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ABUNDANCE AND BIOMASS DISTRIBUTION OF MICROBIAL ASSEMBLAGES AT THE SURFACE IN THE OCEANIC PROVINCE OF ANTARCTIC OCEAN

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Abstract: During the JARE-32 (the 32nd Japanese Antarctic Research Expedition) cruise, bacterioplankton, autotrophic and heterotrophic nanoplankton (2–20 μm in length), and microzooplankton (15–250 μm in length) were collected from the surface of the Indian and Australian sectors of Antarctic Ocean. The average abundance of bacterioplankton was 4.56×10^4 cells/m*l*, and the biomass was 0.25 mg C/m^3. The values of the autotrophic nanoplankton were 0.5×10^3 cells/m*l* and 2.27 mg C/m^3, respectively. There were 1.02×10^2 cells/m*l* of heterotrophic nanoplankton in abundance, and its biomass was 0.71 mg C/m^3. About 30% of the biomass of this assemblage was choanoflagellates. Average abundance and biomass of the microzooplankton were 1.62×10^3 inds./*l* and 3.78 mg C/m^3, respectively. A large portion of the abundance and biomass of microzooplankton was oligotrichs.

Protozoans within micro-sized (15–250 μm) category which ingest preys similar to or larger than themselves were found where biomass ratio of nanoplankton to microzooplankton was low (<0.3). This may indicate adaptability of the protozoan to a severe food environment. The biomass (log-scale) decreased linearly with increasing individual body weight (log-scale) with a slope of –0.21. This result suggests that smaller protozoans such as heterotrophic nanoplankton and oligotrichs have an important role in the Antarctic Ocean's energy flow.

1. Introduction

In the Antarctic pelagic ecosystem, a simple and short food chain consisting of the predator/prey relationships among diatoms, krill and whales has been widely recognized (*e.g.* EL-SAYD, 1971). In recent years, however, a more complicated food web including a microbial food chain has been proposed by several scientists. HEWS *et al.* (1985) suggested that the energy flow from pico- and nanophytoplankton to higher consumers through microzooplankton might be important in the Antarctic waters. CLARKE (1985) showed a food web of Antarctic ecosystems in which the concept of microbial food web was introduced. His statement was made on the basis of previous knowledge of individual groups of microorganisms. More recently, GARRISON and BUCK (1989) and GARRISON *et al.* (1991) clarified the importance of protozoan assemblage in energy flow from primary producers to higher consumers across the ice-edge zone in the Weddell Sea, a high productive area of the Antarctic Ocean. In order to

understand the whole ecosystem of the Antarctic Ocean, microbial structure in oceanic province should also be elucidated. In the oceanic area, some restricted groups of microorganisms such as bacterioplankton (HOBBIE *et al.*, 1977; SAMYSHEV, 1986; KOGURE *et al.*, 1986), and small protozoans (HARA and TANOUE, 1984; HARA *et al.*, 1986; NISHIDA, 1986; SUSHIN *et al.*, 1986) had been individually investigated. However, the whole microbial assemblage including the nanoplankton has been little studied.

With the purpose of estimating the role of the microbial food web, bacterioplankton, nanoplankton and microzooplankton were collected from the surface waters of Indian and Australian sectors of the oceanic province of the Antarctic Ocean during the JARE-32 cruise. In this paper, we report the distribution, abundance and biomass of these plankton, and discuss the energy flow in the Antarctic pelagic ecosystem.

2. Materials and Methods

Water samples were collected in the eastward leg (from 64°–35.8'S, 47°–19.3'E to 61°–59.8'S, 149°–14.8'E) of the JARE-32 (the 32nd Japanese Antarctic Research Expedition) cruise of the "SHIRASE" from March 5 to March 14, 1991 (Fig. 1). The surface water was pumped up from *ca.* 8 m depth with the surface water monitoring system (FUKUCHI and HATTORI, 1987). Samples for counting bacterioplankton and microzooplankton were fixed with 1% of Lugol iodine solution (POMROY, 1984), and stored in the dark. For nanoplankton, seawater was fixed with glutaraldehyde (GA) (final concentration 2%), and stored at a temperature lower than 4°C in the dark.

Bacterioplankton were stained by the 4'6-diamidino-2-phenylindole (DAPI) (IMAI, 1984; POMROY, 1984). Heterotrophic- (HNP) and autotrophic nanoplankton (ANP) were treated following the DAPI-FITC (fluorescein isothiocyanate) double stain method (IMAI and ITO, 1984). To collect bacterioplankton and nanoplankton, the Sudan black B stained (ZIMMERMAN, 1987) 0.2 μm and 1.0 μm pore sized Nuclepore fil-

