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PRIMARY RESEARCH PAPER

Impacts of large-bodied crustaceans on the microbial loop

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Abstract We conducted a mesocosm experiment to assess the impacts of large-bodied crustaceans on microbial communities. Three alien crustacean species (*Daphnia pulex*, *Simocephalus vetulus* and *Macrocyclops albidus*) were collected from the regional species pool and added to mesocosms that were filled with water from a eutrophic lake (Masurian Lake District, Poland). We then analysed chemical (total phosphorus and nitrogen concentrations) and biological (algae, bacteria, nanoflagellates, ciliates, rotifers, crustaceans) parameters over the course of the 40 day experiment. Alien crustacean species constituted 59–88% of the total crustacean biomass throughout the experiment. The final biomass of bacteria and copepods were not affected by the addition of alien species. However, rotifer and native cladoceran biomass tended to be lower while nanoflagellate and ciliate biomass were higher in mesocosms with alien species. Our research suggests that the large-bodied crustaceans altered the structure of the microbial loop. In the control, nanoflagellates were likely the main consumers of bacteria and thus constituted the main link between bacteria and higher trophic levels. In the mesocosms with large-bodied crustaceans, protists were likely not important as bacterial grazers because of strong top-down control of nanoflagellates by crustaceans. Combined, our results provide evidence that alien large-bodied crustaceans can significantly impact the microbial loop.

Keywords Crustaceans · Microbial loop · Trophic relations · Invasive species

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Introduction

Metazooplanktons including rotifers, cladocerans and copepods can influence the major components of the microbial loop (i.e., bacteria, nanoflagellates and ciliates). Metazooplanktons not only affect the abundance and species composition of microbial communities, but they also alter the structure and trophic

relationships of the microbial loop (Pace et al., 1984; Arndt, 1993; Sanders & Wickham, 1993; Gasol et al., 1995; Bec et al., 2003). As such, the microbial loop is tightly coupled to classic aquatic food web ecology through many direct and indirect pathways (Riemann & Christoffersen, 1993).

The impacts of crustacean zooplankton, especially *Daphnia* species, on planktonic microbial communities are well described from laboratory and field experiments (reviewed by Jürgens, 1994). Due to their ability to filter a wide range of food particle sizes, large-bodied *Daphnia* can control most biotic components of the microbial loop (DeBiase et al., 1990; Kopylov & Kosolapov, 2011), and sometimes they are more important as bacterial consumers than protists (Jürgens & Stolpe, 1995). The presence of large-bodied *Daphnia* can lead to reductions in autotrophic and heterotrophic nanoflagellates and the exclusion of protists as the main bacterial consumers. Under this situation, energy and organic carbon are transferred directly from bacteria to cladocerans, thereby shortening the food chain (Jürgens et al., 1994).

Compared to *Daphnia*, less research has been conducted on the influences of other large-bodied cladocerans on the microbial loop. For example, Bec et al. (2003) showed that *Simocephalus vetulus* (Daphnidae) feeds on autotrophic (*Cryptomonas ovata*) and heterotrophic (*Paraphysomonas vestita*) flagellates as well as on ciliates (*Cyclidium glaucoma*) and particulate amorphous organic matter. Individual *S. vetulus* exhibited high fecundity and growth when fed the autotrophic flagellate *C. ovata* (Bec et al., 2003). Therefore, food quality, in terms of the variety of the microbial loop components that a cladoceran is able to feed upon, may be more important than food quantity for survival, growth and reproduction (Norsker & Støttrup, 1994; Goedkoop et al., 1998; Bec et al., 2003).

It is also important to note that large-bodied cyclopoid copepods such as *Macrocyclops albidus* impact microbial communities differently than cladocerans. Large-bodied copepods are not important as bacterial grazers (Sanders et al., 1989), but can effectively consume protists (heterotrophic nanoflagellates and ciliates) and rotifers and cladocerans (Laybourn-Parry et al., 1988; Sanders & Wickham, 1993; Jack & Gilbert, 1997). Little is known about the combined impacts of different large-bodied crustacean species (both cladocerans and copepods) on the major components of the microbial loop and native zooplankton communities in general. The consequences of such impact are very difficult to predict due to higher feeding efficiency of large-bodied crustaceans (Gliwicz, 2004). While a large body of research has shown that non-native species often have strong impacts on aquatic ecosystems through various processes including predation, disturbance, habitat modification and competition (Hall & Mills, 2000; Ricciardi & MacIsaac, 2011), we know of no literature data on the role of alien species in structuring communities within the microbial loop in freshwater ecosystems.

