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Seasonal Fluctuation in Abundance of Bacteria, Heterotrophic Nanoflagellates, Autotrophic Nanoflagellates and Nanodiatoms in Hiroshima Bay, the Inland Sea of Japan[1,2)]

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Abstract

The seasonal and vertical fluctuations in abundance of bacteria (BA), heterotrophic nanoflagellates (HNF), autotrophic nanoflagellates (ANF), and nanodiatoms (ND) were investigated weekly over the course of a year at a sampling station in Hiroshima Bay. They were usually most abundant in the surface layer and their numbers decreased with depth. The average densities of these microbes in the water column showed similar seasonal patterns, being high in summer and low in winter. The seasonal ranges of their average densities were: 1.01–4.89×10^6 cells ml^{-1} for BA, 1.01–10.0×10^3 cells ml^{-1} for HNF, 0.43–19.2×10^3 cells ml^{-1} for ANF and 0.07–52.8×10^3 cells ml^{-1} for ND, showing that nanoautotrophs (ANF and ND) fluctuated more widely than BA and HNF. BA and HNF fluctuated in parallel, and the annual mean ratio of BA to HNF was 881. Among nanoautotrophs, ND were more abundant than ANF in spring and summer, while it was vice versa in winter. Based on multiple regression analysis, temerature, salinity and chlorophyll (plus BA or HNF) accounted for 45–73% of the variation of these microbes.

Keywords: bacteria, heterotrophic nanoflagellates, Hiroshima Bay, microbial food webs, nanoautotrophs

Apart from the classical paradigm of the marine planktonic food webs, i.e. so-called "grazing food webs" (e.g. STEELE 1974), an alternative paradigm which emphasizes the importance of the microheterotrophic processes, being termed "microbial food webs", was presented by POMEROY (1974) some 20 years ago. Since then, numerous studies have been carried out about the microbial food webs (e.g. WILLIAMS 1981, AZAM et al. 1983, DUCKLOW et al. 1986, SHERR et al. 1987), and at present we have a considerable amount of information on this formerly poorly-defined complex processes.

In order to evaluate the roles of the microbial process in the planktonic food webs, it is essential to determine the biomass and the rates of production, grazing (or uptake) and grazing loss of heterotrophic bacteria and nanoprotozoa, since they are key organisms in the lower trophic levels of the microbial food chains.

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Numerous works have been conducted on this subject, so that we now know the general picture about quantitative relationships between phytoplankton, bacteria and heterotrophic nanoflagellates (LINLEY et al. 1983, SANDERS et al. 1992) and between primary production and bacterial production (COLE et al. 1988).

However, studies on the microbial food webs are scarce in the coastal waters of Japan. Only a few studies have been undertaken to measure the density of bacteria and heterotrophic nanoflagellates at the same time (IMAI & ITOH 1984, YAMAMOTO & EZURA 1985, IWAMOTO et al. 1993). We have investigated the spatio-temporal variations in abundance and activities of members of the planktonic microbial food webs in Hiroshima Bay. Previously, we reported the short-term variations of bacteria, heterotrophic nanoflagellates and autotrophic nanoplankton during summer of 1990. In this paper, we describe the longer-term variations over the course of a year.

Materials and Methods

A series of samplings were made weekly for a year from 4 July 1990 to 2 July 1991, at a pier of Nansei National Fisheries Research Institute, Ohno-cho, Hiroshima Prefecture (Figure 1). Water samples were collected from 3 depths usually in mid-morning, with a bucket from the surface and with a Niskin bottle from 3 m deep and 1 m above the bottom (depth range: 6.1–8.7 m). Temperature and salinity were measured with a portable S-T meter (Yeo-Kal, Model 602). From each

