1999) observed some non-motile round cells, about 2–3  $\mu\text{m}$  in size, containing unusual phycoerythrin (fluorescence emission peaks; 494, 564 nm), in the subtropical Pacific Ocean. These yellow cells that we observed, and those observed by NEVEUX *et al.* (1999) are possibly the same organisms as those observed previously by ISHIZAKA *et al.* (1994) and CAMPBELL *et al.* (1997), which were tentatively identified as *Synechocystis* (PARTENSKY *et al.* 1999a). *Prochlorococcus* was identified by its pale red to pale orange fluorescence, a coccid shape with a size of 0.6 to 1  $\mu\text{m}$ . Our method provided not only high contrast, but also low background light, thus *Prochlorococcus*, after the fluorescence was optically bleached, could be detected as a white spot shown in Fig. 2. This method was proved to distinguish a cultured *Prochlorococcus* strain GP2 from the western Pacific (Shimada *et al.*, 1995b). Eukaryotic phytoplankton were identified by the existence of a single red or deep red, cup-like or oval shaped chloroplast with green protoplasm and flagella, with cell size in the range of 1.2 to 3  $\mu\text{m}$ .

### 3. Results

#### *Water masses*

We categorized our study area into three regions: the neritic water on the landward side of the Kuroshio front (LKF), the Kuroshio Current body (KCB) and the water on the south

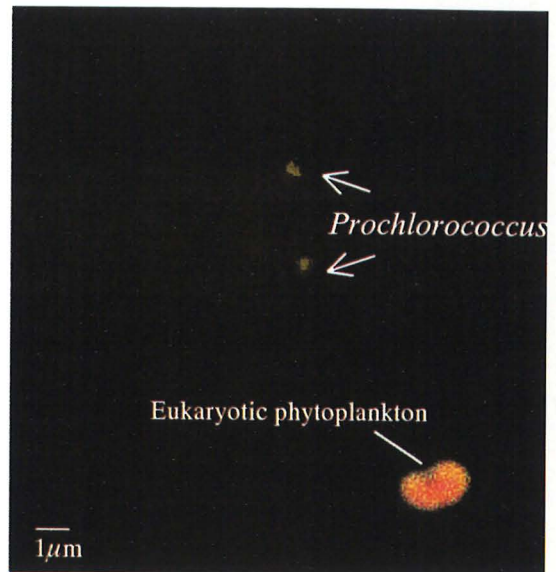


Fig. 2. Bleached *Prochlorococcus* cells recognized under an epifluorescence micrograph.

side of the Kuroshio seaward boundary (WSK). These water masses were determined by examination of vertical structure of temperature for each cruise (Figs. 3A, 4A and 5A). A good indicator of the cross-axis center of the Kuroshio Current body at the surface is the cross-axis position of where the 15°C isotherm intersects a depth of 200 m (*e.g.* KAWABE, 1985). The Kuroshio is also well defined by a sharp southward deepening of isotherms near the northern section in all years (*e.g.* HANAWA and HOSHINO, 1988). Thus, the Kuroshio axis is located between BO2 and BO3 at OG6 and between PL1 and PL2 for the three cruises, respectively. Our determination of the location of the Kuroshio Current axis coincides well with the locations reported in the Bulletin of the Kuroshio (Hydrographic Department of Japan Coast Guard, 1994, 1995 and 1998). During cruises BO and PL, the Kuroshio Current flowed between Miyake-jima and Hachijyo-jima, then passed straight out to the Bousou Peninsula. During cruise OG, the Kuroshio meandered around the Izu Islands and then turned off Aoga-shima. Stations BO1 and BO2 were LKF and stations BO4 and BO5 were WSK during the fall cruise. Stations OG1 to OG5 were LKF, and OG7 to OG10 were WSK in the

