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Vertical planktonic structure in the central basin of Lake Baikal in summer 1999, with special reference to the microbial food web

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Abstract Planktonic microbial interactions in the central basin of Lake Baikal were examined on a summer day in 1999. The subsurface maxima of bacterial abundance and chlorophyll concentration were recorded at the same depth, whereas the vertical distribution of heterotrophic nanoflagellates was the inverse of those of bacteria and picophytoplankton. Release of extracellular organic carbon (EOC) from phytoplankton was estimated by the $NaH^{14}CO_3$ method as $2.4\mu g\,C\,l^{-1}\,day^{-1}$. Bacterial production ($4.3\mu g\,C\,l^{-1}\,day^{-1}$), estimated in a bottle incubation experiment using size-fractionated water samples, exceeded the EOC released. Thus, other supplying sources of organic matter are needed for the bacterial production. Grazing ($2.6\mu g\,C\,l^{-1}\,day^{-1}$) was also estimated in the experiment and accounted for 60% of the bacterial production. This is the first report on the microbial food web in the central basin of Lake Baikal.

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Introduction

Lake Baikal is the deepest (1637 m) and largest lake in the world by volume (23 000 km^3). It is located in southeastern Siberia between 51°28′N and 55°56′N, and between 103°40′E and 110°05′E (Fig. 1). A geological cataclysm created the lake: tectonic movements produced faults and rifts, both of which characterize the morphometry of the lake. Because of its extremely steep shore, the littoral zone is limited, and pelagic organisms such as plankton are thus important in the biogeochemistry of the lake.

Previous studies have demonstrated that planktonic primary production in Lake Baikal is the highest during summer (Kozhova 1987), and that phytoplankton smaller than 10 μm are responsible for a significant proportion (60%–100%) of total primary production in the epilimnion (Votinsev et al. 1972; Popovskaya 1979; Bondarenko and Guselnikova 1989; Back et al. 1991; Nagata et al. 1994). It has recently been shown using epifluorescent microscopy that phytoplankton smaller than 2 μm, i.e., picophytoplankton, such as *Synechococcus* (Cyanophyceae), are the most important primary producers (Boraas et al. 1991; Nagata et al. 1994). Nagata et al. (1994) noted that primary production in the picophytoplankton-size fraction accounted for about 80% of the total on a summer day at one sampling station.

Microbial food webs in which the food linkage between heterotrophic bacteria and protists is the major route for matter cycling have been intensively studied during the past decade. Because the size of picophytoplankton overlaps that of bacteria, they are also consumed by protists such as heterotrophic nanoflagellates (HNF) (Nagata 1988; Nagata et al. 1994; Simek et al. 1997) and ciliates (Weisse 1988; Simek et al. 1995, 1997). Hence, the major supply route of organic matter to metazoan zooplankton in Lake Baikal may be via the microbial food web, because picophyto-

plankton are dominant, as mentioned above. However, the microbial food web in the lake is not yet fully understood.

In Lake Baikal, there have been some studies that examined the microbial food web (Nagata et al. 1994) or the ecology of bacteria (Boraas et al. 1991; Ahn et al. 1999) and picophytoplankton (Votinsev et al. 1972; Popovskaya 1979; Bondarenko and Guselnikova 1989; Back et al. 1991; Boraas et al. 1991), but they were all conducted only in the southern basin where there is eutrophication (Watanabe and Drucker 1999), and we do not have information about the microbial food web in the central and northern basins, where the trophic status is considered to be oligotrophic.

In the present study, we examined the vertical distribution of bacteria, picophytoplankton, and protists in the central basin of Lake Baikal on a summer day in 1999 to collect basic information about the microbial food web. This is the first report on the microbial food web in the central basin of Lake Baikal.

Materials and methods

Water samples were collected on July 31, 1999, at a pelagic station (53°24′36″N and 108°40′40″E; water depth, ca. 700 m) in the central basin of Lake Baikal (Fig. 1). Water samples were collected from depths of 0, 5, 10, 15, 20, 40, and 80 m using a Niskin sampler. Water temperature was measured using a thermistor.

