Vol. 16: 153–161, 1998 | AQUATIC MICROBIAL ECOLOGY Aquat Microb Ecol | Published November 27

Trophic roles of heterotrophic nanoflagellates and ciliates among planktonic organisms in a hypereutrophic pond

Shin-ichi Nakano*, Nobuyoshi Ishii, Pathmalal M. Manage, Zen'ichiro Kawabata

Department of Environmental Conservation, Ehime University, Tarumi 3-5-7, Matsuyama 790-8566, Ehime, Japan

ABSTRACT: We followed seasonal changes in abundance of bacteria, heterotrophic nanoflagellates (HNF), ciliates and crustaceans, and consumption of bacteria by the protozoans, to investigate trophic interactions among these organisms in a hypereutrophic pond from March to October 1997. Densities of HNF and ciliates were high and attained a maximum of 1.4×10^5 and 3500 cells ml^{-1}, respectively. However, the high densities decreased as chlorophyll concentration increased. Since the predominant phytoplankton species was *Microcystis aeruginosa* (Cyanophyceae), toxin produced by the alga possibly affected growth of protozoans. Not only HNF but also ciliates were important consumers of bacteria, and consumption of bacteria by ciliates varied at the same level as that of HNF from August to October. Bacterial turnover rate (% d^{-1}) due to consumption by the protozoa ranged between 5.6 and 112 (mean 25), and there were significant relationships between densities of bacteria and specific ingestion rates (bacteria protozoan $cell^{-1}$ h^{-1}) of the protozoans. These results suggest that the food linkage between bacteria and the protozoans is substantial in the pond. We could not find any significant trophic relationships between HNF and ciliates, and between the protozoans and crustaceans.

KEY WORDS: Bacteria · Heterotrophic nanoflagellates · Ciliates · Microbial food web · Hypereutrophic pond

INTRODUCTION

It is well known that heterotrophic nanoflagellates (HNF) serve as important bacterial consumers in lakes (Nagata 1988, Bloem & Bär-Gilissen 1989, Sanders et al. 1989), while consumption of bacteria by ciliates temporarily dominates among all heterotrophic links in a system (Šimek et al. 1990, 1995, 1998, Šimek & Straskrabova 1992). Thus, there may be competition between HNF and ciliates for bacterial food, but the partitioning of bacterial food between HNF and ciliates is still poorly understood.

Although a great abundance of the genus *Daphnia* sometimes makes the microbial loop less important (Pace & Funke 1991, Nakano et al. 1998), HNF (Sanders & Porter 1990, Carrick et al. 1991, Jürgens 1994, Sanders et al. 1994) and ciliates (Tezuka 1974, Carrick et al. 1991, Sanders et al. 1994) are also considered to be important links in the transfer of bacterial biomass to metazoan zooplankton. Since HNF (Nakano 1994) and ciliates (Stoecker & Capuzzo 1990) are rich in nitrogen and phosphorus, as are bacteria (Nagata 1986, Vadstein et al. 1988), they can be qualitatively good food for their predators. Previous studies have demonstrated efficient predation on HNF by oligotrichous ciliates (Jürgens et al. 1996), high predation pressure on HNF by rotifers (Dolan & Gallegos 1991, Sanders et al. 1994) and on ciliates by copepods (Burns & Gilbert 1993, Dobberfuhl et al. 1997). However, further studies on consumption of protozoa are needed to verify the role of the microbial loop in an aquatic ecosystem.

Predation on protozoa may have some effect on the consumption of bacteria by those protozoa, and the presence of cascading trophic interactions among bac-

*E-mail: shin@agr.ehime-u.ac.jp

teria, protozoa and metazoan zooplankton has been pointed out. For example, Dolan & Gallegos (1991) reported that the rotifer *Synchaeta* reduced consumption of bacteria by HNF through its predation on the HNF. Conversely, Pace & Funke (1991) have reported that such cascading trophic interactions among bacteria, HNF, ciliates and *Daphnia* were not found in 2 temperate lakes. Although some studies have reported trophic interactions among bacteria, HNF and ciliates (Berninger et al. 1993, Stensdotter-Blomberg 1998), information about the microbial interactions is still limited.

In the present study, we focused on the food linkage among bacteria, HNF and ciliates and followed seasonal changes in abundance of these microorganisms and the consumption of bacteria by the protozoans in a hypereutrophic pond, to examine the trophic interactions among the microorganisms. In addition, we also followed the seasonal abundance of crustaceans to see interactions between microbial and classical food chains.

