

Diel changes in phagotrophy by *Cryptomonas* in Lake Biwa

Jotaro Urabe,¹ Tek Bahadur Gurung, Takehito Yoshida, Tatsuki Sekino, and Masami Nakanishi
Center for Ecological Research, Kyoto University, Kamitanakami-Hirano cho 509-3, Otsu, 520-2113 Japan

Masahiro Maruo and Eiichiro Nakayama

Environmental Science, University of Shiga Prefecture, 2500 Hassaka, Hikone, 522-8533 Japan

Abstract

Diel changes in bacterial ingestion by a mixotrophic flagellate, *Cryptomonas* sp., and heterotrophic nanoflagellates (HNF) were examined in situ at 4-h intervals for 2 d in the epilimnion and metalimnion of Lake Biwa using bacteria-sized fluorescent microspheres as a tracer food. Clearance rates of HNF for the microspheres ranged between 1.3 and 4.5 nl cell⁻¹ h⁻¹, but the average rate did not differ between day and night. In contrast, clear diel changes were observed in the clearance rate of *Cryptomonas* sp. in the epilimnion from <0.5 nl cell⁻¹ h⁻¹ at midnight to >3 nl cell⁻¹ h⁻¹ at noon. In the metalimnion where light intensity was lower, however, the clearance rate of *Cryptomonas* sp. was always <0.5 nl cell⁻¹ h⁻¹ through the study period. Thus, bacterial ingestion of *Cryptomonas* sp. is not to acquire supplementary energy or carbon at low phototrophic activities. During the study period, both inorganic phosphorus and nitrogen concentrations were less than or close to the detection limits (10 nM P and 1 μM N) in the epilimnion, but much higher in the metalimnion. The results strongly support the idea that *Cryptomonas* sp. utilizes N and P from bacteria as substitutable nutrients when photosynthesis takes place under conditions of nutrient depletion. To assess the grazing effect of mixotrophic algae on bacterial populations, it is essential to consider diel changes in their phagotrophic mode of nutrition that are induced by light regime and nutrient concentrations in ambient water.

Algal species able to ingest bacteria are one of the important components in planktonic communities. These algae are called mixotrophic because of their dual mode of nutrition, phototrophy, and phagotrophy. Because of ecological and evolutionary interests, a large number of studies have examined the function and rate of bacterial ingestion of mixotrophic algal species (e.g., Raven 1997; Isaksson 1998). Recently, Jones (1997) pointed out that examining the response of bacterial ingestion rate to light conditions would be the best way to understand why mixotrophic algae utilize bacteria as a resource. If the function of phagotrophy is directly or indirectly related to light conditions via photosynthesis, we could expect that mixotrophic algae change their bacterial ingestion rate with diurnal changes in light. However, no previous study has examined diel changes in the bacterial ingestion rate of mixotrophic algae in situ. Such knowledge is essential to assess strength in the trophic interaction between bacteria and their consumers (Weisse 1989).

Cryptomonas are common planktonic algae in freshwater and coastal habitats. It has been reported that they can ingest bacteria-sized particles (Sanders and Porter 1988; Tranvik et al. 1989; Roberts and Laybourn-Parry 1999) and are therefore mixotrophic algae. However, the function of phagotrophy in this genus has been unclear. Tranvik et al. (1989)

suggested that phagotrophy in *Cryptomonas* is induced by low light conditions but concluded that bacteria are not an important energy source for *Cryptomonas* because their specific ingestion rate for bacteria was very limited even under low light. In other studies, *Cryptomonas* did not show any indication of phagotrophy (Sanders et al. 1989; Gasol et al. 1993; Gervais 1997). It should be noted, however, that these studies examined phagotrophy under laboratory conditions, where light intensity was controlled artificially or in situ at an unspecific time during the day. *Cryptomonas* may alter their phagotrophic mode of nutrition according to a circadian rhythm induced by, for example, diurnal changes in light intensity. In addition, the function of phagotrophy may not be to acquire energy or carbon. There is considerable evidence that some mixotrophic algal species ingest bacteria to acquire nutrients and other essential substances (Kimura and Ishida 1986; Nygaard and Tobiesen 1993; Rothhaupt 1996; Urabe et al. 1999).

In the present study, we examined diel changes in bacterial ingestion rate of *Cryptomonas* sp. in Lake Biwa, the largest lake in Japan. This species is morphologically similar to *C. ovata*, but their cell size was 10–18 μm and much smaller than *C. ovata*. Therefore, we treated this species as *Cryptomonas* sp. The bacterial ingestion rate was estimated in situ at 4-h intervals for 2 d at two different depths using bacteria-sized fluorescent beads as tracers. The results provide some evidence that *Cryptomonas* sp. change their phagotrophic mode of nutrition diurnally and that the function of bacterial ingestion is related to light and nutrient regimes in ambient water.

