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Infection of filamentous phytoplankton by fungal parasites enhances herbivory in pelagic food webs

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Abstract

Chytrid fungal parasites are ubiquitous in aquatic ecosystems and infect a wide array of aquatic organisms, including all phytoplankton groups. In addition to their role as parasites, chytrids serve as food to zooplankton, thereby establishing an alternative trophic link between primary and secondary production in pelagic food webs, the so-called mycoloop. We hypothesized that, in addition to the mycoloop, chytrid infection facilitates grazing of filamentous phytoplankton by rendering it more edible to zooplankton consumers through infection-induced fragmentation. We undertook grazing assays to compare the ability of the key zooplankter *Daphnia* to graze on a filamentous cyanobacterium in the presence or absence of chytrid infection. A near doubling in mean clearance rates was consistently recorded when *Daphnia* were fed with infected cultures of the cyanobacterium as compared to uninfected ones. Infected filaments were shorter than noninfected ones, indicating that infection-induced fragmentation undermines resistance of filamentous phytoplankton to grazing. We propose an extended conceptualization of the mycoloop that includes both direct effects (i.e., transfer via grazing of chytrid zoospores) and indirect effects (i.e., trophic upgrading and facilitated grazing on phytoplankton via fragmentation) of chytrid infection on trophic transfer at the base of pelagic food webs.

Pelagic ecosystems occupy over 70% of the Earth's surface. Primary production in these systems is dominated by phytoplankton, accounting for over 50% of total carbon fixation on a global scale, thereby playing a key role in biogeochemical cycling and climate regulation (Falkowski 2012). These large-scale processes are profoundly modulated by fluctuations in phytoplankton production, which are in turn controlled by a complex matrix of abiotic (e.g., nutrients, light, temperature) and biotic factors. Traditionally, the most studied biotic factors are competition for light and nutrients among phytoplankton species, and grazing of phytoplankton by zooplankton (e.g., Lampert et al. 1986; Sommer et al. 1986). However, some phytoplankton taxa, for example colonial diatoms or filamentous cyanobacteria, display morphological and biochemical features which make them particularly resistant to grazing (e.g., Wilson et al. 2006). Other biotic factors, such as infections by parasites or pathogens, can also exert strong top-down control of phytoplankton and elicit significant effects on ecosystem carbon cycling (e.g., Bratbak et al. 1994; Fuhrman 1999; Wommack and Colwell 2000).

Parasitic fungi of the phylum Chytridiomycota (i.e., chytrid parasites) are lethal infective agents of all major phytoplankton groups (reviewed in Frenken et al. 2017*a*). Although chytrid parasites of phytoplankton have long been described already (Braun 1856; Canter 1946; Sparrow 1960), a growing number of environmental molecular surveys in marine, brackish, and freshwater ecosystems are documenting their ubiquitous distribution and so-far underestimated diversity (e.g., Lefèvre et al. 2008; de Vargas et al. 2015; Comeau et al. 2016). Chytrid infection is now regarded as an omnipresent phenomenon that often leads to the development of epidemics capable of driving

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Additional Supporting Information may be found in the online version of this article.

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phytoplankton succession and bloom dynamics (Rasconi et al. 2012; Gerphagnon et al. 2015; Haraldsson et al. 2018).

Chytrids' most characteristic feature is a free-living infectious life stage as flagellated zoospores that actively seek new hosts to infect (Canter and Jaworski 1980; Muehlstein et al. 1988). Besides infecting their hosts, chytrid zoospores can serve as food for zooplankton consumers, including cladocerans, copepods, and rotifers (Kagami et al. 2007*b*, 2011; Agha et al. 2016; Frenken et al. 2018). This observation led to the formulation of the mycoloop, a trophic link (alternative to the herbivory pathway) mediated by chytrids that connects primary and secondary production (Kagami et al. 2007*a*). Model approximations estimate chytrid infection to channel as much as 20% of total primary production (in terms of carbon), providing up to 40% of zooplankton dietary requirements (Grami et al. 2011; Rasconi et al. 2014). Trophic transfer via the mycoloop seems to be particularly relevant when herbivory is constrained by the dominance of large inedible and/or nutritionally inadequate phytoplankton, such as cyanobacteria, whose biomass typically accumulates as massive algal proliferations. These blooms alter the functioning of ecosystems and often lead to reduced water quality and human health hazards due to the production of toxic metabolites (Havens 2008).

In its original formulation, the mycoloop operates by repacking otherwise inaccessible carbon from completely inedible phytoplankton (i.e., colonial diatoms) in the form of readily ingestible chytrid zoospores (Kagami et al. 2007*b*). More recently, experiments have generalized these findings to other phytoplankton groups, revealing additional facets of the mycoloop: under dominance of poorly edible phytoplankton, such as filamentous cyanobacteria, zooplankton displayed higher fitness and population growth when cyanobacteria were infected by chytrids, as compared to quantitatively equal diets in the absence of parasites (Agha et al. 2016). In addition to their high resistance to grazing, cyanobacteria represent a nutritionally suboptimal food source, as they lack lipids essential to zooplankton (von Elert et al. 2003; Martin-Creuzburg et al. 2008). Analyses of lipid compositions of chytrids and their hosts have demonstrated de novo synthesis of sterols and long-chain polyunsaturated fatty acids by chytrid parasites (Kagami et al. 2007*b*; Gerphagnon et al. 2018). Thereby, in addition to conveying carbon from inaccessible primary producers, chytrids supply lipids essential to zooplankton that might be otherwise absent in their hosts, leading to trophic upgrading of cyanobacterial carbon and enhanced carbon transfer efficiency up the food web.

An additional consequence of chytrid infection is the fragmentation of filamentous phytoplankton, leading to reductions of about 50% in mean filament length (Gerphagnon et al. 2013; Agha et al. 2016). Filamentous morphologies of cyanobacteria confer grazing resistance by interfering with zooplankton filtering apparatus (De Bernardi and Giussani 1990), with shorter filaments being easier to ingest by grazers (DeMott et al. 2001; Kurmayer 2001; Oberhaus et al. 2007). Therefore, fragmentation of filaments by chytrid infection may undercut cyanobacterial resistance to grazing and thus enhance trophic transfer through the herbivory pathway, but so far this has not been empirically evaluated.

