Role of *Daphnia* in the decomposition of organic matter in the surface layer of Lake Biwa

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Abstract
To examine the role of *Daphnia* in the decomposition and elimination of organic carbon at the surface layer, organic matter from Lake Biwa was incubated with and without *Daphnia* at 20°C in the dark for 15 days in May, July and October. In all of the experiments, total organic carbon decreased during the incubation, regardless of treatment; however, the net effect of *Daphnia* on this decrease differed among experiments. *Daphnia* stimulated the decrease in particulate organic carbon in two out of three experiments. Bacterial abundance was higher in treatments with *Daphnia* than in treatments without *Daphnia* in all experiments. Higher bacterial abundance in treatments with *Daphnia* could not be explained fully by zooplankton grazing effects on phagotrophic protozoans, rather *Daphnia* seemed to stimulate bacterial growth by supplying substrates for that growth. The results of the present study suggest that *Daphnia* reduces sinking flux, not only by direct grazing, but also by the channelling of particulate organic carbon into dissolved forms that are then available for bacterial growth.

Key words
bacteria, *Daphnia*, decomposition, heterotrophic nanoflagellates, organic matter, sinking flux.

INTRODUCTION
In thermally stratified lakes, increases in the sinking flux of organic matter promote anoxia at the lake bottom by increasing the consumption of oxygen. Under such conditions, benthic habitats deteriorate and the release of nutrients from the bottom sediment is accelerated (Horne & Goldman 1994). If these nutrients are diffused to the surface euphotic layer, the trophic state of the lake is likely to be altered dramatically (Carpenter *et al*. 1999). Thus, the elimination of organic matter at the surface layer by decomposition and grazing is crucial for the ecosystem health of lakes, because this reduces the flux of organic matter discharged to the lake bottom.

In the north basin of Lake Biwa, primary production is nearly balanced with the community respiration rate, and more than 80% of primary production is eliminated above the thermocline (Nakanishi *et al*. 1992; Takahashi *et al*. 1995). Recently, Yoshimizu *et al*. (2001) showed that zooplankton play a significant role in the elimination of suspended particles within the epilimnion during the stagnant period. However, details are not clear regarding the role of zooplankton in the elimination of organic matter in Lake Biwa. It is probable that zooplankton eliminate organic matter in a variety of ways. First, they are known to eliminate particulate organic matter directly by grazing on suspended particles, including phytoplankton. Second, they affect the decomposition rate of organic matter indirectly by changing bacterial abundance and activity through grazing and nutrient recycling. In Lake Biwa, some zooplankton species graze directly on bacteria, but others increase the bacterial abundance by reducing the numbers of bacterial grazers and releasing limiting nutrients (Nakano *et al*. 1998; Gurung *et al*. 2000, 2001). Thus, zooplankton can have both positive and negative effects on the decomposition rate of organic matter by bacteria. In Lake Biwa, *Daphnia* is one of the dominant zooplankton (Yoshimizu *et al*. 2001). In the present study, laboratory experiments were performed using *Daphnia* and organic matter from Lake Biwa to clarify the role of *Daphnia* in the elimination of organic matter from the epilimnion of Lake Biwa.

MATERIALS AND METHODS
Experiments were carried out on 17 May and 28 October 1999 and 19 July 2000. For each experiment, lake water was sampled from a depth of 10 m using a 10 L modified Van Dorn water sampler at a pelagic station (off Wani, which

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was approximately 53 m deep) in the north basin of Lake Biwa. After passing through a 150 μm mesh net to remove zooplankton, lake water was brought to a laboratory within 2 h and poured into six acid-washed polycarbonate bottles (6.5 L capacity). To half of these bottles, *Daphnia* were added (*Daphnia* treatment) and the remaining bottles were used as controls. The daphnids were originally collected from Lake Biwa and cultured in a laboratory. The initial density of *Daphnia* was six individuals/L in experiments conducted in May and October 1999, and 10 individuals/L in July 2000. All bottles were incubated at 20°C under dark conditions for 15 days and shaken by hand once per day. At days 0 (initial), 1, 2, 3, 5, 7 (or 8), 10 (or 11) and 15, samples (550 mL each) were withdrawn. For enumeration of bacteria and heterotrophic nanoflagellates (HNF), 50 mL samples were fixed with 1% glutaraldehyde (final concentration) and stored at 4°C. To determine the concentration of particulate organic carbon (POC), suspended particles in the experimental water were concentrated onto the precombusted (450°C, 2 h) Whatman GF/F glass-fibre filters (Maidstone, UK). Filtrates of experimental water were used to determine the concentrations of nutrients and dissolved organic carbon (DOC).

