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The Impacts of Crustacean Zooplankton on a Natural Ciliate Community: a Short-term Incubation Experiment

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Abstract. Direct and indirect effects of crustacean zooplankton (cladocerans and copepods) are important regulators of ciliate communities, especially in eutrophic systems. However, it is not clear whether pseudodiaptomids (e.g., *Schmackeria*), one of the dominant calanoid copepods in Chinese lakes, effectively impacts natural ciliate communities. The impacts of small-bodied cladocerans (e.g., *Bosmina*) on ciliates are also controversial.

We performed an incubation experiment using winter lake water from Lake Chaohu to assess the structuring effects that crustacean zooplankton have on natural ciliate populations. The presence and absence of cladocerans (*Bosmina* sp.) and copepods (*Schmackeria inopinus*) were alternated in four treatments.

Both *Bosmina* sp. and *Schmackeria inopinus* had substantial impacts on ciliate abundance, biomass, and community structure. The response of ciliates was different in the presence of *Bosmina* sp. compared with *Schmackeria inopinus* and varied among categories such as the ciliate population, relative body size and functional feeding group. Our results also highlight the importance of interference and exploitative competition among metazooplankton groups.

Key words: ciliates, small-bodied cladocerans, pseudodiaptomid copepods, suppression impacts, functional groups

INTRODUCTION

Protozoa, especially ciliates, effectively utilize the production of bacteria and phytoplankton and may play an important role in transferring energy and materials from their prey to larger zooplankton (e.g., Weisse and Scheffel-Möser 1990; Zubkov and Leakey 2009). Genera belonging to Oligotrichida (e.g., *Rimostrombidium* and *Hateria*), Prostomatida (e.g., *Balanion* and *Urotricha*), Scuticociliatida (e.g., *Cyclidium*), and Peritrichida (e.g., *Vorticella*) typically dominate planktonic ciliate communities in eutrophic Chinese lakes (Li *et al.* 2013, 2014, 2016). Species dominance and total ciliate abundance are determined by different factors in lakes (van Wichelen *et al.* 2013). In eutrophic freshwater ecosystems, food resources (bottom-up effect) for ciliates are relatively plentiful; thus, predator-mediated (top-down) effects are vital to the ciliate communities (Sanders and Wickham 1993; Burns and Schallenberg 2001; Galbraith and Burns 2010; Agasild *et al.* 2013).

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Crustacean zooplankton (copepods and cladocerans) can potentially impact ciliate assemblages through direct predation and (or) exploitative and interference competition (see reviews, e.g., Sanders and Wickham 1993; Jürgens 1994; Jack and Gilbert 1997). Relationships between copepods and ciliates have been widely studied in both freshwater lakes and marine environments (e.g., Hansen 2000; Zöllner *et al.* 2009; Dhanker *et al.* 2013; Rollwagen-Bollens *et al.* 2013). It has been shown that the predation rate of copepods on ciliates is high (Adrian and Schneider-Olt 1999; Burns and Gilbert 1993; Kamjunke *et al.* 2012) and that predation by both cyclopoid (e.g., *Cyclops*, *Diacyclops*, and *Thermocyclops*) and calanoid copepods (e.g., *Boeckella*, *Epischura*, *Eudiaptomus*, and *Diaptomus*) can strongly influence the abundance, biomass, and species composition of ciliates (Wiackowski *et al.* 1994; Wickham 1998; Adrian and Schneider-Olt 1999; Hansen 2000; Balseiro *et al.* 2001). Although many species are capable of grazing on ciliates, it is not clear whether pseudodiaptomids (e.g., *Schmackeria*), one of dominant calanoid copepods in Chinese lakes, can cause significant impacts on ciliate populations. Both large (e.g., *Daphnia pulex*) and intermediate-sized daphnids (e.g., *Daphnia galeata mendotae*) are known to suppress ciliate assemblages (Gilbert 1989; Wickham and Gilbert 1991; Jürgens 1994), but the impacts of small-bodied cladocerans (e.g., *Bosmina*, *Chydorus*) on ciliates are controversial (Wickham and Gilbert 1991; Ventelä *et al.* 2002; Agasild *et al.* 2012).

The effects of crustacean zooplankton on ciliates are dependent on the species and both the grazer and prey sizes (e.g., Burns and Gilbert 1993; Jack and Gilbert 1993; Adrian and Schneider-Olt 1999; Agasild *et al.* 2012). Copepod clearance rates were higher on oligotrichs than other ciliates species (Burns and Gilbert 1993; Hansen 2000). The ciliate ingestion rates of several common species of both copepods (e.g., *Eudiaptomus graciloides*, *Diacyclops bicuspidatus*, and *Thermocyclops oithonoides*) and *Daphnia* (e.g., *Daphnia hyalina* and *Daphnia cucullata*) were always higher for ciliates in the 20–55 μm size category than for smaller ciliate species (10–20 μm) (Adrian and Schneider-Olt 1999). The abundance of grazers is also a key factor (Burns and Gilbert 1993; Burns and Schallenberg 1996). For example, the growth of oligotrich (*Strobilidium velox*) (ca. 43 μm) populations was halted by the presence of approximately 1.6 adult *Epischura* L^{-1} or sixteen adult female *Diaptomus* L^{-1} in summer (Burns and Gilbert 1993).

In the present study, we performed an incubation (15 days) experiment and manipulated the presence and absence of both *Bosmina* sp. and *Schmackeria inopinus* to test (1) whether small cladocerans (*Bosmina* sp.) and a pseudodiaptomid copepod (*Schmackeria inopinus*) effectively impact natural winter ciliate communities and (2) how the ciliates, at species and community levels, respond to the presence of crustacean zooplankton groups.

MATERIALS AND METHODS

Study site

Lake Chaohu (31°25′–31°43′N, 117°16′–117°5′E) is a semi-closed (artificially controlled), shallow, eutrophic lake situated in eastern China. The lake has an area of 770 km^2, a mean depth of 2.7 m and a maximum depth of 3.8 m. Submerged vegetation is sparse. Cyanobacterial blooms first appeared in the 1950s and have occurred in the lake every summer and autumn since the 1980s. The monthly mean total phytoplankton biomass varies between 5.05 and 19.70 mg L^{-1}, and bacillariophytes (mainly *Melosira*, *Cyclotella*, *Synedra*, and *Surirella*) and cyanophytes (mainly *Anabaena* and *Microcystis*) dominate the winter algal assemblages compared with the other seasons (Deng *et al.* 2007). The cladoceran community is dominated by *Daphnia* spp. in spring and by small-sized *Bosmina coregoni* in summer, autumn, and winter. *Limnoithona sinensis*, *Sinocalanus dorri* and *Schmackeria inopinus* are the main species of copepods (Deng *et al.* 2008). The mean ciliate abundance was 27.5, 13.4, and 5.6 cells mL^{-1} in July 2009, December 2009, and April 2010, respectively (Li *et al.* 2013). The ciliate communities were dominated by small-bodied species, e.g., oligotrich *Rimostrombidium brachykinetum*, prostomatids *Balanion planctonicum* and *Urotricha farcta*, and scuticociliatid *Cyclidium* spp.