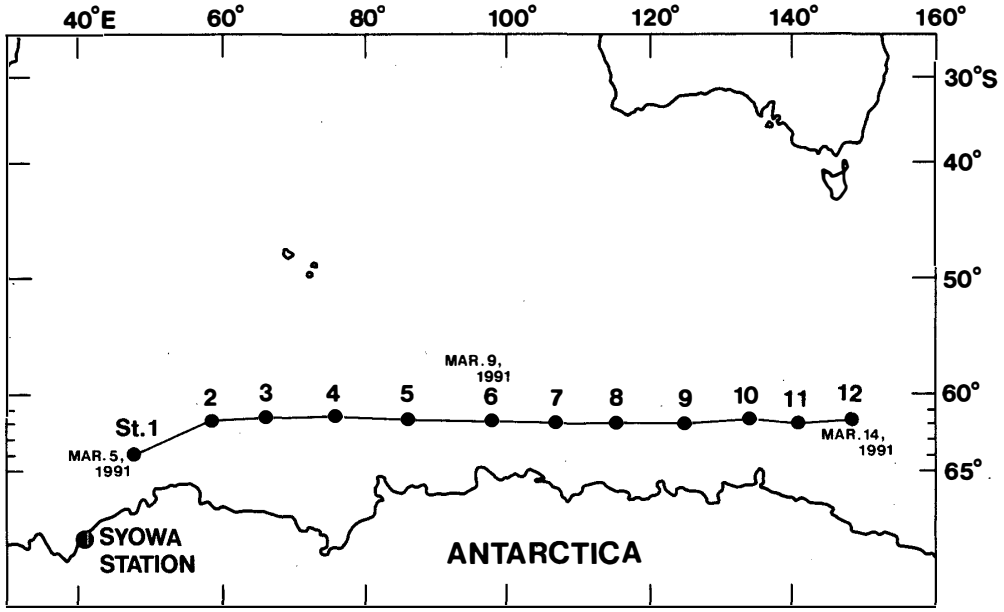

Fig. 1. Map showing the sampling stations of the JARE-32 cruise, March 1991.

ters (25 mm diameter) were used respectively with a damp Millipore filter (25 mm diameter, 0.45 μm pore size) as a backing (POTER and FEIG, 1980). The magnitude of the vacuum was less than 10 cm Hg (CARON, 1983). The damp Nuclepore filters were mounted with immersion oil (Olympus, for fluorescence microscopy), and stored at –20°C in the dark.

Bacterioplankton were enumerated at 1000× using an epifluorescence microscope (Olympus microscope BH2-RFK, with ×100UKVFL objective lens, UG1 exciter filter, and KM 400+L420 dichroic mirror). Bacterial cells were counted at least 75 fields using a 10×10 graticulated eyepiece. Several photos of bacterioplankton were taken with ASA 400 color film (Kodak Co.). Cell volume was analyzed using the "Personal Image Analysis System LA-525; PIAS Co.". A conversion factor of 0.087 g C/cm^3 (FERGUSON and RUBLEE, 1976) was used to estimate bacterial carbon biomass. The trophic type of nanoplankton cells (2–20 μm in length) was examined separately and counted at 1000 × (IMAI and ITO, 1984) at least 65 fields of 10×10 graticulated eyepiece. A DAPI-stained nuclear was observed under UV excitation. And for the observation of FITC-stained cell's shape and autofluorescence of chlorophyll, an IF410-485 exciter filter, a DM505 dichroic mirror and a 515W barrier filter were used. Among HNP, only choanoflagellates and larger dinoflagellates (larger than *ca.* 5 μm) could be identified under the epifluorescence microscope. Diatoms were not included in the ANP data because it was difficult to know their correct size and number with the epifluorescence microscope. Cell volumes were calculated by assuming spherical or prolate spheroid shape. Carbon biomass of HNP and ANP were estimated using the relationship proposed by EPPLEY *et al.* (1967);

$$\mathrm{Log_{10}Carbon(pg)}=0.094\mathrm{Log_{10}[Cell\ volume}(\mu m^3)]-0.60.$$

Prior to the counting of microzooplankton, Lugol iodine fixed samples were settled for longer than 24 h. Microzooplankton were counted and measured using an inverted microscope. Microzooplankton are in general classified into the size group ranging from 20–200 μm (DUSSART, 1965). However, the heterotrophs of 15–250 μm size in length were here defined as microzooplankton for the following reasons: Ciliates in about 15–20 μm length should be missed in the process of heterotrophic nanoplankton counting method at high magnifications (×1000) (SHERR *et al.*, 1986), and also, in this study, most heterotrophs up to 250 μm in length could be considered to belong to the same groups of some largest microzooplankton. But *Mesodinium* spp. were not included in the microzooplankton (SIEBURTH, 1979), nor were heterotrophic microflagellates for the trophic types of flagellates were hardly distinguished by the present counting method without an epifluorescence microscope. We referred to KOFOID and CAMPBELL (1929) for identification of tintinnids. The volume of the naked ciliates was converted to carbon weight using a conversion factor of 0.19 pg C/μm^3 (PUTT and STOECKER, 1989). For the other microzooplankton, conversion factor of 0.04 pg C/μm^3 was used (TANIGUCHI, 1977; BEERS *et al.*, 1975).

3. Results

3.1. Bacterioplankton

Cell density of bacterioplankton in the eastward leg was relatively constant with an average of 4.56×10^4 cells/m*l* (Fig. 2 upper). With the exception of Stn. 1, no significant variation of cell volume was observed at any of the stations (mean; 0.0827 μm^3/cell). Therefore, bacterial biomass was directly proportional with abundance, and the average was 0.25 mg C/m^3 (Fig. 2 lower).

