

Abundance and bacterivory of heterotrophic nanoflagellates in the meromictic Lake Suigetsu, Japan

Takahiko Okamura^{1,*}, Yumi Mori¹, Shin-ichi Nakano², Ryuji Kondo^{3,*}

¹Graduate School of Bioscience and Biotechnology and ³Department of Marine Bioscience, Fukui Prefectural University, Obama, Fukui 917-0003, Japan

²Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113, Japan

ABSTRACT: The abundance and bacterivory of heterotrophic nanoflagellates (HNF) were seasonally followed in the oxic and anoxic layers of the meromictic Lake Suigetsu between May 2008 and November 2010. The HNF abundance in the anoxic layer was always lower than in the oxic layer during the study period. Ingestion of fluorescently labeled 0.5 μm diameter beads by the anaerobic HNF in the anoxic layer indicated bacterivory by HNF. The specific ingestion rates in the anoxic layers were generally similar to those taken from the oxic layer, with some exceptions. Our data thus suggested that anaerobic HNF were bacterial consumers with high potential bacterivory comparable to that of aerobic HNF in Lake Suigetsu. Bacterial turnover rates by HNF grazing in the oxic layer were estimated to be as high as $\sim 10\% \text{ d}^{-1}$ of the bacterial standing stock. In contrast, the rates in the anoxic layer were $< 1\% \text{ d}^{-1}$ due to the low density of HNF in the anoxic layer. Our data thus provide a valuable contribution to understanding the structure and function of microbial food webs in anoxic aquatic environments.

KEY WORDS: Anoxic environments · Grazing rate · Heterotrophic nanoflagellates · Meromictic lake

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INTRODUCTION

The 'microbial loop' is known as a dynamic component of the planktonic food web in marine and lacustrine systems (Azam et al. 1983, Sanders et al. 1989). Heterotrophic nanoflagellates (HNF) and ciliates are significant consumers of bacterioplankton in oxic waters (Sanders et al. 1989, Vaqué & Pace 1992, Nakano et al. 1998a, Ichinotsuka et al. 2006). In contrast, little is known about the ecology of HNF and ciliates in the microbial loop of anoxic waters. The protozoa in the anoxic deeper layer of Mariager Fjord, Denmark, were dominated by bacterivorous ciliates, and the community structures of the ciliates in the anoxic layer were different from those in the

oxic layer (Fenchel et al. 1990). Massana & Pedrós-Alió (1994) reported that anaerobic ciliates were of minor importance as bacterial consumers due to their low abundance in spite of high specific ingestion rates, while anaerobic ciliates were important grazers on phototrophic sulfur bacteria dominant in the anoxia of a chemically stratified Italian lake (Saccà et al. 2009). Thus, anaerobic ciliates may act as important bacterial grazers in anoxic waters.

Unlike anaerobic ciliates, data concerning HNF in anoxic waters are limited. Previous studies demonstrated HNF abundance in the anoxic layer of a fjord and meromictic lakes (Fenchel et al. 1990, Kopylov et al. 2002, Saccà et al. 2008). Lower abundances of HNF, ranging from 10^1 to 10^4 cells ml^{-1} , were de-

*Emails: s1194001@fpu.ac.jp (T. Okamura),
rykondo@fpu.ac.jp (R. Kondo)

tected from the anoxic waters compared to the abundance (10^3 to 10^4 cells ml^{-1}) in the oxic surface waters. Transmission electron microscopy detected bacteria ingested into the food vacuoles of anaerobic HNF (Brugerolle 1991, Fenchel & Finlay 1995), indicating HNF bacterivory in anoxic waters. However, there are no quantitative data concerning bacterivory by anaerobic HNF.

The aim of the present study was to determine the spatiotemporal distribution and bacterivory of HNF in oxic and anoxic layers of the meromictic Lake Suigetsu. The present study is the first to report grazing rates for bacteria by HNF in sulfidogenic anoxic aquatic environments.

MATERIALS AND METHODS

Sample collection and physico-chemical analyses

Lake Suigetsu is located near the Sea of Japan in Fukui, Japan, on the coast of Wakasa Bay. Lake Suigetsu is a meromictic lake characterized by a permanent oxic-anoxic interface at a depth of ~5 m separating the oxic freshwater surface layer from the anoxic saline sulfidogenic deeper layer. Seawater enters Lake Suigetsu through Lakes Hiruga and Kugushi at high tide. Freshwater enters Lake Suigetsu through Lake Mikata. Thus, the deeper layer of Lake Suigetsu stagnates and has become anoxic and a sulfidogenic environment (Matsuyama 1973, Kondo et al. 2000).