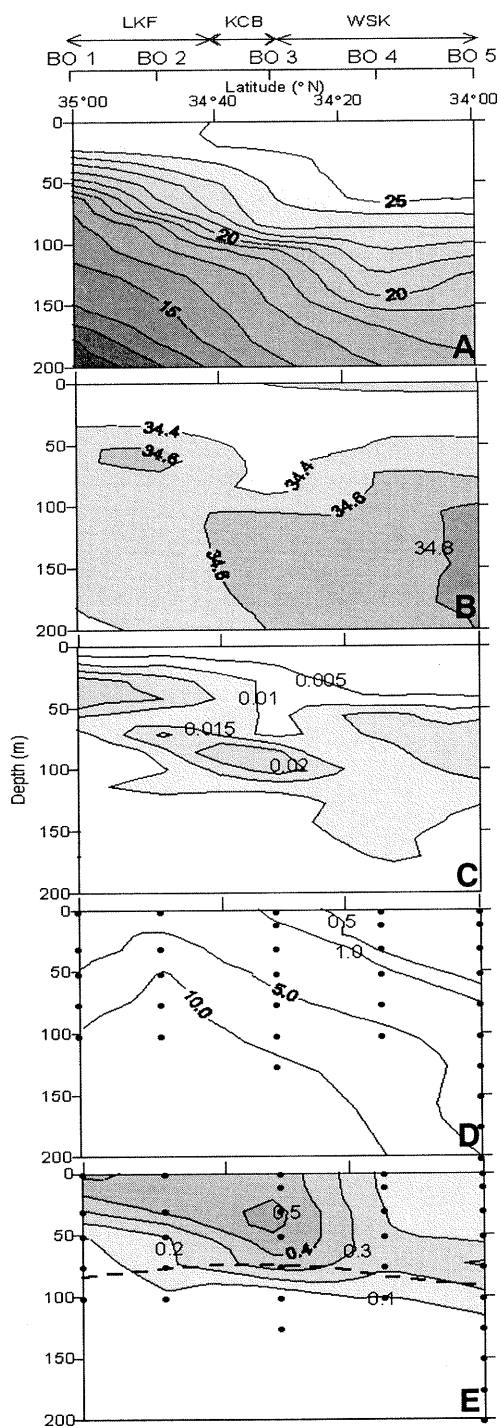


Fig. 3. Vertical sections of A) temperature ( $^{\circ}\text{C}$ ), B) salinity (PSU), C) stability based on Brunt-Väisälä frequency ( $\text{s}^{-1}$ ), D) nitrate ( $\mu\text{M}$ ), and E) Chl *a* ( $\mu\text{g l}^{-1}$ ) with the depth of the 1% relative light intensity level (dotted line) in the western Pacific Ocean in Cruise BO, October 1994.

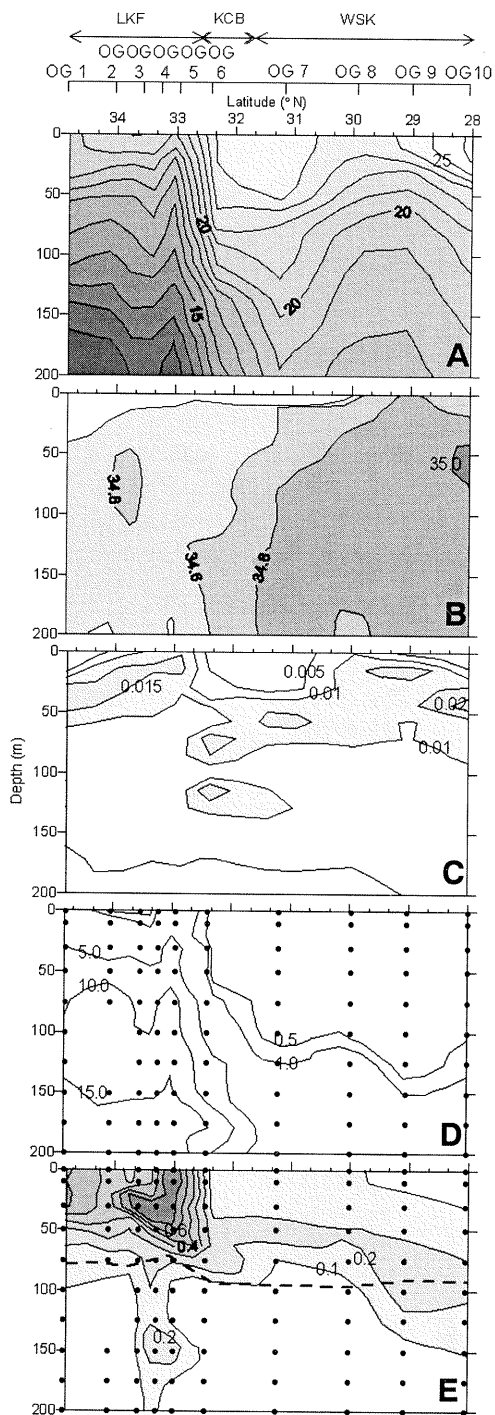


Fig. 4. Vertical sections of A) temperature ( $^{\circ}$ C), B) salinity (PSU), C) stability based on Brunt-Väisälä frequency ( $s^{-1}$ ), D) nitrate ( $\mu$ M), and E) Chl a ( $\mu$ g l $^{-1}$ ) with the depth of the 1% relative light intensity level (dotted line) in the western Pacific Ocean during Cruise OG, June 1995.