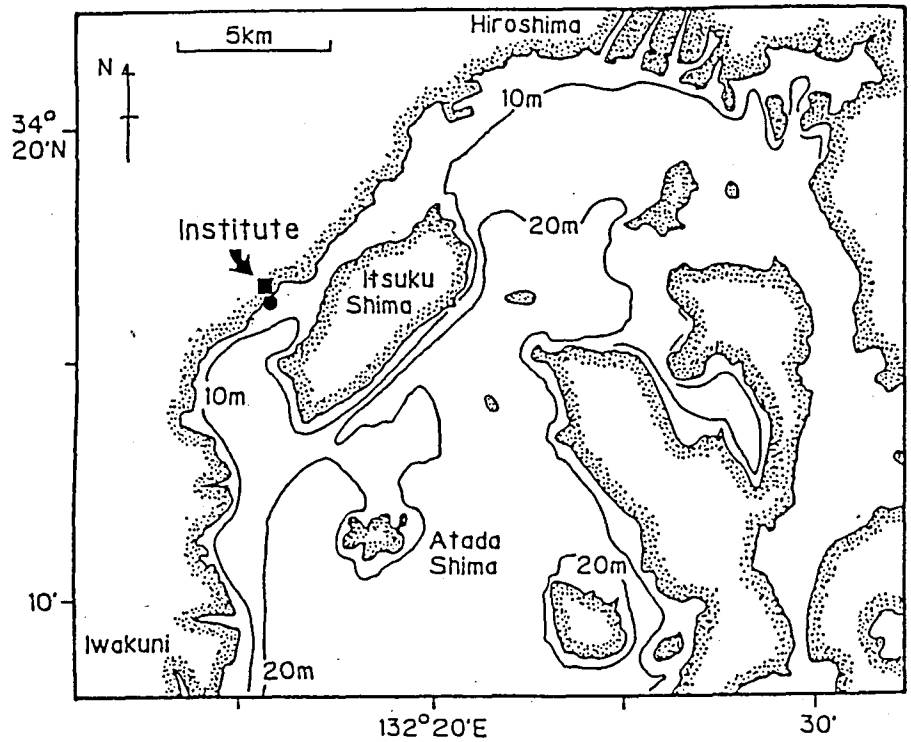

Fig. 1. Location of the sampling station (●) in Hiroshima Bay, the Inland Sea of Japan, and Nansei National Fisheries Research Institute (■).

depth, approximately 100 ml seawater samples were taken into two 100 ml-volume plastic bottles, fixed immediately with glutaraldehyde (final concentration: 1 %), and they were kept refrigerated (ca. 5 ℃) until counting, which was made within 3 days. For chlorophyll determination, 500 ml of seawater was filtered through a glassfiber filter (Whatman GF/C) and the filters were kept at ca. −20 ℃. Later, these filters were extracted in 90% acetone, and chlorophyll concentration was measured fluorometrically (Turner Designs).

Bacteria (BA), heterotrophic nanoflagellates (HNF), autotrophic nanoflagellates (ANF) and nanodiatoms (ND) were quantified by epifluorescence microscopy. BA were stained with 4'6-diamidino-2-phenylindole (DAPI, final concentration: 0.5 μg ml^{-1}) and filtered on Sudan Black B-stained Nuclepore polycarbonate filters (pore size: 0.2 μm) (ZIMMERMANN et al. 1978), according to the procedure of PORTER & FEIG (1980) with some modification by IMAI (1984). At least ca. 200 cells were counted for no less than 10 fields at×1250 magnification. HNF, ANF and ND were double stained with fluorescein isothiocyanate (FITC, final concentration: 1 μg ml^{-1}) and DAPI (final concentration: 0.1 μg ml^{-1}) and filtered on Sudan black B-stained Nuclepore filters (pore size: 1.0 μm) following the methods described by SHERR & SHERR (1983a, b). At least 50 cells were counted in a minimum of 25 fields. Autotrophs and heterotrophs were separated by the presence/absence of red or orange autofluorescence derived from photosynthetic pigments. Separation between ANF and ND was made morphologically. Since our preliminary investigations revealed that the size of HNF falls within the range of 2–10 μm equivalent spherical diameter (ESD), the size of not only HNF but also ANF and ND were confined to the 2–10 μm ESD in the present study.

On each sampling date, the average of environmental variables and of densities of microbes in the water column were determined by dividing the integrated values for the water column by the depth. Based on these data, simple multiple regression analyses were performed between dependent and independent variables.

Results

Environmental Variables

The seasonal variations in vertical temperature, salinity and chlorophyll profiles are shown in Figure 2. Although salinity was stratified more or less during most of the year, temperature and chlorophyll were stratified only in summer. Salinity varied widely at the surface from 13.6 to 32.4, but varied less at the bottom from 30.1 to 33.1. The annual range in average temperature within the water column was 9.7–26.3℃ (Figure 3). The average chlorophyll varied from 0.7 to 14.7 μg l^{-1}, and it was higher and more fluctuated in summer and fall (Figure 3).

