"Depth Distribution, Biomass and Taxonomic Composition of Subtropical Microbial Community in Southwestern Japan"

Depth Distribution, Biomass and Taxonomic Composition
of Subtropical Microbial Community
in Southwestern Japan

KOBARI Toru[1*], HIJIYA Kayo[1], MINOWA Masato[2] and KITAMURA Minoru[3]

1: Fisheries Biology and Oceanography Division, Faculty of Fisheries,
Kagoshima University
4-50-20 Shimoarata, Kagoshima 890-0056, Japan
2: Graduate School of Fisheries, Kagoshima University
4-50-20 Shimoarata, Kagoshima 890-0056, Japan
3: Institute of Biogesciences, Japan Agency for Marine-Earth Science and Technology
Natsushima-cho, Yokosuka 237-0061, Japan
*corresponding author
Email: kobari@fish.kagoshima-u.ac.jp

Abstract

We compared the depth distribution, biomass and taxonomic composition of subtropical microbial community among the three stations in the Kuroshio waters around southwestern Japan. While subsurface chlorophyll maximum was evident for all stations, it was not corresponded to depth distributions of carbon-based biomass for phytoplankton and heterotrophic bacteria, nanoplankton and ciliates. The predominant component was diatoms for phytoplankton biomass at all stations, and naked ciliates at St. A and St. C or heterotrophic nanoplankton at St. B for microzooplankton biomass. Although microbial standing stock and size composition were much different among the three stations, biomass of heterotrophic bacteria and respiratory requirement of heterotrophic ciliates and nanoplankton showed a small fluctuations. These results suggest that heterotrophic bacteria are major food resources and pico- to nano-sized phytoplankton are supplement for heterotrophic ciliates and nanoplankton in the Kuroshio waters around southwestern Japan.

Key words: biomass, carbon flow, depth distribution, microbial community, taxonomic composition

Introduction

In pelagic plankton ecosystem, two major pathways are proposed for carbon flow. Grazing food web is a simple structure, showing major carbon pathways from large phytoplankton like diatoms and dinoflagellates to mesozooplankton (RYTHER 1969). On the other hand, microbial food web includes smaller components, such as pico- to nano-sized pigmented cells, heterotrophic bacteria and protozoans (AZAM *et al.* 1983). Microbial food

web is believed to be a minor contribution to biological productions at higher trophic levels because of the more trophic cascades and dependent on recycled resources compared with the grazing food web. Thereby, structure of plankton food web is greatly associated with biological productivity and carbon flow of pelagic ecosystems.

Plankton biomass and productivity have been considered to be temporally and spatially uniform and the observed variability was explained by stochastic events in the oligotrophic waters, such as equatorial to subtrophical Pacific Oceans (MCGOWAN and HAYWARD 1978, HAYWARD et al. 1983). However, recent findings from the subtropical Pacific Oceans show temporal and spatial variations in abundance, biomass and taxonomic composition of plankton community (KARL et al. 1995, ROMAN et al. 1995, CAMPBELL et al. 1997, ISHIZAKA et al. 1997).

Kuroshio forms the western boundary of the subtropical gyre in the North Pacific flowing northeastward along the edge of the continental shelf along the East China Sea (KAWAI 1972). During this journey, the properties of the Kuroshio waters are modified by continental shelf waters of the East China Sea and by coastal waters around archipelago in southwestern Japan (CHEN et al. 1994). It has been long believed that biological productivity is low in the Kuroshio waters. Some researches described temporal and spatial variations in standing stocks and taxonomic composition of metazoans (HIROTA 1995, NAKATA et al. 2001, NAKATA and HIDAKA 2003, NAKATA and KOYAMA 2003). However, we have little knowledge on standing stock and productivity of microbial community in the Kuroshio waters.

In the present study, we compared the depth distribution, biomass and taxonomic composition of microbial community among the three stations in the Kuroshio water around southwestern Japan. From these results, we describe the properties of carbon flow in the microbial community.

Materials and Methods

Oceanographic observations and samplings

Oceanographic observations and samplings were conducted at the three stations around southwestern Japan during summer of 2008 (Fig. 1). Stations A and C and Stations A and B were for spatial and temporal comparisons, respectively. Temperature and salinity were recorded from 200 m to sea surface using a CTD system. Water samples for chlorophyll a measurements and microscopic analyses were collected at nine depths with Niskin bottle attached on CTD-CMS and at sea surface with plastic bucket. Pico- to nano-sized plankton was fixed with glutaraldehyde (1% final concentration). Micro-sized plankton was preserved with acid Lugol's solution (3% final concentration). Water samples for chlorophyll a concentrations were filtered through a plankton net (20 μm mesh opening), a Millipore polycarbonate membrane filter (5 μm pore size) and a Whatman GF/F filter ($\sim$0.7 μm nominal pore size) under lower vacuum pressure than 20kPa. Thereafter, chlorophyll pigments on the filter were immediately extracted by direct immersion into N, N-dimethylformamyde (DMF) at -5°C under dark condition for more than 24 hours