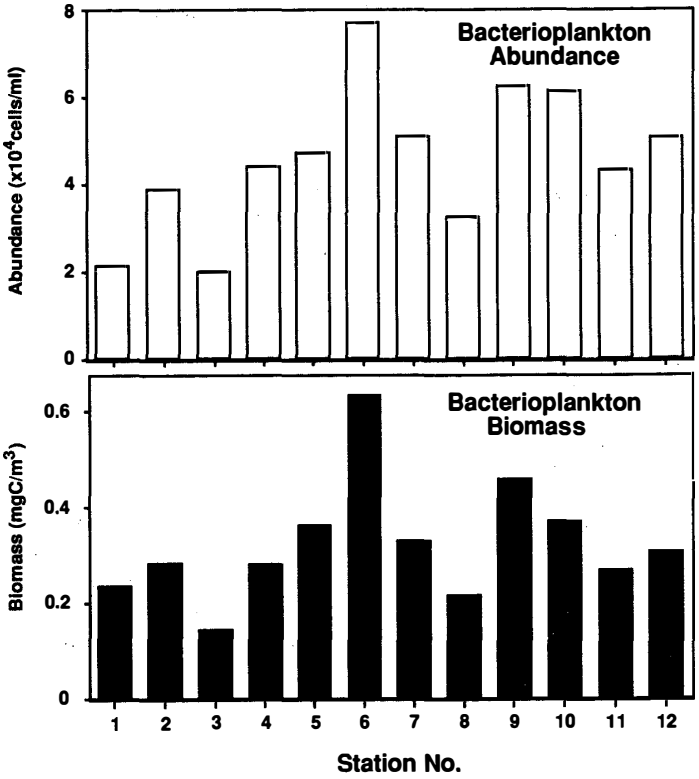

Fig. 2. Distribution of abundance (upper) and biomass (lower) of bacterioplankton.

3.2. Autotrophic nanoplankton (ANP)

About 85% of the total ANP density was in 1–3 μm size fraction in equivalent spherical diameter (ESD) (Fig. 3 upper). Most of them were naked flagellates which were *ca.* 2.5 μm in cell diameter with two or three flagella. A mode of the biomass was also found in 2–3 μm size (26.3%) (Fig. 3 lower). With the highest value (1.48×10^3 cells/m*l*) at Stn. 5, ANP occurred in the order of 10^2 cells/m*l* at most stations, with an average of 0.5×10^3 cells/m*l* (Fig. 4 upper). Remarkably high biomass (8.00 mg C/m^3) was observed at Stn. 5. At other stations, biomass ranged from 0.55 to 2.82 mg C/m^3 (Fig. 4 lower). The average value of the biomass through the all stations was 2.27 mg C/m^3.

3.3. Heterotrophic nanoplankton (HNP)

Cells in 1–5 μm (ESD) accounted for 85.8% of total HNP density (Fig. 5 upper). In terms of biomass, there were two peaks, one between 3–6 μm (49.6%) and the other

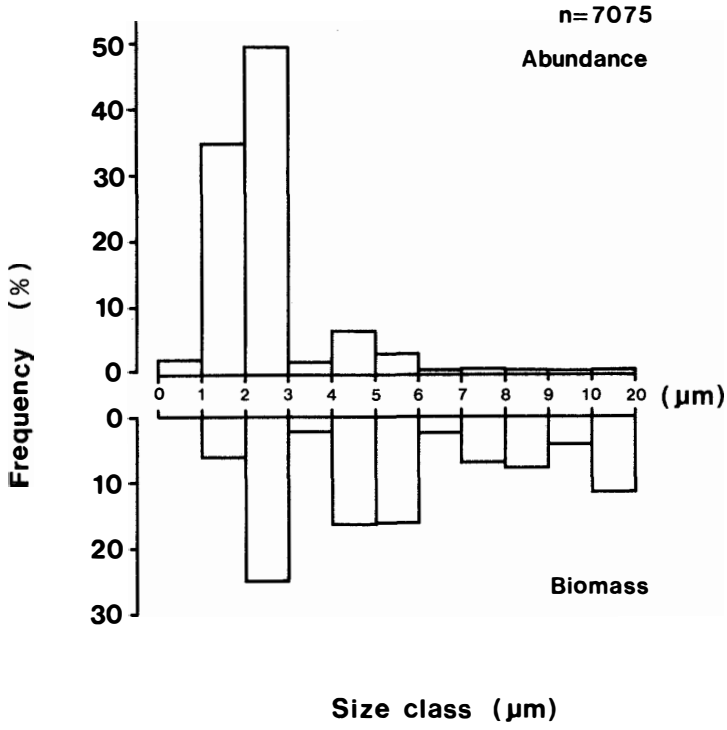

Fig. 3. Relative frequency of autotrophic nanoplankton of each size class in terms of abundance (upper) and biomass (lower).

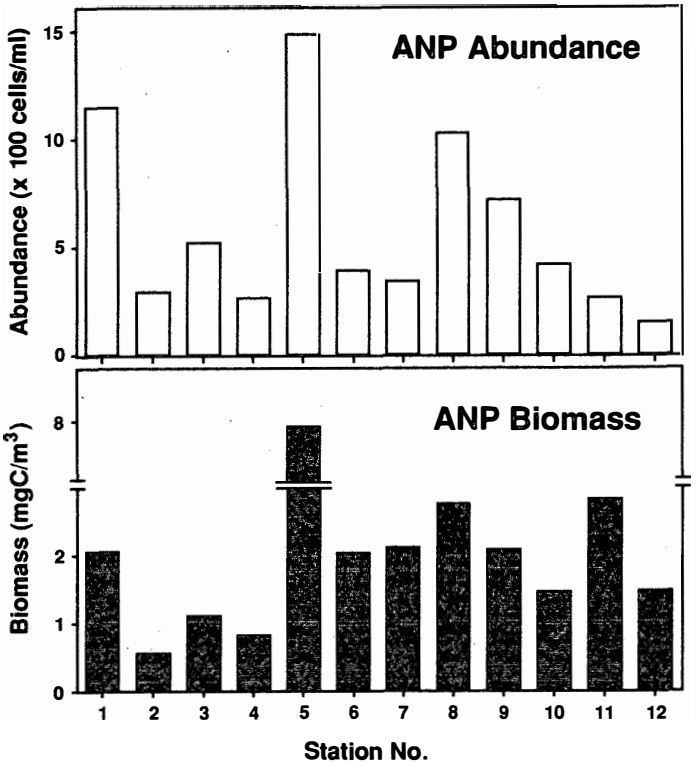

Fig. 4. Distribution of the abundance (upper) and biomass (lower) of autotrophic nanoplankton.