Experimental design

The incubation experiment was conducted to assess the impacts of cladocerans and copepods on natural ciliate communities. Lake water from a depth of 0.5–1 m was collected from Lake Chaohu (N31°38′20″, E117°22′18″) on 24 December 2013 with a Patalas sampler (5 L). A qualitative zooplankton sample was collected using a zooplankton net (64 μm) at the same site. Lake water and zooplankton were stored in plastic carboys and transported to the laboratory as soon as possible, which is ca. 28 km away from Lake Chaohu. At the sampled site, the water temperature was 5.5°C, the Secchi depth was 55 cm, the dissolved oxygen was 8.49 mg L^{-1}, the pH was 8.4, and the chlorophyll a was 21.2 μg L^{-1}. The total suspended solids, the total nitrogen and the total phosphorus concentrations were 30.5, 3.7 and 0.17 mg L^{-1}, respectively.

Four treatments were set and named FILTER, CONTROL, CLAD and COPE. Each treatment was triplicated. The CONTROL treatment contained natural lake water with both cladocerans and copepods. The lake water was preliminarily filtered through the zooplankton net (64 μm) in the other three treatments. Neither cladocerans nor copepods were added to the FILTER treatment;

additionally, only cladocerans (*Bosmina* sp.) were added to the CLAD treatment and only copepods (*Schmackeria inopinus*) were added to the COPE treatment. Both *Bosmina* sp. and *S. inopinus* were selected from the qualitative sample using a stereoscopic microscope (Olympus SZX10, Tokyo, Japan) in the laboratory (ca. 5°C). Active animals were selected and put into 1-L beakers with filtered (64-μm net) lake water. Then, the beakers containing the water and the selected animals were placed into containers corresponding to each appropriate treatment. In CLAD and COPE treatments, the abundances of added *Bosmina* sp. and *S. inopinus* were the same as the corresponding abundances in lake water (Table 1). All the containers with 5 L of water were incubated in an uncovered cement pond (1 m in depth and 3 × 3 m square) near the laboratory, approximately 30 cm below the surface. The experimental treatments were set up within 4 h from when the collected samples arrived at the laboratory. The experiment began on 25 December 2013 and lasted for 15 days.

Biological sampling and analysis

Samples (200 mL) for analysing phytoplankton were taken separately from each container on days 1 and 15 and fixed with acid Lugol's solution (final concentration 1.5%). Ciliate samples (200 mL) were collected separately from each container on days 1, 5, 10, and 15 and fixed with Bouin's solution (final concentration 5%). Samples were then concentrated from 200 mL to 50 mL by settling prior to further analysis. Algal cells were identified, enumerated and measured with a microscope (Olympus BX53, Tokyo, Japan) at 400 × magnification in 0.1-mL counting chambers (Hu and Wei, 2006). Ciliate samples were manipulated separately using the quantitative protargol staining (QPS) approach (Skibbe 1994; Li *et al.* 2013). Ciliate species identification was based on Kahl (1930–1935), Corliss (1979), Foissner and Berger (1996), Foissner *et al.* (1999), and Lynn (2008), while biomass (as wet weight) calculation referred to the literature (e.g., Foissner *et al.* 1999).

Metazooplankton screened (64-μm mesh) from 10 L of lake water were preserved and used as the abundance estimates of cladocerans, copepods, and rotifers allocated to containers on day 1. On the last day, the metazooplankton samples were collected separately from each container by pouring all of the water through a 64-μm-mesh nylon net. Metazooplankton samples were preserved in 40% formaldehyde, for a final concentration of 2% (v/v), and measured and analysed with a light microscope (Olympus BX53; Japan) at 100 × magnification. The identification keys for cladocerans, copepods, and rotifers were from Chiang and Du (1979), Sheng (1979), and Wang (1961), respectively. The biomass of rotifers and crustacean plankton was estimated according to Huang *et al*. (2000).

Data analysis

The normality and homogeneity of the variables were tested with the Shapiro-Wilk test and Levene's test, respectively, using the IBM SPSS Statistics package (SPSS 19.0). Several variables (abundance of mixotrophs, *Askenasia chlorelligera* and *Tintinnidium pusillum* and biomass of prostomatids, algae, and rotifers) showed slight heterogeneity that we were unable to correct with transformations; thus, results should be considered with caution. Repeated-measures ANOVA (GLM procedure in the SPSS Statistics package) was used to test the differences among the treatments and the variance over time for the abundance and biomass of algae and ciliate groups (total ciliates, each ciliate species, small ciliates, medium ciliates, large ciliates, oligotrichs, prostomatids, algivores, bacterivores, mixotrophs, total algae, cyanophytes, bacillariophytes, and chlorophytes). Differences in rotifers among treatments were tested with one-way ANOVA. The significance level (further referred to as "P_{adj}") was corrected using the Bonferroni technique (Rice 1989).

RESULTS

Dynamics of phytoplankton and metazooplankton

The algal assemblages were initially dominated by cyanophytes (mainly *Microcystis* and *Anabaena*) and finally by bacillariophytes (e.g., *Cyclotella* and *Synedra*), respectively contributing 59.7 and 86.5% of the total biomass (mg L^{-1}, as wet weight) in the FILTER treatment, 66.3 and 83.2% of the total biomass in the CLAD treatment, 33.6 and 94.6% of the total biomass in the COPE treatment, and 57.2 and 86.6% of the total biomass in the CONTROL. The biomass of cyanophytes, bacillariophytes, and total phytoplankton significantly increased in every treatment (all $P < 0.001$, Fig. 1). However, there was no significant difference among the four treatments. The proportion of chlorophyte biomass (e.g., *Ankistrodesmus* and *Pediastrum*) slightly increased in the CLAD treatment (10.8 to 15.5%) and decreased rapidly from 24.8 to 4.7%, 38.3 to 5.0%, and 36.2 to 7.8% in the FILTER, COPE, and CONTROL treatments, respectively.