Here, we tested putative modulation of phytoplankton edibility as a result of chytrid infection. Specifically, we performed grazing assays to quantitatively evaluate the ability of a key zooplankter to graze on a filamentous cyanobacterium over the course of a chytrid epidemic, as compared to control conditions without parasites. Changes in cyanobacterial filament length distributions were analyzed to disentangle the effects of chytrid infection and zooplankton grazing on filament fragmentation.

Materials and methods

Study system and culture conditions

The experimental host-parasite system consisted of the commonly occurring, bloom-forming, filamentous cyanobacterium *Planktothrix rubescens* and its obligate parasite, the chytrid *Rhizophydium megarrhizum* (Sønstebø and Rohrlack 2011). The cyanobacterium (strain NIVA-CYA98) and its parasite (strain Chy-Kol2008) were isolated from the Norwegian Lakes Steinsfjorden in 1982 and Kobotnvannet in 2008, respectively. *Planktothrix* was routinely maintained as batch cultures in Z8 medium (Kotai 1972) at 16°C under continuous 10 μmol photons m^{-2} s^{-1} light. The chytrid parasite was maintained at 16°C and 20 μmol photons m^{-2} s^{-1} by transferring infected cultures into uninfected *Planktothrix* cultures every 3 weeks. Three *Daphnia* clones belonging to the *Daphnia longispina* complex, some of the most common cladoceran herbivores in permanent lakes (e.g., Yin et al. 2014; Ma et al. 2019), were used as grazers: AMME-51, AMME-12, and AMME-3, isolated from Lake Ammersee (Germany) in 2008. *Daphnia* cultures were kept in a synthetic medium (Saebelfeld et al. 2017) at 20°C with a 16:8 light–dark period and fed three times per week with > 1.0 mg C L^{-1} of the green alga *Scenedesmus obliquus*.

Experiment

A stock culture of exponentially growing *Planktothrix* with a final biomass of 25 μg Chl *a* L^{-1} was split into two flasks of 1.2 L volume each. Twenty milliliters of a highly infected *Planktothrix* culture were added into one of them to obtain an infected culture. After a 3 h incubation period, the uninfected and infected cultures were split as 15 mL aliquots into 30 mL glass vials, resulting in a total of 70 replicate cultures of either uninfected or infected cyanobacteria. Glass vials were incubated at 16°C and 10 μmol photons m^{-2} s^{-1}, and mixed daily by gentle manual swaying, and their position within the incubator randomized every day during the experiment (18 d). At days 0, 3, 6, 9, 12, 15, and 18, sets of 20 bottles (10 uninfected, 10 infected) were randomly selected and used destructively to

perform seven grazing assays over the course of the experiment. All grazing assays followed the same full factorial design, with four treatments that combined (1) presence or absence of chytrid infection and (2) presence or absence of zooplankton grazers, resulting in a total of 20 experimental units (4 treatments × 5 replicates) for each grazing assay. For every grazing assay, 5 mL of the replicate cultures containing either uninfected or infected cyanobacteria (the remaining 10 mL were used for analytical measurements, see below) were incubated for 3 d in the presence of ten 10-d old *Daphnia* in the above described glass vials (three individuals from clones AMME-51 and AMME-12 and four from clone AMME-3). Whereas these high *Daphnia* densities might not be representative of natural conditions, they aimed at detecting changes in algal biomass that provide proof of concept for the effect of chytrid infection in zooplankton clearances rates. All *Daphnia* were washed thrice with Z8 medium, left in this medium overnight, and washed thrice again with the respective culture before being transferred to the grazing assay vials. The number of surviving *Daphnia* was recorded daily. Control assays without grazers were subject to a mock pipetting procedure, using the medium in which *Daphnia* was previously grown, but otherwise identical to that performed for vials with grazers. All grazing assays were performed at 16°C under continuous 10 μmol photons m^{-2} s^{-1} light.

Sample processing

Duplicate measurements of chlorophyll *a* (Chl *a*) concentration at the start and end of every grazing assay were performed on all 20 experimental units (10 grazed, 10 ungrazed) using a Phyto-PAM with an ED-101US/MP Optical Unit (Heinz Walz GmbH, Effeltrich, Germany) to calculate zooplankton clearance rates. At the start and end of every grazing assay, 2 mL of culture was fixed with formaldehyde to a final concentration of 2% and stored in the dark at 5°C. These samples were analyzed (blinded) for prevalence of infection and filament length distribution.

Zooplankton clearance rates

Clearance rates were used as a proxy of the ability of zooplankton to feed on the filamentous cyanobacteria and, thereby, of trophic transfer through the herbivory link. *Daphnia* clearance rates (CR, in mL ind^{-1} d^{-1}) under presence or absence of chytrid infection were calculated for every grazing assay according to Coughlan (1969),

$$CR = \frac{V}{N \cdot T} \cdot \left(\ln \frac{B_0}{B_t} - \ln \frac{B_{0c}}{B_{tc}} \right)$$

where V is the volume of the grazed suspension (mL), N is the average number of grazing *Daphnia* over the grazing experiment, and T is the time interval over which grazing took place (days). B_0 and B_t stand for the Chl *a* concentrations at the start and end of the grazing assay in grazed vials, respectively. B_{0c} and B_{tc} are the average Chl *a* concentrations at the start and end of the grazing assay in the control vials, respectively. *Daphnia* survival was recorded over the course of each grazing assay (*see* Supporting Information Fig. S1) and *Daphnia* densities were included in the CR calculations as monitored. Clearance rates were calculated for each grazed biological replicate as the mean CR calculated iteratively from all possible combinations with control (ungrazed) biological replicates, resulting in five CR values per treatment. Within single grazing assays, clearance rates in uninfected and infected treatments were compared using *t*-tests. Sequential Bonferroni correction was applied to correct for multiple comparisons.

