



Relative importance of nanoflagellates and ciliates as consumers of bacteria in a coastal sea area dominated by oligotrichous *Strombidium* and *Strobilidium*

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ABSTRACT: To examine the relative importance of nanoflagellates and ciliates as grazers of bacteria, we examined seasonal changes in their consumption of bacteria in a coastal sea, where the oligotrichous ciliates *Strombidium* and *Strobilidium* are dominant throughout the year. The numbers of these 2 dominant taxa accounted for 16 to 71 % (average 55 %) of the total ciliate numbers. Other ciliate taxa which seasonally dominated were photosynthetic *Myrionecta rubra*, the aloricate oligotrichs *Laboea* and *Lohmaniella*, the loricate oligotrichs *Tintinnopsis*, *Cyttarocyclus*, and scuticociliates. Fluorescently labelled 0.5 μm diameter beads were used to determine grazing rates. Bacterivory was detected for nanoflagellates, *Strombidium*, *Strobilidium*, *Lohmaniella*, *Tintinnopsis* and *Cyttarocyclus*. Ingestion rates on bacteria by nanoflagellates ranged between 0.3 and 2.2 bacteria protistan cell⁻¹ h⁻¹. Ingestion rates of the dominant ciliate taxa *Strombidium* and *Strobilidium*, when feeding on bacteria, varied from 7 to 34 bacteria protistan cell⁻¹ h⁻¹, though the rates were low relative to those of other dominant ciliate taxa (14 to 50 bacteria protistan cell⁻¹ h⁻¹). Significant relationships ($p < 0.01$) were found between the cell numbers of bacteria and ingestion rates of nanoflagellates, and between the cell numbers of bacteria and ingestion rates of *Strombidium*, whereas the relationship between the cell numbers of bacteria and ingestion rates of the total ciliates was not significant. Turnover rates of bacteria due to grazing by the total ciliates (0 to 0.63 % d⁻¹) were lower than those by nanoflagellates (1 to 15 % d⁻¹). Thus, it is likely that the dominant ciliates in the bay are not important grazers of bacteria, and that the main grazers of bacteria are nanoflagellates.

KEY WORDS: Microbial food web · Nanoflagellates · Oligotrichous ciliates · Grazing · Coastal sea · Japan

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INTRODUCTION

In pelagic marine environments, it is now commonly accepted that nanoflagellates are the most important grazers of planktonic bacteria (Caron & Finlay 1994), and some planktonic ciliates also consume a significant portion of bacterial abundance (Rassoulzadegan et al. 1988). Fluorescently labeled surrogates (FLS) such as fluorescently labeled beads (McManus & Fuhrman 1986) and bacteria (Sherr et al. 1987) have been widely used to determine grazing rates of protists on bacteria. The advantage of the method is that we can identify which protistan cells ingest bacteria and determine rates of grazing on bacteria for each protistan taxon.

Since elucidating the major channels for the biological transfer of organic matter within an ecosystem is one of the key issues in ecology, the FLS method is useful for identifying the dominant transfer pathways of organic matter within a microbial food web.

Consumption of bacteria by nanoflagellates and ciliates has been intensively studied in lakes by the FLS method (Sanders et al. 1989, Vaqué & Pace 1992, Šimek et al. 1995, Nakano et al. 1998a,b), and it has been demonstrated that the relative importance of nanoflagellates and ciliates as grazers of bacteria changes seasonally in lakes (Šimek et al. 1990, 1995, Šimek & Straskrabova 1992, Nakano et al. 1998a). In oceans and coastal seas, grazing on bacteria by nanoflagellates

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(Sherr et al. 1987, Gonzalez et al. 1990, Epstein & Shiaris 1992, James et al. 1996) or ciliates (Albright et al. 1987, Sherr et al. 1987, 1988, 1989, Bernard & Rassoulzadegan 1990, Gonzalez et al. 1990, Epstein & Shiaris 1992, James et al. 1996) has also been studied using the FLS method. However, only a few studies have made comparisons between rates of grazing on bacteria by nanoflagellates and ciliates, and information on the relative importance of these groups of grazers in oceans and coastal seas is still limited (Sherr et al. 1987, Epstein & Shiaris 1992) relative to that in freshwater.

For marine ciliates, experiments to investigate food selection have been conducted using latex beads, live food, wheat starch particles; the oligotrichous ciliates *Strombidium* and *Strobilidium* were also frequently used in such studies (Lessard & Swift 1985, Jonsson 1986, Kivi & Setälä 1995). These studies have demonstrated that the 2 ciliate taxa showed size-selective grazing on particles between 1 and 10 μm (Jonsson 1986, Kivi & Setälä 1995). However, $<30 \mu\text{m}$ ciliates are probably consumers of bacteria (Rassoulzadegan et al. 1988). Some species of *Strombidium* are small, and can ingest bacteria-sized particles (Rivier et al. 1985, Sherr et al. 1988). Moreover, since freshwater species of the genus *Strobilidium* graze on bacterial prey (Kisand & Zingel 2000), it is likely that marine species of this genus do also. Finally, these 2 ciliate taxa often numerically dominate in many marine areas (Gonzalez et al. 1990, James et al. 1996), suggesting their importance as grazers of bacteria. However, there is a shortage of information about grazing by these 2 ciliate taxa on bacteria in a marine system. Therefore, we examined the relative importance of nanoflagellates and ciliates as grazers of bacteria by following seasonal changes in

their grazing in a bay of the Uwa Sea, where *Strombidium* and *Strobilidium* are dominant throughout the year.