Rotifers were dominated by *Keratella* (mainly *K. cochlearis* and *K. quadrata*) and *Brachionus* spp., which initially contributed 60.3 and 32.8% of the total abundance, respectively (Fig. 1). At the end of the experiment, the former increased to 77.2, 87.5, 92.4, and 82.5% in FILTER, CLAD, COPE, and CONTROL, respectively, while the latter decreased to 5.5, 7.8, 6.3, and 13.1% in FILTER, CLAD, COPE, and CONTROL, respectively. Both the abundance and biomass of rotifers were significantly different among the four treatments (both $P_{adj} < 0.001$). On average, there was significantly higher abundance (94.8 individuals L^{-1}) and biomass (73.3 μg L^{-1}) in the CONTROL group than in the treatment groups (all $P_{adj} < 0.001$).

There was very low abundance of cladocerans and copepods (both 0.1 individuals L^{-1}) in the FILTER treatment at the end of the experiment. The final abundance of cladocerans decreased in the CONTROL group, while no obvious change in copepod abundance occurred in the CONTROL group or the COPE treatment. In the CLAD treatment, the abundance of cladocerans

Table 1. Initial and final abundance of crustacean zooplankton groups in the four treatments

	Initial abundance (individuals L^{-1})				Final abundance (individuals L^{-1})			
	FILTER	CLAD	COPE	CONTROL	FILTER	CLAD	COPE	CONTROL
Cladocerans								
Bosmina sp.	–	26.0	–	25.6	0.1	5.1	1.1	10.7
Chydorus ovalis	–	–	–	3.2	–	0.5	–	–
Copepods								
Schmackeria inopinus adults & larva	–	–	12.0	11.7	–	0.7	7.2	18.0
Limnoithona sinensis adults & larva			–	4.5	–	0.3	0.9	2.0
Cyclops sp. adults & larva	–	–	–	2.0	0.1	0.5	2.5	1.8
Copepod nauplii	–	–		5.3	–	0.2	0.4	0.8

decreased by the end of the experiment, and few copepods remained in the CLAD treatment (Table 1).

Ciliate community structures

Ciliate community structures were largely different in the different treatments. By the end of the experiment, the total abundance of ciliates increased in FILTER (from 21.9 to 68.7 cells mL^{-1}) and CONTROL (from 21.1 to 31.9 cells mL^{-1}) and decreased in CLAD (from 28.3 to 22.5 cells mL^{-1}) and COPE (from 16.9 to 13.9 cells mL^{-1}). The total ciliate biomass, however, increased in all treatments. In FILTER, CONTROL, CLAD, and COPE, the mean total ciliate abundance was 50.7, 33.9, 20.8, and 18.3 cells mL^{-1}, respectively, and mean biomass was 817, 599, 344, and 255 $\mu g\ L^{-1}$ (as wet weight), respectively (Table 2). Removal of crustaceans initiated an increase in ciliate abundance, which was significantly higher in FILTER than in CLAD and COPE (both $P_{adj} < 0.008333$). In the CONTROL group containing natural lake water, the ciliate abundance was not significantly lower than the abundance in the FILTER treatment; however, the ciliate abundance in the CONTROL group was higher than the abundances in the other two treatments (CLAD, nonsignificant; COPE, $P_{adj} < 0.008333$). There was a significant interaction between time and treatment for both total abundance ($P_{adj} < 0.000167$) and total biomass ($P_{adj} < 0.001667$).

A total of 44 ciliate species from 36 genera were observed in this study. Oligotrichs (mainly *Rimostrombidium brachykinetum*, *R. hyalinum*, and *Limnostrombidium viride*) and prostomatids (mainly *Balanion planctonicum* and *Urotricha farcta*) were the main species in all treatments (Fig. 2). In all containers, abundance and biomass of oligotrichs increased, while those of prostomatids decreased by the end of the incubation period. There was no significant difference in oligotrich density, while prostomatid abundance was significantly higher in FILTER and CONTROL than in the other two treatments ($P_{adj} < 0.008333$) (Table 2).

In terms of body size, small (biovolume < 3000 μm^3) ciliates, including *B. planctonicum*, *Cyrtolophosis mucicola*, *R. brachykinetum*, *U. farcta*, and *Cyclidium* sp., initially composed most of the total ciliate abundance in all treatments, while medium- (biovolume 3000–5000 μm^3, e.g., *R. hyalinum*) and large-bodied (biovolume > 5000 μm^3, e.g., *Askenasia acrostomia*, *A. chlorelligera*, *Balantidium pellucidum*, *Lagynophrya acuminate*, and *R. lacustris*) species dominated the ciliate communities at the end of the experiment (Fig. 3). In regard to biomass, large ciliates occupied the majority of the total ciliate biomass from the beginning to the end of the experiment due to their large individual biovolume. Abundance of small ciliates was significantly higher in FILTER than in CLAD and COPE (both $P_{adj} < 0.008333$), while abundances of medium and large species showed no significant differences among these three treatments (Table 2). In the CONTROL group, neither total ciliate abundance nor biomass were significantly lower than FILTER; however, both were significantly higher than COPE (both $P_{adj} < 0.008333$).

Algivores (e.g., *R. Brachykinetum* and *B. planctonicum*) occupied most the total abundance and biomass in the four treatments (Fig. 4). Algivore abundance increased in FILTER and CONTROL and decreased in the other two treatments. There were significant differences in algivore abundance ($P_{adj} < 0.008333$), with relatively high values in FILTER (40.5 cells mL^{-1}) and

Table 2. Effects of crustacean zooplankton on ciliates (repeated-measures ANOVA, GLM). TREAT = treatment, TIME*TREAT = interaction between time and treatment. Small, medium, and large ciliates refer to ciliate biovolumes of < 3000, 3000–5000, and > 5000 μm^3, respectively. P_{adj} refers to significance level corrected by the Bonferroni technique (Rice 1989). NS, not significant at $P_{adj} < 0.008333$ level