The number of bacteria was determined by the acridine orange direct count method (Hobbie et al. 1977): two subsamples were taken by filtering 1.0 mL of the sample suspension onto 0.2 μm pore size black Nuclepore filters (Corning, Corning, USA). Bacteria were enumerated using an epifluorescence microscope (1250×) with a standard B-excitation system (100 W mercury lamp, BP420-480; Olympus, Tokyo, Japan). Epifluorescence microscopic counts for HNF were also made with fluorescein isothiocyanate (FITC) according to the methods of Sherr and Sherr (1983). An aliquot of 15–40 mL was filtered onto a 0.8 μm pore size black Nuclepore filter and stained with FITC, and HNF cells were counted. The POC concentration was determined with a CHN analyser (PE–2400II; Perkin Elmer, Shelton, USA). The DOC concentrations were determined with a total organic carbon analyser (TOC–5000A; Shimadzu, Kyoto, Japan) after being acidified with HCl and purged with CO₂-free air for 10 min to remove inorganic carbon. Ammonium nitrogen and dissolved inorganic phosphorus (DIP) were determined by the indophenol method (Sagi 1966) and the ascorbic-molydbdate method (Murphy & Riley 1962), respectively. Repeated measures ANOVA were used to examine significant effects of time and treatments on measured variables.

Fig. 1. Temporal changes in (a) bacterial, and (b) heterotrophic nanoflagellate abundance, and (c) dissolved organic carbon, (d) particulate organic carbon, (e) total organic carbon, (f) ammonium nitrogen, and (g) dissolved inorganic phosphorus concentrations at the May experiment. (○) *Daphnia* treatments, (<) control treatments. The vertical bars show standard deviations of the mean.
RESULTS
In all experiments, *Daphnia* individuals were active with no dead individuals found during the incubation period. In all experiments, bacterial abundance tended to decrease toward the end of the experiment, both in *Daphnia* treatments and in controls, but was higher in the former than in the latter treatments throughout the experimental period (Figs 1–3). An ANOVA found that interaction effects of time and treatment on bacterial abundance was significant in all experiments ($P < 0.0005$), indicating that temporal changes in bacterial abundance differed between the controls and *Daphnia* treatments. The abundance of HNF during the May experiment decreased markedly in the *Daphnia* treatments compared with the controls at the beginning of the experiment, but this difference disappeared after day 5 (Fig. 1). In contrast to the May experiment, the July experiment resulted in an increase in HNF abundance within the first 3 days, followed by a decrease thereafter in both treatments (Fig. 2). In both experiments, the abundance of HNF was on average significantly lower in the *Daphnia* treatments than in the controls ($P < 0.05$). Similarly, the abundance of HNF tended to decrease toward the end of the experiment in October (Fig. 3). However, in contrast to the first two experiments, the abundance of HNF was significantly higher in the *Daphnia* treatments than in the controls during the October experiment ($P < 0.005$).

In all experiments, the DOC decreased rapidly in the first 5 days and did not show any large changes thereafter, regardless of treatment (Figs 1–3). Throughout the experiments, 0.1–0.2 gC/m$^3$ disappeared from the DOC pool. These values corresponded to 5–15% of the initial DOC. The average DOC concentration during the incubation was at the same level between the treatments in July and October, but was significantly higher in the *Daphnia* treatments in May ($P < 0.05$). The POC concentration also decreased in all experiments. As a result, 0.1–0.4 gC/m$^3$ was removed from the POC pool throughout the incubation, which corresponded to 25–55% of the initial POC. In May, no significant difference was detected in either temporal changes or average values of POC between the treatments. However, the POC concentration was on average lower in the *Daphnia* treatments in July and October ($P < 0.05$). Total organic carbon concentration (TOC = DOC + POC) was significantly higher in the *Daphnia* treatments in May (Fig. 1; $P < 0.005$) but significantly lower in the *Daphnia* treatments in July ($P < 0.05$). The TOC concentration was at the same level between treatments in October.