(SUZUKI and ISHIMARU 1990). Chlorophyll *a* concentration was measured with a Turner Designs fluorometer (Turner Designs, TD-700) based on the non-acidified fluorometric method (WELSCHMEYER 1994).
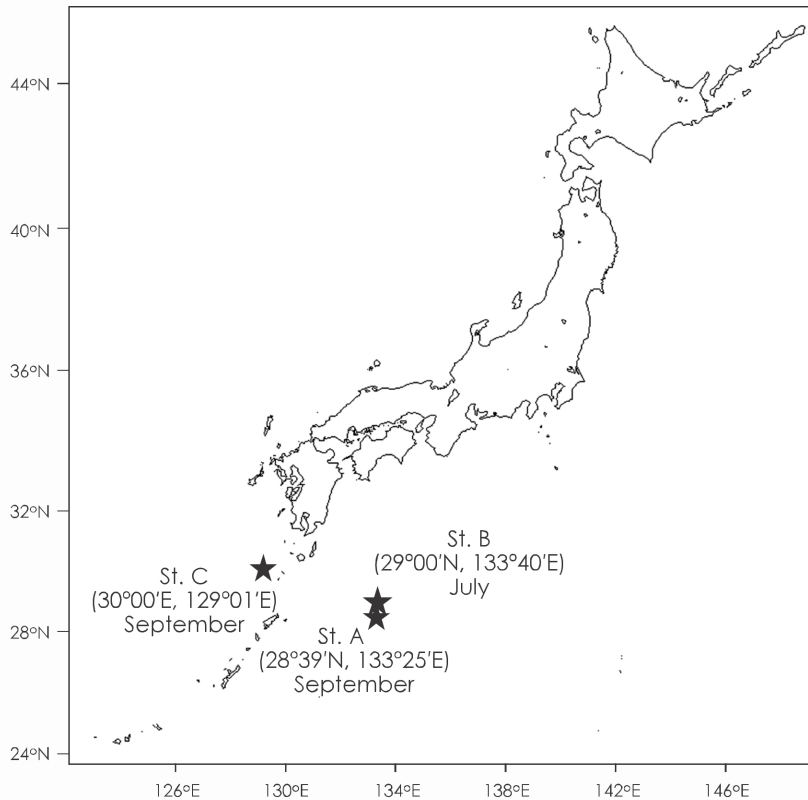

Fig. 1. Sampling stations in the present study.

Enumeration and biomass estimation

We distinguished the pico-plankton into 3 groups (BAC: heterotrophic bacteria, CYN: coccoid cyanobacteria, PE: pico-eukaryotes) and 2 groups for nano-plankton (ANP: autotrophic nanoplankton, HNP: heterotrophic nanoplankton) (Table 1). Heterotrophic bacteria were stained with 4', 6-diamidino-2-phenylindole (DAPI) and filtered onto 0.2-µm black polycarbonate filters (TURLEY 1993), and distinguished with a difference of autofluorescense by switching between UV and violet-blue lights from cyanobacteria. At least 400 cells on each filter were counted by an epifluorescence microscopy. Pico-sized phytoplankton and nano-plankton were filtered onto 0.4-µm black polycarbonate filters after double staining with DAPI and proflavine hemisulphate (HAAS 1982). At least 100 cells or cells at 100 sights on each filter were counted under violet-blue light by an

Table 1. Taxonomic groups identified in the present study and their conversion factors. Abbrevia-
tions with asterisks show heterotrophic protists. C: carbon weight (pgC). N: cell number
(cells). V: cell volume (μm^3).

Taxonomic group	Abbreviation	Conversion factor	Resource
Picoplankton			
Heterotrophic bacteria	BACT	C=0.0128N	Fukuda et al.(1998)
Cyanobacteria	CB	C=0.220N	Mullin et al.(1966)
Pico eukaryotes	PE	C=0.220N	Mullin et al.(1966)
Nanoplankton			
Autotrophic nanoplankton	ANP	$Log_{10}C=0.866Log_{10}V-0.460$	Strathmann(1967)
Heterotrophic nanoplankton	HNP*	C=0.12V	Fenchel(1982)
Microplankton			
Diatoms	DT	$Log_{10}C=0.811Log_{10}V-0.541$	Menden-Deuer and Lessard (2000)
Dinoflagellates	DF	$Log_{10}C=0.864Log_{10}V-0.353$	Menden-Deuer and Lessard (2000)
Naked ciliates	NC*	C=0.19V	Putt and Stoecher(1989)
Tintinnid ciliates	TC*	C=444.5+0.053V	Verity and Langdon(1984)