From the central basin of Lake Suigetsu (Fig. 1; $35^{\circ} 52.32' \text{N}$, $135^{\circ} 52.98' \text{E}$) between May 2008 and November 2010, water samples were collected bi-monthly from the oxic surface layer (1 m depth) and from the anoxic bottom layer (10 m depth) using a Kitahara's water sampler (Rigo). The oxic-anoxic interface was usually found at 5 to 7 m depth where the dissolved oxygen (DO) concentration decreased to 0 mg l^{-1} ; we also collected water samples at the suboxic layer (1 m below the interface). For enumeration of HNF and bacteria, autoclaved BOD bottles were filled, fixed with glutaraldehyde (1%, v/v), and immediately stoppered to prevent contact with air. For sulfide analysis, samples were fixed with a small spoonful (~0.2 g) of zinc acetate powder. All samples were stored on ice and transported to the laboratory within 2 h. Water column temperatures and salinity were measured using a portable combined meter (Model 85, YSI). The DO concentration was measured using an oxygen meter (Model HQ30d, Hach).

Total sulfides in the water fixed with zinc acetate were measured spectrophotometrically using the methylene blue method (Kondo 2006).

Bacterial and HNF enumeration

For total bacterial counts, 1 ml of the fixed samples was stained overnight with 4',6-diamidino-2-phenylindole (DAPI; final concentration $1 \mu\text{g ml}^{-1}$). The stained cells were filtered onto 3 separate black $0.2 \mu\text{m}$ polycarbonate membrane filters (Advantec) and counted using epifluorescence microscopy (Porter & Feig 1980). Filamentous bacterial cells were defined as those 10 times longer than the cell widths. Filamentous and non-filamentous cells were counted separately.

To enumerate HNF cells, 7 ml of fixed water were filtered onto 3 separate $0.8 \mu\text{m}$ polycarbonate membrane filters (Advantec) stained with Sudan Black B solution (0.2%, w/v). A 1 ml aliquot of primulin solution ($250 \mu\text{g ml}^{-1}$) was added, and the sample was incubated for 5 min. At least 100 microscopic random fields were counted per filter to reach >30 cells of HNF using epifluorescence microscopy (Caron 1983).

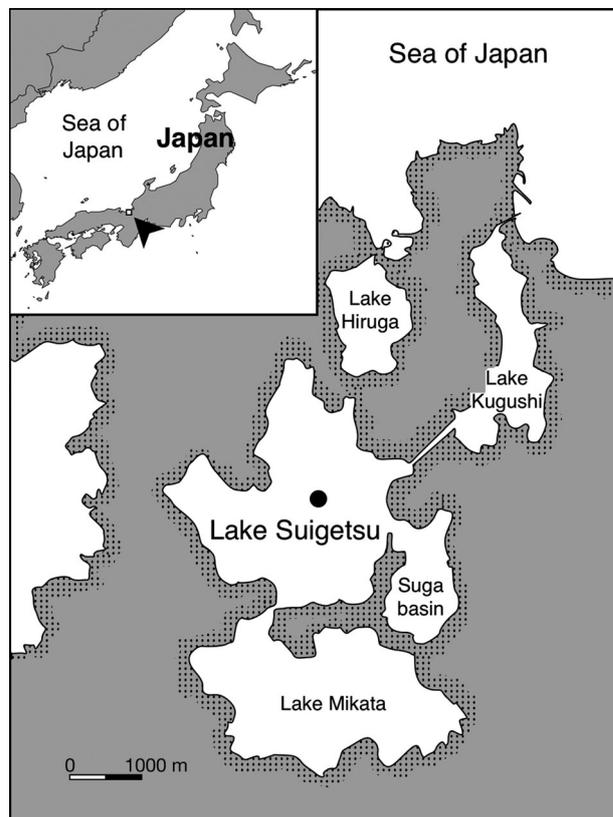


Fig. 1. Map of the Mikata Lake Group. ●: sampling station

HNF were defined based on a cell length of 2 to 20 μm with flagella and without clear autofluorescence under green excitation.

HNF bacterivory

Bacterivory by HNF was determined bimonthly from November 2009 to November 2010. Immediately after sampling, triplicate 100 ml water samples were added to autoclaved glass bottles with butyl rubber stoppers using a needle and syringe. For the anoxic layer samples, autoclaved glass bottles whose headspace was replaced with N_2 were used. We used 0.5 μm diameter fluorescently labeled beads (FLBeads, Polysciences) to simulate ingestion of bacteria by HNF. An FLBeads working solution was prepared using autoclaved distilled water flushed with N_2 gas. FLBeads were added to the bottles at <10% of the *in situ* total bacterial abundance. In preliminary experiments, the total number of ingested FLBeads was found to increase linearly within 15 min of incubation (data not shown). Thus, the bottles were incubated for 15 min at the depth where the water sample was taken. After incubation, a 10 ml sub-sample was taken from the bottles with a needle and syringe and fixed immediately with an equal volume of ice-cold 4% (v/v) glutaraldehyde buffered with Tris-HCl (0.1 mol l^{-1} , pH 8.0), effective to stop the egestion of surrogates taken into food vacuoles of the nanoflagellates (Sanders et al. 1989). To account for FLBeads adsorbed on the cell surface of HNF, a time-zero control was fixed as described above. The fixed samples were treated the same as for the enumeration of HNF. At least 300 HNF cells were inspected for each sample using epifluorescence microscopy under UV excitation, and the FLBeads in the HNF food vacuoles were counted under blue light excitation (Sanders et al. 1989, Ichinotsuka et al. 2006).

