Vol. 29: 173–179, 2002 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published September 23

Grazing on *Microcystis aeruginosa* by the heterotrophic flagellate *Collodictyon triciliatum* in a hypertrophic pond

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ABSTRACT: Temporal changes in abundance of the heterotrophic flagellate *Collodictyon triciliatum* and its grazing rates on *Microcystis aeruginosa* were examined in a hypertrophic pond during a bloom of the cyanobacterium in 2000. The rates were estimated from ingested cells of *M. aeruginosa* in food vacuoles of *C. triciliatum* and experimentally determined digestion rates of the flagellate feeding on the cyanobacterium. Cell densities of *C. triciliatum* fluctuated between 10 and 510 cells ml^{-1}, and the changing pattern was different from that of *M. aeruginosa*. Single cells and small colonies of *M. aeruginosa* were found in food vacuoles of the flagellate, ranging between 2.9 and 16.7 cells $flagellate^{-1}$. The digestion rate of *C. triciliatum* was determined as 0.74 ± 0.02% cell contents min^{-1}. Estimates of grazing rate on *M. aeruginosa* by *C. triciliatum* ranged between 1.1 and 9.9 *M. aeruginosa* cells $flagellate^{-1}$ h^{-1}, and grazing impact on the cyanobacterial standing stock fluctuated between 0.002 and 0.09% h^{-1} in the pond. It is estimated that consumption of *M. aeruginosa* supplies carbon to the flagellate at a rate of 5.5 ± 3.2% to its cellular carbon content per hour. The growth rates of the flagellate are estimated as 0.016 ± 0.009 h^{-1}, which overlap those in previous laboratory studies. Hence, grazing on *M. aeruginosa* by *C. triciliatum* is probably of minor importance for the loss of cyanobacterial abundance in the pond, though the cyanobacterium is possibly an important food for the flagellate.

KEY WORDS: *Microcystis aeruginosa* · Heterotrophic flagellate · Digestion · Grazing rate

INTRODUCTION

Cyanobacterial blooms have been reported in many eutrophic and hypertrophic lakes, most commonly as *Microcystis aeruginosa* (Reynolds et al. 1981). The physiological and ecological characteristics of *M. aeruginosa* have been intensively studied, and the bloom-forming mechanisms clarified (e.g. Reynolds et al. 1981, Paerl et al. 1983, Takamura et al. 1985, Zohary & Robarts 1989, Nakano et al. 2001a). However, the processes of loss from *M. aeruginosa* populations are not yet fully understood.

Protists can be important consumers of bloom-forming cyanobacteria (e.g. Dryden & Wright 1987). Previous studies have noted that some species of flagellates graze on *Microcystis aeruginosa* (Cole & Wynne 1974, Sugiura et al. 1992, Klaveness 1995, Zhang et al. 1996). In particular, Cole & Wynne (1974) and Sugiura et al. (1992) have reported active grazing by flagellates on *M. aeruginosa* in laboratory experiments. Hence, flagellate grazing may contribute to the decline of *M. aeruginosa* biomass, but little is known about the abundance of flagellates which graze on *M. aeruginosa* and their grazing rates on the cyanobacterium in natural environments. Even less is known about the role of cyanobacteria in the natural diet of flagellates.

There are various functional groups of protists, based on their different feeding modes, which sug-