MATERIALS AND METHODS

This study was conducted in Uchiumi Bay, Iegushi, Uchiumi Village, Ehime Prefecture, Japan (Fig. 1). Pearl oysters *Pinctada fucata* are cultivated in this area, the production level of which is the highest in Japan. Oyster larvae graze on bacteria and picocyanobacteria (Tomaru et al. 2000) and, hence, studies on the ecology of the microbial food web in this area are important not only to assess food web structure and function, but also to assess fisheries management. The bay is oligo- to mesotrophic, based on its chlorophyll levels, which are usually $<2 \mu\text{g l}^{-1}$ (Tomaru et al. 2002). Thermal stratification in the bay usually develops from May to October.

Monthly monitoring was conducted at Stn Ub (33° 2' N, 132° 28' E; ca. 53 m) (Fig. 1), from May 2000 to July 2001. Water column temperature was measured using a Chlorotech profiler (Areck Electronics). Water samples were collected monthly from 10 m depth using a 6L Van-Dorn water sampler. To determine the chlorophyll a concentration, 300 ml of the water sample was filtered through a Nuclepore filter (pore size 0.2 μm) and analyzed following the *N,N*-dimethylformamide fluorometric method (Moran & Porath 1980).

For enumeration of heterotrophic bacteria and nanoflagellates, 100 ml were fixed with glutaraldehyde to a final concentration of 1%; 1 to 2 ml of the fixed water sample were for enumerating hetero-

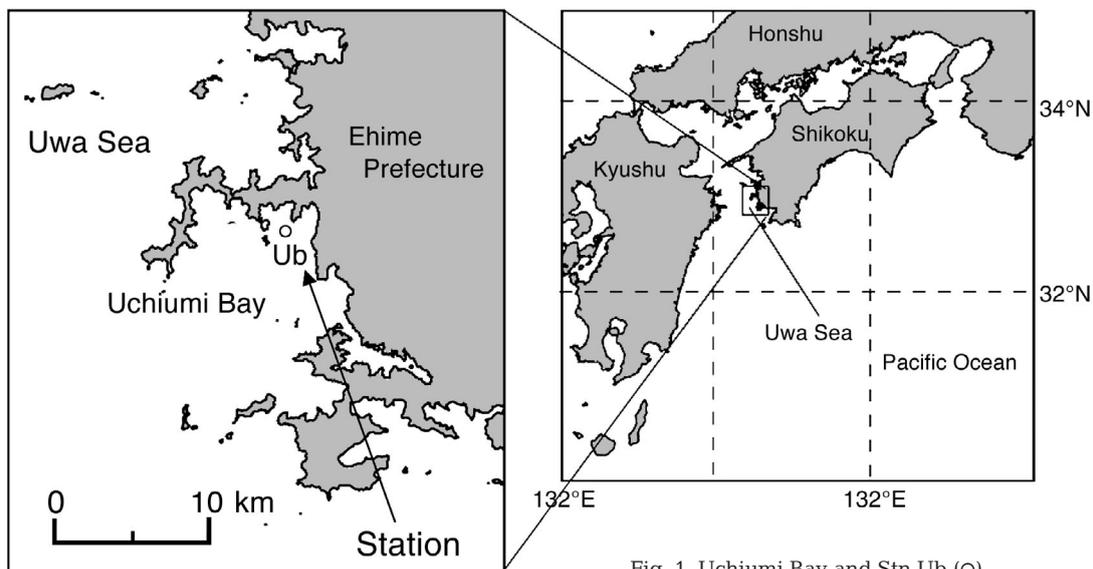


Fig. 1. Uchiumi Bay and Stn Ub (O)