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The seasonal vertical profiles of BA, HNF, ANF and ND are shown in Figure 4. Generally, the density of each group was highest at the surface and decreased with depth. The vertical heterogeneity was prominent in summer. The seasonal variations in their average densities within the water column followed a similar pattern, being high in summer and low in winter (Figure 5). Seasonal ranges of the densities were 1.01–4.89×10^6 cells ml^{-1} for BA and 1.01–10.0×10^3 cells ml^{-1} for

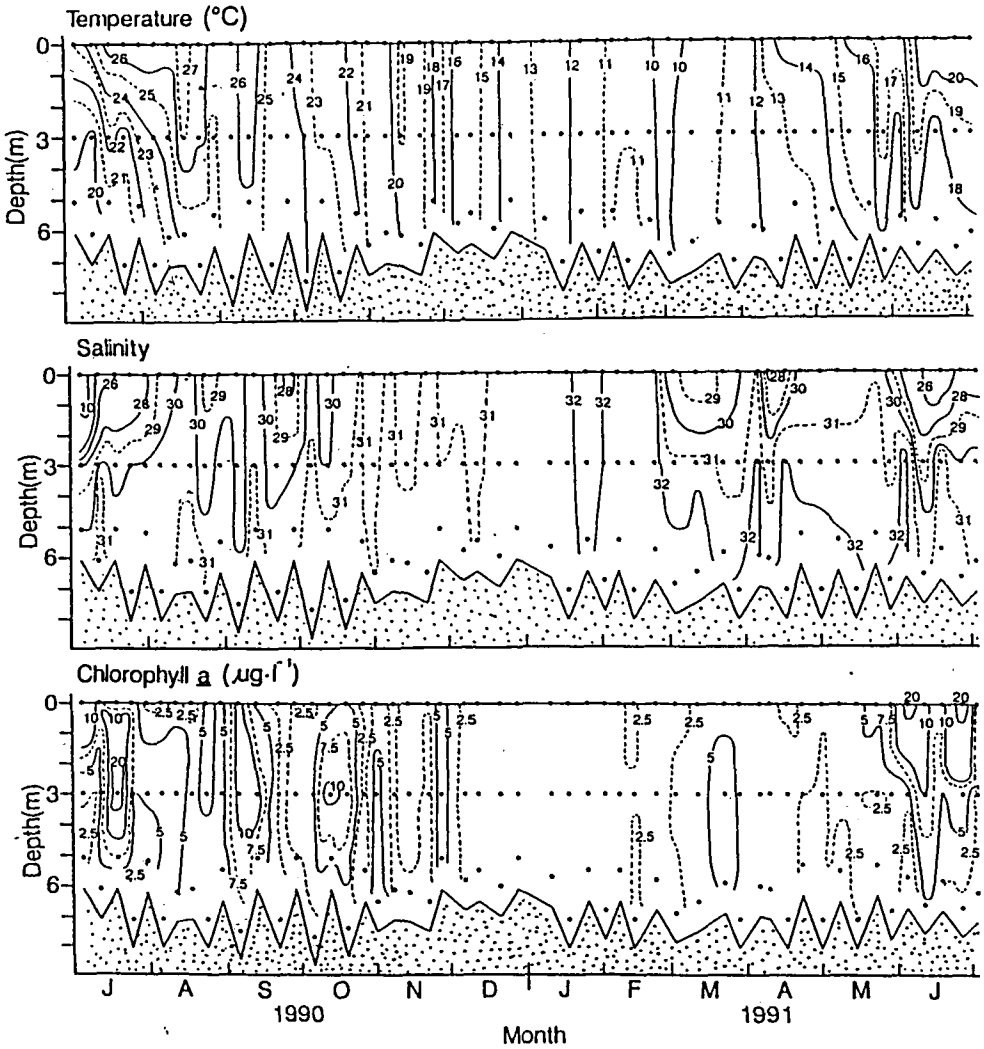

Fig. 2. Seasonal variations in vertical profile of temperature, salinity and chlorophyll *a* concentration. The depth varied with a neap-spring tidal cycle.

HNF. Seasonal fluctuations of ANF and ND were larger than HNF; 0.43–19.2×10^3 cells ml^{-1} for ANF and 0.07–52.8×10^3 cells ml^{-1} for ND.