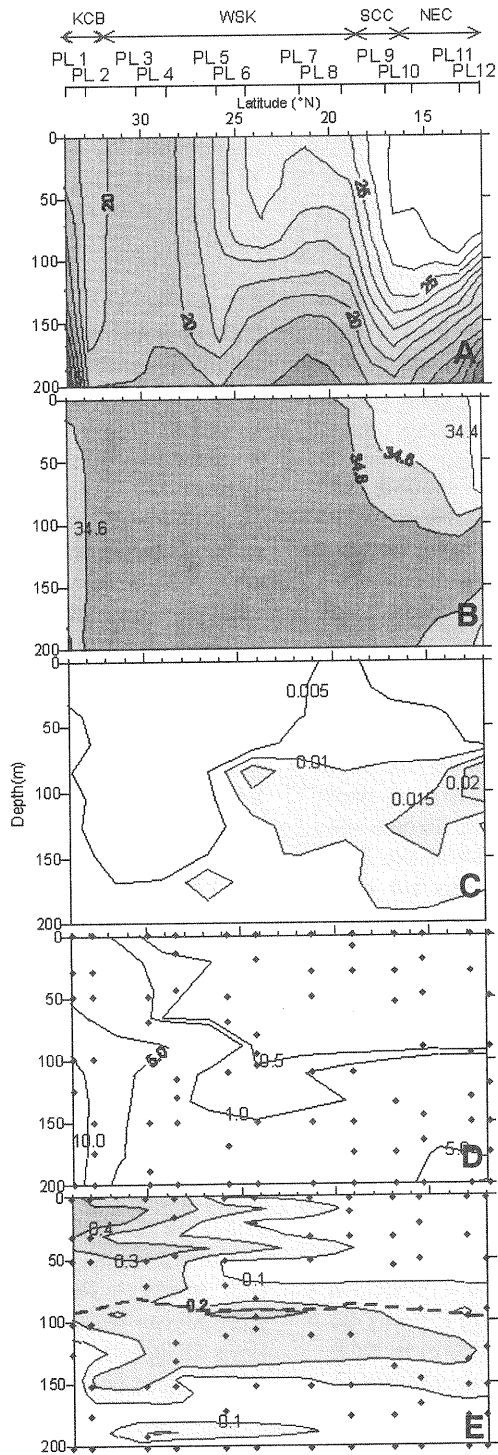


Fig. 5. Vertical sections of A) temperature ( $^{\circ}\text{C}$ ), B) salinity (PSU), C) stability based on Brunt-Väisälä frequency ( $\text{s}^{-1}$ ), D) nitrate ( $\mu\text{M}$ ), and E) Chl  $a$  ( $\mu\text{g l}^{-1}$ ), with the depth of the 1% relative light intensity level (dotted line) in the western Pacific Ocean in Cruise PL, March 1998.

spring cruise. During cruise PL, our stations extended southward, ranging from 12 to 32° N, and therefore transected two other currents, the Subtropical Countercurrent and the North Equatorial Current.

#### *Hydrography, Irradiance, and Nitrate and Chl a concentrations*

The distributions of temperature, salinity, Brunt-Väisälä frequency ( $N$ ), nitrate and Chl  $a$  concentrations, and the depth of the 1% relative light intensity at cruise BO, OG, and PL are shown in Figs. 3, 4 and 5, respectively.

#### Cruise BO

Surface water temperature was around 24°C in LKF and higher than 25°C in KCB and WSK. Deepening of the surface mixing layer had not yet occurred. The strong seasonal thermocline, shown by a value of  $N$  greater than  $0.02 \text{ s}^{-1}$ , was located between 30 and 50 m in LKF, around 80 m in KCB (a high  $N \sim 0.015 \text{ s}^{-1}$  here), and between 70 and 100 m in WSK. We found upwelled water with high salinity and high nitrate concentration at 50-m depth at BO2, which was probably caused by frontal eddy pumping such as reported for Gulf Stream meanders (Yoder *et al.*, 1981). However, the high nitrate concentration was not reflected by a high Chl  $a$  concentration. Nitrate was not depleted at Sta. BO3 in KCB, probably by the influence of upwelling water around BO2, and Chl  $a$  was high ( $0.4 \mu\text{g l}^{-1}$ ) at the surface with a subsurface chlorophyll maximum layer (SCM) above the strong thermocline. In WSK, nitrate was depleted in the surface layer and the SCM with a low Chl  $a$  concentration ( $< 0.2 \mu\text{g l}^{-1}$ ) was observed just above the thermocline.

#### Cruise OG

Surface temperature was about 20°C in LKF, and higher than 24°C in KCB and WSK. A thermocline indicated by  $N > 0.015 \text{ s}^{-1}$  was between 10 to 30 m in LKF, around 80 m in KCB, and 20 to 60 m in WSK (Fig. 4C). Upwelling occurred in regions shallower than 600 m (data not shown), located at Sta. OG4 and OG5 off the southwest of Hachijyo-jima. Regional upwelling is known to occur near the Izu Islands (*e.g.* FURUYA *et al.*, 1986). Low temperature (18°C)

and high nitrate concentrations ( $> 5 \mu\text{M}$ ) were seen at 25-m depth of OG5 with coincident high concentrations of Chl  $a$  ( $> 0.7 \mu\text{g l}^{-1}$ ). Chl  $a$  concentrations higher than  $0.4 \mu\text{g l}^{-1}$  were measured from the surface to 50 or 60 m in LKF. A SCM with  $0.2 \mu\text{g l}^{-1}$  Chl  $a$  was observed at 60 m in KCB (OG6) and deepened to 90 m toward the south.