Materials and methods

During the stagnant period in Lake Biwa, vertical profiles of water temperature, and thus the thickness of the surface

¹ Corresponding author.

Acknowledgments

We thank T. Koitabashi, T. Ueda, and T. Ishikawa for their field assistance. We also thank T. Kimoto for his valuable suggestions. Comments by W. Vincent, S. Flöder, and anonymous reviewers improved the manuscript.

This study was supported by Grants-in-Aid for Scientific Research (B) 10440234 to J.U. and (A) 10308025 to N.M.

mixing layer, are changed in a 40–48-h cycle by an internal wave (Kanari 1975; Hayami et al. 1996). This study was performed as a part of the research program that examined the effects of short-term changes in the vertical profile of water temperature on chemical and biological processes in this lake.

Sampling and experiments were conducted at 4-h intervals for 2 d in July 1998 at a pelagic site (53 m deep) in the north basin of Lake Biwa. Lake water for feeding experiments was collected at two depths: one in the epilimnion and the other in the metalimnion using a 10-L modified Van Dorn sampler. Before each sampling, vertical profiles of water temperature and light intensity were measured by a CTD profiler (SBE-25; SeaBird Electronics) equipped with an underwater quantum meter (QSP-200L, Biospherical Instruments). The sampling depth in the epilimnion was fixed at 2.5 m. Because the thermocline moved vertically during the study period, we changed the sampling depth in the metalimnion according to vertical profiles of water temperature. At each depth, 100 ml of lake water was fixed with glutaraldehyde (2% final concentration [conc.]) and stored at 4°C in the dark. The sample was used to estimate concentrations of bacteria, nonpigmented heterotrophic nanoflagellates (HNF), and *Cryptomonas* sp. The other 100-ml sample was frozen (–20°C) and used to analyze nutrient concentrations after filtration through 0.2- μm Nuclepore filters.

Microscopic analysis was made within a week of the sampling. Bacteria in the lake water were estimated using the acridine orange direct count method (Hobbie et al. 1977). A 0.5- to 1-ml aliquot of the sample was filtered onto 0.2- μm pore size, black Nuclepore filters, and bacteria were enumerated using an Olympus epifluorescence microscope ($\times 1,250$) equipped with a standard B-excitation system. *Cryptomonas* sp. and HNF were also enumerated by filtering 20 to 30 ml of the sample onto 0.8- μm pore size, black Nuclepore filters and staining them for 30 s with fluorescein isothiocyanate (0.004%, dissolved in sodium phosphate buffer of pH 7.2) according to Sherr and Sherr (1983).

Chemical analysis was made within a month after the sampling. Soluble reactive phosphorous (SRP) and nitrite-nitrogen were analyzed with a two-channel continuous-flow system (AACS II, Bran+Luebbe Co.). The detection limit of this automated wet chemistry was 10 nM both for SRP and nitrite-nitrogen. Nitrate-nitrogen was analyzed by an ion chromatography system (DX-AQ1111, DIONEX Corp.) equipped with a DIONEX IonPac AS-14A anion exchange column and an electrochemical autosuppressor. Ammonium-nitrogen was analyzed on a continuous-flow system (MCF-1000 high-sensitivity ammonia analyzer, KIMOTO Electric Co.), which measures fluorescent products from a ternary reaction of ammonia with *O*-phthalaldehyde and sulfite ion in phosphate buffer solution (pH 11.0) at 85°C (Maruo et al. 1996). The detection limits of nitrate- and ammonium-nitrogen in these methods were 0.6 μM and 2 nM, respectively. The sum of nitrate-, nitrite-, and ammonium-nitrogen was expressed as dissolved inorganic nitrogen (DIN).

Feeding experiments were carried out in situ using 0.5- μm -diameter fluorescent microspheres (Fluoresbrite YG Microspheres, Polyscience, Inc.) as a tracer food, because the size of these microspheres was comparable to that of bacteria

in Lake Biwa (Nagata 1986). To initiate the feeding experiment, three 165-ml polycarbonate bottles were filled with lake water immediately after the sampling, and microspheres were added to each bottle to a final concentration of 4.02×10^5 spheres ml^{-1} . Soon after, the bottles were gently shaken, and an aliquot was sampled from one bottle for time = 0 sampling and fixed with ice-cold glutaraldehyde (2% final conc.) as in Sanders et al. (1989). The remaining two bottles were incubated for 15 min at the depth where lake water was collected. After withdrawing these bottles, an aliquot was sampled from the bottles and fixed with ice-cold glutaraldehyde as above.

