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Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms

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Abstract Diatoms form large spring blooms in lakes and oceans, providing fuel for higher trophic levels at the start of the growing season. Some of the diatom blooms, however, are not grazed by filter-feeding zooplankton like *Daphnia* due to their large size. Several of these large diatoms are susceptible to chytrid infections. Zoospores of chytrids appeared to be excellent food for *Daphnia*, both in terms of size, shape, and quality (PUFAs and cholesterol). Thus, zoospores of chytrids can bridge the gap between inedible diatoms and *Daphnia*. In order to examine the effects of diatoms and chytrids on the survival of copepods, we performed one grazing and one survival experiment. The grazing experiment revealed that the diatom, *Asterionella formosa*, was not grazed by the copepod, *Eudiaptomus gracilis*, even after being infected by the chytrid *Zygorhizidium planktonicum*. However, carbon and nitrogen concentrations were significantly reduced by *E. gracilis* only when *A. formosa* was infected by *Z. planktonicum*, indicating that the chytrids might facilitate material transfer from inedible diatoms to the copepods. The survival experiment revealed that *E. gracilis* lived shorter with *A. formosa* than with the cryptophyta *Cryptomonas pyrenoidifera*. However, the survival of *E. gracilis* increased significantly in the treatment where *A. formosa* cells were infected by *Z. planktonicum*. Since *E. gracilis* could not graze *A. formosa* cells due to their large colonial forms, *E. gracilis* may acquire nutrients by grazing on the zoospores, and were so able to survive in the presence of the *A. formosa*. This provides new insights into the role of parasitic fungi in aquatic food webs, where chytrids may improve copepod survival during diatom blooms.

Keywords Parasite · Food web · Chytrid · *Eudiaptomus* · Mycoloop

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Introduction

The efficiency with which biomass and energy are transferred from primary producers to grazers is highly variable in ecosystems (Lindemann, 1942; Cebrian, 1999). The trophic transfer efficiency depends on

various factors such as plant morphology (palatability) and nutritional quality (Porter, 1977; Elser et al., 2000). The two trophic levels can even become uncoupled. This is especially true when inedible species dominate the primary producers or when plants produce toxins.

In lakes and oceans, diatoms may form large spring blooms. Spring blooms of diatoms have been considered to be a fuel for zooplankters, such as daphnids and copepods (Ryther, 1969; Sommer et al., 1986). Some of the diatom blooms, however, are not grazed by zooplankton due to their large (colonial) size (Knisely & Geller, 1986). Furthermore, some marine diatoms have been found to have deleterious effects on copepod reproduction (Ban et al., 1997). This might be related to chemical substances, PUA (polyunsaturated aldehydes), produced by mechanically damaged diatom cells (Miralto et al., 1999; Pohnert, 2000; Ianora et al., 2004). Some freshwater diatoms also produce PUA when damaged (Pohnert, 2000; Watson et al., 2001; Carotenuto et al., 2005). PUA produced by freshwater diatoms were found not to affect *Daphnia* populations (Carotenuto et al., 2005), yet their effects on freshwater copepods fitness have not been tested.

In lakes, diatoms are often infected by parasitic chytrids (Van Donk & Ringelberg, 1983; Ibelings et al., 2004; Kagami et al., 2007a). Chytrids infections seem to be most common in large phytoplankton species that are fairly resistant to grazing by zooplankton (Sommer, 1987; Kagami et al., 2007a). Parasitic chytrids of phytoplankton play an important role in aquatic food webs (Kagami et al., 2007a; Gleason et al., 2008). Chytrids take up energy and nutrients from their host and produce abundant zoospores, which are released into the water. Contrary to the host phytoplankton cells, these zoospores are excellent food for zooplankton in terms of size and shape (Kagami et al., 2004). In addition, zoospores are rich in polyunsaturated fatty acids (PUFAs) and cholesterol, which are essential nutrition for crustaceans (Kagami et al., 2007b). By grazing on the zoospores, the zooplankton, *Daphnia galeata hyalina*, acquired important supplementary nutrients and was able to grow in the presence of the inedible diatom, *Asterionella formosa* (Kagami et al., 2007b). Thus, chytrids can improve zooplankton production and enhance the trophic transfer.