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gests that there may be a limited number of species which could graze on *Microcystis aeruginosa*. Thus, it is important to examine the grazing rates on *M. aeruginosa* by a particular species. Grazing rates on phytoplankton by particular protistan species have so far been estimated using 2 methods: tracer techniques with fluorescently labeled algae (Rublee & Gallegos 1989, Sherr et al. 1991, Mischke 1994, Šimek et al. 1996, Kamiyama et al. 2001), and the vacuole content method (Fenchel 1975, Kopylov & Tumantseva 1987, Dolan & Šimek 1997, 1998, Li et al. 2001). For the former, fluorescent staining of colonies of *M. aeruginosa* is required to determine the grazing rate of the protists; however, *M. aeruginosa* in culture usually forms small colonies and/or becomes unicellular, which is different from the colonies formed by *M. aeruginosa* in natural environments and may thus yield grazing rates different from those on *M. aeruginosa* in natural environments, since protistan grazing is in most cases size-selective (Chrzanowski & Šimek 1990, Gonzalez et al. 1990, Šimek & Chrzanowski 1992). In contrast, in the second method, grazing rates on *M. aeruginosa* by protists is based on digestion rates determined in laboratory experiments and the food vacuole contents of protists collected from natural environments. We therefore consider that the latter method is more appropriate for estimating grazing rates of protists feeding on *M. aeruginosa*.

In the fall of 1998, based on microscopic observations of food vacuole contents, we found that the heterotrophic flagellate *Collodictyon triciliatum* Carter was a grazer of *Microcystis aeruginosa* in a hypertrophic pond. Although it has been reported that this flagellate grows on *M. aeruginosa* (Klaveness 1995), grazing rate has never been examined. In the present study, we estimated grazing rates of *C. triciliatum* feeding on *M. aeruginosa* during a bloom of the cyanobacterium in a hypertrophic pond, by examining the ingested cells of *M. aeruginosa* in its food vacuoles, and determining the digestion rates of the flagellate feeding on the cyanobacterium in laboratory experiments. Grazing impacts by *C. triciliatum* on *M. aeruginosa* populations and importance of the cyanobacterium as food for the flagellate were also examined.

MATERIALS AND METHODS

Field investigation. Investigations were conducted in Furuike Pond which is located in Sancho, Matsuyama City, Ehime Prefecture, Japan. The pond has a surface area of ca. 7400 m^2, and its maximum depth was 1.5 m; it is hypertrophic due to anthropogenic loading from the watershed, and *Microcystis aeruginosa* forms heavy blooms from early summer to fall every year (Nakano et al. 1998, Manage et al. 1999, 2001). Physical and chemical characterizations of this pond are described in Nakano et al. (2001b).

Water samples were collected from the surface with a 15 l plastic bucket at the near-shore station (Nakano et al. 1998), twice a week from 24 August to 17 October 2000. All samples were taken at around the same time of day (09:30 to 10:30 h) and water temperature was measured simultaneously with a thermistor (TOA Electronics).

To determine chlorophyll *a* (chl *a*) concentration, samples were filtered through a 0.2 µm Nuclepore filter to retain seston. The filter was then transferred into a glass tube containing 6 ml of N,N-dimethylformamide to extract the chlorophyll and kept in a freezer at –20°C. The chl *a* concentration was determined by the fluorometric method (Moran & Porath 1980).

Immediately after collection, a 100 ml portion of water sample was fixed with acidified Lugol's solution, at a final concentration of 1%, and concentrated by natural sedimentation for enumeration of *Microcystis aeruginosa*. The concentrated sample was slightly sonicated (50 kw, 3 min) to disintegrate colonies of *M. aeruginosa*, and cells of the cyanobacterium were counted in a Burker-Turk type haemacytometer under a microscope at a magnification of 400× at least 3 times.

For enumeration of *Collodictyon triciliatum*, another 100 ml portion of water sample was fixed and concentrated as described above. *C. triciliatum* cells were counted in a Fuchs-Rosenthal type haemacytometer under a microscope at a magnification of 400× at least 3 times.