The aim of the present study was to determine how the introduction of three large-bodied crustacean species (*D. pulex*, *S. vetulus* and *M. albidus*) affected the structure, function and relationships in the microbial loop in a series of experimental mesocosms. We predicted that large-bodied alien crustaceans would affect the structure of the microbial loop through top-down control. Specifically, large-bodied crustaceans would graze more effectively on algae and protists than small-bodied crustaceans.

Materials and methods

A 40 day mesocosm experiment was conducted from 20 July to 30 August, 2011. Water for the experiment was taken from 1 m below the surface of the pelagic zone of eutrophic Lake Mikołajskie (Masurian Lake District, northeastern Poland; area 498 ha, max. depth 26 m, mean depth 11 m). Mesocosms (internal dimensions 940 × 640 × 500 mm; 300 l) were filled with unfiltered lake water (270 l) using an electrical pump and placed on the shore of Lake Mikołajskie.

The experimental design consisted of two treatments with three replicates each. Three of the mesocosms served as a control and were filled with unfiltered water that contained only in situ zooplankton and microbial communities from the source water. An Alien Species (AS) treatment was created by adding a mixture of zooplankton that was dominated by three species of alien crustaceans: *Daphnia pulex* Leydig, *Simocephalus vetulus* (O. F. Müller) and *Macrocyclops albidus* (Jurine). The initial biomass of alien crustaceans in the AS treatment was 0.037 mg l^{-1} for *D. pulex*, 0.354 mg l^{-1} for *S. vetulus* and 0.235 mg l^{-1} for *M. albidus* (Fig. 1). These three

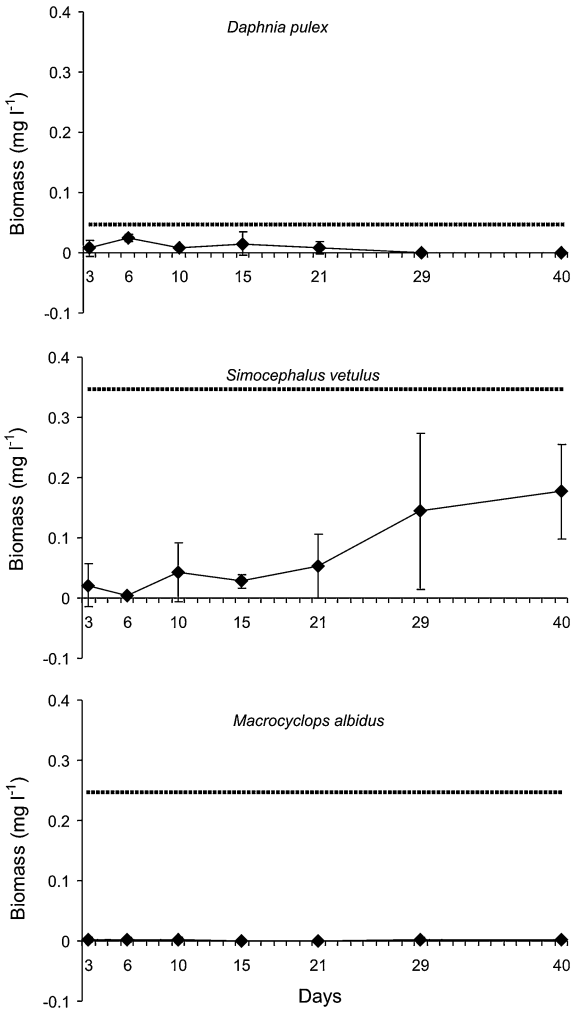

Fig. 1 Average biomass of alien species in the Alien Species (AS) treatment over the course of the experiment. *Error bars* represent standard deviations. The initial biomass (day 0) at which each species was added to the mesocosms before the first sample collection are represented by the *vertical lines*

species of large-bodied crustaceans were not present in the source water, but were part of the regional zooplankton pool. They were collected from ponds that were located near the lake and within the distance that zooplankton are thought to actively disperse in nature (approximately 100 km; Shurin, 2000), provided that biotic or abiotic factors do not hinder their development (Shurin, 2000). The sizes of *D. pulex* (1.3–2.5 mm), *S. vetulus* (2.0–3.0 mm), and *M. albidus* (1.5–2.5 mm) are very similar. The food requirements for large-bodied *Daphnia* spp. and *S. vetulus* overlap (algae, bacteria, protists), and they compete in nature, e.g. in Lake Naroch (Semenchenko et al., 2007). Adult *M. albidus* are commonly predators (Rey et al., 2004); however, early nauplii and copepodites stages can graze on phytoplankton and ciliates (Adrian & Schneider-Olt, 1999) such that they also likely compete with the other two alien taxa at least during some life stages. After the alien species were added, the mesocosms were allowed to stabilize for 3 days before the first samples were collected. Water samples were collected from the mid-depth of each mesocosm using a 2.6 l Limnos sampler and then analysed for the chemical and biological parameters described below. Samples were collected on day 3 (3 days after addition of the alien species), 6, 10, 15, 21, 29 and 40 of the experiment. The trophic state index (TSI), calculated from chlorophyll *a* and total phosphorus (TP) concentrations according to Carlson (1977), indicated that waters in both the control (TSI from 44.6 to 59.1; mean 51.6) and AS (TSI 45.2–53.8; mean 51.9) treatments were meso/eutrophic.