To estimate clearance rate for microspheres, 20 to 30 ml from the experimental bottles was filtered onto 0.8- μm pore size, black Nuclepore filters and stained with fluorescein isothiocyanate as above. At least 100 cells filter^{-1} were counted for both *Cryptomonas* sp. and HNF with an Olympus epifluorescence microscope ($\times 1,250$). We also counted fluorescent microspheres that completely overlapped with these cells in the microscope image as ingested particles. The specific clearance rate (CR) for microspheres ($\text{nl cell}^{-1} \text{h}^{-1}$) was calculated according to Urabe et al. (1999) as

$$\text{CR} = (N_f - N_i)/M \times 60/t$$

where N_f and N_i are the number of fluorescent microspheres overlapped with a flagellate cell (*Cryptomonas* sp. or HNF) in final and initial samples, respectively (time = 0); M is the concentration of fluorescent microspheres in the feeding suspension (spheres nl^{-1}); and t is the feeding time. Bacterial ingestion rate was calculated from CR and bacterial abundance, assuming that the bacterial cell and the fluorescent microspheres were grazed at the same rate. Grazing rates of HNF and *Cryptomonas* populations were calculated by multiplying the specific clearance rate by population density.

Results

At the experimental depth in the epilimnion (2.5 m), water temperature was stable at 26.5°C throughout the study period. Because the thermocline moved vertically through the study period and because we could not exactly catch the isothermal depth, water temperature at the experimental depth in the metalimnion was not constant but ranged from 15.1° to 19.8°C (Fig. 1). Hereafter, we will denote the experimental depths in the epilimnion and the metalimnion as shallow depth and deep depth, respectively.

During the study period, sunset was at 1815 h and sunrise was at 0535 h. Underwater light intensity reached its maximum at noon every day because of fine weather conditions. The maximum light intensity exceeded 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ at the shallow depth but was below 20 $\mu\text{E m}^{-2} \text{s}^{-1}$ at the deep depth (Fig. 1). At the shallow depth, both SRP and DIN concentrations were close to or below the detection limits, indicating that this depth was under nutrient-depleted conditions. At the deep depth, SRP was sometimes >30 nM but close to the detection limit in the majority of the cases. DIN at this depth also varied widely from 0.6 to 21 μM . On average, however, these nutrient concentrations were much higher at the deep than at the shallow depth.

Bacterial abundance was within a range between 4.4 and

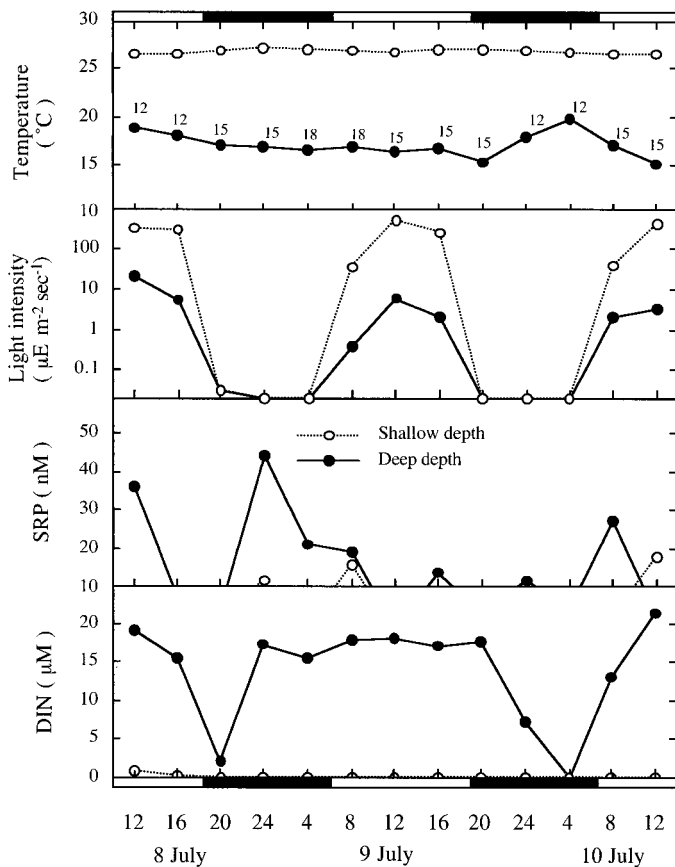


Fig. 1. Diurnal changes in temperature, light intensity, soluble reactive phosphorus (SRP), and dissolved inorganic nitrogen (DIN: ammonium + nitrite + nitrate) at the shallow and deep depths. The shallow depth was fixed at 2.5 m, but the deep depth changed temporally within the metalimnion and is indicated by the numbers on the top panel.