Prevalence of infection and filament length distribution

Prevalence of infection in infected vials was determined at the beginning of every grazing assay in 5 (out of 10) randomly chosen replicates by inspecting 100 random filaments for infection (i.e., attached chytrid sporangia) under a Leica DM IL inverted microscope. Filament length distribution with and without grazers, and with and without infection, was analyzed for the grazing assay performed on day 6. Specifically, in each

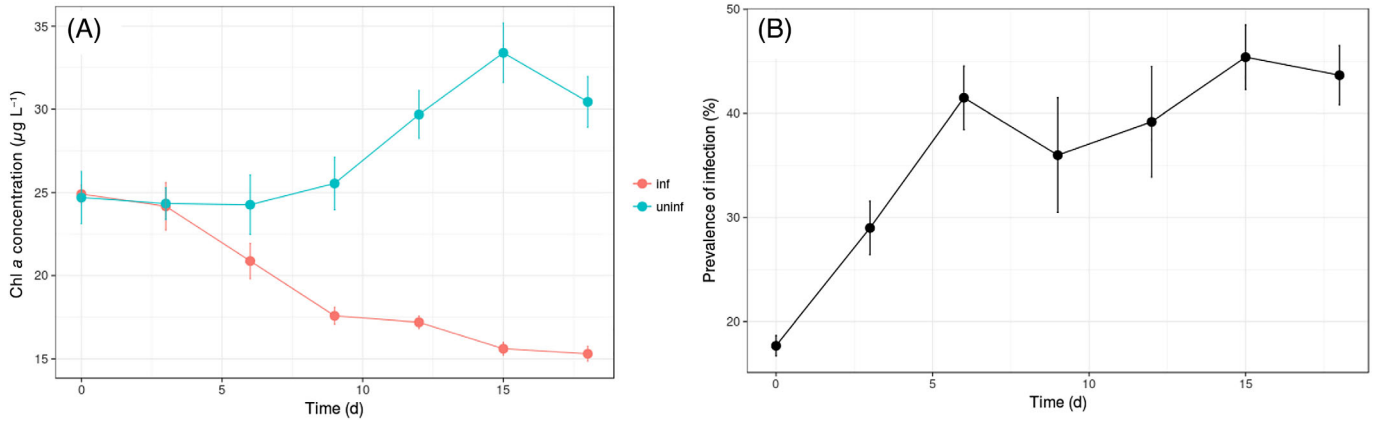

Fig. 1. Changes over time of (**A**) Chl *a* concentration in uninfected and infected cyanobacterial cultures (n = 10) and (**B**) prevalence of infection in the infected cultures (n = 5). Data are shown as means ± SE.

experimental unit, the length and infection status (hereafter referred to as filament health) of at least 100 individual filaments was analyzed before and after the 3-d grazing incubation period, using a Nikon Ti Eclipse inverted microscope with the Nikon NIS-Element Br 4.5 software. Linear models were used to evaluate the effect of experimental treatments on mean filament length, testing for fixed and interactive effects of (1) presence or absence of chytrid infection in the treatment, (2) presence or absence of grazers, (3) filament health (uninfected/infected), and (4) time (before/after the 3-d grazing assay). Model assumptions were confirmed by visual inspection of the residuals. In order to account for repeated measures on biological replicates before and after the grazing assay, experimental unit (from which repeated measures were taken) was included as a random factor in the model. Within-group variability was not sufficient to justify incorporating the random factor, as shown by the poorer quality of the mixed model (higher Akaike information criterion values) and zero sum of its variance components. Consequently, the output of the degenerate model was reported. The proportion of the total variance explained by the individual terms was determined as sum of squares quotients.

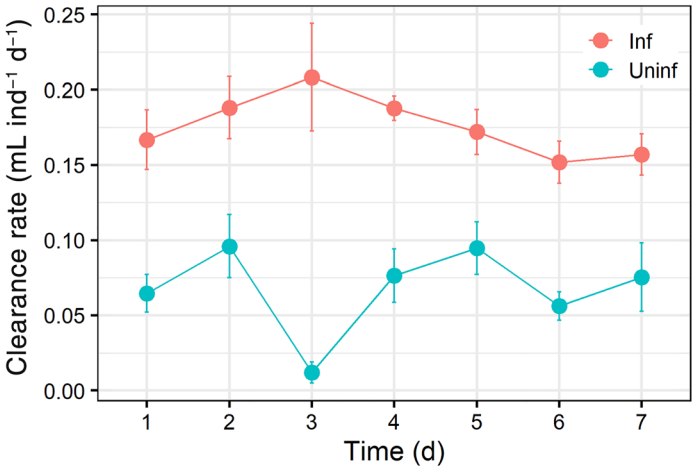

Fig. 2. Mean clearance rates (± SE) in infected and uninfected cultures ($n = 5$) for each of the seven grazing assays conducted. All pairwise *t*-tests comparing mean clearance rates between infected and uninfected cultures showed significant differences ($p < .05$ after sequential Bonferroni correction).

Results

Uninfected *Planktothrix* cultures showed positive growth over the study period, whereas total Chl *a* concentrations declined in infected cultures (Fig. 1A) reflecting cyanobacterial decay as a result of chytrid infection, which spread gradually among the population over the course of the experiment (Fig. 1B). Mean *Daphnia* clearance rates on infected cyanobacterial cultures were consistently higher (about twofold) than on uninfected cultures (Fig. 2).

Mean filament lengths and their frequency distribution were studied for the grazing assay performed on day 6. Infected filaments were conspicuously shorter than uninfected ones, as indicated by differences in the skewness of the histograms of uninfected and infected filaments and their mean