In order to investigate the effects of the freshwater diatom, *Asterionella formosa*, and the chytrid on the copepod, *Eudiaptomus gracilis*, we conducted two types of laboratory experiments. First, a grazing experiment was performed to assess the vulnerability of *A. formosa* to *E. gracilis* grazing, both when cells are healthy and when cells are infected by chytrid. Second, a survival experiment was conducted to examine the effects of *A. formosa* on the survival of the *E. gracilis*. We also tested the hypothesis whether survival of copepods increases when chytrids are present.

Materials and methods

The diatom, *Asterionella formosa*, was isolated from Lake Maarsseveen during 2002 (strain MS07702-5). The strain was maintained in non-axenic batch cultures with modified Chu-10 medium (Kagami et al., 2004). The parasitic chytrid, *Zygorhizidium planktonicum*, was isolated from Lake Maarsseveen during 2006 and was maintained on its host (*A. formosa*, strain MS07702-5) in a non-axenic culture. The alga *Cryptomonas pyrenoidifera* (strain NIVA-2/81) was cultured with modified Chu-10 medium and used as good edible food for the copepod.

A. formosa and *A. formosa* infected by *Z. planktonicum* (60% of the cells were infected) were grown in 10 l batch cultures with magnetic stirrers. *C. pyrenoidifera* was grown in 250 ml batch cultures. *Eudiaptomus gracilis* was isolated from Lake Maarsseveen in 2006 and was maintained in 500 ml flasks containing filtered lake water from Lake Maarsseveen (<0.2 μm). *E. gracilis* was fed with *C. pyrenoidifera*. The juvenile copepodites (C5) and adult copepods (C6) were collected in a 1 l beaker and rinsed three times with filtered lake water and finally kept for 2 h in the medium to wash away the algae.

Grazing experiment

We prepared two food treatments, with *A. formosa* only (diatom) and with *A. formosa* infected by *Z. planktonicum* (diatom + fungi). 30 ml of uninfected *A. formosa* cells were inoculated into 30-ml bottles (diatom treatment) at a concentration of 0.6 mg C l^{-1} (0.6×10^4 cells ml^{-1}). 30 ml of *A. formosa* cells infected by *Z. planktonicum* were inoculated into other 30-ml bottles (diatom + fungi) again at a concentration of 0.6 mg C l^{-1} (2×10^4 cells ml^{-1}).

For each treatment (diatom, diatom + fungi), two types of bottles were prepared; bottles without and with copepods. Four and eight bottles were incubated with copepods in the "diatom" and "diatom + fungi" treatment, respectively. For both treatments, three other bottles without copepods were prepared. In total, seven bottles were used for the diatom treatment, and 11 bottles for the diatom + fungi treatment. All copepod-containing bottles contained a pair of male and female *E. gracilis.*

All bottles were incubated for 2 days at 18°C under a 12:12 L:D cycle at 100 μmol quanta m^{-2} s^{-1}, mixed with a rotator. After 2 days incubation, we counted the abundance of *A. formosa* cells in the bottles with and without copepods for each treatment (diatom, diatom + fungi). Particulate carbon and nitrogen concentrations were also determined with a Calanus universal carbon analyzer (Uniquant OY). 15-ml of a subsample were centrifuged at 3,000*g* for 15 min, and the pellet was put into a precombusted silver cup (200 μl).