6.5×10^6 cells ml⁻¹ at the shallow depth (Fig. 2). When we divided the data into day (0800–1600 h) and night (2000–0400 h), the mean abundances were significantly higher in the night than in the day at the shallow depth ($t = 9.45$, $P < 0.001$). At the deep depth, the bacterial concentration was on average 35% lower than that at the shallow depth and did not differ between day and night. Concentrations of HNF ranged from 1.5 to 4.1×10^3 cells ml⁻¹ at the shallow depth and from 0.3 to 4.6×10^3 cells ml⁻¹ at the deep depth. However at both depths, no significant difference was detected in their concentration between day and night.

Concentrations of *Cryptomonas* sp. also changed temporally from 250 to 1,100 cells ml⁻¹ for 2 d, and the mean concentration throughout the study period was similar at the two depths (Fig. 2). At the deep depth, however, the concentration of *Cryptomonas* changed more drastically and increased at nighttime. Indeed, the mean concentration of this species was significantly higher at night than in day at the deep depth ($t = 2.99$, $P < 0.01$), whereas such a difference was not detected at the shallow depth.

Specific clearance rates of HNF for microspheres changed from 1.3 to 4.5 nl cell⁻¹ h⁻¹ for 2 d (Fig. 3). Temporal changes in the clearance rate were similar between the two depths

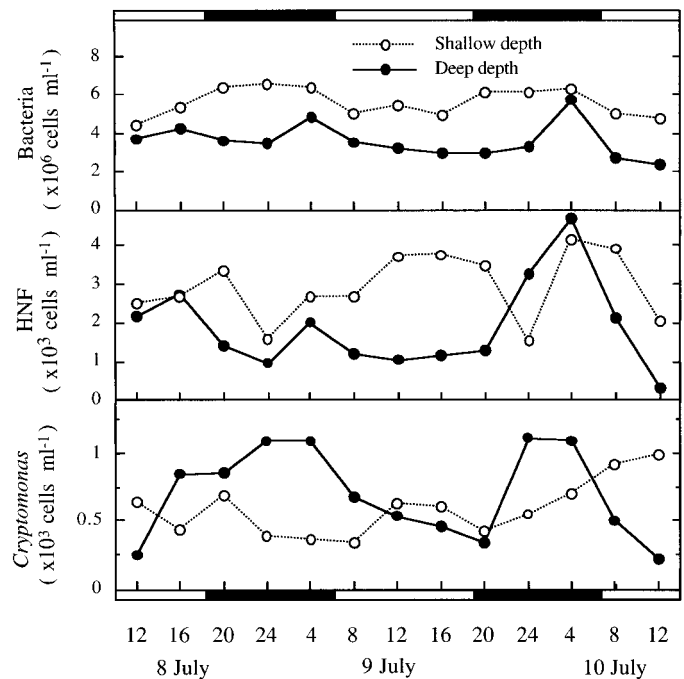


Fig. 2. Diurnal changes in concentrations of bacteria, heterotrophic nanoflagellates (HNF), and *Cryptomonas* sp. at the shallow and deep depths.

and tended to decrease at night, although the rate did not differ significantly between day and night. More drastic temporal changes in the specific clearance rates were found in *Cryptomonas* (Fig. 3). Their clearance rate was lower than 0.5 nl cell⁻¹ h⁻¹ at midnight but reached more than 3 nl cell⁻¹ h⁻¹ at noon at the shallow depth. On average, the specific clearance rate differed significantly between day and night ($t = 6.38$, $P < 0.001$). In contrast, the clearance rate of *Cryptomonas* sp. was, at most, 0.5 nl cell⁻¹ h⁻¹ at the deep depth regardless of changes in light intensity. The temporal pattern of the bacterial ingestion rate was almost the same as that of the clearance rate because bacterial abundance was less varied compared with the clearance rate. In HNF, the specific ingestion rate was 8.6–26.7 bacteria cell⁻¹ h⁻¹ at the shallow depth and 5.5–17.5 bacteria cell⁻¹ h⁻¹ at the deep depth. In *Cryptomonas* sp., it varied from 0.59 at midnight to 22.7 bacteria cell⁻¹ h⁻¹ at noon at the shallow depth but was less than 2.9 bacteria cell⁻¹ h⁻¹ at the deep depth.

