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## Annual Fluctuations of Phytoplankton and Bacterial Communities in Maizuru Bay and their Interrelationship

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**Abstract:** The annual changes in bacterial community were studied as related to those of phytoplankton in Maizuru Bay, Japan from May 1985 to April 1986. Five phytoplankton blooms were observed through the year. Dominant algal species for three blooms were diatoms, *Cerataulina pelagica*, *Asterionella japonica* and *Leptocylindrus danicus*, and for the other two, dinoflagellate *Prorocentrum micans* and Raphidophycean *Chattonella marina*, respectively. The analysis of the bacterial community revealed that in these blooming periods the diversity of bacterial flora decreased. In particular the bacteria of genus *Vibrio* were not found in the bloom of phytoplankton. A positive correlation was found between abundance of free-living bacteria and chlorophyll *a* concentrations. The results suggest that phytoplankton in the blooming period influences the bacterial flora and the bacterial concentration is closely related to the abundance of phytoplankton.

**Key words:** bloom, phytoplankton, bacteria, interaction, annual cycle.

### Introduction

In marine and freshwater ecosystems it is generally recognized that bacteria utilize the extracellular organic carbon (EOC) released by algae as an energy and carbon source (Williams and Yentsch, 1976; Larsson and Hagstrom, 1982; Wolter, 1982; Coveney, 1982; Cole *et al.*, 1982; Lancelot, 1984; Brock and Clyne, 1984; Bell, 1984; Sondergaard *et al.*, 1985). The information in the literature has shown that the contribution of dissolved organic carbon released by phytoplankton to bacterial production varied from 20 to 90% (Coveney, 1982; Larsson and Hagstrom, 1982; Sondergaard *et al.*, 1985). All of those studies have demonstrated that EOC is important for supporting heterotrophic bacterial growth in natural aquatic environments. However, little is known about the changes of the bacterial flora in response to phytoplankton "bloom" *in situ* (Jones, 1973). In the present study we investigated the seasonal changes in the communities of phytoplankton and bacteria in Maizuru Bay, and attempted to examine their interrelation in an annual cycle.

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### Materials and Methods

The sampling station of about 8 m depth was located at the head of the pier (21 m off shore) at the Fisheries Research Station of Kyoto University in Maizuru Bay.

Water samples were collected from 1.0 m depth with a Kitahara sampler monthly from May 1985 to April 1986. The sampler was cleaned with ethanol before use. *In situ* water temperature was measured. All samples were processed immediately after collection. One sample was preserved with formalin (final con. 2%) for counting bacteria and another sample was with Lugol's solution for phytoplankton examination. The concentration of chlorophyll *a* was determined by the method of SCOR/UNESCO (1966) and/or Lorenzen (1967) and the pheopigments was after Lorenzen (1967). The concentrations of nitrate, nitrite, ammonia and

silicate were measured using a Technicom autoanalyzer. The dissolved organic carbon (DOC) was determined with a total carbon analyser (Shimadzu, TOC-500).

The phytoplankton abundance was microscopically determined using a Sedgwick-Rafter counting chamber.

Number of total culturable bacteria in seawater was determined by the spread method using duplicate plates of modified ST  $10^{-1}$  medium (Ishida *et al.*, 1986) containing 0.5 g trypticase (BBL), 0.05 g yeast extract (Difco) and  $2.5 \times 10^{-5}$  g ferric citrate in 1 l of aged seawater. For counting the attached culturable bacteria, a subsample of collected seawater was filtered through a sterilized 5  $\mu$ m Nucleopore polycarbonate filter, and the filter sample was homogenized by a sterile blender with sterile artificial seawater containing 5 ppm of Tween 80 to disperse the attached or clumped bacteria. The bacterial suspension prepared was inoculated in the plate medium mentioned above. The plates were incubated at 20°C for 2 weeks. For the identification of bacteria about 20 strains were isolated at random from each plate. These bacterial strains were identified according to the

simplified chart of Shewan *et al.* (1960), and classified into 7 genera/groups as follows: *Pseudomonas* (Ps), *Acinetobacter-Moraxella* (Ac), *Vibrio* (V), *Aeromonas* (A), Enterobacteriaceae (E), Chromogenic bacteria (C), and Gram positive bacteria (Po).