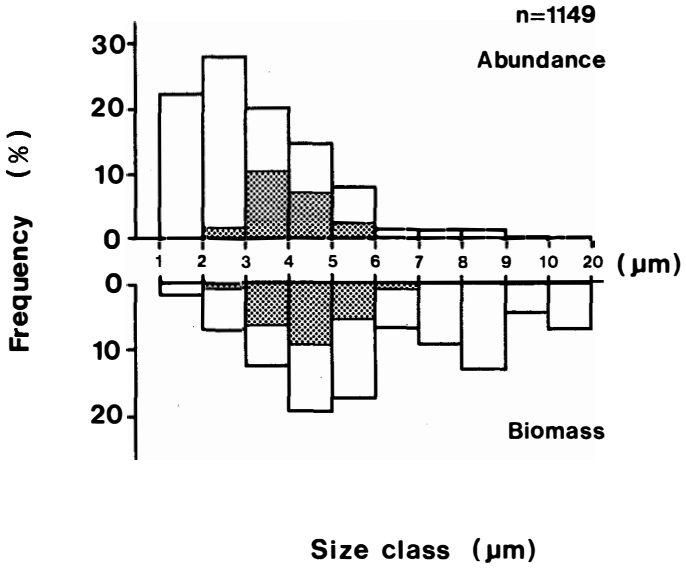

Fig. 5. Relative frequency of heterotrophic nanoplankton of each size class in terms of abundance (upper) and biomass (lower). Dotted fractions mean chanoflagellates.

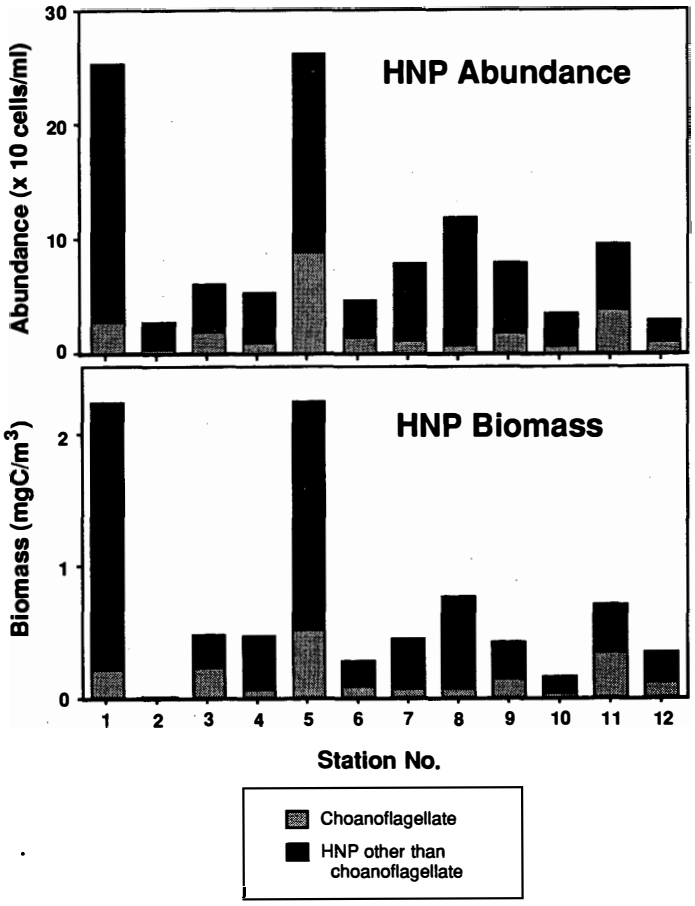

Fig. 6. Distribution of abundance (upper) and biomass (lower) of heterotrophic nanoplankton.

in 8–9 μm (13.1%) classes (Fig. 5 lower). In the 3–6 μm fraction of the HNP, 47% of the total abundance of organisms and 42% of the total biomass were choanoflagellates (Fig. 5). Larger dinoflagellates (10–20 μm) were rare, which ranged from 3.3 to 9.7 cells/m*l*, and 0.42–0.61 mg C/m^3. Cells in 10–20 μm size class might have been underestimated because the HNP cells in this size fraction were so scarce when counted by an ordinary HNP counting method. Abundances of HNP were in order of 10^2 cells/m*l* at Stns. 1, 5, 8, and in order of 10 cells/m*l* at the other stations (Fig. 6 upper). The average abundance of HNP was 1.02×10^2 cells/m*l*, while the average biomass was 0.71 mg C/m^3 with a maximum value of 2.23 mg C/m^3 at Stn. 5 (Fig. 6 lower). The percentage of HNP biomass in total nanoplankton ranged from 3.3 to 52.1% throughout the stations, with an average of 21.8%. Percentage of choanoflagellates abundance on the average was 23.5% of the total HNP, and 29.1% in biomass. Choanoflagellates cells ingesting bacteria or forming colonies were observed frequently, though the frequency was not calculated.

