Combined Impacts of Algal Density and Copepod Predation on the Community of Small-Sized Zooplankton

TAKAMARU NAGATA1* and TAKAYUKI HANAZATO2

1Lake Biwa Environmental Research Institute/5–34 Yanagasaki, Otsu, Shiga 520–0022, Japan
2Institute of Mountain Science Shinshu University, Division of Science for Inland Water Environment /5–2–4 Kogandori, Suwa, Nagano 392–0027, Japan

Abstract

We assessed the combined impacts on the community of small-sized herbivorous zooplankton using mesocosms, in which their populations were exposed to copepod Mesocyclops pehpeiensis predation and/or food shortage. The densities of the small-sized zooplankton were depressed in the tanks with M. pehpeiensis and a low algal density, probably because the prey populations were unable to compensate for the population loss by copepod predation. In contrast, most of the herbivorous zooplankton, especially rotifers, established large populations, despite their exposure to predation in the tanks with high algal density. This might be due to their high reproductivity under the conditions. In addition, the release from competitive pressure by cladocerans, whose populations were suppressed by intensive predation by M. pehpeiensis, would be favorable for the rotifers. Our experimental findings may explain the herbivorous zooplankton community structure in the shallow eutrophic lakes inhabited by abundant copepods. The results may become valuable information for reducing biological interference with rotifers within zooplankton assemblages, leading to effective use of the animals for water treatment systems.

Keywords: community structure, copepod predation, food abundance, small sized zooplankton

**INTRODUCTION**

Predacious copepods often appear abundantly in lakes, and impose high predation pressure on small-sized herbivorous zooplankton such as rotifers4). Copepods prey effectively on small-sized, soft-bodied, and non-spined prey zooplankton species2,9). This is probably the main factor accounting for the phenomenon that, in the field, copepods exert different degrees of predation pressure on each of herbivorous zooplankton populations, and that they alter species composition of the prey community3,4).

A factor other than predation that controls population dynamics of small-sized herbivorous zooplankton is food abundance (algal, bacterial and detrital resources), which governs the reproduction of zooplankton populations5–8). Herbivorous zooplankters compete with one another for food resources because most of them utilize particles within a common size range of up to 25 µm (4–17 µm for rotifers8–10), 1–25 µm for cladocerans7,11,12). Many studies have shown that population densities of the small zooplankters, such as rotifers, decline in the presence of large cladocerans such as Daphnia8,11). Cladocerans generally have large body and food competition advantage over rotifers. This advantage is attributed mainly to the fact that large zooplankters have a high clearance rate and tolerance to starvation8). In addition, the wider size range

---

*Corresponding author
Phone number: +81 77 526 4800
E-mail: nagata-t@lberi.jp
The community structures of herbivorous zooplankton would be controlled in a complex manner by food resources and invertebrate predation. It is expected that the populations of small-sized zooplankton species, especially rotifers, which dominate zooplankton communities in eutrophic lakes (generally fish-abundant lakes) are imposed strong effect by the combined effects. Predaceous copepods play significant role in determining community structures of herbivorous zooplankton even in such lakes because they are able to maintain its populations by their escape response, diel vertical migration which is effective to reduce predation pressure by fish. Chang and Hanazato have performed a community-level laboratory experiment to evaluate the combined impacts of food abundance and copepod predation on zooplankton assemblage. However, they were unable to evaluate the combined impact on planktonic rotifer species since the densities of such species were low, but the benthic species Lepadella sp. was abundant during the experiment. Thus, we tried to assess the combined impacts of algal concentration and copepod predation on small-sized herbivorous zooplankton, particularly planktonic rotifer populations in a community-level experiment.