For direct counts of the total and attached bacteria the fixed samples were stained with DAPI (4' 6-diamidino-2-phenylindole) and were observed with a Nikon epifluorescence microscope by the modified method of Porter and Feig (1980). More than 500 cells were counted for each sample. The clumped bacteria of more than 5  $\mu$ m in size and the bacteria attached on particles of more than 5  $\mu$ m in size were counted as attached bacteria. The number of free-living bacteria was estimated as the difference in the count between the total and attached bacteria.

## Results

In the Maizuru Bay the surface water temperature ranged from 7°C to 29°C during the study period and there was a gradual decrease from the highest summer value to the lowest winter ones as

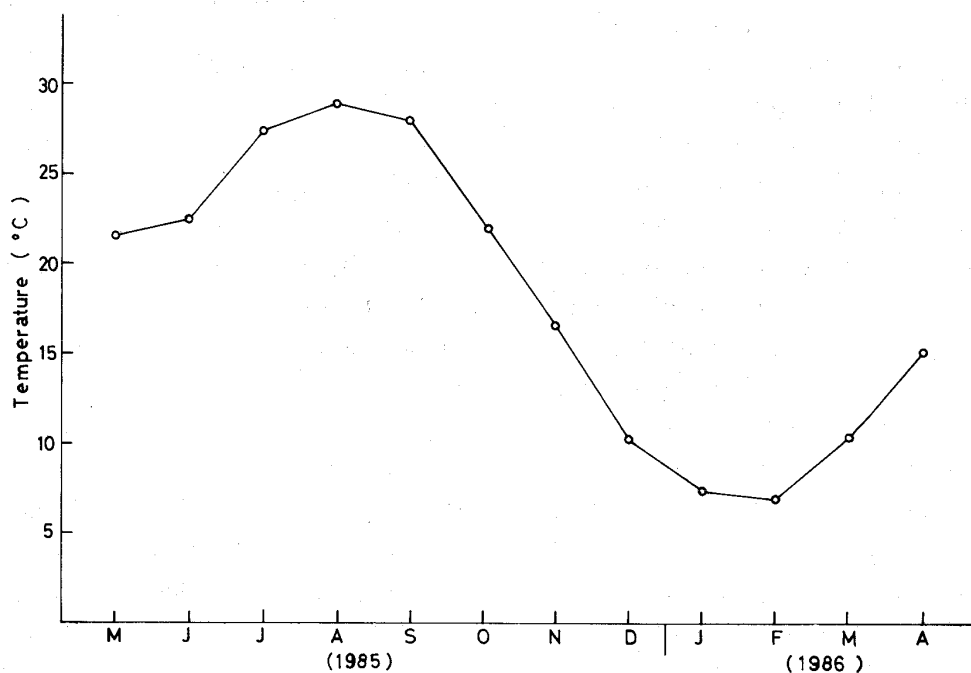


Fig. 1. Annual fluctuations of surface water temperature.