The specific ingestion rate (I_{si} ; bacteria $\text{HNF}^{-1} \text{h}^{-1}$) for HNF was calculated as follows:

$$I_{si} = (G_f \times N_b) / (P \times N_f \times T) \quad (1)$$

where G_f is the number of FLBeads ingested by HNF, and N_b and N_f are the total bacterial and FLBead densities, respectively. P is the number of the HNF inspected, and T is the incubation time.

Specific ingestion rates were converted to community ingestion rates (I_{ci} ; bacterial cells $\text{ml}^{-1} \text{h}^{-1}$) by multiplying by the density of HNF, assuming that the HNF we enumerated were bacterivores. Bacterial turnover rate by HNF grazing (TR ; % d^{-1}) was calcu-

lated on the basis of community ingestion rates and bacterial standing stock as follows:

$$TR = [(I_c \times 24) / N_b] \times 100 \quad (2)$$

Statistics

For correlations of abundance and bacterivory of HNF with environmental factors, we used Spearman's rank correlation. These statistics were computed with R software (<http://cran.r-project.org>).

RESULTS

Water column profiles

The salinity decreased during the winter to summer and then increased into the autumn in the oxic layer of Lake Suigetsu (Fig. 2A). The water temperature increased into the summer and then decreased as winter approached in the oxic layer (Fig. 2B), while below 15 m depth, the temperature was stable at $\sim 15^\circ\text{C}$. The DO concentration decreased rapidly from 2 to 4 m depth and was at the detection limit at 5 to 6 m (Fig. 2C). The oxic-anoxic interface was 6 m except for July 2009 (5 m) and May 2010 (7 m) (Fig. 2C). The total sulfide concentration ranged from 0.01 to 0.41 mmol l^{-1} in the suboxic layer and 1.3 to 2.4 mmol l^{-1} in the anoxic layer, while sulfide was not detected in the oxic layer. Therefore, the water column below the interface was anoxic and sulfidogenic.

Distribution of bacteria and HNF

The total bacterial abundance was between 4.7×10^6 and 21×10^6 cells ml^{-1} in the oxic layer, between 5.0×10^6 and 18×10^6 cells ml^{-1} in the suboxic layer, and between 2.0 and 8.9×10^6 cells ml^{-1} in the anoxic layer (Fig. 3A). The abundance of total bacteria in the anoxic layer was lower than at the other sampling depths. Filamentous bacterial abundance varied from 0.3×10^6 to 1.5×10^6 cells ml^{-1} in the anoxic layer, from below the detection limit to 0.6×10^6 cells ml^{-1} in the oxic layer, and from 0.1×10^6 to 1.7×10^6 cells ml^{-1} in the suboxic layer (Fig. 3B). Unlike the total bacterial abundance, filamentous bacteria were in most cases more abundant in the anoxic layer than in the other 2 layers.

HNF abundance in the anoxic layer (2.3×10^2 to 7.1×10^2 cells ml^{-1}) was always lower than in the oxic layer (4.0×10^2 to 52×10^2 cells ml^{-1}) or in the suboxic