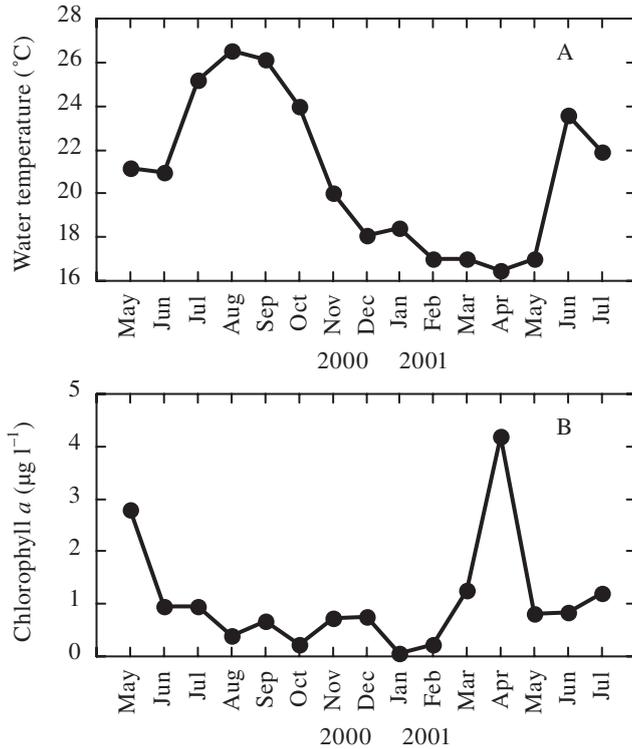


Fig. 2. Seasonal changes in (A) water temperature and (B) chlorophyll *a* concentration at Stn Ub

trophic bacteria and 30 to 50 ml were for enumerating nanoflagellates. Both were counted using an epifluorescence microscope under ultraviolet excitation by the DAPI (Porter & Feig 1980) and primulin (Caron 1983) methods, using black-stained 0.2 and 0.8 µm Nuclepore filters, respectively. We did not discriminate flagellates into autotrophic and heterotrophic groups; hence, cells <20 µm with flagellae were all counted as nanoflagellates and considered potential bacterivores. At least 300 cells of heterotrophic bacteria and 30 nanoflagellate cells were counted in each sample: the CVs of the counts were respectively 12 and 19%.

For enumeration of ciliates, a 500 ml portion of the water sample was fixed with acid Lugol's solution to a final concentration of 1%, and the ciliates were concentrated by sedimentation. Ciliate cells were enumerated in a haematocytometer at 200× or 400× magnification. We identified ciliate taxa using the classification guides of Foissner (1994), Chihara & Murano (1997), Strüder-Kypke & Montagnes (2002) and Strüder-Kypke et al. (2002).

Grazing rates of nanoflagellates and ciliates feeding on bacteria were determined using 0.5 µm fluorescently labeled beads (FLB; fluoresbrite yellow green microspheres, Polysciences) following the methods of McManus & Fuhrman (1986). For this, 250 ml water samples were poured into triplicate polycarbonate

bottles and FLB were added to each bottle (0.5×10^5 FLB ml⁻¹ to 1.0×10^5 FLB ml⁻¹) at <10% of the *in situ* bacterial cell numbers (McManus & Okubo 1991). The FLB-spiked samples were then incubated for 15 min at the original depth at which the water sample had been collected. Note preliminary experiments indicated ingestion of FLB by nanoflagellates and ciliates was saturated after 20 and 40 min, respectively (data not shown). After the incubation, subsamples were taken and fixed with 4% ice-cold, buffered glutaraldehyde, an effective fixative to reduce the egestion of bacteria which have been taken into a food vacuole of nanoflagellates and ciliates (Sanders et al. 1989). To account for any FLB adsorbed to cell surfaces of the protists, a time-zero control was taken and fixed as described above. Nanoflagellates and ciliates thus fixed were, respectively, retained on 0.8 and 5 µm black Nuclepore filters and stained with primulin, as previously described. The FLB in their food vacuoles were then counted under an epifluorescence microscope. At least 100 nanoflagellate cells and 20 to 40 ciliate cells were examined from each sample. Daily ingestion rates were then determined assuming no biases for FLBs and a constant grazing rate over 24 h. Bacterial turnover rates (% d⁻¹) were estimated by expressing the bacteria ingested by the nanoflagellates or ciliates as percentages of the corresponding bacterial cell numbers, with the assumption that the bacterial cell numbers remained at steady-state.

RESULTS

The water temperature at 10 m depth in Uchiumi Bay rapidly increased from July (25.2°C) to August 2000 (26.5°C), decreased from September (26.1°C) to April 2002 (16.5°C) and increased again from May 2002 (Fig. 2A). Thermal stratification developed from May to October. Chlorophyll *a* concentrations decreased from June 2000 (0.95 µg l⁻¹) to January 2001 (0.05 µg l⁻¹) with fluctuations increased to a maximum in April 2001 (4.20 µg l⁻¹) and decreased again from May 2001 (Fig. 2B).