#### Cruise PL

Steep southward deepening of isotherms was found around PL9, suggesting eastward flow. This is identified as the Subtropical Countercurrent (SCC), although it is shifted southward compared to its average position (around 25° N at 140° E; see TSUCHIYA, 1982). South of SCC was the North Equatorial Current (NEC) region characterized by low salinity (Fig. 3 in QUI and JOYCE, 1992). The eastward flow around 17° N and low salinity ( $< 34.5$ PSU) south of 16° N in 137° E transect were also observed by *Ryofu-maru* cruise during January and March in 1998 (Meteorological Agency of Japan, 1998).

Temperature in the mixing layer was 20°C or lower at stations north of 29° N (PL1 to PL4), where no clear thermocline was present. Strong thermoclines defined by  $N$  of  $> 0.01 \text{ s}^{-1}$  were observed below 80 m at stations south of 25° N (PL6 to PL12), where nitrate was depleted in the mixing layer and a SCM was observed at 100–120 m.

#### *Picophytoplankton Distribution*

#### Cruise BO

*Synechococcus* was found at high concentrations ( $\sim 5 \times 10^4$  cells  $\text{ml}^{-1}$ ) at all the stations from Cruise BO (Fig. 6A), with the maximum concentration ( $> 3 \times 10^5$  cells  $\text{ml}^{-1}$ ) within KCB at Sta. BO3. Generally, *Synechococcus* concentrations were highest within the mixed layer in this study. *Prochlorococcus* concentrations were higher than  $5 \times 10^3$  cells  $\text{ml}^{-1}$  at all the stations (Fig. 6B). Toward the south, maximum abundances occurred at deeper depths. Typically, this subsurface maximum was located within the seasonal thermocline, as defined by a  $N$  of  $> 0.01 \text{ s}^{-1}$ , except at Sta. BO5. At Sta. BO5, cell concentration at 150 m was more than  $2.5 \times 10^4$  cells  $\text{ml}^{-1}$ , although Chl  $a$  was less than



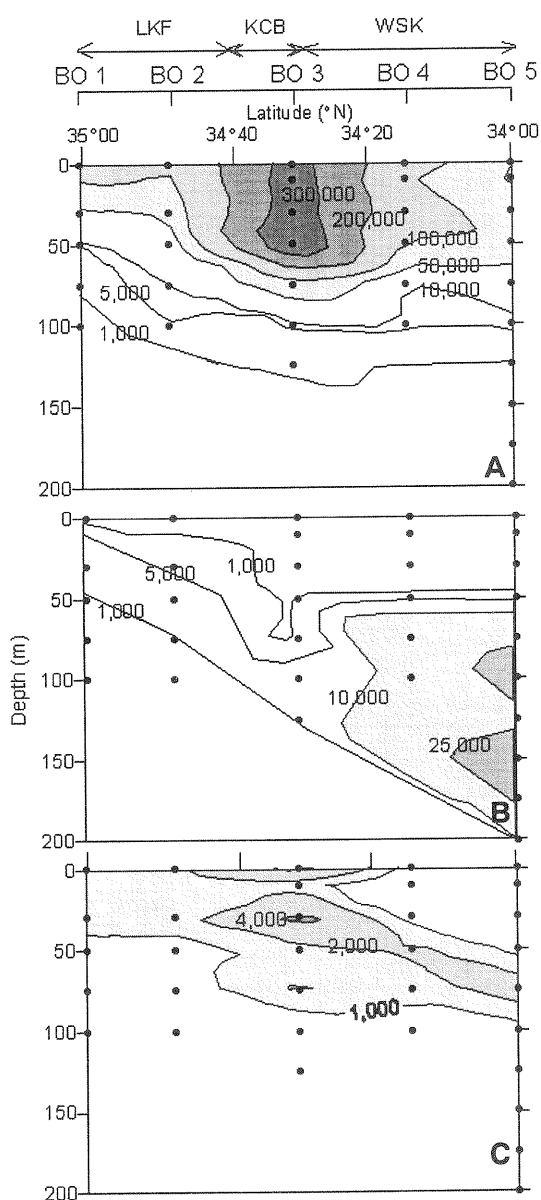


Fig. 6. Vertical sections of A) *Synechococcus* spp., B) *Prochlorococcus* spp., and C) eukaryotic picophytoplankton (cells ml<sup>-1</sup>) in the western Pacific Ocean during Cruise BO, October 1994.