MATERIALS AND METHODS

Twelve cylindrical plastic tanks (volume 100 L, diameter 40 cm, height 73 cm) were used for the experiment. To establish a zooplankton community in each tank, the bottom sediments of the eutrophic Lake Suwa, Japan, including resting eggs of various zooplankton species, was collected at the center of the lake (6 m depth; 36°2’N, 138°5’E) with an Ekman grab sampler. About 1.5 kg of sediment was placed in each tank, which was then filled with 80 L of tap water, and the experiment was started (day 0). All of the tanks were kept in the laboratory in a controlled environment (temperature 20°C, photoperiod 16-h light/8-h dark). On day 10, the mesocosm tanks were divided into two groups of six tanks each: low-food tanks and high-food tanks (Fig. 1). On the same day, to create different food levels for the herbivorous zooplankton in the tanks, we added the green alga Chlorella vulgaris (purchased from Chlorella Industry Co. Ltd., Fukuoka, Japan) to the low-food and high-food tanks to create algal densities of approximately 1×10³ cells/mL and 1×10⁵ cells/mL, respectively. Algae were added repeatedly at 1- or 2-day intervals, thereafter. On day 19, 120 adults of Mesocyclops pehpeiensis (body length, > 1000 µm) were introduced into each of three low-food tanks and three high-food tanks (low-food Mesocyclops tanks, high-food Mesocyclops tanks, respectively). The predators were obtained from Lake Suwa one month before the start of the experiment and maintained in the laboratory with a zooplankton assemblage collected from the

Figure 1 The experimental protocol.
lake. No invertebrate predators were introduced into the remaining three low-food tanks and three high-food tanks, which served as controls (low-food control tanks, high-food control tanks, respectively). The experiment was terminated on day 27.

Water temperature and phytoplankton biomass indicated by chlorophyll $a$ (chl. $a$) in the tanks were measured at 1- or 2-day intervals. Water temperature was measured with a thermister (YSI 55 dissolved oxygen meter; YSI Co., Ohio, USA). For measurement of chl. $a$, 100 mL of water was collected from each tank and filtered through a Whatman GF/C filter, and then chl. $a$ in the particles that remained on the filter was measured using methyl alcohol and fluorescence according to Marker et al$^{19}$.

Zooplankton were sampled from all the tanks on day 16 and at 1- or 2-day intervals thereafter. To collect zooplankton samples, the water column (3.0 L in volume) from the surface to 32 cm depth (approximately 20 cm above the bottom sediment) in the tanks was collected using a column sampler (diameter 5 cm, length 50 cm) with a hydraulically operated bottom flap. Since some zooplankters might have been distributed unequally in the tanks, the water was gently mixed with a wooden rod before sampling to make their distribution uniform. Zooplankton were then collected by filtering the sampled water through a 40-$\mu$m mesh net, and preserved in sugar-formalin containing a final concentration of 4% formalin$^{19}$. The mesh size (40 $\mu$m) of the net was sufficiently smaller than the body width of small species such as the rotifers Filinia and Polycarthra$^{8,19}$. Therefore, all the crustacean and rotifer individuals (except eggs) should have been collected with the net. The water that had filtered through the net was returned to the tank to reduce water loss. Zooplankton in the samples were identified to species or genus level and counted with a microscope at ×100 magnification. For the predator M. pehpeiensis, the body lengths of individuals were measured and divided into two life stages according to Chang and Hanazato$^{16}$: early copepodite stages (body length < 800 $\mu$m) and late copepodite stages including adults (body length $\geq$ 800 $\mu$m). The feeding habits of the early and late copepodite stages are generally known to be herbivorous and omnivorous, respectively, and therefore the latter stage is considered to be when M. pehpeiensis feeds mainly on herbivorous crustaceans and rotifers.

The data sets were analyzed using 2 × 2 factorial ANOVA with the difference in algal density and the introduction of M. pehpeiensis as the main factors, and day as repeated measures. The differences in density of zooplankton species among tanks subjected to different treatments were analyzed statistically with two-way repeated-measures ANOVA using StatView ver.5 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Water temperature did not differ markedly between the control and the Mesocyclops tanks under the respective food conditions (Fig. 2). Under the high-food conditions, the values of chl. $a$ tended to be lower in the control tanks than those in the Mesocyclops tanks, but the difference was not significant (repeated-measures ANOVA, treatment: $F = 1.211, P = 0.2820$). The values of chl. $a$ in the low-food tanks were very low and were therefore hardly determined during most of the experimental period.

