

The parasitic chytrid, *Zygorhizidium*, facilitates the growth of the cladoceran zooplankter, *Daphnia*, in cultures of the inedible alga, *Asterionella*

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In food-web studies, parasites are often ignored owing to their insignificant biomass. We provide evidence that parasites may affect trophic transfer in aquatic food webs. Many phytoplankton species are susceptible to parasitic fungi (chytrids). Chytrid infections of diatoms in lakes may reach epidemic proportions during diatom spring blooms, so that numerous free-swimming fungal zoospores (2–3 µm in diameter) are produced. Analysis shows that these zoospores are rich in polyunsaturated fatty acids and sterols (particularly cholesterol), which indicates that they provide excellent food for zooplankters such as *Daphnia*. In life-table experiments using the large diatom *Asterionella formosa* as food, *Daphnia* growth increased significantly in treatments where a parasite was present. By grazing on the zoospores, *Daphnia* acquired important supplementary nutrients and were able to grow. When large inedible algae are infected by parasites, nutrients within the algal cells are consumed by these chytrids, some of which, in turn, are grazed by *Daphnia*. Thus, chytrids transfer energy and nutrients from their hosts to zooplankton. This study suggests that parasitic fungi alter trophic relationships in freshwater ecosystems and may be the important components in shaping the community and the food-web dynamics of lakes.

Keywords: parasite; food web; chytrids; *Daphnia*; diatom; cholesterol

1. INTRODUCTION

Parasites are ubiquitous and have strong effects on their host populations. They alter interspecific competition and are important in the maintenance of genetic polymorphism and biodiversity (Thomas *et al.* 2005). In ecosystem and food-web studies, however, parasites have often been ignored (Polis & Strong 1996), mainly owing to their insignificant biomass (this has been referred to as ‘insignificant biomass yet significant effects’; Marcogliese & Cone 1997). Recent studies have indicated that parasites are nevertheless important to food-web topology (Hudson 2006; Lafferty 2006; Lafferty *et al.* 2006). Of specific interest to our studies are cross links between host–parasite and predator–prey interactions. These occur when predators eat infected prey or the free-living stages of parasites (Lafferty *et al.* 2006). One possible consequence of predation of parasites is that it may decrease the overall severity of the infection in prey populations, as has been demonstrated in several recent studies (Kagami *et al.* 2004; Hall *et al.* 2005; Johnson *et al.* 2006). Despite growing interest in ecological interactions between predators and pathogens, few studies have experimentally examined how predation of parasites affects the growth of the predator, energy flow between trophic levels and food-web dynamics. This might partly

be owing to the difficulties associated with manipulating predators, pathogens and their hosts experimentally.

In lakes, many phytoplankton species are susceptible to fungal parasitism, mainly by chytrids (Ibelings *et al.* 2004). Chytrids are primitive fungi (James *et al.* 2006) characterized by a motile zoosporic stage. These zoospores actively find and infect new host cells, extract nutrients and develop into a mature sporangium that releases new zoospores (Canter 1967). Chytrids frequently cause significant algal mortality, since the prevalence of infection during epidemics may exceed 90% (e.g. Van Donk & Ringelberg 1983; Kagami *et al.* 2006), and every infection kills the host cell. Chytrid infections seem to be most common in large phytoplankton species that, owing to their size, are fairly resistant to grazing by zooplankton such as *Daphnia* (Sommer 1987; Kagami *et al.* 2007). Even if chytrid and zooplankton consume different size classes of phytoplankton, this does not necessarily mean that there is no interaction between them. Instead, zooplankton are found to graze free-swimming zoospores of chytrids, which are of a suitable size (2–5 µm in diameter) and shape to be eaten (Kagami *et al.* 2004). In this way, *Daphnia* could decrease the fungal infection rate of phytoplankton (Kagami *et al.* 2004).

One of the significant morphological characteristics of chytrid zoospores is the presence of a large central lipid globule in their body (Canter 1967; Beakes *et al.* 1988). Grazing on chytrid zoospores might facilitate the growth of zooplankton due to the high nutritional quality.