Because of large changes in concentration and specific clearance rate, the community grazing rate (HNF plus *Cryptomonas* sp. populations) decreased at midnight in comparison with that at daytime (Fig. 4). The temporal changes in this community grazing rate reflected mainly the activity of HNF because of their numerical dominance. Although *Cryptomonas* sp. contributed less than 30% of the grazing rate, they substantially increased the community grazing rate during the day at the shallow depth. In contrast, *Cryptomonas* did not contribute to temporal changes in the grazing rate at the deep depth because of their low specific clearance rate.

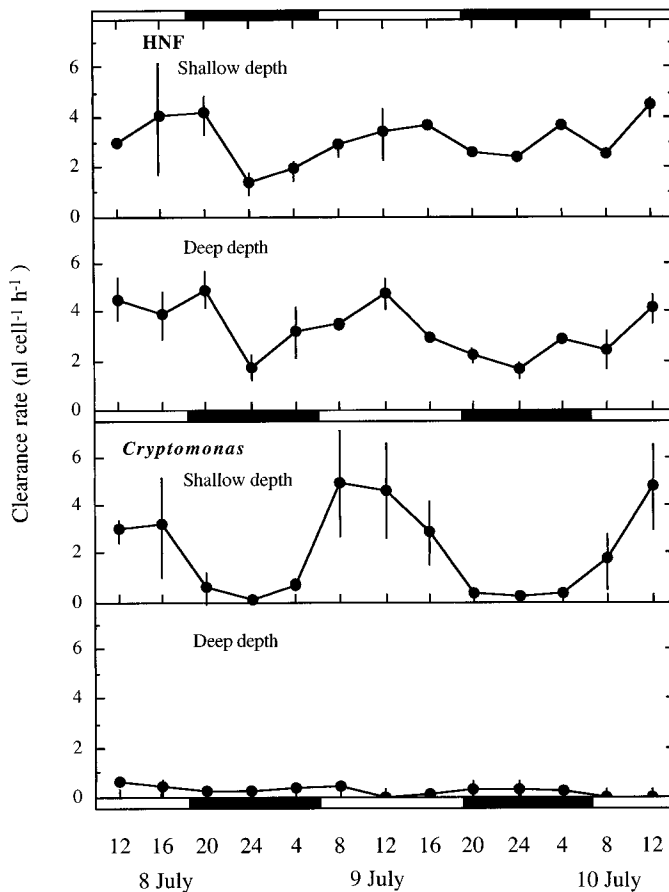


Fig. 3. Diel changes in specific clearance rate of heterotrophic nanoflagellates (HNF) and *Cryptomonas* sp. at the shallow and deep depths. Vertical bars are the range of values measured.

Discussion

The present study clearly showed that *Cryptomonas* sp. diurnally changed the clearance rate for bacteria-sized particles at the epilimnion in Lake Biwa. Ingestion of bacteria-sized particles by cryptophyte species has been reported in several studies (Sanders and Porter 1988; Tranvik et al. 1989; Roberts and Laybourn-Parry 1999). In these studies, cryptophytes ingested 0.3–4 bacteria cell⁻¹ h⁻¹. Ingestion rate of *Cryptomonas* sp. in Lake Biwa, however, reached 22.7 bacteria cell⁻¹ h⁻¹. This maximum ingestion rate may still underestimate the in situ rate, because phagotrophic flagellates are believed to prefer bacteria to fluorescent microspheres (e.g., Sherr et al. 1987). Thus, cryptophytes can ingest bacteria at a higher rate than expected in the previous studies. To our knowledge, the present study provides the first evidence that mixotrophic cryptophytes have a daily cycle in their phagotrophic mode of nutrition. Stoecker et al. (1997) also showed that *Prorocentrum minimum*, a mixotrophic dinoflagellate, have a daily pattern in their particle ingestion rate. Diurnal changes in feeding activities, therefore, seem not to be limited in mixotrophic cryptophytes alone.

In the daytime, *Cryptomonas* sp. increased their specific clearance rate at the shallow depth, but not at the deep depth

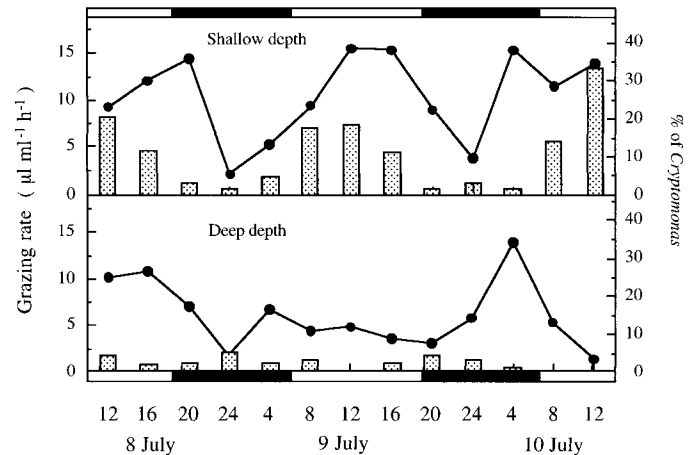


Fig. 4. Temporal changes in community grazing rate (HNF + *Cryptomonas* sp., solid line) and percent contribution of *Cryptomonas* sp. (vertical bar) at the shallow and deep depths.