epifluorescence microscopy. Micro-plankton was distinguished into 4 groups (DT: diatom,
DF: dinoflagellate, NC: naked ciliate, TC: tintinnid ciliate). At least 100 cells or cells at 100
sights were enumerated under an inverted microscope after allowing samples to settle
overnight. Although part of marine planktonic ciliates and dinoflagellates are known to be
mixotrophs (GAINES and ELBRŠCHTER 1987, PIERCE and TURNER 1992, JONES 1994), we
assumed diatoms and dinoflagellates as autotrophs and naked and tintinnid ciliates as
heterotrophs in the present study.

For pico-sized plankton, the cell number was directly converted to carbon values using
appropriate conversion factors (Table 1). For nano- to micro-sized plankton, biovolumes or
lorica volumes were estimated from size measurement of 30 arbitrary selected organisms,
assuming simple geometrical shapes. Digital images were captured by CCD camera and
each cell size was measured by Lumina vision (Mitani co.). The biovolumes or lorica
volumes were converted to carbon value by using appropriate formulae or conversion
factors.

Respiratory requirements of heterotrophic microbes at 20°C (R_{20}: nLO_2 $cell^{-1}$ $hour^{-1}$)
were estimated from the following equation (FENCHEL and FINLAY 1983):

$$logR_{20} = 0.75 \times logV - 4.09 \qquad (1)$$

where V is cell or lorica volumes (μm^3). R_{20} was transformed into RC_{20} (mgC $cell^{-1}$ day^{-1}) as
follows:

$$RC_{20} = RQ \times R_{20} \times 12/22.4 \times 24 \times 10^{-6} \qquad (2)$$

where respiratory quotient (RQ) is assumed to be 0.97 (GNAIGER 1983). Respiratory
requirement (RC_t) were adjusted by Q_{10} (2.5: CARON et al. 1990) using the following
equation:

$$Q_{10} = (RC_{20}/RC_t)^{10/(20-t)} \qquad (3)$$

Respiratory requirement of heterotrophic community (RC: mgC m^{-2} day^{-1}) was estimated summing cumulative cell numbers in the water column above 200 m (N: cells m^{-2}):

$$RC = RC_t \times N \qquad\qquad (4)$$

Carbon flow in microbial food web was estimated with the following assumptions.

1. Microbes are categorized into three size groups (i.e. micro-, nano- and pico-sized).
2. Some autotrophic nanoflagellates need carbon resources other than photosynthesis.
3. Heterotrophic ciliates and nanoplankton feed on particles smaller than their cell sizes.
4. Major carbon flow is formed between larger standing stock and larger respiratory requirement.

Results

Oceanographic conditions

Mixed layer depth was the shallowest at Station A (St. A) and the deepest at Station B (St. B) (Fig. 2). Although locations and seasons were very close among the stations, mixed layer depth was different between St. A and St. B. Mean temperature in the mixed layer ranged from 28 to 29 °C and showed no substantial difference among the three stations. Thermocline was developed at a depth range of 10 to 20 m at St. A and 40 to 50 m at St. B and Station C (St. C). Mean salinity in the mixed layer was lower at St. C (34.0 PSU) compared with those at the other stations (34.4PSU). Mean chlorophyll *a* concentrations in the mixed layer were lower at St. A and St. B compared with those at St. C (Fig. 3).
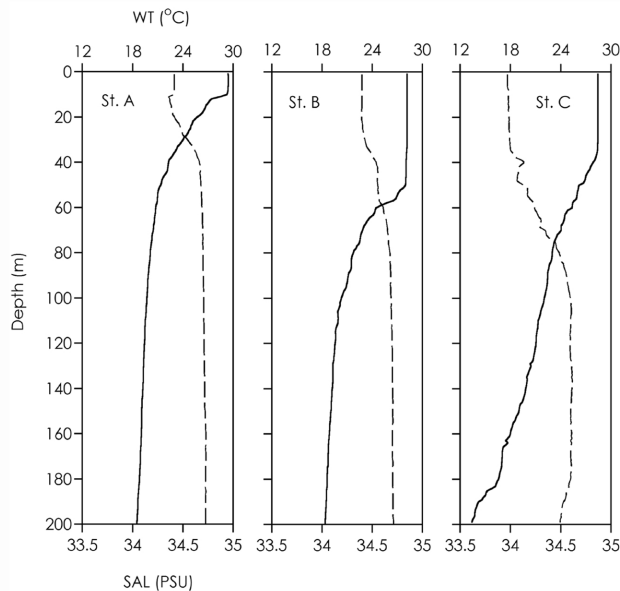