It was impossible to completely exclude M. pehpeiensis from the control tanks, because their resting stages were contained in the bottom sediment, and hence some copepods appeared in the tanks (Fig. 3). However, the densities of their early and late copepodite stages were higher in the Mesocyclops tanks than in the control tanks during the experiment (Table 1). In the experiment, unexpected invertebrate predators such as Leptodora kindtii (except for M. pehpeiensis) did not appeared in the mesocosm tanks.

Three cladoceran (Bosmina longirostris, B. fatalis and Bosminopsis deitersi) and four rotifer species (Brachionus angularis, Filinia longiseta, Hexarthra mira, and Keratella valga) appeared commonly in the mesocosm tanks (Figs. 4 and 5). Their densities were higher under high-food conditions than under low-food conditions. The results of two-way repeated measures ANOVA showed that the effects of food treatment were significant for
Figure 2  Changes in water temperature and chl. a (average ± SE) in the mesocosm tanks with low food density (left panel) and high food density (right panel) during the experiment. Clear circles, control tanks; Solid circles, *Mesocyclops* tanks.

Figure 3  Changes in density (average ± SE) of *M. pehpeiensis* in the mesocosm tanks with low food density (left panel) and high food density (right panel) during the experiment. Clear circles, control tanks; Solid circles, *Mesocyclops* tanks. Note that the scales of density (vertical axes) in the panels are different from one another.
Table 1  Results of two-way repeated-measures ANOVA used to estimate the differences in the densities of each zooplankton species among the treatments after introduction of *M. pehpeiensis* (days 21-27)

<table>
<thead>
<tr>
<th></th>
<th>Food</th>
<th>Predator</th>
<th>Food × Predator</th>
<th>Day</th>
<th>Food × Day</th>
<th>Predator × Day</th>
<th>Food × Predator × Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cyclopoid copepods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. pehpeiensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>early copepodite stages</td>
<td>22.346</td>
<td>&lt;0.001</td>
<td>16.964 &lt;0.001</td>
<td>11.996</td>
<td>0.002</td>
<td>8.421 &lt;0.001</td>
<td>8.637 &lt;0.001</td>
</tr>
<tr>
<td>late copepodite stages</td>
<td>1.385</td>
<td>0.248</td>
<td>7.538 0.010</td>
<td>0.000</td>
<td>1.000</td>
<td>2.154 0.113</td>
<td>2.103 0.119</td>
</tr>
<tr>
<td><strong>Cladocerans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. longirostris</em></td>
<td>64.324</td>
<td>&lt;0.001</td>
<td>4.978 0.033</td>
<td>1.924</td>
<td>0.175</td>
<td>15.461 &lt;0.001</td>
<td>7.354 0.001</td>
</tr>
<tr>
<td><em>B. fatalis</em></td>
<td>16.095</td>
<td>&lt;0.001</td>
<td>8.595 0.006</td>
<td>0.595</td>
<td>0.446</td>
<td>1.924 0.175</td>
<td>1.000 0.406</td>
</tr>
<tr>
<td><em>B. deitersi</em></td>
<td>1.877</td>
<td>0.180</td>
<td>1.855 0.183</td>
<td>1.810</td>
<td>0.188</td>
<td>0.770 0.519</td>
<td>0.758 0.526</td>
</tr>
<tr>
<td><strong>Rotifers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. angularis</em></td>
<td>115.197</td>
<td>&lt;0.001</td>
<td>12.840 0.001</td>
<td>14.382</td>
<td>0.001</td>
<td>1.971 0.138</td>
<td>2.031 0.129</td>
</tr>
<tr>
<td><em>F. longiseta</em></td>
<td>4.920</td>
<td>0.034</td>
<td>1.087 0.305</td>
<td>1.435</td>
<td>0.240</td>
<td>0.830 0.487</td>
<td>0.773 0.518</td>
</tr>
<tr>
<td><em>H. mira</em></td>
<td>27.814</td>
<td>&lt;0.001</td>
<td>6.227 0.018</td>
<td>8.722</td>
<td>0.006</td>
<td>4.152 0.014</td>
<td>2.885 0.051</td>
</tr>
<tr>
<td><em>K. valga</em></td>
<td>6.725</td>
<td>0.014</td>
<td>3.666 0.065</td>
<td>3.826</td>
<td>0.059</td>
<td>0.983 0.413</td>
<td>0.934 0.435</td>
</tr>
</tbody>
</table>

The treatment food and predator show difference in food density and with or without *M. pehpeiensis*, respectively. Day was the repeated measure. Significant level in bold.
the abundances of most zooplankters, except for late copepodite stages of *M. pehpeiensis* and *B. deitersi* (Table 1).