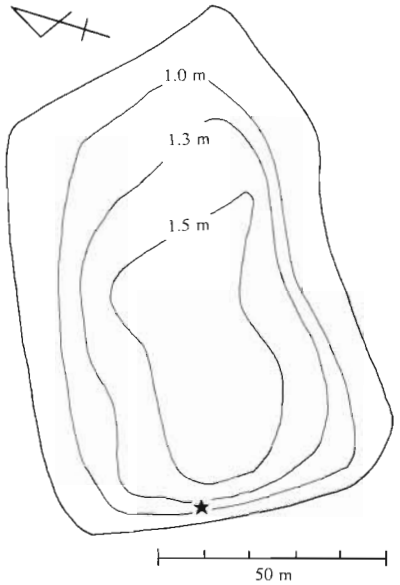

Fig. 1. Map of Furuike Pond (Matsuyama City, Ehime Prefecture, Japan) showing the sampling site

MATERIALS AND METHODS

Furuike Pond (altitude 40 m) is an impoundment located in Sancho, Matsuyama City, Ehime Prefecture, Japan (Fig. 1). It has a surface area of ca 7400 m^2, and its depth was 0.88 ± 0.15 m (mean ± SD) during the study period. The pond is hypereutrophic, due to anthropogenic loading from the watershed. Field observations with 5 drifting buoys showed that there is a horizontal, clockwise eddy which mixes pelagic and near-shore waters. The eddy is caused by a sea breeze even if the wind is weak and thus its presence was probably continuous during the study period.

Water samples for determination of abundance of organisms were collected twice a month, from the surface with an 8 l bucket, at a near-shore station (Fig. 1) from March to October 1997. To determine consumption of bacteria by protozoa, grazing experiments were also conducted at the same intervals over the same period. All samples were taken at around the same time of the day (09:30 to 10:30 h). Water temperature was measured with a thermistor (TOA Electronics Co. Ltd). To measure chlorophyll *a* concentration a measured portion of each water sample was filtered through a Nuclepore filter with pore size of 0.2 µm to retain seston. The filter was then placed into a glass test tube and 6 ml of N, N-dimethylformamide (DMF) was added to extract chlorophyll *a*. The amount of chlorophyll *a* thus extracted was determined by the fluorometric method (Rami & Porath 1980).

Immediately after collection, two 50 ml portions of the water sample were fixed, one with glutaraldehyde to a final concentration of 1% for enumeration of bacteria and heterotrophic nanoflagellates (HNF), and the other with acidified Lugol's solution to a final concentration of 1% for enumeration of ciliates. Bacteria were counted using the fluorescent dye diamidinophenylindole (DAPI), direct count method (Porter & Feig 1980) with an epifluorescence microscope. HNF were enumerated under ultraviolet excitation using the primulin staining method (Caron 1983). We counted nanoflagellates as HNF if they showed no obvious red chlorophyll fluorescence under green excitation. Since the cell shapes of some ciliate species may change due to fixation, an unfixed portion of the water sample was brought to our laboratory, and the dominant ciliate species were identified under a microscope within 2 h of collection using the classification guides of Mizuno & Takahashi (1991) and Patterson & Hedley (1992). We used 10% methylcellulose (15 cp) solution to slow down swimming ciliates. The ciliate sample fixed with acid Lugol's solution was concentrated by natural sedimentation, and ciliate cells were enumerated for each species in a haematocytometer under a microscope.

For counting crustacean zooplankton, a measured volume of between 10 and 20 l of water was filtered through an approximately 200 µm mesh (NXX 7) plankton net to concentrate the zooplankton, which were then fixed with acid Lugol's solution at a final concentration of 1%. The fixed samples were concentrated by natural sedimentation for more than 24 h before a 0.2 to 1 ml aliquot of the concentrated sample was put on a scaled glass slide, and all the

zooplankters found there were counted at least twice.

Grazing rates of HNF and ciliates feeding on bacteria were determined with fluorescently labeled bacteria (FLB) (Sherr et al. 1987) obtained from a minicell-producing mutant strain of *Escherichia coli*. After cultivation of the bacterium in a liquid medium, minicells were separated using the method of Christen et al. (1983) and stained with the fluorescent dye 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF) following Sherr et al. (1987). FLB thus prepared are spherical with a diameter of 0.5 to 1 µm, and the size range overlaps that of *in situ* bacteria in Furuike Pond (Nakano & Kawabata unpubl.).