Fig. 2. Vertical profiles of water temperature (WT: solid line, °C) and salinity (SAL: broken line, PSU).
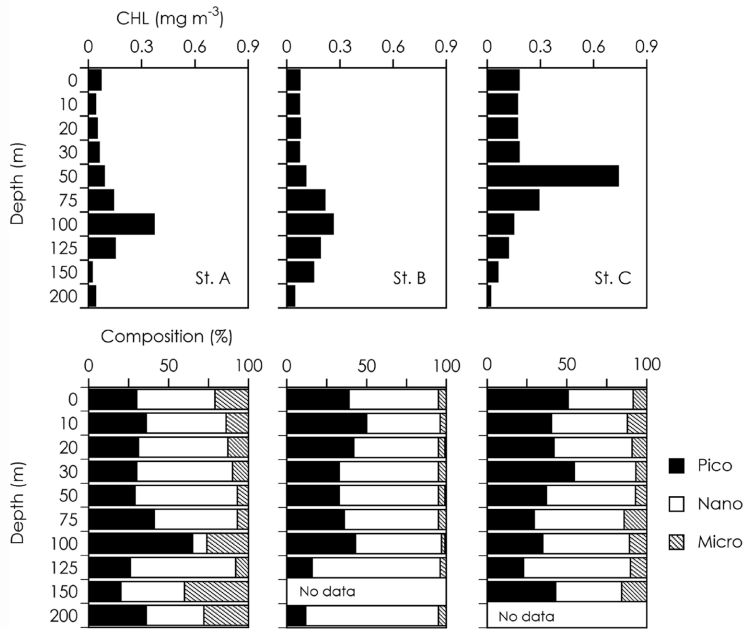
Fig. 3. Depth distribution of chlorophyll *a* concentration (CHL: mg m⁻³) and its size composition (%). Pico: 0.7-5 μm. Nano: 5-20 μm. Micro: >20 μm.
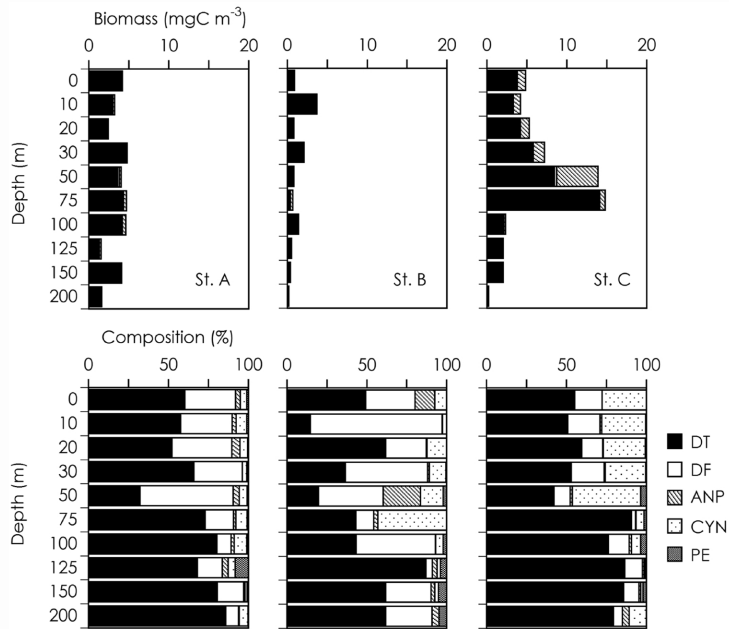
Fig 4. Depth distribution of biomass of autotrophic plankton (mgC m⁻³) and relative composition (%). DT: Diatom. DF: Dinoflagellate. ANP: Autotrophic nanoplankton. CYN: Cyanobacteria. PE: Pico-eukaryote.

Subsurface chlorophyll maximum was evident for all three stations. It was developed in the layers much deeper than the thermocline at St. A and St. B (100 m) and at the bottom of the mixed layer depth at St. C (50 m). Pico to nano-sized chlorophyll *a* was predominant throughout the water column at all stations.

Depth distribution of microbes

Phytoplankton biomass was high on the thermocline at St. C, but such pattern was obscure at St. A and St. B (Fig. 4). Depth distributions of phytoplankton biomass were not consistent with subsurface chlorophyll maximum at all stations. The most predominant group was diatoms throughout the water column at all stations, while dinoflagellates occurred abundantly at 50 m at St. A and at 10 m at St. B. Cyanobacteria appeared abundantly at subsurface chlorophyll maximum at all stations.