Temperature and oxygen concentration were measured daily in the mesocosms using a WTW multiparameter probe 3410 with optical sensor FDO 925. TP, total Kjeldahl nitrogen (TKN) and nitrate–nitrogen ($N–NO_3$) concentrations were analysed by the standard analytical procedures described in Standard Methods (2005). We used the sum of Kjeldahl nitrogen and nitrate–nitrogen to represent total nitrogen (TN).

Concentrations of chlorophyll *a* and major carotenoids (peridinin, fucoxanthin, alloxanthin, zeaxanthin) were measured by quantitative high-performance liquid chromatography (HPLC). Water samples were filtered through Whatman GF/F filters (0.7 µm, 2.5 cm diameter) and immediately frozen. The pigments were extracted ultrasonically (Sonoplus HD 2070) with methanol and analysed using Shimadzu HPLC System equipped with a UV–Vis and fluorescence detector on a Waters Spherisorb C_{18}ODS2 column. The gradient method recommended by SCAR (Wright et al., 1991) was used to separate pigments. The pigments were identified by comparison of their retention times and absorption spectra with standards (DHI LAB products) and also with literature data (Jeffrey et al., 1997). Calibration curves were made using external standards.

Bacteria were enumerated in formalin-fixed samples (1.5% final concentration) after DAPI staining (Porter & Feig, 1980) under an epifluorescence

microscope equipped with a digital camera. The size of bacterial cells was measured using Nikon Nis-Elements image analysis system. A minimum of about 1,000 cells were counted on each filter (black, 0.2 μm). Bacterial biomass was calculated from the bacterial number per percentage share of mean cell volume in each length class (0.2–0.5; 0.5–1.0; 1.0–3.0 μm).

Nanoflagellate (NF) samples were fixed with formaldehyde (final concentration 2%), stained with DAPI (Porter & Feig, 1980), filtered through 1.0 μm pore size polycarbonate membrane filters and enumerated by epifluorescence microscopy (Nikon Optiphot 2). The NF biovolume was calculated from measurements of cells size and their approximations to simple geometric forms. Autotrophic (ANF) and heterotrophic nanoflagellates (HNF) were differentiated on the basis of chlorophyll *a* autofluorescence.

Ciliate samples were fixed with Lugol's solution and examined with a light microscope (Nikon Optiphot 2). Biovolume was calculated from measurements of cell dimensions and simple geometric shapes. Species identifications of ciliates were based mainly on Foissner et al. (1991–1995).

Rotifers and crustaceans were collected with a 2.6-l Limnos sampler and then concentrated using a 30 μm mesh plankton net and preserved with Lugol's solution and 4% formalin. At the end of the experiment, a larger volume of water (60 l) was sampled from each mesocosm. Rotifers and crustaceans were identified and enumerated under the microscope after sedimentation. Length:wet weight relationships were used to derive the mean body weights. Approximately 10 individuals of each species were measured to determine the body length, and length:weight relationships were used to determine the biomass of rotifers using Ejsmont-Karabin (1998) and the biomass of crustaceans using Balushkina & Vinberg (1978).

We used Repeated Measures Analysis of Variance (RM-ANOVA) to determine if the AS treatment affected the physical, chemical and biological parameters described above. We were particularly interested in the main treatment effect or interactions between the treatment and time to determine if there were differences between the control and AS mesocosms. Data were log transformed if necessary to help meet the assumptions of normality. All RM-ANOVAs were conducted using Sigma Stat (3.5). When significant differences were detected with RM-ANOVA, we used Tukey's HSD post hoc comparisons to determine on which dates the differences occurred ($P < 0.05$). Pearson's correlation coefficients were also calculated between pairs of biological variables in the control and AS treatments to determine trophic relationships among the studied groups of organisms. Data from the three replicate mesocosms on each sample date ($n = 21$ for each treatment) were used to perform correlations for the control and AS treatments.