To examine the food vacuoles of *Collodictyon triciliatum*, a 100 ml water sample was immediately fixed with 4% buffered glutaraldehyde at a final concentration of 2%. The temperature of the fixative was adjusted to be the same as *in situ* water temperature. A preliminary experiment had shown that cell breakage due to fixation was minimal with fixation using 4% temperature-adjusted, buffered glutaraldehyde compared to use of 4% ice-cold, buffered glutaraldehyde (Sanders et al. 1989), 20% temperature-adjusted glutaraldehyde or 20% ice-cold glutaraldehyde. Each fixed sample was concentrated by natural sedimentation, a 0.05 ml aliquot of the concentrated sample containing *C. triciliatum* was mounted on a glass slide, 30 to 40 cells of *C. triciliatum* were observed under a microscope at a magnification of 400×, and *Microcystis aeruginosa* in their food vacuoles were counted. Average numbers of ingested *M. aeruginosa* cells per flagellate were calculated for each sample.

Digestion experiment. *Collodictyon triciliatum* were isolated from the surface water of Furuike Pond in late

June 2000. The strain was clonal but not axenic. Cultures were kept in sterile, filtered (0.2 µm Nuclepore filter) pond water with *Microcystis aeruginosa* NIES-298 (2.0 to 6.0 × 10^6 cells ml^{-1}) provided by the National Institute of Environmental Studies, Japan. All cultures were maintained in 30 ml borosilicate tubes at 25°C under a light intensity of 48.8 µE m^{-2} s^{-1} with a 12:12 h light:dark cycle.

We followed Dolan & Šimek (1998) to determine the digestion rates of *Collodictyon triciliatum* on *Microcystis aeruginosa*. A 6 ml portion of the flagellate culture in its early stationary phase was inoculated into a 50 ml polypropylene tube containing 12 ml of *M. aeruginosa* culture (4.0 × 10^6 cells ml^{-1}). The flagellate was allowed to ingest the cyanobacterium for 12 h at 25°C in the dark. To halt ingestion, the flagellate culture was diluted by adding 6 ml of the mixture to 600 ml of filtered pond water (GF-75 glass fiber filter, Advantec) in triplicate 1 l Erlenmeyer flasks. The triplicate, diluted mixtures were incubated at 25° in the dark. The incubation temperature was equal to the average water temperature (25°C) measured in the field investigation. Fifty milliliter subsamples were taken from each flask at 30 to 120 min intervals for 360 min, and fixed with 4% temperature-adjusted (25°C), buffered glutaraldehyde at a final concentration of 2%. Each fixed sample was concentrated by natural sedimentation, and a 0.05 ml aliquot of the concentrated sample containing *C. triciliatum* was mounted on a glass slide. One hundred cells of *C. triciliatum* were observed under an epifluorescence microscope at a magnification of 640× under green excitation, and *M. aeruginosa* in the food vacuoles of the flagellates were enumerated using chlorophyll autofluorescence.

The slope of the linear regressions of ln (% initial average numbers of ingested *M. aeruginosa* per flagellate) versus time yield the digestion rate, and multiplying the slope by 100 gives a digestion rate constant, with units of % cell contents min^{-1} (Dolan & Šimek 1998).

Estimation of grazing rate. To estimate *in situ* grazing rates of *Collodictyon triciliatum* on *Microcystis aeruginosa*, a steady state between ingestion and digestion (Dolan & Šimek 1999) of the flagellate was assumed, and the digestion rate at 25°C was corrected for *in situ* temperature using Q_{10} = 2.1 calculated from Mischke (1994). Estimates of grazing rate of *C. triciliatum* (G_c, *M. aeruginosa* cells flagellate^{-1} h^{-1}) were calculated by multiplying the corrected digestion rate by the average numbers of ingested *M. aeruginosa* cells per flagellate for each sampling day, respectively. Estimates of clearance rate of *C. triciliatum* (nl flagellate^{-1} h^{-1}) were calculated by dividing the grazing rate by the cell densities of *M. aeruginosa* for each sampling day. Hourly grazing impact on the standing stock of *M. aeruginosa* by *C. triciliatum* (G_i, % standing stock h^{-1}) was estimated as follows:

$$G_i = 100 \times (G_c \times N_c)/N_m$$

where N_c (cells ml^{-1}) and N_m (cells ml^{-1}) are the cell densities of *C. triciliatum* and *M. aeruginosa* for each sampling day, respectively.