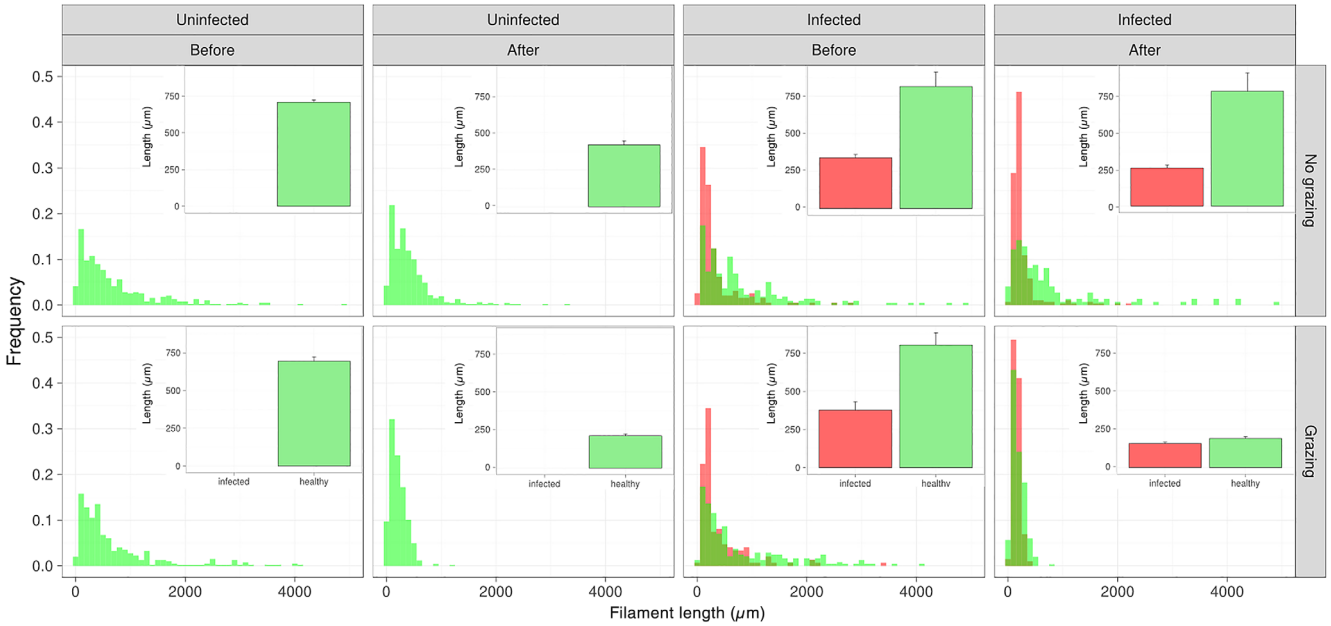

Fig. 3. Frequency distribution of uninfected (green) and infected filaments (red) across uninfected and infected treatments, in presence or absence of a grazer, and before and after the grazing assay. Histograms depict length frequency distribution of all (pooled) measured filaments across replicates. Bar graphs in the upper-right corner of each panel show mean lengths ± SE of uninfected (green) and infected (red) cyanobacterial filaments in the respective treatment ($n = 5$).

Table 1. Output of the linear model for fixed and interactive effects of (1) infection (uninfected/infected culture), (2) grazing (grazing/no grazing), (3) filament health (uninfected filament/infected filament), and (4) time (before/after grazing assay) on mean cyanobacterial filament length. Significant *p* values are depicted in bold. Variance explained on individual terms stems from sum of squares quotients.

	df	*F* ratio	*p* value	Variance explained (%)
Infection	1	1.632	0.207	0.6
Grazing	1	21.400	**<0.001**	7.4
Filament health	1	85.486	**<0.001**	29.5
Time	1	79.208	**<0.001**	27.4
Infection × grazing	1	0.71	0.403	0.2
Grazing × filament health	1	11.795	**0.001**	4.1
Infection × time	1	4.949	**0.031**	1.7
Grazing × time	1	22.198	**<0.001**	7.7
Filament health × time	1	4.849	**0.032**	1.7
Infection × grazing × time	1	1.467	0.231	0.5
Grazing × filament health × time	1	7.630	**0.008**	2.6
Residuals	48			16.6

lengths (Fig. 3). Filament health was in fact the best linear predictor of filament length in the model (29.5% variance explained; Table 1). Filament length was reduced over the incubation period (Fig. 3; significant effect of Time, Table 1). However, in the presence of grazers, reduction in filament lengths was even more pronounced, both in uninfected and infected treatments (Fig. 3; significant effects of Grazing and Grazing × Time, jointly explaining 15% of the variance, Table 1). Interestingly, uninfected filaments in the presence of chytrids underwent length reduction only in the presence of grazers (Fig. 3; significant Grazing × Filament health × Time, Table 1).

Discussion

Parasites are more and more recognized for their ability to structure food webs and increase the stability of ecosystems (Lafferty et al. 2008; Poulin 2010). Parasites can establish new trophic links and modulate existing ones, providing alternative pathways for carbon and energy to flow up the food web. This also applies to aquatic food webs, where fungal parasites of phytoplankton constitute an additional high quality food source to consumers, especially when primary production is dominated by inedible or biochemically inadequate phytoplankton (Kagami et al. 2007*b*; Agha et al. 2016; Gerphagnon et al. 2018). In addition to providing new trophic links, parasites often have the ability to modulate existing ones (Amundsen et al. 2009). Our findings exemplify this by showing that trophic transfer through the herbivory link is enhanced when cyanobacteria are infected by chytrid parasites, as demonstrated by higher zooplankton clearance rates recorded under infection relative to conditions without parasites.

Our analyses point toward infection-induced filament fragmentation as the cause (although not necessarily the only cause) behind the twofold increase in clearance rates. Infected cyanobacteria were conspicuously shorter than noninfected ones, as also reported in previous laboratory and field studies (Gerphagnon et al. 2013; Agha et al. 2016). Additionally, length of uninfected filaments was reduced both in infected and uninfected treatments in the presence of grazers, confirming that *Daphnia* is capable of mechanically fragmenting cyanobacteria, as has been reported elsewhere for *Daphnia* and other zooplankton, and also at lower zooplankton densities than those provided in our experiment (Burns and Xu 1990; Dawidowicz 1990; Sikora and Dawidowicz 2017). One could reason that fragmentation of infected filaments was facilitated by zooplankton. However, analyses indicate that the effect of grazers and fungal infection on filament length is additive, not synergistic (Infection × Grazing × Time Interaction and Infection × Grazing interaction were not significant, Table 1). If grazers are able to fragment cyanobacterial filaments, one could argue that the presence of zooplankton alone (i.e., in the absence of parasites) can increase *Planktothrix* edibility and thus neutralize its grazing resistance. Whereas this may be true, filament fragmentation is likely energetically costly to *Daphnia*, leading to higher respiration and rejection rates which in turn reduce net carbon assimilation (Conover 1966; Porter and McDonough 1984). Instead, in the presence of parasites, chytrid infection contributes further to filament length reduction, making cyanobacteria more edible and facilitating zooplankton grazing, as reflected by higher *Daphnia* clearance rates. This speaks for more efficient carbon assimilation and hence enhanced trophic transfer via the herbivory pathway under conditions of chytrid epidemics.

