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PLANT ANIMAL INTERACTIONS

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Direct and indirect effects of zooplankton on algal composition in in situ grazing experiments

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Abstract To examine both direct and indirect effects of macrozooplankton on phytoplankton species in Lake Biwa, we conducted in situ grazer-gradient experiments under different nutrient levels in summer, when *Daphnia galeata* dominated, and in autumn, when *Eodiaptomus japonicus* dominated. The experiments revealed that grazing pressure on phytoplankton was highly dependent on zooplankton species composition. Smaller phytoplankton species such as *Stephanodiscus carconensis* were more grazed when *D. galeata* was abundant, whereas large colonial diatom species such as *Aulacoseira granulata* were preferentially grazed when *E. japonicus* dominated. In addition, indirect effect of macrozooplankton through nutrient regeneration was suggested, although the magnitude of nutrient regeneration effects seemed to differ between *D. galeata* and *E. japonicus*. Specifically, growth rates of *Sphaerocystis schroeteri* were stimulated more by *E. japonicus* than by *D. galeata*. Macrozooplankton also enhanced the growth rates of colonial cyanobacteria such as *Microcystis incerta*, probably through decreasing the density of microzooplankton grazers (ciliates and rotifers). The results suggest that the effects of large zooplankton on phytoplankton populations are species-specific and cannot be understood without consideration of changes in abundance of other components of plankton communities.

Keywords Grazing · Lake Biwa · Nutrient regeneration · Species-specific response

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Introduction

The abundance and species composition of phytoplankton change seasonally according to species-specific differences in the growth and loss rates in response to temporal changes in abiotic (e.g. light, temperature and nutrient) and biotic (e.g. grazing) factors (Reynolds 1984a). Among these, grazing by large zooplankton such as daphnids and diaptomus copepods is known to play a crucial role in the seasonal succession of phytoplankton populations in lakes and ponds (Sommer et al. 1986; Sterner 1989). However, zooplankton affect phytoplankton populations not just through direct grazing alone. They also affect phytoplankton populations indirectly by altering the nutrient conditions through nutrient regeneration (Lehman and Sandgren 1985; Sterner 1986, 1989; Urabe 1995). Furthermore, since daphnids and copepods influence the ciliates and rotifers negatively through consumption, interference, and exploitative competition (Gilbert 1989; Wickham and Gilbert 1991; Pace and Vaque 1994; Brett et al. 1994), they may affect the phytoplankton populations indirectly by modulating the abundance of these small zooplankton grazers.

Many studies have examined the effects of large zooplankton on phytoplankton abundance and composition in various lakes., Most of them, however, have focused on *Daphnia* as macrozooplankton grazers (Lynch and Shapiro 1981; Lehman and Sandgren 1985; Bergquist and Carpenter 1986; Elser et al. 1987; Sterner 1989; Elser and Goldman 1991; Elser 1992), because *Daphnia* are herbivorous and frequently dominate in zooplankton communities. Although diaptomus copepods also are large and herbivorous and are abundant in lakes, their effects on phytoplankton populations have been less well examined. Their effects probably differ from those of daphnids, because the two groups differ greatly in their feeding mode and nutrient-recycling efficiencies (DeMott 1986; Vanderploeg 1990; Hessen 1997; Elser and Urabe 1999). Thus, the results based on daphnids may not be applicable to periods when diaptomus copepods dominate (Brett et al. 1994; Burns and Schallen-

berg 1998). Indeed, Sommer et al. (2001) showed a large difference in net effects of zooplankton on phytoplankton species composition between *Daphnia*-dominated and *Diaptomus*-dominated communities: *Daphnia* suppressed the small phytoplankton while diaptomus copepods (*Eudiaptomus* spp.) suppressed large phytoplankton. However, the reasons for the difference were not clear, because most previous studies did not separately examine the direct and indirect effects of macrozooplankton.