Microzooplankton (heterotrophic ciliates and nanoplankton) showed high biomass in the mixed layer at St. C, while no clear pattern was found for those at St. A and St. B. Depth distributions of microzooplankton biomass were not correspondent with those of chlorophyll *a* concentrations and phytoplankton biomass. The most predominant group was naked ciliates at St. A and St. C and heterotrophic nanoplankton at St. B (Fig. 5).
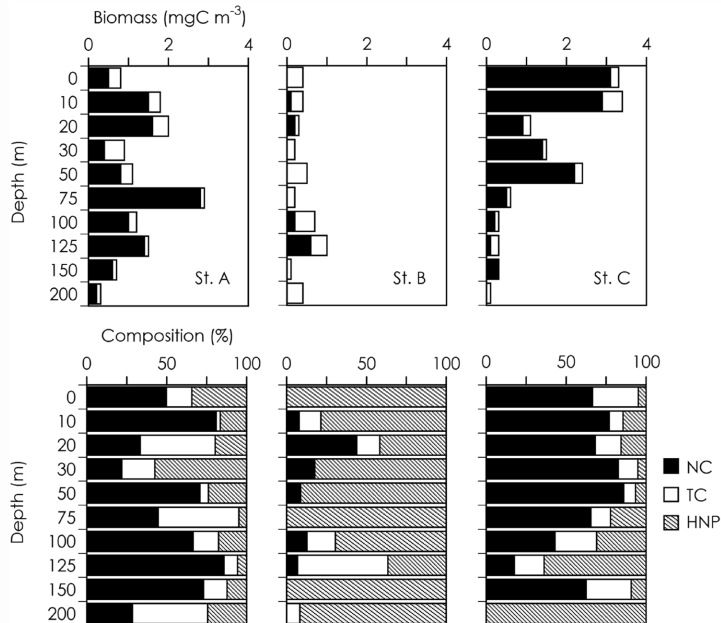

Fig 5. Depth distribution of biomass of heterotrohic protests (mgC m^{-3}) and relative composition (%). NC: Naked ciliate. TC: Tintinnid ciliate. HNP: heterotrophic nanoplankton.
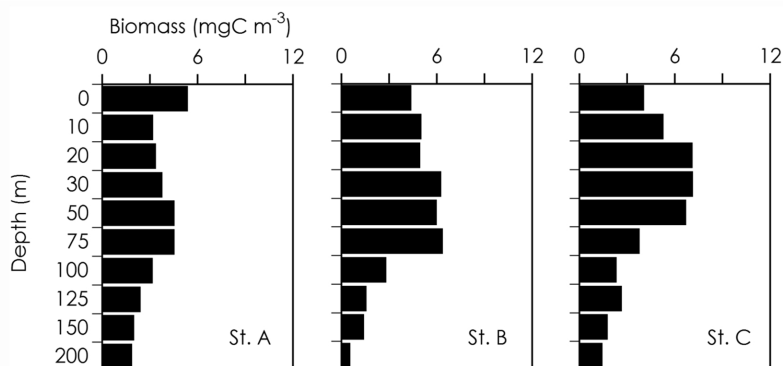
Fig 6. Depth distribution of biomass of heterotrophic bacteria (mgC m^{-3}) and relative composition (%).

Heterotrophic bacteria showed high biomass in the layers shallower than 100 m at all three stations (Fig. 6). The distribution pattern was not consistent with those of chlorophyll *a* and biomasses of phytoplankton and microzooplankton.

Carbon flow

Figure 7 demonstrated standing stock and possible carbon flow among microbial community above 200 m at the three stations. Based on chai-square test, significant difference of biomass among the stations was evident for each taxonomic group of both auto- ($\chi^2=237.8$, df=8, $p<0.05$) and heterotrophs ($\chi^2=162.3$, df=6, $p<0.05$). At St. A, standing stock of ciliates was the highest among the three stations. Phytoplankton community showed moderate standing stock compared with those at the other stations. Respiratory requirements of ciliates were higher than that of heterotrophic nanoplankton. St. B was characterized by the lowest standing stock for phytoplankton among the three stations. Thereby, heterotrophic bacteria showed the highest standing stock among all microbial components. Respiratory requirement of heterotrophic nanoplankton were higher than ciliates. At St. C, phytoplankton demonstrated the highest standing stock among the three stations due to diatoms and cyanobacteria. Respiratory requirement of naked ciliates was higher than those of tintinnid ciliates and heterotrophic nanoplankton.

