Live and Let die

How climate change affects bottom-up and top-down factors regulating phytoplankton disease

Theodorus Henricus Martha Frenken

Thesis committee	Prof. dr. E. Decaestecker
	Prof. dr. H.P. Grossart
	Prof. dr. J. Huisman
	Prof. dr. G.A. Kowalchuk
	Prof. dr. H.A.B. Wösten

Design cover: Loes Kema

Printed by: GVO drukkers & vormgevers B.V.

ISBN: 978-94-6332-328-4

This thesis should be cited as:

Frenken, Thijs (2018) Live and Let die. How climate change affects bottom-up and top-down factors regulating phytoplankton disease. PhD thesis. Utrecht University, Utrecht, the Netherlands

Live and Let die

How climate change affects bottom-up and top-down factors regulating phytoplankton disease

Leef en Laat sterven

Hoe klimaatsverandering bottom-up en top-down factoren beïnvloedt die fytoplankton ziekten reguleren

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 18 april 2018 des ochtends te 10.30 uur

door

Theodorus Henricus Martha Frenken

geboren op 5 april 1990 te Roermond

Promotor:	Prof. dr. E. Van Donk
Copromotoren:	Dr. ir. D.B. Van de Waal Dr. A.S. Gsell

Table of Contents

Chapter 1

General introduction

The role of phytoplankton – Food for thought

Almost all life on Earth relies on them: primary producers. These organisms are responsible for photosynthesis, the process that converts CO_2 with light and water into organic carbon such as carbohydrates that serve as energy for higher trophic levels. Phytoplankton in lakes and oceans only account for less than 1% of the photosynthetic biomass on Earth. However, they contribute to almost half of Earth's primary production, making them equally important in modifying the global carbon cycle as all terrestrial plants combined (Falkowski 2012; Field et al. 1998).

Phytoplankton biomass is regulated by a combination of growth and mortality related factors. Even though their densities are highly dynamic, seasonal succession of plankton is to some extent a predictable process of community assembly (Sommer et al. 2012; Sommer et al. 1986). These mechanisms are summarized in the plankton ecology group (PEG) model, which emphasizes the role of physical factors, grazing and nutrient limitation as the main regulators of phytoplankton growth (Sommer et al. 1986). Other phytoplankton loss factors, such as parasitism, are included in the updated PEG model (Sommer et al. 2012). Although the role of parasites in driving changes in for instance species replacements is well established now, the generality of higher level effects of parasites (size structure, biomass of entire functional groups and trophic levels) thus far remains an open question (Sommer et al. 2012).

In the past decades, global temperatures have risen as a result of human induced climate change (Stocker et al. 2013). Additionally, human activities have led to eutrophication, a substantial increase in the amount of nutrients running off into aquatic systems (Smith 2003; Smith et al. 2006). Both warming and eutrophication may promote mass development (i.e. blooms) of phytoplankton (O'Neil et al. 2012; Paerl et al. 2011; Paerl and Huisman 2009; Paerl and Paul 2012). Algal blooms not only result from growth, but also by reduced mortality caused by a lack of top down control. Algal blooms often consist of relatively large species, such as filamentous or colonial cyanobacteria, large diatoms, as well as dinoflagellates, which can be resistant to zooplankton grazing (Gliwicz 1990; Paerl et al. 2001; Sommer et al. 2012; Sommer et al. 1986). In these cases, other top down factors, such as parasites, may become more important in regulating phytoplankton densities. Examples of parasites that commonly occur with phytoplankton are viruses, bacteria and parasitic fungi (Gachon et al. 2010; Gerphagnon et al. 2015).

The role of parasites - Live and let die?

Parasitism is the most common consumer lifestyle, yet food web studies rarely include parasites (Lafferty et al. 2006). Since the (relatively recent) discovery of the ecological relevance of viruses for phytoplankton and bacterial dynamics (Bergh et al. 1989; Proctor and Fuhrman 1990; Suttle et al. 1990), parasites have gained an increased scientific interest as top-down components in aquatic ecosystems. As parasites can be very host specific, infection of one algal species may favour the development of other algae (Canter and Lund 1951). This 'kill the winner' hypothesis describes that once a host becomes dominant, the increased abundance will also increase its encounter rate with pathogens leading to higher infection rates and enhanced host mortality, preventing the host of winning competition (Thingstad and Lignell 1997).

Accumulating evidence highlights the role of viruses in controlling phytoplankton dynamics through reduction of host populations, preventing blooms of hosts, or by infecting specific hosts only (Brussaard 2004b). Upon infection, organic carbon and nutrients are released from the host back into the environment, which is also referred to as the 'viral shunt' (Wilhelm and Suttle 1999). This shows that presence of parasites can affect phytoplankton species succession, the flow of organic carbon and nutrients (Brussaard 2004b; Middelboe et al. 1996; Middelboe and Lyck 2002; Wilhelm and Suttle

1999), as well as steer community structure and maintenance of diversity (Sønstebø and Rohrlack 2011).

Besides viruses, another important but less studied group of parasites represent the Chytridiomycota, usually referred to as chytrids (fig. 1.1). These fungal parasites are often involved in the decline of blooms of phytoplankton (Gerphagnon et al. 2015; Rasconi et al. 2011; Van Donk 1989; Van Donk and Bruning 1992). Parasitic chytrids can be host specific, highly infective, and extremely virulent. Up to 90 percent of the total host population may be infected, and every infection will lead to death of the host cell (Canter and Lund 1951). Models suggest that during a bloom of inedible algae, up to 20 percent of the total primary production can be directed through the chytrids into production of zoospores (Rasconi et al. 2014). Chytrids are characterized by a free-living motile stage in the form of single-flagellated zoospores (Canter and Jaworski 1980; Van Donk and Ringelberg 1983) which actively search for new hosts, presumably via chemotactic attraction (Bruning 1991c; Muehlstein et al. 1988). In lake water, densities of up to a billion zoospores per litre have been reported (Kagami et al. 2014). These vast numbers not only indicate a high potential for top down control on their phytoplankton hosts, but also a putative source for carbon and nutrients for higher trophic levels. Indeed, research has shown that fungal zoospores can serve as a nutritious food source for *Daphnia*, due to their elemental composition and fatty acid contents (Kagami et al. 2004). If large inedible phytoplankton species are infected, carbon and nutrients within host cells can be transferred to zooplankton via the zoospores of the chytrids and disrupted host colonies and cells (fig 1.2), hereby forming the so-called 'mycoloop' (Kagami et al. 2007a). In this thesis, I will use chytrids as a model system to investigate the effects of climate change on bottom-up and top-down control of phytoplankton disease.

Figure 1.1: Chytrid infections (indicated by arrow). Left: the diatom Synedra sp. Right: the filamentous cyanobacterium Planktothrix

The role of a changing environment - Does warmer mean sicker?

Environmental conditions are rapidly changing as a result of global change. These changes may have effects on pathogen spread, transmission and incidence. Changes in the climate are already affecting phenology of consumer-resource interactions and lead to climate-induced range shifts (Chen et al. 2011; Ogden et al. 2006; Parmesan and Yohe 2003). These movements in time and space will likely affect disease dynamics as well (Altizer et al. 2011; Morgan et al. 2004; Paull and Johnson 2014).

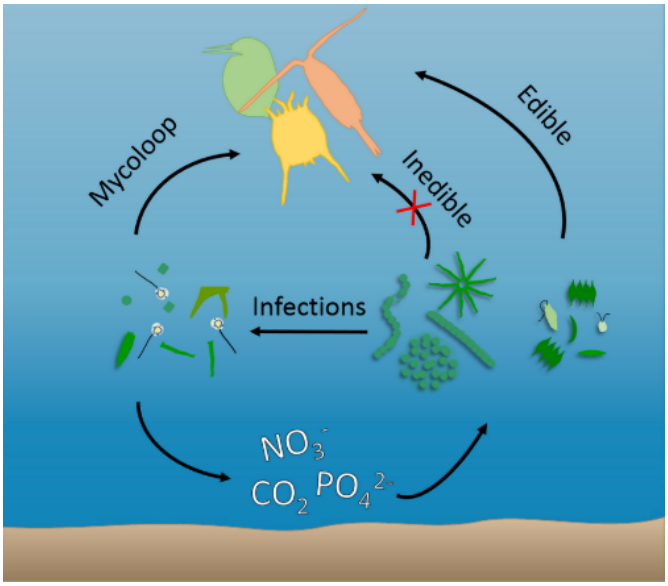

Figure 1.2: The mycoloop: when large inedible phytoplankton species are infected by chytrids, nutrients from these cells are consumed by parasitic fungi, which in turn are grazed by zooplankton in the form of zoospores.

Disease spread will depend on the thermal tolerance of both the host and the pathogen (Altizer et al. 2013). Outbreaks by West Nile virus (WNV) infections in humans, for instance, have been limited to southern and central European countries. However, competent mosquito vectors and susceptible bird hosts are already present in northern Europe. Recent work has shown that, for now, the low average summer temperature of 18 °C in northern Europe is still a limiting factor for WNV transmission (Vogels et al. 2017a; Vogels et al. 2017b). The question remains when this threshold will be exceeded.

Generally, it is expected that disease agents are sensitive to warming (Harvell et al. 2002; Marcogliese 2008). Some diseases are for instance not very well able to survive winter periods (Burdon and Elmqvist 1996; Ibelings et al. 2011). Thus, conditions that enhance pathogen winter survival or extend host breeding periods should increase the abundance of many parasites (Harvell et al. 2002). The impacts of global warming on diseases, however, is not unambiguous., Although diseases have increased in some cases, there is no clear evidence of a widespread climate change-driven disease expansion (Altizer et al. 2013; Karvonen et al. 2010; Lafferty 2009; Lafferty and Mordecai 2016).

Climate change affects freshwater ecosystems in numerous ways, including increasing temperatures and pCO_2, as well as changes in precipitation, wind and nutrient loading (Mooij et al. 2005; Woodward et al. 2010). In this thesis I will focus on the effects of rising temperatures and changes in nutrient availability. In freshwater ecosystems, increasing temperatures affect phyto- and zooplankton peak biomass, size and timing (Sommer and Lengfellner 2008; Sommer and Lewandowska 2011; Velthuis et al. 2017). Temperature not only affects hosts, it also affects the growth of their parasites. Earlier work on chytrids has indicated the existence of a cold water refuge (<1-3 °C) for phytoplankton hosts (Gsell et al. 2013b; Ibelings et al. 2011). At low temperatures, development time of chytrid infections is increased, or they form resting stages (Bruning 1991b; Gsell et al. 2013b; Van Donk and Ringelberg 1983). Thus, during cold winters the activity of chytrids is inhibited, which provides phytoplankton with an opportunity to bloom. As a result of global warming, the frequency of

these ‘disease free’ cold winters may disappear. During warmer winters chytrid infections are facilitated, preventing the host to form a bloom (Gsell et al. 2013b; Ibelings et al. 2011).

Besides these more direct effects of rising temperatures, climate change also affects the amount of available nutrients in the natural environment. Although aquatic systems generally suffer from an increased nutrient input (Smith et al. 1999), nitrogen (N) and phosphorus (P) limitation are still widespread (Bracken et al. 2014; Burson et al. 2016; Elser et al. 2007). Warming may for instance reduce nutrient input from deep water to the surface by enhanced stratification (Behrenfeld et al. 2006; Polovina et al. 2008). These changes in the availability of nutrients alter the elemental composition of phytoplankton, and as such change the availability of nutrients to higher trophic levels as well (Hessen et al. 2013; Hessen et al. 2002; Sterner and Elser 2002). Since chytrids are completely dependent on their host for nutrition, the outcome of infection could depend on the overlap in nutrient requirements of the host and the parasite (Aalto et al. 2015).

In general, autotrophic organisms such as phytoplankton are flexible in their elemental stoichiometry, since their carbon and nutrient uptake mechanisms are separated (Sterner and Elser 2002). However, heterotrophic organisms, such as zooplankton, usually actively regulate their stoichiometry within a fixed range, also known as homeostasis (Meunier et al. 2014; Van de Waal et al. 2010b). To which extent chytrids are homeostatic is unknown (fig. 1.3). But, since chytrids are highly sensitive to phosphorus (P) limitation and have high demands for P (Bruning 1991a; Bruning and Ringelberg 1987; Kagami et al. 2007b), it can be expected that chytrids have a low C:P ratio. Consequently, chytrids may provide a nutritious food source for zooplankton (Agha et al. 2016; Kagami et al. 2007b).