Since BA and HNF are coupled trophically in prey-predator relationship, the ratio of BA to HNF was examined (Figure 6). It varied from 374 to 2171 with overall mean of 881. It was relatively low in July/August (mean: 554) and increased gradually to November/December (mean: 1276). After the unusual peak on 11 December, it varied in rather cyclic pattern with a center of ca. 1000. Among nanoautotrophs, ND were more abundant (ca. 60% of autotrophs) than ANF in spring and summer, but they were less abundant (ca. 20% of autotrophs) in winter.

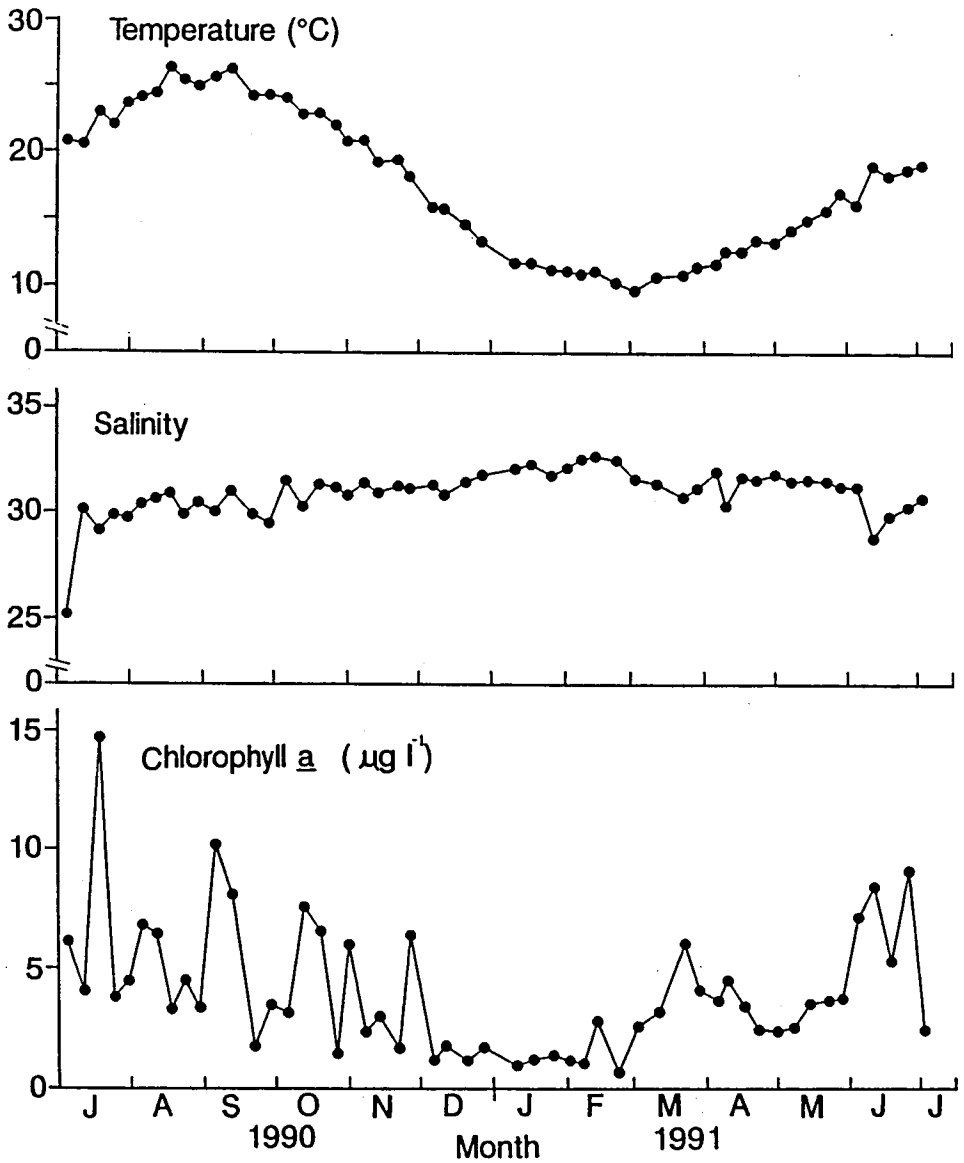

Fig. 3. Seasonal variations in average temperature, salinity and chlorophyll *a* concentration in the water column.

Statistical Analysis

Table 1 presents partial correlation coefficients for the multiple regressions which give significant ($p<0.05$) results for the variation of BA, HNF, ANF and ND. Although not shown in Table 1, BA, HNF, ANF and ND are significantly ($p<0.05$) positively correlated each other. They are positively correlated with temperature and chlorophyll, but negatively with salinity.