The change rates of *A. formosa* and of particulate carbon and nitrogen were estimated assuming exponential growth. Grazing rates of *E. gracilis* on *A. formosa* cells, and particulate carbon and nitrogen, were calculated from the difference in the change rates between the bottles with and without copepods. Effects of *E. gracilis* grazing and chytrid infection on change rates of *A. formosa* cells and particulate carbon and nitrogen, were assessed by two-way factorial ANOVA. The differences in grazing rates of *E. gracilis* between diatom and diatom + fungi treatments were assessed by a *t* test. Before the analysis, normality of the data (change rates) was verified by Kolmogorov–Sminov normality test.

Survival experiment

A pair of male and female copepods were randomly selected and transferred into 30 ml bottles containing 30 ml food suspension in filtered lake water. 12 pairs (12 males and 12 females) were prepared for each three treatments, with *A. formosa* only (diatom), with *A. formosa* infected by *Z. planktonicum* (diatom + fungi), and with *C. pyrenoidifera* (control). For each treatment, 400 ml of food suspension was prepared and food concentrations were adjusted to 0.6 mg C l^{-1} (0.5–2 × 10^4 cells ml^{-1}), by diluting cultures with filtered lake water. Thirty-six of 30 ml bottles were inoculated with 30 ml of each food suspensions.

All bottles were incubated at 18°C under a 12:12 L:D cycle at 100 μmol quanta m^{-2} s^{-1} for 10 days. Bottles were mixed with a rotator (one rotation per minute) to suspend the food throughout the experiments. The animals were transferred every 1–2 days to new bottles with fresh food. Dead individuals and number of females with eggs were counted under a microscope at 25 × magnification.

Survival analysis was carried out using the R procedure "survreg" (Crawley 2007). Survival data were fitted to Weibull distribution. A likelihood-ratio test, based on log-likelihoods given by R "survreg", was used to determine the statistical significance of the treatments. A pairwise comparison of survival function were made between diatom + fungi treatment and the other two treatments (control, diatom treatments). The time needed to kill 50% of the animals (LT_{50}) was calculated from survival function estimation.

Results

Grazing experiments

The change rates of *A. formosa* in the diatom treatment are significantly higher than those of infected *A. formosa* in the diatom + fungi treatment in both bottles with/without copepods (Fig. 1).

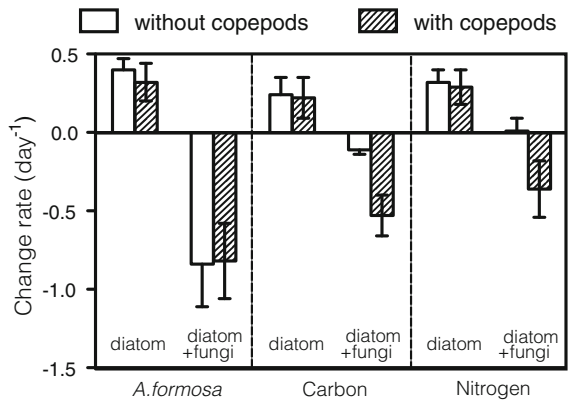

Fig. 1 The change rates of *Asterionella formosa* and of particulate carbon and nitrogen concentrations in bottles with and without copepods in the diatom and the diatom + fungi treatments

Between bottles with/without copepods, the change rate of *A. formosa* did not differ significantly, either in diatom and diatom + fungi treatments. ANOVA showed the effect of fungal infection on change rates of *A. formosa* was significant ($F_{1,14} = 140.34$, $P < 0.0001$), but neither effects of copepod grazing ($F_{1,14} = 0.05$, $P = 0.813$) nor effects of copepod grazing × fungal infection interaction ($F_{1,14} = 0.222$, $P = 0.644$) on change rates were significant. These data indicates that copepod *E. gracilis* did not affect change rates of *A. formosa*, and the effects of copepod grazing did not differ between diatom and diatom + fungi treatments.