An important consideration refers to the fact that *Planktothrix*, like many other cyanobacterial taxa, can synthesize the cyanotoxin microcystin, together with a wide array of intracellular oligopeptides with diverse bioactive properties (Welker and von Dohren 2006; Agha and Quesada 2014). These compounds might cause toxic effects on zooplankton upon ingestion (e.g., Rohrlack et al. 1999, 2004; Czarnecki et al. 2006). Increased grazing on infected cyanobacteria might thus lead to higher exposure to these potentially harmful compounds, which might ultimately reduce *Daphnia* carbon assimilation in the long term. Moreover, given the indication that cyanobacterial oligopeptides, including microcystins, might be involved in defense against chytrid parasites (Rohrlack et al. 2013), chytrid infection could result in the upregulation of metabolite production, potentially exposing *Daphnia* to higher amounts of oligopeptides and toxins.

Yet, our data are not in line with this hypothesis: *Daphnia* mortality was relatively stable over the course of the experiments and was not higher under conditions of chytrid infection (Supporting Information Fig. S1), as also observed in previous experiments using the exact same host-parasite system (Agha et al. 2016). In fact, chytrids penetrate and digest their host's cells, including their intracellular metabolites, as indicated by lower toxin (microcystin) cell quota observed in infected *Planktothrix* (Frenken et al. 2017*b*). Chytrid infection might hence hypothetically reduce exposure of *Daphnia* to cyanobacterial metabolites after ingestion, as compared to noninfected cyanobacteria. Exploring the fate and production of cyanotoxins and other bioactive secondary metabolites under chytrid infection will provide valuable insights into this issue.

The analysis of filament length distributions sheds light on the mechanism of chytrid infection. At present, it is unclear whether overrepresentation of short filaments in the infected filament cohort is the consequence of, or a cause for, chytrid infection. On the one hand, chytrid rhizoids penetrating and digesting the filament over the course of the infection may structurally compromise host filaments, resulting in fragmentation. On the other hand, it is also possible that shorter, freshly divided (or broken) filaments are more susceptible to infection and, once infected, their growth is arrested. Cyanobacteria are covered by a sheath of complex carbohydrates (e.g., lipopolysaccharides, glycans) and lectins, which represents a barrier between the filament and its immediate environment and plays a role in a number of intra- and interspecies interactions (Kehr and Dittmann 2015). This sheath is the first contact surface between a chytrid and its host and likely constitutes a barrier defense against infection (sensu Dybdahl et al. 2014). Upon filament division during growth, a sheath-free surface is temporarily produced where the host's cell wall is exposed and might be particularly susceptible to chytrid encystment. The fact that chytrid zoospores encyst only at the tips of *Planktothrix* filaments is compatible with this possibility (Agha et al. 2018). Overrepresentation of short filaments among infected individuals might be the result of the arrested growth of recently divided (and therefore shorter and more susceptible) filaments upon infection. Indeed, uninfected cyanobacterial filaments in the presence of chytrids were longer than in the treatments without parasites (Fig. 3), supporting the hypothesis that filaments that grow without dividing might have a better chance to evade infection. These mechanistic considerations aside, our experiment shows that chytrid-induced reductions in

