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RESEARCH ARTICLE

Selective zooplanktivory of an invasive Ponto-Caspian mysid and possible consequences for the zooplankton community structure of invaded habitats

Patrick Fink · Anke Kottsieper · Martina Heynen · Jost Borcherding

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Abstract The impact of invasive alien species on native species is of increasing global concern. Invasive species can cause food-web shifts that have severe consequences for native species and ecosystems. However, the mechanisms by which the invaders influence the native communities are poorly understood. Here we investigated the interactions of the invasive Ponto-Caspian mysid *Limnomysis benedeni* with native freshwater zooplankton in laboratory and mesocosm experiments. This mysid migrates between benthic and pelagic zones and thereby forms a potential trophic link between these habitats. In laboratory predation experiments, *L. benedeni* fed both on *Daphnia galeata* and *D. magna*, and predation rates depended on the sizes of predator and prey but not on the availability of light. However, no predation was observed at prey sizes greater than 2 mm, which appears to be the upper size limit for a successful prey capture by *L. benedeni*. In outdoor mesocosm experiments, *L. benedeni* strongly decreased the densities of cladocerans, rotifers and copepod nauplii within a few days, while the densities of copepods were unaffected. Prey selection indices provide further evidence for strongly selective predation of *L. benedeni* on different zooplankton taxa. The presence of phytoplankton as an additional resource for the omnivorous mysid led to a lower predation pressure of *L. benedeni* on Cladocera and rotifers, indicating that the presence of alternative prey modulates the strength of the top-down effect of invasive mysids on the zooplankton community. These results suggest that the invasion of *L. benedeni* can have profound and complex impacts on the community structure of the native zooplankton fauna and that mysid invasions potentially have whole-ecosystem consequences.

Keywords Invasive species · *Limnomysis benedeni* · Mysida · Neozoa · Omnivory · Zooplankton

Introduction

The invasion of non-native species is an increasing problem since the second half of the twentieth century. Invasive species can cause food-web shifts that can have severe consequences for native species and ecosystems (Williamson 1996). In aquatic ecosystems, Crustacea, and among those the Mysida, are frequently observed groups of invasive species (Cristescu and Hebert 2005). Several mysid species have been intentionally released in water bodies throughout Eastern (Arbaciauskas 2002) and Northern Europe (Koksvik et al. 1991; Langeland et al. 1991) and North America (e.g., Chess and Stanford 1998) in attempts to increase fishery yields. From those original sites of introduction, the mysids have rapidly spread and are claimed to have caused perturbations in the native food-webs of the invaded habitats (Chess and Stanford 1998; Koksvik et al. 1991; Langeland et al. 1991), although quantitative data are scarce.

The invasive mysid *Limnomysis benedeni* (Czerniavsky 1882) originates in the Ponto-Caspian region (Cristescu and Hebert 2005). Since the opening of the Rhine–Main–Danube-Channel, it has rapidly spread throughout the large

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European river systems and adjacent lakes (e.g., Wittmann 2007). In particular along the Danube River and gravel pit lakes along the River Rhine, *L. benedeni* has developed mass abundances and therefore can be classified as an invasive species (Williamson 1996). Furthermore, it is predicted to reach North America in the near future (Ricciardi and Rasmussen 1998). Thus, *L. benedeni* is one of the most recent invaders from the Ponto-Caspian region. Interestingly, this region, comprising the Caspian, Black and Azov Sea (Arbaciauskas 2002), is the source of most of the aquatic neozoa that have invaded not only Western and Central Europe (Bij De Vaate et al. 2002) but also many aquatic systems in North America (Pothoven et al. 2007; Ricciardi and MacIsaac 2000). Despite this rapid spread of many Ponto-Caspian mysids, in particular *L. benedeni*, there is little knowledge about the potential consequences of their invasion on the native ecosystems.

Mysis relicta, a mysid species native to Central Europe, was deliberately introduced to lakes in Scandinavia and North America. (Chess and Stanford 1998; Koksvik et al. 1991; Langeland et al. 1991). This pelagic species is known to feed both on zooplankton and phytoplankton (Grossnickle 1982; Lasenby and Langford 1973; Viherluoto et al. 2000) and its introduction in non-native habitats had a strong negative impact on the populations of Cladocera in those lakes (Goldman et al. 1979; Langeland et al. 1991). While *M. relicta* and the Ponto-Caspian *Hemimysis anomala* are known to have modified the food webs of invaded lakes (Branstrator et al. 2000; Ketelaars et al. 1999), the more benthic oriented *L. benedeni* was so far considered to have no or little impact on native ecosystems (Wittmann and Ariani 2000). Without knowledge on the behaviour and feeding strategies of *L. benedeni*, its real impact on native communities cannot be estimated. It is crucial to investigate the role of *L. benedeni* in ecosystems that have been invaded by this species, since it can act both as a consumer (top-down effects) as well as novel prey for native predators (bottom-up effects).

Mysids generally have relatively large eyes and those eyes are the only non-transparent part of their bodies, thus enhancing their visibility for visual oriented predators such as fish. It should therefore be expected that these large eyes fulfil essential functions for the survival of the mysids, such as prey capture or predator avoidance. However, previous experimental studies on the importance of visual signals for the prey capture of mysids have yielded inconsistent results: While Ramcharan and Sprules (1986) found light to enhance the prey capture rate of *M. relicta*, Viherluoto and Viitasalo (2001a) concluded that mechanoreception and not light determined the prey capture for a pelagic and a littoral mysid species from the Baltic Sea.

The aim of this study was to investigate the interactions of *L. benedeni* with the native herbivorous zooplankton, since omnivores such as *L. benedeni* can simultaneously act as a resource competitor (for phytoplankton) and as a predator on small zooplankton. The recent invasion pattern of *L. benedeni* and the development of mass abundances in the invaded habitats are very similar to other invasive Ponto-Caspian mysids (Borcherding et al. 2006; Lesutiene et al. 2007; Pothoven et al. 2007). Therefore, we assume that the effects of this invasive species on native zooplankton communities are also more similar to those of other Ponto-Caspian mysids than to the food web effects of a purely pelagic, cold-adapted species like *M. relicta*.

Specifically, we tested the following hypotheses: (1) *L. benedeni* can be an effective predator of small zooplankton; (2) the efficiency of this predation depends on the size of both the predator and the prey, as well as on the prey species; (3) the strength of the predation pressure of *L. benedeni* on zooplankton depends on the presence of light and of alternative resources for the omnivorous mysids (such as phytoplankton); and (4) size- and species-selective predation of *L. benedeni* on native zooplankton communities can influence the community structure of the native zooplankton and thus potentially has whole-ecosystem consequences.

