LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 63, 2018, 2384–2393

doi: 10.1002/lno.10945

Fungal parasites of a toxic inedible cyanobacterium provide food to zooplankton

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Abstract

During the end of spring and throughout summer, large-sized phytoplankton taxa often proliferate and form dense blooms in freshwater ecosystems. In many cases, they are inedible to zooplankton and prevent efficient transfer of energy and elements to higher trophic levels. Such a constraint may be alleviated by fungal parasite infections on large-sized phytoplankton taxa like diatoms and filamentous cyanobacteria, as infections may provide zooplankton with a complementary food source in the form of fungal zoospores. Zoospores have been shown to support somatic growth of large filter feeding zooplankton species. Here, we tested if selectively feeding zooplankton, more specifically rotifers, also can use fungal zoospores as a food source. Our results show that chytrid fungal parasites can indeed support population growth of rotifers (*Keratella* sp.). Specifically, in cultures of an inedible filamentous cyanobacterium (*Planktothrix rubescens*), *Keratella* populations rapidly declined, while in *Planktothrix* cultures infected with chytrids, *Keratella* population growth rate equaled the growth observed for populations fed with a more suitable green algal diet (*Chlorella sorokiniana*). Feeding of *Keratella* on zoospores was furthermore indicated by a reduced number of zoospores during the last sampling day. These findings not only imply that rotifers may survive on zoospores, but also that the zoospores can support high rotifer population growth rates. We thus show that fungal parasites of inedible cyanobacteria can facilitate grazers by providing them alternative food sources. Together, these results highlight the important role that parasites may play in the aquatic plankton food web.

During the onset of spring, phytoplankton communities in freshwater ecosystems usually contain a relatively large amount of small-sized edible algae, which are available as food to herbivorous zooplankton (Lampert et al. 1986; Sommer et al. 1986). Later in the season increased herbivory or competition for nutrients result in the replacement of the phytoplankton community by larger-sized groups, e.g., dinoflagellates and/or filamentous cyanobacteria (Sommer et al. 1986; De Senerpont Domis et al. 2013). Since these taxa may be toxic and are generally less easily ingested by zooplankton, they may represent trophic bottlenecks (Yasumoto and Murata 1993; Havens and East 1997; Sivonen and Jones 1999; Havens 2008). As a result, zooplankton may be forced to graze on other food sources such as bacteria (Christoffersen et al. 1990). Bacteria, however, generally represent low quality food (Demott and Müller-Navarra 1997; Schmidt and Jonasdottir 1997) because they lack essential sterols to sustain zooplankton growth (Martin-Creuzburg et al. 2011).

Besides grazing, other top-down factors can regulate phytoplankton growth and population densities. Viruses and fungal parasites are among the most common pathogenic agents involved in the regulation of phytoplankton blooms (Canter and Lund 1951; Brussaard et al. 1995; Brussaard 2004*b*; Ibelings et al. 2004; Tijdens et al. 2008; Sime-Ngando 2012; Gerphagnon et al. 2015; Frenken et al. 2016, 2017*a*). Through lysis of inedible phytoplankton cells, parasites may unlock nutrients that otherwise would remain unavailable to higher trophic levels. Fungal parasites belonging to the phylum Chytridiomycota, usually referred to as chytrids, are often involved in the decline of blooms of diatoms (Van Donk and Ringelberg 1983; Van Donk

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1989; Frenken et al. 2016) or filamentous cyanobacteria (Rasconi et al. 2012; Gerphagnon et al. 2013, 2015). They are host-specific zoosporic fungi that parasitize on phytoplankton cells and completely rely on them for nutrition and energy.