From the multiple regression analysis, the variation in natural logarithm of BA ($\times 10^6$ cells ml^{-1}) and HNF ($\times 10^3$ cells ml^{-1}) can be expressed by:

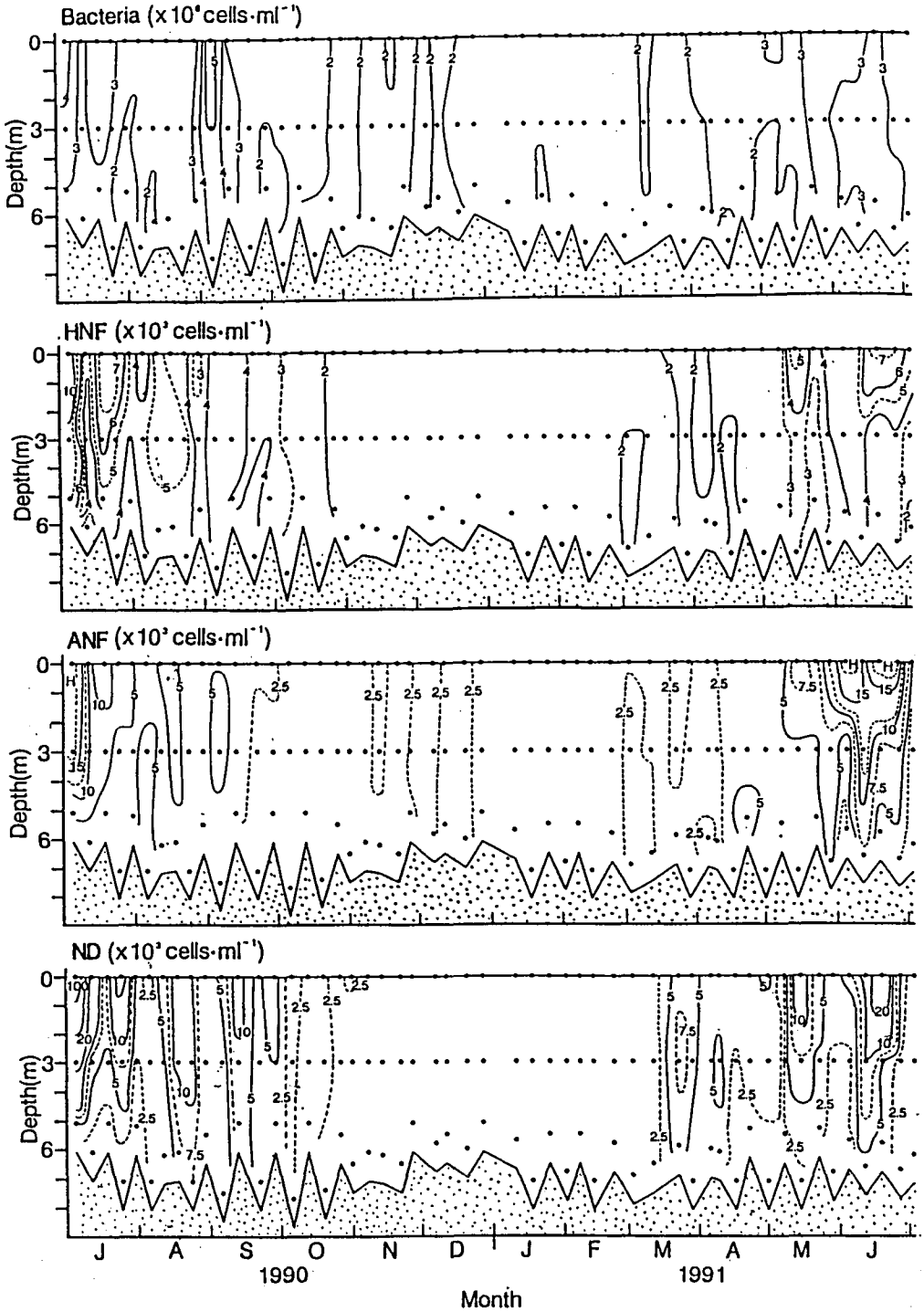

Fig. 4. Seasonal variations in vertical profile of bacteria (BA), heterotrophic nanoflagellates (HNF), autotrophic nanoflagellates (ANF) and nanodiatoms (ND). The depth varied with a neap-spring tidal cycle.