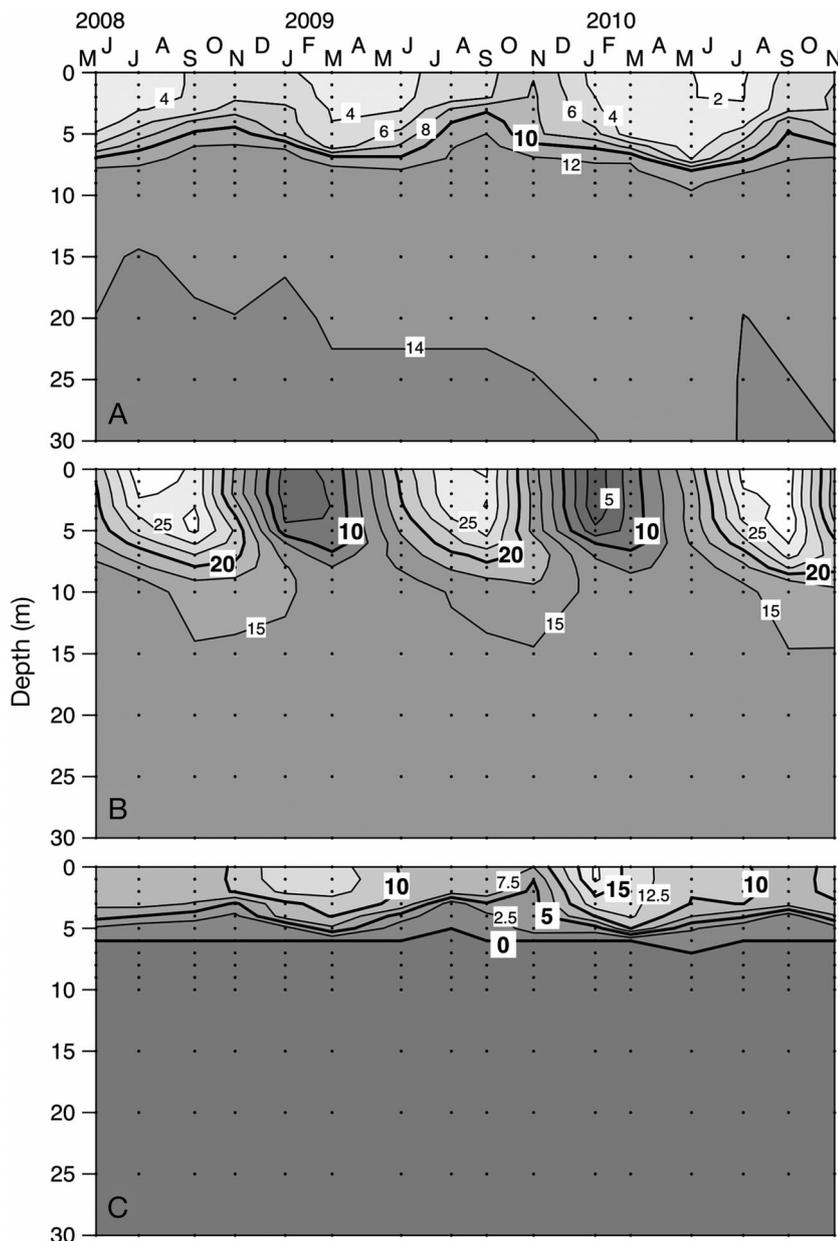


Fig. 2. Seasonal changes in the vertical distribution of (A) salinity (psu), (B) water temperature ($^{\circ}\text{C}$), and (C) dissolved oxygen (mg l^{-1}) in Lake Suigetsu between May 2008 and November 2010. Dots: measured dates and depths

layer (4.4×10^2 to 31×10^2 cells ml^{-1}). HNF abundance in the oxic layer showed an annual cyclic pattern: the numbers decreased from May to November and then increased into March (Fig. 4). Similar seasonal patterns for HNF abundance appeared in the suboxic layer. In contrast, no seasonal patterns of HNF abundance were observed in the anoxic layer.

We made rough determinations of the average cell volumes of HNF, non-filamentous bacteria, and filamentous bacteria with an image analyzing sys-

tem (ImageJ; <http://rsbweb.nih.gov/ij/>) using 77 to 97 cells of HNF, 149 to 152 cells of non-filamentous bacteria, and 35 to 98 cells of filamentous bacteria for each sampling depth between May 2008 and January 2009. The carbon biomass of those microorganisms was calculated using conversion factors of $106 \text{ fg C } \mu\text{m}^{-3}$ for bacteria (Nagata 1986) and $220 \text{ fg C } \mu\text{m}^{-3}$ (Børshiem & Bratbak 1987) for HNF with 47% cell shrinkage due to fixation (Choi & Stoecker 1989). Finally, we estimated the biomass ratios between HNF and bacteria in Lake Suigetsu, treating bacterial biomass as the sum of the non-filamentous and filamentous bacterial biomass. The ratios between HNF and bacterial biomass were 0.19 ± 0.16 (mean \pm SD) in the oxic layer, 0.18 ± 0.15 in the suboxic layer, and 0.04 ± 0.02 in the anoxic layer.

HNF bacterivory

Specific ingestion rates in the oxic layer ranged from 0.20 to 10 bacteria $\text{HNF}^{-1} \text{ h}^{-1}$, and those in the anoxic layer ranged from under the detection limit to 2.9 bacteria $\text{HNF}^{-1} \text{ h}^{-1}$. The specific ingestion rates in the anoxic and oxic layers in March 2010 were significantly higher than those on other sampling dates (pairwise *t*-test, $p < 0.05$; Fig. 5A). The specific ingestion rates in the suboxic layer largely fluctuated between 0.12 and 16 bacteria $\text{HNF}^{-1} \text{ h}^{-1}$. There were no significant differences in the specific ingestion rates among the 3 layers in November 2009 and March, July, and November 2010.

Community ingestion rates varied from 7.3×10^1 to 5.4×10^4 bacteria $\text{ml}^{-1} \text{ h}^{-1}$ in the oxic layer, from 1.0×10^2 to 4.2×10^4 bacteria $\text{ml}^{-1} \text{ h}^{-1}$ in the suboxic layer, and from the detection limit to 1.7×10^3 bacteria $\text{ml}^{-1} \text{ h}^{-1}$ in the anoxic layer (Fig. 5B). Community ingestion rates in the oxic layer appeared to be higher than those in the suboxic and anoxic layers except for in September 2010. The rates in March and July 2010 for each sampling occasion were not significantly different among sampling depths (pairwise *t*-test, $p > 0.05$),