Bacterial cell numbers (Fig. 3A) decreased from June 2000 (1.4×10^6 cells ml⁻¹) to February 2001 (0.6×10^6 cells ml⁻¹), increased from March (1.2×10^6 cells ml⁻¹), attained a maximum in May (1.7×10^6 cells ml⁻¹) and then gradually decreased to July (1.4×10^6 cells ml⁻¹) (Fig. 2A). Nanoflagellate densities (Fig. 3B) ranged between 770 cells ml⁻¹ (January 2002) and 4300 cells ml⁻¹ (August 2001), and high densities tended to be recorded during the stratified period (May to August). There were large fluctuations in the ciliate numbers, varying from 1.2 (December 2001)

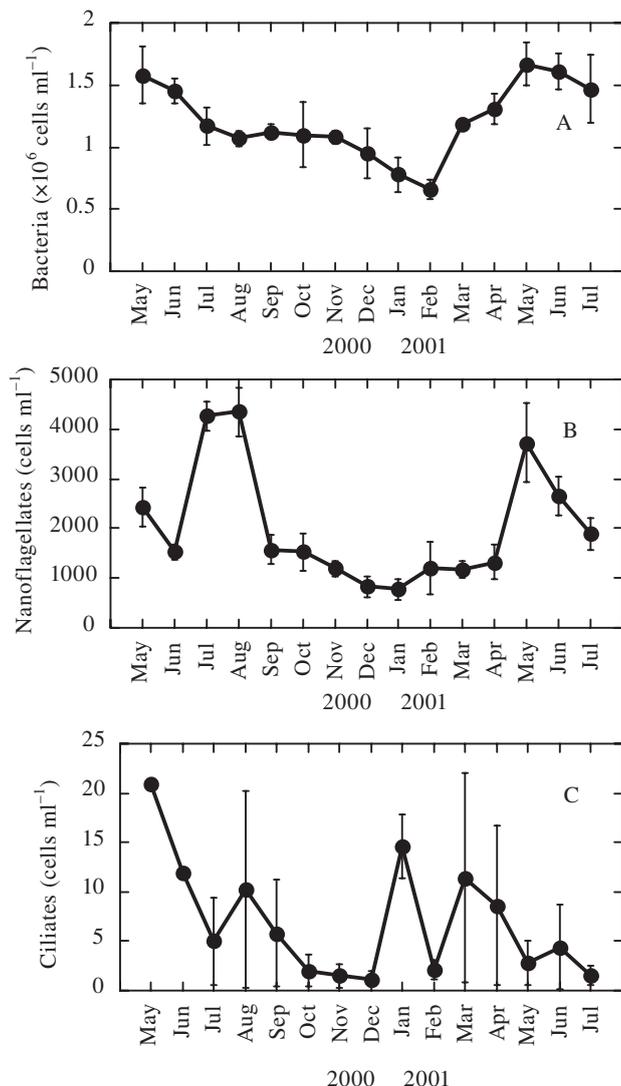


Fig. 3. Seasonal changes in cell numbers (mean \pm SD) of (A) bacteria, (B) nanoflagellates and (C) ciliates at Stn Ub

to 21 (January 2002) cells ml^{-1} (Fig. 3C). The dominant taxa were the oligotrichous genera *Strombidium* (dominated by *S. conicum*, *S. epideum*, *S. lynni*, *S. emergens*, *S. wuffi* and *S. dalum*) and *Strobilidium* (dominated by *S. neptuni* and *S. spiralis*) (Fig. 4). The numbers of these 2 dominant taxa accounted for 16 to 71% (average 55%) of the total ciliate numbers. The photosynthetic *Myrionecta rubra* (Gymnostomatida) was detected throughout the study period, dominating between December 2000 and February 2001 (Fig. 4). The aloricate oligotrichs *Laboea* and *Lohmaniella*, loricate oligotrichs *Tintinnopsis* and *Cyttarocylis* and Scuticociliates were also seasonally abundant (Fig. 4), although their abundance was still low relative to *Strombidium* and *Strobilidium*.

Table 1. Ingestion rates on bacteria by some ciliate taxa dominated in the present study

	Average ingestion rate (bacteria protistan $\text{cell}^{-1} \text{h}^{-1}$)	Range	n
<i>Codonella</i>	29	14–44	30
<i>Cyttarocylis</i>	42	27–76	45
<i>Lohmaniella</i>	39	39	3
<i>Strobilidium</i>	23	7–34	102
<i>Strombidium</i>	18	7–34	342
<i>Tintinnopsis</i>	47	43–50	20

Ingestion rates of nanoflagellates ranged between 0.3 and 2.2 bacteria protistan $\text{cell}^{-1} \text{h}^{-1}$, decreasing from May to December and increasing from January 2001 onwards (Fig. 5A). Changes in the ingestion rates of the total ciliates (Fig. 5B) did not show any clear seasonal trend, and the maximum rate (31 bacteria protistan $\text{cell}^{-1} \text{h}^{-1}$) was found in May 2002 (Fig. 5B). Ingestion rates of the dominant ciliate taxa *Strombidium* spp. and *Strobilidium* spp. varied within the same range from 7 to 34 bacteria protistan $\text{cell}^{-1} \text{h}^{-1}$ (Table 1), which were lower than those of other ciliate taxa (Table 1).