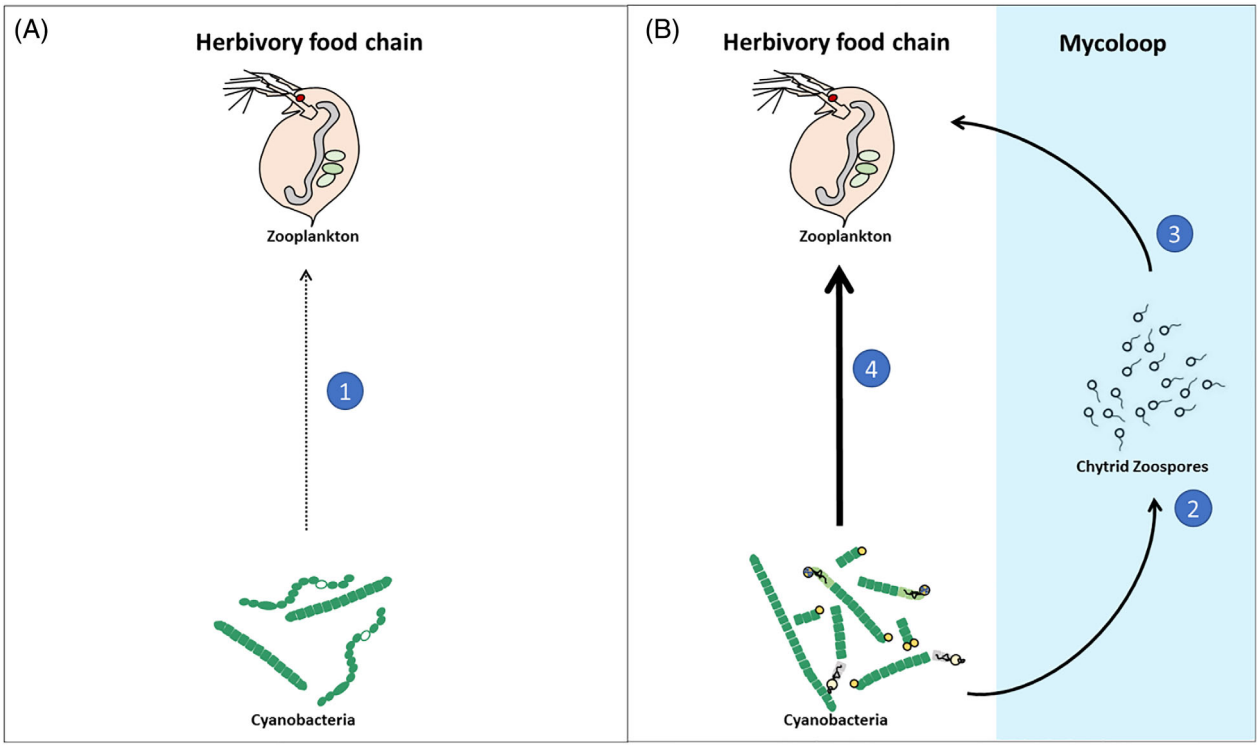

Fig. 4. Schematic representation of the trophic pathways under (**A**) absence and (**B**) presence of chytrid infection. For simplicity, the microbial loop is not depicted. Under the dominance of cyanobacteria, (effect 1) trophic transfer to zooplankton consumers is hampered by phytoplankton resistance to grazing. As a result of chytrid infection, (effect 2) carbon from phytoplankton is repacked in the form of chytrid zoospores (i.e., traditional mycoloop, sensu Kagami et al. 2007*a*,*b*). In addition, (effect 3) chytrids synthesize essential lipids de novo (i.e., trophic upgrading of cyanobacteria; Gerphagnon et al. 2018). Last, (effect 4) length reductions in cyanobacterial filaments increase their edibility and enhance the flow of carbon and energy through the herbivory link.

cyanobacterial filament length lead to doubled *Daphnia* clearance rates, in comparison to conditions without infection. Clearance rates recorded in our assays were lower than reported elsewhere and likely do not represent maximal rates (Hessen 1985; Kurmayer and Juttner 1999). We attribute this to partial sedimentation of cyanobacterial biomass at the bottom of the vials despite daily resuspension, which might make cyanobacteria less available for grazing. Yet, the significant differences between uninfected and infected treatments demonstrate increased grazing under conditions of chytrid infection. This indicates a subsequent enhancement of trophic transfer through the herbivory pathway. In addition to increased cyanobacterial edibility, an alternative, nonexclusive cause for increased zooplankton clearance rates might be an improvement in *Daphnia* physiological conditions, as a result of a dietary upgrade in the presence of chytrid zoospores. While cyanobacteria alone are a nutritionally inadequate food source lacking long-chain polyunsaturated fatty acids and sterols, chytrids synthesize these essential lipids de novo (Gerphagnon et al. 2018). Several fitness traits, including age at maturity, growth rate, and body size, improved when *Daphnia* were fed with infected cyanobacteria relative to feeding conditions without parasites (Agha et al. 2016). The increased clearance rates recorded here may hence reflect higher cyanobacterial edibility due to shorter filaments under infection, or improved physiological status of *Daphnia* as a result of better nutrition, or a combination of the two. Direct effects of chytrid dietary supplements on zooplankton intrinsic filtering rates remain to be evaluated.

When integrating our findings with those reported elsewhere, it becomes evident that chytrid infection counteracts two of the main features that endow cyanobacteria with increased resistance to grazing. While the poor nutritional quality of cyanobacteria is countered by the production of essential lipids and subsequent trophic upgrading by their chytrid parasites (Gerphagnon et al. 2018), we show that morphological features constraining grazing are minimized by reductions in filament length upon chytrid infection. This subsequently led to an about twofold increase in zooplankton clearance rates, which reflects enhanced herbivory in the presence of parasites. Thereby, this work reveals an additional indirect effect of chytrid infection on the functioning of aquatic food webs, which, together with the recently described trophic upgrading of cyanobacterial carbon (Gerphagnon et al. 2018), depicts novel facets of the mycoloop (Fig. 4): in the absence of chytrid infection, carbon transfer is constrained by the limited edibility and poor nutritional quality of cyanobacteria, which act as a trophic bottleneck. In contrast, chytrid infection establishes a direct link to consumers via the mycoloop (Kagami et al. 2007*b*; Agha et al. 2016), which can elicit trophic upgrading of phytoplankton carbon if this is of poor nutritional quality (e.g., cyanobacteria; Gerphagnon et al. 2018). In addition, poorly edible or inedible phytoplankton becomes fragmented and thereby more susceptible to grazing, enhancing trophic transfer via the herbivory link. These findings contribute to a better understanding of the effects of chytridiomycosis on carbon flows at the base of pelagic food webs, and exemplify how parasites can create new trophic pathways and boost existing ones, facilitating carbon and energy flows in the ecosystem and increasing its resilience against perturbations.

References

Agha, R., and A. Quesada. 2014. Oligopeptides as biomarkers of cyanobacterial subpopulations. Toward an understanding of their biological role. Toxins **6**: 1929–1950. doi:10.3390/toxins6061929

Agha, R., M. Saebelfeld, C. Manthey, T. Rohrlack, and J. Wolinska. 2016. Chytrid parasitism facilitates trophic transfer between bloom-forming cyanobacteria and zooplankton (*Daphnia*). Sci. Rep. **6**: 35039. doi:10.1038/srep35039

Agha, R., A. Gross, M. Gerphagnon, T. Rohrlack, and J. Wolinska. 2018. Fitness and eco-physiological response of a chytrid fungal parasite infecting planktonic cyanobacteria to thermal and host genotype variation. Parasitology **145**: 1279–1286. doi:10.1017/S0031182018000215

Amundsen, P. A., K. D. Lafferty, R. Knudsen, R. Primicerio, A. Klemetsen, and A. M. Kuris. 2009. Food web topology and parasites in the pelagic zone of a subarctic lake. J. Anim. Ecol. **78**: 563–572. doi:10.1111/j.1365-2656.2008.01518.x

Bratbak, G., F. Thingstad, and M. Heldal. 1994. Viruses and the microbial loop. Microb. Ecol. **28**: 209–221. doi:10.1007/BF00166811

Braun, A. 1856. Ueber einige neue Arten der Gattung *Chytridium* und die damit verwandte Gattung *Rhizidium*. Monatsber. Berlin Akad. 587–592.

Burns, C. W., and Z. Xu. 1990. Calanoid copepods feeding on algae and filamentous cyanobacteria: Rates of ingestion, defaecation and effects on trichome length. J. Plankton Res. **12**: 201–213. doi:10.1093/plankt/12.1.201

Canter, H. M. 1946. Studies on British chytrids: I. *Dangeardia mammillata* schröder. Trans Br Mycol Soc **29**: 128–134. doi:10.1016/S0007-1536(46)80036-6

Canter, H. M., and G. Jaworski. 1980. Some general observations on zoospores of the chytrid *Rhizophydium planktonicum* Canter emend. New Phytol. **84**: 515–531.