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Table 1. Food concentrations in three treatments of the *Daphnia* growth experiment. ((i) uninfected *Asterionella* (diatom), (ii) *Asterionella* infected by chytrid (diatom + chytrid), and (iii) chytrid zoospores (chytrid).)

	diatom	diatom + chytrid	chytrid
carbon ($\mu\text{g ml}^{-1}$)	1.97 ± 0.14	1.75 ± 0.19	0.13 ± 0.04
nitrogen ($\mu\text{g ml}^{-1}$)	0.17 ± 0.01	0.16 ± 0.01	0.01 ± 0.00
phosphorus ($\mu\text{g ml}^{-1}$)	0.46 ± 0.02	0.48 ± 0.02	0.04 ± 0.00
<i>Asterionella</i> (cells ml^{-1})	$39\,900 \pm 5830$	$57\,320 \pm 11\,580$	1040 ± 14
zoospores (spores ml^{-1})		$18\,290 \pm 3220$	$11\,200 \pm 530$
bacterial abundance (10^7 cells ml^{-1})	1.423 ± 0.328	1.210 ± 0.050	1.210 ± 0.050

Fungal epidemics occur mainly in spring (Van Donk & Ringelberg 1983; Kudoh & Takahashi 1990) when diatoms form blooms. In lakes, diatoms potentially offer food of good quality for zooplankton like filter feeding *Daphnia*, and diatoms would offer the food that *Daphnia* requires to start its growing season, were it not that these diatoms are too large to be eaten (Sommer *et al.* 1986). Using a host-parasite system consisting of the freshwater diatom, *Asterionella formosa*, and its obligate parasite, *Zygorhizidium planktonicum*, we conducted laboratory growth experiments in which *Daphnia galeata* \times *hyalina* was fed on both uninfected and infected *Asterionella* cells as well as a purified suspension of zoospores. We tested the hypothesis that *Daphnia* fitness increases when chytrids are present, since nutrients are transferred from inedible algae to easily grazable fungal zoospores.

2. MATERIAL AND METHODS

(a) Growth experiments

Asterionella formosa was isolated from Lake Maarsveen I 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 Maarsveen I during 2001 (strain FMS34600) and maintained on its host (*A. formosa*, strain MS07702-5) in a non-axenic culture (Kagami *et al.* 2004).

Asterionella formosa and *A. formosa* infected by *Z. planktonicum* (80% of the cells were infected) were grown in batch cultures (10 l Techne vessels with magnetic stirrers were used). Fungal zoospore suspensions, free of host cells, were obtained by filtration of the infected culture over 33 μm and subsequently over 5 μm filters. In this way, a suspension of fungal zoospore was obtained. Their swimming activity was not affected by the filtration procedure.

Juveniles of *Daphnia galeata* \times *hyalina* born on the same day were placed individually in separate 125 ml test tubes containing filtered lake water from Lake Maarsveen (less than 0.2 μm ; Kagami *et al.* 2004). *D. galeata* \times *hyalina* was fed every 2 days with *Scenedesmus obliquus* cultured in WC (Woods Hole Chu-10) medium at a concentration of 5 mg C l^{-1} . The newborns (780 μm) were collected in a 1 l beaker and rinsed three times with Chu-10 medium and finally kept for 2 h in the medium to wash the algae (*S. obliquus*) away. For each treatment, six neonates were randomly selected and transferred into 125 ml test tubes containing 100 ml food suspension in Chu-10 medium. The treatment with *A. formosa* (diatom) only and with *A. formosa* + *Z. planktonicum* (diatom + chytrid) received food concentrations well above the incipient limiting

concentration for *Daphnia* growth (Lampert 1987), 2.0 mg C l^{-1} (diatom 1.97 ± 0.14 (s.d.) mg C l^{-1} , diatom + chytrid 1.75 ± 0.19 (s.d.) mg C l^{-1}). The treatment with only zoospores of *Z. planktonicum* (chytrid) received lower food concentrations (0.13 ± 0.04 (s.d.) mg C l^{-1}), comparable to the amount of fungal zoospores in diatom + chytrid treatment (table 1). Bacterial abundance was not significantly different between the treatments ($p = 0.3284$; table 1).