0.1  $\mu\text{g l}^{-1}$ . Eukaryotic picophytoplankton concentration was more than  $10^3$  cells ml<sup>-1</sup> at all stations. Their distribution was similar to the distribution of Chl *a*, with a maximum ( $4 \times 10^3$  cells ml<sup>-1</sup>) at 25 m in KCB (Fig. 6C).

#### Cruise OG

*Synechococcus* was present at all stations, however, high concentrations ( $>10^5$  cells ml<sup>-1</sup>) were only found at shallow depths in LKF and KCB, and concentrations decreased to the south in WSK. The highest concentration ( $>2 \times 10^5$  cells ml<sup>-1</sup>) was found above the seasonal thermocline in KCB (Fig. 7A). *Prochlorococcus* was not detected at LKF, and had only very low concentrations in KCB. High concentrations ( $>2.5 \times 10^4$  cells ml<sup>-1</sup>) were found between 75 and 150 m depths in WSK (Fig. 7B) where the water temperatures were higher than 18°C. Eukaryotic picophytoplankton were abundant ( $>6 \times 10^3$  cells ml<sup>-1</sup>) at around 30-m depth at Stas. OG3 to OG5 in LKF, where upwelling was observed (Fig. 7C). Within KCB and WSK, eukaryotic picophytoplankton concentration at SCM depths decreased ( $\sim 2 \times 10^3$  cells ml<sup>-1</sup>).

#### Cruise PL

*Synechococcus* occurred at much lower concentration than in the previous cruise. *Synechococcus* was evenly distributed within the mixed layer, down to 100 m in KCB with high concentrations ( $\sim 10^4$  cells ml<sup>-1</sup>, Fig. 8A). Also, high concentrations were observed at northern stations (PL3 to PL5) in WSK at shallower depths, which corresponded to shallower mixing depth in these stations. Cell concentrations decreased to the south in WSK and *Synechococcus* was not detected in SCC and NEC waters. *Prochlorococcus* was not found within KCB, but within WSK, SCC and NEC were found below the 1% relative light level. Low concentrations ( $\sim 10^3$  cells ml<sup>-1</sup>) of eukaryotic picophytoplankton were found at the surface of KCB, and below the SCM in SCC and NEC.

## 4. Discussion

### Distribution of *Synechococcus*

During Cruise BO (fall) and OG (spring), *Synechococcus* cell concentrations reached their maximum level ( $2\text{--}3 \times 10^5$  cells ml<sup>-1</sup>) within the upper mixed layer when the water column was stratified, with a rapid decrease in concentration below the top of the thermocline, which was around the 1% relative light depth.

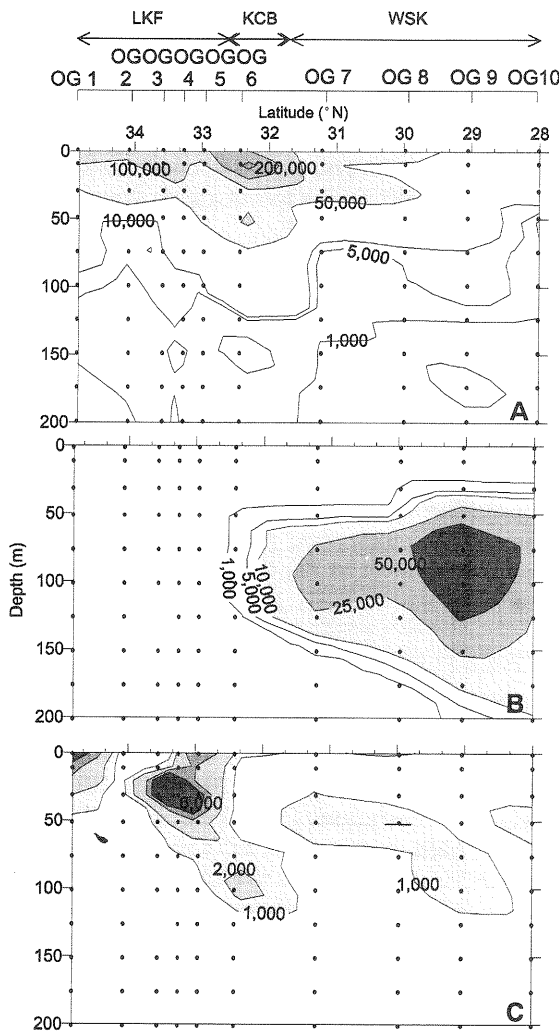


Fig. 7. Vertical sections of A) *Synechococcus* spp., B) *Prochlorococcus* spp., and C) eukaryotic picophytoplankton (cells ml<sup>-1</sup>) in the western Pacific Ocean during Cruise OG, June 1995.