Table 1 Basic physical and chemical parameters in the control and Alien Species (AS) treatments over the course of the experiment (range with mean values and standard deviations in parentheses)

Parameter	Control	AS
Temperature (°C)	16.3–19.3	16.5–19.2
	(17.6 ± 1.2)	(17.7 ± 1.2)
Oxygen (mg l^{-1})	8.46–10.68	8.78–9.93
	(9.38 ± 0.84)	(9.18 ± 0.50)
Total P (μg l^{-1})	46–113	47–90
	(70.7 ± 23.6)	(65.0 ± 18.4)
Total N (mg l^{-1})	1.21–1.49	1.29–1.52
	(1.39 ± 0.12)	(1.39 ± 0.08)

Results

Physical and chemical characteristics

The physical and chemical characteristics of the mesocosms were not affected by the addition of alien species. Water temperature, dissolved oxygen, total phosphorus (TP) and total nitrogen (TN) did not differ between the control and AS treatments (all RM-ANOVAs, $P > 0.25$ for main treatment effect). The range with mean values of the physical and chemical variables in the two treatments is presented in Table 1.

Crustacean biomass and composition

Among the alien species, *S. vetulus* dominated throughout most of the experiment (Fig. 1). However, by the end of the experiment the mean biomasses of all three alien species were lower than the initial biomasses at which they were added to the mesocosms.

The addition of alien species had a significant negative effect on the biomass of native cladocerans in

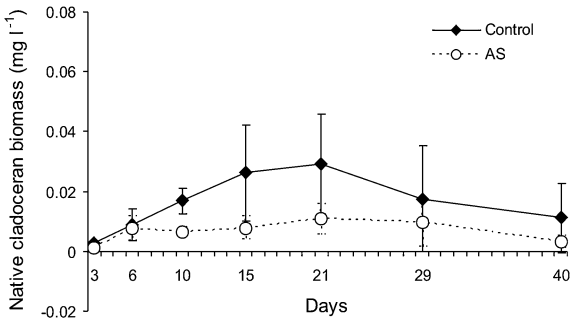

Fig. 2 Changes in native cladoceran biomass in the control and Alien Species (AS) treatments over the course of the experiment. *Error bars* represent standard deviations. Note the difference in scale between Figs. 2 and 1

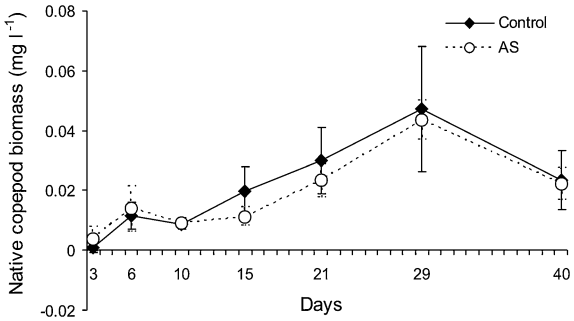

Fig. 3 Changes in native copepod biomass in the control and Alien Species (AS) treatments over the course of the experiment. *Error bars* represent standard deviations. Note the difference in scale between Figs. 3 and 1

the AS mesocosms (RM-ANOVA, treatment effect, $P < 0.033$). In the control, native cladoceran biomass increased gradually from 0.0026 ± 0.0007 to 0.0292 ± 0.0168 mg l^{-1} on day 21 and then decreased (Fig. 2). In the AS mesocosm, cladoceran biomass increased at the start of the experiment (from 0.0010 ± 0.0003 to 0.0075 ± 0.0041 mg l^{-1}), remained at a relatively constant level during next days and then decreased to 0.0031 ± 0.0020 mg l^{-1} at the end of the experiment. There were no significant differences in native copepod biomass between mesocosms with and without alien species (RM-ANOVA, $P > 0.05$). Native copepod biomass showed almost identical trends in the two treatments (Fig. 3). Biomass increased markedly on day 29 (from 0.0009 ± 0.0016 to 0.0472 ± 0.0208 mg l^{-1} and from 0.0037 ± 0.0040 to 0.0434 ± 0.0065 mg l^{-1} in the control and AS mesocosms, respectively) and then distinctly decreased.

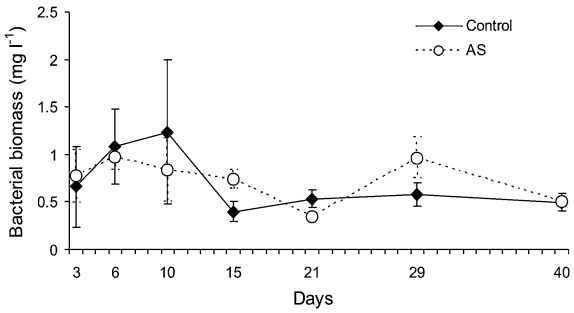

Fig. 4 Changes in bacterial biomass in the control and Alien Species (AS) treatments over the course of the experiment. *Error bars* represent standard deviations

A total of 16 crustacean taxa were identified in the control while 19 were identified in the AS mesocosms over the course of the experiment. Three species dominated the biomass in the control mesocosms—*Bosmina coregoni* Baird at the start of the experiment, then *Mesocyclops leuckarti* (Claus) and from day 15 to the end—*Eudiaptomus graciloides* Lilljeborg. Different community structure of native crustaceans was found in the AS mesocosms, where *Acanthocyclops vernalis* (Fischer) and *Diaphanosoma brachyurum* (Lievin) dominated during the first half of the experiment and then after 18 days the community was dominated by *Eudiaptomus graciloides*.