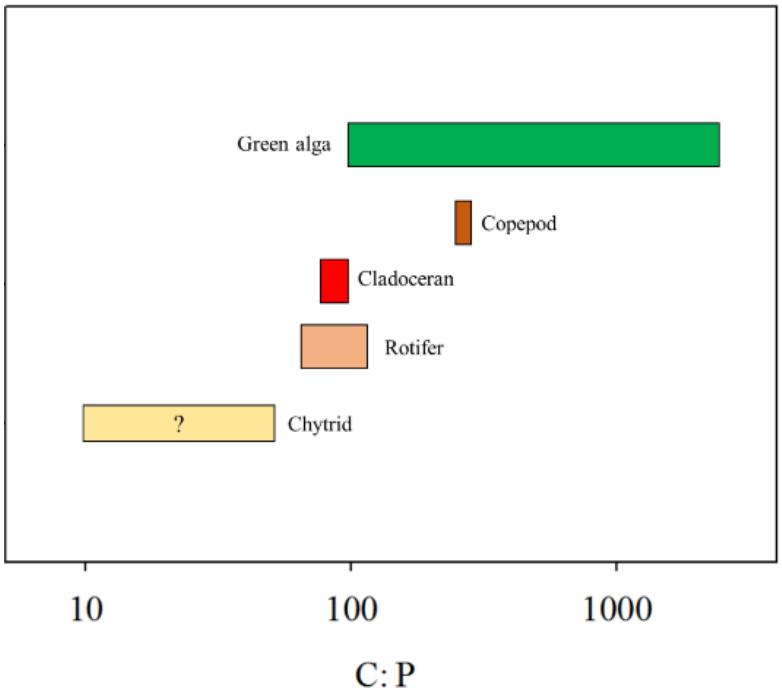

Figure 1.3: Carbon to phosphorus (C:P) stoichiometry of phytoplankton, zooplankton and chytrids. Different colours represent different species. Data extracted from Jensen et al. (2006); Kagami et al. (2007b) and Van de Waal et al. (2010b).

Aims of this thesis

The overall aim of this thesis is to investigate how climate change affects bottom-up and top-down factors regulating phytoplankton disease. Specifically, I will focus on the effects of warming, nutrient availability and grazing on chytrid infection dynamics.

The main aims of this thesis are:

1) Identify potential knowledge gaps in the existing literature on phytoplankton chytridiomycosis
2) Determine how warming directly and indirectly affects bloom dynamics of a natural phytoplankton-chytrid community
3) Assess the effect of nutrient limitation on phytoplankton-chytrid interactions and their stoichiometry
4) Test if the mycoloop can sustain zooplankton population growth.

To achieve these aims, different research approaches will be combined. These include a literature review, a large-scale indoor mesocosm experiment with a diverse, natural freshwater plankton community and single species microcosm experiments with hosts and parasites.

In **chapter two** I present a review which covers the state of the art of research on parasitic chytrids of phytoplankton ranging from taxonomy, physiology to ecology. This paper aims to identify future research directions, stimulate further experimentation, and highlights the importance of incorporating this often neglected trophic level into traditional plankton ecology.

In **chapter three** I assess the effects of warming on the prevalence of chytrid infections under the full complexity of a natural plankton community including bottom-up (host density and stoichiometry) and top-down (grazing by zooplankton) control in a large-scale mesocosm experiment. I hypothesize that warming will lead to increased infection rates by the chytrid, a lower host biomass and earlier termination of the host bloom. Since more infections will provide more zoospores as food to zooplankton, I expect that the chytrid infections will favour zooplankton biomass build-up and advance their timing.

In **chapter four** I investigate the effects of changing nutrient availability on phytoplankton-chytrid interactions. Changes in nutrients affect phytoplankton elemental stoichiometry, which might have cascading effects on the reproduction and stoichiometry of the parasite as well. I expect that chytrids are more vulnerable to nutrient limitation than their hosts, because they have a relatively high demand for nutrients. With other words, their carbon to nutrient ratio will be lower and less flexible than that of their host.

Chapter five explores the potential of zoospores as food for zooplankton. Specifically, I test the hypothesis that the mycoloop can sustain survival and reproduction of zooplankton.

Lastly, in **chapter six**, I will place the findings of the different experiments in a broader perspective and point out future research directions. In short, I will identify key drivers underlying chytrid infection dynamics, and highlight the impacts of global change on the role of chytrid infections in plankton ecology. Additionally, I evaluate if chytrids can be regulated by top-down control.

Chapter 2

Integrating chytrid fungal parasites into plankton ecology. Research gaps and needs

Published as

Frenken, T., Alacid, E., Berger, S. A., Bourne, E. C., Gerphagnon, M., Grossart, H.-P., Gsell, A. S., Ibelings, B. W., Kagami, M., Küpper, F. C., Letcher, P. M., Loyau, A., Miki, T., Nejstgaard, J. C., Rasconi, S., Reñé, A., Rohrlack, T., Rojas-Jimenez, K., Schmeller, D. S., Scholz, B., Seto, K., Sime-Ngando, T., Sukenik, A., Van de Waal, D. B., Van den Wyngaert, S., Van Donk, E., Wolinska, J., Wurzbacher, C. and Agha, R. (2017), Integrating chytrid fungal parasites into plankton ecology: research gaps and needs. Environ Microbiol. doi:10.1111/1462-2920.13827

Abstract

Chytridiomycota, often referred to as chytrids, can be virulent parasites with the potential to inflict mass mortalities on hosts, causing e.g. changes in phytoplankton size distributions and succession, and the delay or suppression of bloom events. Molecular environmental surveys have revealed an unexpectedly large diversity of chytrids across a wide range of aquatic ecosystems worldwide. As a result, scientific interest towards fungal parasites of phytoplankton has been gaining momentum in the past few years. Yet, we still know little about the ecology of chytrids, their life cycles, phylogeny, host specificity and range. Information on the contribution of chytrids to trophic interactions, as well as co-evolutionary feedbacks of fungal parasitism on host populations is also limited. This paper synthesizes ideas stressing the multifaceted biological relevance of phytoplankton chytridiomycosis, resulting from discussions among an international team of chytrid researchers. It presents our view on the most pressing research needs for promoting the integration of chytrid fungi into aquatic ecology.

Introduction

Phytoplankton constitute the base of most aquatic food webs and play a pivotal role in biogeochemical cycles, accounting for more than half of the global carbon fixation (Falkowski 2012) Phytoplankton can be infected by a number of parasites, which have the potential to regulate their abundance and dynamics and, thereby, modulate large scale ecological and/or biogeochemical processes. Parasitism constitutes an important evolutionary driver, which can promote genetic diversity in host populations and speciation (Evison et al. 2013; Hamilton 1982; Weinbauer and Rassoulzadegan 2004). Parasites are involved in most trophic links within aquatic food webs, and can contribute significantly to the transfer of carbon and energy between trophic levels (Amundsen et al. 2009). Moreover, diverse phytoplankton taxa are also increasingly used in aquaculture industry for the production of food supplements, biofuels and pharmaceuticals (Skjånes et al. 2013). Parasite epidemics can be especially devastating in such commercial scale monocultures, posing severe monetary risk for the algal industry (Carney and Lane 2014).

Common parasites of phytoplankton include viruses, fungi, protists, and pathogenic bacteria (Gachon et al. 2010; Gerphagnon et al. 2015; Park et al. 2004). Among these, viruses raised the most interest in the previous decades (Bergh et al. 1989) and their profound ecological implications were recognized soon after (Bratbak et al. 1993; Bratbak et al. 1994; Fuhrman and Suttle 1993; Fuhrman 1999; Proctor and Fuhrman 1990; Suttle et al. 1990). In a similar way, we perceive that scientific interest toward fungal parasites of phytoplankton has gained momentum in recent years. This is in large part attributable to molecular environmental surveys revealing unexpected diversity of uncultured aquatic fungal organisms, – i.e. the so-called Dark Matter Fungi (Grossart et al. 2016) – which is often dominated by members of the early diverging fungal phylum Chytridiomycota (Comeau et al. 2016; Jobard et al. 2012; Lefèvre et al. 2012; Monchy et al. 2011). Following initial work by Canter and Lund (Canter 1946; Canter and Lund 1948; Canter and Lund 1951) and some later studies (Reynolds 1973; Van Donk and Ringelberg 1983), chytrids are raising renewed interest, as further evidence accumulates for their widespread distribution across climatic regions, in both marine and freshwater ecosystems (De Vargas et al. 2015; Gutiérrez et al. 2016; Hassett et al. 2016; Hassett and Gradinger 2016; Lefèvre et al. 2007; Lepère et al. 2008; Wurzbacher et al. 2014).

Due to their inconspicuous morphological features, chytrids have been often misidentified as bacterivorous flagellates and their role as parasites or saprobes in aquatic ecosystems have thus often been neglected. However, some chytrid taxa are lethal parasites (i.e. parasitoids) and have the potential to inflict mass mortalities on their hosts, causing changes in phytoplankton size distributions, promotion of r-strategist hosts with fast turnover, delay or suppression of bloom formation, and successional changes (Gerphagnon et al. 2015; Gleason et al. 2015; Noble and Fuhrman 1998; Rasconi et al. 2012; Van Donk 1989; Van Donk and Ringelberg 1983). Parasitism by chytrids mediates inter- and intraspecific competition (Rohrlack et al. 2015) and might promote diversity and polymorphisms in host populations (Gsell et al. 2013b). Chytrids are characterized by a free-living motile stage in the form of single-flagellated zoospores that are assumed to actively search for their hosts by chemotaxis (Canter and Jaworski 1980; Muehlstein et al. 1988). Upon settlement on their host, chytrids penetrate the cell and develop rhizoids to extract nutrients from it. Encysted zoospores develop into epibiotic sporangia which, once mature, release new zoospores (Canter 1967). Zoospores have been found to constitute a highly nutritional food source for zooplankton and chytrids may hence establish alternative trophic links between primary and secondary production in pelagic ecosystems (Kagami et al. 2007b; Rasconi et al. 2011)a(Agha et al. 2016).

Despite accumulating evidence for their ecological importance, studies addressing phytoplankton-chytrid interactions are limited by the availability of model systems and empirical data. We know relatively little about the life cycles of chytrids, their phylogeny, and their host specificity and range. Information regarding their mechanisms of infection, as well as the co-evolutionary effects of chytrid parasitism on host populations is also missing. This paper aims to synthesize novel notions stressing the biological relevance of phytoplankton chytridiomycosis, keeping a focus on the immediate research needs. Our intent is not to recreate existing reviews on the topic (Gerphagnon et al. 2015; Gleason et al. 2015; Gleason et al. 2008; Gleason et al. 2011; Gleason et al. 2014; Ibelings et al. 2004; Jephcott et al. 2015; Kagami et al. 2014; Sime-Ngando 2012). Rather, we aim to (i) briefly highlight the profound and multifaceted impact of chytrid parasitism on phytoplankton dynamics, (ii) identify the current major gaps in knowledge, and (iii) propose future directions to bridge them. We intend to stimulate experimentation in different aspects of the biology of chytrids and their hosts and, thereby, contribute integrating chytrid parasitism of phytoplankton into traditional aquatic (microbial) ecology.

Life-cycle and ecological strategies

Parasitic chytrids obtain their nutrients and energy from living organisms, mainly phyto- and zooplankton, whereas saprophytic taxa generally use other organic substrates (Longcore et al. 1999). Currently, chytrids are categorized as (i) **obligate parasites**, which need a living host to reproduce and complete their life cycle, e.g. *Rhizophydium planktonicum* parasitizes the diatom *Asterionella formosa* (Canter and Jaworski 1978); (ii) **obligate saprophytes**, which can use a broad spectrum of organic materials as a substrate to reproduce and complete their life cycle, e.g. *Rhizoclosmatium globosum* grows on pollen, keratin, cellulose and chitin (Sparrow 1960); and (iii) **facultative parasites**, which are able to infect and reproduce on living hosts, but are also able to exploit senescent hosts or other dead organic material, e.g. *Dinochytrium kinnereticum* is parasitic on weakened cells of the dinoflagellate *Peridinium gatunense*, but also grows saprophytic on pollen (Table 2.1) (Leshem et al. 2016).