Discussion

There is little knowledge on microbial food web in the Kuroshio waters around southwestern Japan, while many papers are published from the equatorial to subtropical Pacific Oceans (e.g. CAMPBELL *et al.* 1997, SUZUKI *et al.* 1997, MATSUMOTO *et al.* 2004, YANG *et al.* 2004). These authors mention that oligotrophic waters like equatorial to subtropical Pacific Oceans are characterized by subsurface chlorophyll maximum formed
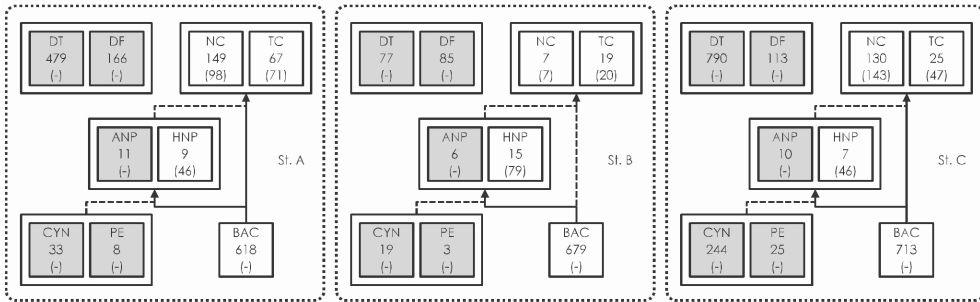
Fig. 7. Components (boxes) and provable carbon flows (arrows) of microbial food web at each station. Numbers with and without parentheses indicate respiratory requirement cumulated in the water column above 200 m (mgC m^{-2} day^{-1}) and standing stock (mgC m^{-2}), respectively. Shaded boxes mean autotrophic plankton. Bottom boxes: Pico-sized plankton. Middle boxes: Nano-sized plankton. Upper boxes: Micro-sized plankton. DT: Diatom. DF: Dinoflagellate. ANP: Autotrophic nanoplankton. CYN: Cyanobacteria. PE: Picoeukaryote. NC: Naked ciliate. TC: Tintinnid ciliate. HNP: Heterotrophic nanoplankton. BAC: Heterotrophic bacteria. -: no data.

Table 2. Comparisons of ocean conditions, biomasses of heterotrophic bacteria, phytoplankton and microzooplankton, and respiratory requirements of microzooplankton among the three stations in the Kuroshio waters around southwestern Japan. Asterisks show the numbers cumulated in the water column in 0-200 m.

Parameter	St. A	St. B	St. C
Sampling season	September	July	September
Mixed layer depth (m)	11	53	44
Mean temperature (°C)	20.9	22.3	22.2
Chlorophyll *a* (mg m^{-2})*	1.0	1.3	2.1
Bacterial biomass (BAC: mgC m^{-2})*	618.2	678.6	712.9
Phytoplankton biomass (PHY: mgC m^{-2})*	696.8	192.0	1181.6
Size composition (BAC+PHY: %)*			
Micro	49.0	18.7	47.7
Nano	0.8	0.7	0.5
Pico	50.2	80.6	51.8
Zooplankton biomass (mgC m^{-2})*	225.0	40.8	161.7
Size composition (%)*			
Micro	96.1	64.4	96.0
Nano	3.9	35.6	4.0
Respiratory requirement (mgC m^{-2} day^{-1})*	215.3	105.4	235.3

by pico-sized phytoplankton. It has been believed that they appear abundantly at the subsurface depths where nutrient concentrations were high (CAMPBELL *et al.* 1997; MATSUMOTO *et al.* 2004). In recent years, their depth distributions were sometimes inconsistent with subsurface chlorophyll maximum while they dominated phytoplankton community (TAYLOR *et al.* 2011). They described that such distribution pattern was resulted from enhancement of pico-phytoplankton in the upwelling edges and larger diatoms in the downwelling edges. In the present study, cyanobacteria appeared more abundantly at subsurface chlorophyll maximum than at the surface layers, while diatoms and dinoflagellates composed more than half of phytoplankton biomass throughout the water column at all stations (Fig. 4). These results suggest that larger phytoplankton cells contribute to biomass but do not form subsurface chlorophyll maximum.