After chytrids complete their infection cycle, zoospores are released into the water. As zoospores can be grazed by zooplankton, they may allow the transfer of nutrients from primary producers to higher trophic levels (Kagami et al. 2004, 2007*a*, *b*; Agha et al. 2016) and as such alleviate trophic bottlenecks associated to blooms of diatoms or filamentous cyanobacteria (Rasconi et al. 2012; Frenken et al. 2016). Through the provisioning of zoospores, chytrid infections may thus support higher trophic levels during blooms of inedible algae (Agha et al. 2016; Frenken et al. 2016), also referred to as the "mycoloop" (Kagami et al. 2007*a*). Additionally, these zoospores can complement the zooplankton diet, since they are rich in polyunsaturated fatty acids and sterols (Kagami et al. 2007*b*). Modeling efforts have shown that epidemic growth of chytrids can channel up to 20% of the primary production to higher trophic levels through the production of grazer exploitable zoospores, and can represent up to 50–57% of the zooplankton diet (Grami et al. 2011; Rasconi et al. 2014). Chytrids were shown to support somatic growth of cladocerans and facilitate an increased survival of copepods and cladocerans (Kagami et al. 2004, 2007*b*, 2011; Agha et al. 2016). Feeding modes of cladocerans are generally none-selective (Peters 1984; Turner and Tester 1989), so they presumably do not intentionally predate on zoospores. For copepods, which are selective feeders (Richman and Rogers 1969; Meunier et al. 2016), the mechanisms underlying improved survival in infected phytoplankton cultures remains unresolved (Kagami et al. 2011).

Previous work has suggested that chytrid zoospores may also serve as a food source to selectively feeding rotifers (Schmeller et al. 2014; Frenken et al. 2016). Experimental evidence that zoospores can effectively support rotifer population growth, however, is lacking. Rotifers are important predators of the microbial food web (Arndt 1993; Reynolds et al. 2006) and thereby play a key role in grazing, nutrient regeneration, and secondary productivity in aquatic ecosystems (Pace and Orcutt 1981). Here, we tested the hypothesis that population growth of the rotifer *Keratella* can be supported by chytrid infections of the inedible filamentous cyanobacterium *Planktothrix*. To this end, *Keratella* was fed with a suitable food source consisting of the green alga *Chlorella*, with infected *Planktothrix* and with noninfected *Planktothrix*. Additionally, treatments without *Keratella* were included as a control. We then compared the growth of rotifers, phytoplankton, chytrids, and bacteria among these treatments.

Materials and methods

Test organisms and stock culture conditions

Keratella were isolated during spring (05 April 2016) from a small pond in Wageningen, The Netherlands (co-ordinates in DMS: 51°59′16.3″N 5°40′06.0″E), 5 weeks prior to the start of the experiment. For culturing, 20 L of water was filtered over a 30 μm plankton mesh from which individuals of the species *Keratella* cf. *cochlearis* were picked and inoculated into sterile 24-well cell culture plates (Greiner Bio-One, Alphen aan den Rijn, The Netherlands). Each well contained 15 randomly picked individuals in 2 mL sterile WC medium (Guillard and Lorenzen 1972) fed ad libitum with the green alga *Chlorella sorokiniana* (CCAP 211/8K). *Chlorella* was maintained in exponential growth by diluting the cultures once a week with WC medium. *Keratella* and *Chlorella* were grown at 16°C in a temperature and light controlled incubator (Snijders Labs, Tilburg, The Netherlands), at a 14 : 10 light : dark cycle with 10 μmol photons m^{-2} s^{-1}. To homogenize *Keratella* populations, all wells were mixed weekly into one larger volume, from which individuals were picked and washed randomly and inoculated into new well plates. *Keratella* were counted and picked using a dissecting microscope (magnification of 15X, LeicaWILD MZ8, Leica Microsystems B.V., Son, The Netherlands) and a 20–200 μL pipette (Mettler-Toledo B.V., Tiel, The Netherlands).