To measure the chlorophyll *a* concentration, a measured portion of each water sample was filtered through a

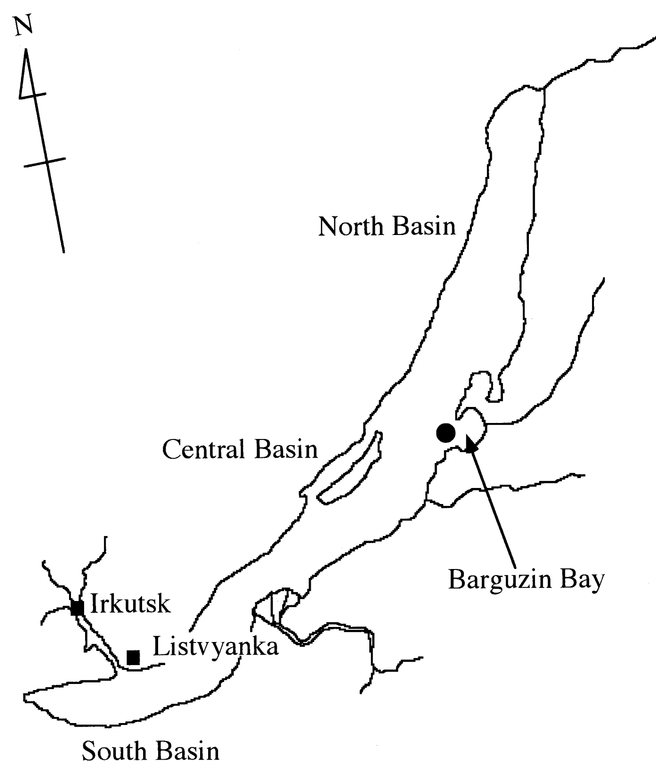

Fig. 1. Map of Lake Baikal showing the sampling station (*circle*)

Whatman (Whatman International, Maidstone, UK) GF/F filter to retain seston. The filter was placed in a glass test tube and 6 ml of *N*,*N*-dimethylformamide was added to extract the chlorophyll *a*, which was then quantified by the fluorometric method (Suzuki and Ishimaru 1990).

A 500-ml portion of the water sample was fixed with acid Lugol's solution at a final concentration of 1%, and the phytoplankton were concentrated by natural sedimentation. Phytoplankton cells were enumerated with a hematocytometer under a microscope at ×200 or ×400 magnification.

Immediately after collection, 100-ml portions of the water sample were fixed with glutaraldehyde to a final concentration of 1% for enumeration of microorganisms. Bacteria and HNF were enumerated using an epifluorescence microscope under ultraviolet excitation using the 4′,6-diamidino-2-phenylindole (DAPI, Porter and Feig 1980) and primulin (Caron 1983) staining methods, respectively. We counted nanoflagellates as HNF if they showed no obvious chlorophyll fluorescence, and the detection limit of HNF enumeration was 50 cells ml^{-1}. Picophytoplankton were enumerated using autofluorescence under green excitation.

We enumerated ciliates using the same sample and method as described above for phytoplankton. We identified ciliate genera or species using the classification guides of Taylor and Sanders (1991), Patterson and Hedley (1992), Foissner and Berger (1996), and Timoshkin (1995).

Primary production was measured by the radiocarbon technique of Steemann Nielsen (1952). The water samples collected from the surface were poured into two series of bottles (transparent and dark) and inoculated with radioactive bicarbonate solution. The bottles were incubated in a water tank on the deck of the research vessel Obrachev, at a water temperature similar to the surface water temperature of the sampling station. After incubation (12:35 to 20:35), biological activity was terminated by adding formaldehyde solution. Each sample was then filtered through a membrane filter (Millipore HA, Billerica, USA) before the filtrate was acidified with sulfuric acid solution and bubbled with purified N_2 to remove the radioactive inorganic carbon. Ten milliliters of Bray scintillation fluid (Bray 1960) was added to the filter and the filtrate in a scintillation vial. The radioactivity in the phytoplankton cells and extracellular organic carbon (EOC) was measured with a liquid scintillation spectrometer (LSC-651, Aloka, Mitaka, Japan). The concentration of total CO_2 in the sample water was determined with an infrared CO_2 analyzer (Model 864, Beckman, Palo Alto, CA, USA), as described by Satake et al. (1972).

Three-hundred-milliliter aliquots of water samples filtered through 0.8-μm Nuclepore (Whatman) filters (<0.8-μm fraction) and untreated water samples (total fraction), were poured into polycarbonate bottles. We prepared duplicate bottles for each fraction. On board, they were incubated for 25 h in the dark at a water temperature similar to the surface water temperature of the sampling station. At approximately 5-h intervals we took a 20-ml subsample from each bottle and fixed it with glutaraldehyde at a final concentration of 1% for the enumeration of bacteria.