However, it is not clear whether the degree of parasitism or saprophytism is bound to individual taxa, or if chytrids display a continuum of consumer strategies, ranging from obligate parasitic to obligate saprophytic life styles, depending on environmental conditions (Fig. 2.1). Some chytrids that exploit phytoplankton, can also be found on organic substrates (Alster and Zohary 2007; Leshem et al. 2016). It is unclear whether these facultative parasites can only infect physiologically senescent hosts as an "extension" of saprophytism, or whether they are also adapted to parasitism. Parasitism likely grants access to higher quality resources compared to most other (dead) organic substrates, but the costs associated with parasitism are usually high, given the necessity of evading host immune response (Frank 1996; Schmid-Hempel 2008). On the other hand, saprophytism in facultative parasites can serve as a survival strategy in the absence of a host. Exploring the continuum between parasitic and saprophytic lifestyles of chytrids and their trade-offs is still needed for a functional and ecological characterization of chytrid diversity.

The ecological role of chytrid hyper-parasites, taxa that infect other parasitic chytrids (e.g. *Chytridium parasiticum* or *Septosperma spp.*), represent a unique case (Gleason et al. 2014), which remains largely unknown (see section 8). To estimate the proportion of parasitic species relative to total chytrid diversity and to determine general patterns that can explain their lifecycles and host range remains challenging. However, this would allow us to better understand their functional diversity and establish hypotheses about the divergence of chytrid lineages and the evolution of parasitism.

Table 2.1: List of isolated parasitic chytrid taxa, including their lifecycle strategy and host taxa. (LCS = lifecycle strategy, O=Obligate parasite, F=Facultative parasite)

Species	LCS	Host(s)	Reference
Chytridium olla	O	*Oedogonium* spp.	Sparrow (1960), Vélez et al. (2011)
Dinochytrium kinnereticum	F	*Peridinium gatunense*	Leshem et al. (2016)
Phlyctochytrium planicorne	F	*Asterococcus* sp., *Cladophora* sp., *Cosmarium contractum* var. *ellipsoideum*, *Oedogonium* sp., *Peridinium cinctum*, *Rhizoclonium hieroglyphicum*, *Sphaerocystis schroeteri*, *Spirogyra* spp., *Staurastrum* spp., *Staurodesmus curvatus*, *Vaucheria* sp.	Canter (1961), Letcher and Powell (2005), Sparrow (1960)
Rhizophydium planktonicum	O	*Asterionella formosa*	Canter (1969), Seto et al. (2016)
Gromochytrium mamkaevae	O	*Tribonema gayanum*	Karpov et al. (2014)
Chytridium polysiphoniae	O	Only macroalgal hosts described: *Acinetospora crinita*, *Ectocarpus* spp., *Feldmannia* spp., *Hincksia* spp., *Pilayella littoralis*, *Spongonema tomentosum*, *Myriotrichia clavaeformis*, *Haplospora globosa*, *Eudesme virescens*, *Carpomitra costata*, *Endarachne binghamiae*, *Scytosiphon lomentaria*,	Kupper et al. (2006), Müller et al. (1999)
Mesochytrium penetrans	O	*Chlorococcum minutum*	Karpov et al. (2010)
Aquamyces chlorogonii	F	*Chlorogonium* spp., *Oedogonium cardiacum, Spirogyra* spp., *Tribonema bombycinum, Ulothrix subtilissima*, *Vaucheria* sp., *Zygnema* sp.	Barr (1973), Letcher et al. (2008), Sparrow (1960)
Dinomyces arenysensis	O	*Alexandrium* spp., *Ostreopsis* spp.	Lepelletier et al. (2014)
Gorgonomyces haynaldii	F	*Chlorogonium elongatum, Oedogonium* spp., *Spirogyra* spp., *Tribonema bombycinum, Ulothrix* spp., *Vaucheria* sp., *Zygnema* sp.	Barr (1973), Letcher et al. (2008), Sparrow (1960)
Protrudomyces laterale	F	*Ulothrix* spp., *Stigeoclonium* sp.	Barr (1973), Letcher et al. (2008), Sparrow (1960)
Rhizophydium globosum	F	*Cladophora lomerate, Closterium* spp., *Navicula* sp., *Penium digitus*, *Pinnularia viridis*, *Pleurotaenium trabecula*, *Spirogyra* sp., *Staurastrum* sp., *Ulothrix* sp.	Letcher et al. (2006), Sparrow (1960)
Rhizophydium megarrhizum	O	*Lyngbya* sp., *Oscillatoria* spp., *Planktothrix* sp.	Sønstebø and Rohrlack (2011), Sparrow (1960)
Staurastromyces oculus	O	*Staurastrum* sp.	Van den Wyngaert et al. (2017)
Zygorhizidium planktocnium	O	*Asterionella formosa*, *Synedra* spp.	Canter (1967), Doggett and Porter (1995), Seto et al. (2016)
Zygorhizidium melosirae	O	*Aulacoseira* spp.	Canter (1967), Seto et al. (2016)

Chytrids combine asexual and sexual modes of reproduction (Doggett and Porter 1996b), but so far sexual reproduction has only infrequently been documented (Canter and Lund 1948; Seto et al. 2016; Van Donk and Ringelberg 1983). Zoospores are produced asexually and can survive for only short periods of time (hours to a few days (Fuller and Jaworski 1987)) in absence of a suitable host. Therefore, some chytrid species probably rely on resting/resistant stages to survive periods of host absence (Doggett and Porter 1996a). Studies on the abundance of resting spores in sediments and the water column, as well as the stimuli and/or mechanisms triggering resting spore formation and germination are still needed. This, together with accurate estimates of the lifetime of zoospores in the absence of hosts will help increase our understanding of the life cycles of chytrids and their survival strategies during periods of host absence in the water column.

Taxonomy and molecular phylogeny

Much effort has been devoted to unravelling the molecular phylogeny of chytrids and other zoosporic fungi. However, phylogenies in the early branches of the fungal tree still remain an open question. Traditionally, the taxonomic assignment of these organisms was based on morphology and host affiliation. Yet, identification by morphology alone has proven a difficult task, given their small and inconspicuous thalli and considerable morphological variation under changing environmental conditions and substrates (Paterson 1963).

The application of transmission electron microscope (TEM) techniques, allows for analysis and characterization of zoospore ultrastructural features and has proven to be a powerful tool for identification purposes (Beakes et al. 1988; Beakes et al. 1993; Letcher et al. 2012), especially when integrated with molecular data (James et al. 2006). However, some studies have indicated that cryptic species might exist at both the genetic and ultrastructural levels (Letcher et al. 2008; Simmons 2011).

Ultrastructural analyses require concentrated suspensions of zoospores, but given the limited number of chytrids strains available in culture (especially obligate parasitic chytrids), relatively few taxa are currently available for study. However, the use of molecular tools for identification of

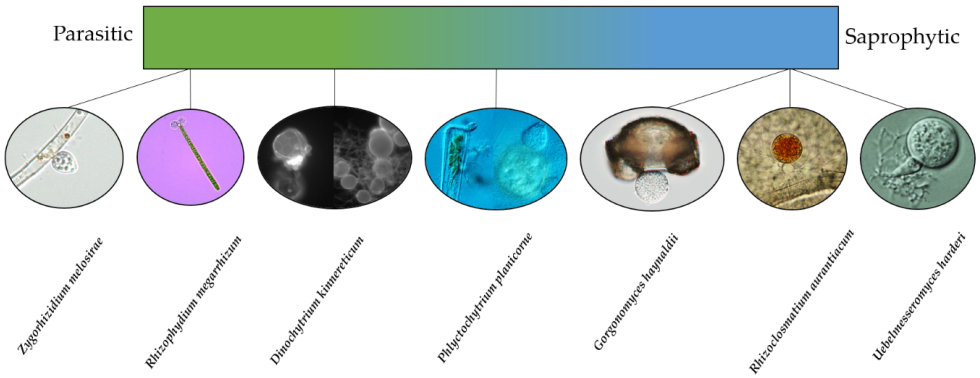

Figure 2.1: Examples of chytrid taxa with different consumer strategies ranging from parasitic to saprophytic. From left to right: Z. melosirae parasitising Aulacoseira granulata (Kensuke Seto), R. megarrhizum parasitising Planktothrix rubescens (Thijs Frenken), D. kinnereticum parasitising Peridinium gatunense (left) and growing on pollen (right) (Tamar Leshem), P. planicorne parasitising an unindentified diatom (left) and growing on pollen (right) (Martha J. Powell), G. haynaldii growing on pollen (Kensuke Seto), R. aurantiacum growing on chitin (Martha J. Powell), U. harderi growing on agar (Martha J. Powell and Peter Letcher).

sequences originating from environmental DNA by reference to sequence databases (Hibbett et al. 2016) can overcome many limitations of traditional microscopic approaches, not only to discover, classify, and name fungal species according to their phylogenetic relationships and taxonomy, but also to perform ecological studies. In this context, two key considerations must be taken into account: (i) there does not appear to be a universal genetic marker able to discriminate among distant taxa, and simultaneously provide adequate resolution to identify organisms at the species level, and (ii) current representation of Chytridiomycota, and especially parasitic chytrids, in sequence databanks is limited.

The nuclear rRNA gene region, consisting of three genic markers evolving at different rates, has been instrumental for fungal identification by molecular barcoding. First, the small ribosomal subunit (SSU), which can be aligned across the breadth of the phylum level due to its conservative nature, allows the placement and identification of a broad and divergent range of taxa. Such analyses can result in phylogenies with strongly supported lineages, but may suffer a poorly supported backbone due to many polytomies with little or no indication of relative relationships among clades (Letcher et al. 2008; Longcore and Simmons 2012; Wakefield et al. 2010). Hence, the SSU can provide an adequate molecular framework at the phylum level for Chytridiomycota, but a higher resolution can only be achieved using other markers.

Second, and to the goal of achieving higher resolution, the large ribosomal unit (LSU) has proven a promising genetic marker for chytrids delineation, as it exhibits more variability than the SSU. Thus, it has been used to delineate new orders such as Rhizophydiales, Rhizophlyctidales, Cladochytriales, Lobulomycetales, and Polychytriales, and to confirm existing orders (Spizellomycetales, Chytridiales), and for delineation at family, genus and species level (Davis et al. 2015; Leshem et al. 2016; Letcher et al. 2015b; Powell et al. 2015).

Third, of the rRNA markers, the intergenic transcribed spacer (ITS) has been proposed as the most suitable molecular marker for fungal barcoding (Schoch et al. 2012). Yet, for the early diverging Chytridiomycota, the unconstrained and rapidly evolving ITS1 and ITS2 portions of the ITS region are difficult to align, and may suffer saturation (i.e. reduced signal of sequence divergence rate), thereby ruling out its use as the only marker for phylogenetic studies. However, the ITS region has been successfully used in conjunction with LSU to delineate closely related taxa, which was not possible using the LSU alone (Letcher et al. 2006; Letcher et al. 2015a; Vélez et al. 2013). Consequently, resolution in phylogenetic studies of Chytridiomycota would benefit from combining more than one molecular marker.

Recent developments in sequencing technologies pave the way for promising new alternatives such as the use of the complete ribosomal operon. This long read can be readily covered by novel sequencing methods like Pacific Biosciences (www.pacb.com) and Oxford Nanopore (https://nanoporetech.com). Additionally, phylogenetic analyses could be complemented by the use of other novel fungal markers such as the elongation factor TEF1α and the single-copy protein-coding gene RPB2 (Stielow et al. 2015; Větrovský et al. 2016). Another promising approach could involve the development of a large number of new candidate loci from sequencing different chytrid genomes from divergent lineages (e.g. through single cell genomics) and the development of specific primers for these regions (Gawad et al. 2016; Rutschmann et al. 2016).

The second major constraint for the taxonomy of this group is a general lack of representatives, especially parasitic species (or those described as such), in sequence databases. A survey of the most important databases for fungal taxonomic assignment reveals that Chytridiomycota represent between 0.1-4 % of the fungal sequences, where the number of those that are parasitic species is difficult to estimate, but not larger than a few dozen (Table 2.2). It is therefore not surprising that some

species of parasitic chytrids were recently found to be related to sequences of novel lineages only characterized by environmental sequences (Karpov et al. 2014; Seto et al. 2016). The use of culture-independent molecular methods, e.g. single cell/colony/spore PCR (Ishida et al. 2015), as well as sequencing of bulk phytoplankton samples, will likely improve chytrid representation in future sequence databases.