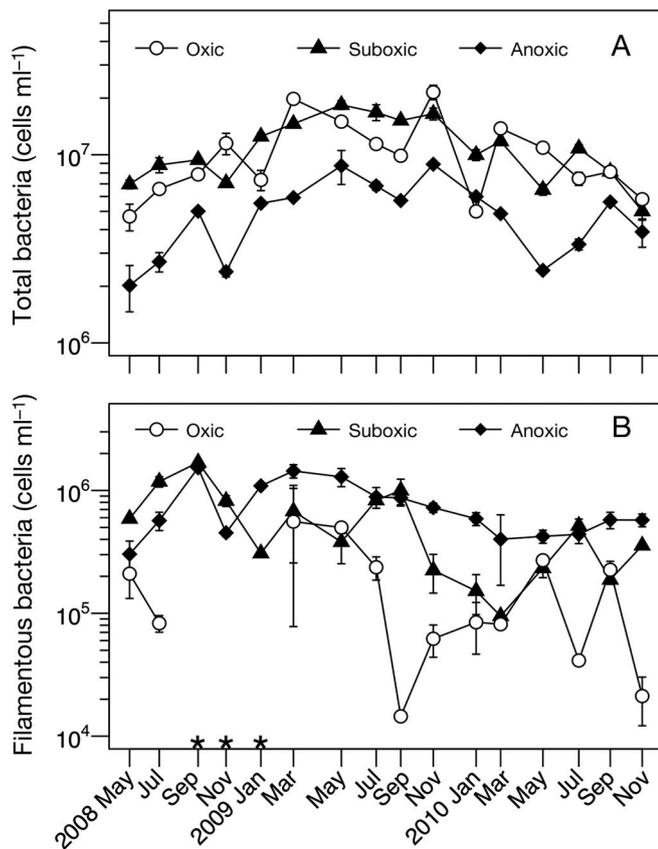


Fig. 3. Seasonal changes in (A) total bacteria and (B) filamentous bacteria in Lake Suigetsu between May 2008 and November 2010. Error bars are standard errors ($n = 3$). *: under the detection limit

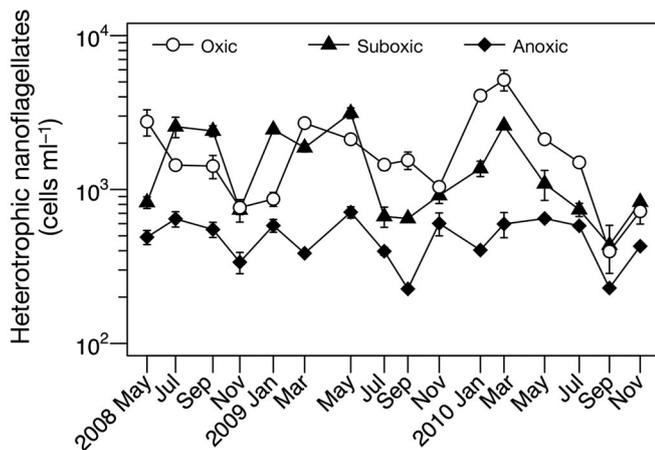


Fig. 4. Abundance of heterotrophic nanoflagellates (HNF) in Lake Suigetsu between May 2008 and November 2010. Error bars are standard error ($n = 3$)

while those in November 2009 and January, May, and November 2010 were significantly higher in the oxic layer than in the suboxic and/or the anoxic layers for each sampling date (pairwise t -test, $p < 0.05$).

Low bacterial turnover rates ($< 1\% \text{ d}^{-1}$) by HNF grazing based on bacterial standing stock were estimated in the water column of Lake Suigetsu during our investigation except for oxic and suboxic samples in September 2010. The highest rates of bacterial turnover were found in September 2010 at $9.4\% \text{ d}^{-1}$ and $8.6\% \text{ d}^{-1}$ in the oxic and the suboxic layers, respectively (Fig. 5C).

Correlations for HNF abundance, bacterivory, and environmental properties

In the suboxic layer, HNF abundance was significantly and negatively correlated with water temperature ($p < 0.01$), and no other measured factors had significant correlations with HNF abundance. In the anoxic layer, no factors significantly correlated with HNF abundance. In the oxic layer, HNF abundance was significantly and negatively correlated with salinity and positively correlated with community ingestion rate (Table 1). At all 3 depths, the specific ingestion rates did not correlate with any of the measured abiotic factors and microbial abundance. The community ingestion rate correlated with the bacterial abundance in the suboxic layer (Table 1).

DISCUSSION

The oxic surface water of Lake Suigetsu is meso- to eutrophic with a high concentration of chlorophyll *a* (4.0 to $81 \mu\text{g l}^{-1}$; R. Kondo et al. unpubl. data) and a low Secchi disc transparency ($< 1.8 \text{ m}$). HNF were detected from the surface layer at 4.0×10^2 to $5.2 \times 10^3 \text{ cells ml}^{-1}$, which falls into the lower range of the values reported for other meso- and eutrophic lakes (Berninger et al. 1991). HNF were also detected in the sulfidogenic suboxic and anoxic layers of Lake Suigetsu at 2.3×10^2 to $3.1 \times 10^3 \text{ cells ml}^{-1}$. These HNF abundances were within the range of those in anoxic hypolimnia of stratified lakes (Bloem et al. 1989, Hadas & Berman 1998, Kopylov et al. 2002, Saccà et al. 2008). Previous studies demonstrated that abiotic factors regulate HNF abundance (McManus & Fuhrman 1990, Choi et al. 2002). Therefore, our data using Spearman's rank correlation analysis suggest salinity and temperature are the factors that affect HNF abundance in the oxic and suboxic layers, respectively, whereas no significant correlation was found between the abundances of HNF and bacteria (Table 1). The data in Table 1 suggest that bottom-up control was of minor importance for HNF abundance. Predation