Lake Biwa is the largest lake in Japan. It consists of a large, deep northern basin (surface area 616 km^2, mean depth 45.5 m) and a small, shallow southern basin (surface area 58 km^2, mean depth 3.5 m). In the north basin, the abundance and species composition of phyto- and zooplankton change seasonally (Nakanishi 1976; Yoshida et al. 2001a; Kagami et al., in preparation). Among the zooplankton, *Daphnia galeata* occurs abundantly from spring to early summer, but the diaptomus copepod *Eodiaptomus japonicus* dominates from summer to autumn. Among the phytoplankton, *Fragilaria crotonensis*, a colonial diatom species, forms large blooms in spring, but *Staurastrum dorsidentiferum*, a large desmid alga, predominates from summer to autumn. Several studies have examined the effects of zooplankton on phytoplankton in Lake Biwa, including the effects of direct grazing (Okamoto 1984; Kawabata 1987; Urabe et al. 1996), nutrient regeneration (Urabe et al. 1995; Elser et al. 2001), and both grazing and nutrient regeneration (Yoshida et al. 2001b). However, these studies focused mainly on total phytoplankton abundance, as quantified by chlorophyll-*a* or seston concentration. Thus, it is unclear how different zooplankton species can differentially affect the abundance of each phytoplankton species.

To examine species-specific differences in direct and indirect effects of macrozooplankton on other planktonic organisms (bacteria, protozoans, rotifers, and algae) in Lake Biwa, we performed in situ bottle incubation experiments in early summer and autumn. We assessed the effects by incubating plankton with and without nutrient enrichment, as described by Lehman and Sandgren (1985). The results for bacteria and protozoans are reported elsewhere (Yoshida et al. 2001b). Here, we show the effects of *D. galeata* and *E. japonicus* on phytoplankton.

Materials and methods

We conducted in situ experiments from 27 June to 2 July (July experiment) and from 26 September to 1 October (October experiment) 1997 at an open-water site (55 m deep, 3 km off the western coast) in the northern basin of Lake Biwa. In each experiment we measured nutrient concentrations and water temperature as described by Gurung et al. (2001). Lake water for the experiments was collected from 2.5 m depth and passed through a 200-μm-mesh net to remove large zooplankton. This filtrate was poured into eighteen 4.25-l polycarbonate bottles. Nine of these bottles received no nutrients and served as controls. The other nine were spiked with 18 μmol l^{-1} NH_4Cl and 2.5 μmol l^{-1} KH_2PO_4 (enriched treatment). These concentrations were determined from previous reports (Lehman and Sandgren 1985; Urabe 1993). Aliquots of lake water were fixed with 0.4% acid Lugol to determine initial cell numbers of phytoplankton species. Macrozooplankton, mainly *D. galeata* and *E. japonicus*, were collected by vertical tows of a conical net with a 300-μm mesh from 15 m depth to the surface, concentrated, rinsed, and used as we report elsewhere (Yoshida et al. 2001b).

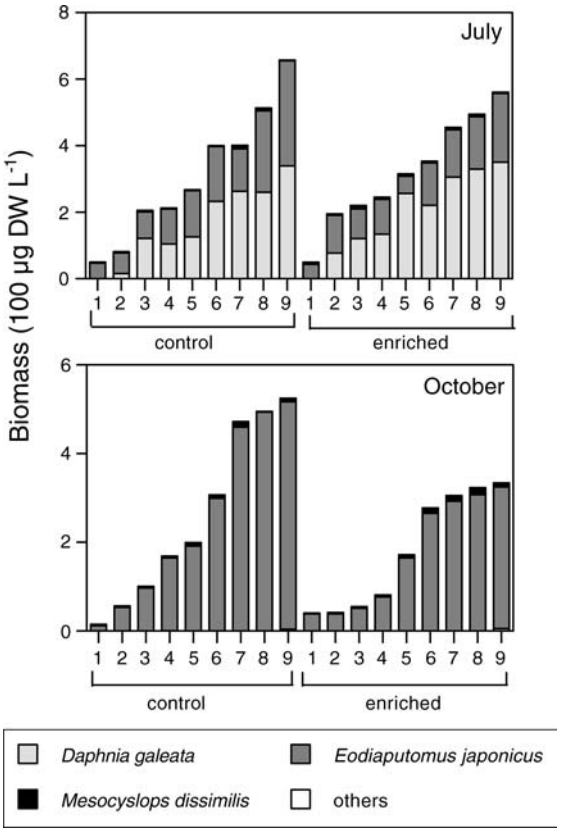

Fig. 1 Zooplankton abundance and species composition in July and October experiments

To initiate the experiment, we added different amounts of macrozooplankton to the nine bottles for each treatment to create a biomass gradient with a more than tenfold difference (Fig. 1). The bottles were incubated in situ for 5 days at 2.5 m depth. The experiments were terminated by filtration of the water from each bottle through a 200-μm-mesh screen. The macrozooplankton were preserved in 2% formalin. For the sample of phytoplankton, 500 ml of the 200-μm filtrate was fixed as above and stored in the dark. Macrozooplankton in the bottles were counted under a dissecting microscope and their biomass was estimated (Yoshida et al. 2001b). Phytoplankton in the 500-ml samples were concentrated to 20 ml by sedimentation for at least 4 days, and counted under a microscope in a 1-ml Sedgwick-Rafter chamber at 40–200× magnification. The biovolume of each phytoplankton species was estimated according to Kagami and Urabe (2001).