![Fig. 2. Temporal changes in (a) bacterial, and (b) heterotrophic nanoflagellate abundance, and (c) dissolved organic carbon, (d) particulate organic carbon, (e) total organic carbon, (f) ammonium nitrogen, and (g) dissolved inorganic phosphorus concentrations at the July experiment.](image-url)
In all experiments, both ammonium nitrogen and dissolved inorganic phosphorus increased through the experimental period, and both were higher in the Daphnia treatments (Figs 1–3; \( P < 0.05 \)).

**DISCUSSION**

The present study showed that TOC decreased regardless of treatment, and the net effect of Daphnia on this decrease differed among experiments. The difference among the experiments was largely due to an inconsistent pattern in effects of Daphnia on DOC and POC. In May, the DOC decreased less in the Daphnia treatments, while no significant effects of Daphnia on DOC were detected on the other experimental dates. In contrast, the amount of POC disappearing during the incubation was significantly greater in the Daphnia treatments in July and October, but similar between the treatments in May. The effect of Daphnia on POC implies that Daphnia can reduce the vertical flux of organic matter, as the sinking flux of organic matter depends mainly on the particulate forms, rather than the dissolved forms. It should be noted that, although the initial concentration of the DOC was much higher than the POC, a higher fraction of organic carbon disappeared from the POC pool during the incubation, regardless of treatment. The results suggest that in a comparison between the particulate pool and the dissolved pool, the particulate pool contained a greater fraction of organic carbon, which was more easily available to heterotrophic organisms in Lake Biwa.

In all experiments, bacterial abundance decreased less in the Daphnia treatments compared to the controls. These results differ somewhat to those in previous studies. Nakano et al. (1998) and Gurung et al. (2001) suggest that bacterial abundance was depressed by Daphnia in Lake Biwa because this species grazes efficiently on bacteria-sized particles (Nagata & Okamoto 1988). This discrepancy implies either that the bacterial growth rate was high enough to compensate for the grazing loss rate in the Daphnia treatments, or that the mortality rate of the bacteria was greater in the controls. The most important bacterial consumers are HNF (Pace et al. 1990; Nakano et al. 1998; Gurung et al. 2000) and HNF are, in turn, grazed by Daphnia (Nakano et al. 1998; Yoshida et al. 2001). Thus, the less intense decrease in bacterial abundance in the Daphnia treatments may be due to a suppression of HNF by Daphnia grazing. This possibility is
strengthened by the results in May and July that showed that the abundance of HNF was on average lower in the *Daphnia* treatments, while this was not the result in October.

In October, the abundance of both bacteria and HNF was somewhat higher in the *Daphnia* treatments, suggesting that there was compensation for the grazing loss rate due to *Daphnia* by an increase in the growth rate. In Lake Biwa, the bacterial growth rate is limited mainly by phosphorus supply (Gurung & Urabe 1999). Thus, one may suspect that *Daphnia* stimulates bacterial growth by releasing phosphorus. However, both DIP and ammonium nitrogen concentrations increased during the incubation, even in the controls, although the increased levels were much greater in the *Daphnia* treatments. Such an accumulation of inorganic nutrients is an unlikely occurrence when bacterial growth is highly limited by these nutrients (Tezuka 1990). Therefore, bacterial growth may have been limited by the supply of labile organic carbon rather than by inorganic nutrients during the present study. In experiments with a long dark incubation period, the production of organic carbon by algae does not take place. Nonetheless, decreases in the DOC concentration during the incubation were very small, regardless of treatment in all experiments, suggesting that the majority of DOC was refractory. It is known that organic carbon released from zooplankton is an efficient substrate for bacterial growth (Olsen *et al.* 1986). Thus, the DOC from the *Daphnia* may have stimulated bacterial growth and thus the abundance of HNF through a bottom-up effect. This implies that the pool of organic carbon available for bacterial growth was small, relative to the demand of the bacteria in Lake Biwa, resulting in rapid depletion when lake water was incubated in closed containers for long periods under dark conditions.

In conclusion, a substantial fraction of organic carbon in Lake Biwa was decomposed and respired, regardless of the presence of *Daphnia*. However, *Daphnia* were able to reduce and channel the POC into DOC, making it more available to bacteria. In this carbon flow, particle size is reduced from detritus and algae, to bacteria, which would sink more slowly according to Stokes’ law. Thus, *Daphnia* appear to reduce sinking flux not only by direct grazing, but also by changing the size distribution of suspended particles.

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**REFERENCES**