Microzooplankton biomass is corresponded to subsurface maximum of chlorophyll *a* and phytoplankton biomass in the equatorial Pacific Ocean (YANG *et al.* 2004). They mentioned that the subsurface maximum of heterotrophic microbes was caused by high food availability. However, such correspondence was obscure for the depth distributions of biomass for heterotrophic bacteria, nanoplankton and ciliates in the present study (Figs. 5 and 6). Heterotrophic ciliates and nanoplankton are known to feed on smaller particles than their body sizes (HANSEN *et al.* 1994). In the present study, pico- to nano-sized phytoplankton biomass was much different among the three stations, indicating that spatial and temporal fluctuations are large for smaller phytoplankton as food resources for microzooplankton. Moreover, standing stocks of cyanobacteria and pico-eukaryotes were lower than respiratory requirement of heterotrophic nanoplankton at St. A and St. B (Fig. 7). Contrary to them, such spatial and temporal fluctuations were obscure for biomass of heterotrophic bacteria, which was comparable to the previous estimates at the subtropical sites in the eastern North Pacific (4.3 to 8.9 mgC m^{-3}: TAYLOR *et al.* 2011). They can support respiratory requirement of heterotrophic nanoplankton (Table 2, Fig. 7). In spite of the large difference of biomass and its size composition of heterotrophic nanoplankton and ciliates, their respiratory requirements were nearly equal among the three stations (Table 2, Fig. 7). These results suggest that heterotrophic bacteria are major food resources and smaller phytoplankton cells are supplement for heterotrophic nanoplankton and ciliates in the Kuroshio waters around southwestern Japan. Such trophic relationship is probable explanation for the discrepancies in depth distributions between subsurface chlorophyll maximum and heterotrophic microbes in the present study.

Acknowledgements

Reference

AZAM, F., FENCHEL, T., FIELD, J. G., GRAY, J. S., MEYER-REIL, L. A. and THINGSTAD, F. 1983. The Ecological Role of Water-Columm Microbes in the Sea. Marine Ecology and Progress Series, 10: 257-263.

CAMPBELL, L., HONGBIN, L., NOLLA, H. A., and VAULOT, D. 1997. Annual Variability of Phytoplankton and Bacteria in the Subtropical North Pacific Ocean at Station ALOHA during the 1991-1994 ENSO event. Deep-Sea Research I, 44: 167-192.

CARON, D. A., GOLDMAN, J. C. and FENCHEL, T. 1990. Protozoan Respiration and Metabolism. In: Ecology of Marine Protozoa (Ed. CAPRIULO, G. M.), 307-322, Oxford University Press, New York.

CHEN, C., BEARDSLEY, R. C., LIMEBURNER, R. and KIM, K. 1994. Comparison of Winter and Summer Hydrographic Observations in the Yellow and East China Seas and Adjacent Kuroshio during 1986. Continental Shelf Research, 14: 909-929.

FENCHEL, T. 1982. Ecology of Heterotrophic Microflagellates. I. Some Important Forms and Their Functional Morphology. Marine Ecology Progress Series, 8: 211-223.

FENCHEL, T. and FINLAY, B. J. 1983. Respiration Rates in Heterotrophic, Free-Living Protozoa. Microbial Ecology, 9: 99-122.

FUKUDA, R., OGAWA, H., NAGATA, T. and KOIKE, I. 1998. Direct Determination of Carbon and Nitrogen Contents of Natural Bacterial Assemblages in Marine Environments. Applied Environmental Microbiology, 64: 3352-3358.

GAINES, G. and ELBRŠCHTER, M. 1987. Heterotrophic Nutrition. In: The Biology of Dinoflagellates (Ed. TAYLOR, F. J. R.), 224-268, Blackwell, Oxford.

GNAIGER, E. 1983. Calculation of Energetic and Biochemical Equivalents of Respiratory Oxygen Consumption. In: Polarographic Oxygen Sensors (Eds. GNAIGER, E. and HORSTNER, H.), 337-347, Springer-Verlag, Berlin.

HAAS, L. W. 1982. Improved Epifluorescence Microscopy for Observing Planktonic Micro-Organisms. Annales de l'Institut Oceanographique, Paris, 58: 261-266.

HANSEN, B., BJORNSEN, P. K. and HANSEN, P. J. 1994. The Size Ratio between Planktonic Predators and Their Prey. Limnology and Oceanography, 39: 395-403.

HAYWARD, T. L., VENRICK, E. L. and MCGOWAN, J. A. 1983. Environmental Heterogeneity and Plankton Community Structure in the Central North Pacific. Journal of Marine Research, 41: 711-729.

HIROTA, Y. 1995. The Kuroshio. Part III. Zooplankton. Oceanography and Marine Biology: Annual Review, 33: 151-220.

ISHIZAKA, J., HARADA, K., ISHIKAWA, K., KIYOSAWA, H., FURUSAWA, H., WATANABE, Y., ISHIDA, H., SUZUKI, K., HANDA, N. and TAKAHASHI, M. 1997. Size and Taxonomic Plankton Community Structure and Carbon Flow at the Equator, 175°E during 1990-1994. Deep-Sea Research II, 44: 1927-1949.

JONES, R. I. 1994. Mixotrophy in Planktonic Protists as a Spectrum of Nutritional Strategies. Marine Microbial Food Webs, 8: 87-96.

KARL, D. M., LETELIER, R. M., HEBEL, D., TUPAS, L., DORE, J., CHRISTIAN, J. and WINN, C. 1995. Ecosystem Changes in the North Pacific Subtropical Gyre Attributed to the 1991-1992 El Niño. Nature, 373: 230-234.