	F	P	P_{adj} ※	Multiple comparisons results※※			
				FILTER	CLAD	COPE	CONTROL
Ciliate abundance							
TREAT	27.23	0.0001	***	50.7^{ad}	20.8^{bcd}	18.3^{bc}	33.9^{abd}
TIME	6.69	0.0019	*	+	–	–	+
TIME*TREAT	7.02	0.0001	***				
Ciliate biomass							
TREAT	28.99	0.0001	***	817^{abd}	344^{abc}	255^{bc}	599^{ad}
TIME	89.61	0.0000	***	+	+	+	+
TIME*TREAT	5.68	0.0003	**				
Oligotrich abundance							
TREAT	6.66	0.0145	NS	32.8	12.3	13.0	20.2
TIME	40.22	0.0000	***	+	+	+	+
TIME*TREAT	7.86	0.0000	***				
Oligotrich biomass							
TREAT	22.51	0.0003	**	662^{abd}	276^{abc}	204^{bc}	450^{ad}
TIME	110.99	0.0000	***	+	+	+	+
TIME*TREAT	3.87	0.0038	*				
Prostomatid abundance							
TREAT	25.94	0.0002	**	16.0^{ad}	6.8^{bc}	4.6^{bc}	12.6^{ad}
TIME	66.73	0.0000	***	–	–	–	–
TIME*TREAT	11.25	0.0031	*				
Prostomatid biomass							
TREAT	15.01	0.0012	**	42^{abd}	23^{abcd}	13^{bcd}	28^{abcd}
TIME	37.11	0.0000	***	–	–	–	–
TIME*TREAT	3.16	0.0117	NS				
Small ciliate abundance							
TREAT	12.55	0.0022	*	35.2^{ad}	13.8^{bcd}	13.8^{bcd}	24.0^{abcd}
TIME	33.40	0.0000	***	–	–	–	–
TIME*TREAT	4.33	0.0019	*				
Small ciliate biomass							
TREAT	10.76	0.0035	*	99^{abd}	46^{abcd}	41^{bcd}	63^{abcd}
TIME	19.83	0.0000	***	–	–	–	–
TIME*TREAT	2.92	0.0173	NS				
Medium ciliate abundance							
TREAT	7.63	0.0099		5.6	2.7	1.5	1.5
TIME	176.45	0.0000	***	+	+	+	+
TIME*TREAT	2.31	0.0495	NS				
Medium ciliate biomass							
TREAT	8.58	0.0070	*	28^{ab}	13^{abcd}	8^{bcd}	8^{bcd}
TIME	196.43	0.0000	***	+	+	+	+
TIME*TREAT	3.09	0.0132	NS				

Large ciliate abundance							
TREAT	10.54	0.0037	*	9.9^{abcd}	4.3^{abcd}	3.0^{abc}	8.4^{abd}
TIME	152.61	0.0000	***	+	+	+	+
TIME*TREAT	6.96	0.0001	***				
Large ciliate biomass							
TREAT	16.72	0.0008	**	690^{abcd}	284^{abcd}	206^{abc}	528^{abd}
TIME	69.14	0.0000	***	+	+	+	+
TIME*TREAT	4.02	0.0031	*				
Algivore abundance							
TREAT	12.01	0.0025	*	40.5^{ad}	14.0^{bcd}	14.9^{bcd}	25.8^{abcd}
TIME	6.74	0.0019	*	+	–	–	+
TIME*TREAT	9.17	0.0000	***				
Algivore biomass							
TREAT	12.39	0.0022	*	524^{abd}	224^{abcd}	176^{bcd}	279^{abcd}
TIME	27.96	0.0000	***	+	+	+	+
TIME*TREAT	2.10	0.0708	NS				
Bacterivore abundance							
TREAT	5.50	0.0240	NS	6.5	5.6	2.7	3.8
TIME	0.30	0.8265	NS	+	–	–	–
TIME*TREAT	1.19	0.3474	NS				
Bacterivore biomass							
TREAT	10.06	0.0043	*	43^{abd}	38^{abd}	22^{cd}	31^{abcd}
TIME	0.72	0.5486	NS	+	–	–	+
TIME*TREAT	1.90	0.1011	NS				
Mixotroph abundance							
TREAT	12.36	0.0023	*	3.3^{abcd}	1.1^{abcd}	0.6^{abc}	4.0^{abd}
TIME	38.60	0.0003	**	+	+	+	+
TIME*TREAT	2.85	0.1054	NS				
Mixotroph biomass							
TREAT	18.27	0.0006	**	187^{abcd}	55^{abcd}	27^{abc}	196^{abd}
TIME	85.60	0.0000	***	+	+	+	+
TIME*TREAT	4.11	0.0027	*				

P_{adj} refers to a significance level of $*P_{adj} < 0.008333$, $**P_{adj} < 0.001667$, $***P_{adj} < 0.000167$.

Different letters (a, b, c, d) within the same line refer to significant differences among treatments (abundance, cells mL^{-1}; biomass, µg L^{-1}). Symbols + (–) within the 'TIME' line refer to an increase (decrease) in ciliate assemblage by the end of the experiment.

CONTROL (25.8 cell mL^{-1}) and low values in CLAD (14.0 cells mL^{-1}) and COPE (14.9 cells mL^{-1}). By the end of the experiment, the composition of the total abundance shifted; in all treatments, the percentage of bacterivores (e.g., *U. farcta*) decreased, and the percentage of mixotrophs (e.g., *L. viride* and *A. chlorelligera*) increased. Together, omnivores (e.g., *B. pellucidum*, *Linostomella vorticella*, and *Hypotrichidium conicum*) and predators (e.g., *Actinobolina radians* and *Litonotus cygnus*) composed less than 1% of the total ciliate abundance and approximately 10% of the total biomass in every treatment.

Twelve taxa, each with an abundance greater than 0.1 cells mL^{-1}, composed 99.0% of the total abundance, including *R. brachykinetum* (average 11.1 cells mL^{-1}, 36.1%), *B. planctonicum* (average 68.2 cells mL^{-1}, 22.1%), *U. farcta* (average 3.2 cells mL^{-1}, 10.2%), *R. hyalinum* (average 2.8 cells mL^{-1}, 9.1%), *R. lacus-*

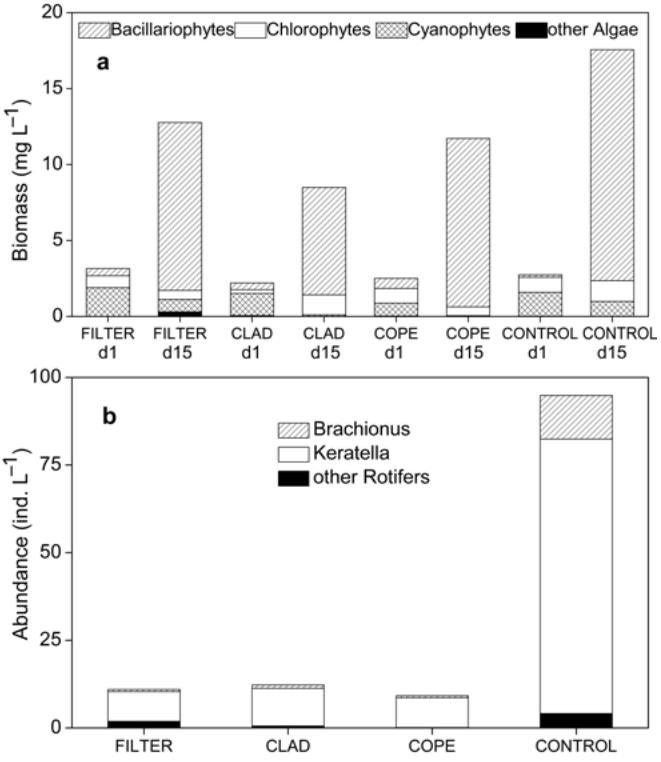

Fig. 1. The initial (d1) and final (d15) biomass of algae **(a)** and final abundance of rotifers **(b)** in the four treatments.