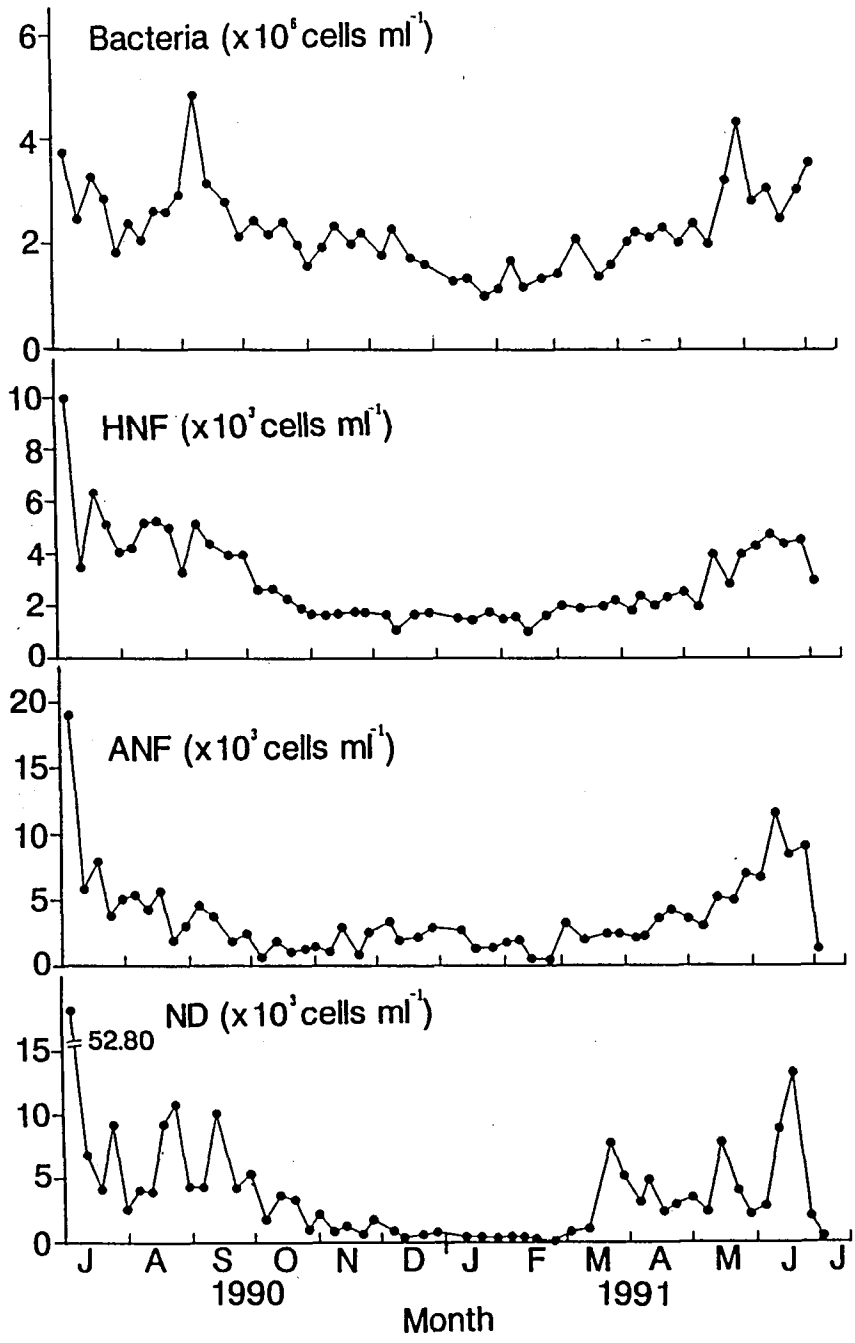

Fig. 5. Seasonal variations in average density of bacteria (BA), heterotrophic nanoflagellates (HNF), autotrophic nanoflagellates (ANF) and nanodiatoms (ND) in the water column.