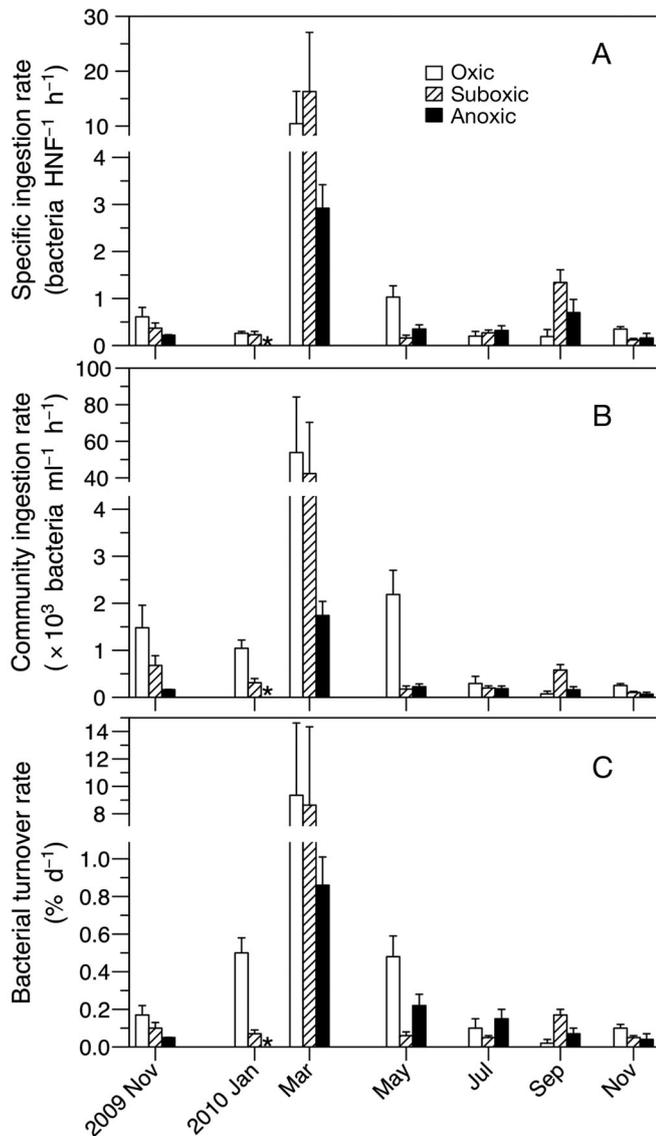


Fig. 5. Seasonal changes in (A) the specific ingestion rate of total bacteria by heterotrophic nanoflagellates (HNF), (B) community ingestion rates, and (C) bacterial turnover rates in Lake Suigetsu between November 2009 and November 2010. Error bars are standard errors ($n = 3$). *: under the detection limit

pressure (top-down) and/or other loss factors, such as viral lysis, may be important for controlling HNF abundance. In oxic layers of meso- to eutrophic lakes, previous studies have demonstrated that predation by *Daphnia* (Cladocera) is one of important factors that control HNF abundance (Jürgens 1992, Nakano et al. 1998b). By contrast, in eutrophic to hypertrophic lakes, HNF abundance may be regulated by ciliate predation because of negligible abundance of *Daphnia* in those lakes (Nakano et al. 2001). In the oxic layer of Lake Suigetsu, the dominant meso-zooplankton were rotifers and cyclopoid copepods (Kikuchi 1931, Watanabe 1967). In the present study, only the rotifer genus *Brachionus* spp. and unidentified cyclopoid copepods were detected in November 2008 and January 2009 (S. Nakano unpubl. data). Therefore, the case of the present study is different from the case in meso- to eutrophic lakes where *Daphnia* dominates, and top-down control by ciliates may be important for the regulation of HNF abundance in the oxic layer of Lake Suigetsu. This may be also the case for ciliates. To determine the abundance and composition of ciliates, we collected 500 ml of water samples, fixed with acid Lugol's solution at the final concentration of 2%, concentrated the samples thus fixed by natural sedimentation, and observed the samples microscopically. The ciliate genera *Strombidium*, *Strobilidium*, *Cyclidium*, *Urotricha*, and *Cinetochilum*, all of which are commonly found in eutrophic to hypertrophic lakes, were also abundant in the oxic layer of the lake (S. Nakano unpubl. data.) In the anoxic layer of Lake Suigetsu, however, the abundance of ciliated protozoa was negligible or below the detection limit (S. Nakano unpubl. data), and metazoan zooplankton were absent in the sulfidogenic hypolimnion (Kikuchi 1931, S. Nakano unpubl. data). Therefore, the biological system in Lake Suigetsu may be unusual, different from other meromictic lakes with anoxic hypolimnia where large ciliates are found (Sorokin & Donato 1975, Zingel & Ott 2000, Saccà et al. 2008). This suggests HNF may