Methods

Field sampling

Adult *L. benedeni* were collected by dip-net sampling in the littoral of the Reeserward II (51°47′20 N and 6°20′2E) a gravel pit lake temporarily connected to the River Rhine near Rees-Grietherbusch, Germany. After transportation to the lab in Cologne (under cooling to minimize oxygen limitation), the animals were kept in a climate-controlled chamber at 12 ± 3°C with a 12:12 h light:dark cycle and fed ground TetraMin fish food (Tetra, Melle, Germany) ad libitum. Body size of *L. benedeni* was determined using a dissecting microscope equipped with a digital camera and an image analysis system as described by Gergs et al. (2008).

Laboratory predation trials

In the predation experiments in the laboratory, *L. benedeni* was fed a clone of *Daphnia magna* (Lampert 1991) and a clone of *Daphnia galeata* (Stich and Lampert 1984), respectively. Both clones had been kept in the laboratory for several years prior to the experiments at constant temperature (20°C) and food (the green alga *Scenedesmus obliquus*). For the experiments, the body length of one *L. benedeni* and one *Daphnia* sp. was determined as described above to allow the determination of predator–prey size ratios. Subsequently, the two crustacean individuals were placed into a 100 ml jar with membrane filtered (0.2 µm), aerated tap water in the climate-controlled chamber at 12 ± 3°C. After 18 h (6 light:12 dark), predation success was determined. A total of 802 predation trials (449 with *D. galeata* and 353 with *D. magna*) were performed. Obviously, the chosen experimental setup in a relatively small volume is not suited for the determination of real capture rates that might depend on the habitat/container size (Gorokhova and Hansson 1997). Rather, our intention was to determine the possible range of predator–prey size ratios in a small and simple setup with high predator–prey encounter rates. The maximum *D. magna* body sizes in trials with variable *L. benedeni* body sizes that resulted in successful predation (filled symbols in Fig. 1) were subsequently used to fit a sigmoidal (dose–response like) function with 95% confidence intervals using GraphPad Prism v.4. This allowed us to mathematically define the function of the predator–prey size relationship as well as the maximum ingestible prey size in the interaction of *L. benedeni* with *D. magna*. This function was then compared to the predator–prey size relationship in the interaction of *L. benedeni* with *D. galeata*. Successful predation trials with *L. benedeni* with *D. galeata* above the upper confidence interval of the maximum predator–prey size ratio defined by the above mentioned function were interpreted as a significant difference between the two prey species.

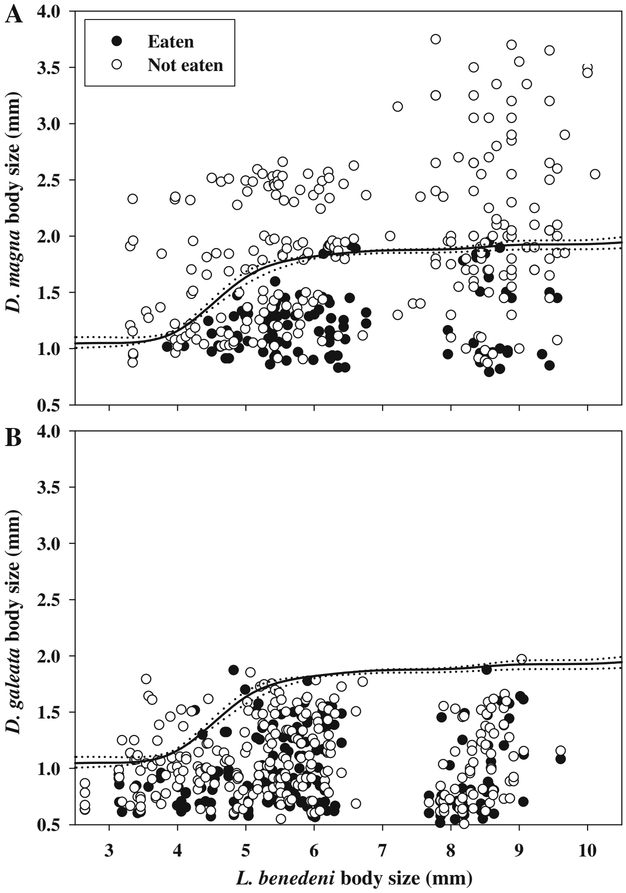


Fig. 1 Results of the 18 h predation trials with *L. benedeni* and *Daphnia magna* (353 trials, **a**) and *Daphnia galeata* (449 trials, **b**); the *solid line* in both plots represents the sigmoidal function fitted to the maximum *Daphnia magna* body size vulnerable to predation by *L. benedeni* of a given size. The *dashed lines* give the upper and lower 95% confidence intervals of the model

In another set of laboratory predation trials, we investigated the effects of light and an alternative phytoplankton food source on the predation success of *L. benedeni* on *Daphnia* sp. For this experiment, daphnids (*D. galeata* and *D. hyalina*) as well as *L. benedeni* were collected from Lake Speldrop, a small gravel pit lake near the Ecological Field Station in Rees-Grietherbusch (Borcherding et al. 2007). In contrast to Lake Reeserward II, this lake is not connected to the River Rhine. Nevertheless, it contains high abundances of *L. benedeni* that were probably introduced via waterfowl from the nearby River Rhine or its floodplain lakes. For the experiments, 5 *L. benedeni* and 20 *Daphnia* sp. of known body size (see above) were placed into a 400 ml jar with membrane filtered (0.2 µm), aerated water from Lake Speldrop. The control treatments contained 20 *Daphnia* sp. but no *L. benedeni*. The jars were incubated for 24 h either with a 16:8 L:D cycle (light treatment) or in complete darkness (dark treatment). We further added the green alga *S. obliquus* at carbon concentrations equivalent to 2 mg particulate organic carbon (POC) l^{-1} to half of the jars in the light and dark treatments, respectively. This resulted in a three-factorial design with the factors light (light/dark), mysids (presence/absence) and phytoplankton (presence/absence) in full-factorial treatment combinations with seven replicates each (56 jars in total). *L. benedeni* used in this experiment had a mean (± SD) body size of 7.23 ± 1.19 mm (which was in a similar size range as *L. benedeni* stocked in the mesocosm experiments). After 24 h, the number of remaining live daphnids per jar was counted and the *Daphnia* mortality calculated as percent of the originally stocked (20) daphnids that had disappeared from the containers (i.e. that had been eaten by *L. benedeni*) or individuals that were found dead on the bottom of the jars (which occurred in very few replicates only). A three-way analysis of variance (ANOVA with the General Linear Model of SPSS 17.0) was performed to compare the effects of *L. benedeni*, light and phytoplankton density (as three independent variables) on the mortality of *Daphnia* (dependent variable, normalised by the square root arcsine transformation and tested for homoscedasticity with Levene's test) in the laboratory experiments.