The net growth rate (r; day^{-1}) of each phytoplankton species during the 5-day incubation was estimated as $r=(\ln n_5 - \ln n_0)/5$, where n_0 and n_5 are the number of phytoplankton cells per millilitre at the start and end of the experiment, respectively. Growth rate was estimated for phytoplankton species that gave counts of >30 cells or colonies per single sample. For each treatment, the effect of zooplankton on the net growth rate of phytoplankton species was assessed by a simple regression analysis against macrozooplankton biomass. When the regression was significant in both control and enriched treatments, the effects of nutrient enrichment were examined by analysis of covariance (ANCOVA). When the

regression was not significant in one or both treatments, significant differences between treatments were determined by *t*-test.

Results

July experiment

The water temperature at the start of the experiment was 21.7°C. The concentrations of soluble reactive phosphorus and total dissolved inorganic nitrogen (ammonium+nitrite+nitrate) were 0.04 and 11.1 μmol l^{-1}, respectively. The biomass of macrozooplankton in the bottles ranged from 53 to 703 μg dry weight (DW) l^{-1}, and no large difference was found in their composition among the bottles (Fig. 1). *D. galeata* dominated, contributing on average 56±14% of the total macrozooplankton biomass, followed by *E. japonicus* (43±14%). Nine phytoplankton species occurred abundantly enough for us to examine their response to experimental manipulations. Among these, *Stephanodiscus carconensis*, a small diatom, dominated the phytoplankton biovolume (Fig. 2). Large green algae, including *Staurastrum dorsidentiferum, S. pingue*, and *Closterium aciculare*, and colonial green algae including *Oocystis parva* and *Sphaerocystis schroeteri*, were also commonly found, but their biomass was lower than that of *Stephanodiscus carconensis*.

The response to experimental manipulations differed among phytoplankton species. The net growth rates of *Stephanodiscus carconensis* and *Staurastrum pingue* decreased significantly with increasing macrozooplankton biomass in both the control and enriched treatments, and was higher in the enriched treatments at any macrozooplankton biomass (Table 1). Nutrient enrichment also stimulated the net growth rate of *C. aciculare*, but a significant decrease in the net growth rate versus macrozooplankton biomass was detected only in the enriched treatments (Fig. 3). *Stephanodiscus carconensis* had the steepest regression slope (Table 1). In contrast, the net growth rate of *O. parva* and *Sphaerocystis schroeteri* increased significantly with macrozooplankton biomass in the control treatments, but not in the enriched treatments (Table 1). No significant relationships between net growth rate and macrozooplankton biomass were detected in any treatments for *Selenastrum* sp., *Ceratium hirundinella*, or *Staurastrum dorsidentiferum*. As for *Stephanodiscus carconensis* and *C. aciculare*, net growth rates of *Sphaerocystis schroeteri*, *Selenastrum* sp. and *Staurastrum dorsidentiferum* were stimulated by the nutrient enrichment. In contrast, the net growth rates of *O. parva* and *C. hirundinella* were lowered by the nutrient enrichment.

October experiment

The water temperature was 22.4°C. The concentrations of soluble reactive phosphorus and dissolved inorganic nitrogen were 0.03 and 5.4 μmol l^{-1}, respectively. In this experiment, 96±2% of the macrozooplankton biomass was made up of late copepodite stages of *E. japonicus*, while *Daphnia* abundance was very limited (Fig. 1). The biomass of macrozooplankton in the bottles ranged from 16 to 562 μg DW l^{-1}, and again no large difference was found in their composition among the bottles (Fig. 1). Twenty common phytoplankton species were found. Among these, *Staurastrum dorsidentiferum* dominated, accounting for >85% of the phytoplankton biomass (Fig. 2). Large diatoms, including *Aulacoseira granulata* and *Fragilaria crotonensis*, the colonial cyanobacteria