The introduction of *M. pehpeiensis* significantly affected the zooplankton densities (except for *B. deitersi*) in the mesocosm tanks (Table 1). The densities of *B. longirostris* and *B. fatalis* were lower in the *Mesocyclops* tanks than in the control tanks, especially under low food-conditions (Fig. 4). Although the abundances of two cladoceran species were significantly influenced by the predator treatment, their abundances under high-food conditions were not different between the *Mesocyclops* tanks and the control tanks (repeated measures ANOVA: *B. longirostris*, F = 0.2, P = 0.63; *B. fatalis*, F = 4.5, P = 0.05). On the other hand, the effects of predator (introduction of *M. pehpeiensis*) were not significant for *B. deitersi*, but the density of the cladoceran in the *Mesocyclops* tanks was low as compared with in the control tanks during the experiment. Two-way repeated measures ANOVA represented that the effects of food × predator interactions were not significantly for the densities of cladocerans (Table 1).

The densities of rotifers were also significantly affected by the predator treatment (Table 1). Under low-food conditions, like the cladocerans, the rotifer abundances were lower in the *Mesocyclops* tanks than in the control tanks (Fig. 5). In contrast to the cladocerans and the rotifer populations under low-food conditions, the densities of rotifers in the high-food control tanks were lower than those in the high-food *Mesocyclops* tanks (Fig. 5), although significant differences in the densities between the control and the *Mesocyclops* tanks were observed only for *B. angularis* and *H. mira* (Table 1, food × predator interaction: *B. angularis*, F = 14.38, P = 0.0001; *H. mira*, F = 8.72, P = 0.006).
DISCUSSION

In contrast to Chang and Hanazato\(^\text{10}\), most rotifers in the present study were planktonic species. This might be due to the difference of lapsed days from the addition of lake sediments to the end of the experiment. Because mesocosm tanks have walls, periphytic and/or benthic species often dominate zooplankton community when many days elapse after the addition of lake sediments to the tanks. However, we succeeded in examining the combined impacts of algal density and copepod predation on planktonic species before the dominance of periphytic and/or benthic species. The combined impacts played important roles in structuring the community of small-sized herbivorous zooplankton. The results suggest that the responses against the effects differ among zooplankton populations.

All of the herbivorous zooplankton species without Mesocyclops tanks established larger populations under high-food conditions than under low-food conditions. This might be because the zooplankters attained high

Figure 5 Changes in density (average ± SE) of rotifers in the mesocosm tanks with low food density (left panel) and high food density (right panel) during the experiment. Clear circles, control tanks; Solid circles, Mesocyclops tanks. Note that the scales of density (vertical axes) in the panels are different from one another.
reproductivity under plenty food conditions. These results can be expected by huge previous literatures. On the other hand, most herbivorous zooplankton populations were negatively affected by *M. pehpeiensis* under low-food conditions, in agreement with the results of Chang and Hanazato\(^{16}\). The values of chl. *a* under low-food conditions were close to zero during the experiment (Fig. 2). Therefore, zooplankton would have been exposed to both food shortage and *M. pehpeiensis* predation in the *Mesocyclops* tanks. The food shortage would have reduced their reproductivity and probably made it difficult for their populations to compensate for the population loss due to predation, thus increasing the impact of predation. This might explain why the zooplankton populations were strongly suppressed by *M. pehpeiensis* predation under low-food conditions.

In contrast, it seemed that the prey populations under high-food conditions were hardly affected by copepod predation. Interestingly, rotifers established larger populations in the *Mesocyclops* tanks than in the control tanks (Fig. 5, Table 1). However, this did not mean that the preys were not eaten by copepods, because many studies have shown that they are greatly susceptible to copepod predation\(^{19}\). In general, rotifers are considered to have much higher population growth rates than cladocerans when food is plentiful\(^{19}\). Thus, rotifers might be able to compensate for any population loss due to copepod predation by their high reproductivity.