Microbial loop components

Bacterial biomass fluctuated throughout the experiment (Fig. 4). In the control, biomass increased rapidly from 0.66 ± 0.42 to 1.24 ± 0.75 mg l^{-1} during the first 10 days of the experiment, decreased to approximately 0.4 mg l^{-1} on day 15, and then remained relatively constant until the end of the experiment. In the AS mesocosms, bacterial biomass decreased gradually at first, but later increased reaching maximum of 0.97 ± 0.21 mg l^{-1} on day 29, and then dropped. Similar trends were observed in the bacterial abundances that fluctuated between 3.85 ± 1.04 and $11.00 \pm 3.45 \times 10^6$ ml^{-1} in the control and between 5.17 ± 0.11 and $11.20 \pm 2.46 \times 10^6$ ml^{-1} in the AS treatments (data not shown). Despite these variations, there were no significant differences in bacterial biomass or abundance between the control and AS treatments (RM-ANOVA, $P > 0.05$).

Variations in nanoflagellate biomass were similar to those observed for bacteria (Fig. 5). In the control, after

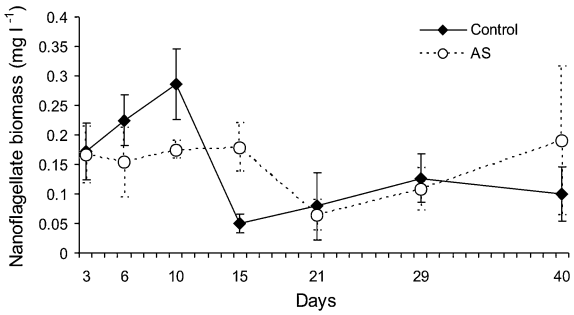

Fig. 5 Changes in total nanoflagellate biomass in the control and Alien Species (AS) treatments over the course of the experiment. *Error bars* represent standard deviations

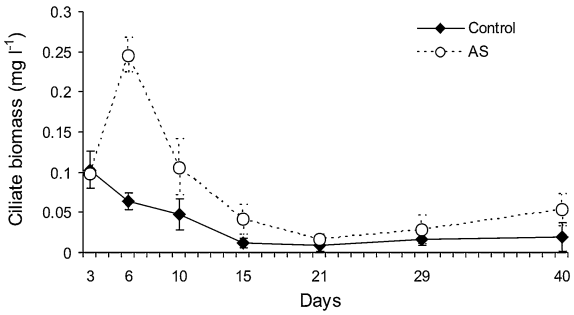

Fig. 6 Changes in total ciliate biomass in the control and Alien Species (AS) treatments over the course of the experiment. *Error bars* represent standard deviations

a distinct increase from 0.172 ± 0.048 to 0.286 ± 0.060 mg l^{-1} during the first 10 days of the experiment, nanoflagellate biomass drastically decreased to 0.049 ± 0.016 mg l^{-1} on day 15, and then gradually increased until the end of the experiment. In the AS mesocosms, nanoflagellate biomass remained at a relatively constant level during the first half of the experiment
(0.153 ± 0.058 to 0.179 ± 0.041 mg l^{-1}), decreased to 0.064 ± 0.026 mg l^{-1} during next few days, and then increased to the end of the experiment. In the control, autotrophic forms (ANF) dominated during approximately the first half of the experiment, while heterotrophic cells (HNF) dominated during the second half of the experiment. In the AS mesocosms, HNF dominated throughout most of the study with ANF representing a greater proportion of the biomass at the start of the experiment. However, there were no significant differences in nanoflagellate biomass between the control and AS treatments (RM-ANOVA, $P > 0.05$).