where light intensity was lower. This result suggests that its phagotrophic mode of nutrition is in some way linked with the light condition. Jones (1994) pointed out that mixotrophy is not a single strategy but should be viewed as an array of strategies because its function differs and is species specific, depending on the environmental condition. Jones (1997) recently divided mixotrophic protists into four categories based on the response of the phagotrophic mode to the light condition: Group A, protists that rely mainly on phagotrophy but utilize the phototrophic mode when prey density is limited; Group B, protists that rely mainly on phototrophy but utilize bacteria to supplement energy (carbon) at low light; Group C, protists that gain energy by the phototrophic mode but ingest bacteria to acquire essential nutrients or substances for growth; and Group D, protists that rely on the phototrophic mode but utilize the phagotrophic mode to survive under prolonged dark conditions. According to these definitions, we would expect that protists in Group D as well as in Group B increase bacterivory at night or in deeper layers and those in Group A keep their bacterial ingestion rate constant under low light conditions. The present results do not match with these expectations. Thus, *Cryptomonas* sp. in Lake Biwa seem to belong to Group C. This inference is supported by Tranvik et al. (1989), who showed that although *Cryptomonas* could ingest bacteria, the energetic gain by the phagotrophy was very limited.

What nutritional substance motivates *Cryptomonas* to ingest bacteria? Some chrysophyte species are known to rely on bacteria for the acquisition of organic substances, such as phospholipids and vitamins, that they cannot synthesize by themselves (Kimura and Ishida 1986; Sanders and Porter 1988; Isaksson 1998). If *Cryptomonas* have to acquire some "essential" substances from bacteria, it is likely that they have to ingest bacteria to some extent regardless of depth. In Lake Biwa, however, the clearance rate of *Cryptomonas* sp. was very limited at the deep depth. Gasol et al. (1993) showed that *C. phaseolus* maintained a high population density in the metalimnion without any sign of bacterial ingestion. Gervais (1997) also showed that several species of *Cryptomonas* could grow without bacterial ingestion in lab-

oratory cultures. Thus, phagotrophy by *Cryptomonas* seems not for acquisition of some substance essential to their growth.

It should be noted that not only the light intensity but also the nutrient conditions in the ambient water differed greatly between the shallow and deep depth in Lake Biwa. At the shallow depth, both DIN and SRP were severely depleted. Under such a condition, it is advantageous for mixotrophic algae to utilize bacteria as a substitutable N and P source (Nygaard and Tobiesen 1993; Rothhaupt 1996; Urabe et al. 1999). If this is true, we can expect mixotrophic algae to decrease their bacterial ingestion rate when the concentrations of dissolved nutrients are high in the ambient water. In accordance with this expectation, the specific clearance rate of *Cryptomonas* sp. was very low regardless of the light condition at the deep depth where DIN concentration was much higher and a pulse of high SRP concentration was sometimes found. Tranvik et al. (1989) showed that the specific clearance rate of *Cryptomonas* for bacteria was less than $0.6 \text{ nl cell}^{-1} \text{ h}^{-1}$ at 1 and 4 m deep in Barber Pond, which was the same level as the clearance rate at the deep depth in the present study. Although they did not mention the ambient nutrient concentrations, Tranvik et al. (1989) may have measured the clearance rate at relatively high nutrient levels.

A fundamental question arising from the present study is why *Cryptomonas* sp. at 2.5 m deep ceased bacterial ingestion at night. One may suspect that nutrient supply rates from, for example, zooplankton increase at night. We cannot reject this possibility because we have no data on the diurnal changes in the nutrient supply rate. During the study period, however, no increase in zooplankton biomass at night was observed at 2.5 m deep. Recently, Li et al. (1999) found that the mixotrophic dinoflagellate *Gyrodinium galatheanum* decreased its particle ingestion rate with decreasing light intensity. Similarly, it is known that some mixotrophic chrysophyte species require light for sustaining their high bacterial ingestion rate (Caron et al. 1993; Keller et al. 1994). Thus, the decrease in phagotrophic rate under low light conditions is not limited to *Cryptomonas* sp. Li et al. (1999) speculated that the light-stimulated phagotrophy reflects a dependency on photosynthesis to meet energy requirement associated with phagotrophy. *Cryptomonas* sp. may ingest bacteria to balance their stoichiometry for growth. If the energetic cost of the phagotrophic mode is high and if significant amounts of N and P, but not C, can be gained from bacteria, it is likely that *Cryptomonas* sp. cease to ingest bacteria at night when there is no C gain from photosynthesis.