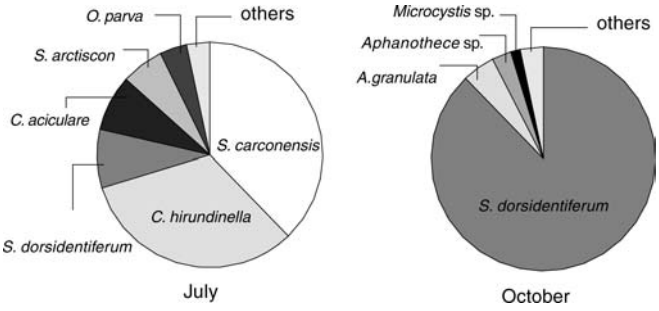

Fig. 2 Initial composition of phytoplankton species in terms of biovolume in July and October experiments. *O. parva Oocystis parva*, *S. arctiscon Staurastrum arctiscon*, *C. aciculare Closterium aciculare*, *S. dorsidentiferum Staurastrum dorsidentiferum*, *S. carconensis Stephanodiscus carconensis*, *C. hirundinella Ceratium hirundinella*, *A. granulata Aulacoseira granulata*

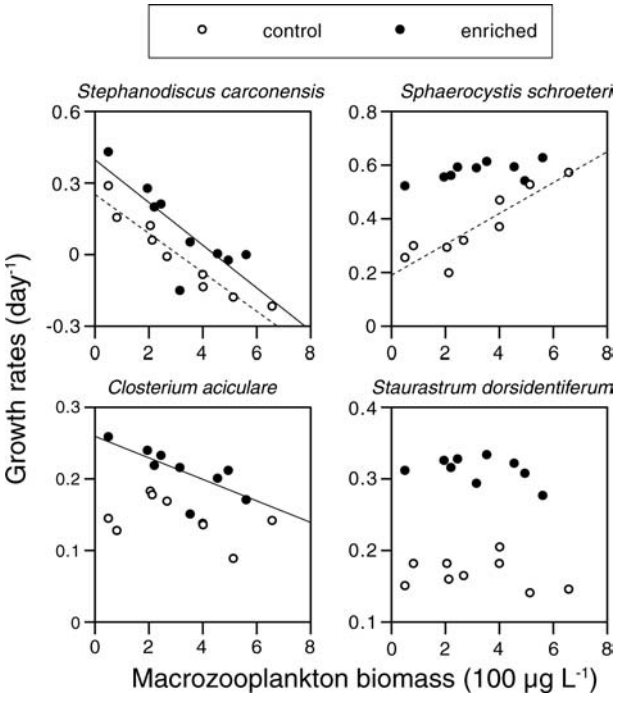

Fig. 3 Typical response of net growth rates of phytoplankton populations to changes in macrozooplankton biomass with (*filled circles*, *thin line*) and without (*open circles*, *dotted line*) nutrient enrichment in the July experiment. A regression line is shown if the net growth rate is significantly related to macrozooplankton biomass at $P<0.05$

Table 1 Results of statistical analyses testing the effects of macrozooplankton (*G*) and nutrient enrichment (*N*) on net growth rate of phytoplankton species in July experiment. Slope of the regression line is shown if the net growth rates are related significantly to macrozooplankton biomass at the $P<0.05$ level. Analysis of covariance (*ANCOVA*) and/or *t*-test was used to examine the effect of nutrient enrichment. Biovolume of each phytoplankton cell is also shown. + Positive response of the net growth rate to nutrient enrichment, – negative response of the net growth rate to nutrient enrichment, *n.s.* no significant effects

		G				N				Biovolume (μm^3)
		Enriched		Control		Interaction (G×N)	ANCOVA (N)	*t*-test	Response	
		Slope	r^2	Slope	r^2					
Bacillariophyceae	*Stephanodiscus carconensis*	–0.089	0.64	–0.081	0.92	n.s.	$P<0.05$		+	4,200
Chlorophyceae	*Closterium aciculare*	–0.015	0.53	n.s.	0.16			$P<0.01$	+	9,200
	Oocystis parva	n.s.	0.32	0.047	0.64			$P<0.01$	–	100
	Selenastrum sp.	n.s.	0.36	n.s.	0.04			$P<0.01$	+	12
	Sphaerocystis schroeteri	n.s.	0.37	0.057	0.81			$P<0.01$	+	74
	Staurastrum dorsidentiferum	n.s.	0.19	n.s.	0.05			$P<0.01$	+	32,000
	Staurastrum pingue	–0.072	0.81	–0.045	0.71	n.s.	$P<0.05$		+	25,000
Dinophyceae	*Ceratium hirundinella*	n.s.	0.31	n.s.	0.43			$P<0.05$	–	30,000