100 ml portions of the water samples were poured into triplicate polycarbonate bottles. FLB were added to the bottles at between 1.0 and 1.5×10^6 cells ml^{-1}. These densities represented between 2.0 and 9.5% of the *in situ* bacterial densities, being appropriate surrogate densities (McManus & Okubo 1991). The FLB-spiked water samples were incubated at *in situ* temperature. After 8 min of incubation, subsamples were fixed with 4% ice-cold, buffered glutaraldehyde, which is an effective fixative to reduce the egestion of bacteria ingested into a food vacuole of HNF and ciliates (Sanders et al. 1989). HNF and ciliates thus fixed were respectively retained on 0.8 µm and 5 µm black Nuclepore filters and stained with primulin as mentioned before, and FLB in the food vacuoles of protozoa were counted under an epifluorescence microscope. Protozoa were detected under ultraviolet excitation, and FLB in their food vacuoles were observed after switching to blue excitation. At least 100 cells of HNF and 20 to 40 ciliate cells were examined for each sample. A time-zero control was prepared to account for FLB adsorbed to cell surfaces of the protozoa.

Specific ingestion rate (I_s, bacteria protozoan $cell^{-1}$ h^{-1}) of HNF or ciliates was calculated as follows

$$I_s = (G_f \times N_b)/(P \times N_f \times T)$$

where G_f is the number of FLB ingested by protozoa, N_b and N_f are respectively the densities of bacteria (cells ml^{-1}) and FLB (particles ml^{-1}), P is the number of protozoa examined to determine their ingestion of FLB and T is time (hours). Consumption rate (I_c, bacteria ml^{-1} d^{-1}) of HNF or ciliates was calculated as

$$I_c = I_s \times N_p \times 24$$

where N_p is the density of protozoa (cells ml^{-1}).

Total consumption rates of bacteria (bacteria ml^{-1} d^{-1}) by protozoa were calculated as the sum of the ingestion rates of HNF and ciliates, and bacterial turnover rates (% d^{-1}) were estimated by expressing the ingested bacteria as percentages of the corresponding bacterial densities.

RESULTS

Water temperature at the surface of Furuike Pond gradually increased from 24 March (12.8°C) to 10 August (28.9°C) and decreased from 24 August (27.3°C) to 24 October (20.2°C) (Fig. 2). At the beginning of the study, chlorophyll *a* concentration (Fig. 2) was high (466 µg l^{-1}) due to a bloom of *Phormidium mucicola* (Cyanophyceae). It decreased rapidly to 96.3 µg l^{-1} on 24 March as the bloom collapsed but increased again from 24 April (145 µg l^{-1}) to its maximum on 10 July (367 µg l^{-1}) except for a sharp dip on 24 June (36.6 µg l^{-1}). Thereafter, it fluctuated between 236 and 333 µg l^{-1}. Dominant phytoplankton species during the study period were the Cyanophyceae *Microcystis aeruginosa*, *P. mucicola* and *Anabaena flos-aquae*, the Cryptophyceae *Cryptomonas* spp., Chlorophyceae, *Acanthosphaera* spp., *Scenedesmus* spp. and Bacillariophyceae, *Synedra* spp. *M. aeruginosa* was quantitatively dominant during most of the study period.

Bacterial densities (Fig. 3A) increased from 24 March (2.0×10^7 cells ml^{-1}) to 24 April (4.6×10^7 cells ml^{-1}), decreased sharply to 9 May (1.3×10^7 cells ml^{-1}) then gradually increased to 24 September (4.9×10^7 cells ml^{-1}), with minor fluctuations, before decreasing again.

Heterotrophic nanoflagellates (HNF) showed a sharp peak (1.4×10^5 cells ml^{-1}) between 10 March and 10 April (Fig. 3B) and, although their density was again relatively high on 9 May (7.8×10^4 cells ml^{-1}) from then onwards it tended to decrease, reaching 2.4×10^3 cells ml^{-1} on 10 August. There was again a slight increase from 24 August (5.1×10^3 cells ml^{-1}) to 24 September (2.1×10^4 cells ml^{-1}) but numbers decreased again from 10 October.

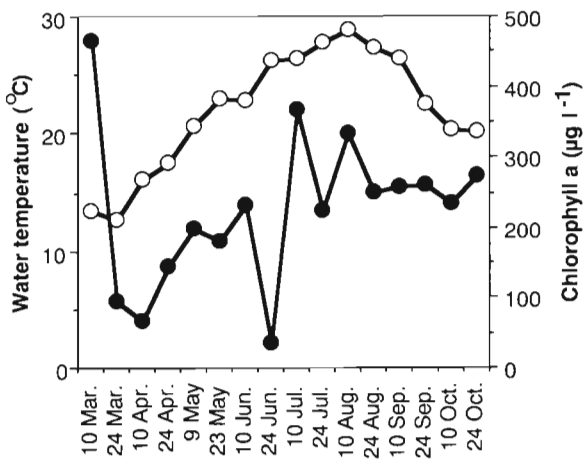

Fig. 2. Seasonal changes in water temperature (○) and chlorophyll *a* concentration (●) in Furuike Pond during the study period