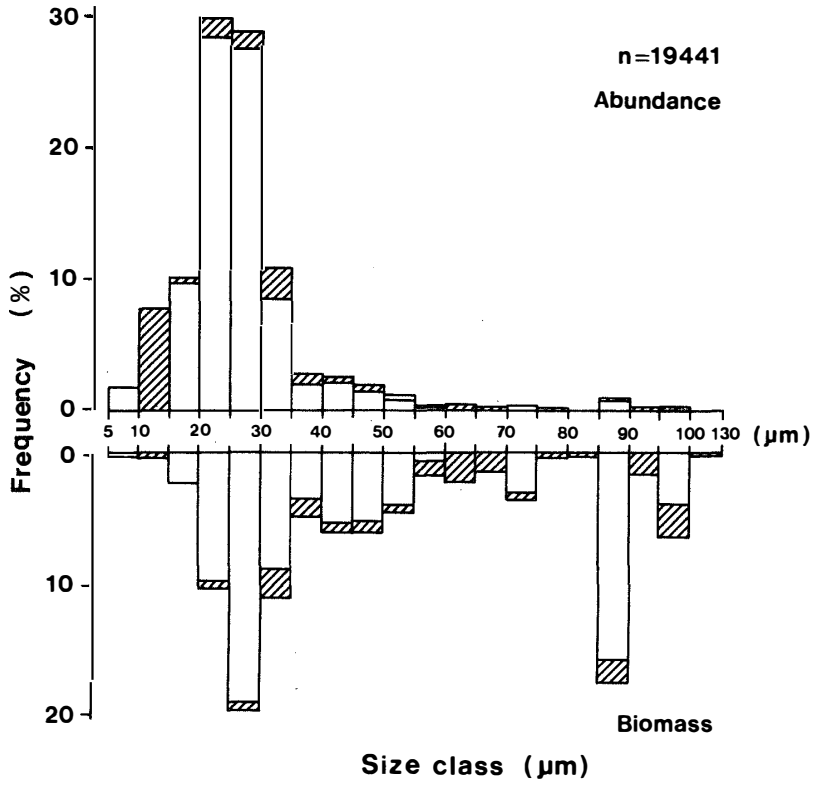

Fig. 7. Relative frequency of microzooplankton of each size class in terms of abundance (upper) and total biomass (lower). Shaded parts indicate microzooplankton other than oligotrichs.

3.4. Microzooplankton

The average abundance and biomass of total microzooplankton were 1.62×10^3 inds./*l* and 3.78 mg C/m^3, respectively. 83.5% of total abundance of microzooplankton was occupied by oligotrichs (Fig. 7 upper). Tintinnids were the second most abundant group, and were usually composed of smaller species of *Salpingella* spp. (74.4% of total tintinnid abundance). Larger tintinnid species such as *Laackmanniella* sp., *Codonellopsis* spp., *Cymatocylis calyciformis*, and *Cymatocylis* spp. were sometimes observed. Oligotrichs were also dominant in the biomass of microzooplankton (80.8% on average, Figs. 7, 8 lower). The regional variation was not always consistent with that of abundance (Fig. 8). Sporadic occurrence of the cells of an unidentified largest oligotrich (cell volume; 2.25×10^5 μm^3) determined the total biomass of oligotrichs at some stations; this oligotrich at Stns. 3 and 4 took up 62.2% and 49.3% of total oligotrich biomass, respectively (Fig. 9). Although copepod nauplii, Hydrachnellae, radioralians and *Fritillaria* did not appear frequently, their biomass contribution was significant at some stations. At Stn. 9, planktonic Hydrachnellae and *Fritillaria* accounted for 36.5% of the total biomass of microzooplankton (Fig. 8 lower).

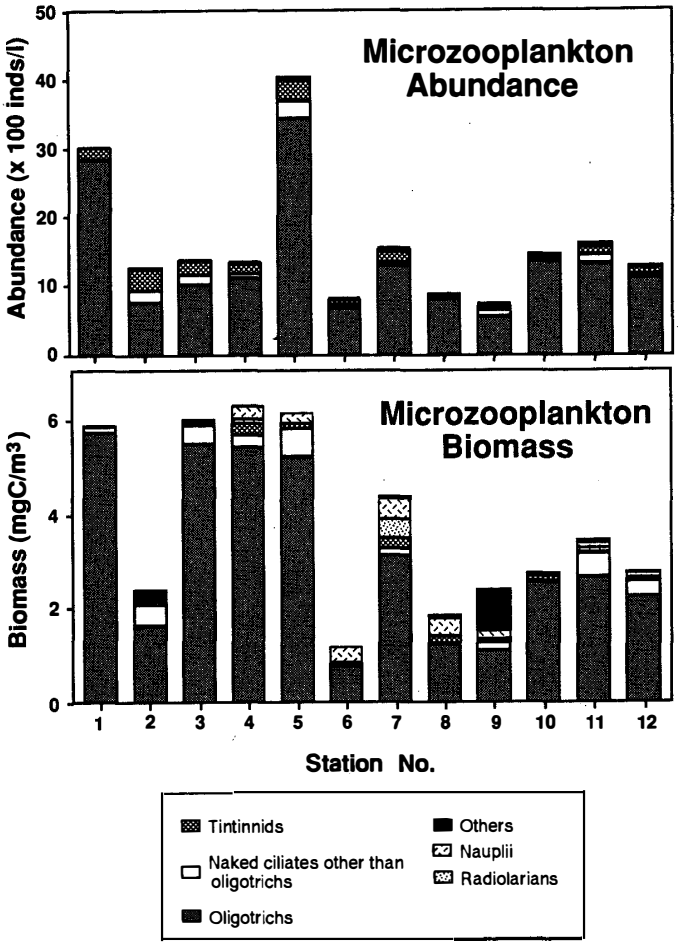

Fig. 8. Distribution of abundance (upper) and the biomass (lower) of microzooplankton.