Comeau, A. M., W. F. Vincent, L. Bernier, and C. Lovejoy. 2016. Novel chytrid lineages dominate fungal sequences in diverse marine and freshwater habitats. Sci. Rep. **6**: 30120. doi:10.1038/srep30120

Conover, R. J. 1966. Factors affecting the assimilation of organic matter by zooplankton and the question of superfluous feeding. Limnol. Oceanogr. **11**: 346–354. doi:10.4319/lo.1966.11.3.0346

Coughlan, J. 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. **2**: 356–358.

Czarnecki, O., M. Henning, I. Lippert, and M. Welker. 2006. Identification of peptide metabolites of *Microcystis* (Cyanobacteria) that inhibit trypsin-like activity in planktonic herbivorous *Daphnia* (Cladocera). Environ. Microbiol. **8**: 77–87. doi:10.1111/j.1462-2920.2005.00870.x

Dawidowicz, P. 1990. The effect of *Daphnia* on filament length of blue-green algae. Hydrobiologia **191**: 265–268. doi:10.1007/BF00026061

De Bernardi, R. D., and G. Giussani. 1990. Are blue-green algae a suitable food for zooplankton? An overview Hydrobiologia, **200**: 29–41. doi:10.1007/BF02530326

de Vargas, C., and others. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science **348**: 1261605. doi:10.1126/science.1261605

DeMott, W. R., R. D. Gulati, and E. Van Donk. 2001. *Daphnia* food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. Limnol. Oceanogr. **46**: 2054–2060. doi:10.4319/lo.2001.46.8.2054

Dybdahl, M. F., C. E. Jenkins, and S. L. Nuismer. 2014. Identifying the molecular basis of host-parasite coevolution: Merging models and mechanisms. Am. Nat. **184**: 1–13. doi:10.1086/676591

Falkowski, P. 2012. Ocean science: The power of plankton. Nature **483**: S17–S20.

Frenken, T., and others. 2017*a*. Integrating chytrid fungal parasites into plankton ecology. Research gaps and needs. Environ. Microbiol. **19**: 3802. doi:10.1111/1462-2920.13827

Frenken, T., J. Wierenga, A. S. Gsell, E. van Donk, T. Rohrlack, and D. B. Van de Waal. 2017*b*. Changes in N:P supply ratios affect the ecological stoichiometry of a toxic cyanobacterium and its fungal parasite. Front. Microbiol. **8**: 1015. doi:10.3389/fmicb.2017.01015

Frenken, T., and others. 2018. Fungal parasites of a toxic inedible cyanobacterium provide food to zooplankton. Limnol. Oceanogr. **63**: 2384–2393. doi:10.1002/lno.10945

Fuhrman, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature **399**: 541–548. doi:10.1038/21119

Gerphagnon, M., D. Latour, J. Colombet, and T. Sime-Ngando. 2013. Fungal parasitism: Life cycle, dynamics and impact on cyanobacterial blooms. PLoS One **8**: e60894. doi:10.1371/journal.pone.0060894

Gerphagnon, M., and others. 2015. Microbial players involved in the decline of filamentous and colonial cyanobacterial blooms with a focus on fungal parasitism. Environ. Microbiol. **17**: 2573–2587. doi:10.1111/1462-2920.12860

Gerphagnon, M., and others. 2018. Comparison of sterol and fatty acid profiles of chytrids and their hosts reveals trophic upgrading of nutritionally inadequate phytoplankton by fungal parasites. Environ. Microbiol. **21**: 949–958. doi:10.1111/1462-2920.14489

Grami, B., S. Rasconi, N. Niquil, M. Jobard, B. Saint-Béat, and T. Sime-Ngando. 2011. Functional effects of parasites on food web properties during the spring diatom bloom in Lake Pavin: A linear inverse modeling analysis. PLoS One **6**: e23273. doi:10.1371/journal.pone.0023273

Haraldsson, M., and others. 2018. Microbial parasites make cyanobacteria blooms less of a trophic dead end than commonly assumed. ISME J. **12**: 1008–1020. doi:10.1038/s41396-018-0045-9

Havens, K. E. 2008. Cyanobacteria blooms: Effects on aquatic ecosystems, p. 733–747. *In* Hudnell H. K. (eds) Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Advances in Experimental Medicine and Biology, vol 619. New York, NY, Springer. doi:10.1007/978-0-387-75865-7_33

Hessen, D. O. 1985. Filtering structures and particle size selection in coexisting Cladocera. Oecologia **66**: 368–372. doi:10.1007/BF00378300

Kagami, M., A. de Bruin, B. W. Ibelings, and E. Van Donk. 2007*a*. Parasitic chytrids: Their effects on phytoplankton communities and food-web dynamics. Hydrobiologia **578**: 113–129. doi:10.1007/s10750-006-0438-z

Kagami, M., E. von Elert, B. W. Ibelings, A. de Bruin, and E. Van Donk. 2007*b*. The parasitic chytrid, *Zygorhizidium*, facilitates the growth of the cladoceran zooplankter, *Daphnia*, in cultures of the inedible alga, *Asterionella*. Proc. R. Soc. Lond. B Biol. Sci. **274**: 1561–1566. doi:10.1098/rspb.2007.0425

Kagami, M., N. R. Helmsing, and E. van Donk. 2011. Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms. Hydrobiologia **659**: 49–54. doi:10.1007/s10750-010-0274-z

Kehr, J.-C., and E. Dittmann. 2015. Biosynthesis and function of extracellular glycans in cyanobacteria. Life **5**: 164–180. doi:10.3390/life5010164

Kotai, J. 1972. Instructions for preparation of modified nutrient solution Z8 for algae. Norwegian Institute for Water Research.