Table 1. Spearman's rank correlation ρ and level of significant values. Suboxic: data are from 1 m below the interface; na: data not available, the correlation ρ could not be calculated because the dissolved oxygen (DO) concentration was always 0. * $p < 0.05$, ** $p < 0.01$

Parameter	HNF abundance			Specific ingestion rate			Community ingestion rate		
	Oxic	Suboxic	Anoxic	Oxic	Suboxic	Anoxic	Oxic	Suboxic	Anoxic
Salinity	-0.64**	-0.19	-0.14	-0.04	0.07	-0.22	-0.29	0.2	-0.38
Temperature	-0.32	-0.62**	-0.35	-0.54	-0.14	-0.5	-0.57	-0.29	-0.61
DO	0.31	na	na	0.14	na	na	0.18	na	na
Total bacteria	0.06	0.19	-0.06	0.57	0.71	-0.29	0.57	0.82*	-0.46
HNF abundance				0.61	0.07	0.21	0.82*	0.32	0.71
Specific ingestion rate	0.61	0.07	0.21				0.89*	0.93**	0.79*
Community ingestion rate	0.82*	0.32	0.71	0.89*	0.93**	0.79*			

have the highest position in the food chain in the anoxic hypolimnion of the lake and that top-down control is less important for HNF abundance.

Fenchel & Finlay (1990) report that the biomass ratios between protozoa and bacteria in anoxic waters were 3 to 7 times lower than those in aerobic systems, suggesting a low growth efficiency of anaerobic protozoa. In Lake Suigetsu, the biomass ratios between HNF and bacteria in the anoxic layer (mean \pm SD: 0.04 ± 0.02) were lower than those in the oxic (0.19 ± 0.16) and the suboxic layer (0.18 ± 0.15). Thus, our observations corroborate those of Fenchel & Finlay (1990), suggesting that the growth efficiency of HNF in the anoxic layer is low.

The difference in specific ingestion rates between water layers for each sampling occasion was examined using a pairwise *t*-test; there were no significant differences in November 2010 or March, July, and November 2011. Additionally, the rates in the suboxic and anoxic layers in September 2011 were significantly higher than in the oxic layer ($p < 0.05$). This suggests the anaerobic HNF in the anoxic layer of Lake Suigetsu potentially have bacterivorous activity equal to the aerobic HNF in the oxic layer. The specific ingestion rates in the oxic layer of the lake were low compared to those in previous studies (Table 2), though it is not possible to exclude the possibility that variations in specific ingestion rates among aquatic environments are the result of methodological differences.

A common method to measure HNF grazing on bacteria uses fluorescently labeled bacteria in short-term incubations (Weisse 2002 and references therein). This method is a refinement of an earlier approach using inert fluorescent latex beads as a surrogate for bacterial uptake by HNF and ciliates (Pace & Bailiff 1987, Sherr et al. 1987). However, no general pattern of preference has been found for either heat-killed bacteria or artificial latex beads (Sanders et al. 1989, Montagnes & Lessard 1999). It is difficult to evaluate the use of artificial 0.5 μm fluorescent latex beads to determine the HNF grazing rate on bacteria in the present study because of the technical simplicity. Previous studies demonstrate protozoan prey preference for living over non-living microorganisms (Landry et al. 1991, Montagnes & Lessard 1999, Massana et al. 2009). Consequently, the grazing rates shown here may be underestimated due to the use of artificial beads as surrogates.

The community ingestion rates by HNF were generally higher in the oxic layer than in the suboxic and/or anoxic layers of Lake Suigetsu. This was due to the high HNF abundance in the oxic layer during our study period. High turnover rates of bacteria due to protozoan grazing on bacterial standing stock have been reported in eutrophic and hypertrophic waters, while low rates are found in oligotrophic waters (Table 2). The turnover rates in the oxic layer of Lake Suigetsu were low relative to those of other eutrophic environments despite the meso- to eutro-

Table 2. Specific ingestion rate of heterotrophic nanoflagellates (HNF) and bacterial turnover rate by HNF (rate due to HNF grazing calculated on the basis of bacterial standing stock) found in the literature and the present study