The change rates of particulate carbon/nitrogen concentrations in the diatom treatment are significantly higher than those in the diatom + fungi treatment in both bottles with/without copepods (Fig. 1). The change rates of particulate carbon/nitrogen concentrations between bottles with and without copepods did not differ significantly in the diatom treatment (Fig. 1). The change rates of those in the diatom + fungi treatment, however, were lower in the bottles with copepods than those without copepods. For the particulate carbon and nitrogen concentrations, the effects of fungal infection ($F_{1,14} = 134.45$, $P < 0.0001$ for carbon, $F_{1,14} = 91.36$, $P < 0.0001$ for nitrogen), copepod grazing ($F_{1,14} = 17.81$, $P = 0.001$ for carbon, $F_{1,14} = 14.28$, $P = 0.002$ for nitrogen), and copepod grazing × fungal infection interactions ($F_{1,14} = 11.47$, $P = 0.044$ for carbon, $F_{1,14} = 8.68$, $P = 0.010$ for nitrogen) were significant. These results indicate that copepod grazing did affect the change rates of particulate carbon/nitrogen concentrations and the effects did differ significantly between diatom and diatom + fungi treatments.

Grazing rates of the copepod *E. gracilis* on *A. formosa* did not differ significantly between diatom and diatom + fungi treatments (Fig. 2, $t = 0.707$, $P = 0.489$). Grazing on particulate carbon and nitrogen, however, did differ significantly between treatments, and they are higher in diatom + fungi treatment than in diatom treatment ($t = 5.874$, $P < 0.0001$ for C, $T = 4.08$, $P = 0.0007$ for N).

Survival experiment

The survival experiment showed that *E. gracilis* survived significantly different among treatments ($\chi^2 = 53.07$, df = 2, $P < 0.0001$, Fig. 3). Only a few animals died in the control treatment with *C. pyrenoidifera*. *E. gracilis* in diatom + fungi treatment (with infected *A. formosa*) survived significantly shorter than the animals in the control treatment ($P = 0.0003$), but longer than in diatom treatment ($P = 0.02$). The time needed to kill 50% of the animals (LT_{50}) was 3.1 days in the diatom treatment and 4.5 days in the diatom + fungi treatment, and more than 10 days in the control. In the control treatment, 4 females among 12 females produced eggs (Table 1). One female produced eggs

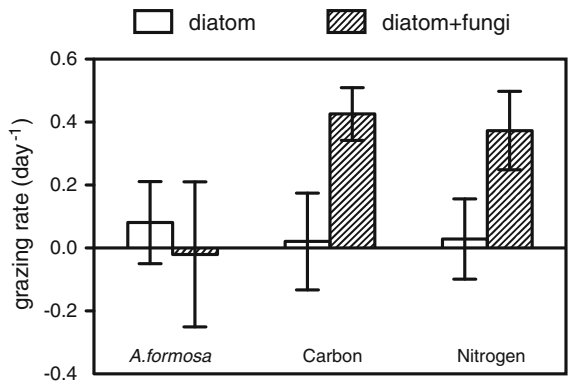

Fig. 2 Grazing rates of *Eudiaptomus gracilis* on *Asterionella formosa* cells and on the particulate carbon and nitrogen concentrations in the diatom and diatom + fungi treatments

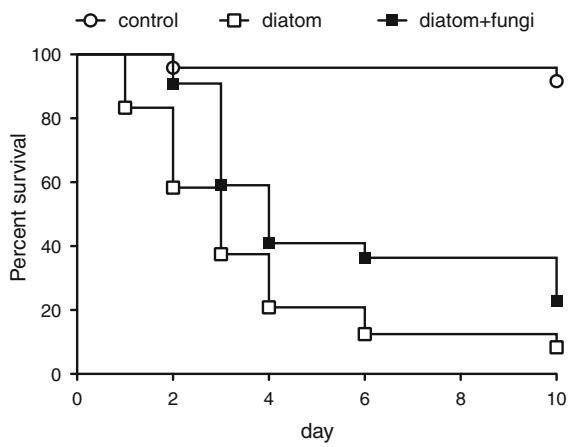

Fig. 3 Kaplan–Meier survival curves of *Eudiaptomus gracilis* in the three food treatments (Control, diatom, diatom + fungi). Control: feeding on *Cryptomonas pyrenoidifera*, Diatom treatment: feeding on *Asterionella formosa* only, Diatom + fungi treatment: feeding on *A. formosa* infected by the chytrid *Z. planktonicum*. Initial number of copepods was 24 for each treatment