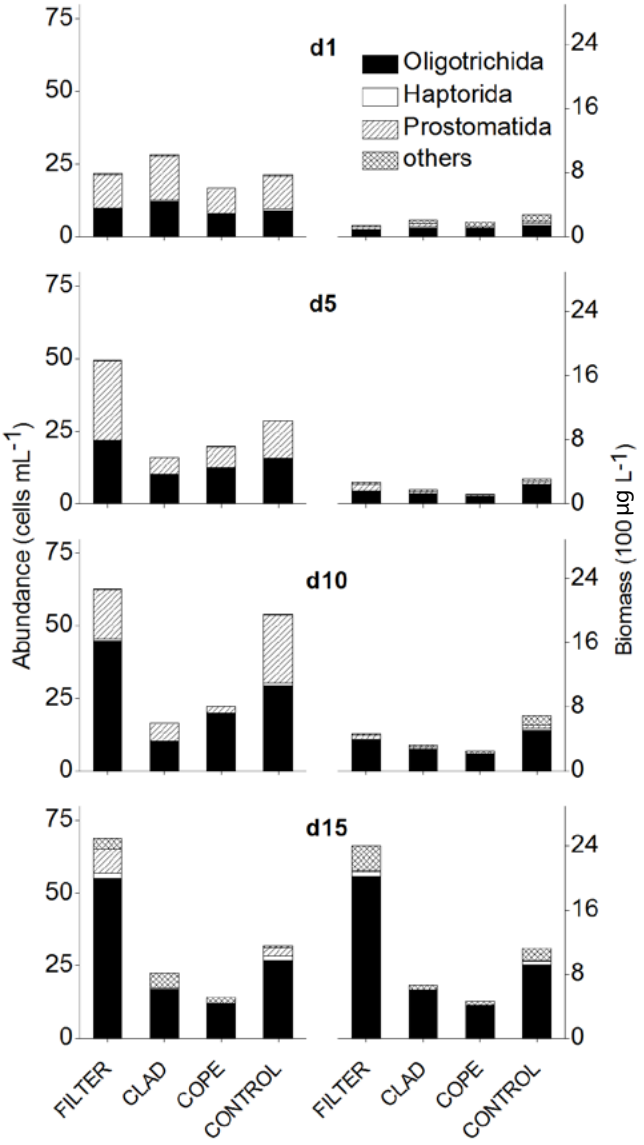

Fig. 2. The abundance and biomass of ciliate taxa in the four treatments. The samples were collected on days 1, 5, 10, and 15.

tris (average 2.5 cells mL^{-1}, 8.2%), *L. viride* (average 2.1 cells mL^{-1}, 6.9%), *Halteria* spp. (average 0.8 cells mL^{-1}, 2.5%), *Cyclidium* sp. (average 0.6 cells mL^{-1}, 1.8%), *A. acrostomia* (average 0.3 cells mL^{-1}, 1.1%), *Tintinnidium pusillum* (average 0.1 cells mL^{-1}, 0.4%), *A. chlorelligera* (average 0.1 cells mL^{-1}, 0.3%), and *Vorticella* spp. (*V. campanula* + *V. aquadulcis* complex, average 0.1 cells mL^{-1}, 0.3%) (Fig. 5). We also found some species that were very common but far less abundant, e.g., *B. pellucidum*, *Codonella cratera*, *H. conicum*, *L. acuminata*, *L. vorticella*, *Pseudostrombidium planctonticum*, and *Pelagostrombidium mirabile* (Fig. 6). With the exception of *A. acrostomia* and *T. pusillum*, each species listed above showed a significant temporal change in abundance. Only five species, however, experienced significant changes in abundance among the different treatments (for *B. planctonicum*, *A. chlorelligera*, and *T. pusillum*, $P_{adj} < 0.001667$; for *R. hyalinum* and *L. viride*, $P_{adj} < 0.008333$). There was no significant interaction between time and treatment for those five species.

DISCUSSION

Our results clearly show that both cladocerans (*Bosmina* sp.) and copepods (pseudodiaptomid *Schmackeria inopinus*) had substantial impacts on the abundance, biomass, and winter ciliate community structure in Lake Chaohu, and we highlight the importance of interference and exploitative competition among metazooplankton groups. Total ciliate abundance significantly increased in response to the removal of crustacean

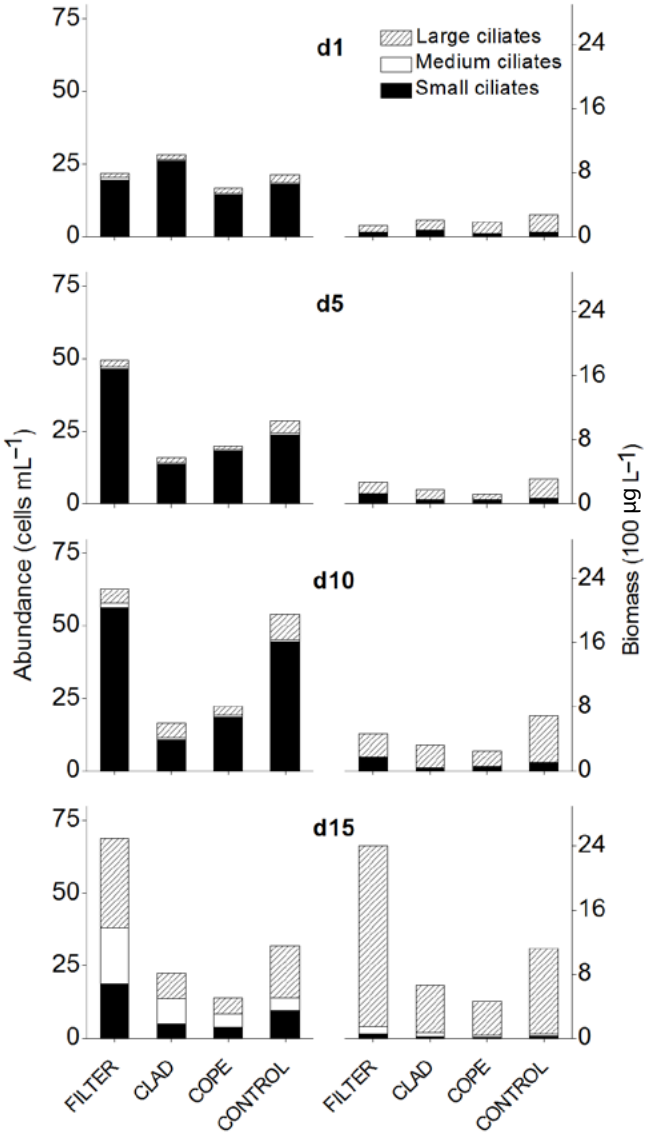

Fig. 3. Ciliate abundance and biomass by classification of body size. Small, medium, and large ciliates refer to a ciliate biovolume of < 3000, 3000–5000, and > 5000 µm^3, respectively.