During the incubation, the bacterial cell density in the <0.8-μm fraction doubled, and that of the total fraction increased by 20%. Thus, we calculated the population growth rate as follows: $\mu = (\ln Nt - \ln N_0)/t$ where μ is growth rate (day^{-1}); N_0 and Nt are, respectively, cell densities of bacteria at the beginning and end of incubation; and t is incubation time. We assumed that bacterial growth in the <0.8-μm fraction was exponential, and that predation rates on bacteria in the total fraction were constant. The means of the growth rates of bacteria in each size fraction were calculated.

We determined rates of production (P, cells ml^{-1} day^{-1}) and loss to predation (L, cells ml^{-1} day^{-1}) as follows:

$$P = \left(\frac{\mu_{<0.8}}{\mu_{tot}}\right) \times N_0[\exp \mu_{tot} - 1]$$

$$L = \left(\frac{\mu_{<0.8} - \mu_{tot}}{\mu_{tot}}\right) \times N_0[\exp \mu_{tot} - 1]$$

where $\mu_{<0.8}$ is the growth rate (day^{-1}) of bacteria in the <0.8-μm fraction, μ_{tot} is the net bacterial growth rate in the total fraction, and N_0 is the cell density at the start of incubation of the total. These equations have previously been used for calculation of production and consumption of bacteria in size-fractionated water (Nagata 1988). Production and consumption of bacteria were converted into carbon valves using a bacterial cell volume of 0.0212 μm^3, which was determined by measuring the sizes of 100 bacterial cells according to the method of Nakano and Kawabata (2000), and a carbon-to-volume ratio of 0.12 pg C μm^{-3} (Nagata and Watanabe 1990).

Results

The epilimnion, where water temperature was about 17°C, extended from the surface to 5 m, and the thermocline developed down to 20 m (Fig. 2). There was a subsurface chlorophyll maximum at 15 m (Fig. 2). The maximum cell density of picophytoplankton (1.0×10^6 cells ml^{-1}) was recorded at 10 m (Fig. 3A). Other dominant phytoplankton taxa were the dinoflagellate *Gymnodinium* sp. in the 0- to 20-m layer (Fig. 3B) and the diatoms *Stephanodiscus* and *Synedra* from 40 to 80 m (Fig. 3C).

Bacterial cell density (Fig. 4A) remained low from the surface to 5 m and became higher within the thermocline. HNF cell density (Fig. 4B) was high at the surface and decreased sharply below the surface. Ciliate cell density (Fig. 4C) was relatively low at the surface, but high densities of ciliates were detected at depths of 5 m (40 cells ml^{-1}) and 10 m (36 cells ml^{-1}), where the genera *Vorticella*, *Strobilidium*, and *Askenasia*, all of which can ingest picoplankton, (Foissner and Berger 1996) were dominant.

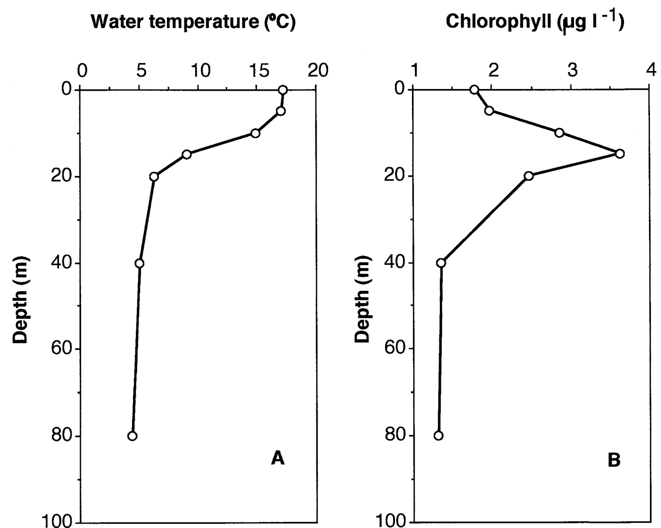

Fig. 2. Vertical distributions of water temperature (**A**) and chlorophyll concentration (**B**) at the sampling station on Lake Baikal on July 31, 1999