Mesocosm experiment 1

To test the effect of *L. benedeni* on the composition of a natural zooplankton community, outdoor mesocosms were stocked with a low density of *L. benedeni* (1 ind l^{-1}), a high density of *L. benedeni* (5 ind l^{-1}), and a control treatment without *L. benedeni* in quadruplicates. The mesocosm experiment was carried out in the Botanical Gardens of the University of Cologne. Twelve containers (volume 90 l) were placed into a half-shaded area (PAR = 11 ± 19 μmol photons s^{-1} m^{-2} during the day, depending on weather conditions) and filled with 50 l of tap water each. After 3 days of ageing and temperature adjustment of the water, the mesocosms were stocked with *L. benedeni* and a mixed zooplankton community with a density of approx. 100 ind l^{-1}. Mysids for the experiment had been collected in the field 1 day prior to the start of the experiment as described above. Zooplankton for the mesocosm experiment was collected in a pond near the University of Cologne (Aachener Weiher) dominated by *Daphnia* sp. and a nearby lake (Fühlinger See) dominated by copepods. The mixed zooplankton community used for the stocking of the mesocosms thus consisted mainly of *Daphnia* sp. and cyclopoid copepods, but also included rotifers, bosminid Cladocera, calanoid copepods and copepod nauplii (for details of the zooplankton composition see Table 1).

Zooplankton was collected by trawls with a 30 μm plankton net from jetties at the shore of both lakes during the morning of the day on which the experiment was started. A representative sub-sample (with a defined volume) of both zooplankton communities was initially counted under a dissecting microscope. From the density of the zooplankton taxa in this subsample, the volume of zooplankton suspension required to reach the intended zooplankton starting density in the mesocosms was calculated. Subsequently, this volume of water was first removed from the mesocosms and then replaced with exactly the same volume from the dense zooplankton suspension to yield the starting density of the experiments of 100 zooplankton individuals per litre of mesocosm volume (Table 1). No additional phytoplankton was supplied as a food source for *L. benedeni* or for the herbivorous zooplankton in this experiment, and the inocolum of the mesocosms with lake phytoplankton introduced together with the zooplankton inocolum is considered to be minor as the majority of phytoplankton in the two lakes is smaller than 30 μm.

On day 0 (just after the start of the experiment), day 1 and day 3 (end of the experiment), zooplankton samples were drawn from each of the experimental containers by mixing the whole water body with a plastic rod and subsequent scooping of 2 l of the (well-mixed) water from each of the containers. This 2 l sample was poured through a 30 μm mesh and the zooplankton retained by the mesh fixed with 4% formaldehyde solution. Both the stocking of the mesocosms and the daily samplings were performed approximately mid-day to avoid confounding factors such as diurnal changes in feeding activity or temperature. Taxonomic composition of the zooplankton samples and the body sizes of daphnids and copepods were determined using an image analysis system (see above) at the beginning and end of the experiments. Seston samples were collected at the beginning (day 0) and end (day 3) of the experiment. Two litres of water from each experimental container were poured through a 30 μm mesh and 400–1,000 ml of this “edible” sestonic size fraction were filtered on precombusted glass-fibre filters (GF/F, Whatman, Maidstone, UK). After drying of the filters, the particulate organic carbon per filter was analysed with an Carlo Erba NCS-2500 analyser (for details see Fink et al. 2006). The experiment was started on September 5, 2008 (d_0) and terminated on September 8, 2008 (d_3). During the course of both mesocosm experiments, conductivity

Table 1 Composition of the zooplankton community (mean ind l^{-1} ± SD, $n = 3$) used for the stocking of the mesocosms; abundances given are final densities in the mesocosms

Taxon	Experiment 1		Experiment 2	
	Zooplankton density (ind l^{-1})	Proportion of total (%)	Zooplankton density (ind l^{-1})	Proportion of total (%)
Rotifera	14.2 ± 1.4	16.4	29.9 ± 2.7	22.2
Crustacea: Cladocera				
Daphnia sp.	17.2 ± 2.3	19.6	75.9 ± 8.7	56.4
Bosmina sp.	1.9 ± 0.8	2.2	3.6 ± 1.4	2.7
Crustacea: Copepoda				
Cyclopoid copepods	25.6 ± 3.0	29.1	7.6 ± 2.3	5.7
Calanoid copepods	6.6 ± 1.5	7.5	10.8 ± 1.6	8.0
Copepod nauplii	22.2 ± 2.0	25.2	6.8 ± 1.3	5.1
Sum zooplankton	87.9 ± 5.8	100.0	134.6 ± 27.8	100.0

(887 ± 30 μS cm^{-2}) and temperature (15 ± 1°C) of the water in the containers varied only slightly and independently of the experimental treatments.

Mesocosm experiment 2

To investigate the effect of *L. benedeni* on the zooplankton community composition in the presence of phytoplankton as alternative resource, a second mesocosm study was performed in the same experimental containers as described above. In contrast to the first mesocosm experiment, the zooplankton community was collected from the *Daphnia*-dominated Aachener Weiher only and no zooplankton from Fühlinger See was included (for details of the zooplankton composition see Table 1). The factorial design combined the presence of *L. benedeni* (1 ind l^{-1}, all treatments) with one set of experimental units with addition of phytoplankton only (PHYTO), zooplankton only (ZOO), and one set of experimental containers with both phytoplankton and zooplankton (MIX) in quadruplicates. Zooplankton (in the treatments ZOO and MIX) was added at a density of 100 ind l^{-1}. In the treatments PHYTO and MIX, a laboratory-grown culture of the green alga *Chlamydomonas* sp. was added as additional resource for both *L. benedeni* (PHYTO and MIX) and the herbivorous zooplankton (MIX) at a biomass equivalent to 0.3 mg POC l^{-1}. This experiment was started on October 14, 2008 (d_0) and terminated on October 16, 2008 (d_2) after a drastic reduction of the initial zooplankton abundances could be observed. The analysis of the zooplankton and phytoplankton (on days 0, 1 and 2) was performed as described above.