The tubes containing *D. galeata* \times *hyalina* and food were incubated in a water bath at 18.5°C under 14 : 10 L : D cycles at 4 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Low light intensity is used to prevent the growth of algae during experiments. The animals were transferred every 2 days to new tubes with fresh food. At the end of the incubation period (day 6), the body length of each individual was measured under a microscope at a magnification of 25 \times . Survival (%) of *D. galeata* \times *hyalina* was calculated from the ratio of dead animals to total number of animals. The differences in the body length of *D. galeata* \times *hyalina* between the treatments were assessed by one-way factorial ANOVA and *post hoc* test (Tukey–Kramer test).

(b) Analyses

Particulate carbon and nitrogen concentrations in each food type were determined with a Calanus universal carbon analyzer (Uniquant OY). Fifteen millilitres of a subsample were centrifuged at 3000 g for 15 min, and the pellet was put into a precombusted silver cup (200 μl). Particulate phosphorus was collected by concentrating on Whatman GF/F filters. Particulate phosphorus was determined by the ascorbic acid–molybdenum blue method after digestion with potassium persulphate at 120°C for 60 min (American Public Health Association 1992). The abundances of *A. formosa* cells and zoospores of *Z. planktonicum* were counted under an inverted microscope at a magnification of 400 \times . Bacterial abundance was counted after staining with SYBR green (Noble & Fuhrman 1998) under a fluorescence microscope with UV fluorescence at a magnification of 1000 \times . Lipids were determined from aliquots of *A. formosa* concentrated on precombusted Whatman GF/F filters or from pellets of *Z. planktonicum*. Fatty acids were extracted and analysed as their methyl esters on a gas chromatograph equipped with a flame ionization detector (von Elert 2002). Sterols were identified after extraction and saponification as free sterols and after silylation with BSTFA on a gas chromatograph–mass spectrometer (Finnigan MAT GCQ) and quantified on a gas chromatograph equipped with a flame ionization detector (von Elert *et al.* 2003).

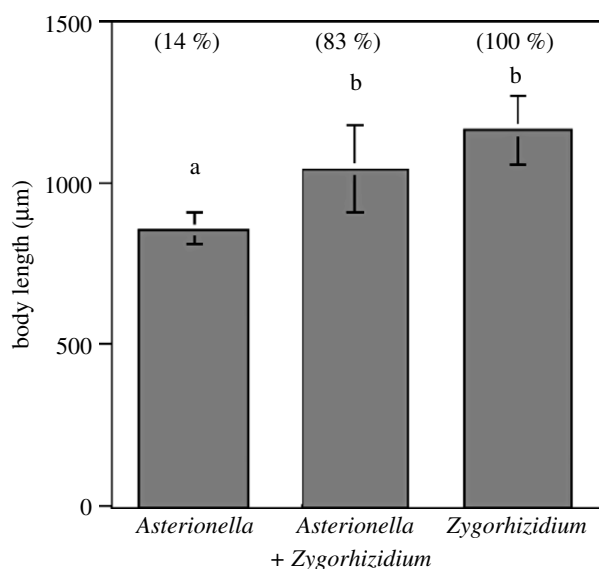


Figure 1. Body length of *Daphnia galeata hyalina* in the growth experiments. There are three treatments: (i) uninfected *Asterionella* (diatom), (ii) *Asterionella* infected by chytrid (diatom + chytrid), and (iii) chytrid zoospores (chytrid). Values are means of six replicates with s.d. The percentages of survival in the different treatments are given in parenthesis in the graph. Treatments that were significantly different ($p < 0.05$) are denoted by different letters on the bars.