However, a high population growth rate alone cannot explain the higher rotifer densities that were observed in the *Mesocyclops* tanks than in the control tanks. Presumably, the lower densities in the control tanks resulted from competition with cladocerans. Rotifers and cladocerans feed on particles (algae, bacteria, and detritus) within similar size ranges (4–17 µm for rotifers\(^{9–11}\); 1–25 µm for cladocerans\(^{7,12,13}\)). In the present experiment, we used the green alga *Chlorella vulgaris* as food source for zooplankton. Since the clearance rate of cladocerans on the alga is higher than that of rotifers\(^{20,21}\), then the former animals are competitively superior to the latter. Therefore, the low densities of rotifers in the control tanks might have been due to competitive pressure by cladocerans.

With a mesocosm experiment, Chang and Hanazato\(^{16}\) reported that most herbivorous cladoceran populations were subjected to high predation pressure by *M. pehpeiensis* irrespective of algal density. In contrast to the cladocerans, the rotifer populations in their experiment were not affected negatively by the copepod, irrespective of food abundance. In the study of Chang and Hanazato\(^{16}\), periphytic animals (the cladoceran *Chydorus* sp. and the rotifer *Lepadella* sp.) appeared abundantly in the mesocosm tanks. Because copepods possess mechanoreceptors that detect vibrations and water displacements created by swimming prey\(^{22}\), and periphytic animals are generally more sessile than planktonic ones, it is difficult to assess the relationships between food abundance and prey-predator interactions (planktonic zooplankton prey-invertebrate predators) in pelagic food web by their study. In the present experiment, any periphytic species were hardly observed. Therefore, we have succeeded in demonstrating the predation impact of a cyclopoid copepod on a pelagic zooplankton community in the presence of different phytoplankton biomasses, and the results indicate that zooplankton community structures are shifted in a complex manner by the combined impacts of invertebrate predation and food abundance.

The differences were also evident between the present study and Chang and Hanazato\(^{16}\) with regard to the densities of the early and late copepodite stages of *M. pehpeiensis* in the low-food tanks. The early copepodite stages maintained low densities in the low-food tanks (0.22–0.89 indiv./L) in the former study whereas they hardly appeared in the latter. On the other hand, the late copepodite density in the low-food tanks was lower in the former experiment (max. density: 0.33
indiv./L) than in the latter (max. density: approx. 2.0 indiv./L). In case of the genus *Mesocyclops*, the early copepodite stages feed on rotifers in addition to algae. Thus, it is possible that the early copepodite stages of *M. pehpeiensis* have imposed competitive and predation pressure on rotifers in our experiment. This might explain why the rotifer populations were suppressed by copepods in the present study than in the study by Chang and Hanazato under low-food conditions.

In eutrophic lakes, small zooplankters especially rotifers are principal grazers, and consume a lots of organic particles such as phytoplankton and bacteria, inducing increase in water transparency. Due to their sharp appetite, they are used generally for water treatment systems. Our results have shown that the community structures of small-sized zooplankton depend on biological interactions (predation and competition) as well as food abundance. A particularly intriguing result was that rotifers might be suppressed by competitive pressure given by crustaceans (including the early stages of copepods) rather than by predation under the high-food conditions. Such dynamics of rotifers are often observed in eutrophic lakes. Our results would supply information to understand the structuring of small-sized zooplankton communities, especially rotifer ones in eutrophic lakes. In addition, such information may be valuable for reducing biological interference with rotifers within zooplankton assemblages, leading to effective use of the animals for water treatment systems.

**ACKNOWLEDGEMENT**

I would like to thank Mr. K. Watanabe and Ms. H. Inoue for technical assistance with the data analyses. This study was supported by the Environment Research and Technology Development Fund (5–1607) of the Ministry of the Environment, Japan.

**REFERENCES**


13) Nadin-Hurley, C. M., and Duncan, A.: A


(Submitted 2017. 8. 24)

(Accepted 2017. 11. 14)