Ciliate biomass was significantly higher in the AS treatment than it was in the control (RM-ANOVA, $P < 0.001$ for both treatment effect and the time × treatment interaction). Specifically, post hoc comparisons found that ciliate biomass was higher in the AS treatment on days 6, 10 and 15. In the control mesocosms, ciliate biomass decreased gradually from 0.103 ± 0.023 to 0.009 ± 0.007 mg l^{-1} throughout the experiment. In the AS mesocosms, it increased initially from 0.098 ± 0.007 to 0.246 ± 0.021 mg l^{-1}, but subsequently declined to lower levels (Fig. 6). The two treatments also differed in the community structure of ciliates. Three ciliate orders were dominant in the control mesocosms including Oligotrichida, represented by small species from the genus *Rimostrombidium* and *Halteria* (this order dominated at the start and on days 15 and 29 of the experiment), Prostomatida, mainly composed of *Coleps* (dominated on day 6), and Peritrichida, mainly *Vorticella* (dominated on days 10, 21 and 40). The contribution of these orders to the total biomass was 42–77%. In the AS mesocosms, Oligotrichida (mainly *Strombidium* sp. and *Halteria grandinella*) dominated throughout most of the experiment, accounting for 51–86% of the total biomass. On days 15 and 40 of the experiment, Haptorida (mainly *Askenasia volvox*) constituted substantial part of the total biomass (34 and 46%).

Rotifer biomass and composition

Rotifer biomass tended to be lower in the AS treatment than it was in the control; however, this effect was only marginally significant (treatment effect, $P = 0.056$). Rotifer biomass showed similar trends in both treatments (Fig. 7). Biomass increased during the first half of the experiment (from 0.151 ± 0.031 and 0.126 ± 0.051 mg l^{-1} in the control and AS mesocosms, respectively), reached maximal values on days 15 and 21 and then rapidly dropped to very low levels of 0.086 ± 0.068 mg l^{-1} in the control and 0.005 ± 0.004 mg l^{-1} in the AS mesocosms. Maximum rotifer biomass in the control (2.271 ± 1.459 mg l^{-1}) was two times higher than in the AS mesocosms (1.185 ± 0.452 mg l^{-1}).

Rotifer community structure was very similar in both treatments. At the start of the experiment, algivorous *Polyarthra vulgaris* dominated, constituting 24% of the

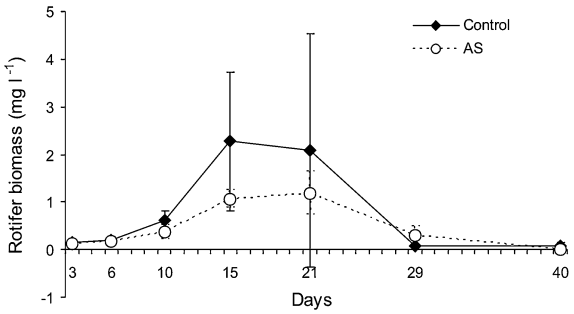

Fig. 7 Changes in total rotifer biomass in the control and Alien Species (AS) treatments over the course of the experiment. *Error bars* represent standard deviations

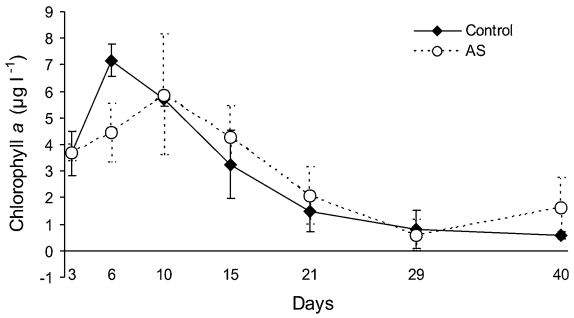

Fig. 8 Changes in chlorophyll *a* concentrations in the control and Alien Species (AS) treatments over the course of the experiment. *Error bars* represent standard deviations

total biomass. During the next days of the experiment, the predatory *Asplanchna priodonta* was the most abundant rotifer (30–96% of the total biomass). At the last day of the experiment, bacterivorous *Lepadella patella* dominated in the control mesocosms while algivorous *P. vulgaris*—in the AS mesocosms, constituting 26 and 27% of the total biomass, respectively.

Phytoplankton biomass and pigment composition

The addition of large zooplankton had little effect on algal biomass in the mesocosms. There were significant time × treatment interactions (RM-ANOVA, $P < 0.05$) for chlorophyll *a* and peridinin (typical of Dinophyta) concentrations (data not shown). However, post hoc tests (Tukey's HSD, $P < 0.05$) showed that concentrations were lower in the AS treatment on day 6 only for both pigments. There were no significant differences in the biomass of fucoxanthin (typical of Bacillariophyceae), alloxanthin (typical of cryptophytes) and zeaxanthin (typical of cyanobacteria) between mesocosm with and without AS species (all treatment effects, $P > 0.25$).

Chlorophyll *a* concentrations showed similar trend in both treatments. Concentrations increased during the first days of the experiment, reaching maximal values of 7.2 ± 0.6 µg l^{-1} on day 6 and 5.9 ± 0.3 µg l^{-1} on day 10, in the control and AS mesocosms, respectively, and then gradually decreased to low levels (Fig. 8).