3. RESULTS

(a) Growth of *D. galeata* × *hyalina*

The body length of *D. galeata* × *hyalina* after 6 days was significantly higher ($F_{2,16} = 11.74$, $p = 0.001$) in the treatment of 'diatom + chytrid' or in the treatment with the zoospores of *Z. planktonicum* as sole food 'chytrid' than in the treatment with *A. formosa* only 'diatom' (figure 1). *Daphnia galeata* × *hyalina* did not grow well and hardly survived with uninfected *A. formosa* (diatom; figure 1), although food (carbon) concentrations were above the incipient limiting level (table 1). Similar results were found for the survival of *D. galeata* × *hyalina* (figure 1), i.e. survival was significantly higher in the diatom + chytrid and chytrid treatments than in the diatom treatment.

(b) CNP and lipid composition of *A. formosa* and *Z. planktonicum*

Carbon, nitrogen and phosphorus (CNP) concentrations in food suspensions were higher in diatom and diatom + chytrid treatments than in the chytrid treatment (table 1). From these concentrations, the efficiencies of elemental transfer from diatom to free-swimming zoospores of chytrid were calculated by dividing CNP concentrations in the chytrid treatment by those in the diatom treatment. Transfer efficiency of carbon was estimated to be 6.6%, that of nitrogen 5.8% and that of phosphorus 8.6% (table 1).

CNP contents in single *A. formosa* cells and in single zoospores of *Z. planktonicum* were estimated from food concentrations and cell densities. CNP contents were higher in single *A. formosa* cells than in single zoospores of *Z. planktonicum* (table 2), since the cell volume of *A. formosa* cells ($200 \mu\text{m}^3$) is larger than that of zoospores ($20 \mu\text{m}^3$). The carbon to nitrogen ratio was significantly higher in zoospores than in *A. formosa* cells ($p = 0.038$),

Table 2. Carbon, nitrogen, phosphorus contents and CNP ratios of *Asterionella formosa* and zoospore of *Zygorhizidium planktonicum*.

	<i>Asterionella</i>	zoospore
carbon (pg cell^{-1})	46.0 ± 0.0	10.7 ± 1.7
nitrogen (pg cell^{-1})	4.0 ± 0.1	0.60 ± 0.1
phosphorus (pg cell^{-1})	9.8 ± 1.1	2.40 ± 0.0
C : N ratio	11.6 ± 0.2	18.4 ± 1.3
C : P ratio	4.7 ± 0.5	4.45 ± 0.7

but the carbon to phosphorus ratio was not significantly different ($p = 0.775$). Carbon content in single zoospores was 23.2% of that in single *A. formosa*, nitrogen content was 14.6% and phosphorus content was 24.4%.

Lipid analysis revealed that both host (*A. formosa*) cells and the zoospores of *Z. planktonicum* contained large amounts of fatty acids and sterols. Host and parasites resembled each other, in that both are rich in polyunsaturated fatty acids (PUFAs), although *A. formosa* is slightly richer in PUFAs (figure 2). But, while *A. formosa* cells were rich in brassicasterol (ergosta-5,22-dien-3-ol), which is typical for diatom cells, zoospores of *Z. planktonicum* were rich in cholesterol.

4. DISCUSSION

The trophic transfer efficiency from plants to herbivores is highly variable in lakes, and the two trophic levels can even become uncoupled especially when inedible phytoplankton species dominate, as may be the case during diatom spring or summer blooms dominated by cyanobacteria. Instead of being grazed, large inedible phytoplankton species are thought to sink to the bottom of the lake without being incorporated into the food web (Legendre & Fevre 1991). Here, we argue that this view is not always correct, since parasites have a great potential to modify the trophic structure of food webs and enhance the trophic transfer efficiency from plants to herbivores. More specifically, we claim that predation of parasites of inedible prey renders nutrients available for higher trophic levels. The growth experiments showed that only in the presence of algal parasites (chytrid fungi), is *Daphnia* able to grow well on large inedible diatoms, *A. formosa*. We attribute the higher growth rates and survival of *D. galeata* × *hyalina* in the diatom + chytrid treatment mainly to the grazing on fungal zoospores (Kagami et al. 2004), not on infected *A. formosa* itself (Kagami et al. 2005) or bacteria. *A. formosa* retained its colony form even after being infected and was still not grazed efficiently by *D. galeata* × *hyalina* (Kagami et al. 2005). Bacterial abundance was not significantly different between the uninfected and infected treatments ($p = 0.328$; table 1). Our results demonstrate that the parasite supported *Daphnia* growth through transfer of carbon and nutrients from large inedible diatoms to zoospores, which are of the right size and shape, thus making material and energy available to zooplankton.