The phytoplankton-chytrid system used in this experiment is the filamentous cyanobacterial host *Planktothrix rubescens* NIVA-CYA97/1 with its fungal parasite, the chytrid Chy-Lys2009. This chytrid possesses identical morphological characteristics and infection patterns to *Rhizophydium megarrhizum*, described earlier by Canter and Lund (1951). Earlier work on host specificity and virulence of the chytrid can be found in Rohrlack et al. (2013), Sønstebø and Rohrlack (2011), and Frenken et al. (2017*b*). All cultures used in this study were monoclonal and non-axenic. The host and parasite cultures were prepared as described in Frenken et al. (2017*b*). In short, the *Planktothrix* and Chy-Lys2009 cultures were grown in a temperature and light controlled incubator (Snijders Labs, Tilburg, The Netherlands) at 5 μmol photons m^{-2} s^{-1} in a 14 : 10 light : dark cycle, at 24°C and 16°C, respectively. These low light conditions resemble the conditions where *Planktothrix* and its chytrid were isolated. All *Planktothrix* and *Chlorella* cultures were grown in batch in 100 mL Erlenmeyer's with 50 mL suspension and were diluted 10 times every other week using WC-medium (Guillard and Lorenzen 1972). Prior to the experiment, all cultures were acclimated to 16°C.

Experiment

The experimental design consisted of six treatments in which three food types, i.e., *Chlorella*, uninfected *Planktothrix* and *Planktothrix* infected by chytrids were cultured in the presence as well as absence of *Keratella*. *Chlorella* is a spherical shaped green alga with a diameter that can range between 2 μm and 8 μm (Azaman et al. 2017). *Planktothrix* is a filamentous cyanobacterium that can reach lengths of over 2500 μm with a diameter of 6–8 μm (John et al. 2002; Davis et al. 2003; Zeder et al. 2010). Therefore, *Chlorella* is an easily ingestible food source, while *Planktothrix* was supposedly inedible to *Keratella*.

Experiments were performed in six series of five 12-well plates (VWR, Amsterdam, The Netherlands). The second and third column of each well plate were left empty. The

remaining wells were randomly assigned to one of the six treatments using a random number generator (https://www.random.org/). Over the course of the experiment, five series of five replicates were used to sample *Keratella*, phytoplankton and bacteria every second day (further referred to as "destructive samples"). The sixth series of five replicates was maintained over the entire course of the experiment in which *Keratella* numbers were counted every day (referred to as "continuous samples"). See Supporting Information for a schematic overview of the experimental setup.

One day before the start of the experiment, *Keratella* individuals were picked from the stock cultures and washed three times in sterile WC medium to remove *Chlorella*. The wells that received grazing treatments were filled with 1 mL of WC-medium and 10 randomly picked individuals of *Keratella*. These individuals were left to starve overnight, after which the different food treatments were added reaching a total culture volume of 4 mL per well. All plates were incubated on a randomly assigned location within a temperature and light controlled incubator (Snijders Labs, Tilburg, The Netherlands) at 16°C and 5 μmol photons m^{-2} s^{-1} in a 14 : 10 light : dark cycle.

Sample analysis

In the continuous samples, *Keratella* abundance was determined daily using a dissecting microscope at a magnification of 15X (LeicaWILD MZ8, Leica Microsystems B.V., Son, The Netherlands). In the destructive samples, *Keratella* abundances were determined every second day after which the entire sample was preserved immediately using 25% glutaraldehyde (Merck, Darmstadt, Germany) to a final concentration of 0.5% (v/v). We used these samples to assess biovolume and population densities of the phytoplankton, zoospore density, prevalence of chytrid infection, and bacteria density. *Planktothrix* biovolume and *Chlorella* population density were determined in triplicate on a CASY Cell Counter (Schärfe System GmbH, Reutlingen, Germany).

Density of zoospores was determined using a protocol modified from Gsell et al. (2013), where every sample was counted in duplicate until at least 250 counts or 20 fields of view were reached, using an inverted microscope (DMI 4000B, Leica Microsystems CMS GmbH, Mannheim, Germany). Infections were counted as a categorical variable: at least 100 filaments were inspected, which were either infected (*i*) or uninfected (*ui*). The prevalence of infection was subsequently calculated as $P = i/(i + ui)$. All microscopic counting was carried out at a magnification of 200X on an inverted microscope. To stain bacteria for flow cytometric counting, we used a protocol originally developed for viruses (Marie et al. 1999; Brussaard 2004*a*). In short, samples were diluted at least 10 times in a Tris-EDTA buffer (pH 8.2) and stained with the DNA stain SYBR® Green I nucleic acid gel stain (Molecular Probes, Invitrogen, Paisley, UK) for 10 min in the dark at 80°C (final concentration of 5×10^{-5} of commercial stock). Just before analyses, the sample was left to cool down at room temperature for 5 min after which 5 μL of a red fluorescent bead solution was added as a control (Fluoresbrite® Polychromatic Red Microspheres 2.0 μm, Polysciences, Hirschberg an der Bergstraße, Germany). This suspension was analyzed on a MoFlo XDP cell sorter (Beckman Coulter Nederland BV, Woerden, The Netherlands). On the last day of the experiment, all *Keratella* individuals were photographed on the inverted microscope, after which body length and width were measured using the program ImageJ 1.48v (ImageJ, National Institutes of Health). Rotifer body volume was calculated assuming a cylindrical shape.