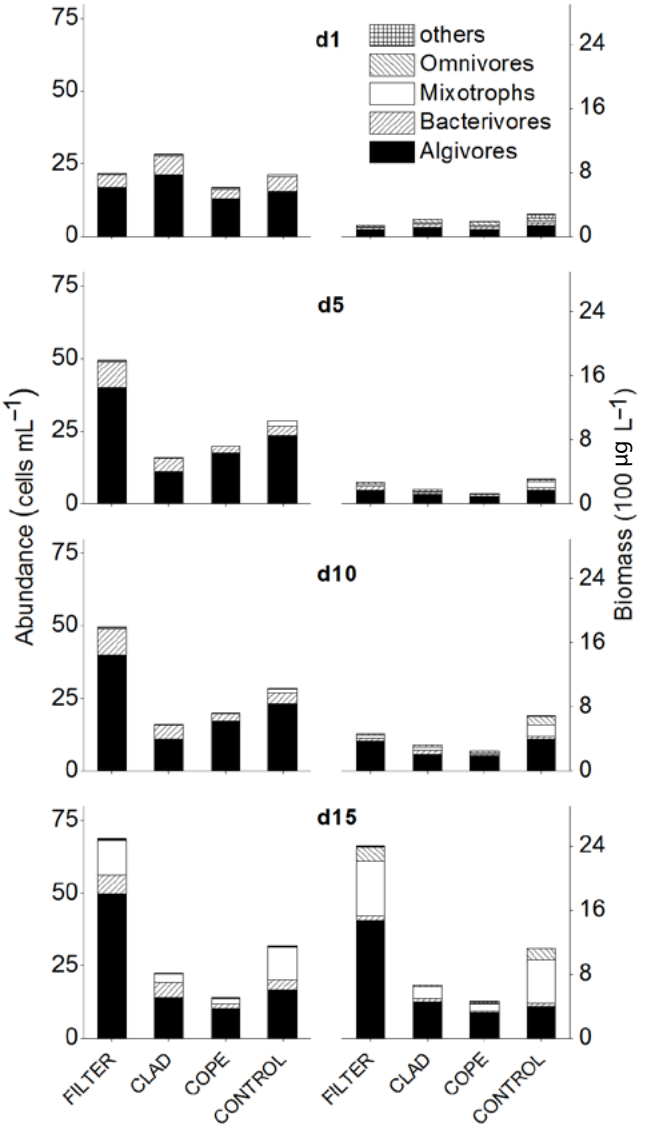

Fig. 4. Abundance and biomass of ciliate functional feeding groups.

zooplankton, mainly *Bosmina* sp. and pseudodiaptomid *Schmackeria inopinus*, while other comparable studies commonly used other crustacean zooplankton species, e.g., *Daphnia*, cyclopoids, and diaptomids (e.g., Burns and Gilbert 1993; Jürgens 1994; Wiackowski *et al.* 1994; Wickham 1995; Adrian and Schneider-Olt 1999; Agasild *et al.* 2013). This study is in line with several other studies, demonstrating that ciliate response is dependent on both the ciliate and crustacean zooplankton species being studied (Jürgens 1994; Wiackowski *et al.* 1994). Here, the response of ciliates was different in the presence of copepods (*Schmackeria inopinus*) compared with cladocerans (*Bosmina* sp.) and varied among categories including ciliate population, relative body size, and functional feeding groups (Table 2).

The few copepods remaining in the CLAD treatment at the end of the experiment may have been caused by nauplii passing through the plankton net (64 µm), and the same mechanism may explain the *Bosmina* sp. abundance in the COPE treatment (small individuals slipped through the plankton net) and the results of the FILTER treatment. These animals, how-

ever, composed very small proportions of the total abundance of crustacean zooplankton in the CLAD and COPE treatments. Screening may also remove rotifers (mainly *Keratella* spp. and *Brachionus* spp.); thus, there was significantly low rotifer density in the FILTER, CLAD, and COPE treatments. For the small cladocerans, *Bosmina* sp. always made up more than 89% of the cladoceran abundance, and the *Bosmina* sp. impacted the ciliates by suppressing their population. Similarly, the pseudodiaptomid *Schmackeria inopinus* is believed to be the main contributor to the suppression of ciliates by copepods. This short-term and small-capacity experimental incubation may overestimate the impacts of crustacean zooplankton on ciliates occurring in lakes over longer spatial scales (Sarnelle 1997). Still, the response of ciliates to the presence and absence of crustacean zooplankton groups can provide extensive information on the interactions between crustacean zooplankton and ciliate trophic levels.

Previous studies have documented that medium- and large-bodied crustaceans (e.g., daphnids) were the most effective grazers that might strongly impact ciliate populations, while small forms (e.g., *Bosmina* and *Chydorus*) were challenged by their top-down control on these unicellular heterokaryotic organisms (Gilbert 1989; Wickham and Gilbert 1991; Ventelä *et al.* 2002). In the present study, we identify the suppression effects of small *Bosmina* sp. on ciliates based on two pieces of evidence. First, total ciliate abundance decreased in the treatment that only included cladocerans (CLAD), while it increased in the FILTER treatment. Second, the total number of ciliates was significantly higher in FILTER than in CLAD (Table 2). The suppression effects, however, may also depend on the abundance of *Bosmina* sp. During the incubation period, the total ciliate abundance decreased quickly in the first five days and then increased by a small margin starting on day 10 in the CLAD treatment. This trend was likely caused by the decline in *Bosmina* sp. abundance, from 26.0 to 5.1 individuals L^{-1}. Suppression impacts of *Bosmina* on ciliates were also observed in an *in situ* investigation in Lake Chaohu (Li *et al.* 2016). The results from this study also suggested that the dominance of *Bosmina* sp. may contribute to the relatively low ciliate abundance in Lake Chaohu in winter.