The carbon to nitrogen and carbon to phosphorus ratios in single zoospores were higher than, or similar to, those in the host cell (table 2), indicating that zoospores do not contain much more nitrogen and phosphorus per unit of carbon than *A. formosa* cells. Lipid analysis revealed that

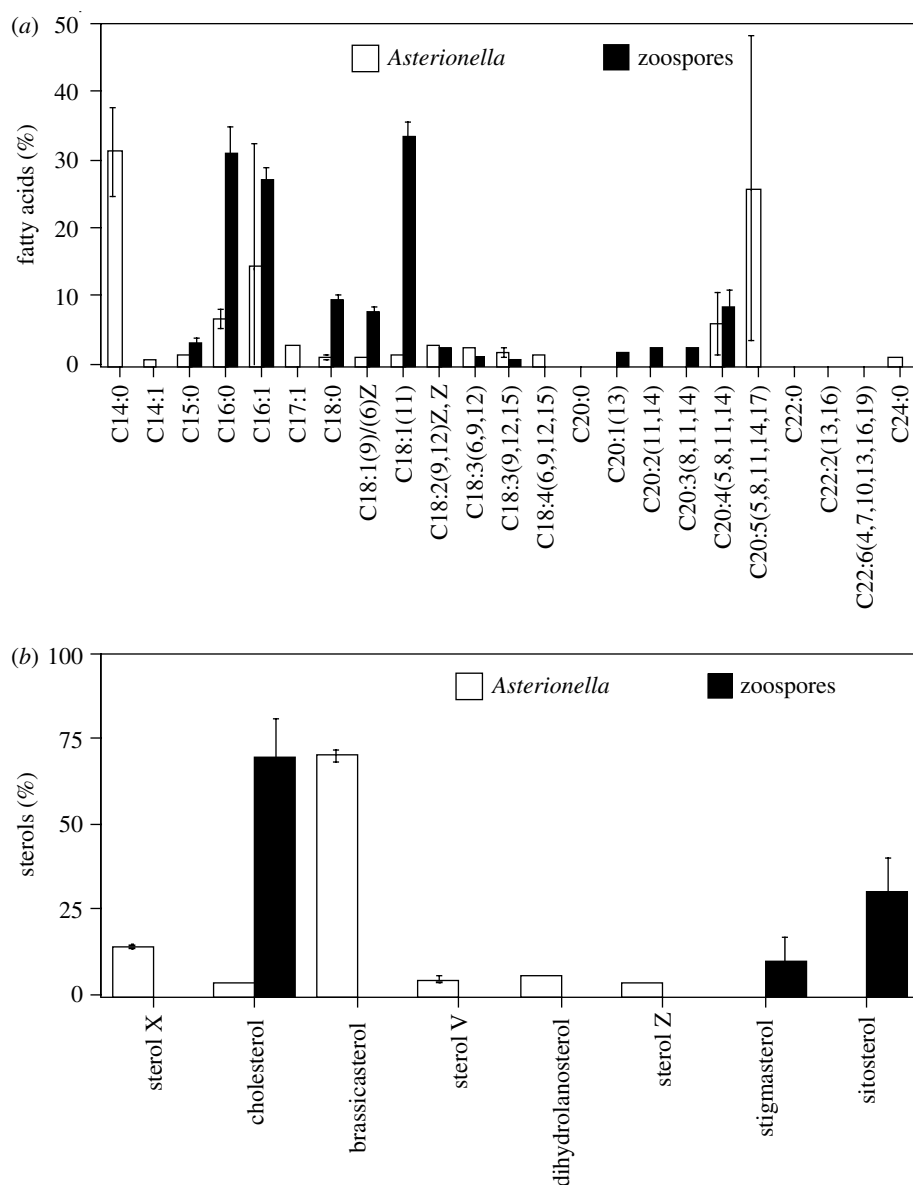


Figure 2. Composition of (a) fatty acids and (b) sterols of the host diatom, *Asterionella formosa*, and the zoospores of its parasite, *Zygorhizidium planktonicum*.