Trophic relationships

Trophic relationships among the studied organisms differed between the two treatments (Table 2). In the control, bacterial biomass was positively correlated with nanoflagellate and peritrich ciliate biomass. Nanoflagellates, both autotrophic and heterotrophic forms, were positively correlated with ciliates, while only ANF was negatively correlated with rotifers. Ciliates were negatively correlated with both cladocerans and copepods.

In the AS mesocosms, nanoflagellates showed numerous correlations with crustaceans (Table 2). Heterotrophic nanoflagellates were negatively correlated not only with the total biomass of crustacean, but also with the biomass of cladocerans, copepods and alien species (*S. vetulus*). There were not, however, significant correlations between crustaceans and autotrophic nanoflagellates. Ciliates were negatively correlated with predatory rotifers, but positively correlated with *D. pulex*. Rotifers, both algivorous and bacterivorous species, were negatively correlated to cladocerans, copepods and alien species whereas algivorous species showed positive correlation with heterotrophic nanoflagellates.

In both treatments, ciliates and rotifers were positively correlated with algal pigments, mainly chlorophyll *a* and peridinin, while cladocerans and copepods were negatively correlated with those pigments. However, there was a positive correlation between bacteria and algae in the control mesocosms only (Table 2).

Discussion

We studied the top-down effects of large-bodied metazooplankton (*D. pulex*, *S. vetulus*, and *M. albidus*) on the structure of the microbial loop using experimental mesocosms. *D. pulex* and *M. albidus* were not

Table 2 Pearson's correlation coefficients among the studied groups of organisms in the control and Alien Species (AS) treatments

	Bacteria	NF	Ciliata	Rotifera	Cladocera	Copepoda
Control						
Algae	0.53*	–	0.53*	0.63**	−0.47*	−0.54*
Bacteria	–	0.56**	0.51*	NS	NS	NS
NF		–	0.46*	−0.45*	NS	NS
Ciliata			–	NS	−0.57**	−0.62**
Rotifera				–	NS	NS
Cladocera					–	–
Copepoda						–
AS						
Algae	NS	–	0.46*	0.78***	−0.50*	−0.71***
Bacteria	–	NS	NS	NS	NS	NS
NF		–	0.61**	0.47*	−0.50*	−0.50*
Ciliata			–	−0.55*	NS	NS
Rotifera				–	−0.63**	−0.58**
Cladocera					–	–
Copepoda						–

NS not significant

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; $n = 21$

able to survive in the ambient conditions of the mesocosms. In contrast, *S. vetulus* was able to successfully persist in the new environment, and native communities were not able to provide biotic resistance against its invasion. Although the final biomass of alien species was substantially lower in comparison to their initial biomass, they made up 59–88% (mean = 74%) of the total crustacean biomass throughout the experiment in the AS treatment. Therefore, among the large-bodied alien species, *S. vetulus* appears to have had a negative impact on the native crustacean assemblages, and it may play an important role in structuring the components of the microbial loop. It is important to note, however, that competition can play an important role in structuring crustacean communities, and it is possible that the three alien taxa competed with each other after they were added to our mesocosms (DeMott, 1989). Specifically, research has shown that *Daphnia* and *Simocephalus* can influence each other through competitive interactions, but that the outcome of this competition varies based on environmental conditions and priority effects (Loureiro et al., 2013). As such, in the current experiment we do not know how our results would have varied had we introduced individual alien cladoceran species into the mesocosms individually. Therefore, additional studies are needed to better determine how individual species of alien crustaceans and competition between multiple alien species influence the microbial loop.

While the total biomass of crustaceans (native + alien) was significantly higher in the AS mesocosm, the biomass of native crustaceans was reduced by the presence of the alien zooplankton. Furthermore, rotifers were also generally lower in AS mesocosms than in control mesocosms which may be due to interactive effects of the large alien cladocerans on ciliates and rotifers. For example, experimental studies by Wickham & Gilbert (1991) showed that the largest cladocerans depressed the growth rates of ciliates and rotifers that were vulnerable to interference competition. The addition of alien crustacean species increased the biomass of ciliates as well as altered ciliate community composition from large oligotrichs *Strombidium* to small *H. grandinella* that can use defensive mechanisms against predation such as jumping movements (Gilbert, 1994). The decrease in rotifer biomass in the AS treatments may explain the higher biomass of ciliates which is due to the lower grazing pressure from rotifers in this treatment. The biomass of bacteria (about 0.50 mg l^{-1}) was similar in the control and AS treatments, suggesting combined compensatory effects of grazing losses and supply of organic substrates (Jürgens, 1994; Modenutti et al., 2003). The above results suggest that alien crustacean species significantly affected the biomass of the

microbial assemblages and altered the trophic relationships within the microbial loop. The same and relatively high ratio (from 2.5 to 6.0) of microbial (bacteria, nanoflagellates and ciliates as prey) to metazoan (rotifers and crustaceans as predator) biomass in both treatments at the first sampling points of the experiment may indicate weak grazing pressure. In contrast, the relatively low ratio (0.9 in the AS mesocosm and 0.2 in the control) on day 15 may imply that grazing activity was very strong. The experimental duration of 40 days may lead to ecological stability of planktonic communities i.e. higher grazing pressure was observed in the mesocosms with higher crustacean biomass (the ratio = 3.6) than in the control (the ratio = 5.2). However, we also acknowledge the fact that in our experiment predation and top-down effects likely occurred among the components of the microbial loop, which can complicate the interpretations of the effect of the alien species.