During Cruise PL (winter), during which the mixed layer extended down to about 100 m or deeper, *Synechococcus* was still distributed throughout the mixed layer in KCB and WSK, although the cell number was thirty times less from the fall and spring levels. In previous works, ODATE *et al.* (1990) observed *Synechococcus* in the western North Pacific Ocean (36.5 to 44°N, 155°E), and noted high concentrations ( $\sim 10^5$  cells ml<sup>-1</sup>) only in the surface of the subtropical water (extension of the Kuroshio

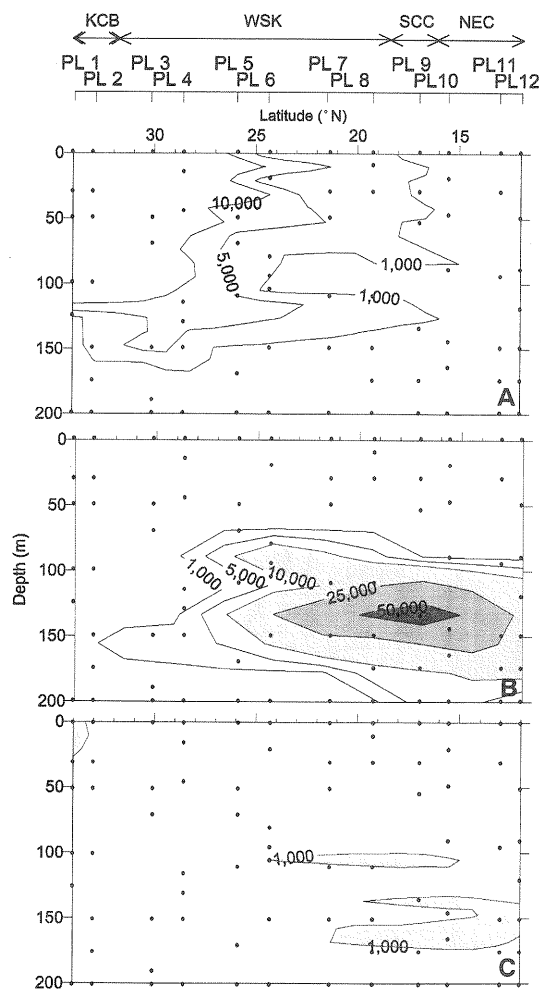


Fig. 8. Vertical sections of A) *Synechococcus* spp., B) *Prochlorococcus* spp., and C) eukaryotic picophytoplankton (cells ml<sup>-1</sup>) in the western Pacific Ocean during Cruise PL, March 1998.

Current,  $>18^{\circ}\text{C}$ ). This is similar to the maximum cell abundance in oceanic water of  $10^5$  cells ml<sup>-1</sup> as suggested by MURPHY and HAUGEN (1985).

During our study, *Synechococcus* abundance in the Kuroshio Current was always fairly high ( $\sim 10^5$  cells ml<sup>-1</sup>). Previous studies of the near shore coastal areas and certain bays have shown both high and low *Synechococcus* abundances at various times. HAMASAKI *et al.* (1999) found low pico-cyanobacteria concentration ( $\sim 10^3$  cells ml<sup>-1</sup>) during winter when

sea surface temperature was low ( $<21^{\circ}\text{C}$ ) in the inner Sagami Bay, while they found up to  $8 \times 10^4$  cells  $\text{ml}^{-1}$  in summer. In Suruga Bay, SHIMADA *et al.* (1995a) found that *Synechococcus* concentrations were low normally, but increased (up to  $10^5$  cells  $\text{ml}^{-1}$ ) when a branch of the Kuroshio flow into the bay in the summer. High concentrations of *Synechococcus* were also reported off the Izu Peninsula in June (TSUJI *et al.*, 1986) when the Kuroshio Current came very close to the coastal area, judging from its main path at that time (Hydrographic Department of Coast Guard, 1982). The fact that our measurements of *Synechococcus* concentrations within KCB were similar to those reported at particular times within these near shore areas, supports the hypothesis that the Kuroshio Current is responsible for supplying *Synechococcus* to these regions. Therefore, when temperatures are warmer in these areas—an indication of influence by the Kuroshio Current—*Synechococcus* concentrations are typically higher.

The factors which lead to *Synechococcus* dominance are not completely related to the higher temperatures within the Kuroshio waters, but also to nitrate levels. When nitrate levels were low ( $<0.5 \mu\text{M}$ ), *Synechococcus* concentration within WSK waters decreased, such as at Stas. BO4, BO5, OG9 and OG10. At these stations, the *Synechococcus* vertical distribution was strongly influenced by the mixing condition of the water column. During cruises BO and OG, higher *Synechococcus* cell concentrations were observed in KCB than in WSK, when remarkably high nitrate concentrations were observed in KCB (around  $1 \mu\text{M}$ ) compared to previous studies around the Kuroshio Current region (TAKAHASHI *et al.*, 1985). Therefore, the increase of *Synechococcus* cell concentration within KCB in this study could be induced by the start of stratification in spring (spring bloom) or by deepening of mixing depth (autumn bloom). Nutrient supply by upwelling during the stratified season may therefore be an important component of *Synechococcus* blooms in KCB along the Izu Ridge.