RESULTS

Surface water temperature (Fig. 1A) gradually decreased from 24 August (29.6°C) to 17 October (20.8°C). Chl *a* concentrations (Fig. 1A) increased from 24 August (389.0 µg l^{-1}) to 1 September (581.2 µg l^{-1}), but decreased sharply from 5 (546.4 µg l^{-1}) to 8 September (213.2 µg l^{-1}). Concentrations of chl *a* fluctuated between 242.4 and 326.5 µg l^{-1} from 11 - September onwards.

Microcystis aeruginosa was the predominant phytoplankton species during the study period, and the pattern of changes in cell densities of the cyanobacterium was similar to that of the chl *a* concentrations (Fig. 1A,B). The cell densities of *M. aeruginosa* varied from 0.96 to 2.6 × 10^6 cells ml^{-1} during the study period.

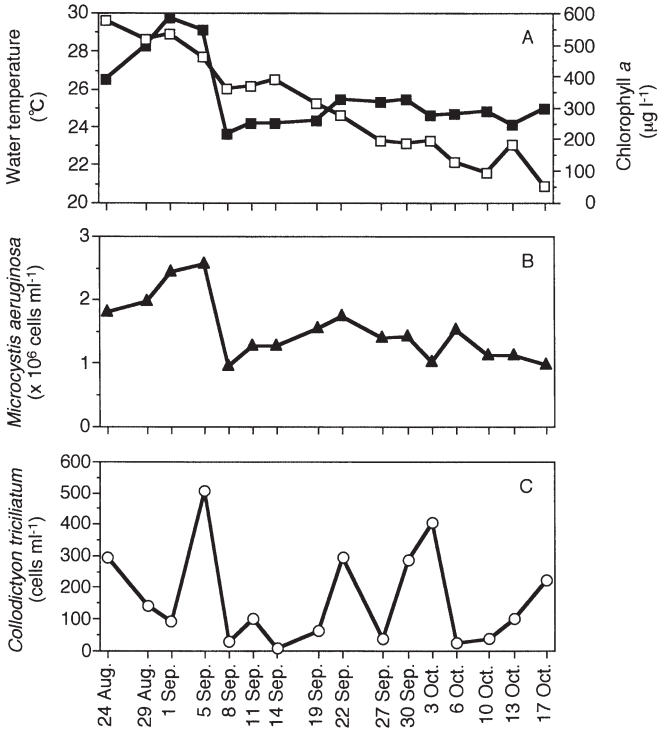

Fig. 1. Changes in (A) water temperature (□), chl *a* concentration (■), and cell densities of (B) *Microcystis aeruginosa* and (C) *Collodictyon triciliatum* in Furuike Pond during the study period

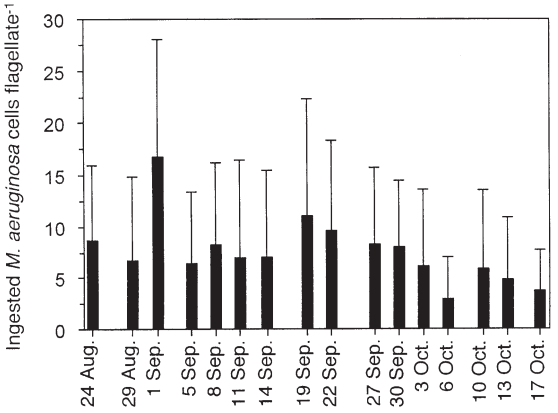

Fig. 2. Changes in average numbers of ingested *Microcystis aeruginosa* in the food vacuoles of *Collodictyon triciliatum* in Furuike Pond during the study period. Error bars indicate +SD