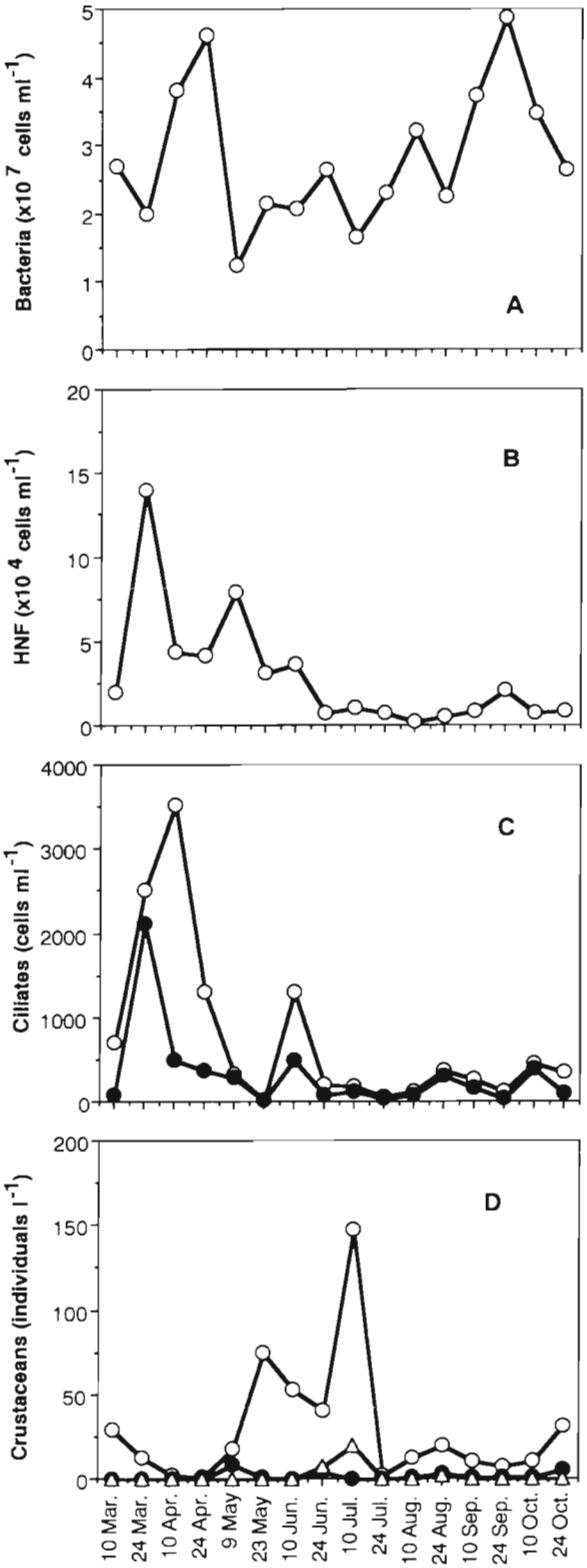

Fig. 3. Seasonal changes in densities of (A) bacteria, (B) heterotrophic nanoflagellates (HNF), (C) total ciliates (○) and bacteria-consuming ciliates (●), (D) cyclopoid copepods (○), *Moina* sp. (●) and *Diaphanosoma* sp. (△) in Furuike Pond during the study period

Table 1 Dominant genera of ciliates during the study period in Furuike Pond

Date	Ciliate genera
10 Mar	*Urotricha*, *Cyclidium*
24 Mar	*Cyclidium*, *Urotricha*
10 Apr	*Cinetochilum*, *Halteria*
24 Apr	*Cinetochilum*
9 May	*Halteria*
23 May	*Cyclidium*, *Urotricha*
10 Jun	*Cyclidium*, *Cinetochilum*
24 Jun	*Cinetochilum*, *Cyclidium*
10 Jul	*Cyclidium*, *Cinetochilum*, *Urotricha*
24 Jul	*Cyclidium*, *Cinetochilum*
10 Aug	*Cyclidium*, *Cinetochilum*
24 Aug	*Cinetochilum*, *Cyclidium*
10 Sep	*Cinetochilum*, *Cyclidium*
24 Sep	*Cinetochilum*, *Cyclidium*, *Strombidium*
10 Oct	*Cyclidium*
24 Oct	*Cinetochilum*, *Cyclidium*

During the study period, 5 dominant genera of ciliates were found (Table 1). Judging from the microscopic observation of FLB ingestion by ciliates, we confirmed that *Cyclidium* spp. and *Halteria* spp. were consumers of bacteria. Although *Cinetochilum* sp. is classified as a bacterial consumer in the literature (Patterson & Hedley 1992, Šimek et al. 1995), the food vacuole of this ciliate contained some cyanobacterial cells, and we seldom detected FLB ingestion. Hence, we regarded *Cyclidium* spp. and *Halteria* spp. as bacteria consumers, and *Cinetochilum* sp., *Urotricha* sp. and *Strombidium* sp. as algivores in Furuike Pond.