Table 2.2: Current number of sequences of Fungi and Chytridiomycota across various databases and according to different molecular markers (April 2017).

Database	Marker	Fungi	Chytridiomycota	Percentage
GenBank	SSU	546,728	1,243	0.23
GenBank	ITS	983,576	978	0.10
GenBank	LSU	507,270	1,097	0.22
Silva Ref128	SSU	23,721	862	3.63
Silva Ref128	LSU	2,925	124	4.24
UNITE* v7.1	ITS	21,607	124	0.57
RDP**	LSU	8,993	249	2.77

**Representative sequences for 97% similarity clustering;*

*** Training set 11*

Mechanisms of infection

The process of chytrid infection has been primarily documented by microscopic observations. However, the underlying mechanisms still remain largely unknown. In general, infection consists of four main phases comprising (i) attraction of zoospores to a host; (ii) interactions on the host´s surface leading to chytrid encystment (i.e. attachment); (iii) germination and formation of infection structures by the parasite and penetration of host cell wall, and (iv) maturation of infection, during which new zoospores are formed and finally released.

Observations that some chytrids are unable to complete their infection cycle in darkness, or at very low light intensities, indicate that chemical cues driving attraction of zoospores to their host, and host recognition might be closely related to photosynthetic exudates (Barr and Hickman 1967; Bruning 1991c; Canter and Jaworski 1981). This idea is further supported by a lowered ability of a chytrids taxon to infect its diatom host in the presence of photosynthesis-inhibiting compounds, such as herbicides (Van den Wyngaert et al. 2014). A range of phytoplankton exudates, including photosynthesis by-products, have been reported as attractants for different zoosporic parasites. These compounds include amino acids, saccharides and other carbohydrates (Donaldson and Deacon 1993; Halsall 1976; Mitchell and Deacon 1986; Moss et al. 2008; Muehlstein et al. 1988; Orpin and Bountiff 1978). Whole-cell extracts attracted more zoospores as compared to single compounds alone (Scholz et al. 2017), suggesting that multiple attractants drive chemotaxis. Altogether, this suggests that taxis in zoosporic parasites might not be specific in terms of host selection and is consistent with observations that zoospore attachment to hosts can be reversible in some taxa (Doggett and Porter 1995).

Upon encounter, zoospores encyst on suitable hosts. Parasite-host recognition traits are likely mediated by chemical interactions at the host´s surface and arguably constitute one of the determining factors controlling host-parasite compatibility. Knowledge of other zoosporic parasites (e.g. oomycetes) suggests lectin-carbohydrate interactions as likely chemical mechanisms driving zoospore encystment (Hinch and Clarke 1980; Jacobson and Doyle 1996; Levitz 2010; Petre and Kamoun 2014), as well as interactions with antibodies or exopolysaccharides in the host mucilage. Particularly in the chytrids *Entophlyctis apiculata* and *Zygorhizidium planktonicum*, adhesive materials between fungal and host cells were observed by TEM (Beakes et al. 1992; Shin et al. 2001). Analysing host and parasite

surface characteristics using laboratory chytrid-phytoplankton systems is needed to elucidate the triggers of zoospore encystment. In particular, comparative studies of conspecific susceptible and resistant host isolates can potentially help to pinpoint cellular surface traits that determine host-parasite compatibility.

Upon zoospore encystment on the host cell, a germ tube is formed which, in most cases, penetrates the host cell immediately after germination. Rhizoids are then produced, which expand through the host cell, enabling transfer of material into the host cell (Gromov et al. 1999; Karpov et al. 2014; Lepelletier et al. 2014; Shin et al. 2001; Van Rooij et al. 2012). However, host penetration mechanisms likely differ between host species. For instance, diatom infecting chytrids use a germ tube that enters the host cell through the girdle region of the frustule (Beakes et al. 1992; Van Donk and Ringelberg 1983), whereas in other algal hosts, the germ tube penetrates the cell through the mucilage surrounding the host (Canter 1950; Canter and Lund 1951), or directly through the cell wall in absence of such mucilage (Gromov et al. 1999; Karpov et al. 2014; Lepelletier et al. 2014; Shin et al. 2001). Despite these observations, the underlying molecular mechanisms of the penetration process are largely unknown. It has been shown that some fungal plant pathogens degrade enzymatic polysaccharides of the host cell wall (Jones et al. 1972) and penetrate the cell by using the internal turgor pressure of the plant (Howard and Ferrari 1989). More studies should be performed to observe successive stages of the infection process (encystment to penetration) including the study of structures by Scanning Electron Microscopy (SEM) and TEM.

Regarding the colonization of host cells, it has been shown that zoosporic plant pathogens, such as oomycetes, deliver effector proteins inside the cells to facilitate host colonization (Petre and Kamoun 2014). One class of secreted pathogen effectors comprises the modular CRN (Crinkling and Necrosis) family of proteins that alter the host cell physiology by targeting and cleaving DNA. CRN proteins contain a conserved N84 terminal domain specifying translocation into host cells and diverse C-terminal regions harbouring effector functions (Stam et al. 2013). Notably, CRN proteins have been identified in the genome of the amphibian chytrid *Batrachochytrium dendrobatidis* (Joneson et al. 2011). The presence of CRNs genes in phylogenetically distant eukaryotic pathogens suggests that eukaryotic effectors might display a conserved mode of action and might also be present in phytoplankton-infecting chytrids. The use of transcriptomic, proteomic and metabolomic approaches will contribute to a mechanistic understanding of all infection phases, which is crucial for unravelling the bases of host-parasite specificity.

Host specificity and range

Host specificity, defined as the extent to which parasites can exploit different host species, is a fundamental trait of parasites both from an ecological and evolutionary perspective (Poulin et al. 2011). Most field studies have concluded, solely based on morphological identification of phytoplankton-chytrid pairs, that these interactions are highly species-specific (Holfeld 1998; Rasconi et al. 2012) and that some chytrids are even specialized on specific cell types or even proteins (Gerphagnon et al. 2013a; Marantelli et al. 2004; Vélez et al. 2011). Molecular analyses based on single spore/cell PCR revealed the presence of specialists, but also of generalists capable of infecting multiple host species (Ishida et al. 2015). Cross-infection assays under laboratory conditions often expose an even more complex picture, with some chytrids infecting specific host strains only (Canter and Jaworski 1979; De Bruin et al. 2004) and others capable of infecting different species, although within single host species both susceptible and resistant strains occur (Gromov et al. 1999; Gutman et al. 2009; Lepelletier et al. 2014).

Our current knowledge of host range and chytrid specificity is greatly biased by the fact that morphological identification often does not provide enough resolution to identify chytrids (and sometimes also phytoplankton) at the species level (Letcher et al. 2008; Van den Wyngaert et al. 2015). This potentially masks several hidden host-chytrid interactions and their dynamics. As seen in many other host-parasite systems, it is likely that within a single chytrid species both specialist and generalist strains coexist (Koehler et al. 2012). Extrapolations of results from cross-infection assays between single chytrid and host strains to the population level have, therefore, to be taken with caution. Moreover, whereas most infection assays have been conducted under constant environmental conditions (De Bruin et al. 2008; Gutman et al. 2009; Lepelletier et al. 2014), temperature can alter host-genotype specific susceptibility to chytrid infection (Gsell et al. 2013b), implying that heterogeneous environments might provide different outcomes in specificity tests (Wolinska and King 2009).

Similarly to the continuum between saprophytic and parasitic consumer strategies (see section 2), the occurrence of generalist and specialist parasitic chytrids raises questions about the conditions promoting different strategies. Commonly assumed costs associated with generalists have not been investigated yet in parasitic chytrids. Elucidating the mechanisms underlying host specificity and their associated costs will allow formulation of more targeted hypotheses about the conditions that promote specialist or generalist strategies. For example, if host specificity does not operate at the attraction stage, specialists are expected to suffer more from a "dilution effect" (i.e. reduced host densities) under conditions of high host diversity, since generalists may have higher probability to encounter suitable hosts (Alacid et al. 2016; Keesing et al. 2010).

Whereas field studies capture the "contextual" host range and specificity of chytrids in their natural settings, experimental cross-infection assays can capture the potential host range. By examining the different steps of the infection process across a range of potential host species and environmental conditions, we can test which infection steps drive specificity and contribute to shaping host ranges, as well as to what extent genetics and environment determine and modulate host and parasite compatibility (Ebert et al. 2016). Such assays are important for making predictions on the spread and persistence of chytrids in novel environments - as driven by climate change - but also in mass cultivation systems.

Host-parasite co-evolution and host diversity

Maintenance of genetic diversity in populations has been linked to strong reciprocal selection between hosts and their parasites, resulting in co-evolution. Host-parasite co-evolution can occur through successive fixation of beneficial mutations (selective sweeps) or through sustained genotype frequency oscillations as parasites adapt to the most common genotypes, conferring a selective advantage to rare genotypes (Red Queen dynamics) (Woolhouse et al. 2002). While selective sweeps lead to fast evolution in genes but low levels of genotype standing variation, Red Queen dynamics lead to long-term maintenance of genotype diversity. To show potential for co-evolution, we need evidence for (i) strong reciprocal selective pressure, (ii) genotype-specific infectivity and resistance, and (iii) a genetic basis for differences in infectivity and resistance. Conclusive proof of co-evolution in chytrid-phytoplankton systems is lacking, but some of the above points are supported. Phytoplankton-infecting chytrids are often obligate parasites (but see section 2), and phytoplankton hosts cannot recover from infection, resulting in strong reciprocal selection pressure. Host geno- and/or chemotypes (i.e. differentiated by cellular oligopeptide fingerprints) can differ in resistance (Gsell et al. 2013a; Sønstebø and Rohrlack 2011). Experimental evolution of chytrids shows fast adaptation in genetically homogeneous host cultures, but not in heterogeneous ones, indicating that host genetic

diversity restricts parasite evolution (De Bruin et al. 2008). Genotype-specific differences in parasite infectivity, however, remain understudied.

To understand how co-evolution shapes host-parasite dynamics and diversity, we need insight into the extent of specificity in phytoplankton-chytrid relationships and the genetics underlying infectivity and resistance. Moreover, alternative mechanisms affecting co-evolutionary trajectories need to be evaluated, e.g. fluctuating selection in variable environments (Wolinska and King 2009) or selection for facultative parasites in non-host refuges. Effects of spatiotemporal variation in competition between parasites (multiple infections) raise questions on the importance of priority effects, i.e. effects caused by the first-infecting parasite. The occurrence of chytrids infecting subsets of genotypes across several host species (Kagami, personal comm.) allows exploration of co-evolutionary trajectories leading to subdivision of host species and possibly sympatric speciation. Before we can gauge the potential for co-evolution in the field, we need to map the extent of refuges for parasites and hosts (see section 8) reducing reciprocal selection and therefore slowing co-evolution.

To disentangle the mechanisms of co-evolution in phytoplankton-chytrid model systems, we need experiments that test evolutionary responses based on mutations (selective sweep scenario) and/or standing genetic variation (Red Queen scenario). Further efforts are needed to assess spatial and temporal co-evolutionary trajectories through local adaptation experiments (Greischar and Koskella 2007) or experiments on asymmetric evolution i.e. one of the antagonists is not allowed to evolve (Schulte et al. 2010). As the genetic basis for differences in infectivity and resistance remain unresolved, proteomics of infected and uninfected cultures may help to identify proteins involved in the response to infection and elucidate the nature of host defence and resistance. Modelling host-parasite interactions can help to constrain expectations for different co-evolution scenarios when exploring the effect of specialist/generalist or obligate/facultative parasitism (or graduations thereof) on the maintenance of host genetic diversity, and, conversely, the effect of host genetic diversity on disease spread (King and Lively 2012).

Host defence and parasite counter-defence

Host defences can be classified in three main groups: barrier defences, immune defences and behavioural defences. Barrier defences guard against the entry of the parasite into the cell prior to contact with the immune defences (Parker et al. 2011). The genetic and biochemical mechanisms of zoospore encystment remain essentially unknown (but see section 4). Within a single host species, zoospores encyst on certain strains only (Sønstebø and Rohrlack 2011), indicating that host surface traits may grant resistance in some cases. In turn, parasites often evade barrier defences by molecular mimicry of host receptors and, therefore, host and parasite active binding sites often show convergent evolution (Sikora et al. 2005). However, whether this is the case for chytrids remains currently unknown.