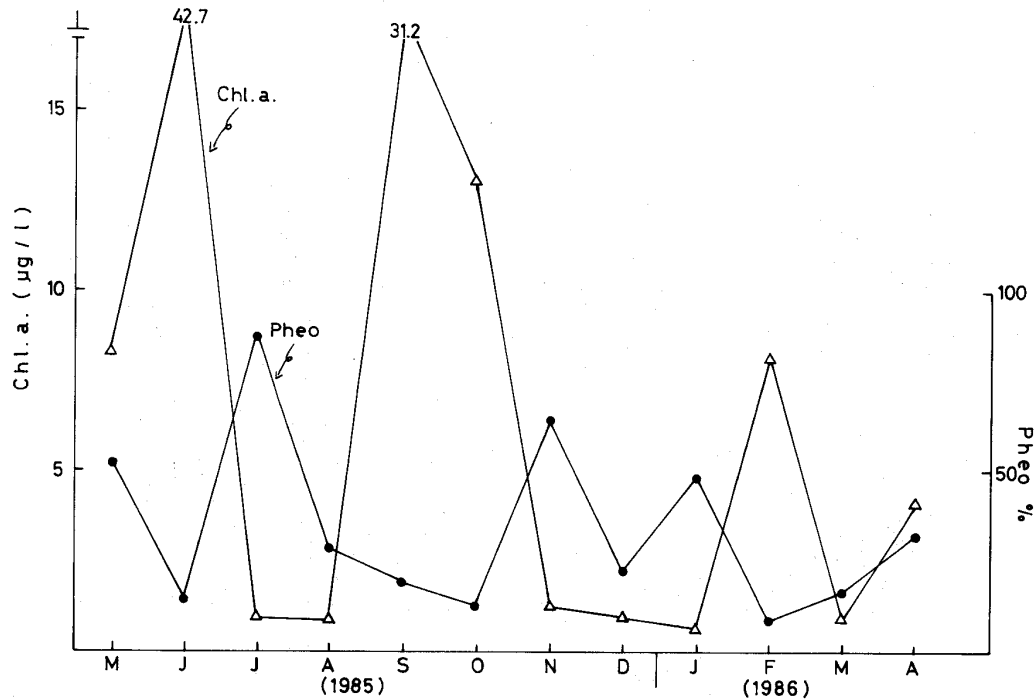


Fig. 2. Annual fluctuations of chlorophyll *a* and pheopigments in surface water.

shown in Fig. 1.

The change of chlorophyll *a* values obtained by Lorenzen's method showed a similar pattern to that of SCOR/UNESCO chlorophyll *a*. The percentage of pheopigments in the total pigments were in low during the blooms, indicating that phytoplankton were mostly active during the blooming period (Fig. 2).

For the period from May 1985 to April 1986 five blooms of phytoplankton were observed as indicated by the chlorophyll *a* concentrations and the phytoplankton cell numbers (Fig. 3). The concentration of chlorophyll *a* was approximately corresponded with the cell number of phytoplankton. Considering that the chlorophyll background in Maizuru Bay oscillated below  $5 \mu\text{g} \cdot \text{l}^{-1}$  (Fig. 2), the "blooming period" were defined as the period with chlorophyll *a* of more than  $8 \mu\text{g} \cdot \text{l}^{-1}$ . During the blooming period were observed only one species of phytoplankton as dominant (Fig. 3).

The phytoplankton community in Maizuru Bay showed great variability during the annual cycle and the dominant group of algae changed month by month (Fig. 4). Centric diatoms were detected

in all seasons. Raphidophyceae were observed only in September as a major component.

Seasonal change of cell number of dominant algal species is shown in Fig. 3. The first bloom in May was dominated by the centric diatom *Cerataulina pelagica* which accounted for about 79% of total number of phytoplankton; the second bloom in early summer (June) was dominated by the dinoflagellate *Prorocentrum micans*, accounted for about 88% of total phytoplankton number. In this bloom the secondary abundant species was other dinoflagellate *Prorocentrum triestinum* accounting for about 9% of total phytoplankton. The third bloom in September was dominated by the Raphidophyceae *Chattonella marina*, which accounted for about 99% of total phytoplankton. The October bloom was dominated by the pennate diatom *Asterionella japonica* (76%) which gave the highest cell number ( $4.5 \times 10^6 \text{ cells} \cdot \text{l}^{-1}$ ) observed during the study period. In winter (February) the fifth bloom which was predominated by the centric diatom *Leptocylindrus danicus*, accounting for about 94% of total phytoplankton, occurred.