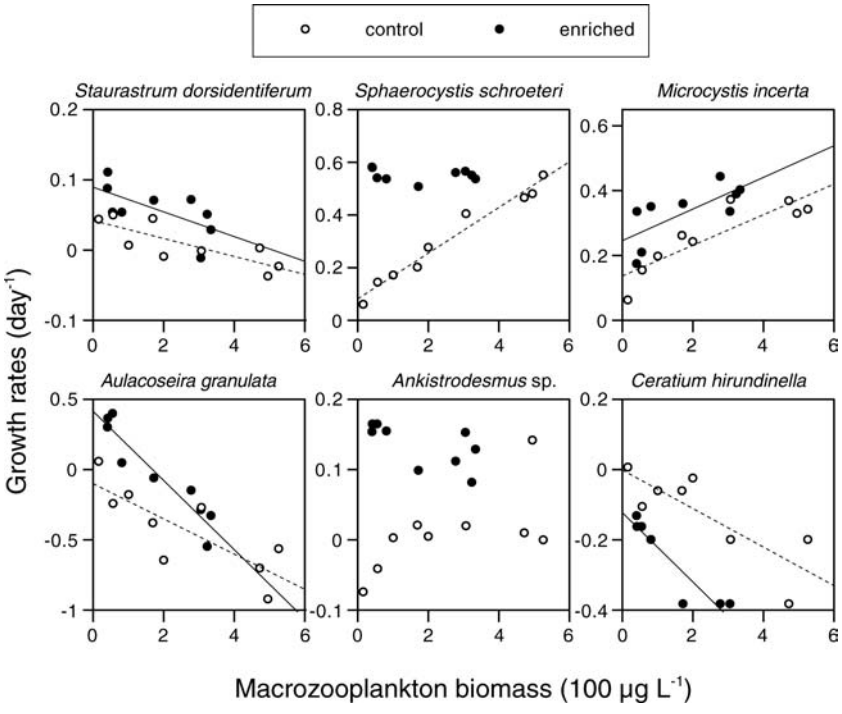

Fig. 4 Typical response of net growth rates of phytoplankton populations to changes in macrozooplankton biomass with (*filled circles*, *thin line*) and without (*open circles*, *dotted line*) nutrient enrichment in the October experiment. A regression line is shown if the net growth rate is significantly related to macrozooplankton biomass at $P<0.05$

Microcystis spp. and *Aphanothece* sp., and colonial green algae such as *Oocystis* spp. and *Sphaerocystis schroeteri* were also found, but their abundance was much lower than that of *Staurastrum dorsidentiferum*.

Significant effects of nutrient enrichment and zooplankton were detected also in this experiment, but responses to the experimental manipulation again differed among phytoplankton species. Net growth rates of *F. crotonensis*, *A. granulata*, *Pediastrum* spp., *Staurastrum dorsidentiferum*, and *C. hirundinella* decreased with increasing macrozooplankton biomass in both the control and enriched treatments (Table 2). *Pediastrum* spp. and *Staurastrum dorsidentiferum* showed no significant difference between treatments in regression slopes, and their net growth rates were higher in the enriched treatments at any macrozooplankton biomass (Table 2). Similarly, no significant difference was found in the slope of *C. hirundinella* between treatments. However, as in the July experiment, the net growth rate of this species was lower in the enriched treatment (Fig. 4). In