Another possible reason for the decrease in the bacterial ingestion rate at night is that the physiological state of the *Cryptomonas* cells may differ between day and night. Gasol et al. (1993) observed that the ratio of polyglucose to protein in *Cryptomonas phaseolus* decreased at night because this alga consumed starch that was accumulated during the day. The result implies that the physiological condition of *C. phaseolus* is different between day and night. The accumulation of carbohydrate during the day and synthesis of protein at night are frequently described for algae (e.g., Cuhel et al. 1984; Miyazaki et al. 1987). *Cryptomonas* sp. in Lake Biwa may use the day to assimilate carbon with nutrients

and use the night to catabolize biochemicals for cell division by ceasing assimilation (bacterial ingestion). Several studies showed that HNF decreased feeding activity at night in marine environments (Fuhrman et al. 1985; Weisse 1989). This diurnal change in feeding rate of HNF is believed to relate to cell division cycles (Weisse 1989).

In the present study, however, no significant difference was detected in the clearance rate of HNF between day and night, although the rate tended to decrease at night. It should be noted that HNF in the present study was a mixture of species. The absence of clear diurnal changes in the clearance rate of HNF may, therefore, merely reflect the lack of synchronization in the diurnal feeding rhythms among HNF species. Unlike *Cryptomonas* sp., the clearance rate of HNF at the deep depth was as high as that at the shallow depth. This result can be easily explained by the fact that nonpigmented nanoflagellates have to ingest particulate food to gain energy and carbon even if nutrient concentration is high in the ambient water.

Finally, we would like to point out that the bacterial grazing rate of flagellates varies largely within a day because of temporal changes in their density and specific clearance rate. Indeed, community grazing rates for bacteria decreased to a low level at midnight. Relatively high bacterial densities at the shallow depth in the night might be due to the decrease in the community grazing rate. If this is true, we should consider diurnal changes in the feeding activities of flagellates to assess their effect on bacterial populations.

It is apparent that clearance rates of mixotrophic algae for bacteria change diurnally. Diurnal patterns of bacterial ingestion probably differ among species for whom the function of phagotrophy is different. Depending on the function and diurnal rhythm of phagotrophy, the strength of trophic interaction between bacteria and mixotrophic species would change within a day according to light and nutrient regimes. To assess the ecological importance of mixotrophic algae in aquatic communities, therefore, it is essential to examine their heterotrophic mode of nutrition at a diurnal time scale.

References

- CARON, D. A., R. W. SANDERS, E. L. LIM, C. MARRASÉ, L. A. AMARAL, S. WHITNEY, R. B. AOKI, AND K. G. PORTER. 1993. Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. *Microb. Ecol.* **25**: 93–111.
- CUHEL, R. L., P. B. ORTNER, AND D. R. S. LEAN. 1984. Night synthesis of protein by algae. *Limnol. Oceanogr.* **29**: 731–744.
- FUHRMAN, J. A., R. W. EPPLEY, A. HAGSTROM, AND F. AZAM. 1985. Diel variations in bacterioplankton, phytoplankton, and related parameters in the Southern California Bight. *Mar. Ecol. Prog. Ser.* **27**: 9–20.
- GASOL, J. M., J. GARCIA-CANTIZANO, R. MASSANA, R. GUERRERO, AND R. PEDROS-ALIO. 1993. Physiological ecology of a metalimnetic *Cryptomonas* population: relationship to light, sulfide and nutrients. *J. Plankton Res.* **15**: 255–275.
- GERVAIS, F. 1997. Light-dependent growth, dark survival, and glucose uptake by Cryptophytes isolated from a freshwater chemocline. *J. Phycol.* **33**: 18–25.
- HAYAMI, Y., T. AOKI, T. FUJIWARA, H. MUKAI, AND Y. TANAKA. 1996. Internal surge transporting nutrients and sediments in the north basin of Lake Biwa. *Jpn. J. Limnol.* **57**: 39–48.