Data analysis

To test if the time course of the different measured variables showed differences between treatments, a repeated measurements ANOVA (RM ANOVA) was performed in the statistical package Statistica 12.5 (Statsoft Europe, Hamburg, Germany). Variables were tested for normality and equal variance using the Shapiro–Wilk and Levene's test, respectively. Bacteria density was square root transformed before analyses to improve normality. *Keratella* net population growth rates (μ) were calculated as the change in the population count between the start and end of the experiment according to $\mu = \ln(N_{end}/N_{start})/(t_{end} - t_{start})$, where N_{end} and N_{start} refer to the population counts at time points t_{end} and t_{start}, respectively (Weisse and Frahm 2002). Growth rates, body length, body width, and body volume were compared between treatments using one-way ANOVA in the statistical program SigmaPlot for Windows version 13 (Systat Software, London, UK). Pairwise comparisons were conducted using the Tukey post hoc test.

Results

Keratella population development differed significantly between the different food treatments (Fig. 1). *Keratella* density in the uninfected *Planktothrix* treatment showed a strong

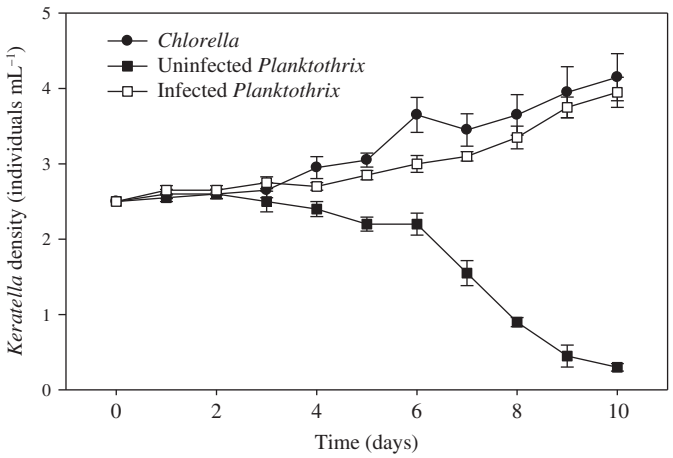

Fig. 1. *Keratella* density in the treatments fed with either *Chlorella*, uninfected *Planktothrix*, or infected *Planktothrix*. Values denote mean ± standard error (n = 5).

Table 1. *Keratella* population growth rate (μ), body length (L), body width (W), and body volume (V). Values denote average with standard deviation between brackets. Superscript letters represent summary output of the pairwise comparison between treatments (Tukey test, μ: $F_{2,14} = 106.6$, $p < 0.001$; L: $F_{2,14} = 0.259$, $p = 0.776$; W: $F_{2,14} = 2.281$, $p = 0.145$; and V: $F_{2,14} = 1.636$, $p = 0.235$).

Treatment	μ (d^{-1})	L (μm)	W (μm)	V (10^3 μm^3)
Chlorella	0.07 (0.02)[a]	107.9 (2.8)[a]	61.8 (3.3)[a]	325 (44)[a]
Planktothrix	–0.03 (0.08)[b]	109.4 (3.7)[a]	65.3 (4.1)[a]	369 (56)[a]
Infected *Planktothrix*	0.06 (0.01)[a]	108.6 (3.1)[a]	65.6 (1.6)[a]	368 (24)[a]

Table 2. Output of the RM ANOVA reporting the significance, degrees of freedom, and the F value of treatment effect on different variables. Values in bold denote a significant effect ($\alpha < 0.05$).