A significant correlation ($n = 14$, $r = 0.826$, $p < 0.01$) occurred between bacterial cell numbers and nanoflagellate ingestion rates (Fig. 6A). There was no significant correlation between cell numbers of bacteria and ingestion rates of ciliates (Fig. 6B). However, there was a significant correlation ($n = 13$, $r = 0.757$, $p < 0.01$) between cell numbers of bacteria and ingestion rates of *Strombidium* (Fig. 6C), while no significant correlation occurred between cell numbers of bacteria and ingestion rates of other individual ciliate taxa.

Daily consumption of bacteria by nanoflagellates varied from 0.06×10^5 ingested bacterial cells $\text{ml}^{-1} \text{d}^{-1}$ (December 2000) to 1.6×10^5 bacteria $\text{ml}^{-1} \text{d}^{-1}$ (May 2001). Bacterial turnover rates ($\% \text{d}^{-1}$), due to grazing by nanoflagellates, were determined as between 1 and 15% d^{-1} (Fig. 7A), tending to be higher during the stratified period. Daily consumption of bacteria by ciliates was lower than that of nanoflagellates (310 to 7500 bacteria $\text{ml}^{-1} \text{d}^{-1}$), and bacterial turnover rates due to the total ciliates were estimated to be 0 to 0.63% d^{-1} (Fig. 7B). The turnover rates due to ciliate grazing had no clear seasonal trend (Fig. 7B).

DISCUSSION

In the original concept of microbial loop (Azam et al. 1983), both nanoflagellates and ciliates consume a portion of bacterial abundance. Successive microbiological studies in marine environments have supported the concept, though the importance of nano-

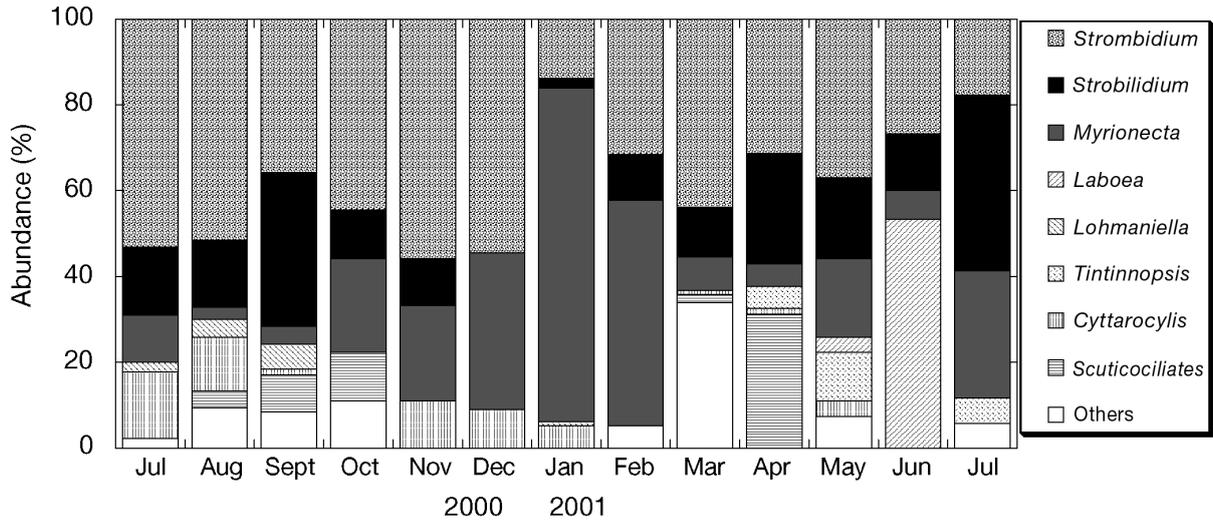


Fig. 4. Seasonal changes in abundance (%) of dominant ciliate taxa at Stn Ub

flagellates as grazers of planktonic bacteria tends to be higher than that of ciliates (Rassoulzadegan et al. 1988, Caron & Finlay 1994). Thus, there may be competition between nanoflagellates and ciliates for bacterial food, but the partitioning of bacterial food between nanoflagellates and ciliates is still poorly understood. In the present study, we did find a significant functional response between cell numbers of bacteria and inges-

tion rates of nanoflagellates (Fig. 6A). This relationship indicates subsaturation of grazing on bacteria by flagellates. Thus, the rate at which nanoflagellates graze on bacteria in Uchiumi Bay is limited by bacterial cell numbers. By contrast, we did not find a significant functional response between cell numbers of bacteria and ingestion rates of ciliates (Fig. 6B). Usually, ciliates consist of diverse species with various feeding modes which allow grazing on variety of food items. We also found seasonal changes in composition of dominant ciliate taxa during the study period in Uchiumi Bay (Fig. 4). Hence, it is probably inappropriate to treat whole ciliate community as grazers of bacteria. Indeed, we detected significant correlation between cell numbers of bacteria and ingestion rate of *Strombidium* (Fig. 6C).