Kurmayer, R. 2001. Competitive ability of *Daphnia* under dominance of non-toxic filamentous cyanobacteria. Hydrobiologia **442**: 279–289. doi:10.1023/A:1017598518952

Kurmayer, R., and F. Juttner. 1999. Strategies for the coexistence of zooplankton with the toxic cyanobacterium *Planktothrix rubescens* in Lake Zurich. J. Plankton Res. **21**: 659–683. doi:10.1093/plankt/21.4.659

Lafferty, K. D., and others. 2008. Parasites in food webs: The ultimate missing links. Ecol. Lett. **11**: 533–546. doi:10.1111/j.1461-0248.2008.01174.x

Lampert, W., W. Fleckner, H. Rai, and B. E. Taylor. 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. Limnol. Oceanogr. **31**: 478–490. doi:10.4319/lo.1986.31.3.0478

Lefèvre, E., B. Roussel, C. Amblard, and T. Sime-Ngando. 2008. The molecular diversity of freshwater picoeukaryotes

reveals high occurrence of putative parasitoids in the plankton. PLoS One **3**: 2324. doi:10.1371/journal.pone.0002324

Ma, X., W. Hu, P. Smilauer, M. Yin, and J. Wolinska. 2019. *Daphnia galeata* and *D. dentifera* are geographically and ecologically separated whereas their hybrids occur in intermediate habitats: A survey of 44 Chinese lakes. Mol. Ecol. **28**: 785–802. doi:10.1111/mec.14991

Martin-Creuzburg, D., E. von Elert, and K. H. Hoffmann. 2008. Nutritional constraints at the cyanobacteria—*Daphnia magna* interface: The role of sterols. Limnol. Oceanogr. **53**: 456–468. doi:10.4319/lo.2008.53.2.0456

Muehlstein, L. K., J. P. Amon, and D. L. Leffler. 1988. Chemotaxis in the marine fungus *Rhizophydium littoreum*. Appl. Environ. Microbiol. **54**: 1668–1672.

Oberhaus, L., M. Gélinas, B. Pinel-Alloul, A. Ghadouani, and J.-F. Humbert. 2007. Grazing of two toxic *Planktothrix* species by *Daphnia pulicaria*: Potential for bloom control and transfer of microcystins. J. Plankton Res. **29**: 827–838. doi:10.1093/plankt/fbm062

Porter, K. G., and R. McDonough. 1984. The energetic cost of response to blue-green algal filaments by cladocerans. Limnol. Oceanogr. **29**: 365–369. doi:10.4319/lo.1984.29.2.0365

Poulin, R. 2010. Parasite manipulation of host behavior: An update and frequently asked questions, p. 151–186. *In* Advances in the study of behavior. Elsevier. doi:10.1016/S0065-3454(10)41005-0

Rasconi, S., N. Niquil, and T. Sime-Ngando. 2012. Phytoplankton chytridiomycosis: Community structure and infectivity of fungal parasites in aquatic ecosystems. Environ. Microbiol. **14**: 2151–2170. doi:10.1111/j.1462-2920.2011.02690.x

Rasconi, S., B. Grami, N. Niquil, M. Jobard, and T. Sime-Ngando. 2014. Parasitic chytrids sustain zooplankton growth during inedible algal bloom. Front. Microbiol. **5**: 229. doi:10.3389/fmicb.2014.00229

Rohrlack, T., E. Dittmann, M. Henning, T. Börner, and J.-G. Kohl. 1999. Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by the cyanobacterium *Microcystis aeruginosa*. Appl. Environ. Microbiol. **65**: 737–739. doi:10.1128/AEM.65.2.737-739.1999

Rohrlack, T., K. Christoffersen, M. Kaebernick, and B. A. Neilan. 2004. Cyanobacterial protease inhibitor microviridin J causes a lethal molting disruption in *Daphnia pulicaria*. Appl. Environ. Microbiol. **70**: 5047–5050. doi:10.1128/AEM.70.8.5047-5050.2004

Rohrlack, T., G. Christiansen, and R. Kurmayer. 2013. Putative antiparasite defensive system involving ribosomal and nonribosomal oligopeptides in cyanobacteria of the genus *Planktothrix*. Appl. Environ. Microbiol. **79**: 2642–2647. doi:10.1128/AEM.03499-12

Saebelfeld, M., L. Minguez, J. Griebel, M. O. Gessner, and J. Wolinska. 2017. Humic dissolved organic carbon drives oxidative stress and severe fitness impairments in *Daphnia*. Aquat. Toxicol. **182**: 31–38. doi:10.1016/j.aquatox.2016.11.006

Sikora, A., and P. Dawidowicz. 2017. Breakage of cyanobacterial filaments by small-and large-sized *Daphnia*: Are there any temperature-dependent differences? Hydrobiologia **798**: 119–126. doi:10.1007/s10750-015-2436-5

Sommer, U., Z. M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. Arch. Hydrobiol. **106**: 433–471.

Sønstebø, J. H., and T. Rohrlack. 2011. Possible implications of chytrid parasitism for population subdivision in freshwater cyanobacteria of the genus *Planktothrix*. Appl. Environ. Microbiol. **77**: 1344–1351. doi:10.1128/AEM.02153-10

Sparrow, F. K. 1960. Aquatic phycomycetes. Univ. of Michigan Press, p. 36–39.

von Elert, E., D. Martin-Creuzburg, and J. R. Le Coz. 2003. Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (*Daphnia galeata*). Proc. R. Soc. Lond. Ser. B Biol. Sci. **270**: 1209–1214. doi:10.1098/rspb.2003.2357

Welker, M., and H. von Dohren. 2006. Cyanobacterial peptides - nature's own combinatorial biosynthesis. FEMS Microbiol. Rev. **30**: 530–563. doi:10.1111/j.1574-6976.2006.00022.x

Wilson, A. E., O. Sarnelle, and A. R. Tillmanns. 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. Limnol. Oceanogr. **51**: 1915–1924. doi:10.4319/lo.2006.51.4.1915

Wommack, K. E., and R. R. Colwell. 2000. Virioplankton: Viruses in aquatic ecosystems. Microbiol. Mol. Biol. Rev. **64**: 69–114. doi:10.1128/MMBR.64.1.69-114.2000

Yin, M., S. Gießler, J. Griebel, and J. Wolinska. 2014. Hybridizing *Daphnia* communities from ten neighbouring lakes: Spatio-temporal dynamics, local processes, gene flow and invasiveness. BMC Evol. Biol. **14**: 80. doi:10.1186/1471-2148-14-80

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Conflict of Interest

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