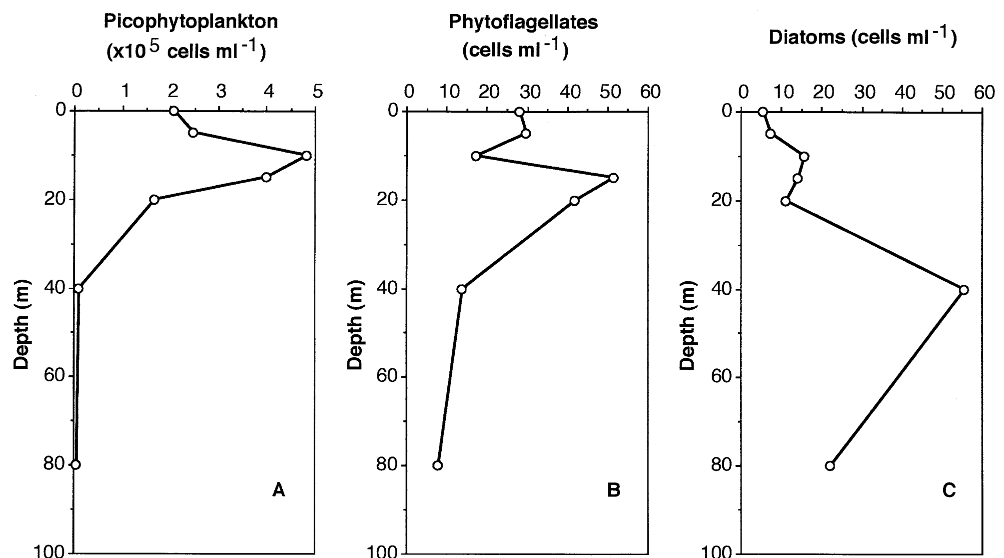

Fig. 3. Vertical cell densities of picophytoplankton (**A**), phytoflagellates (**B**), and diatoms (**C**) at the sampling station on Lake Baikal on July 31, 1999

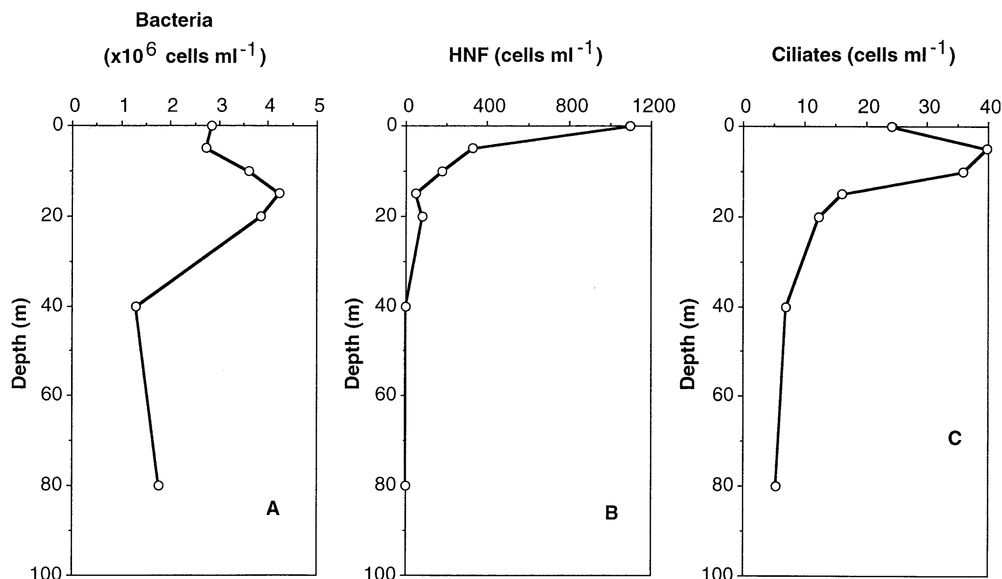

Fig. 4. Vertical cell densities of bacteria (**A**), heterotrophic nanoflagellates (*HNF*) (**B**), and ciliates (**C**) at the sampling station on Lake Baikal on July 31, 1999

Table 1. Primary production, extracellular organic carbon (EOC) release, bacterial production, and grazing loss in the surface water of the central basin of Lake Baikal

Date	Primary production	EOC release	Bacterial production	Grazing loss
July 31, 1999	9.7	2.4	4.3	2.6

All data are $\mu g C l^{-1} day^{-1}$

The abundance of rotifers was high in the epilimnion and decreased markedly below that layer (data not shown). Dominant rotifer taxa were *Synchaeta* and *Polyarthra*. *Filinia* was also frequently found. The biomass of crustaceans was high between 5 and 15m. Cyclopoid copepods dominated at 5m and 10m, while the cladoceran *Leptodora kindtii* was also dominant at 5m, and calanoid copepods became dominant at 15m (data not shown).

Net primary production in the present study was determined as $9.7 \mu g C l^{-1} day^{-1}$, and release of EOC as $2.4 \mu g C l^{-1} day^{-1}$ (Table 1). Bacterial production was estimated as $4.3 \mu g C l^{-1} day^{-1}$, while the estimate of bacterial loss to grazing was $2.6 \mu g C l^{-1} day^{-1}$ (Table 1).