It is well known that the interactions between the components of the microbial loop are influenced by metazooplankton community structure (Jack & Gilbert, 1997). Literature data reviewed by Jürgens (1994) indicate that microbial food webs differ considerably when large *Daphnia* dominate compared to when the metazoan community is dominated by small-bodied taxa. When *Daphnia* dominate, autotrophic and heterotrophic nanoplankton are grazed to extremely low levels, HNF and ciliates become insignificant as picoplankton consumers, and the abundance and biomass of bacteria decline (Güde, 1988). The dominance of small-bodied metazooplankton leads to higher diversity and higher abundance of bacteria, phytoplankton and protists (Güde, 1989). Under this situation, heterotrophic and mixotrophic nanoflagellates and small ciliates are the main grazers of bacteria (Sherr & Sherr, 1992).

Significant correlations between the components of the microbial loop in the control suggest that the main bacterial consumers were heterotrophic and autotrophic nanoflagellates as well as bacterivorous ciliates. Nanoflagellates in turn were likely grazed mainly by ciliates and rotifers. Ciliates, which showed negative correlations with crustaceans in our experiment, can effectively transfer energy from bacteria to higher trophic levels. Significant positive correlation between chlorophyll *a* concentration and bacteria may indicate that substrates released by algae were the source of considerable amounts of organic carbon for bacteria. In addition, correlations between chlorophyll and/or pigment concentrations and ciliates, rotifers and crustaceans show that, in addition to bacteria and nanoflagellates, phytoplankton were an important food resource. Finally, analysis of the control treatment revealed links between bacteria and protists, nanoflagellates and ciliates/rotifers, and ciliates and crustaceans.

Trophic relations in the AS treatment were different than those observed in the control. Significant correlations between nanoflagellates (especially heterotrophic forms) and crustaceans (total biomass of crustaceans and copepods, the biomass of native crustaceans, and alien species—mainly *S. vetulus*) may indicate high grazing pressure that may lead to the exclusion of nanoflagellates as the main consumers of bacteria. The lack of correlations between bacteria and protists as well as between bacteria and phytoplankton may imply that inorganic nutrients are more important in regulating the abundance and production of bacteria than protistan grazing and organic substrates produced by phytoplankton. Significant negative correlations between bacteria and TP ($r = -0.44$, $P = 0.047$) and TN ($r = -0.59$, $P = 0.005$) support this conclusion. Similarly, Jürgens et al. (1994) found that in holomictic Lake Ciso, the temporary invasion of *D. pulex* created a situation where heterotrophic nanoflagellates and other protists were not important in controlling bacteria. A positive correlation between ciliates and crustaceans and a negative correlation between ciliates and predatory rotifers indicate that the predation by these two groups of metazooplankton might be a major force for structuring the ciliate community in the mesocosms. The high biomass of rotifers on days 15 and 21 of the experiment may have had an important effect on all of the studied groups of organisms. During this time, we observed low nanoflagellate, ciliate and crustacean biomasses, that increased after the peak in rotifer biomass. Similarly, as in the control, the negative correlation between the total biomass of crustaceans and both chlorophyll *a* and peridinin (Dinophyta) concentrations may suggest that crustaceans (copepods and cladocerans) were strongly controlled by algal food resources, in addition to nanoflagellates. In contrast, ciliates and rotifers positively responded to chlorophyll *a*, peridinin and fucoxanthin concentrations.

In conclusion, the large-bodied crustacean species *S. vetulus* was able to establish in the AS mesocosms

and dominate the native small-bodied crustacean communities in the mesocosms. More importantly, *S. vetulus* also appeared to alter trophic relationships in the microbial loop. For example, our results suggest that protozoan grazing pressure on bacteria was reduced in the AS treatment, causing a shift from top-down control to bottom-up control and changing the efficiency of carbon transfer from bacteria to higher trophic levels. Combined, our results highlight the importance of considering how large-bodied crustaceans other than *Daphnia* affect the microbial loop. Furthermore, if large-bodied crustaceans invade new habitats, they have the potential to impact the microbial loop.

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