The temporal dynamic pattern of ciliate abundance in COPE was different from that in CLAD. In the presence of copepods only, the total abundance of ciliates initially increased slightly and then largely decreased after day 10, probably because of the change in developmental instar composition of the copepod population. The importance of ciliates as a food resource for copepods has been highlighted by several indoor studies (e.g., Hartmann *et al.* 1993; Kamjunke *et al.* 2012; Dhanker *et al.* 2013), and clear suppression of ciliate populations by copepods, including both cyclopoids (e.g., *Cyclops*, *Diacyclops*, and *Thermocyclops*) and calanoids (e.g., *Eudiaptomus* and *Diaptomus*), has been verified in many *in situ* experiments (e.g., Wiackowski *et al.* 1994; Adrian and Schneider-Olt 1999; Jürgens *et al.* 1999; Hansen 2000). This study provides further evidence for strong top-down control effects on ciliates by copepods (mainly pseudodiaptomids *Schmackeria inopinus*), as we found significantly lower total ciliate abundance and biomass in the treatment containing only copepods (COPE) than in the FILTER treatment. Nevertheless, when cladocerans and copepods were simultaneously abundant, their top-down control on ciliates might have declined, as the total of both ciliate abundance and biomass were lower in CONTROL than in FILTER but were relatively higher than those in CLAD and COPE. This may have been caused by the interference and exploitative competition among the metazoan zooplankton groups (copepods, cladocerans, and rotifers) (Gilbert and MacIsaac 1989; MacIsaac and Gilbert 1991).

Taxonomic replacements occurred in every treatment. Generally, small *Balanion planctonicum* and *Urotricha farcta* were replaced by omnivorous – bacteria and picocyanobacterial feeders belonging to Oligotrichida (mainly small *Rimostrombidium brachykinetum*, medium *R. hyalinum*, and large *R. lacustris* and *Limnostrombidium viride*); however, they varied in their susceptibility to crustacean zooplankton groups. As shown in Fig. 2, we found a nearly two-fold increase in prostomatid abundance during the first five days after crustacean zooplankton were removed, but prostomatid numbers rapidly decreased by ca. 60% in CLAD during the first five days and by ca. 70% in COPE from the fifth to the tenth day. Statistical analysis showed that prostomatid abundance was significantly suppressed by cladocerans (*Bosmina* sp.) and copepods (*Schmackeria inopinus*), and this suppression effect significantly interacted with incubation time. Several studies found that suppression effects by crustacean zooplankton were higher on oligotrichs compared with prostomatids and other ciliate species (Hansen 2000; Zöllner *et al.* 2003). In this study, the total number of oligotrichs was not restrained by crustaceans; however, their biomass was significantly suppressed by copepods, and species replacements occurred within oligotrich ciliates. Final-

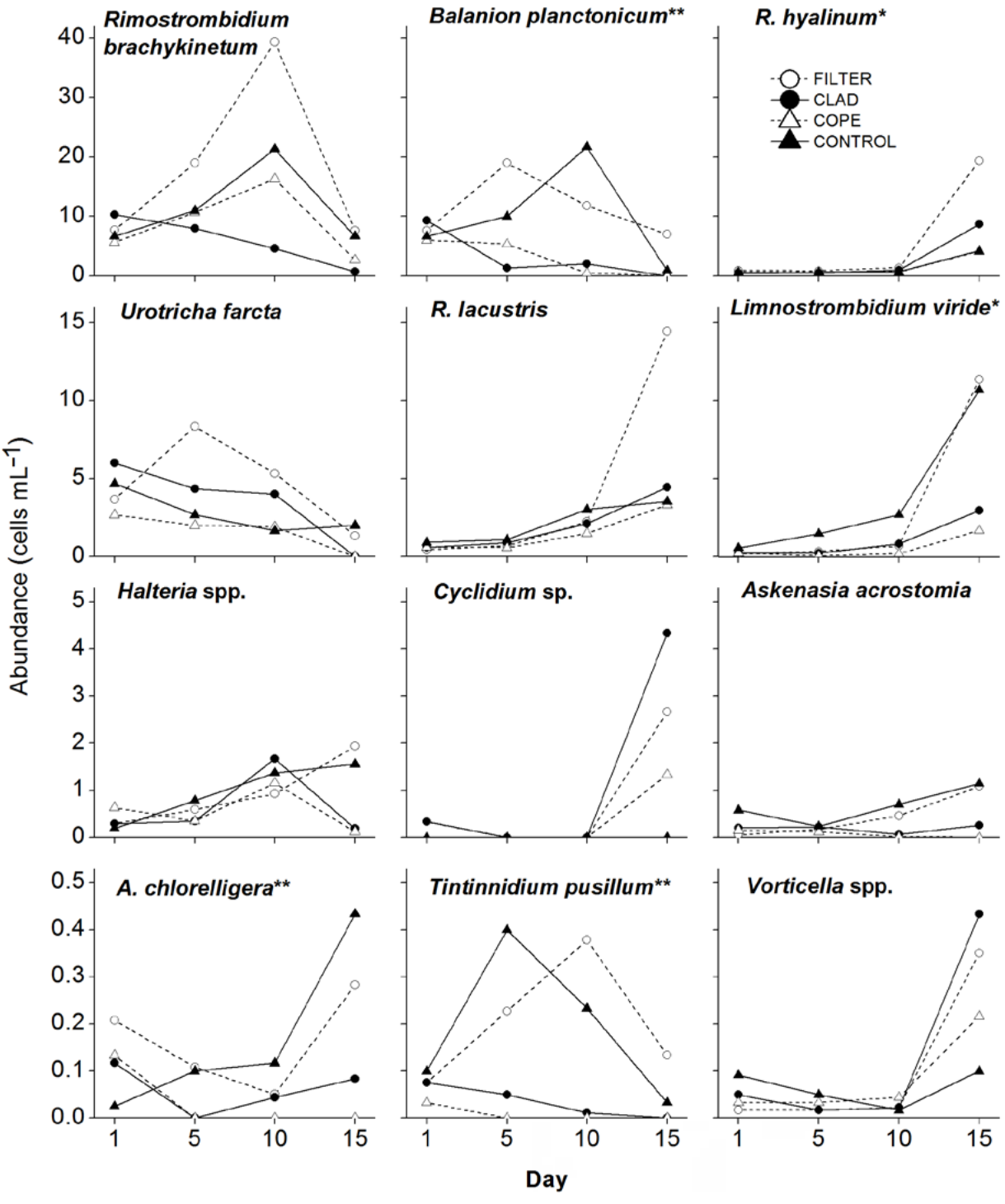

Fig. 5. Dynamics of some dominant ciliate species during the experiment. The samples were collected on days 1, 5, 10, and 15. *$P_{adj} < 0.008333$, **$P_{adj} < 0.001667$

