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

KOBARI Toru¹*, HIJIYA Kayo¹, MINOWA Masato² and KITAMURA Minoru³

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, nanoplanктон and ciliates. The predominant component was diatoms for phytoplankton biomass at all stations, and naked ciliates at St. A and St. C or heterotrophic nanoplanктон 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 nanoplanктон 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 nanoplanктон 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 subtropical 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 (~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-dimethylformamide (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).

![Sampling stations in the present study.](image)

**Enumeration and biomass estimation**

We distinguished the pico-plankton into 3 groups (BAC: heterotrophic bacteria, CYN: coccolid cyanobacteria, PE: pico-eukaryotes) and 2 groups for nano-plankton (ANP: autotrophic nanoplanckton, HNP: heterotrophic nanoplanckton) (Table 1). Heterotrophic bacteria were stained with 4', 6-diamidino-2-phenylinodole (DAPI) and filtered onto 0.2-μm black polycarbonate filters (Turley 1993), and distinguished with a difference of autofluorescence 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. Abbreviations with asterisks show heterotrophic protists. C: carbon weight (pgC). N: cell number (cells). V: cell volume (µm³).

<table>
<thead>
<tr>
<th>Taxonomic group</th>
<th>Abbreviation</th>
<th>Conversion factor</th>
<th>Resource</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picoplankton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterotrophic bacteria</td>
<td>BACT</td>
<td>C=0.0128N</td>
<td>Fukuda et al. (1998)</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>CB</td>
<td>C=0.220N</td>
<td>Mullin et al. (1966)</td>
</tr>
<tr>
<td>Pico eukaryotes</td>
<td>PE</td>
<td>C=0.220N</td>
<td>Mullin et al. (1966)</td>
</tr>
<tr>
<td>Nanoplankton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autotrophic nanoplankton</td>
<td>ANP*</td>
<td>Log₂C=0.866Log₀V-0.460</td>
<td>Strathmann (1967)</td>
</tr>
<tr>
<td>Heterotrophic nanoplankton</td>
<td>HNP*</td>
<td>C=0.12V</td>
<td>Fenchel (1982)</td>
</tr>
<tr>
<td>Microplankton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diatoms</td>
<td>DT</td>
<td>Log₂C=0.811Log₀V-0.541</td>
<td>Menden-Deuer and Lessard (2000)</td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td>DF</td>
<td>Log₂C=0.864Log₀V-0.353</td>
<td>Menden-Deuer and Lessard (2000)</td>
</tr>
<tr>
<td>Naked ciliates</td>
<td>NC*</td>
<td>C=0.19V</td>
<td>Putt and Stoecker (1989)</td>
</tr>
<tr>
<td>Tintinnid ciliates</td>
<td>TC*</td>
<td>C=444.5+0.053V</td>
<td>Verity and Langdon (1984)</td>
</tr>
</tbody>
</table>

epi fluorescenc 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 (R₂₀: nLO₂ cell⁻¹ hour⁻¹) were estimated from the following equation (FENCHEL and FINLAY 1983):

\[
\log R_{20} = 0.75 \times \log V - 4.09
\]

(1)

where V is cell or lorica volumes (µm³). R₂₀ was transformed into RC₂₀ (mgC cell⁻¹ day⁻¹) as follows:

\[
RC_{20} = RQ \times R_{20} \times 12/22.4 \times 24 \times 10^6
\]

(2)

where respiratory quotient (RQ) is assumed to be 0.97 (GNAIGER 1983). Respiratory requirement (RC₁) were adjusted by Q₁₀ (2.5: CARON et al. 1990) using the following equation:

\[
Q_{10} = (RC_{20}/RC_{1})^{10/20-1}
\]

(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\(^{-3}\)):

\[
RC = RC_c \times N
\]

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 (34.4 PSU). 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).](image)
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 nanoplanckton. 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).

![Graphs showing depth distribution of biomass and composition](image)

*Fig 5. Depth distribution of biomass of heterotrophic protests (mgC m⁻³) and relative composition (%). NC: Naked ciliate. TC: Tintinnid ciliate. HNP: heterotrophic nanoplankton.*
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 chia-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$^{-3}$), 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 nanoplanктон. CYN: Cyanobacteria. PE: Picoeukaryote. NC: Naked ciliate. TC: Tintinnid ciliate. HNP: Heterotrophic nanoplanктон. 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>St. A</th>
<th>St. B</th>
<th>St. C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling season</td>
<td>September</td>
<td>July</td>
<td>September</td>
</tr>
<tr>
<td>Mixed layer depth (m)</td>
<td>11</td>
<td>53</td>
<td>44</td>
</tr>
<tr>
<td>Mean temperature (°C)</td>
<td>20.9</td>
<td>22.3</td>
<td>22.2</td>
</tr>
<tr>
<td>Chlorophyll α (mg m$^{-2}$)*</td>
<td>1.0</td>
<td>1.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Bacterial biomass (BAC: mgC m$^{-2}$)*</td>
<td>618.2</td>
<td>678.6</td>
<td>712.9</td>
</tr>
<tr>
<td>Phytoplankton biomass (PHY: mgC m$^{-2}$)*</td>
<td>696.8</td>
<td>192.0</td>
<td>1181.6</td>
</tr>
<tr>
<td>Size composition (BAC+PHY: %)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micro</td>
<td>49.0</td>
<td>18.7</td>
<td>47.7</td>
</tr>
<tr>
<td>Nano</td>
<td>0.8</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Pico</td>
<td>50.2</td>
<td>80.6</td>
<td>51.8</td>
</tr>
<tr>
<td>Size composition (%)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micro</td>
<td>96.1</td>
<td>64.4</td>
<td>96.0</td>
</tr>
<tr>
<td>Nano</td>
<td>3.9</td>
<td>35.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Zooplankton biomass (mgC m$^{-2}$)*</td>
<td>225.0</td>
<td>40.8</td>
<td>161.7</td>
</tr>
<tr>
<td>Size composition (%)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micro</td>
<td>96.1</td>
<td>64.4</td>
<td>96.0</td>
</tr>
<tr>
<td>Nano</td>
<td>3.9</td>
<td>35.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Respiratory requirement (mgC m$^{-2}$ day$^{-1}$)*</td>
<td>215.3</td>
<td>105.4</td>
<td>235.3</td>
</tr>
</tbody>
</table>
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, nanoplanckton and ciliates in the present study (Figs. 5 and 6). Heterotrophic ciliates and nanoplanckton 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 nanoplanckton 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 nanoplanckton (Table 2, Fig. 7). In spite of the large difference of biomass and its size composition of heterotrophic nanoplanckton 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 nanoplanckton 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

We are grateful to two anonymous reviewers for valuable comments on our manuscript. We thank Dr. A. Nishina and the crews of T/S “Tansei-Maru” and “Kagoshima-Maru” for help with oceanographic observations and plankton samplings. A part of the present study was supported by grants from the Japan Society for the Promotion of Science (21710012) and from Kagoshima University.
Reference