Dissolved chemical nutrient concentrations in

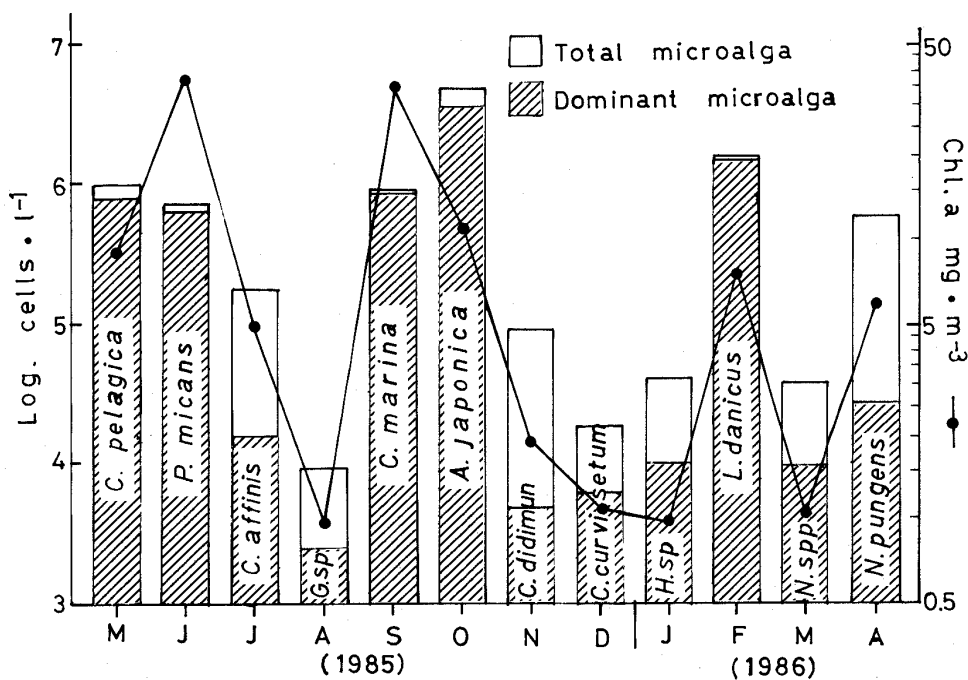


Fig. 3. Changes of the microalgal cells numbers, chlorophyll *a* concentrations and dominant species of microalgal. In order from May 1985, *Cerataulina pelagica*, *Prorocentrum micans*, *Chaetoceros affinis*, *Gymnodinium* sp., *Chattonella marina*, *Asterionella japonica*, *Chaetoceros didymun*, *Chaetoceros curvisetum*, *Heterosigma* sp., *Leptocylindrus danicus*, *Nitzschia* spp., *Nitzschia pungens*.