Table 2 As Table 1. but for October experiment

		G				N				Biovolume (μm^3)
		Enriched		Control		Interaction (G×N)	ANCOVA (N)	*t*-test	Response	
		Slope	r^2	Slope	r^2					
Bacillariophyce	*Aulacoseira granulata*	–0.244	0.89	–0.125	0.66	$P<0.05$			+	1,500
	Fragilaria crotonensis	–0.266	0.71	–0.106	0.69	$P<0.05$			+	755
Chlorophyceae	*Ankistrodesmus* sp.	n.s.	0.43	n.s.	0.42			$P<0.01$	+	11
	Closterium aciculare	–0.042	0.52	n.s.	0.44			$P<0.01$	+	9,200
	Coelastrum cambricum	n.s.	0.03	n.s.	0.17			$P<0.05$	+	610
	Cosmocladium constrictum	n.s.	0.92	n.s.	0.20			n.s.		1,600
	Crucigenia sp.	n.s.	0.32	n.s.	0.10			n.s.		120
	Elakatothrix gelatinosa	0.066	0.61	0.055	0.90	n.s.	n.s.			260
	O. parva	n.s.	0.07	0.042	0.09			$P<0.05$	+	100
	Oocystis solitaria	–0.046	0.58	n.s.	0.01			$P<0.05$	+	530
	Pediastrum spp.	–0.041	0.68	–0.051	0.95	n.s.	$P<0.01$		+	1,000
	Quadrigula i chodatt	n.s.	0.19	n.s.	0.11			n.s.		210
	Sphaerocystis schroeteri	n.s.	0.04	0.087	0.97			$P<0.01$	+	74
	Staurastrum arctiscon	–0.048	0.61	n.s.	0.10			n.s.		82,000
	Staurastrum dorsidentiferum	–0.018	0.65	–0.013	0.42	n.s.	$P<0.01$		+	32,000
Cyanophyceae	*Aphanothece* sp.	0.023	0.68	0.01	0.49	n.s.	n.s.			2
	Microcystis incerta	0.049	0.52	0.047	0.77	n.s.	$P<0.01$		+	10
	Microcystis sp.	n.s.	0.01	n.s.	0.27			n.s.		14
	Microcystis wesenberguii	–0.168	0.64	n.s.	0.06			n.s.		110
Dinophyceae	*Ceratium hirundinella*	–0.097	0.87	–0.055	0.66	n.s.	$P<0.01$		–	30,000

contrast, the regression slopes of *F. crotonensis* and *A. granulata* were significantly steeper in the enriched treatments than in the control (Table 2), indicating that the net growth rate of those species was more sensitive to changes in macrozooplankton abundance under nutrient-rich conditions.

The net growth rates of seven other species increased significantly in response to the nutrient enrichment (Table 2). Among them, a significant decrease in the net growth rate with macrozooplankton biomass was found for *O. solitaria*, *C. aciculare*, *Staurastrum arctiscon*, and *M. wesenberguii* in the enriched treatments. In contrast, an increase was detected for *Aphanothece* sp., *M. incerta*, and *E. gelatinosa* in both treatments (Table 2). Net growth rates of *Sphaerocystis schroeteri* and *O. parva* also increased with macrozooplankton biomass in the control (Table 2). Net growth rates of *Aphanothece* sp., *M. incerta*, and *E. gelatinosa* also increased with macrozooplankton biomass in both the control and enriched treatments (Table 2). The remaining species, *Ankistrodesmus* sp. and *Crucigenia* sp., did not respond to changes in macrozooplankton biomass in any treatments.

Discussion

The response of phytoplankton species to changes in macrozooplankton biomass differed largely between July and October experiments. We cannot attribute this difference to water temperatures and nutrient concentrations because these were almost the same in both experiments. We cannot rule out the possibility that the differences between the two experiments were caused by differences in other physical and chemical factors such as light conditions and dissolved organic matters because these were not controlled in our experimental set up. However, the zooplankton species composition was completely differ-

ent between the two experiments (Fig. 1). Thus, we argue that the differences in the results are most likely attributable to zooplankton composition. In July, when *D. galeata* dominated, the net growth rate of *Stephanodiscus carconensis* decreased markedly with macrozooplankton biomass, indicating that this small diatom alga was one of the phytoplankton most vulnerable to grazing by *D. galeata*. In October, when *E. japonicus* was abundant, however, larger diatom algae, such as *F. crotonensis* and *A. granulata*, had the greatest decrease in net growth rate versus macrozooplankton biomass. In addition, net growth rates of *C. hirundinella* and *Staurastrum dorsidentiferum* decreased with increasing macrozooplankton biomass in October, but they did not respond in July. Thus, grazing effects of *E. japonicus* on phytoplankton populations differed from those of *D. galeata*. These results accord well with the knowledge that *Daphnia* ingests mainly small algae (Lampert 1974), while diaptomus copepods ingest selected food and can graze on large colonial diatoms (Okamoto 1984; DeMott 1986). Recently, Sommer et al. (2001) also showed that a *Daphnia*-dominant community suppressed small phytoplankton species, whereas a diaptomus-dominant community preferentially removed large phytoplankton species.