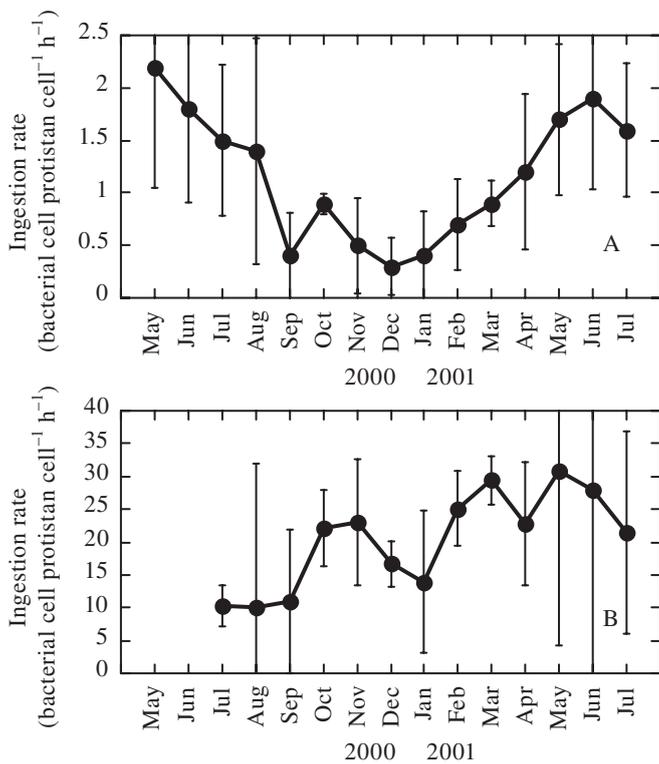


Fig. 5. Seasonal changes in ingestion rates (mean ± SD) of bacteria by (A) nanoflagellates and (B) ciliates at Stn Ub

We used artificial fluorescent beads to determine protistan grazing rate on bacteria (see 'Materials and methods'). Heat-killed and fluorescently stained microcell producing bacteria were used in our previous studies (Nakano 1994, Nakano et al. 1998a,b), but no general pattern of preference has been found for either heat-killed bacteria or artificial beads (Sanders et al. 1989, Montagnes & Lessard 1999). However, previous studies have demonstrated preference on prey types, living or non-living, by protists (Landry et al. 1991, Montagnes & Lessard 1999), and thus the methods using fluorescent surrogates to determine grazing rates by protists have limitations (Vaqué et al. 1994, Strom 2000). Hence, grazing rates in the present study may be underestimated due to the use of artificial beads as surrogates.

On an individual basis, ciliates may graze more bacterial cells than nanoflagellates do (Table 1, Figs. 5 & 6). However, abundance of ciliates is usually less than that of nanoflagellates in marine environments (Carlough & Meyer 1989, James et al. 1996), and

Table 2. Turnover rates of bacteria due to protistan grazing (% d⁻¹), from the literature

	Trophic state	Protists	Range	Average	Source
Marine					
Hudson Estuary, USA		Flagellates and ciliates	3–16	10	Vaqué et al. (1992)
Off South Island, New Zealand		Flagellates and ciliates	0.2–2.3		James et al. (1996)
Sapelo Island tidal creek, USA		Flagellates		15	Sherr et al. (1987)
Sapelo Island tidal creek, USA		Ciliates		90	Sherr et al. (1987)
Savin Hill Cove embayment, USA	Eutrophic	Flagellates		10	Epstein & Shiaris (1992)
Savin Hill Cove embayment, USA	Eutrophic	Ciliates		6	Epstein & Shiaris (1992)
Uchiumi Bay, Japan	Oligo- to mesotrophic	Flagellates	1–15	5.5	This study
Uchiumi Bay, Japan	Oligo- to mesotrophic	Ciliates	0.05–0.65	0.1	This study
Freshwater					
Furuike Pond, Japan	Hypereutrophic	Flagellates and ciliates	5–112	25	Nakano et al. (1998a)
Grosser Binnensee, Germany	Eutrophic	Flagellates	1–89		Jürgens & Stolpe (1995)
Lake Biwa (North Basin), Japan	Mesotrophic	Flagellates	0.6–16	3.5	Nakano (1994)
Lake Biwa (South Basin), Japan	Eutrophic	Flagellates	1.3–23	7.7	Nakano (1994)
Lake Biwa (North Basin), Japan	Mesotrophic	Flagellates	0.7–24	5.9	Nakano et al. (1998b)
Lake Oglethorpe, USA	Eutrophic	Flagellates	3–35	12	Sanders et al. (1989)
Ogeechee River, USA	Dystrophic	Flagellates	0.2–59	15	Carlough & Meyer (1991)
Ogeechee River, USA	Dystrophic	Ciliates	0.0044–3.5	0.58	Carlough & Meyer (1991)
Paul Lake, USA	Oligotrophic	Flagellates	0.8–2.3	1.3	Vaqué & Pace (1992)
Plusssee, Germany	Eutrophic	Flagellates		33	Fukami et al. (1991)
Tuesday Lake, USA	Oligotrophic	Flagellates	0.2–5.9	3.5	Vaqué & Pace (1992)

therefore grazing pressure on bacteria by ciliates would be lower than that by nanoflagellates. In the present study, consumption of bacteria due to grazing by ciliates was negligible relative to that by nanoflagellates (Fig. 7, Table 2), and this was due to low abundance of ciliates (Fig. 3).