Effect	Variable	Effect	dF	F	p
Keratella	*Chlorella* density	Treatment	1	643.3	**<0.001**
		Time × treatment	3	96.0	**<0.001**
	Planktothrix biovolume	Treatment	1	0.2	0.702
		Time × treatment	4	0.3	0.886
	Bacteria density	Treatment	1	7.0	**0.015**
		Time × treatment	3	1.4	0.266
	Zoospore density	Treatment	1	5.2	0.052
		Time × treatment	3	7.9	**<0.001**
	Prevalence of infection	Treatment	1	1.8	0.217
		Time × treatment	3	0.6	0.594
Food source	*Keratella* density	Treatment	2	59.2	**<0.001**
		Time × treatment	18	54.3	**<0.001**
	Bacteria density	Treatment	2	1305.3	**<0.001**
		Time × treatment	6	2.8	**0.017**
Chytrid	*Planktothrix* biovolume	Treatment	1	146.8	**<0.001**
		Time × treatment	4	90.2	**<0.001**
	Keratella density	Treatment	1	156.4	**<0.001**
		Time × treatment	9	130.6	**<0.001**
	Bacteria density	Treatment	1	109.6	**<0.001**
		Time × treatment	3	2.6	0.068

decrease, especially after 6 d and reached very low levels by the end of the experiment (< 0.3 individuals mL^{-1}). In contrast, population densities of *Keratella* increased similarly in the treatments with *Chlorella* and infected *Planktothrix* (Tables 1, 2). Body length of *Keratella*, body width, and volume showed no differences between treatments (Table 1).

When *Keratella* was absent, *Chlorella* density increased strongly during the course of the experiment (Fig. 2a; Table 2). In presence of *Keratella*, *Chlorella* growth was lower ($F_{1,8} = 643.3$, $p < 0.001$). Population growth of infected *Planktothrix* was lower than that of uninfected *Planktothrix* ($F_{1,16} = 146.8$, $p < 0.001$). *Keratella* had no influence on *Planktothrix* biovolume (Fig. 2a,b; Table 2) in either of these treatments ($F_{1,16} = 0.2$, $p = 0.702$). Chytrid zoospore density increased strongly 5 d after the start of the experiment (Fig. 3a). As a result, infection prevalence of *Planktothrix* increased rapidly from about 20% to almost 70%. Overall, *Keratella* presence had no significant effect on prevalence of infection ($F_{1,8} = 1.8$, $p = 0.217$). Zoospore density remained unaffected by *Keratella* ($F_{1,8} = 5.2$, $p = 0.052$) except during the last time interval when densities were lower in the presence than in the absence of *Keratella* (Fig. 3a; Table 2).

Bacteria density showed significant differences between treatments (Fig. 4; Table 2). Generally, bacteria densities were lowest in the *Chlorella* treatment, higher in the *Planktothrix* treatment and highest in the infected *Planktothrix* treatment. Bacteria density increased in time in both the *Planktothrix* treatments. In the *Planktothrix* cultures, bacteria density was higher during infections ($F_{1,15} = 109.6$, $p < 0.001$). In the presence of *Keratella*, bacteria density was lowered in the *Chlorella* treatment ($F_{1,21} = 7$, $p = 0.015$) but not in the *Planktothrix* treatments.

Discussion

Our results clearly demonstrate that chytrid infections supported *Keratella* population growth when the food source is