Data analysis

To compare the effect of different *L. benedeni* densities on plankton abundance during the first mesocosm experiment, we fitted a separate mixed-effect model for each plankton species (copepod nauplii, calanoid Copepoda, cyclopoid Copepoda, *Daphnia* sp., rotifers and *Bosmina* sp.). The abundance of the targeted plankton species (ind l^{-1}) was included as a response variable, and treatment (control, low-*Limnomysis* and high-*Limnomysis* density), sampling day (day 0, 1 and 3) and their interaction were included as categorical fixed effects. As each mesocosm was sampled several times during a 4 day period, a nested design was created to avoid temporal pseudoreplication in the analysis. Within-container sampling day (day 0, 1 and 3) was added as random effect at the innermost level and between containers at the next outer level. To analyze how the presence of phytoplankton influenced the effect of *L. benedeni* on the zooplankton community in the second mesocosm experiment, we included the abundance of each examined zooplankton species (copepod nauplii, calanoid Copepoda, cyclopoid Copepoda, *Daphnia* sp., rotifers and *Bosmina* sp.) as response variables in a separate mixed-effect model approach. Treatment (zooplankton only or a mixture of phyto- and zooplankton), sampling day (day 0, 1 and 2) and their interaction were included as categorical fixed effects, with the same hierarchical structure of the random effects as stated above. The most parsimonious models were derived by testing the fixed effects using Wald statistics using significance level of 0.1 (Pinheiro and Bates 2000). The free software R for statistical computing (R Development Core Team 2009) and the library *nlme* v.3.1.-90 were used to run the mixed effect models. Prior to the analyses, the homoscedasticity of the data was confirmed with Levene's test using Statistica v.6. Statistica 6 was also employed to compare the size distribution of *Daphnia* sp. and copepods in the mesocosm experiments via one-way ANOVA.

We calculated a prey selection index (according to Pearre 1982) for *L. benedeni* and each of its potential zooplankton prey taxa in the mesocosm experiment. The selection index C using Yate's correction for continuity (Pearre 1982) is: $C = [(A_d\,B_e - B_d\,A_e - n/2)^2/(a\,b\,d\,e)]^{1/2}$, where the amount of species A (the focal species) in the diet is A_d, the amount of all other species in the diet is B_d, the amount of species A in the environment is A_e, the amount of all other species in the environment is B_e. Further, d is the sum of the organisms in the diet ($A_d + B_d$), e the sum of the organisms in the environment ($A_e + B_e$) and n the total ($A_d + A_e + B_d + B_e$). The start values (i.e. the initial zooplankton densities in the mesocosms were considered as the "environment" abundances, and the difference between start and end values (i.e. the net mortality of the respective taxon) as the abundances in the diet. The selection index ranges from +1 (maximum preference) to −1 (minimum preference) and has already successfully been applied to mysids in a previous study (Viherluoto and Viitasalo 2001b).

Results

Laboratory experiments

In the first set of predation trials in the laboratory, *L. benedeni* consumed both *Daphnia magna* (Fig. 1a) and *Daphnia galeata* (Fig. 1b) in about 40% of the trials. We were able to fit a sigmoidal dose–response curve to the upper size limit of the successful predation trials dependent on both the predator (*L. benedeni*) and prey (*Daphnia* sp.) body size for the trials with *D. magna* (Fig. 1a). The best-fit function for the maximum ingestible prey (*D. magna*) body size was $Y = 1.96/(1 + 10^{(5.123-X)})$ with Y being the prey body length and X the predator body length (in mm, Fig. 1a). A comparison of this function obtained from the *D. magna* predation trials with the successful predation trials with *L. benedeni* and *D. galeata* indicated that the maximum predator–prey size ratios required for a successful predation might differ (Fig. 1b). More specifically, a few relatively larger *D. galeata* were successfully captured and ingested by smaller *L. benedeni* than would have been expected from the predation trials with *D. magna*, which is indicated by some successful predation trials with *L. benedeni* and *D. galeata* (filled symbols) above the upper 95% confidence intervals of the function fitted to the *D. magna* predation trials in Fig. 1b.

The second laboratory predation experiment was designed to investigate the role of light and alternative resources (phytoplankton) on the predation efficiency of *L. benedeni* on *Daphnia* sp. Similar to the 1:1 predation trials described above, the mortality of *Daphnia* sp. was significantly higher in the treatments where *L. benedeni* was present as revealed by an ANOVA (explaining 61.8% of the total variance; $F_{(1,36)} = 14.38$, $p = 0.001$, Figs. 2, 3). The availability of light had no significant influence on the predation rate of *L. benedeni* on *Daphnia* sp. (ANOVA, $F_{(1,36)} = 0.001$, $p = 0.97$, Fig. 2). The presence of *S. obliquus* as an additional resource for *L. benedeni* led to a significantly lower predation rate on the daphnids ($F_{(1,36)} = 11.98$, $p = 0.001$, Fig. 3). Furthermore, no size-selective predation on a natural assemblage of *Daphnia galeata*/*hyalina* could be found (*t* test on the average size of surviving daphnids in the presence and absence of *L. benedeni*: $p = 0.66$). This was to be expected, since all daphnids in this experiment had a body size of 0.5–2.0 mm and were thus almost all below the critical size range determined in the first set of predation trials (1.96 mm, Fig. 1).

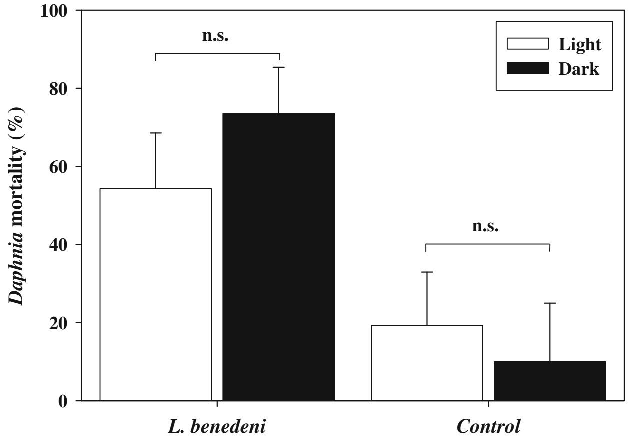


Fig. 2 Impact of *L. benedeni* on the mortality of *Daphnia* sp. in the presence and absence of light; values are given as mean ± SD ($n = 7$), *n.s.* no significant difference in the one-way ANOVA followed by a post-hoc Tukey HSD test