Table 1 Final abundance of live/dead *Eudiaptomus gracilis* in the three treatments (control, diatom, diatom + fungi)

	Control	Diatom	Diatom + fungi
Live	22	0	6
Male	11	0	3
Female	11 (4)	0	3 (1)
Dead	2	24	18
Male	1	12	9
Female	1	12	9

Number of females producing eggs during incubation are shown in *parentheses*

in the diatom + fungi treatment, while no female did in the diatom treatment.

Discussion

The grazing experiment showed that *E. gracilis* did not reduce *A. formosa* cells, either in the diatom and the diatom + fungi treatment (Figs. 1 and 2). The colonies of *A. formosa* must be too large to be grazed by the copepod, *E. gracilis* (Knisely & Geller, 1986). Even after being infected by chytrids, *A. formosa* retained its colony form and became aggregated, as already reported by Kagami et al. (2005). Thus, the vulnerability of infected *A. formosa* to copepod grazing might be the same or even less than those of healthy ones.

E. gracilis could not reduce the particulate carbon and nitrogen concentrations in the diatom treatment, indicating there were no available food sources for *E. gracilis* in the diatom treatment. While, particulate carbon and nitrogen were significantly reduced by *E. gracilis* in the diatom + fungi treatment (Figs. 1, 2), though *A. formosa* cells were not reduced. This indicates that other carbon/nitrogen sources except *A. formosa* cells, such as zoospores of chytrids and bacteria, might have been grazed by copepods.

The survival experiment showed that survival of *E. gracilis* was significantly lower in the diatom treatment with only *A. formosa* as food (Fig. 3). The lowest survival must be explained by less vulnerability of *A. formosa* to copepod grazing. While, the survival of *E. gracilis* was significantly higher in diatom + fungi treatment (Fig. 3). The slight improvement of copepod survival in the diatom + fungi treatment cannot be due to grazing on infected *Asterionella*, but due to other carbon sources including zoospores of chytrids and bacteria. Thus, the transfer of carbon and nitrogen between *A. formosa* and *E. gracilis* was increased by chytrid, which resulted in higher survival.

In some diatom species, allelopathic compounds such as polyunsaturated aldehydes (Ianora et al., 2004) have been found to be responsible for growth inhibition in copepods (Miralto et al., 1999; Pohnert et al., 2002). Some freshwater diatoms including *A. formosa* also release PUA, 12-ODTE, and 9-ONDE (Pohnert, 2000; Carotenuto et al., 2005). But they are not feeding deterrents for *Daphnia* (Carotenuto & Lampert, 2004), and did not affect any fitness parameters of *Daphnia* (Carotenuto et al., 2005). Yet, the effects of these compounds on freshwater copepods are not yet clear. Contrary to *Daphnia*, *Eudiaptomus* can ingest larger diatoms, such as *Aulacoseira granulata* (Kagami et al., 2002). In our experiments, the lowest survival of *E. gracilis* with *A. formosa* cells was most likely to be due to their unpalatability. Yet, the effects of PUAs on freshwater copepods need to be evaluated more by using different diatom species.

Our experiments showed that chytrids improved the survival of copepod in the presence of diatoms. The mechanism behind this is not clear, but might be due to transferring nutrients and carbon from the less edible diatoms to copepods via the small fungal zoospores and bacteria. This evidence also supports the existence of a "mycoloop" (Kagami et al., 2007a), suggesting that inedible algae and herbivores are connected via abundant zoospores of chytrids.

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