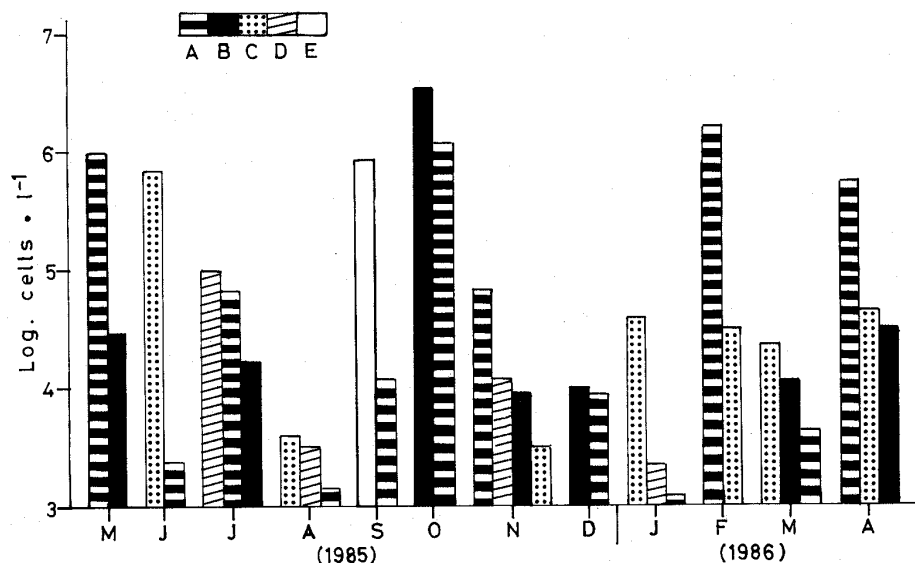


Fig. 4. Occurrence of the different microalgal group. (A) Centric Diatoms, (B) Pennate Diatoms, (C) Dinoflagellates, (D) Chlorophyceas, (E) Raphidophyceas.

the study period are shown in Fig. 5. Concentrations of nitrite and ammonia were in the ranges of 0.06–0.37 mg-at · m<sup>-3</sup> and 0.33–8.35 mg-at · m<sup>-3</sup>, respectively. The nitrate levels were in the ranges

of 0.06–15.9 mg-at · m<sup>-3</sup>, having higher concentrations in July, November and March, without phytoplankton bloom. The silicate showed great variation in concentration in the range of 0.3–86.1

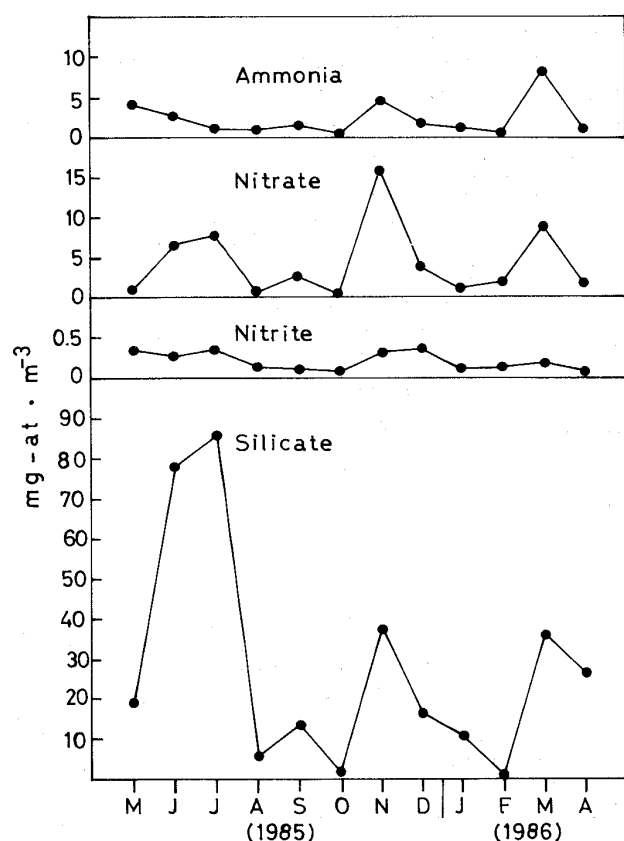


Fig. 5. Annual fluctuations of  $\text{NH}_4$ ,  $\text{NO}_2$ ,  $\text{NO}_3$  and Si concentration in surface water.

$\text{mg-at} \cdot \text{m}^{-3}$  and was completely depleted during the diatom blooms in October and February, although the higher values were observed in June, July and November. The pattern of seasonal changes in silicate level corresponded with that in nitrate.

Annual fluctuations of the direct counts of attached and free-living bacteria and the numbers of attached and free-living culturable bacteria are shown in Fig. 6 with those of the chlorophyll *a* and DOC values. The numbers of free-living bacteria, in both directly counted and culturable bacteria, were more than one order of magnitude higher than those of attached bacteria. The attached bacteria were drastically decreased in winter in both direct count and culturable bacterial numbers. Annual changes in the bacterial number of both two counting methods showed as similar pattern to those of chlorophyll *a* and DOC concentrations. DOC concentration increased at

the time of blooms of phytoplankton. The exceptionally high peak of DOC value in February seems to be due to the supply of terrestrial organic matter by thawing snow. The correlation coefficient values between bacterial abundance and chlorophyll *a* concentration are summarized in Table 1. Chlorophyll *a* had a higher correlation to free-living and total bacterial numbers than to attached bacterial number.