#### Identification of *Prochlorococcus*

*Prochlorococcus* is difficult to enumerate under the epifluorescence microscopy, especially in surface waters where the fluorescence of the cells is weak and decays very fast (ISHIZAKA *et al.*, 1994). This is in direct contrast to oceanic *Synechococcus*, the majority of which are phycourobilin rich cells with bright orange fluorescence and are easily detected (see PARTENSKY *et al.*, 1999a). SUZUKI *et al.* (1995) measured divinyl Chl *a* (Chl *a*<sub>2</sub>) concentration and estimated the *Prochlorococcus* cell concentration. The Chl *a*<sub>2</sub> in the layers above the 10 % isolume depths between the Kuroshio extension and more tropical areas was 0.01 to 0.05  $\mu\text{g l}^{-1}$ , which converts to around  $4.5 \times 10^4$  to  $1 \times 10^5$  cells  $\text{ml}^{-1}$  (SUZUKI *et al.*, 1995). However, on the same cruise as this study by SUZUKI *et al.* (1995), ISHIZAKA *et al.* (1994) reported that they could not detect *Prochlorococcus* cells near the surface.

Although we took precise care with our epifluorescence technique described in the methods, we could not detect *Prochlorococcus* from the surface waters covering the subtropical oligotrophic waters where this species has distribution (CAMPBELL *et al.*, 1994, LIU *et al.*, 1997). We did find fairly high concentrations of *Prochlorococcus* cells at 25 to 50-m depth in the coastal water in Cruise BO (Fig. 6A). Because the distribution of *Prochlorococcus* is continuous from off shore subsurface to inshore surface depths along the same isotherms, this may indicate that the cells we sampled all belong to the same population.

Two sub-populations of *Prochlorococcus*—a “dim”, high-light adapted, and a “bright”, low-light adapted strain—have been identified in the past using flow cytometry to measure differential red fluorescence (see PARTENSKY *et al.*, 1999a). The dim subpopulation tends to dominate in the surface depths and the bright population in the subsurface. The high-light adapted form has a low divinyl Chl *b* (Chl *b*<sub>2</sub>)/divinyl Chl *a* (Chl *a*<sub>2</sub>), and the low-light adapted form has a high divinyl Chl *b*<sub>2</sub>/Chl *a*<sub>2</sub> (e.g. MOORE *et al.*, 1998). The cultured strains from both sub-populations keep their pigment ratios after several years of culture (MOORE *et al.*, 1998), and they also fall into two phylogenetic

groups based on 16S RNA sequence analysis (ROCAP *et al.*, 1999).

The fact that the maximal *Prochlorococcus* concentrations found in our study were at or beneath the SCM suggests that the cells we observed belong to the bright fluorescing sub-population. We can count the bright cells by epifluorescence microscope, while it is unlikely that we were able to count the dim cells in this study.

#### *Distribution of Prochlorococcus*

In our study, *Prochlorococcus* was detected at relatively high concentrations beneath the mixed layer within WSK, where the temperature was between 22 and 24°C. Its abundance was around  $10^3$  cells ml<sup>-1</sup> at LKF near the surface during fall (Cruise BO), when temperatures were near 24°C. Similar concentrations were found in the KCB beneath the mixed layer where temperatures were around 23 to 24°C in both the fall (Cruise BO) and spring (Cruise OG). *Prochlorococcus* was not observed when temperature in the mixing layer in KCB was below 20°C in the winter. Thus, temperature seems to be an important limiting factor for the distribution of *Prochlorococcus*, which can only extend to the coastal area when water temperature is higher than 20°C. Supporting this hypothesis, SHIMADA *et al.* (1995) observed *Prochlorococcus* in Suruga Bay in the summer when a branch of the Kuroshio came within the bay.

JIAO and YANG (1999) examined samples from the East China Sea during the winter for picophytoplankton using flow cytometry. *Prochlorococcus* abundances were high (maximum  $5.6 \times 10^6$  cells ml<sup>-1</sup>) in the Kuroshio Current, but appeared only at low concentrations in waters that were characteristic of a mixture of the shelf water and the Kuroshio Current, with surface temperatures lower than 18°C. FURUYA *et al.* (*in press*), found Chl *a*<sub>2</sub> only in the water south of the Kuroshio seaward boundary in the winter, however, with the development of the summer stratification in East China Sea its distribution extended to the shelf water as indicated by Chl *a*<sub>2</sub> concentration. The temperature at the boundary of its distribution in winter was about 18°C, while during

the summer, temperatures on the shelf were usually higher than 20°C.