The density of total ciliates (Fig. 3C) increased rapidly from 10 March (711 cells ml^{-1}) to 10 April (3500 cells ml^{-1}), but declined rapidly to 22 cells ml^{-1} on 23 May. Apart from a sharp peak on 10 June (1300 cells ml^{-1}), it fluctuated between 133 and 460 cells ml^{-1} until the end of the study. The pattern of changes in the numbers of bacteria-consuming ciliates was similar to that of total ciliates, except that they declined earlier. Their densities ranged between 11 and 2100 cells ml^{-1} over the season as a whole (Fig. 3C). Small ciliates, bacteria-consumers, *Cyclidium* spp., and an algivore, *Cinetochilum* sp. (Table 1), were dominant throughout the study period. In April and May, *Halteria* spp. and *Urotricha* spp. were also dominant species, and the latter was detected again on 10 July. *Strombidium* sp. dominated only on 24 September. Large ciliates such as *Monodinium* sp. and *Strobilidium* sp. were sometimes observed, but their densities were low (<0.2 cells ml^{-1}) throughout the study period.

Cyclopoid copepods, most of which probably belong to the genus *Eucyclops*, dominated the crustacean zooplankton throughout the study period (Fig. 3D). Individual densities of cyclopoid copepods decreased from

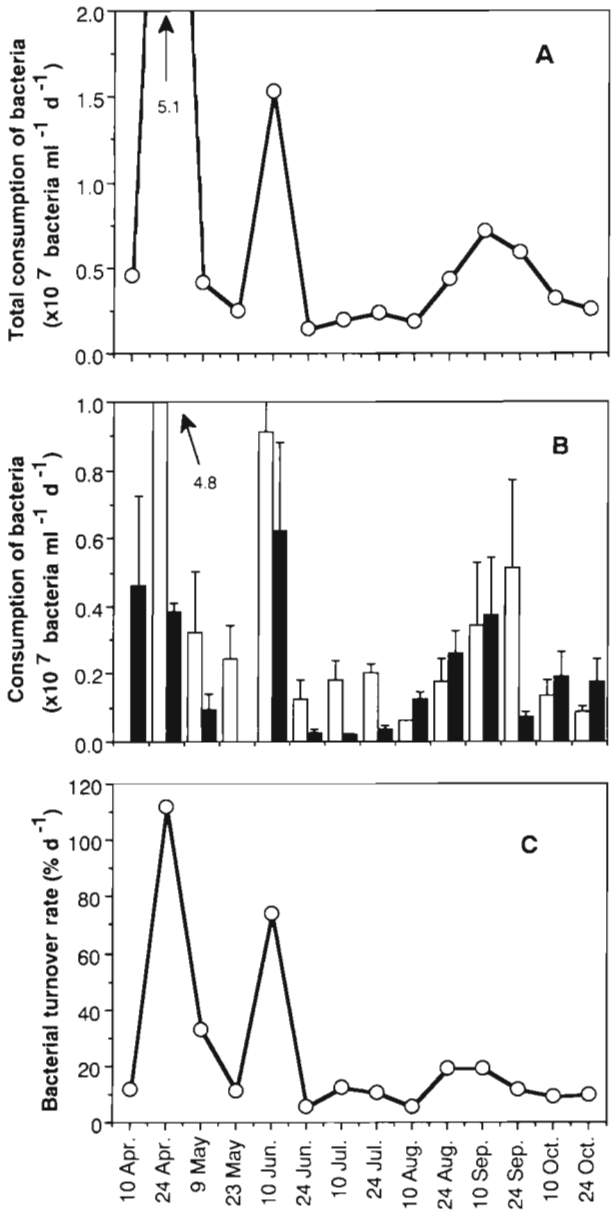

Fig. 4. Seasonal changes in total consumption rate of bacteria by (A) protozoa and (B) heterotrophic nanoflagellates (HNF) (open bars) and ciliates (closed bars) and in (C) bacterial turnover rate in Furuike Pond during the study period. Standard deviations are indicated for consumption rates of HNF and ciliates (B)