However, the response of phytoplankton species to changes in macrozooplankton biomass cannot be explained by direct grazing alone. In October, the decrease in the net growth rate of *F. crotonensis* versus microzooplankton biomass was steeper in the enriched treatments than in the control. In addition, although the net growth rates of *C. aciculare* (in both July and October) and *O. solitaria* (in October) decreased with macrozooplankton biomass in the enriched treatments, no significant response was detected in the control treatments. The results indicate that the direct grazing effects of macrozooplankton were somewhat compensated for under low nutrient conditions. In Lake Biwa, most phytoplankton species are limited by nutrient supply (Urabe et al. 1999; Kagami and Urabe 2001). Our results which showed that most phytoplankton populations responded to nutrient enrichment (Tables 1, 2) confirmed this. Urabe et al. (1995) showed that macrozooplankton could regenerate substantial amounts of nutrients when they were abundant. In addition, macrozooplankton may favour some phytoplankton species by reducing the numbers of competitively superior species. In the control, therefore, it is likely that both nutrient supply and grazing pressure were increased and strength of competitive interactions for nutrients decreased, with macrozooplankton biomass. In the enriched treatments, however, the effects of nutrient regeneration could not operate and strength of competitive interactions should be independent of zooplankton abundance, because we added enough nitrogen and phosphorus to maximize algal growth rates (Lehman and Sandgren 1985). Thus, the difference in the responses between the treatments suggests that macrozooplankton affect phytoplankton populations not only negatively by grazing but also positively by changing the nutrient conditions.

In some phytoplankton species, the positive effects of macrozooplankton exceeded the negative grazing effects. For example, in both July and October, the net growth rates of *Sphaerocystis schroeteri* and *O. parva* increased with macrozooplankton biomass in the control treatments, but they did not relate to macrozooplankton biomass and were consistently higher in the enriched treatments. These results imply that these phytoplankton species are less vulnerable to direct grazing but benefit from increased nutrient return at a higher macrozooplankton biomass. *S. schroeteri* and *O. parva* are colonial green algae that have thick cell walls or gelatinous sheaths. It is well documented that the thick cell walls and gelatinous sheaths protect the algae from digestion by *Daphnia*, and thus, even if they are ingested, most are egested again without damage (Porter 1977; Van Donk 1997). Moreover, they can absorb nutrients during their passage through the gut (Porter 1977). Indeed, the predominance of these digestion-resistant green algae has frequently been reported when *Daphnia* are abundant (e.g. Lynch and Shapiro 1981; Lehman and Sandgren 1985; Bergquist and Carpenter 1986; Elser et al. 1987). However, the effects of diaptomus copepods on these colonial green algae have been unclear. Our results showed that growth of *S. schroeteri* and *O. parva* was enhanced even by *E. japonicus*. It is not clear whether these algae are ingested but not digested by *E. japonicus* or are simply unfavourable food for *E. japonicus*. However, the present results would help to explain the fact that those green algae occur abundantly in Lake Biwa from early summer to autumn when *E. japonicus* were abundant (Kagami et al., in preparation). Interestingly, slopes of net growth rates of *S. schroeteri* versus macrozooplankton biomass in the control treatments were greater in October than in July (ANOVA, $F_{1,14}$=5.70, P<0.05). Since phosphorus is the most deficient nutrient in Lake Biwa (Tezuka 1984; Urabe et al. 1999), and since diaptomus copepods release relatively more phosphorous per unit body weight than *Daphnia* do (Urabe et al. 1995; Elser and Urabe 1999), the growth rate of *S. schroeteri* may be stimulated more by *E. japonicus*. Although data are limited, the result suggests that the magnitude of nutrient-regeneration effects on phytoplankton species differs between *Daphnia* and diaptomus copepods.

The net growth rates of *Selenastrum* sp. and *Staurastrum dorsidentiferum* did not relate to changes in macrozooplankton biomass in either the control or enriched treatments in July, although they were stimulated by nutrient enrichment. The same trend was found for *Ankistrodesmus* sp. and *Coelastrum cambricum* in October. Note that nutrient uptake rate of phytoplankton is highly species-specific and depends on nutrient concentration (Sommer 1989; Sterner 1989). Thus, the nutrient return from macrozooplankton may have not been sufficient for these species to increase their growth rate. In support of this inference, *Staurastrum* are known to take up nutrients slowly and are less sensitive to a short-term change in nutrient concentrations (Reinertsen et al. 1986; Olsen 1989). The implication of this possibility is that the im-

pacts of nutrient regeneration by macrozooplankton also differ among phytoplankton species.