- HOBBIE, J. E., R. D. DALEY, AND S. JASPER. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33**: 1225–1228.
- ISAKSSON, A. 1998. Phagotrophic phytoflagellates in lakes: A literature review. *Arch. Hydrobiol. Spec. Issues Adv. Limnol.* **51**: 63–90.
- JONES, H. L. J. 1997. A classification of mixotrophic protists based on their behavior. *Freshw. Biol.* **37**: 35–43.
- JONES, R. I. 1994. Mixotrophy in planktonic protists as a spectrum of nutritional strategies. *Mar. Microb. Food Webs* **8**: 87–96.
- KANARI, S. 1975. The long-period internal wave in Lake Biwa. *Limnol. Oceanogr.* **20**: 544–553.
- KELLER, M. D., L. P. SHAPIRO, E. M. HAUGEN, T. L. CUCCI, E. B. SHERR, AND B. F. SHERR. 1994. Phagotrophy of fluorescently labeled bacteria by an oceanic phytoplankter. *Microb. Ecol.* **28**: 39–52.
- KIMURA, B., AND Y. ISHIDA. 1986. Possible phagotrophic feeding of bacteria in a freshwater red tide Chrysophyceae, *Uroglena americana*. *Bull. Jpn. Soc. Sci. Fish.* **52**: 697–701.
- LI, AISHAO, D. K. STOECKER, AND J. E. ADOLF. 1999. Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. *Aquat. Microb. Ecol.* **19**: 163–176.
- MARUO, M., N. NAKAYAMA, H. OBATA, AND T. KIMOTO. 1996. Analytical methods and instrumentation, p. 223. *In* H. M. Widmer [ed.], 2nd Symposium on Micro Total Analysis Systems, special issue, v. 96. AMI Editorial Office, Basel.
- MIYAZAKI, T., H. SUYAMA, AND H. UOTANI. 1987. Diel changes of uptake of inorganic carbon and nitrogen by phytoplankton, and the relationship between inorganic carbon and nitrogen uptake in Lake Nakanuma, Japan. *J. Plankton Res.* **9**: 513–524.
- NAGATA, T. 1986. Carbon and nitrogen content of natural planktonic bacteria. *Appl. Environ. Microbiol.* **52**: 28–32.
- NYGAARD, K., AND A. TOBIESEN. 1993. Bacterivory in algae: A survival strategy during nutrient limitation. *Limnol. Oceanogr.* **38**: 273–279.
- RAVEN, J. A. 1997. Phagotrophy in phototrophs. *Limnol. Oceanogr.* **42**: 198–205.
- ROBERTS, E. C., AND J. LAYBOURN-PARRY. 1999. Mixotrophic cryptophytes and their predators in the Dry Valley lakes of Antarctica. *Freshw. Biol.* **41**: 737–746.
- ROTHHAUPT, K. O. 1996. Utilization of substitutable carbon and phosphorus sources by the mixotrophic chrysophyte *Ochromonas* sp. *Ecology* **77**: 706–715.
- SANDERS, W. R., AND K. G. PORTER. 1988. Phagotrophic phytoflagellates, p. 167–192. *In* K. C. Marshall [ed.], *Advanced in microbial ecology*, v. 10. Plenum.
- , ———, S. J. BENNETT, AND A. E. DEBIASE. 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. *Limnol. Oceanogr.* **34**: 673–687.
- SHERR, B. F., E. B. SHERR, AND R. D. FALLON. 1987. Use of Monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory. *Appl. Environ. Microbiol.* **53**: 958–965.
- SHERR, E. B., AND B. F. SHERR. 1983. Double-staining epifluorescence technique to assess frequency of dividing cells and bacterivory in natural populations of heterotrophic microprotozoa. *Appl. Environ. Microbiol.* **46**: 1388–1393.
- STOECKER, D. K., A. LI, D. W. COATS, D. E. GUSTAFSON, AND M. K. NANNEN. 1997. Mixotrophy in the dinoflagellate *Prorocentrum minimum*. *Mar. Ecol. Prog. Ser.* **152**: 1–12.
- TRANVIK, L. J., K. G. PORTER, AND J. M. SEIBURTH. 1989. Occurrence of bacterivory in *Cryptomonas*, a common freshwater phytoplankter. *Oecologia* **78**: 473–476.
- URABE, J., T. B. GURUNG, AND T. YOSHIDA. 1999. Effects of phosphorus supply on phagotrophy by the mixotrophic alga *Uroglena americana* (Chrysophyceae). *Aquat. Microb. Ecol.* **18**: 77–83.
- WEISSE, T. 1989. The microbial loop in the Red Sea: Dynamics of pelagic bacteria and heterotrophic nanoflagellates. *Mar. Ecol. Prog. Ser.* **55**: 241–250.

Received: 28 March 2000

Accepted: 4 July 2000

Amended: 11 July 2000