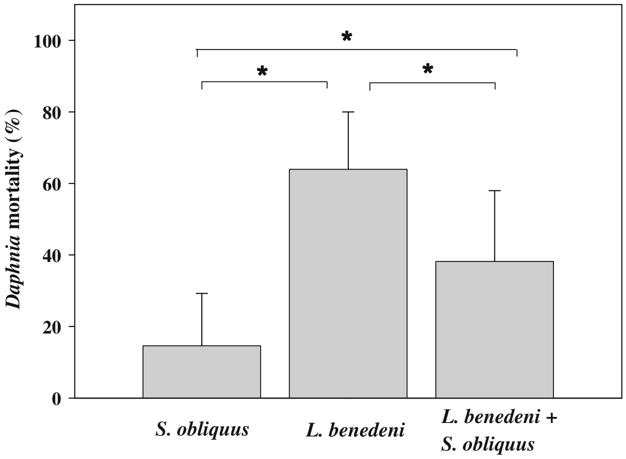


Fig. 3 Impact of the presence of mysids (*L. benedeni*), phytoplankton (*S. obliquus*) or both mysids and phytoplankton on the mortality of *Daphnia* sp. in the laboratory predation experiment. Values are given as mean ± SD ($n = 7$). *Asterisks* indicate significant differences between treatments as revealed by a one-way ANOVA followed by a post-hoc Tukey HSD test

Mesocosm experiment 1

The initial density of zooplankton did not differ between any of the treatments or for any of the taxa investigated. During the mesocosm experiment, the different densities of *L. benedeni* had different effects on the abundance of the examined zooplankton taxa (Table 2): *L. benedeni* reduced the density of *Daphnia* sp., *Bosmina* sp., rotifers and copepod nauplii (Fig. 4). However, the abundance decline of *Daphnia* sp. and *Bosmina* sp. did not depend on the density of *L. benedeni*. In contrast, the density of *L. benedeni* had a significant effect on the abundance of rotifers and copepod nauplii: at the end of the experiment, there were fewer copepod nauplii in containers with the high *L. benedeni* density, compared to the low *L. benedeni* density. There was no significant difference in the density of copepod nauplii between the low *L. benedeni* density and the controls. The abundance of rotifers decreased more when the predators were at high density than when the predators were at low density, while the abundance of adult calanoid and cyclopoid copepods were completely unaffected by the presence of *L. benedeni* (Table 2; Fig. 4).

Calculation of the prey selection index C (Pearre 1982) indicated that *Daphnia* sp. was the preferred prey of *L. benedeni* among the natural zooplankton community in the mesocosm experiment, followed by *Bosmina* sp. and rotifers (Fig. 5). Both calanoid and even more strongly cyclopoid copepods were negatively selected by *L. benedeni*, which is indicated by the negative prey selection indices (Fig. 5). Copepod nauplii were positively selected only at the high *L. benedeni* density, but negatively selected at the low *L. benedeni* density as revealed by a

Table 2 Results of the Wald statistics for the fixed effects [treatment (*L. benedeni* density), sampling day and their interaction] for the density of various zooplankton taxa in the first mesocosm experiment, tested with mixed effects models

	df	*F*	*p*
Daphnia sp.			
Treatment	2	13.517	0.002**
Day	2	7.133	0.006**
Day × treat	4	5.141	0.007**
Copepod nauplii			
Treatment	2	13.232	0.002**
Day	2	56.393	0.000***
Day × treat	4	4.489	0.012*
Rotifera			
Treatment	2	35.522	0.000***
Day	2	58.405	0.000***
Day × treat	4	10.970	0.000***
Calanoid copepods			
Treatment	2	1.884	0.207
Day	2	1.869	0.185
Day × treat	4	0.301	0.874
Cyclopoid copepods			
Treatment	2	2.235	0.163
Day	2	1.321	0.293
Day × treat	4	0.296	0.877
Bosmina sp.			
Treatment	2	10.427	0.005**
Day	2	2.439	0.117
Day × treat	4	1.889	0.159

Asterisks indicate significant differences at $p < 0.1$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)

two-tailed *t* test ($p < 0.05$). In contrast to this observation, there was no statistically significant difference between the prey selection indices in the high and low *L. benedeni* density treatments for daphnids or copepods.

The mean body size of both the daphnids (0.66 ± 0.07 mm) and the copepods (0.59 ± 0.05 mm, determined at the start and at the beginning of the experiment) was unaffected by the presence and density of *L. benedeni* or by the sampling date as revealed by two-way analyses of variance (copepods $F_{(2,27)} = 3.3$, $p = 0.05$; daphnids $F_{(2,27)} = 0.21$, $p = 0.81$). Particulate organic carbon in the size fraction <30 µm was 0.27 ± 0.08 mg C l^{-1}, irrespective of the experimental treatment (one-way ANOVA $F_{(2,10)} = 3.06$, $p = 0.08$).

Mesocosm experiment 2

In the second mesocosm experiment, *Daphnia* sp., *Bosmina* sp., rotifers and copepod nauplii showed a significant decrease in the ZOO and MIX treatments (Table 3; Fig. 6). In contrast to the first mesocosm experiment, the density of the calanoid copepods decreased slightly, but significantly in both treatments of the second mesocosm experiment. However, the presence of additional phytoplankton did not affect the abundance decline for calanoid copepods and copepod nauplii, whereas the density of *Daphnia* sp., *Bosmina* sp. and rotifers were significantly influenced by the presence of phytoplankton. Similar to the findings of the first mesocosm experiment, the density of the cyclopoid copepods was unaffected by *L. benedeni*, irrespective of the presence of phytoplankton (Table 3).

Furthermore, the experimental treatments had no significant effect on the mean body size within the *Daphnia* populations between the start (day 0) and end (day 2) of the experiment (two-way ANOVA, $F_{(1,12)} = 3.88$, $p = 0.07$). The mean body size of the copepods (pooled sample of calanoids and cyclopoids) was significantly higher in the MIX treatments than in the ZOO treatments (two-way ANOVA, $F_{(1,18)} = 16.35$, $p < 0.001$). However, this was probably an effect of an unequal stocking of the experimental units and not related to the experimental conditions, since these unequal abundances did not change over the duration of the experiment. The analysis of particulate organic carbon (<30 µm) revealed that all experimental units contained 0.61 ± 0.16 mg C l^{-1}, irrespective of the experimental treatment (two-way ANOVA $F_{(3,16)} = 0.95$, $p = 0.44$). There was a tendency towards a decrease in seston carbon content in the PHYTO treatment, indicating a consumption of *Chlamydomonas* sp. by *L. benedeni*, but this was not statistically significant ($p = 0.31$).