Site	Trophic status	Specific ingestion rate (bacteria HNF ⁻¹ h ⁻¹)	Bacterial turnover rate (% d ⁻¹)	Source
Freshwater				
Lake Paul	Oligotrophic	0.2–5.2	0.76–2.32	Vaqué & Pace (1992)
Lake Tuesday	Oligotrophic	0.2–24	0.18–5.89	Vaqué & Pace (1992)
Piburger See	Oligomesotrophic	3.1–24	7.7–66	Pernthaler et al. (1996)
Lake Bourget	Mesotrophic	1–45	15–19	Jacquet et al. (2005)
Lake Suigetsu				
Oxic	Meso–eutrophic	0.2–10	0.02–9.4	Present study
Suboxic	–	0.1–16.3	0.05–8.6	Present study
Anoxic	–	≤ 2.9	≤ 0.9	Present study
Back water of River Danube	Eutrophic	2.2–26.5	0.55–16.1	Wieltschnig et al. (1999)
Lake Oglethorpe	Eutrophic	2–53	3–35	Sanders et al. (1989)
Furuike Pond	Hypereutrophic	12 ± 12^a	≤ 104	Nakano et al. (1998)
Marine				
Coast of Taiwan	Oligotrophic	0.2–2.0	0.3–2.8	Tsai et al. (2011)
Mediterranean Sea	Oligotrophic	0.9–4.3	0.2–18	Christaki et al. (2001)
Uchiyumi Bay	Oligomesotrophic	0.3–2.2	1–15	Ichinotsuka et al. (2006)
Chesapeake Bay	Eutrophic	1.8–25	1.1–19	McManus & Fuhrman (1988)
Uranouchi Inlet	Eutrophic	2.2–17	0.8–15	Fukami et al. (1996)

^aMean \pm standard deviation

phic state. The HNF:bacteria ratio was approximately 1:10 000 in the oxic layer of Lake Suigetsu, where this ratio is high compared to that of other aquatic environments (Sanders et al. 1992). The low turnover rates and high HNF:bacteria ratio in the oxic layer of Lake Suigetsu is probably due to the low HNF abundance relative to other meso- or eutrophic lakes (see the first paragraph of the 'Discussion'), and this may also be the case in the suboxic and anoxic layers. However, it is unclear why the HNF abundance in Lake Suigetsu is low. Further research to determine the factor(s) controlling HNF abundance is needed.

There has to date been no report on grazing of bacteria by anaerobic HNF in anoxic sulfidogenic environments; accordingly, our data on bacterivory in the anoxic layer cannot be compared to other studies. It has been reported that bacterial production and grazing showed a positive relationship in oxic environments of marine and freshwater systems (Sanders et al. 1989, Wieltschnig et al. 1999). Christaki et al. (2001) showed that higher bacterial production was always associated with a larger HNF stock in the oligotrophic Mediterranean Sea. Thus, grazing is more closely related to bacterial production than to bacterial abundance. In the anoxic layer of Lake Suigetsu, low bacterial turnover rates by HNF grazing based on the bacterial standing stock ($<1\% \text{ d}^{-1}$) were estimated throughout the year (Table 2). Because we did not measure the bacterial production in this study, it is not possible to state the relationship between bacterial production and grazing. Although it is difficult to make a comparison of *in situ* bacterial production between oxic and anoxic environments, the growth of bacteria by anaerobic respiration, such as metal and sulfate reduction or fermentation, is generally inferior to aerobic growth from the thermodynamic and energetic points of view (Zehnder & Stumm 1988). Anaerobic bacteria, such as sulfate-reducing and fermentative bacteria, dominate in the sulfidogenic hypolimnion of Lake Suigetsu based on analyses using denaturing gradient gel electrophoresis of the 16S rRNA gene (Kondo & Butani 2007) and clone libraries of a functional gene (Kondo et al. 2006, 2009). One possible explanation for the low bacterial turnover rate by HNF grazing in the anoxic layer of Lake Suigetsu might be low production of anaerobic bacteria.

Specific ingestion rates of bacteria into ciliates are usually higher than those of HNF, and this is also the case in anaerobic systems (Massana & Pedrós-Alió 1994, Saccà et al. 2009). However, community ingestion rates and bacterial turnover rates by ciliate graz-

ing in anaerobic systems are usually low due to the low abundance of ciliates (Massana & Pedrós-Alió 1994). Recently, Saccà et al. (2009) reported that pleuronematine ciliates were important grazers of anaerobic picoplankton dominated by brown-colored green sulfur bacteria. Because low bacterial turnover rates by HNF grazing were observed in the suboxic and anoxic layers of Lake Suigetsu, we considered bacterial mortality due to ciliate grazing. However, ciliates were not observed in the anoxic layer of the lake during our investigation (S. Nakano unpubl. data), indicating that bacterial grazing by ciliates was negligible there. The ciliate genera of *Spirostomum* and *Metopus* were usually observed in the suboxic layer (S. Nakano unpubl. data), suggesting that bacterivory by ciliate grazing should be examined to assess the bacterial mortality in the suboxic layer of Lake Suigetsu. Viral lysis has also been reported to account for bacterial mortality in the anoxic hypolimnion (Weinbauer & Höfle 1998, Colombet et al. 2006). However, it has not been determined whether viral lysis is important for bacterial mortality in the anoxic environment of Lake Suigetsu.

In conclusion, the specific ingestion rates in the anoxic and/or suboxic layers were often higher than those in the oxic layer of Lake Suigetsu. This suggests that the anaerobic bacterivorous HNF in the lake have high potential bacterivory comparable to aerobic HNF. Anaerobic bacterivory is generally ignored in the anaerobic microbial ecology literature. Our data may be valuable for understanding the structure and function of microbial food webs in anoxic aquatic environments.

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