the beginning of the study (29 ind. l^{-1}) to 24 April (0.83 ind. l^{-1}) and then increased from 9 (18 ind. l^{-1}) to 23 May (75 ind. l^{-1}) before increasing further to the maximum (147 ind. l^{-1}) on 10 July after decreasing on 10 and 24 June. Densities of cyclopoid copepods decreased precipitously to 24 July (1.9 ind. l^{-1}), and there were small fluctuations between 6.9 (24 September) and 32 ind. l^{-1} (24 October) until the end of the study period. The only cladocerans we found were *Diaphanosoma* sp. and *Moina* sp. Their densities were low throughout the study period (Fig. 3D): <20 ind. l^{-1} for *Diaphanosoma* sp. and <9 ind. l^{-1} for *Moina* sp. Densities of *Diaphanosoma* sp. made 2 peaks: one was from 24 June (7.0 ind. l^{-1}) to 10 July (20 ind. l^{-1}), the other on 24 August (1.9 ind. l^{-1}). Individual densities of *Moina* sp. were relatively high when those of cyclopoid copepods were also high from 9 May (8.8 ind. l^{-1}) to 24 June (4.0 ind. l^{-1}). Thereafter, the densities were occasionally high on 24 August (3.4 ind. l^{-1}) and 24 October (5.3 ind. l^{-1}).

To compare seasonal changes in abundance of organisms we used Spearman rank correlation analysis for concentration of chlorophyll *a* and abundance of bacteria, protozoans and crustaceans. Unfortunately, we could not find any correlations among these parameters.

Total consumption of bacteria by protozoa (the sum of ingestion rates of HNF and ciliates) (Fig. 4A) fluctuated from 10 April to 24 June between 2.5×10^6 (23 May) and 5.1×10^7 bacteria ml^{-1} d^{-1} (24 April). It decreased to a minimum (1.5×10^6 bacteria ml^{-1} d^{-1}) on 24 June, increased until 10 September (7.2×10^6 bacteria ml^{-1} d^{-1}) and decreased again from 24 September. On 10 April only ciliates were consuming bacteria, accounting for 100% to the total (Fig. 4B). From 24 April to 24 July, consumption of bacteria by HNF predominated. On 10 June, the contribution of ciliates (6.2×10^6 bacteria ml^{-1} d^{-1}) was also important. From 10 August, the ciliates generally dominated (>50%) the total consumption of bacteria until the end of the study, although consumption by HNF was the highest on 24 September.

Table 2. Specific ingestion rates (bacteria protozoan $cell^{-1}$ h^{-1}) of heterotrophic nanaflagellates (HNF) and ciliates in the literature (mean values, ranges or mean ± SD) and the present study (mean ± SD)

Protozoa	Specific ingestion rate	n	Source
HNF			
Monas sp.	3–23		Sanders et al. (1989)
Choanoflagellates	8–42		Sanders et al. (1989)
Spumella-like chrysomonads	21 ± 11	102	Šimek et al. (1997)
Bodonids	36 ± 17	50	Šimek et al. (1997)
Choanoflagellates	53 ± 19	50	Šimek et al. (1997)
Mixed HNF	12 ± 12	4284	This study
Ciliates			
Halteria grandinella	67–1276		Sanders et al. (1989)
Halteria grandinella	1580	118	Šimek et al. (1995)
Cyclidium sp.	470	30	Šimek et al. (1995)
Halteria spp.	159 ± 78	37	This study
Cyclidium spp.	321 ± 248	212	This study

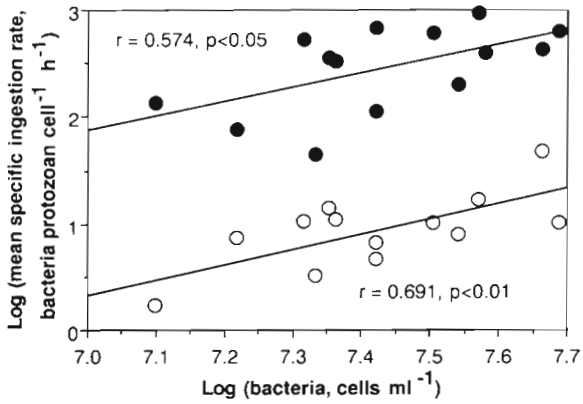

Fig. 5. Logarithmic relationships between mean specific ingestion rates of heterotrophic nanoflagellates (HNF) (○) and bacteria-consuming ciliates (●) and bacterial cell density

The pattern of bacterial turnover rates (% d^{-1}) (Fig. 4C) was similar to that of consumption of bacteria by protozoa (Fig. 4A). In most cases, turnover rates were less than 30% d^{-1}, and very high rates were detected only on 24 April (112% d^{-1}) and 10 June (74% d^{-1}).