As for gelatinous green algae, the net growth rates of *Microcystis incerta*, *Aphanothece* sp., and *E. gelatinosa* increased with macrozooplankton in October. However their response can be attributed to neither an increase in nutrient return nor changes in strength of competitive interactions for nutrients, because the net growth rate of these species increased even in the enriched treatments, where the effects of nutrient regeneration by macrozooplankton were cancelled. The abundance of microzooplankton (ciliates and rotifers) decreased significantly with increasing macrozooplankton biomass in enriched treatments (Yoshida et al. 2001b). Some ciliates and rotifers can feed on colonial cyanobacteria, such as *Microcystis aeruginosa* (Cole and Wynne 1974; Dryden and Wright 1987). Thus, by decreasing microzooplankton, macrozooplankton may have stimulated the net growth rates of *Microcystis* sp., *Aphanothece* sp., and *E. gelatinosa*. In other words, macrozooplankton may affect algal species composition indirectly by modulating the abundance of microzooplankton grazers.

Although nutrient enrichment stimulated the growth rate of most phytoplankton species, it greatly lowered the net growth rate of *C. hirundinella* (Fig. 4). Nutrient enrichment can sometimes kill phytoplankton species by altering ionic conditions in the water (Sandgren 1988). However, the decrease in the growth rate of *C. hirundinella* cannot be attributed to this, because the cells grew well in ambient lake water spiked with nutrients (Kagami and Urabe 2001). *C. hirundinella* is a mixotrophic species that can utilize bacteria as an energy source (Porter 1988). In the present experiments, bacterial abundance was lower in the enriched treatments than in the control because bacterial grazers such as heterotrophic nanoflagellates were more abundant (Yoshida et al. 2001b). Thus, nutrient enrichment may indirectly affect the net growth rate of *C. hirundinella* through causing changes in bacterial abundance.

In Lake Biwa, *Staurastrum dorsidentiferum* is the most dominant phytoplankton from summer to autumn (Nakanishi 1976; Yoshida et al. 2001a; Kagami et al., in preparation). In the July experiment, its net growth rate was not affected by macrozooplankton biomass. In October, however, when *E. japonicus* dominated, the net growth rate of *S. dorsidentiferum* was negatively related to macrozooplankton abundance, although the regression slope was relatively small. Thus, *S. dorsidentiferum* is more or less vulnerable to macrozooplankton. Several studies suggested that *Staurastrum* is inedible because of its large size and thorny form (e.g. Lampert et al. 1986; Reinertsen et al. 1986). However, this low vulnerability alone may not explain the predominance of *S. dorsidentiferum* in Lake Biwa. The ambient macrozooplankton biomass from summer to autumn in this lake varied between 50 and 200 μg DW l^{-1} (Yoshida et al. 2001a). According to the present results, *Sphaerocystis schroeteri* and *M. incerta* should have predominated over *Staurastrum dorsidentiferum* from summer to autumn, since they had the highest net growth rates at the ambient biomass level of macrozooplankton (Figs. 3, 4). However, this was not the case. In the present study, in situ experiments were conducted at 2.5 m depth. In nature, however, vertical mixing transports phytoplankton to deep layers. In Lake Biwa, the surface mixing layer extends to 15–20 m (Gurung et al. 2001). Thus, light intensity received by phytoplankton in the surface mixing layer would be, on average, lower that that at 2.5 m depth. *Staurastrum chaetoceras* has a high photosynthetic capacity at low light intensity (Coesel and Wardenaar 1994), while both *Sphaerocystis schroeteri* and *M. incerta* are known to adapt to high light intensity (Reynolds 1984b; Litchman 2000). In addition to the low vulnerability of zooplankton, therefore, a relatively high growth rate under low light conditions may sustain the predominance of *Staurastrum dorsidentiferum* in this lake.

Note that the net growth rate of *S. dorsidentiferum* was lower in October than in July even in enriched treatments. From late summer to autumn, we found that most *S. dorsidentiferum* cells were infected by pathogenic fungi (*Rhizophydium couchii*) causing the death of this species (Kagami and Urabe 2002). This may explain the low growth of *S. dorsidentiferum* in October experiments.

In conclusion, our results suggest that macrozooplankton affect the abundance and species composition of phytoplankton both directly through grazing and indirectly through changing nutrient conditions. In addition, comparison of experiments conducted in different seasons suggests that the magnitudes of these direct and indirect effects of macrozooplankton are highly species-specific. Furthermore, we found responses of some phytoplankton species to changes in macrozooplankton grazers that could not be explained without considering concurrent changes in the abundance of microzooplankton grazers and bacteria. Thus, to clarify how large zooplankton affect seasonal succession of phytoplankton species, not only macrozooplankton composition should be considered but also other components of the plankton community.

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