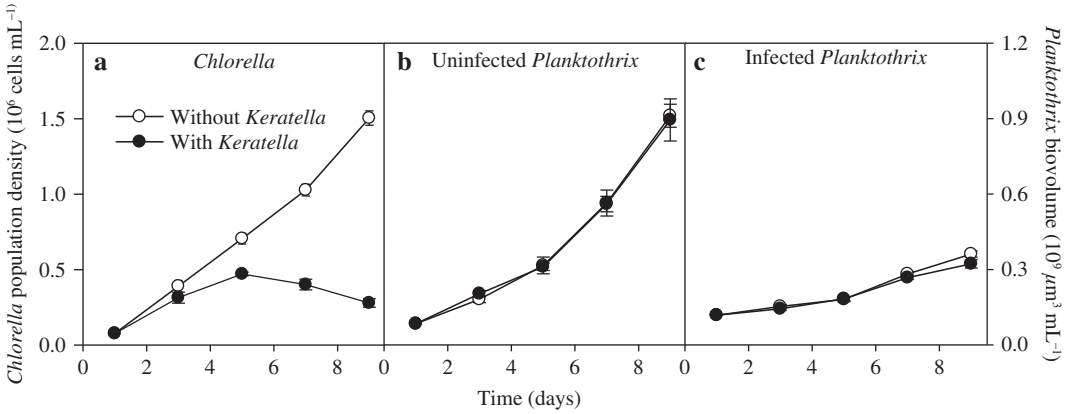

Fig. 2. Population densities of *Chlorella* (**a**) and biovolume of uninfected (**b**) and infected *Planktothrix* (**c**), in treatments with and without *Keratella.* Values denote mean ± standard error (n = 5).

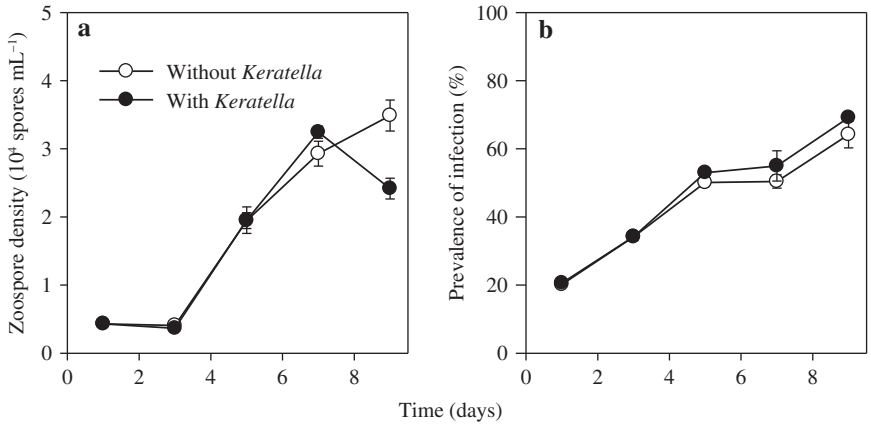

Fig. 3. Zoospore densities (**a**) and prevalence of infection (**b**) in the treatments with and without *Keratella.* Values denote mean ± standard error (n = 5).

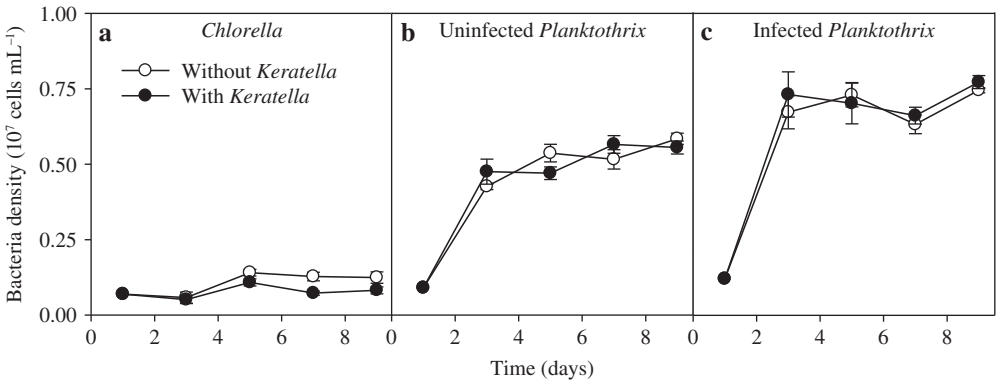

Fig. 4. Bacteria densities in treatments with *Chlorella* (**a**), uninfected *Planktothrix* (**b**) and infected *Planktothrix* (**c**), with and without *Keratella.* Values denote mean ± standard error (n = 5).