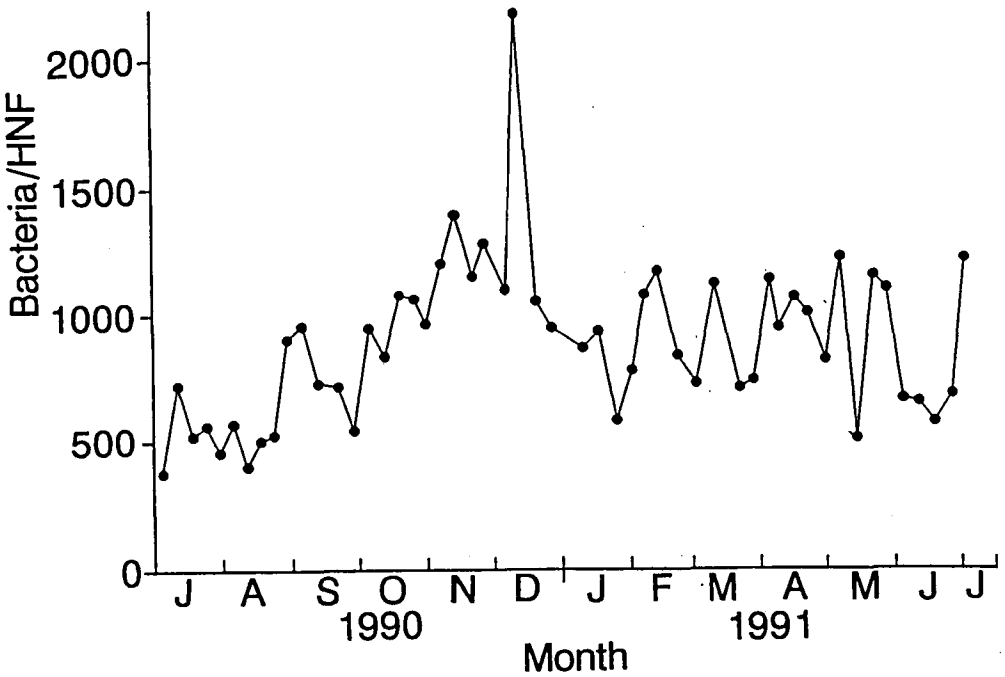

Fig. 6. Seasonal variation in the ratios of density of bacteria (BA) to heterotrophic nanoflagellates (HNF).

Table 1. Partial correlation coefficients for the multiple regressions. All are significant ($p<0.05$).

Independent variables	Dependent variables			
	BA	HNF	ANF	ND
Temperature	0.621	0.647	0.213	0.511
Salinity	−0.583	−0.755	−0.594	−0.701
Chlorophyll	0.551	0.624	0.508	0.538
BA		0.712		
HNF	0.671			
r^2	0.55	0.73	0.45	0.54

$$\ln BA = 0.090 + 0.020T + 0.000028S + 0.021C_{HL} + 0.078HNF$$
$$\ln HNF = 5.50 + 0.018T - 0.17S + 0.030C_{HL} + 0.18BA$$

where, T is temperature (℃), S is salinity and C_{HL} is chlorophyll ($\mu g\ l^{-1}$). These regression equations can account for 55% and 73% of the variability ($F_{13,204}=32.02$ for BA and 14.22 for HNF, $p<0.01$) in $\ln BA$ and $\ln HNF$, respectively (Table 1). Taking temperature, salinity and chlorophyll as independent variables, the equations are:

$$\ln BA = 2.52 + 0.0024T - 0.074S + 0.030C_{HL}$$
$$\ln HNF = 6.71 + 0.026T - 0.21S + 0.045C_{HL}$$

which account for 51% and 69% of the variabilities ($F_{2,102}=16.62$ for BA and 35.19 for HNF, $p<0.01$), respectively. The variation in natural logarithm of *ANF* and *ND* (both $\times 10^3$ cells ml^{-1}) can be expressed as functions of temperature, salinity and chlorophyll as follows:

$$\ln ANF = 12.808 - 0.038T - 0.37S + 0.095C_{HL}$$
$$\ln ND = 17.08 + 0.030T - 0.56S + 0.091C_{HL}$$

which account for 45% and 54% of the variabilities ($F_{2,102}=12.97$ for ANF and 18.91 for ND, $p<0.01$), respectively (Table 1). Strictly speaking, chlorophyll is not a true independent variable for these autotrophs, but this is treated as an independent variable in the multiple regression since it represents the biomass of phytoplankton of whole size range.