Discussion

The subsurface maximum of chlorophyll concentration at a depth of 15m (Fig. 2B) was caused by the abundance of phytoflagellates (Fig. 3B). A high abundance of phytoflagellates was detected in the lower layer of the thermocline (Fig. 3B), and this might be caused by nutrients supplied from the hypolimnion. Using the same water samples, Sugiyama (unpublished) detected high concentrations of dissolved inorganic phosphorus (DIP) in the lower layer of the thermocline relative to those in the upper layer. James et al. (1992) reported that the dinoflagellate *Ceratium hirundinella* has been shown to move below the thermocline to acquire DIP accumulated in the hypolimnion and then move back up to the epilimnion for photosynthesis. The accumulation of phytoflagellates in the present study might also result from migration to the deeper layers where there might be a supply of nutrients near the hypolimnion.

In the pelagic area off Barguzin estuary, Kozhova (1987) determined the cell density of the dominant phytoplankton genus *Stephanodiscus* at depths of 0, 10, 25, and 50m. From depths of 0–25m, the author found decreases in cell density of the alga with increase in depth, and the cell density increased again at a depth of 50m. In the present study, we also detected a high abundance of *Stephanodiscus* at a depth of 40m (Fig. 3C). In contrast, the abundance of the diatom *Cyclotella* decreased with increase in depth (data not shown). Thus, the accumulation of *Stephanodiscus* in the hypolimnion may be independent of passive transport of the diatom. Although the low numbers of *Stephanodiscus* in the epilimnion and thermocline might be caused by heavy grazing by metazoan zooplankton, we do not have any explanation for the accumulation at this stage.

The vertical distribution of bacterial cell density (Fig. 4A) was similar to that of chlorophyll concentration (Fig. 2B), while high cell densities of picophytoplankton (Fig. 3A) and ciliates (Fig. 4C) were detected at shallower depths. The depths where high numbers of the microorganisms were detected correspond to the thermocline (Fig. 2A). Since it is likely that the water is hardly mixed in the thermocline, the vertical distributions of the microorganisms are possibly the result of food linkage among them: for example, bacterial utilization of organic matter derived from phytoplankton, and consumption of picophytoplankton by ciliates. By contrast, the vertical distribution of HNF (Fig. 4B) was different from those of the other microorganisms examined. Bacteria and picophytoplankton in the pelagic area of Barguzin Bay might be abundant where

HNF grazers are scarce; however, the HNF distribution remains unexplained at present.

About 20% of the gross production of phytoplankton was released as EOC at the station on July 31, 1999 (Table 1), but the quantity of EOC released was exceeded by bacterial production. If we assume bacterial growth yield as 30%, bacterial carbon consumption is calculated as $14.3\,\mu g\,C\,l^{-1}\,day^{-1}$ (Table 1). Thus, pelagic bacteria in Barguzin Bay may need an amount of organic matter that exceeds primary production, and it is suggested that there are other carbon sources for bacterial growth (Table 1). Yoshioka et al. (2002) noted that a large amount of dissolved organic matter (DOM) was transported from Barguzin River to the pelagic area off Barguzin Bay. Hence, allochthonous DOM, such as DOM from the watershed, may support production of pelagic planktonic bacteria in Lake Baikal. Consumption of bacteria by grazers accounted for about 60% of bacterial production (Table 1), suggesting that grazing was an important loss process regulating bacterial abundance.

Beaver and Crisman (1982, 1989) arranged chlorophyll concentration and ciliate abundance in order of increasing trophic state. If we consider the chlorophyll concentration in the present study (Fig. 2B), the trophic state of the central basin of Lake Baikal is categorized as oligotrophic. By contrast, the trophic state would be categorized from the ciliate abundance as mesotrophic (Fig. 4C). Some studies have pointed out that the lake has demonstrated some symptoms of eutrophication, such as a spring bloom of diatoms (Kozhova 1987) and summer blooms of picophytoplankton (Back et al. 1991; Nagata et al. 1994) and colony-forming cyanobacteria (Watanabe and Drucker 1999). Thus, the trophic status of the central basin of Lake Baikal is probably in a transitional phase.

Although our study was limited to only one sampling date, the data presented here are important for understanding the planktonic food web in the central basin of Lake Baikal, where information about the microbial food web is still scarce. Further studies are required for elucidation of the whole planktonic food web in Lake Baikal.

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