KAWAI, H. 1972. Hydrography of Kuroshio and Oyashio. In: Physical Oceanography II (Ed. MASUZAWA, J.), 129-320, Tokai University Press, Tokyo.

MATSUMOTO, R., FURUYA, K. and KAWANO, T. 2004. Association of Picophytoplankton Distribution with ENSO Events in the Equatorial Pacific between 145°E and 160°W. Deep-Sea Research I, 51: 1851-1871.

MCGOWAN, J. A. and HAYWARD, T. L. 1978. Mixing and Oceanic Productivity. Deep-Sea Research, 25: 771-793.

MENDEN-DEUER S. and LESSARD, E. J. 2000. Carbon to Volume Relationships for Dinoflagellates, Diatoms, and Other Protist Plankton. Limnology and Oceanography, 45: 569-579.

MULLIN, M. M., SLOAN, P. R., EPPLEY, R. W. 1966. Relationship between Carbon Content, Cell Volume and Area in Phytoplankton. Limnology and Oceanography, 11: 307-311.

NAKATA, K., KOYAMA, S. and MATSUKAWA, Y. 2001. Interannual Variation in Spring Biomass and Gut Content Composition of Copepod in the Kuroshio Current, 1971-89. Fisheries Oceanography, 10: 329-341.

NAKATA, K. and HIDAKA, K. 2003. Decadal-Scale Variability in the Kuroshio Marine Ecosystem in Winter. Fisheries Oceanography, 12: 234-244.

NAKATA, K. and KOYAMA, S. 2003. Interannual Changes of the Winter to Early Spring Biomass and Composition of Mesozooplankton in the Kuroshio Region in Relation to Climatic Factors. Journal of Oceanography, 59: 225-234.

PIERCE, R. W. and TURNER, J. T. 1992. Ecology of Planktonic Ciliates in Marine Food Webs. Reviews in Aquatic Science, 6: 139-181.

PUTT, M. and STOECKER, D. K. 1989. An Experimentally Determined Carbon: Volume Ratio for Marine "Oligotrichous" Ciliates from Estuarine and Coastal Water. Limnology and Oceanography, 34: 1097-1103.

ROMAN, M. R., DAM, H. G., GAUZENS, A. L., URBAN-RICH, J., FOLEY, D. G. and DICKEY, T. D. 1995. Zooplankton Variability on the Equator at 140°W during the JGOFS EqPac Study. Deep-Sea Research II, 42: 673-693.

RYTHER, J. H. 1969. Photosynthesis and Fish Production in the Sea. Science, 166: 72-76.

STRATHMANN, R. R. 1967. Estimating the Organic Carbon Content of Phytoplankton from Cell Volume or Plasma Volume. Limnology and Oceanography, 12: 411-418.

SUZUKI, K., HANDA, N., KIYOSAWA, H. and ISHIZAKA, J. 1997. Temporal and Spatial Distribution of Phytoplankton Pigments in the Central Pacific Ocean along 175°E during the Boreal Summers of 1992 and 1993. Journal of Oceanography, 53: 383-396.

SUZUKI, R. and ISHIMARU, T. 1990. An Improved Method for the Determination of Phytoplankton Chlorophyll Using N, N-dimethylformamide. Journal of the Oceanographical Society of Japan, 46: 190-194.

TAYLOR, A. G., LANDRY, M. R., SELPH, K. E. and YANG, E. J. 2011. Biomass, Size Structure and Depth Distributions of the Microbial Community in the Eastern

Equatorial Pacific. Deep-Sea Research II, 58: 342-357.

TURLEY, C. M. 1993. Direct Estimates of Bacterial Numbers in Seawater Samples without Incurring Cell Loss due to Sample Storage. In: Handbook of Methods in Aquatic Microbial Ecology (Eds. KEMP, P. F., SHERR, B. F., SHERR, E. B. and COLE, J. J.), 143-147, Lewis Publishers, New York.

VERITY, P. G. and LANGDON, C. 1984. Relationships between Lorica Volume, Carbon, Nitrogen, and ATP Content of Tintinnids in Narragansett Bay. Journal of Plankton Research, 6: 859-868.

WELSCHMEYER, N. A. 1994. Fluorometric Analysis of Chlorophyll *a* in the Presence of Chlorophyll *b* and Pheopigments. Limnology and Oceanography, 39: 1985-1992.

YANG, E. J., CHOI, J. K. and HYUN, J. H. 2004. Distribution and Structure of Heterotrophic Protist Communities in the Northeast Equatorial Pacific Ocean. Marine Biology, 146: 1-15.