Annual changes in bacterial community are illustrated in terms of relative contribution of each genus or group to total cell number, in Fig. 7. The genus *Pseudomonas* and *Acinetobacter-Moraxella* group were dominant in all seasons,

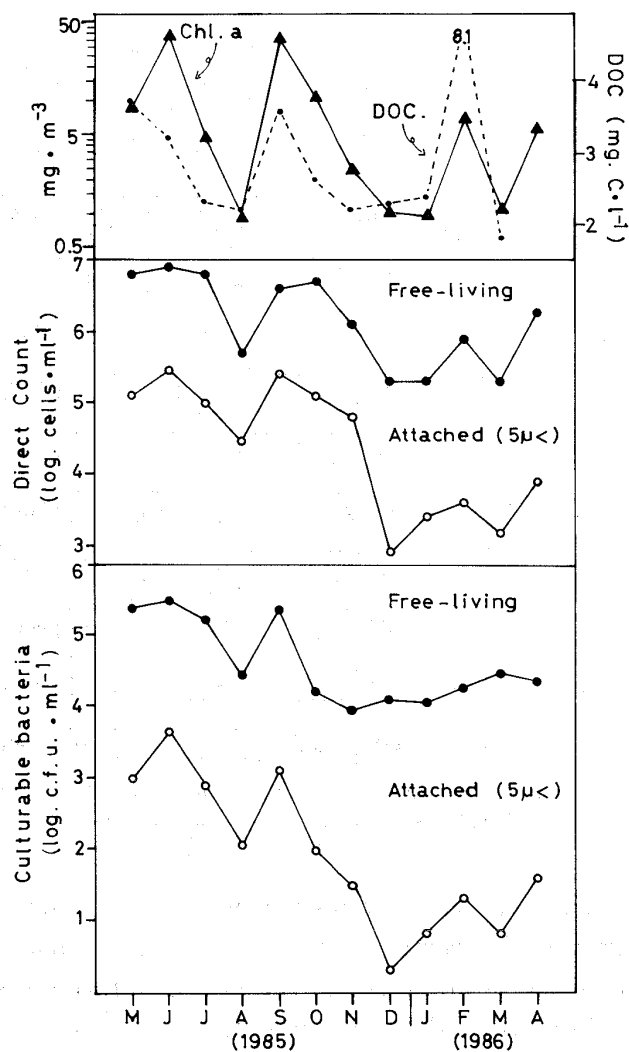


Fig. 6. Changes in the dissolved organic carbon (DOC), Chl. *a* concentration and bacterial numbers.

Table 1. Regression analysis on the data obtained during an annual study in Maizuru Bay. Variables are: Free-living bacteria, attached bacteria and total bacteria by direct count, (DC; no. of bacteria  $\cdot$  ml $^{-1}$ ), free-living culturable bacteria, attached culturable bacteria and total culturable bacteria (CFU  $\cdot$  ml $^{-1}$ ), and Chl. *a* (mg  $\cdot$  M $^{-3}$ ) analyzed according to SCOR/UNESCO (SU) and Chl. *a* (mg  $\cdot$  m $^{-3}$ ) according to Lorenzen (Lor). All of variables were log-transformed.

Variables	Chl. <i>a</i> (S.U.)	Chl. <i>a</i> (Lor)
Free-liv. bact. DC	+0.83	+0.80
Attached bact. DC	+0.66	+0.66
Total bacteria DC	+0.84	+0.81
Free-liv. bact. CFU.*	+0.79	+0.77
Attached bact. CFU.*	+0.79	+0.66
Total bact. CFU.	+0.78	+0.74

n=20, n=12\*, P<0.05.