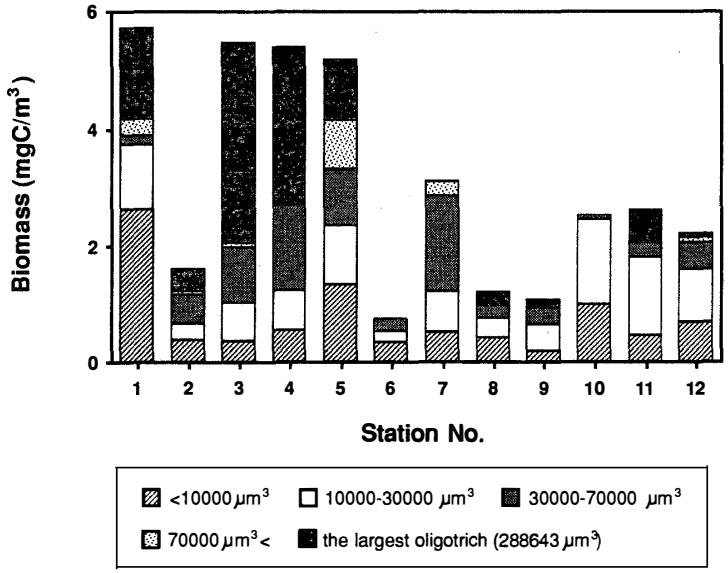

Fig. 9. Changes in the biomass of each size fraction of oligotrichs.

3.5. Correlation of abundance and biomass between different assemblages

While no significant correlations were seen between bacterioplankton and any other assemblages, there can be seen significant correlations among all assemblages except bacterioplankton (Table 1). In terms of biomass, ANP and HNP, and HNP and microzooplankton were correlated significantly, while microzooplankton were not significantly correlated with ANP.

Table 1. Matrix of correlation between different assemblages. The figures are correlation coefficients and the ones with asterisks are statistically significant. N=12.

Parameter	CHOANO	HNP other than CHOANO	HNP	OLIGO	MICRO
BACT (A)	0.070	0.447	0.378	0.298	0.312
(B)	0.124	0.191	0.190	0.532	0.513
ANP (A)	0.668 *	0.877 *	0.896 *	0.710 *	0.692 *
(B)	0.820 *	0.632 *	0.710 *	0.219	0.266
HNP (A)	–	–	–	0.885 *	0.864 *
(B)	–	–	–	0.602 *	0.580 *
CHOANO (A)	–	–	–	0.802 *	0.803 *
(B)	–	–	–	0.473	0.500
NANO (A)	–	–	–	0.749 *	0.730 *
(B)	–	–	–	0.346	0.375

(A): abundance (No./*l*) and (B): biomass (mg C/m^3).
BACT: bacterioplankton, ANP: autotrophic nanoplankton, HNP: heterotrophic nanoplankton, CHOANO: choanoflagellates, NANO: total nanoplankton (the sum of ANP and HNP), OLIGO: oligotrichs, MICRO: total microzooplankton.

4. Discussion

The mean value of bacterioplankton abundance shown in this study, 4.56×10^4 cells/m*l*, was within that of previous reports (10^4–10^5 cells/m*l*) in oceanic area of the Antarctic Ocean (EGUCHI and ISHIDA, 1986; KOGURE *et al.*, 1986; SAMYSHEV, 1986; SHIMIDU *et al.*, 1986).

The abundance of HNP was comparable to the previous studies (10^0–10^3 cells/m*l* order) (MARCHANT, 1985; BUCK and GARRISON, 1988; GARRISON and BUCK, 1989; GARRISON *et al.*, 1991). And that of choanoflagellates was also within the range of previous reports (10^{-1}–10^3 cells/m*l*) (BUCK and GARRISON, 1983; MARCHANT and PERRIN, 1990). FENCHEL (1982), and ANDERSON and FENCHEL (1985) suggested a significant influence of chanoflagellates on the bacterial abundance by judging correlational changes between the two. There was, however, no significant correlation between HNP and bacterioplankton in this study (Table 1). But considering that many choanoflagellate cells ingesting bacterioplankton and forming colonies were observed in this study, bacterioplankton biomass should be sufficiently large for choanoflagellates as well as other bacterivorous HNP in the Antarctic Ocean (FENCHEL, 1982; IMAI and ITO, 1984; BUCK and GARRISON, 1988; MARCHANT, 1990).

Both abundance and biomass of oligotrichs in the present study were similar to those in previous studies which sometimes even included *Mesodinium* spp. (TUMANTSEVA, 1982; BUCK and GARRISON, 1983; HEINBOKEL and COATS, 1985; SUSHIN *et al.*, 1986; GARRISON and BUCK, 1989). The present results are, however, inconsistent with those of HARA and TANOUE (1985) and HARA *et al.* (1986) who showed that total heterotrophs were dominated by naked amoebae, choano- and dinoflagellates, but ciliates were very rare in the oceanic area of Southern Ocean. Their result is uncommon, whereas they did not mention this at all. The abundance of tintinnids was also within the range of previous reports (GARRISON and BUCK, 1989; BOLTOVSKOY *et al.*, 1989; GARRISON *et al.*, 1991), or less (HEINBOKEL and COATS, 1985), but the biomass was a little lower than indicated in literatures. Such a lower biomass is likely due to the predominant occurrence of smaller species in the present study.