zoospores of chytrids are rich in cholesterol. Since the major sterol in host cell (*A. formosa*) is brassicasterol, it is probable that chytrids use it for the synthesis of cholesterol. However, it cannot be excluded that the high cholesterol content of the zoospores is due to selective assimilation of the traces of cholesterol in the host. Cholesterol has been shown to be the major sterol in *Daphnia* (von Elert *et al.* 2003) and a key lipid in food-web dynamics and zooplankton life history (Hassett 2004; Martin-Creuzburg *et al.* 2005). Those results suggest that the chytrid zoospores are a high-quality food for *Daphnia*, and a rich source of sterols. Grazing on fungal zoospores would facilitate the growth of zooplankton via transfer of energy and nutrients from inedible algae to zooplankton with a high efficiency due to the high nutritional quality. These results give some of the first insights into the role of parasitic fungi in aquatic food webs, where they can improve zooplankton production and enhance the efficiency of trophic transfer.

Parasites are ubiquitous in nature, and yet they are commonly ignored in food-web studies (Marcogliese & Cone 1997; Ibelings *et al.* 2004; Thomas *et al.* 2005).

However, fungal zoospores can be highly abundant in lake water during epidemics and have been estimated to exceed 70 000 spores per millilitre (Van Donk & Ringelberg 1983; Bruning 1991). In lakes, phytoplankton cell death due to fungal infection could account for 25% of primary production in lakes (Kagami *et al.* 2006). The transfer of carbon from primary producers to chytrids, and from there to higher trophic levels, is comparable to the fluxes of primary production channelled via the microbial food web (Azam *et al.* 1983) or passing through the viral shunt (Suttle 2005). The efficiency of carbon transfer via chytrids will however be higher than that of the microbial food web and viral shunt. Although the microbial food web does support the metazoan food web (Sherr & Sherr 1988), the return of energy—released as dissolved organic matter (DOM) by phytoplankton—to the metazoan food web via a microbial loop of bacteria, flagellates and microzooplankton is rather inefficient. Viral lysis of phytoplankton has been shown to even decrease macrozooplankton production (Fuhrman 1999), due to flux into DOM, thereby increasing ecosystem respiration and decreasing the efficiency of trophic transfer. Our

experiments showed that fungal parasites indeed support *Daphnia* growth. The transfer efficiencies of carbon, nitrogen and phosphorus from algae to free-swimming zoospores of chytrid in our experiment were estimated to be 6–9% (table 1). Since the prevalence of infection during the experiment was about 60%, transfer efficiency from algae to free-swimming zoospores could be even higher during epidemics in the field when the prevalence of infection may exceed 90%. Carbon, nitrogen and phosphorus concentrations in a single zoospore are comparable to 20% of those in single *A. formosa* cells (table 2), indicating that the release of just five new zoospores may exploit all algal tissues. Usage of host resources by parasitic chytrids may be quite efficient, since chytrids take up nutrients directly from the host tissues by entering their host cell through a germ tube (Van Donk & Ringelberg 1983; Ibelings *et al.* 2004).

Our results show that predation of parasites (free-living stages of parasites) enhances the growth of the predator, indicating that predator–parasite links (Lafferty *et al.* 2006) are important for food-web dynamics. In our case, the zoospores of fungal parasites in phytoplankton can be a very important food source for zooplankton at a critical moment in their life cycle, i.e. at a moment in early spring when the diatom bloom is believed to get the renewed growth of cladoceran filter feeders going (PEG model; see Sommer *et al.* 1986). The results support the existence of a link in the pelagic food webs of lakes between inedible primary producers and herbivores via abundant fungal parasites of the plants. This new link may influence the energy flow, decreasing the sinking flux of material and rendering it available to the food web bypassing the microbial loop. This trophic pathway may be essential for understanding food webs in lentic systems.

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