Once barrier defences are overcome, chytrids encounter host immune defences. Although defence mechanisms likely differ between host organisms, some strategies have been identified. A first type of defence is hypersensitivity, a particular type of apoptosis, which requires the host cell to detect infection in an early stage. Laboratory work showed a hypersensitive response of *Asterionella formosa,* which kills the chytrid parasite before it can complete its life cycle (Canter and Jaworski 1979). Since hypersensitivity kills the infected host cells to protect the unaffected ones, this type of defence suggests that host cells within a population (or at least, within the susceptible subset of the population) collaborate to fight off parasites (Franklin et al. 2006). A second type of immune defence is related to the production of defensive chemical compounds. Planktonic cyanobacteria produce a wide range of

bioactive oligopeptides (Agha and Quesada 2014), that display numerous enzyme inhibitory properties and could contribute to anti-parasite defences (Rohrlack et al. 2013). Also, Pohnert (2000) found that phytoplankton cells release potent fungicides from their cells when mechanically wounded. Whether these repel chytrid parasites has yet to be tested, but if they do, they may also protect host cells that are in tight proximity to the cell that is under attack. The third type is behavioural defence. For instance, by utilizing a buoyancy regulation system, the cyanobacterium *Planktothrix* migrates and accumulates in the metalimnion of clear-water lakes, where low temperatures and light render an environmental refuge against chytrid infection (Kyle et al. 2015). This is analogous to the 'Cheshire cat' escape strategy of the coccolitophore *Emiliania huxleyi* in response to viral infection, whereby the usual diploid host phenotype transforms temporarily into a haploid phenotype, which is invisible to the virus (Frada et al. 2008).

The major problem to directly characterize these defence strategies is that chytrids infecting phytoplankton remain black boxes, both biochemically and genetically. Gathering information on the molecular basis of chytrid infection is hence urgently needed to systematically search for host defence and chytrid counter defence mechanisms and characterize them.

Environmental refuges

Environmental refuges in host-parasite interactions are little understood but thought to be important in shaping co-evolution (Wolinska and King 2009). Chytrid escape from low host density conditions is possible through a "host-free" stage in their lifecycle (Leung et al. 2012), for example by switching to saprophytic interactions (Gleason et al. 2008) or the formation of resting stages (Doggett and Porter 1996a; Ibelings et al. 2004). Hosts, in turn, may escape the worst of an epidemic by `taking shelter` where conditions are not favourable for infection. Besides the active migration of the cyanobacterium *Planktothrix* to colder metalimnetic depths to escape infection, Bruning (1991c) demonstrated that a diatom-infecting chytrid displays greatly reduced capacity for epidemic development under conditions of low temperature and irradiance. The existence of a cold water host refuge (< 1-2^{o}C) was confirmed by Gsell et al. (2013b). This study also found evidence for a warm water refuge above 20°C, where *Zygorhizidium* sporangia no longer fully matured. Warmer winters are expected to cause a gradual disappearance of a cold `window of opportunity` for an early, parasite-free development of *Asterionella.* Early chytrid infections, although at low prevalence, may prevent the host from blooming, and thereby, hamper the opportunity of the parasite to reach epidemic levels of infection (Ibelings et al. 2004). So, perhaps paradoxically, the loss of cold water refuges may ultimately be detrimental to this particular parasite.

Despite these observations, many open questions exist: Can the above observations be generalized to other phytoplankton taxa? What is the relative role of refuges for stabilizing the interaction between host and parasite? If the host fully relies on refuges for seasonal development, how will global drivers of lake-ecosystem change affect the persistence and seasonal succession of phytoplankton? Beside their obvious importance for disease prevalence, infection refuges are arguably important modulators of parasitic pressure on host populations, where co-evolutionary processes decelerate or even cease (Kyle et al. 2015; Rohrlack et al. 2015). How does this affect eco-evolutionary feedbacks between host and parasite? On top of this, we still have inadequate understanding of the nature of chytrid specificity, infectivity, and host defence and their modulation by environmental factors - as formalized by the disease triangle concept (Stevens 1960).

Many of these questions can be approached using host-parasite isolates to undertake laboratory experiments under controlled conditions. For example, in order to study the basis of reduced/absence of infections under cold or low light conditions occurring e.g. in deep stratified lakes,

conditions of temperature and light refugia can be reproduced in the laboratory. Since the rate of photosynthesis by hosts is both light and temperature dependent, cold and low light conditions might result in reduced excretion of dissolved organic carbon (DOC) by the host which might in turn limit the chemotactic ability of chytrid zoospores to locate and infect their hosts. To explore this idea, experiments could be performed, where host taxa putatively exploiting these refuges (e.g. *Planktothrix rubescens*) are grown under a range of environmental conditions representing different depths of deep lakes, where irradiance and temperature decrease, and nutrient availability increases with depth. By mimicking their environmental conditions in the laboratory and using different established host-parasite isolates, the role of environmental refugia on phytoplankton-chytrid interactions can be further elucidated.

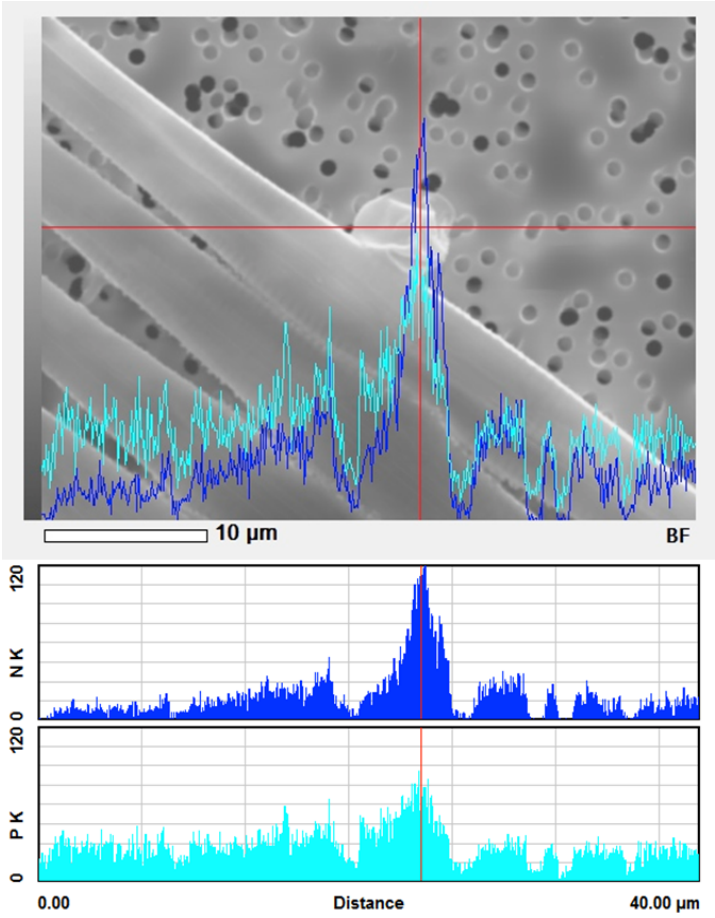

Figure2.2: SEM image of the diatom Fragilaria crotonensis infected by the chytrid strain FRA-CHY1, isolated from Lake Stechlin (Germany) in March 2015. Elemental energy intensities of N (dark blue) and P (light blue) of the diatom and the chytrid sporangium along a transect (horizontal red line) are shown. The graph within and below the SEM image indicates higher N and P contents in the chytrid sporangium compared to its diatom host. Strains were isolated by Silke Van den Wyngaert, IGB, Stechlin. SEM image and energy dispersive x-ray (EDX) analyses, using a SEM microscope (JEOL 6000) equipped with an EDX-system were performed by Reingard Rossberg and Stella A. Berger, IGB Stechlin.

Ecological stoichiometry of chytrid infections

Planktonic organisms experience dynamic changes in resource availability at different temporal and spatial scales, not only as a result of seasonality or changes in mixing regimes, but also due to climate change and anthropogenic impacts (Behrenfeld et al. 2006; Berger et al. 2014). Shifts in the availability of nutrients affect phytoplankton growth and its elemental composition, which may in turn propagate to higher trophic levels (Berger et al. 2006; De Senerpont Domis et al. 2014; Sterner and Elser 2002; Van de Waal et al. 2010b). Specifically, heterotrophs tend to have higher nutrient demands as compared to phytoplankton, reflected by lower C:P and C:N ratios (Hessen et al. 2013; Vrede et al. 1999). Such stoichiometric mismatches may become a bottleneck for the transfer of carbon and nutrients to higher trophic levels (Elser et al. 2010; Urabe et al. 2003).

In analogy to zooplankton grazing, chytrid infections may be stoichiometrically constrained, where the outcome of infection depends on the overlap in stoichiometric requirements of the parasite with its host (Aalto et al. 2015). Although chytrids have been shown to be an important nutritional component of the zooplankton diet (Grami et al. 2011; Kagami et al. 2007a; Kagami et al. 2014; Kagami et al. 2004; Rasconi et al. 2014), surprisingly little is known about the elemental composition of chytrids and their zoospores and, therefore, the stoichiometry of chytrid infections. Initial elemental analyses by single cell SEM-based techniques indicate that chytrid sporangia contain more nutrients (P and N) than its algal host (Fig. 2.2). Furthermore, analyses on zoospore suspensions indicated relatively low C:N and C:P ratios (Table 2.4; Frenken et. al 2017). Low carbon to nutrient ratios may be attributed to relatively high amounts of nucleic acids and lipids, including fatty acids and sterols (Barr and Hadland-Hartmann 1978; Beakes et al. 1988; Elser et al. 1996; Kagami et al. 2007b) and indicate that chytrids have high phosphorus (Kagami et al. 2007b), and nitrogen requirements (Frenken et al. 2017b).

Net effects of nutrient limitation on chytrid epidemics will depend on changes in host growth rates relative to its chytrid parasite (Bruning 1991b; Bruning and Ringelberg 1987; Van Donk 1989) which may result, according to model simulations, in different alternative stable states: one with only the host and one allowing host and parasite coexistence (Gerla et al. 2013). A further understanding of the ecological stoichiometry of chytrid infections and its role in aquatic food webs requires additional analyses of chytrid elemental composition and their interaction with host stoichiometry.

Table2.3: Maximal and average elemental energy intensities of N and P signals recorded on the chytrid sporangium (intersection of red lines in Fig. 2) and its diatom host.

Peak transect	N intensity	P intensity
Chytrid peak	111	85
Chytrid average (SE), n= 67	55 (0.6)	52 (1.7)
Diatom average (SE), n= 191	16 (0.6)	31 (0.6)

Table 2.4. Molar C:N and C:P ratios of zoospores of the chytrid Rhizophydium megarrhizum and its cyanobacterial host (Planktothrix rubescens NIVA-CYA97/1) grown under nutrient replete conditions (Frenken et al. 2017b)

	C:N ratio (molar)	C:P ratio (molar)
Chytrid average (SE), n= 4	4.75 (0.02)	59.9 (0.9)
Cyanobacteria average (SE), n= 4	4.39 (0.01)	48.3 (1.6)

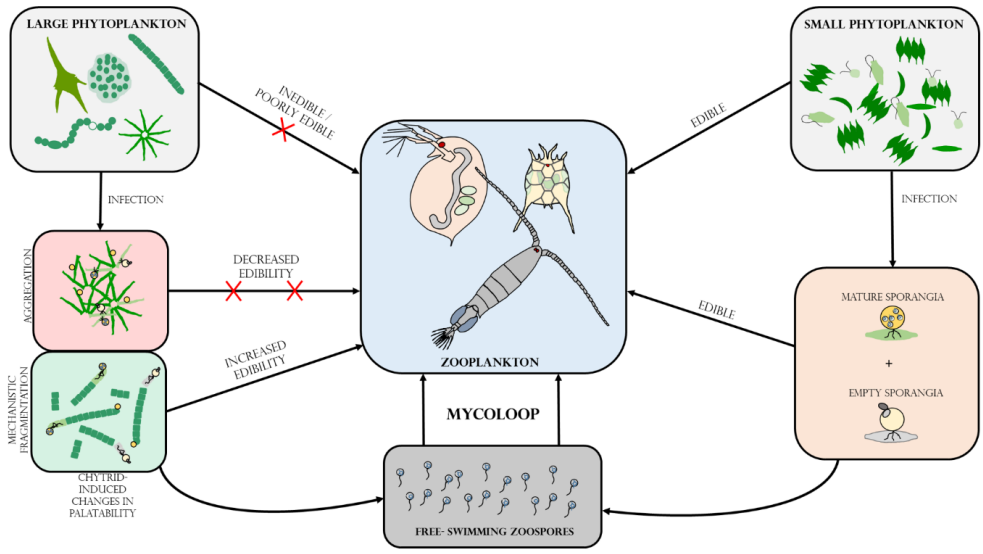

Figure 2.2: Schematic representation of the chytrid-mediated trophic links between phytoplankton and zooplankton (mycoloop). While small phytoplankton species can be grazed upon by zooplankton, large phytoplankton species constitute poorly edible or even inedible prey. Chytrid infections on large phytoplankton can induce changes in palatability, as a result of host aggregation (reduced edibility) or mechanistic fragmentation of cells or filaments (increased palatability). First, chytrid parasites extract and repack nutrients and energy from their hosts in form of readily edible zoospores. Second, infected and fragmented hosts including attached sporangia can also be ingested by grazers (i.e. concomitant predation).