Absence of significant correlation in biomass between microzooplankton and ANP is mainly due to non-correlative variation between two assemblages at Stns. 2, 3, 4 and 7. At the former three stations, biomass ratio of nanoplankton (ANP+HNP)/microzooplankton was very low (<0.3) as compared with that of most stations (Fig. 10). Among these three stations, Stns. 3 and 4 are characterized by the predominance of the cells of an unidentified largest oligotrich (Fig. 9). Therefore, the lower biomass ratio could be interpreted as a result of the depression of nanoplankton (ANP+HNP) density through the vigorous grazing of this largest oligotrichs. The decrease of nanoplankton might induce a competition for nanoplankton between the largest oligotrichs and smaller ciliates and the other microzooplankters within the microzooplankton assemblage. This is because that the biomass ratio of nanoplankton (ANP+HNP)/microzooplankton other than the largest oligotrich is similarly low at Stns. 3 and 4 (Fig. 10). The microbial food chain is stated that the production of picoplankton is transferred to larger consumers through nano- and microzooplankton. It is generally accepted that predators usually utilize organisms one order of magnitude smaller than themselves (SHELDON *et*

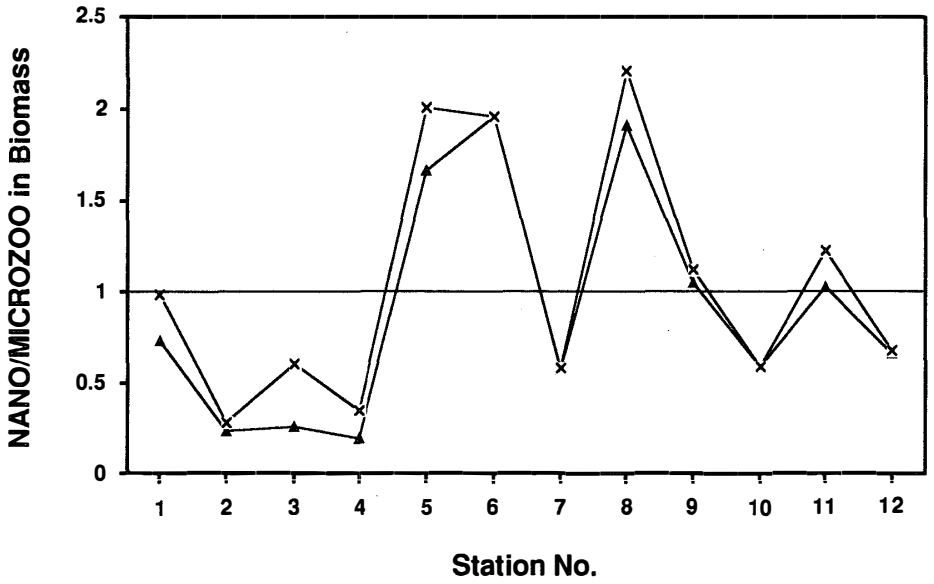

Fig. 10. Biomass ratio of the nanoplankton (ANP+HNP) to microzooplankton at each station. × = nanoplankton/microzooplankton, ▲ = nanoplankton/microzooplankton other than the largest oligotrich.

al., 1972; AZAM *et al.*, 1983). This relationship could be maintained if nano-sized preys are sufficient for micro-sized predators. Therefore at Stns. 3 and 4, microzooplankton are particularly expected to consume micro-sized preys as well as nano-sized ones. In order to examine this hypothesis, we studied selected sample of Stn. 3 focusing on the micro-protozoans ingesting food organisms. Evidences agreeing with the hypothesis were found in the sample (Fig. 11a, b); the percentage of abundance of oligotrichs which were ingesting micro-autotrophs similar to, or larger than the predator was 1.46% of the total number of oligotrichs except for the largest species. On the contrary, the percentage was much lower (0.12%) at Stn. 5 where nanoplankton/microzooplankton ratio was high. Such feeding habit of protozoans has been known in temperate and arctic seas (BURSA, 1961; SMETACEK, 1981; SUTTLE *et al.*, 1986), and from the Weddell Sea (GARRISON and BUCK; 1989). This energy flow within micro-sized category should play an important role as well as the other energy flow from nano- to micro-size, through the oceans when smaller preys are insufficient for larger-sized predators.

We analyzed the biomass of each size category to determine the planktonic community structure in the oceanic province of epipelagic Antarctic Ocean. Figure 12 shows a dry weight biomass spectrum for a variety of organisms with size ranging from nano- to microzooplankton collected from the surface of the Antarctic Ocean. The biomass (log-scale) decreased linearly with increasing individual body weight (log-scale) with a slope of –0.21. This exponent is very close to that (–0.22) predicted by PLATT and DENMAN (1977, 1978). However, this value might have been slightly modified by the employed mass units or weight conversion factors (RODRIGUEZ and MULLIN, 1986). Although this spectrum was obtained solely on the basis of the restricted samples (*ca.* 8 m in depth) within the epipelagic zone, it could be a representative feature of