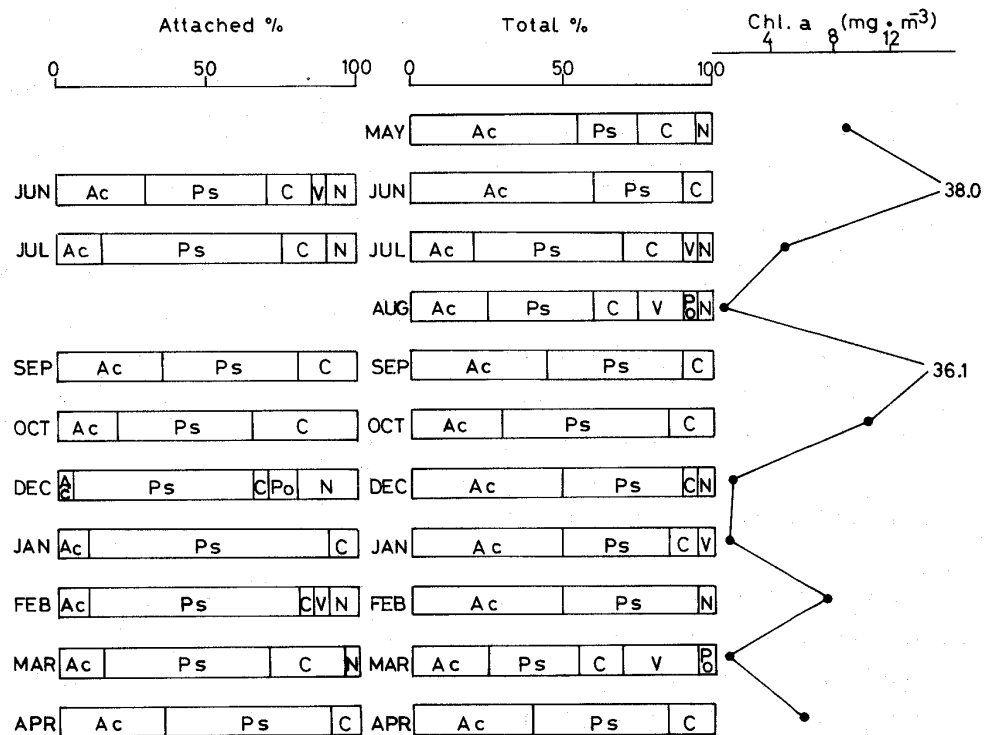


Fig. 7. Changes of bacterial community and Chl. *a* Ac, *Acinetobacter-Moraxella*; Ps, *Pseudomonas*; V, *Vibrio*; C, Chromogenic bacteria; Po, Gram positive bacteria and N, not identified.

especially in the blooming period. In the attached bacteria, the genus *Pseudomonas* predominated. The bacteria of the genus *Vibrio* was not found in the blooms of the phytoplankton. The Shannon-weaver diversity index was calculated based on these data and plotted against the logarithm of chlorophyll *a* (SCOR/UNESCO and Lorenzen)

concentrations. The result shows that chlorophyll *a* had an inverse correlation to the diversity index as follows:  $r = -0.63$  in SCOR/UNESCO method and  $r = -0.69$  in Lorenzen's method,  $n = 11$ ,  $p < 0.05$ .

## Discussion

Nakahara (1978), who studied seasonal variation of phytoplankton community at the same station as in the present study, reported that the concentrations of nitrite, ammonia, nitrate and silicate in the seawater at 2 m depth in 1975 and 1976 were 0.00–0.77, 0.00–3.70, 0.00–7.00 and 0.40–47.3 mg-at  $\cdot$  m<sup>-3</sup>, respectively. The concentrations of the latter three nutrients were about a half of our current results, although nitrite was higher in 1975 and 1976.