Top-down regulation of chytridiomycosis and trophic interactions

While chytrid parasites can exert strong top-down control on phytoplankton, chytrids themselves can also be used as prey in two different ways: they can either be grazed upon by zooplankton, or they serve as a host themselves for hyper-parasites. Chytrids have been shown to constitute a key nutritional component of the zooplankton diet (Grami et al. 2011; Kagami et al. 2014; Kagami et al. 2004; Kagami et al. 2007b; Rasconi et al. 2014). This pictures a three-way trophic link between algal primary producers, zooplankton and chytrid parasites (Fig. 2.3) and implies a potential role of zooplankton as an important top-down control agent of chytrid infections (Kagami et al. 2007a; Kagami et al. 2004; Schmeller et al. 2014). Also these interactions might profoundly affect phytoplankton seasonal dynamics and composition. For example, during blooms of edible algae or other hosts, zooplankton can affect chytrid prevalence and transmission by (i) grazing on the host and/or on the parasite, affecting the chance of host and parasite encounter and (ii) grazing on edible phytoplankton, thereby promoting the dominance of larger inedible phytoplankton species, ultimately reducing the availability of suitable food sources for zooplankton. However, if inedible phytoplankton become infected, produced zoospores can provide an alternative suitable food source to zooplankton, potentially re-coupling primary and secondary production, through the so-called mycoloop (Agha et al. 2016; Frenken et al. 2016; Kagami et al. 2007a; Kagami et al. 2007b). In addition, there are indications that chytrid infections could modify host palatability to zooplankton. For example, large filamentous cyanobacteria get fragmented as a result of infection and might become more edible to zooplankton (Agha et al. 2016; Gerphagnon et al. 2013b), while infected diatom colonies may aggregate and become less edible (Kagami et al. 2005). Additional efforts are needed to better characterize and quantify zooplankton-chytrid-phytoplankton interactions and assimilate them in an ecological context, including their consequences for trophic linkages in aquatic food webs.

Chytrids can also serve as a host for hyper-parasites (Gleason et al. 2014). Hyper-parasitism may reduce disease risk in phytoplankton host populations. For example, the parasitic chytrid *Zygorhizidium affluens* infecting the diatom *Asterionella formosa* is frequently found hyper-parasitized by another early diverging fungus: *Rozella parva* (Canter 1969). Hyper-parasitism of the primary parasite may reduce or suppress the output of spores and therefore arguably results in a reduced parasitic pressure on phytoplankton (Canter-Lund and Lund 1995). Similarly, it is likely that chytrids (like their hosts) are targeted by viral infections, although this research area is virtually unexplored. Parasites, predators and hyper-parasites interact and dynamically shape the phytoplankton community structure. We need to disentangle this complex matrix of multipartite interactions and integrate them with the effects of abiotic variables, which, altogether, modulate the composition, density, and dynamics of the planktonic communities. Since the majority of experiments have been conducted using a single host, chytrid or grazer, interactions at the community level remain largely unexplored. This makes it hard to predict if (and when) top-down control by predators and/or hyper-parasites can override bottom-up mechanisms.

Inclusion of chytrids in food web models

Food web models help to reveal and clarify mechanisms behind food web dynamics (e.g., the effect of parasites on population stability), infer cause-effect relationships between multiple components, estimate standing stocks and fluxes of materials, and/or forecast the future status of food webs (e.g., parasite infection rates one year later).

Theoretical models, such as mass-balance and node food webs, are helpful tools to describe and quantify energy and matter flows via directional trophic linkages. To properly describe trophic food webs, all matter and/or energy flows among nodes need to be known and quantified. However, natural ecosystems are complex and some compartments, such as parasites, are cryptic and difficult to measure directly, preventing a full characterization of all fluxes (Niquil et al. 2011). Inverse analysis (Vézina 1989) is a method based on the mass-balance principle, which allows calculating flows that are not measured directly using linear equations and ecological constraints (van Oevelen et al. 2010; Vézina et al. 2004). For example, inverse analysis was applied successfully to show that chytrid parasites contribute to longer carbon path lengths and loop strength, higher levels of activity and specialization, lower recycling, and enhanced stability of the pelagic food web (Grami et al. 2011; Rasconi et al. 2014).

Alternatively, empirical dynamic modelling approaches, including linear (e.g., Multivariate Autoregressive Models (MAR models; e.g., Hampton et al. (2013)), or nonlinear models (for instance Convergent Cross Mapping (CCM, e.g., Sugihara et al. (2012)), are used to infer interactions between food web and environmental components by regression structure or causal-effect relationship, and for short-term forecasting. The advantage of these models compared to theoretical ones is that no specific assumptions on the underlying driving mechanisms are required. MAR models use long-term data of aggregated taxonomic, trophic or trait-based groups to infer direction and strength of interactions, not only between trophic links, but also between groups connected by indirect interactions (e.g. competition or facilitation). The resulting interaction matrix allows derivation of network stability metrics (Ives et al. 1999) and can be passed on to network analysis (Gsell et al. 2016).

Models have contributed to a better understanding of the quantitative importance of chytrids in trophic food webs (Grami et al. 2011; Kagami et al. 2014), however, they still show limitations. Inverse models provide only a snapshot of the natural complexity, illustrating steady-state webs for a chosen time period, but do not integrate temporal evolution nor allow describing complex dynamics like host-parasite interactions (Miki et al. 2011). In turn, empirical dynamic models require good quality

long-term datasets with a time resolution matching the relevant rates of the biological process in question, i.e. grazing or infection. Hence, they are still limited by the current lack of datasets showcasing long-term dynamics of chytrid infections. Moreover, the interpretation of results from linear models (e.g. MAR models) is not always straightforward. Regression approaches carry a risk of yielding spurious relationships. In addition, linear models assume that the system is linearly fluctuating around the neighbourhood of a stable equilibrium. Instead, nonlinear empirical models (e.g. CCM) are better at excluding spurious relationships and can be also applied to chaotic systems.

Despite current limitations, modelling can contribute to unravelling the influence of parasites on the structure of host populations and its consequences for the rest of the food web, including estimations of the efficiency of matter and energy transfer from hosts to higher trophic levels. Improved methodologies will contribute to more accurate quantifications of ecological processes, which will improve model parameterization. By identifying more realistic ecological constraints, possible model solutions and their associated uncertainties can be effectively reduced, making it possible to draw more generalizable conclusions when comparing models issued for different ecosystems.

Technical and methodological challenges

Despite recent progress, we still have minimal understanding of many fundamental aspects of plankton chytridiomycosis. In spite of their multidisciplinary nature, we perceive that most research gaps we highlight are affected by three main constraints that greatly hamper a deeper knowledge on the biology of chytrid parasites and its implications in plankton ecology.

The first constraint is the **lack of available chytrid-phytoplankton isolates**. Most hypotheses shaping current scientific notions about the importance of chytridiomycosis in ecological processes stem essentially from experimental work with the few available laboratory isolates. Establishing chytrid-host cultures is not an easy task, but so far little effort has been devoted to isolation and cultivation of parasitic chytrids, probably due to the lack of interest this topic raised among aquatic ecologists in the past. To remedy this situation, the application of automated single-cell sorting using flow-cytometry can potentially facilitate the isolation of host-parasite pairs, not only for taxonomic purposes, but also for cultivation. Experimental work with isolates is essential to study chytrid biology and that of their hosts in its numerous facets, such as the ecophysiology of chytrid infection, or its underlying mechanisms at the cellular level. For example, isolates can be used to undertake chemotactic assays based on live-cell imaging coupled with microfluidics (Rusconi et al. 2014; Scholz et al. 2017) to determine zoospore swimming properties or chemotaxis in response to biotic or abiotic factors. Formulation of hypotheses about the interaction between chytrids and other trophic levels (e.g., zooplankton), including food quality and stoichiometric aspects, also demands laboratory work with chytrid isolates. Analyses of chytrids and their hosts using a combination of SEM and x-ray microanalyses can provide accurate estimates of the stoichiometry of host and parasite (Fig. 2.2, Table 2.3), whereas the use of stable isotope probing (SIP) and nanoscale secondary ion mass spectrometry (NanoSIMS) can be a powerful tool to quantify both substrate utilization by parasite, and transfer to upper trophic levels by predators (e.g. *Daphnia*). Lastly, organismal systems based on chytrids and their hosts constitute valuable tools to undertake experimental evolution assays (e.g., De Bruin et al. (2008)) to test evolutionary hypotheses on host-parasite co-evolution and make predictions about the impact of such evolutionary processes in natural communities.

The second constraint is the irrefutable fact that **chytrid parasites represent genetic black boxes**. The lack of a sequenced genome from chytrid isolates represents one of the current most important burdens in chytrid research, as it prevents the application of proteomic and transcriptomic

approaches, which would in turn provide indispensable insights into the mechanisms of infection. Genome sequencing of chytrid parasites will also contribute to the development of improved molecular markers for phylogeny (i.e. markers that provide both discriminatory power among distant taxa and high resolution at the species level) and quantification (i.e. single-copy genes suitable for qPCR applications). In addition, comparative studies between chytrids with different host ranges and preferences will help to identify the molecular basis of host-parasite specificity. These can in turn provide insights into the process of host and parasite co-evolution, while rendering suitable molecular markers to track matching host and parasite genotypes in the wild. A collaborative action among the scientific community could rapidly change this situation, opening new exciting experimentation possibilities.

The third constraint is the lack of **assimilation of hypothesized ecological implications of chytridiomycosis into the context of natural ecosystems**. Information about the diversity of chytrids, their dominant strategies (saprophytism/parasitism and generalism/specialism), and the environmental conditions promoting them, can only be inferred from increased sampling of their natural habitats. However, sampling strategies have to be designed to provide enough temporal resolution to address rapid chytrid dynamics, enabling a better understanding of their life-cycles. Similarly, digital picture based techniques such as FlowCam (FluidImaging, USA) can provide high-throughput, near real-time identification of phytoplankton-chytrid interactions of live samples. In addition, FlowCam in combination with fluorescence staining techniques (e.g. fluorescein diacetate (FDA), SYTOX Green), can be used to estimate prevalence of infection and host viability directly from environmental samples (Dorsey et al. 1989; Franklin et al. 2012). Assisted by flow cytometry and cell sorting applications, single cell (cell, colony or spore) molecular approaches can likewise contribute to the characterization and quantification of chytrids on phytoplankton, thereby facilitating the study of their ecological relationships (Ishida et al. 2015; Maier et al. 2016). With regard to trophic interactions, further direct and model-based quantifications of the relative contribution of chytrid-mediated trophic transfers up the food web are still needed to integrate current experimental hypotheses about the role of chytrids as alternative conveyors of matter and energy between primary and secondary production. Lastly, from an evolutionary perspective, if we can elucidate the traits determining chytrid-host compatibility, these can be used as markers to track matching chytrid and host genotypes in the wild. This would allow studying the intensity of chytrid-mediated selective pressure and its contribution to the maintenance of diversity in host populations, as well as empirically testing different evolutionary scenarios (e.g. Red Queen hypothesis, selective sweeps) in natural settings directly.