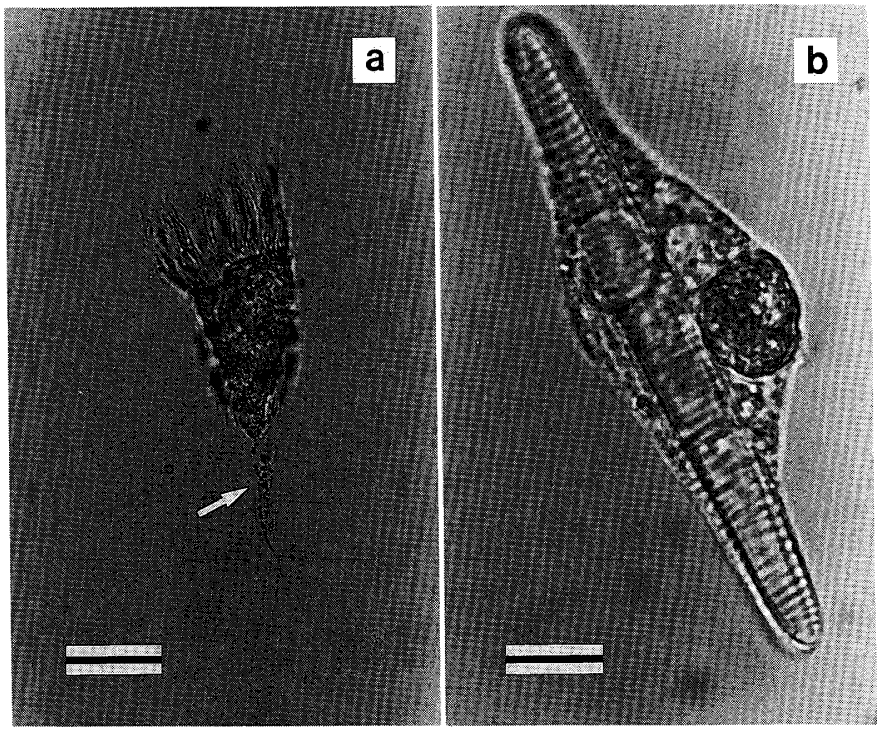

Fig. 11. Micro-protozoans ingesting foods which are similar to or larger than the predator. a = Oligotrich with a stuck out prey (scale bar = 50 μm). b = Heterotrophic flagellate with ingested pennate diatom (scale bar = 10 μm).

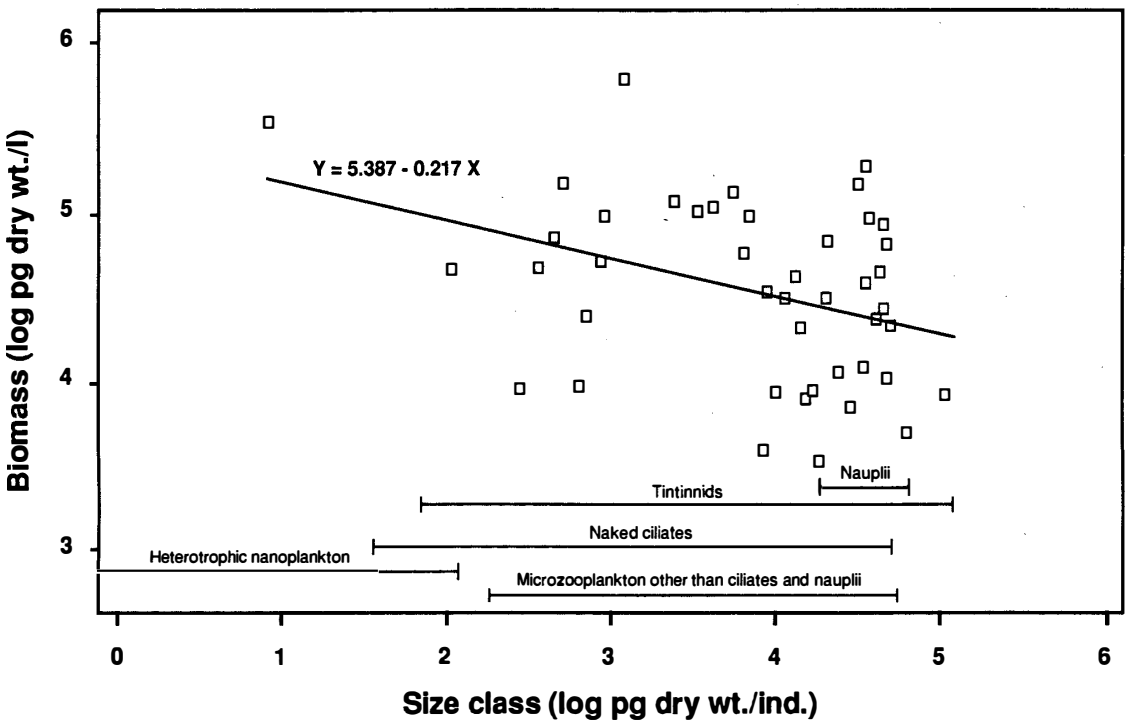

Fig. 12. Biomass spectrum for nano- and micro-sized heterotrophic plankton collected from the surface of oceanic province.

the Antarctic Ocean. This is because the present results of biomass in each size category are all comparable to the previous studies as mentioned above. Furthermore, GARRISON *et al.* (1991) stated the negative correlation between biomass and individual size in protozoan assemblage in the winter Weddell/Scotia Seas. Therefore, these studies suggest that the structure of the Antarctic pelagic ecosystem would be similar to that of subtropical oceans (*e.g.* RODRIGUEZ and MULLIN, 1986). Also, microbial assemblages are more important in the energy and material flows of the open sea of the Antarctic considering that weight specific metabolic rates increase logarithmically with decreasing body size with a slope of –0.25 (*e.g.* MOLONEY and FIELD, 1989). Our analysis is evidently limited to the microbes smaller than 250 μm, thus further studies on the biomass of meso- and macrozooplankton are needed to understand the whole community structure.

Acknowledgments

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