Discussion

In the laboratory feeding trials, adult *L. benedeni* (>8.5 mm) readily ingested *Daphnia galeata* as well as *Daphnia magna* up to a prey body size of approx. 2 mm. This is in marked contrast to the recent findings of Gergs et al. (2008), who found no evidence for predation of *L. benedeni* on *D. magna*. However, in the study of Gergs et al. (2008), *L. benedeni* were offered only adult *D. magna* and no data on the body size of the daphnids was reported. This *Daphnia* species can reach body lengths of up to 5 mm, which is clearly outside the prey size range accessible to *L. benedeni* as reported here. Further, the laboratory experiments revealed that the maximum ingestible prey size is dependent on the body size of the predator (*L. benedeni*) and that this relation can be described by a sigmoidal function. These results support the idea that for juvenile mysids predatory feeding is rather an exception, whereas from a certain age (or body size) on they supposedly switch from a preference for herbivory to predominance of carnivory (e.g., Branstrator et al. 2000;

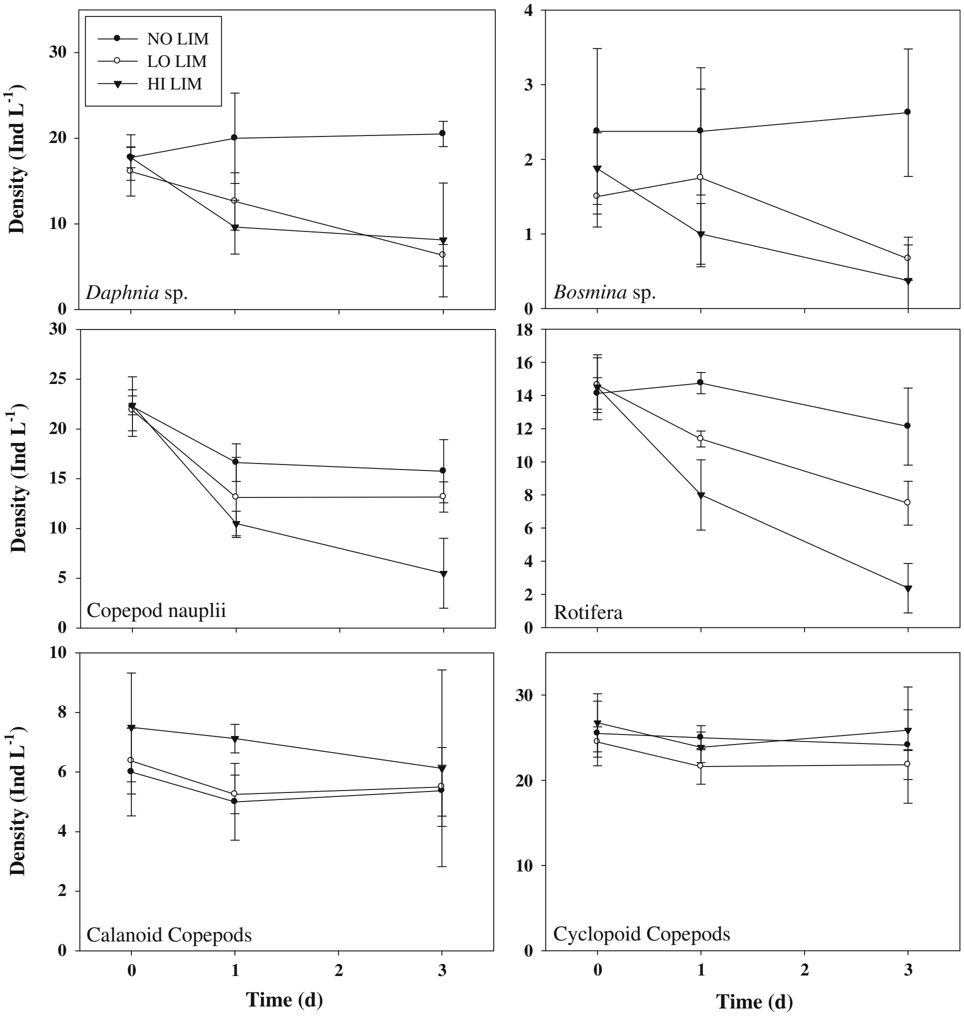


Fig. 4 Population density (ind l^{-1}) of zooplankton organisms in the first mesocosm experiment over the three-day experimental duration in the treatments without *L. benedeni* (NO LIM) or with low (LO LIM, 1 ind l^{-1}) and high (HI LIM, 5 ind l^{-1}) densities of *L. benedeni*. Values are given as mean ± SD ($n = 4$); note the differences in the scaling of the *y* axes

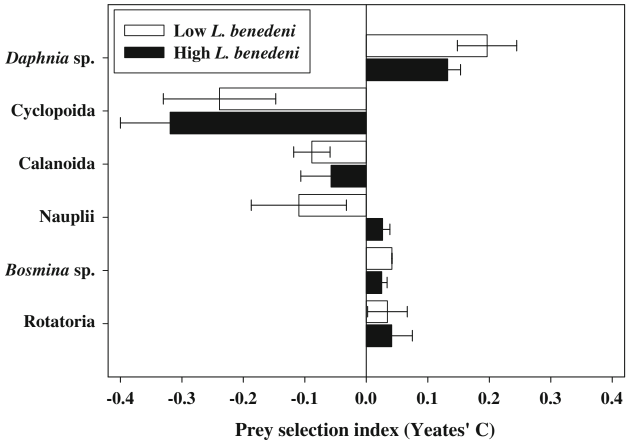


Fig. 5 Selection of *L. benedeni* for different zooplankton prey taxa in the first mesocosm experiment expressed by the prey selection index C ± SD of $n = 4$ (Pearre 1982)

Lesutiene et al. 2007). Since the size-dependence of the herbivory of *L. benedeni* was not tested here, this should be clarified in further studies.

We found evidence for a species-specific effect of the *Daphnia* species on the predation rate of *L benedeni*: Relatively larger *D. galeata* than *D. magna* were ingested by *L. benedeni* of 4–5 mm body length, which is indicated by the successful predation trials (filled symbols) above the 95% confidence interval of the sigmoidal function fitted to the predation of *L. benedeni* on *D. magna* in Fig. 1b. This might be due to *D. galeata* being a more “pelagic” species than *D. magna*, which was often oriented towards the bottom of the experimental containers and was even described to consume benthic resources (Siehoff et al. 2009). There, the encounter rate of *D. magna* and the mysids is probably higher. With the relatively low number of observed successful predation events “above” the function, our ability to generalize from these is somewhat limited.