The above methodological needs, although hampering progress at present, can in most cases be easily overcome. We believe that the scientific community can greatly profit from allocating efforts to resolve them, as it will unlock exciting new avenues for further experimentation that can largely contribute to the integration of chytrid parasites into traditional plankton ecology.

Conclusion

This paper identifies major research gaps in different aspects of the biology of chytrid parasites, as well as their role in the functioning of aquatic ecosystems. Our synthesis shows that the effects of chytrid parasitism on phytoplankton occurs at different scales, ranging from the individual organism, to the community and whole ecosystem levels, integrating physiological, ecological and evolutionary processes. To conclude, we provide our view on the different research aspects of plankton chytridiomycosis and how they relate to each other across complexity levels (Fig. 2.4), which illustrates the idea that progress in certain aspects can enable or stimulate development in others.

At the individual level, three main research areas can be identified. First, elucidating the mechanisms of chytrid infection is crucial to identify the basis of host resistance, specificity and host-parasite compatibility. Increased molecular and phylogenetic characterisation of chytrid parasites and their hosts will allow the development of specific molecular markers that can resolve parasite and host cryptic diversity and thus contribute to a better understanding of chytrid ecological strategies and phytoplankton seasonal dynamics. Thereby, tracking the dynamics of matching host and parasite genotypes cycling in nature would be possible, which would allow researchers to empirically address the role of parasites as evolutionary drivers of the maintenance of genetic diversity at the ecosystem level. Secondly, ecophysiological investigations of chytrid infections will help us to identify potential ecological refuges with putative relevance for both chytrid life cycles and the dynamics of their hosts. In turn, by identifying infection refuges, we can delineate infection hot- and cold-spots and explore their role as modulators of co-evolutionary processes. Lastly, characterization of chytrids in terms of their nutritional value from the elemental stoichiometry and biochemical perspectives, together with data on the intensity, frequency and relative importance of top-down control of chytridiomycosis by zooplankton, can contribute to our understanding of the interrelation between parasitism and predation and its feedback on chytrid epidemics and plankton dynamics. This will result in more accurate estimates of parasite-driven transfer of carbon and nutrients through the food web and their contribution to total nutrient (re)cycling at the ecosystem level. Despite the need for progress in these research areas, we currently face methodological limitations that, although may be easily overcome, hamper further advances in the field. The scarcity of isolated chytrid-host cultures, the lack of genomic information on chytrid parasites of phytoplankton, and the marginal incorporation of chytridiomycosis-related research questions in field investigations represent the most important ones.

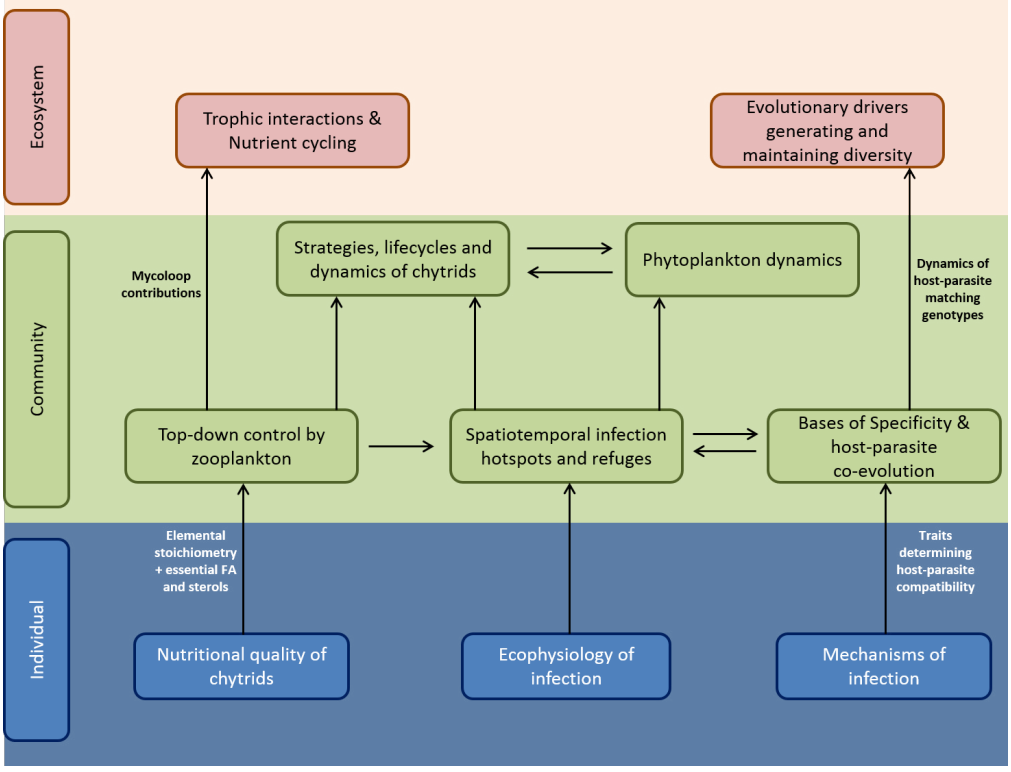

Figure 2.4: Schematic representation of the research areas at different organizational levels and their interrelation. For explanation see text.

However, new methodologies and techniques from other research fields are waiting to be implemented in chytrid research and can be used to overcome most these burdens. Therefore, we expect new exciting research avenues will open in the near future, leading to the integration of chytrid parasitism into aquatic ecology.

Acknowledgements

The authors want to thank all the participants of 1st Plankton Chytridiomycosis Workshop (PCW) in Berlin, Germany (2015) and the 2nd PCW in Skagaströnd, Iceland (2016) for valuable discussions prior to the writing of this paper. The Leibniz Association is acknowledged for supporting the 1st PCW in Berlin (Germany) and the University of Akureyri and the Sóknaráætlun Nordurlands Vestra for their financial support to the 2nd PCW in Skagaströnd (Iceland). HPG and ECB were supported by the Leibniz Pakt/SAW-project "MycoLink" (Pakt/SAW-2014-IGB-1). ASG was funded by the NWO (016.Veni.171.063). SVdW and MG were each funded by the IGB fellowship program. RA was supported by the Alexander von Humboldt Foundation. The authors have no conflict of interest to declare.

Author contributions

Contributions to the individual sections: 1. Introduction: RA, TF; 2. Taxonomy and Molecular Phylogeny: KRJ, PL, KS, CW, FK, MK, SVdW, ECB; 3. Life cycle and strategies: HPG, MK, FK, SVdW, AS, AR; 4. Host specificity and range: SVdW, HPG, PL, KRJ, KS, MK, AS, JW, AR, EA; 5. Mechanisms of infection: RA, BS, KS; 6. Host parasite co-evolution and host diversity: ASG, BWI, TR, JW, SVdW, RA; 7. Host defence and parasite counter defence: RA, TR; 8. Environmental refuges: BWI, TR; 9. Ecological stoichiometry: DVdW, EvD, TF, SAB; 10. Top-down control: MK, RA, TF, MG, DS, AL, JCN, TSN; 11.Inclusion of chytrids in food web models: SR, ASG, TM, MK, JCN; 12.Technical and methodological challenges: KRJ, RA, HPG, ASG, AR, EA, SVdW, JCN, SAB; 13.Conclusion: RA, TF. RA and TF edited the manuscript. All authors commented on the manuscript and approved its final version.

Chapter 3

Warming accelerates termination of a phytoplankton spring bloom by fungal parasites

Published as

Frenken, T., Velthuis, M., de Senerpont Domis, L. N., Stephan, S., Aben, R., Kosten, S., van Donk, E. and Van de Waal, D. B. (2016), Warming accelerates termination of a phytoplankton spring bloom by fungal parasites. Glob Change Biol, 22: 299–309. doi:10.1111/gcb.13095

Abstract

Climate change is expected to favour infectious diseases across ecosystems worldwide. In freshwater and marine environments, parasites play a crucial role in controlling plankton population dynamics. Infection of phytoplankton populations will cause a transfer of carbon and nutrients into parasites, which may change the type of food available for higher trophic levels. Some phytoplankton species are inedible to zooplankton, and the termination of their population by parasites may liberate otherwise unavailable carbon and nutrients. Phytoplankton spring blooms often consist of large diatoms inedible for zooplankton, but the zoospores of their fungal parasites may serve as a food source for this higher trophic level. Here, we investigated the impact of warming on the fungal infection of a natural phytoplankton spring bloom, and followed the response of a zooplankton community. Experiments were performed in ca. 1,000 L indoor mesocosms exposed to a controlled seasonal temperature cycle and a warm (+4˚C) treatment in the period from March to June 2014. The spring bloom was dominated by the diatom *Synedra*. At the peak of infection over 40% of the *Synedra* population was infected by a fungal parasite (i.e. a chytrid) in both treatments. Warming did not affect the onset of the *Synedra* bloom, but accelerated its termination. Peak population density of *Synedra* tended to be lower in the warm treatments. Furthermore, *Synedra* carbon:phosphorus stoichiometry increased during the bloom, particularly in the control treatments. This indicates enhanced phosphorus limitation in the control treatments, which may have constrained chytrid development. Timing of the rotifer *Keratella* advanced in the warm treatments, and closely followed chytrid infections. The chytrids' zoospores may thus have served as an alternative food source to *Keratella*. Our study thus emphasizes the importance of incorporating not only nutrient limitation and grazing, but also parasitism in understanding the response of plankton communities towards global warming.

Introduction

Average global temperatures have increased with an unprecedented rate of approximately 0.11°C per decade since the early seventies (Stocker et al. 2013). This has already been shown to affect a wide range of ecosystems around the world (Walther et al. 2002). Such changes may affect the complex interplay of temperature-dependent chemical and biological processes in freshwater and marine ecosystems (Danovaro et al. 2011). For instance, warming may enhance nutrient loading by increasing mineralization rates and anoxia incidence, but may at the same time reduce nutrient inputs from deep waters into surface mixed layers by enhanced stratification. Such changes in nutrient availability may, in turn, affect primary production by phytoplankton (Behrenfeld et al. 2006; Kudela and Dugdale 2000). Furthermore, phytoplankton growth and biomass build-up have been shown to be directly affected by warming, associated to temperature-dependent changes in their metabolism (De Senerpont Domis et al. 2014; Mooij et al. 2005; Sommer and Lengfellner 2008; Van Donk and Kilham 1990).

Phytoplankton responses to warming not only depend on bottom-up and growth related processes, but also on loss processes that are top-down regulated such as grazing and parasitism (De Senerpont Domis et al. 2013; Hansson et al. 2013; Reynolds et al. 2006). The grazing pressure by zooplankton, for instance, may change due to warming induced changes in zooplankton growth, reproduction and development (Feuchtmayr et al. 2010; Gillooly 2000; Huntley and Lopez 1992; Vidal 1980). Moreover, experimental work indicates that higher temperatures lead to a stronger top-down control of phytoplankton (Kratina et al. 2012). Warming is also expected to favour parasites and infectious diseases, which may have consequences for the structure and functioning of aquatic food webs (Harvell et al. 2002; Lafferty 2009; Marcogliese 2008). Specifically, fungal parasites (chytrids) can play a key role in shaping phytoplankton community structure and host diversity (Kagami et al. 2007a; Sønstebø and Rohrlack 2011; Van Donk and Ringelberg 1983). Transmission of chytrids takes place via free-swimming zoospores that infect a host cell, producing multiple zoospores that can infect new susceptible hosts (Bruning 1991b; Canter 1967; Van Donk and Ringelberg 1983). Consequently, chytrids can control phytoplankton population densities and lead to the termination of spring blooms (Canter and Lund 1951; Van Donk and Ringelberg 1983).

Infections of phytoplankton blooms by chytrids are influenced by environmental conditions, including temperature, resource availability, and other external stressors (Bruning 1991d; Gsell et al. 2013b; Ibelings et al. 2011; Van den Wyngaert et al. 2014; Van Donk and Ringelberg 1983). Transmission rates and virulence of parasites are expected to increase with increasing temperature (Marcogliese 2008). Field studies have shown that relatively cold winters inhibit the activity of chytrids and thereby provide the host with an opportunity to bloom. In contrast, warm winters seem to facilitate chytrids, preventing the host to form a bloom (Gsell et al. 2013b; Ibelings et al. 2011; Van Donk and Ringelberg 1983). Furthermore, laboratory experiments showed enhanced chytrid infections with increasing temperature over a range of 1-16 ˚C, but infection was inhibited at a temperature of 21°C (Gsell et al. 2013b). Not only transmission rates or virulence are important, also chytrid physiological characteristics that are affected by temperature can be important for its success. For instance, higher temperatures shorten development times, which favours chytrids (Bruning 1991b). In contrast, however, increasing temperatures may also negatively affect chytrid development by reducing zoospore production, as well as the zoospore infective lifetime (Bruning and Ringelberg 1987).