dominated by inedible filamentous cyanobacteria. In the absence of chytrid infections, *Keratella* populations that were exclusively fed with *Planktothrix* declined rapidly to very low levels (Fig. 1). In contrast, when exposed to *Planktothrix* that were infected with chytrids, *Keratella* abundances increased and net population growth was comparable to that observed for populations grown on the green alga *Chlorella*. Specifically, population growth rates of *Keratella* ranged between 0.05 d^{-1} and 0.09 d^{-1} when fed on

Chlorella, and between 0.04 d^{-1} and 0.07 d^{-1} when fed with infected *Planktothrix* cultures. These growth rates are largely in line with earlier reported values of 0.08–0.10 d^{-1} for *Keratella* feeding on a good quality food source (Walz 1983, 1987). Zoospore density at the end of the experiment was lower in the treatments where *Keratella* was present, as compared to the treatments where *Keratella* was absent. These results thus support our hypothesis that *Keratella* could directly feed on chytrid zoospores (Frenken et al. 2016), and confirm that the mycoloop may transfer nutrients from large inedible algae to zooplankton (Kagami et al. 2007*a*). As a result, cyanobacterial blooms may not represent trophic bottlenecks, since chytrid parasites recouple primary producers to secondary consumers.

In the *Planktothrix* treatment, rotifer populations rapidly declined over the course of the experiment, with over 50% of the population dying within 2–5 d of exposure. This is comparable to earlier observations, where *Keratella* were fed with the filamentous cyanobacterium *Dolichospermum flos-aquae* (Gilbert and Durand 1990). Filamentous cyanobacteria generally cannot serve as food for rotifers, or at least poorly support rotifer population growth (Gilbert and Durand 1990; Rothhaupt 1991; Weithoff and Walz 1995; Fey et al. 2010). *Keratella* preferentially consume living algae in the size range < 10 μm, but their diets can also include detritus, bacteria, and yeast (Pourriot 1965, 1977; Lindström and Pejler 1975; Pejler 1977; Gilbert and Bogdan 1981; Stemberger 1981; Bogdan and Gilbert 1982; Walz 1983; Ronneberger 1998).

Cyanobacteria may affect higher trophic levels generally in two ways. First, they can directly affect zooplankton grazing by interfering with the feeding process or filtering apparatus (Soares et al. 2009; Panosso and Lürling 2010). Rotifers, however, are considered to be more selective in choosing the particles they ingest, e.g., the rotifer *Brachionus calyciflorus* can actively reject food particles at multiple stages during the feeding process (Gilbert and Starkweather 1977). Consequently, *Keratella* may not have fed on *Planktothrix* and thus suffered from starvation in the uninfected *Planktothrix* treatment.

Second, cyanobacteria have biochemical properties that either lack essential elements needed for zooplankton growth (Elert et al. 2003; Martin-Creuzburg et al. 2005; Wacker and Martin-Creuzburg 2012) or might result in toxic effects on the grazer (Haney 1987; Lampert 1987; De Bernardi and Giussani 1990). When *Keratella* was fed with a small unicellular cyanobacterium (*Microcystis*), survival was lower as compared to an unfed control, which could indicate possible toxic effects of cyanobacteria on *Keratella* (Fulton and Paerl 1987). A major difference with our study, however, is that *Planktothrix* is a large and filamentous cyanobacterium which is likely unsuitable for ingestion by rotifers, since *Keratella* seems to prefer food particles with a diameter < 10 μm (Ronneberger 1998). Furthermore, in our cultures, no negative effect of *Keratella* grazing on *Planktothrix* biomass build-up is apparent.

An earlier study showed that the intracellular microcystin content is lower in *Planktothrix* cultures exposed to chytrids as compared to unexposed treatments, which might indicate microcystin leakage into the water column (Frenken et al. 2017*b*). Negative effects of microcystin on zooplankton growth, however, are not unambiguous. Despite some reports on negative effects (Fulton and Paerl 1987; Rohrlack et al. 1999; Zurawell et al. 2005), other studies reported lack of adverse effects of microcystins on a range of zooplankton species, including rotifers (Porter and Orcutt 1980; Nandini and Rao 1997; Soares et al. 2010). Furthermore, *Keratella* were able to grow in the infected cultures where also uninfected *Planktothrix* filaments were present. Thus, toxic effects of *Planktothrix* on *Keratella* in our experiment are very unlikely.