In our laboratory experiment, the availability of light had no influence on the predation success of *L. benedeni* on *Daphnia* sp. indicating that despite its large eyes, *L. benedeni* is not an optically oriented predator, but equally effective in capturing prey organisms in the absence of

Table 3 Results of the Wald statistics for the fixed effects (phytoplankton presence or absence (ZOO, MIX), sampling day and their interaction) for the density of the examined zooplankton taxa under low abundance of *L. benedeni* (1 ind l^{-1}) in the second mesocosm experiment, tested with mixed effects models

	df	*F*	*P*
Daphnia sp.			
Treatment	1	4.393	0.081*
Day	2	41.228	0.000***
Day × treat	2	2.520	0.122
Copepod nauplii			
Treatment	1	0.089	0.775
Day	2	53.103	0.000***
Day × treat	2	1.555	0.251
Rotifera			
Treatment	1	4.804	0.079*
Day	2	164.118	0.000***
Day × treat	2	13.461	0.001**
Calanoid copepods			
Treatment	1	0.977	0.361
Day	2	7.305	0.084*
Day × treat	2	1.292	0.310
Cyclopoid copepods			
Treatment	1	0.724	0.427
Day	2	2.444	0.129
Day × treat	2	1.421	0.279
Bosmina sp.			
Treatment	1	7.418	0.035*
Day	2	4.525	0.034*
Day × treat	2	0.767	0.486

Asterisks indicate significant differences at $p < 0.1$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)

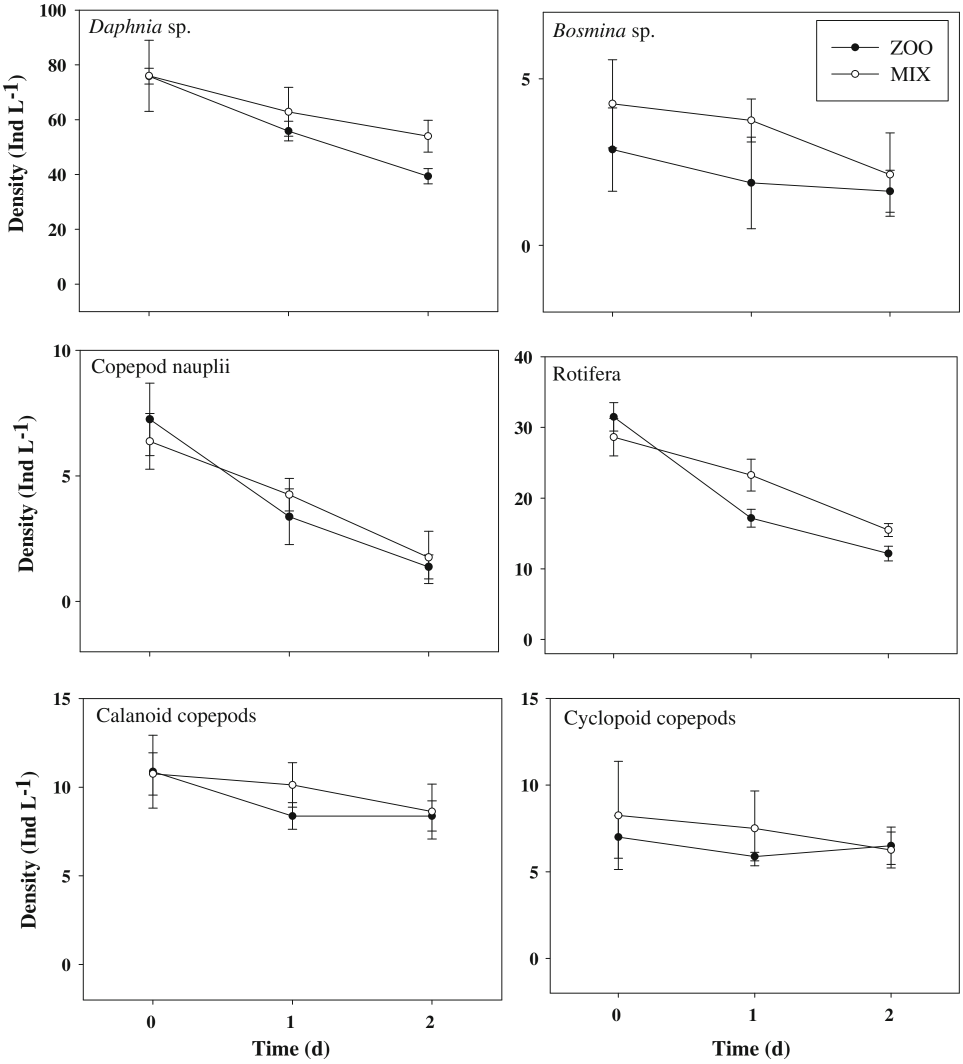
light. This corroborates similar findings for *Praunus flexuosus*, another littoral mysid species (Viherluoto and Viitasalo 2001a). The successful prey detection in darkness suggests that the large second pair of antennae can be used for tactile detection of potential prey items. This finding has important consequences for estimation of the invasion success of *L. benedeni*: If no light is required for a successful predation on zooplankton, the mysids can perform a diel vertical migration (DVM) similar to *M. relicta* (Branstrator et al. 2000; Chess and Stanford 1998) and *H. anomala* (Borcherding et al. 2006), to predate on zooplankton even under turbid conditions or to rest in deep littoral areas to evade the predation pressure of planktivorous fish during the day.

The mesocosm experiments revealed a strong species-specific predation pressure of *L. benedeni* on various taxa of a native zooplankton community. The presence and density of *L. benedeni* had highly significant negative effects on the population density of Cladocera (*Daphnia* sp., *Bosmina* sp.), rotifers and copepod nauplii. The body size of daphnids in the mesocosm experiments varied between 0.5 and 0.8 mm. Hence, the daphnids in these experiments were all considerably smaller than the critical size limit of 2 mm and therefore within the size range vulnerable to the predation by *L. benedeni*. This explains the lack of a size-selective feeding of *L. benedeni* on the *Daphnia* sp. population in both our mesocosm experiments. This is further corroborated by the results of the laboratory predation experiment with the natural *D. galeata/hyalina* assemblage and *S. obliquus*.