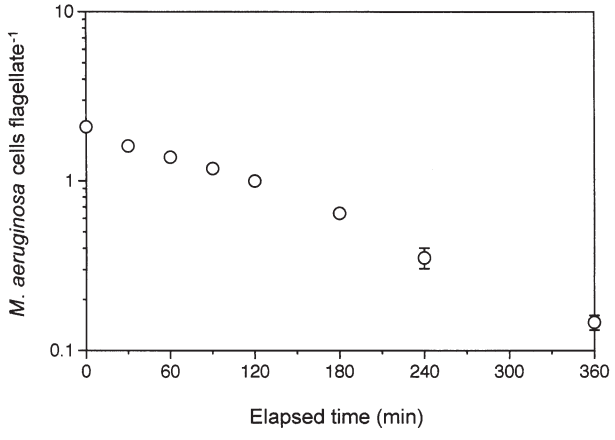

Fig. 3. Time-course of changes in average numbers of *Microcystis aeruginosa* in the food vacuoles of *Collodictyon triciliatum*. Error bars, which indicate ±SE, are shown when they exceed the size of the symbol

Cell densities of *Collodictyon triciliatum* fluctuated largely from 10 to 510 cells ml^{-1} and 3 sharp peaks of that were detected, on 5 and 22 September and from 30 September to 3 October (Fig. 1C). The abundance of *C. triciliatum* was not related to water temperature, chl *a* concentration and cell densities of *M. aeruginosa* (Fig. 1).

The percentage of *Collodictyon triciliatum* which ingested *Microcystis aeruginosa* varied from 56.7 to 97.1%. The contribution of *M. aeruginosa* cells to the total numbers of ingested prey cells in food vacuoles of the flagellate varied from 22.7 to 96.4%. The flagellate ingested not only single cells but also small colonies (2 to 51 cells) of *M. aeruginosa*. Average numbers of ingested *M. aeruginosa* cells by 1 flagellate ranged from 2.9 to 16.7 during the study period (Fig. 2), and

Table 1. Estimates of grazing and clearance rate of *Collodictyon triciliatum* feeding on *Microcystis aeruginosa*. Hourly grazing impacts of *C. triciliatum* on the standing stock of *M. aeruginosa* are also shown

	Range	Mean ± SD
Grazing rate (*M. aeruginosa* cells $flagellate^{-1}$ h^{-1})	1.1–9.9	3.6 ± 2.1
Clearance rate (nl $flagellate^{-1}$ h^{-1})	0.69–4.1	2.3 ± 0.9
Hourly grazing impact (% standing stock h^{-1})	0.002–0.09	0.04 ± 0.03

remained high until 19 September. They tended to decrease from 22 September onwards. Numbers of *M. aeruginosa* cells found in flagellate vacuoles appeared to be independent of *M. aeruginosa* concentration (Figs. 1B & 2).

In the digestion experiment, the average number of *Microcystis aeruginosa* cells per flagellate declined exponentially with time (Fig. 3), and a highly significant relationship was found between the ln (% initial average numbers of ingested *M. aeruginosa* per flagellate) and time ($r = 0.996$, $p < 0.0001$, $n = 8$). The calculated digestion rate constant of *Collodictyon triciliatum* feeding on *M. aeruginosa* was 0.74 ± 0.02% cell contents min^{-1} (mean ± SE).

Estimates of the grazing rate of *Collodictyon triciliatum* ranged between 1.1 and 9.9 *Microcystis aeruginosa* cells $flagellate^{-1}$ h^{-1} and those of clearance rates between 0.69 and 4.1 nl $flagellate^{-1}$ h^{-1} (Table 1). Hourly consumption of *C. triciliatum* on the *M. aeruginosa* population was estimated from 0.002 to 0.09% of the cyanobacterial standing stock.