This result suggests that eutrophication in Maizuru Bay has been progressing during the last ten years. Nakahara (1978) found that nitrate concentration oscillated inversely with the chlorophyll concentrations and was at hardly detectable level in the winter bloom. It is suggested that nitrate is still one of the limiting factors for phytoplankton because this nutrient was depleted during blooming periods of phytoplankton in this study period. In Maizuru Bay during the diatoms bloom of October 1985 and February 1986, the silicate concentrations decreased to 1.80 mg-at  $\cdot$  m<sup>-3</sup> (0.05 mg  $\cdot$  l<sup>-1</sup>-Si) and 0.30 mg-at  $\cdot$  m<sup>-3</sup> (0.0083 mg  $\cdot$  l<sup>-1</sup>-Si), respectively. Considering that diatom growth ceases in concentration below 0.03–0.1 mg  $\cdot$  l<sup>-1</sup>-Si (Lewin, 1963; Aruga, 1973), not only nitrate but also silicate may possibly be the limiting factors for the diatoms bloom in Maizuru Bay. These results are similar to the findings of Morris *et al.* (1985) who reported that nitrate and silicate limited the spring diatom bloom in Loch Ewe.

Only two blooms in a year were observed consecutively in 1975 and 1976 (Nakahara, 1978). Winter-spring bloom was caused by *Chaetoceros* spp. and autumn bloom by *Skeletonema costatum*. In the present study, October bloom was represented by the highest number of *A. japonica*, while the bloom in November 1975 and September 1976 was by *S. costatum*. The Raphidophyceae *C. marina* was not observed in 1975 and 1976, while a bloom of *C. marina* occurred in September 1985. Clearly the major components of phytoplankton in

Maizuru Bay changed noticeably and the frequency of the blooms increased in 1985 as compared to ten years ago.

The DOC concentration increased in the period of phytoplankton blooms. This increase in DOC concentration should be more remarkable considering that heterotrophic utilization by bacteria reduced about 80% of the dissolved organic matter, which is directly associated with phytoplankton release processes (Ittekkot, 1982). Number of free-living bacteria showed better correlation to Chl. *a* concentration. This suggests that they grow by consuming the EOC of algae. This is supported by the fact that almost all isolated bacteria grow well on the EOC of algae such as those of diatoms *A. japonica*, *Coscinodiscus* spp. and the Raphidophyceae, *C. marina* (in preparation). A strong positive statistical relationship between bacterial abundance and chlorophyll *a* has been reported for fresh and marine waters (Aizaki *et al.*, 1981; Bird and Kalff, 1984).

In Maizuru Bay genus *Vibrio* was not detected in phytoplankton blooms period. A possible reason is that this bacteria may be sensitive to antibiotic products released by phytoplankton. In Naraganset Bay and Tokyo Bay some antagonism between *Vibrio* spp. and *S. costatum* growth was reported by Sieburth (1968) and Simidu *et al.* (1977). In the open sea inverse correlation between *Vibrio* spp. and phytoplankton was found by Fukami (1982) and Simidu *et al.* (1987). Another explanation is that this bacteria could not utilize efficiently the EOC of phytoplankton. We have also detected poor growth of *Vibrio* in the EOC of microalgae (in preparation).

In the phytoplankton blooms of Maizuru Bay a decrease in bacterial diversity was observed as compared with the flora in other periods. This suggests that the phytoplankton influenced the composition of bacterial community. In the blooms composed of almost one species of algae, the bacteria utilizing more efficiently the EOC of the dominant phytoplankton would become predominant during the phytoplankton bloom. This

could be responsible for a decreased in bacterial diversity. The experiment will be described in detail in the next report.

Furthermore, we would like to point out in this study that, while bacteria are dependent on the EOC of algae, algae are dependent on bacteria as in: photophagotrophy (Ishida and Kimura, 1986; Bird and Kalff, 1986), bacterial products such as vitamins (Furuki *et al.*, 1985), siderophores (Murphy *et al.*, 1976) and other tracer elements.

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