The specific ingestion rate of HNF in the present study was 12 ± 12 bacteria flagellate^{-1} h^{-1}, that of *Halteria* spp. was 159 ± 78 bacteria ciliate^{-1} h^{-1} and that of *Cyclidium* spp. was 321 ± 248 bacteria ciliate^{-1} h^{-1} (Table 2). There were significant logarithmic relationships between densities of bacteria and mean specific ingestion rates (bacteria flagellate^{-1} h^{-1}) of HNF ($p < 0.01$), and between densities of bacteria and mean specific ingestion rates (bacteria ciliate^{-1} h^{-1}) of bacteria-consuming ciliates ($p < 0.05$) (Fig. 5).

DISCUSSION

Furuike Pond has some features of hypereutrophic lakes as reviewed by Sommaruga & Robarts (1997): for example, high phytoplankton biomass, dominance of cyanobacteria, sudden collapses of algal blooms and low abundance of macrozooplankton. Also, the relationship between the average bacterial abundance (2.8×10^7 cells ml^{-1}) and the average chlorophyll concentration (227 µg l^{-1}) in the present study agrees well with that given by Sommaruga & Robarts (1997). Thus, it is likely that the biological features of Furuike Pond are typical for hypereutrophic lakes. Although we detected extremely high densities of ciliates in the present study (Fig. 3C), such high abundances of ciliates are probably found in other hypereutrophic lakes. Unfortunately, we still have limited information about microbiology and ecology in hypereutrophic environments.

The predominant phytoplankton during most of the study period was *Microcystis aeruginosa*. It is well known that *M. aeruginosa* is inedible for many metazoan zooplankton because of its large colony size and toxin production (Fulton & Pearl 1987). Hence, one may think that the biomass of bacteria, heterotrophic nanoflagellates (HNF) and ciliates is important as food for metazoan zooplankton during the period of *Microcystis* dominance. Indeed, high abundances of bacteria, HNF and ciliates were detected in the present study (Fig. 3). The few zooplankters, however, can subsist on microbial food sources (Kamjunke et al. 1997), and copepods are inefficient predators for bacteria (Nagata & Okamoto 1988, Sanders et al. 1989) and protozoa (Jürgens et al. 1996). Thus, although the microbial loop in Furuike Pond is substantial, the food linkage between microbial and classical food chains is probably small.

We do not have any evidence of toxin production by *Microcystis aeruginosa*. However, densities of HNF and ciliates decreased (Fig. 3) as concentrations of chlorophyll increased (Fig. 2). Effects of algal toxins on growth of HNF have recently been studied (Christoffersen 1996, Pearl & Pinckney 1996). Thus, the decrease in protozoan densities may have been due to *Microcystis* toxin. Šimek & Straskrabova (1992) and Šimek et al. (1998) noted that the genus *Cyclidium* became dominant when the abundance of large or floc-forming phytoplankters was high in a mesoeutrophic reservoir and a dystrophic lake, respectively. However, we could not find any coupled oscillations between abundance of *Cyclidium* spp. and concentration of chlorophyll, and between the abundance of *Cyclidium* and *M. aeruginosa* (data not shown). Since chlorophyll level in the present study (mean 227 µg l^{-1}) is much higher than that of the 2 previous studies (<40 µg l^{-1}), it is likely that toxic effect of *M. aeruginosa* in the present study was more serious than that of the previous studies. By contrast, densities of cyclopoid copepods increased, following the decrease in protozoan density (Fig. 3). Possible planktonic food for the copepods is phytoplankton other than *Microcystis*, HNF, ciliates and rotifers, and we have not yet seen which organism is important for growth of copepods.

Unfortunately, we could not find clear trophic interactions among bacteria, HNF and ciliates, and between microbial and classical food chains. There was a gradual increase in bacterial density from 23 May to 24 September when densities of HNF and ciliates were relatively low (Fig. 3). This may be another type of trophic interaction: bacterial density increases as that of protozoa decreases due to *Microcystis* toxin.