Combining these results, the following conclusions are drawn: 1) the Kuroshio Current is the landward boundary of the distribution of *Prochlorococcus* when the temperature within the Kuroshio is higher than that in the coastal water in the East China Sea, 2) The Kuroshio Current transports *Prochlorococcus* northward in winter while the temperature in the mixing layer remains higher than about 20°C, and 3) *Prochlorococcus* spreads into coastal waters when summer stratification is established along the Japanese coast up to the Boso Peninsula area. Our study indicates that *Prochlorococcus* off the Japanese coast require relatively warm water, such as the waters on the south of the Kuroshio Current, which typically results when there is strong stratification.

The Kuroshio Current system is known as a western boundary current, similar to Gulf Stream in the North Atlantic Ocean. OLSON *et al.* (1990), examined the horizontal distribution of phytoplankton across the Gulf Stream, and found that *Prochlorococcus* occurred where the water temperature was higher than 17°C at the surface during September 1986. Thus, the Kuroshio Current, much like the Gulf Stream, acts as a strong barrier to the shoreward distribution of *Prochlorococcus*. The higher temperature limit in the Kuroshio area than in the Gulf Stream suggests the occurrence of different *Prochlorococcus* ecotypes in these waters.

#### *Differential distribution of Prochlorococcus*

PARTENSKY *et al.* (1999b) discussed the factors controlling the relative distribution of *Prochlorococcus* versus *Synechococcus*. The two main factors appear to be light and nutrient levels. *Prochlorococcus* appears to be better adapted to low light levels than *Synechococcus*, while there appears to be a positive correlation between *Synechococcus* and nitrogen levels, although this may not always be the case in oligotrophic regions. Our findings within the Kuroshio Current system support these two general mechanisms. We found high *Prochlorococcus* concentrations at deeper depths than *Synechococcus*, and we found the highest *Synechococcus* concentrations in areas where

there may have been nutrient input due to recent mixing or frontal circulation. However, *Synechococcus* was not always found in the regions of highest nitrogen. These two factors, acting in conjunction, lead to an apparent "a differential distribution" of *Prochlorococcus* relative to *Synechococcus*. As suggested by PARTENSKY *et al.* (1999b), the maximum water column integrated concentration of *Prochlorococcus* occurs in a different region from that of *Synechococcus*. In our study, *Prochlorococcus* has maximum integrated abundances in the offshore side of the Kuroshio, while that of *Synechococcus* was usually within the KCB or landward within the LKF. Unfortunately, as mentioned earlier, we probably did not detect any high-light adapted *Prochlorococcus* if they were present, thus, we can not address how their distribution relates to *Synechococcus*.

#### *Distribution of eukaryotic picophytoplankton*

The concentrations of the eukaryotic picophytoplankton were much lower than prokaryotic picophytoplankton, and its distribution was fairly different among the different cruises. In general, however, the distribution appears to coincide with chlorophyll distribution. High abundances were found at the surface of coastal stations (PL1 and OG1) and upwelling stations (OG3 to OG5) with high nitrate concentrations (1.0 to 5.0  $\mu\text{M}$ ). Eukaryotic picophytoplankton did not occur in nutrient depleted surface waters but occurred in SCM depths off Kuroshio in each cruise. Thus it might require relatively high nutrient. Comparing vertical distribution among three groups of picophytoplankton, eukaryotic picophytoplankton had its maximum concentrations at depths below the maximum of *Synechococcus* and above that of *Prochlorococcus* (typically found at OG7, OG8, BO4, and BO5). Therefore, eukaryotic picophytoplankton might have a light requirement lower than *Synechococcus* and higher than *Prochlorococcus* (low-light adapted type).

In the present study, we found that the Kuroshio, a strong western boundary current, acts as a barrier to the three types of picophytoplankton that we examined. While

we have tried to assess the importance of picophytoplankton – both prokaryotic and eukaryotic – there is still a lot that remains unknown about the distribution and ecology of these groups. One limitation of our study was due to the epifluorescence microscopic method that we used, which cannot detect high-light adapted *Prochlorococcus*. In the future, we are planning to combine this method with high-sensitivity CCD camera, such as shown in SHIMADA *et al.* (1993), which will enable us to observe high-light adapted *Prochlorococcus*, and also give precise information on size and chloroplast shape in eukaryotic picophytoplankton. Fine structure of picophytoplankton using transmission electron microscopy on the same samples collected during the above cruises will be published elsewhere. Another issue is that SHIMADA *et al.* (1995a) reported the appearance of *Prochlorococcus* in winter (14 °C) from Suruga Bay, which has much lower temperature limit than the populations in this and previous reports. So far, only one strain (strain SB, SHIMADA *et al.* 1995b), a high-light and high-temperature type of *Prochlorococcus*, has been isolated from waters directly adjacent to Japan. Therefore, the isolation of new strains and eco-physiological studies on them are also urgently required.

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