Discussion

The density of BA ($1.01-4.89\times10^6$ cells ml^{-1}) and HNF ($1.01-10.0$ cells ml^{-1}) observed in the present study fall within the range commonly encountered in temperate coastal waters, at the order of 10^6 cells ml^{-1} and of 10^3 cells ml^{-1}, respectively (FENCHEL 1982, IMAI 1989, SANDERS et al. 1992). Assuming that the volume of BA and HNF is 0.098 μm^3 and 43 μm^3 (average volume of BA and HNF from Suo-Nada of the Inland Sea of Japan, IMAI 1984, 1989), respectively, and the carbon-volume coefficient is both 0.12 pg μm^3 (WATSON et al. 1977, FENCHEL 1982), the carbon biomass is calculated as 11.9–57.5 $\mu g\ l^{-1}$ for BA and 5.2–51.8 $\mu g\ l^{-1}$ for HNF. These values are comparable to or slightly higher than the biomass of netzooplankton (with 96 μm-mesh net) in the Inland Sea of Japan (UYE et al. 1986). Since the weight-specific growth rate of BA and HNF is much higher (e.g. biomass doubling time: at the order of hours) than netzooplankton (at the order of days), the energy conveyed through BA and HNF is apparently much higher than the energy through netzooplankton.

We enumerated nanoautotrophs which have cell size similar to HNF, because they have been often overlooked particularly in inshore waters. Strictly speaking, nanoflagellates possessing chloroplasts may include some mixotrophic protists which graze upon BA, although we simply categorized cells with photosynthetic pigments as autotrophs. Hence, the numerical abundance of heterotrophic nanoflagellates determined in our study might be underestimated.

Trophodynamically, nanoautotrophs (ANF and ND) and HNF are grazed together by microzooplankton such as ciliates and larval net zooplankton. The present study demonstrates that nanoautotrophs outnumber HNF, in particular in warm-water seasons. This, in turn, suggests that the microbial food chains through HNF play relatively less important roles in respect to food supply for microzooplankton during warm-water periods.

COLE et al. (1988) examined the relationship between primary production and bacterial production in both fresh-water and marine ecosystems from the data of previous publications, and concluded that bacterial production averages 20–60% of primary production. SANDERS et al. (1992) also examined the data of previous

publications, and found that relative abundances of BA and HNF are similar in both fresh-water and marine environments. These findings suggest that the microbial food webs are ubiquitous and stable relationships exist in the microbial food webs both in fresh-water and marine ecosystem. Interrelations between BA, HNF and environmental variables observed in the present study support these ideas.

There was a significantly ($p<0.05$) negative correlation between chlorophyll and salinity, because primary production was enhanced in less saline water that contained higher nutrient concentrations. Positive correlation of BA to chlorophyll was explained by the increase of BA production with the increase of dissolved organic matter derived from phytoplankton. Further, positive correlation of HNF to BA could be accounted for by the quick growth response of HNF to the increase of BA.

As mentioned above, there exists a fairly tight coupling between BA and HNF. The average numerical ratio of BA to HNF from previous works is approximately 1000 (FENCHEL 1986, SANDERS et al. 1992). IMAI & ITOH (1984) reported that the average ratio is also ca. 1000 in Suo-Nada. In the present study, however, the ratio fluctuated on a seasonal basis; it was lowest in July/August and highest in November/December (Figure 6). This means that the tightness of the prey-predator relationship between BA and HNF varies seasonally in our study area. Since our unpublished data (IMAI et al. in preparation) demonstrate that the production rate of BA was highest and the rate was not presumably affected by any substrate limitation in summer, the low BA:HNF ratio in July/August might be due to heavy grazing pressure by HNF. In other words, BA are under top-down (predation) control in summer. On the other hand, this may not be so in other period of the year. The cyclic (ca. a month intervals) oscillation patterns of BA:HNF ratio in 1991's observations (Figure 6) can be explained by the short-term variation in relative abundance of BA and HNF, as demonstrated by FENCHEL (1982) and ANDERSON & FENCHEL (1985). Weekly samplings may not be frequent enough, and the mechanisms for these oscillations remain to be studied in the future.

The regression equations to describe the variations of BA, HNF, ANF and ND were obtained from data of the present study. Temperature, salinity and chlorophyll (plus BA or HNF) can explain large part of the variability of these microbes. Taking broad structural similarities in the microbial food webs in aquatic ecosystems into consideration, these regression equations are highly useful to approximate the abundance of microheterotrophs from environmental variables, at least for waters of eutrophication level similar to our study area.

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