Most probably, *Keratella* in our experiments fed on a diet of zoospores released from the infections, and bacteria growing on organic cell debris and lysates (Cole 1982; Middelboe et al. 1996; Gobler et al. 1998). Earlier work showed that bacteria can be of significant importance as a source of phosphorus to bacterivore zooplankton species (Hessen and Andersen 1990). The presence of *Keratella* in our experiment, however, had no observable effect on the number of bacteria in the *Planktothrix* treatment. Therefore, it is unclear to which extent *Keratella* grazed on bacteria. However, since the rotifers were unable to survive in the uninfected *Planktothrix* cultures, where the amount of bacteria was relatively high (5.6×10^6 cells mL^{-1}), it seems unlikely that bacteria served as the sole food source for *Keratella*. This is in line to findings by Agha et al. (2016), which show that for cladocerans, survival, and reproduction are higher in cultures with both chytrids and bacteria present as compared to cultures with bacteria alone. This might be explained by the lack of essential sterols in bacteria, and thus their low nutritional quality to zooplankton (Martin-Creuzburg et al. 2011; Freese and Martin-Creuzburg 2013). In cultures where also chytrids were present, the zooplankton diet might have been complemented by chytrid zoospores, which are rich in polyunsaturated fatty acids and sterols, and thus provide a high quality food source supporting zooplankton growth and reproduction via the mycoloop (Kagami et al. 2007*a*,*b*).

Chytrid zoospores are indeed presumed to be a high-quality food source for daphnids and copepods, in terms of size and shape, as well as their nutritional composition (Kagami et al. 2007*b*, 2011, 2017). Moreover, it has been demonstrated that zooplankton can lower the density of zoospores detected in the water (Searle et al. 2013; Schmeller et al. 2014). As hypothesized before (Frenken et al. 2016), chytrid zoospores may serve as an alternative food source to *Keratella* as well. With this experiment, we not only show that *Keratella* abundances increased in the infected treatments resulting in an increased grazing pressure, but also that zoospore concentrations were lower at the end of the experiment. Zooplankton grazing on zoospores may also feedback on the infections, and consequently on new zoospore production. We did not observe a

decrease in prevalence of infection. Yet, this may have occurred if the experiment would have lasted longer. Moreover, we note that our experiments were performed in small chambers without mixing, which may have affected some aspects of the observed dynamics. Future studies under more natural conditions are required to corroborate the relevance of the mycoloop, and quantify trophic transfer of nutrients by for instance stable isotope probing (Frenken et al. 2017*a*).

Until now, it was shown that grazing on zoospores can enhance body length of cladocerans (Kagami et al. 2007*b*) and can sustain a higher percentage of surviving copepods (Kagami et al. 2011). Also, it has been shown that multiple *Daphnia* genotypes can survive and reproduce on a diet of chytrid zoospores and heterotrophic bacteria (Agha et al. 2016). With our study, we provide first evidence that chytrids can support population growth of rotifers, a more selectively feeding group of zooplankton. In other words, our results demonstrate that trophic links between primary producers and secondary consumers that are limited during presence of large inedible cyanobacteria can be re-established by fungal parasites. Our results thus highlight the important function of diseases and the role they can play in food webs. Not only can pathogens strongly regulate their host populations, they can also facilitate growth and reproduction of other trophic levels.

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Acknowledgments

We are grateful to Dennis Waasdorp and Wei Zhang for assistance and general advice during the start-up of the experiment and for help with isolating rotifer cultures. We furthermore thank Suzanne Naus-Wiezer for operating the flow cytometer and Guus van den Heuvel for photographing *Keratella.*

Conflict of Interest

None declared.

Submitted 13 September 2017
Revised 26 April 2018
Accepted 14 May 2018

Associate editor: Takehito Yoshida