DISCUSSION

There is still limited information concerning the digestion of flagellates feeding on cyanobacteria. Dolan & Šimek (1998) reported that the digestion rate constant of *Bodo saltans* feeding on *Synechococcus* at 22°C ranged between 0.9 and 1.1% cell contents min^{-1}. In the present study, the digestion rate constant of *Collodictyon triciliatum* feeding on *Microcystis aeruginosa* was 0.74 ± 0.02% cell contents min^{-1}, roughly agreeing with that of Dolan & Šimek (1998). Furthermore, ingested *M. aeruginosa* cells in *C. triciliatum* did show an exponential decline with time and the pattern is consistent with that of Dolan & Šimek (1998). Thus, we consider that the digestion rate constant obtained in the present study is plausible, although the prey and grazer organisms are different from those of Dolan & Šimek (1998).

We did not examine size-selectivity of *Collodictyon triciliatum* in the present study, but judging from the vacuole contents, the flagellate grazed not only unicellular *Microcystis aeruginosa* but also its colonial form that consisted of from 2 to 51 cells. Mischke (1994) and Klaveness (1995) also noted that this flagellate is capable of grazing on various sizes of phytoplankton, and thus, has a wide size range of edible prey.

The only comparative information available on grazing rates of flagellates feeding on *Microcystis aeruginosa* is from Cole & Wynne (1974). They reported that the grazing rate on *M. aeruginosa* by the chrysomonad *Ochromonas danica* in a laboratory system was on average 1.7 *M. aeruginosa* flagellate^{-1} h^{-1} at 23°C. Thus, estimates of grazing rates in the present study (Table 1) were at the same level as that of Cole & Wynne (1974). However, if we converted the grazing rates into volume-specific clearance rates using the cell volume of *O. danica* in Chrzanowski & Šimek (1990), the clearance rates of *Collodictyon triciliatum* and *O. danica* were respectively estimated as 0.5×10^3 and 1.3×10^4 h^{-1}. Thus, the latter is 26 times higher than the former. These results may be due to the difference in feeding mode of the flagellates: *C. triciliatum* captures its prey using a pseudopodium-like organelle (Mischke 1994), while chrysomonads use their flagella to generate enough water current to transport their prey (Fenchel 1986). *C. triciliatum* needs 10 to 30 min for complete uptake of prey due to its feeding mode (Mischke 1994).

To examine the importance of *Microcystis aeruginosa* as food for *Collodictyon triciliatum*, we estimated growth rates of the flagellate. We calculated the carbon content of the flagellate using the cell volume of 4557 µm^3 determined from our measurements of 165 flagellate cells and the volume-to-carbon conversion of 0.183 pg C µm^3 (Caron et al. 1995). The cell number of ingested *M. aeruginosa* was converted into carbon amount using a factor of 12.89 pg C cell^{-1} (Ichise et al. 1995). Thus, it is estimated that consumption of *M. aeruginosa* supplies carbon to the flagellate at a rate of 1.6 to 15.3% (mean ± SD, 5.5 ± 3.2) to its cellular carbon content per hour. Assuming that growth of the flagellate is based only on carbon ingested from *M. aeruginosa*, we can calculate a potential growth rate. The growth rates of the flagellate are estimated as 0.0047 to 0.045 h^{-1} (mean ± SD, 0.016 ± 0.009), by assuming a gross growth efficiency of 0.42 (Caron et al. 1986), which overlap those in previous laboratory studies where various algal species were used as prey for isolated *C. triciliatum* (Mischke 1994, Klaveness 1995). Hence, we suggest that consumption of *M. aeruginosa* supports a significant portion in carbon uptake of *C. triciliatum* in this pond.