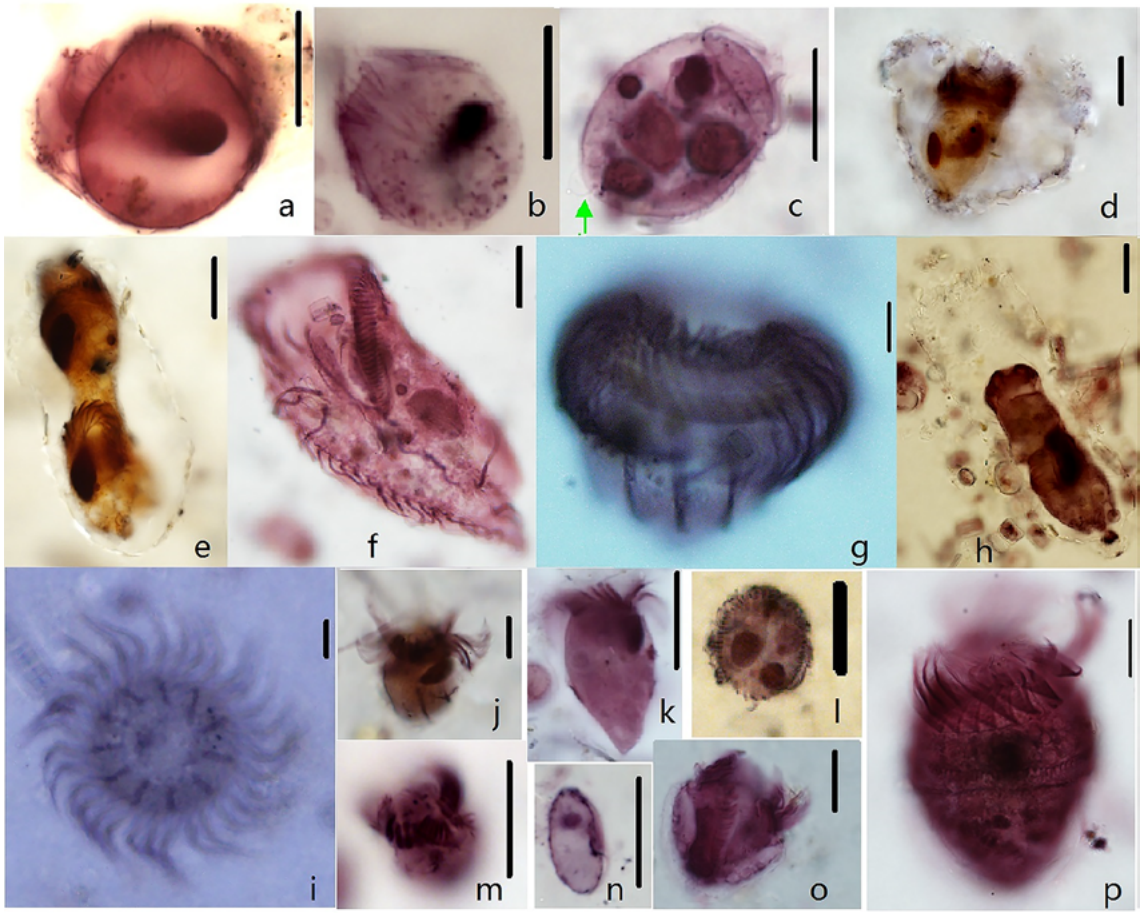

Fig. 6. Microphotographs of some common ciliates stained with the QPS approach during the experiment. **(a)** *Askenasia acrostomia*, **(b)** *Askenasia chlorelligera*, **(c)** *Balanion planctonicum*, **(d)** and **(e)** *Codonella cratera*, **(f)** *Pseudostrombidium planctonticum*, **(g)** and **(i)** *Rimostrombidium lacustris*, **(h)** *Tintinnidium pusillum*, **(j)** *Rimostrombidium hyalinum*, **(k)** *Rimostrombidium brachykinetum*, **(l)** *Urotricha farcta*, **(m)** *Halteria* sp., **(n)** *Cyclidium* sp., **(o)** *Pelagostrombidium mirabile*, **(p)** *Limnostrombidium viride*. All photographs were taken with an Olympic DP73 digital camera mounted on an Olympic BX51 light microscope. Scale bar equals 10 μm.

ly, predominant small *R. brachykinetum* decreased, and medium-bodied *R. hyalinum* and large-bodied *R. lacustris* and *L. viride* dominated the oligotrich ciliates.

At the community level, both small (< 14.4 μm of equivalent spherical diameter) and medium (14.4–17.1 μm of equivalent spherical diameter) ciliates were clearly affected by copepods, while cladocerans only suppressed small ciliates (Table 2). This may be a result of particle selection in the crustacean zooplankton feeding process (Barnett *et al.* 2007). Functional feeding groups, especially algivores, were also strongly affected by both cladocerans and copepods. Algal food competition between ciliates and crustaceans may have induced the significantly lower abundance of algivore ciliates in CLAD (e.g., *R. brachykinetum*) and COPE (e.g., *B. planctonicum*) than that seen in FILTER (Carrick *et al.* 1991; Agasild *et al.* 2007). The decrease in bacterivore (e.g., *U. farcta*) abundance percentage in every treatment may have also been caused by the reduced food resources available in containers than those found in natural lake water, in addition to the suppression effect induced by copepods.

In conclusion, we have verified that both cladocerans (*Bosmina* sp.) and copepods (pseudodiaptomid *Schmackeria inopinus*) had substantial impacts on winter ciliate abundance, biomass, and community structure in Lake Chaohu, and we highlight the importance of interference and exploitative competition among metazooplankton groups. The removal of crustacean zooplankton, mainly *Bosmina* sp. and pseudodiaptomid *Schmackeria inopinus*, initiated a significant increase in ciliate abundance during the incubation period. Pros-

tomatid abundance was significantly suppressed by both cladocerans and copepods, and this suppression effect significantly interacted with incubation time. Oligotrich abundance was not restrained by crustacean groups, but their biomass was strongly impacted by copepods; moreover, taxonomic replacements occurred within these species. In terms of body size, both small and large ciliates were strongly suppressed by copepods, while the suppression effect of cladocerans only affected small ciliates. In contrast to bacterivores and other functional feeding groups, algivores were strongly affected by both cladocerans and copepods.

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