Small ciliates, whose cell sizes are less than 30 µm, are probably consumers of bacteria (Rivier et al. 1985, Rassoulzadegan et al. 1988, Sherr et al. 1988), though larger ciliates may incidentally graze on bacteria. In the present study, some of the dominant ciliate species were small (<30 µm)—*Strombidium epideum*,

S. emergens, and *S. dalum*—and they may prey on bacteria since other small *Strombidium* species, such as *S. sulcatum*, which is now recognized as *S. inclinatum* (Montagnes et al. 1990, Granda & Montagnes 2003), are known to do so (Sherr et al. 1988), although the main foods of our 3 dominant ciliates are currently unknown (Strüder-Kypke et al. 2002). We found a significant correlation between cell numbers of bacteria and ingestion rate of *Strombidium* (Fig. 6C). However, previous studies using other types of surrogates such as latex beads (Jonsson 1986) and wheat starch (Kivi & Setälä 1995) have demonstrated that the

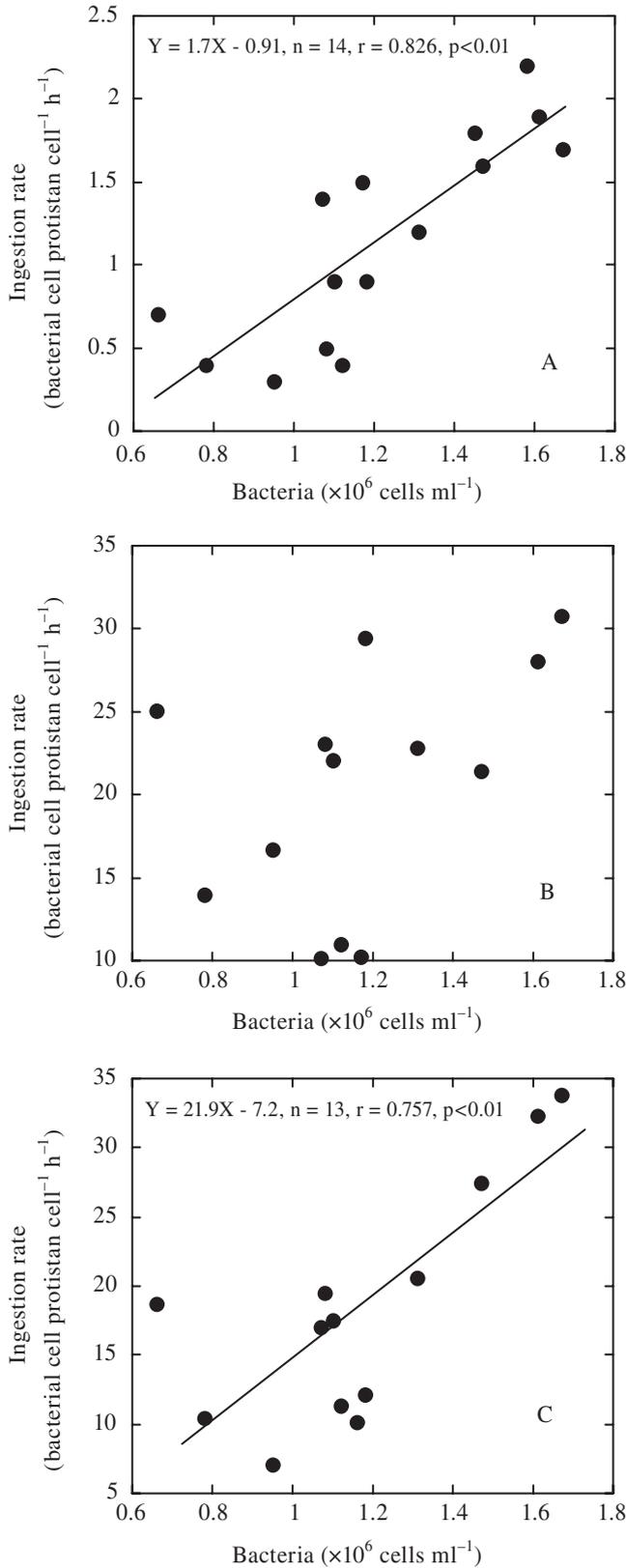


Fig. 6. Relationships between cell numbers of bacteria and ingestion rate of (A) nanoflagellates, (B) ciliates, and (C) *Strombidium*