While the strength of the predation pressure of *L. benedeni* on *Daphnia* sp. was not different between the high and low *L. benedeni* density treatments, the stronger predation pressure on the copepod nauplii in the high versus the low *L. benedeni* density treatment of the first experiment indicates that Cladocera are the preferred prey of *L. benedeni*. This is supported by the strongly positive prey selection index for *Daphnia* and the switch from a negative selection for copepod nauplii at the low *L. benedeni* density to a positive selection for nauplii at the high *L. benedeni* density (Fig. 5). *L. benedeni* probably does not switch to nauplii as prey unless the availability of cladoceran prey has been depleted below a critical level (which was only expected in the treatments with a high *L. benedeni* density). Below this critical abundance, a further search for the preferred prey (Cladocera) is probably energetically no longer adaptive, so that a switch in the prey preference to smaller zooplankton (such as nauplius larvae) occurs. An alternative explanation would be that the sensitivity of the Cladocera to the predation by *L. benedeni* was not constant throughout the duration of the experiment. In laboratory predation experiments, the presence of *L. benedeni* seemed to enhance the swimming speed and predator evasion behaviour of the daphnids (Eric von Elert, unpubl. data). Such behavioural changes might have reduced the predation efficiency of *L. benedeni* on *Daphnia* sp. over the course of the three-day experiment.

In the second mesocosm experiment with phytoplankton offered as an alternative resource for *L. benedeni*, no significant decrease in the density of copepod nauplii could be found. This suggests that nauplii are only chosen as prey by *L. benedeni* when neither preferred zooplankton prey (Cladocera) nor phytoplankton is available in sufficient densities. Nevertheless, the second experiment indicated that the presence of an alternative resource such as phytoplankton (*Chlamydomonas* sp.) for the omnivorous mysids is able to ameliorate the predation pressure of *L. benedeni* on the zooplankton community: The population decreases of daphnids, bosminids and rotifers were significantly smaller than without *Chlamydomonas* as an alternative resource for *L. benedeni*. We cannot completely rule out the possibility that the addition of *Chlamydomonas*

Fig. 6 Population density (ind l^{-1}) of zooplankton organisms in the second mesocosm experiment over the two-day experimental duration in the treatments with addition of zooplankton (ZOO), or phytoplankton and zooplankton (MIX) in the presence of *L. benedeni* (1 ind l^{-1}). Values are given as mean ± SD ($n = 4$); note the differences in the scaling of the *y* axes



sp. also had a direct positive effect on the population growth of the herbivorous zooplankton. This seems unlikely, since the experimental duration of only 2 days appears to be too short to expect strong resource effects on the birth rate of the Cladocera and Rotatoria and the observed egg numbers for both groups were low. Furthermore, these findings are strongly corroborated by the laboratory experiment where the presence of phytoplankton (*S. obliquus*) as an additional resource led to a reduction in the predation pressure of *L. benedeni* on *Daphnia* sp.

In contrast to the strong negative effects on some of the native zooplankton taxa, there was no effect of *L. benedeni* density on the populations of calanoid or cyclopoid copepods. Together with the observation that copepod nauplii seem to be a rather unfavoured prey that is only utilized when other, preferred resources are depleted, this indicates that the potential effects of *L. benedeni* on copepod populations appear to be much lower than the effects on cladoceran taxa. These findings of our mesocosm experiments correspond well to field observations of Ketelaars et al. (1999), who found a strong decrease in the abundances of Cladocera and rotifers, but not of copepods, after the invasion of the Ponto-Caspian *Hemimysis anomala* in a freshwater reservoir in the Netherlands. Furthermore, the introduction of *Mysis relicta* to lakes in the Western United States and Canada is supposed to be responsible for large-scale changes in the native zooplankton communities (Chess and Stanford 1998; Goldman et al. 1979) and a massive reduction of Cladocera was also reported after the introduction of *M. relicta* to Lake Jonsvatn, Norway (Koksvik et al. 1991). In the Baltic Sea, *M. relicta* and *M. mixta* prey on multiple species of phytoplankton and zooplankton, but the selectivity and preference for food organisms changes with mysid age and species (Viherluoto et al. 2000). There, the lower susceptibility of copepods to mysid predation was attributed to the copepods' ability to detect hydrodynamic disturbance from mysids over considerable distances and thereby allowed them effective predator-avoidance behaviour (Viitasalo et al. 1998). This might—at least in part—explain the lower vulnerability of copepods to predation by *L. benedeni* in our experiments.

Several species of omnivorous mysids have invaded Europe within the last two decades (e.g., Arbaciauskas 2002; Bij De Vaate et al. 2002; Wittmann 2007). For example, *Paramysis lacustris* has established high abundances in parts of the Baltic Sea where it influenced the inshore/offshore habitat coupling (Lesutiene et al. 2007, 2008), and the pelagic *Hemimysis anomala* established itself as a new and important link between primary production and higher trophic levels in the food web of its new environments (Borcherding et al. 2006). Here, we focussed on *L. benedeni*, a mysid that has invaded many ecosystems throughout continental Europe in recent years and has since undergone a massive spread in habitat and abundance (Wittmann 2007). Similar to the closely related species *H. anomala* (Holdich et al. 2006; Pothoven et al. 2007), it is predicted to arrive in North America (Ricciardi and Rasmussen 1998) as well as in Britain and the Mediterranean region (Wittmann 2007) within the next few years. This is typical for the euryhaline Ponto-Caspian mysids that have a high dispersion ability (Audzijonyte et al. 2006; Ricciardi and MacIsaac 2000). *L. benedeni* was previously estimated to have only little impact on the native zooplankton communities (Wittmann and Ariani 2000), although this assumption was not based on any quantitative data. For the first time, we here demonstrate potential consequences of the invasion of *L. benedeni* on the native fauna and how these consequences might be modulated by the omnivorous feeding habit of this species. Our results further corroborate the findings from pelagic mysids (Chess and Stanford 1998; Koksvik et al. 1991; Langeland et al. 1991) that the invasion of mysids in general can have profound impacts on the community structure of the native zooplankton fauna and that invasions of *L. benedeni* cannot be considered to be without whole-ecosystem consequences, as suggested by Wittmann and Ariani (2000). Furthermore, mysids are also potential predators of benthic invertebrates (Bailey et al. 2006). This probably is another important aspect with the invasion of *L. benedeni*, since at least parts of the population spends daytimes in littoral refuges close to the bottom (Gergs et al. 2008, Fink and Borcherding, pers. obs.), which probably is a predator avoidance strategy. Hence, the invasion of Ponto-Caspian mysids such as *L. benedeni* can affect the food web structure of invaded habitats at multiple levels and thus potentially have complex whole-ecosystem consequences.

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