In Furuike Pond, another important consumer of *Microcystis aeruginosa* is the rotifer *Brachionus calyciflorus*, which is usually abundant during summer and early fall. This rotifer has been shown by Jarvis et al. (1987) and Fulton & Paerl (1987) to effectively graze *M. aeruginosa* so we made a comparison between hourly grazing impact on *M. aeruginosa* by *Collodictyon triciliatum* and *B. calyciflorus*. To calculate the hourly grazing impact on *M. aeruginosa* by *B. calyciflorus*, we used the following information: the individual density of *B. calyciflorus* during August to October 1998 (35 to 382 ind. l^{-1}, Nishibe et al. unpubl. data), the cell densities of *M. aeruginosa* during the same period (0.48 to 18.9×10^6 cells ml^{-1}, Manage et al. 2001), and the clearance rate on *M. aeruginosa* by *B. calyciflorus* (5 µl ind. h^{-1}, Starkweather & Gilbert 1977). This information provided a grazing impact of 0.02 to 0.2% h^{-1} (mean ± SD, 0.09 ± 0.05), which was similar to that of *C. triciliatum* (Table 1). Thus, we can suggest that the grazing pressure on *M. aeruginosa* by the 2 grazers *C. triciliatum* and *B. calyciflorus* is of minor importance in Furuike Pond, though we do not have a simultaneous determination of grazing impact by the rotifer in the present study. Small ciliates, such as *Cyclidium glaucoma* and *Halteria grandinella*, are frequently abundant in the pond (Nakano et al 1998, 2001b), and are known to be efficient filter feeders of picoplankton (Šimek et al. 1996). Hence, one may think that these ciliates are potential grazers of *M. aeruginosa* in the pond. In the preliminary study where we examined which protists grazed *M. aeruginosa*, however, no cells of the cyanobacterium were found in food vacuoles of these ciliates and, thus, we think that grazing on the cyanobacterium by these ciliates is negligible in this pond. Manage et al. (1999, 2001) have suggested that cyanophages and algicidal bacteria cause the sudden collapse of *M. aeruginosa* blooms in Furuike Pond. Thus, not only grazing but also viral and bacterial attack should be examined to elucidate loss processes of *M. aeruginosa* populations.

We could not explain the changes in abundance of *Collodictyon triciliatum* in relation to water temperature, chl *a* concentration or abundance of *Microcystis aeruginosa*. The concentration of chl *a* was high, ranging between 213.2 and 581.2 µg l^{-1} in the present study, and is sufficient for food requirement of *C. triciliatum* (Mischke 1994). Hence, bottom up control may be of minor importance for changes in abundance of *C. triciliatum*, and we should also examine top down control. In this context it is appropriate that we examine predation on ciliates by metazooplankton since the size of this flagellate is similar in size to the small ciliates which are numerically dominant in the pond (Nakano et al. 1998). In Furuike Pond, predation by metazooplankton is, seasonally, one of the significant

loss processes of ciliates (Nakano et al. 2001b). Hence, the predation by metazooplankton may be responsible for control of *C. triciliatum* abundance.

We have demonstrated a tight food linkage between *Collodictyon triciliatum* and *Microcystis aeruginosa* in Furuike Pond, though the grazing impact of the flagellate may be of minor importance to the decline of the cyanobacterium abundance. It is also suggested that the cyanobacterium is one of the important food sources for the flagellate. Further quantitative studies are required to clarify the trophic interactions between *M. aeruginosa* and protists in natural environments.

Acknowledgements. We are grateful to the National Institute for Environmental Studies in Japan for kindly supplying us with the algal strain. We thank M. M. Pathmalal, M. Hirose, M. Ueki, M. Miyagaki, M. Yokosawa and other members of the Division of Aquatic Biology & Ecology, Center for Marine Environmental Studies, Ehime University, Japan, for their advice and support in the field sampling. Thanks are also due to Dr. Mary Morris for her correction of English. The Laboratory of Marine Biodiversity, Graduate School of Fisheries Sciences, Hokkaido University, also provided logistical and financial support of the data analysis and manuscript preparation, for which Y.N. is grateful.

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Editorial responsibility: Karel Šimek, České Budějovice, Czech Republic

Submitted: November 17, 2001; Accepted: July 8, 2002
Proofs received from author(s): August 27, 2002