The mean specific ingestion rates of HNF, *Halteria* spp. and *Cyclidium* spp. in Furuike Pond (Table 2) overlapped those of previous studies (Sanders et al. 1989, Šimek et al. 1995, 1997). Thus, consumption of

bacteria in the present study is comparable with previous measurement of bacterivory using the FLB method. No ingestion of FLB by HNF was detected on 10 April (Fig. 4C). It has been reported that some HNF do not ingest FLB (Landry et al. 1991), that some even prefer fluorescently labeled prey instead of unlabelled prey (Mischke 1994) and that some HNF species show a variable selectivity depending on their nutritional state (Jürgens & DeMott 1995). Thus, consumption rate on bacteria by HNF can be underestimated by the FLB method. The early dramatic decrease in chlorophyll *a* concentration was due to the collapse of the *Phormidium mucicola* bloom (Fig. 2) and, since most of this alga was single-celled with a cell size of 1 to 5 µm, which was appropriate for grazing by HNF and ciliates, it is likely that the decrease was caused by heavy grazing. The lack of ingestion of FLB on 10 April may have been because the HNF were preferentially grazing the alga at that time. Unfortunately, we did not check whether HNF contained the alga on that date.

In the present study, there are not any data showing that *Cinetochilum* sp. does not ingest bacteria, though we regarded the ciliate as an algivore. Bacteria, if they form flocs or clumps, are probably important food for the ciliates, but the ciliate cannot sieve dispersed bacteria. This is the possible reason for rare FLB ingestion by *Cinetochilum* sp.

Consumption of bacteria by HNF dominated from 10 April to 10 June, while that by ciliates was also important from 10 August to 24 October (Fig. 4C). Thus, not only HNF but also ciliates are important bacteria consumers in Furuike Pond. There is partitioning of bacterial food between HNF, ciliates and cladocerans (Sanders et al. 1989, Šimek et al. 1990, 1998, Šimek & Straskrabova 1992, Nakano et al. 1998), and consumption of bacteria by ciliates occasionally becomes the largest in total consumption of bacteria in mesoeutrophic (Šimek et al. 1990, Šimek & Straskrabova 1992) and dystrophic (Šimek et al. 1998) lakes. However, we still have limited information about bacterial consumption by protozoa in hypereutrophic lakes.

The consumption rates of bacteria (Fig. 4A) were occasionally very high, and the maximum bacterial turnover rate was estimated as 112% d^{-1} (Fig. 4C). Sanders et al. (1992) reported that higher bacterial production was detected in more eutrophicated waters while Jürgens & Stolpe (1995) reported that, in a eutrophic lake, more than 80% of bacterial production was removed per day by HNF alone. Thus, the high bacterial turnover rates in the present study probably mean high bacterial production. The bacterial turnover rates, however, are minimum estimates because they are based only on consumption of bacteria by protozoa. Viral infection also contributes significantly to bacterial mortality (Fuhrman & Noble 1995, Weinbauer & Höfle 1998).

Using data from various aquatic environments, Vaque et al. (1994) have reported that specific ingestion rate of HNF positively correlated with temperature and bacterial abundance. We found a significant relationship between density of bacteria and specific ingestion rate of HNF in Furuike Pond (Fig. 5). The relationship means tight food linkage between bacteria and HNF. A relationship between density of bacteria and specific ingestion rate of ciliates was also significant (Fig. 5). This, however, must be evaluated with some caution, because there are various functional group in ciliates due to their different feeding modes.

In the present study, we found substantial food linkage between bacteria and protozoans in Furuike Pond: high bacterial turnover rates due to protozoan grazing and significant relationships between abundance of bacteria and specific ingestion rate of protozoa. However, trophic interactions between HNF and ciliates, and between microbial and classical food chains, are still unclear. Although the food linkage between the microbial loop and metazoan zooplankton in the pond is probably less important, we need to quantify predation rates of ciliates on HNF and those of rotifers and cyclopoid copepods on both HNF and ciliates to evaluate the linkage. Further, simultaneous measurements of production and loss within both the microbial loop and the classical food chain will be required to elucidate material cycling in order to verify the role of the microbial loop in an aquatic ecosystem.

Acknowledgements. We are grateful to A. Ohshima, A. Yagi, K. Okamoto, M. Ueki and other members of the Ecosystem Conservation Laboratory, Ehime University, for their advice, discussions and encouragement throughout the study. We also thank Dr Y Watanabe for his donation of a minicell-producing mutant of *Escherichia coli*. This study forms a part of Guest Scientist Activities, Center for Ecological Research, Kyoto University. This study was partly supported by a Japanese Ministry of Education, Science and Culture, Grant-in-Aid for Creative Basic Research (09NP1501), 'An interactive study on biodiversity conservation under global change and bioinventory management system', by a grant-in-aid from the Ministry of Education, Science, Sports and Culture, Japan (No. 08308031) and by the Showa Shell Sekiyu Foundation for Promotion of Environmental Research.

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Editorial responsibility: Karel Šimek, České Budějovice, Czech Republic

Submitted: March 23, 1998; Accepted: June 26, 1998
Proofs received from author(s): November 13, 1998