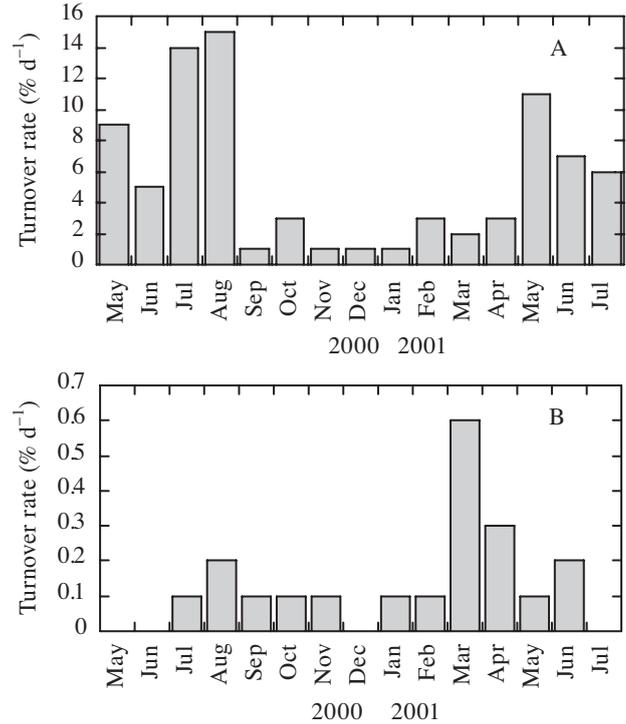


Fig. 7. Seasonal changes in turnover rates of bacteria due to grazing by (A) nanoflagellates and (B) ciliates at Stn Ub

marine ciliate taxa studied so far showed a preference for feeding on particles with a variety of sizes and could not effectively retain particles less than 2 μm . Most of the isolates of *Strombidium* and *Strobilidium* examined so far also could not effectively ingest particles less than 2 μm (Fenchel 1980, Jonsson 1986). In the present study, ingestion rates on bacteria of *Strombidium* and *Strobilidium* were low relative to those of other ciliate taxa such as *Cyrtarocyclus*, *Lohmaniella* and *Tintinnopsis* (Table 1), though there was a significant correlation between bacterial abundance and *Strombidium* ingestion rate (Fig. 6C). It is, therefore, likely that the FLB (0.5 μm) that we used for determination of grazing rates were edible, but inappropriately small, for ingestion by the *Strombidium* and *Strobilidium* examined in the present study.

Cell number of bacteria increased near the time of the spring bloom of phytoplankton and remained high in the summer (Figs. 2B & 3A), possibly due to an increased release of dissolved organic matter from phytoplankton in the spring and to the recycling of organic matter in the summer. There was a commensurate increase in cell number of nanoflagellates and in their grazing rate (Figs. 3B & 5A), resulting in an increase of their grazing pressure (Fig. 7A). Although there was also an increase in grazing pressure on bacteria by ciliates in the spring, this remained low (Fig. 7B). Probably, the ciliates that dominated in the present study mainly

ingested prey larger than bacteria as small nanoflagellates (2.2 to 5 μm) (Lynn & Montagnes 1991). In our previous study, conducted in a hypereutrophic freshwater environment (Nakano et al. 2001), we found that the most important predators for nanoflagellates were ciliates, and that predation by ciliates accounted for about 80% of nanoflagellate production. Low abundance of nanoflagellates between September and April (Fig. 3B) might be explained by predation on nanoflagellates by ciliates, and this is more plausible between January and April when relatively high ciliate abundance was detected (Fig. 3C).

In freshwater systems information about turnover rates due to protistan grazing on bacteria is relatively plentiful (Table 2). Higher turnover rates are found in eutrophic and hypereutrophic lakes, while lower turnover rates are found in oligotrophic lakes (Table 2). In marine environments, a relatively high turnover rate (10%) due to flagellate grazing was also estimated in the eutrophic Savin Hill Cove embayment, USA (Epstein & Shiaris 1992) (Table 2). Turnover rates in mesotrophic lakes are intermediate between those in oligotrophic and eutrophic lakes (Table 2) and the range and average values of turnover rates in the oligo- to mesotrophic Uchiumi Bay are similar to those in mesotrophic lakes (Table 2). The results in the present study support those of previous studies: nanoflagellates are the main first link between bacteria and upper trophic levels, though ciliates may incidentally eat some bacteria. The present study also showed that the transfer of bacterial organic matter up the food web occurs mainly during spring and summer. Thus there appears to be seasonality in the use of bacteria by upper trophic levels. It is likely that ciliates are one of the main next trophic level organisms which consume nanoflagellates, although mezozooplankton will also play a role as predators of nanoflagellates (Lynn & Montagnes 1991, Nakano et al. 2001). As information about grazing on bacteria by nanoflagellates and ciliates is still limited for marine systems, our data provide a valuable contribution towards understanding the structure and functioning of microbial food webs in coastal marine environments.

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