Here, we investigated the impact of warming on the chytrid infection of a natural phytoplankton spring community. We predict an earlier onset of the phytoplankton spring bloom due to higher growth rates at higher temperatures, but lower population densities as a result of earlier and enhanced prevalence of chytrids. Furthermore, since the zoospores of chytrids may function as a food

source for zooplankton (Kagami et al. 2007a), we hypothesize that zooplankton development will follow the chytrid abundance, showing an earlier onset and larger biomass at high temperatures, particularly during a bloom of large inedible diatoms. We tested our hypotheses in large indoor mesocosms (referred to as "limnotrons"; Verschoor et al. (2003)) for a period of thirteen weeks between March and June 2014, with temperatures synchronised with typical Dutch seasonality, and a warmed treatment (+4°C). Systems were inoculated with a mixed natural plankton community and included sediment from the same locations. We followed, amongst others, phytoplankton population dynamics, prevalence of infection, bacteria numbers and zooplankton numbers.

Materials and Methods

Experimental setup

The limnotrons are 988 L indoor mesocosm systems developed at the Netherlands Institute of Ecology (NIOO-KNAW) with an average depth of 1.35 m and an inner diameter of 0.97 m (Verschoor et al. 2003). Data is presented from the start of the experiment on the 3rd of March until the 2nd of June 2014. To enhance gas exchange between water and air, two compact axial fans (AC axial compact fan 4850 Z, ebm-papst St. Georgen GmbH & Co. KG, Georgen, Germany) were installed and set to an air flow rate of 100 m^3 hr^{-1}, and an aquarium pump (EHEIM compact 300, EHEIM GmbH & Co. KG, Deizisau, Germany) at a depth of 6 cm set at a rate of 150 L hr^{-1} provided mixing of the upper water layer and prevented stratification. The temperature scenario resembled natural seasonality (control, n=4) based on average Dutch conditions (see online supplementary), and a +4°C elevation (n=4). The applied light:dark cycle resembled typical Dutch conditions, ranging between 11:13 on March 3 to 16:8 on June 2 (see online supplementary).

Depth-integrated samples for chlorophyll-a concentrations, phytoplankton population densities, chytrid prevalence, and nutrient concentrations were taken twice a week using a 3.5 L Plexiglas cylindrical tube sampler with a length of 1 m and a diameter of 67 mm. For each mesocosm, one depth-integrated sample was taken from the upper meter, and homogenized before subsampling. For seston elemental composition, subsamples were taken once a week, and for bacteria counts subsamples were taken once per week in March and May, and twice a week in April. For zooplankton counts and genus composition, 4 depth-integrated samples were taken once a week from the upper meter (i.e. a total volume of 14 L), and samples were homogenized before subsampling. Additionally, depth gradients of temperature, pH, oxygen concentration and light intensity were measured once a week.

Two weeks prior to the experiment, each limnotron was filled with 80 L of sediment from a small pond in Wageningen, The Netherlands (coordinates in DMS: 51°59'16.3"N 5°40'06.0"E). This is a mesotrophic shallow pond with an estimated average depth of 1.2 m and a surface area of 300 m^2. The sediment was sieved through a 0.5 cm mesh, and homogenized before distribution to the limnotrons. Subsequently, each limnotron was filled with tap water and spiked with a concentrated natural plankton assemblage (≥30µm) retrieved from ~30 L water from the same pond as where the sediment was derived from. In addition, a small amount of plankton inoculum (<15% of spiked inoculum volume) and sediment (<1% of total sediment) was derived from another, more eutrophic pond (coordinates in DMS: 51°58'56.7"N 5°43'34.5"E) to allow for a more diverse plankton community resembling different trophic states. After adding the plankton, nutrient concentrations were analysed in each limnotron, and additional nutrients were added to a final concentration of 88.1±9.4 (mean±SE), 2.3±0.4 and 152±18 µmol L^{-1}, for nitrate (NO_3^-), phosphate (PO_4^{3-}) and total silicon (Si), respectively. To ensure equal starting conditions, all limnotrons were connected and water was circulated for a period of two days prior to the start of the experiment.

Chlorophyll analyses

For all chlorophyll-a measurements, samples were filtered over a 220 µm mesh. Subsequently, the filtrate was used for chlorophyll-a measurements by means of fluorescence. To calibrate these fluorescence data, additional chlorophyll-a extractions were performed at various days during the entire experimental period (R^2=0.68; n=189; P<0.001). For fluorescence measurements, non-fixed samples were measured in triplicate on a Phyto-PAM with an Optical Unit ED-101US/MP (Heinz Walz GmbH, Effeltrich, Germany). Each sample was corrected for background fluorescence by 0.2 µm filtered limnotron sample (CA syringe filter; VWR International B.V. Amsterdam, The Netherlands). *Microcystis aeruginosa* strain PCC 7806, *Scenedesmus obliquus* strain SAG 276-3a, and *Asterionella formosa* isolated from Lake Maarsseveen in 2008 were used as reference fluorescence signals for cyanobacteria (blue channel), green algae (green channel), and diatoms (red channel), respectively. The sum of chlorophyll-a measurements from these different channels was used to calculate total chlorophyll-a concentration. Chlorophyll-a extractions were performed with ethanol according to Nusch (1980). Samples were analysed spectrophotometrically after a single 5 mL 80% ethanol extraction for 10 min at 80°C. Afterwards, samples were centrifuged at 3,500 rpm for 5 min at 4°C where after absorbance was measured at 750 and 665 nm on a dual channel Lambda 800 photospectrometer (PerkinElmer, Groningen, The Netherlands). After acidification with 100 µl of a 0.5 M HCl solution, samples were incubated for 20 min at room temperature, centrifuged and absorbance measurements were repeated. Chlorophyll-a concentration was calculated according to Lorenzen (1967).

Phytoplankton counts and composition

Samples for phytoplankton counts and genus composition were fixed with 1% (v/v) alkaline Lugol's iodine solution, and stored in the dark at room temperature until analysis. Phytoplankton species composition and counts were performed on an inverted microscope (DMI 4000B, Leica Microsystems CMS GmbH, Mannheim, Germany) using Utermöhl counting chambers with a volume of 2.2 mL and a settling time of at least 1 hr. Phytoplankton composition was determined to genus level by counting at least 200 cells or 100 fields of view (FOV). To estimate phytoplankton biovolume, at least 100 cell lengths were measured in each sample using the Soft Imaging System Cell D version 1.20 (Soft Imaging System GmbH, Münster, Germany). Cell biovolumes were calculated from linear measurements after approximation to the most equivalent geometric shape (Hillebrand et al. 1999). Cell width of the diatom *Synedra* was approximated by direct measurements that could be confirmed with earlier reported values (Olenina et al. 2006), and ranged between 3-5.6 µm.

Chytrid determination and counts

Samples for chytrid counts were fixed with 1% (v/v) alkaline Lugol's iodine solution, stored in the dark at room temperature and analysed within two weeks after sampling. Samples for chytrid analyses were stained with 4% (v/v) Calcofluor White and counted on an inverted epifluorescence microscope (DMI 4000B, Leica Microsystems CMS GmbH, Mannheim, Germany). The chytrid species was determined based on the size and shape of 38 sporangia, assessed by using pictures taken on the inverted microscope using the program ImageJ 1.48v (ImageJ, National Institutes of Health, Bethesda, Maryland, U.S.). Samples were counted in duplicate until at least 200 cell counts, or 20 FOV. Infection was counted as a categorical variable: each cell was either infected (i) or uninfected (ui). Prevalence of infection was calculated as P= i/(i+ui), according to a method modified from (Rasconi et al. 2009) and (Gsell et al. 2013a).

Zooplankton counts and composition

For zooplankton counts and genus composition, samples were filtered over a 75 µm mesh to capture macro- and mesozooplankton. After collection, the retentate was fixed immediately with 96% ethanol in plastic containers. Rotifers and cladocerans were identified to genus level on a stereo microscope (Leica WILD MZ8, Leica Microsystems B.V., Son, The Netherlands). Copepods and copepodites were distinguished by order. Copepod nauplii were counted, but not distinguished taxonomically. Large samples were subsampled with a Stempel pipette (Hydrobios GmbH, Altenholz, Germany) to produce subsamples with at least 100 specimens of the most abundant taxa, when possible.

Bacteria counts

For bacteria counts, a 1 mL subsample was taken from the integrated water sample collected for phytoplankton analysis, and fixed with a glutaraldehyde/formaldehyde mixture (6.75/1) to a final concentration of 1% (v/v). Afterwards, samples were incubated at 4°C for about 30 min, and subsequently flash frozen in liquid nitrogen and stored at -80°C until further analyses. Just before analyses, samples were thawed in the dark at 4°C, diluted with a PBS/Tween solution to 50% and incubated for 2 hrs in the dark at room temperature. Next, samples were stained for 1 hr with the DNA stain PicoGreen (1×10^{-3} dilution of commercial stock). Shortly before analysis, 5 µL of a yellow-green fluorescent bead solution (FluoSpheres Polystyrene Microspheres, 1.0 µm, Life Technologies Europe BV, Bleiswijk, The Netherlands) was added. Samples were analysed on a MoFlo XDP cell sorter (Beckman Coulter Nederland BV, Woerden, The Netherlands).

Nutrients and seston C:P stoichiometry

For measurement of dissolved inorganic nutrients, including silicon (Si) and phosphate (PO_4^{3-}), 10 mL of sample was filtered over a prewashed (100 mL distilled water) glass microfiber filter (Whatmann GF/F, Maidstone, U.K.) and stored at -20°C until analyses. Prior to analyses, samples were thawed slowly at 5°C. PO_4^{3-} was measured on a QuAAtro39 AutoAnalyzer (SEAL Analytical Ltd., Southampton, U.K.). Silicon samples were acidified with concentrated nitric acid (69%) until a final concentration of 1% (v/v), and analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Iris Intrepid II, Thermo Fisher Scientific, Waltham, U.S.). Particulate organic carbon (C) and phosphorus (P) were determined in 60 mL <220 µm seston collected on a prewashed GF/F filter (Whatman GF/F, Maidstone, U.K.). Filters were dried overnight at 60˚C, and stored in the dark until further analyses. For C analyses, a subsample (13%) of every filter was folded into a tin cup and analyzed on a FLASH 2000 organic elemental analyser (Brechbueler Incorporated, Interscience B.V., Breda, The Netherlands). Particulate organic P was analysed by first combusting the remainder of the filter (87%) for 30 min at 550°C in Pyrex glass tubes, followed by a digestion step with 5 mL persulfate (2.5%) for 30 min at 120°C. This digested solution was measured for PO_4^{3-} on the QuAAtro39 AutoAnalyzer.

Data analysis

Chlorophyll-a, phytoplankton and zooplankton counts, phytoplankton biovolume, bacteria counts, prevalence of infection, and seston stoichiometry were statistically tested for effect of treatment, time and their interaction by repeated measurements ANOVA (rANOVA) in the statistical package Statistica 12.5 (Statsoft Europe, Hamburg, Germany). If assumptions of normality or homogeneity of variance were not met, data was square root transformed. If variance within one treatment had a value of zero, observations of this time step were left out for statistical analysis. To meet this requirement, statistical

analysis for chlorophyll-a covered the period of 06-03 to 02-06 (dd-MM), *Synedra* counts the period of 06-03 to 12-05, *Synedra* biovolume the period of 13-03 to 30-04, zooplankton counts the period of 09-03 to 26-05, prevalence of infection the period of 13-03 to 19-05, seston stoichiometry the period of 06-03 to 29-05 and bacteria number for the period of 10-03 to 02-06.

To assess the distribution patterns of the phytoplankton spring bloom over time, and their accompanying infections, data from each limnotron were fitted with a Weibull function for the period of 06-03 to 02-06 (dd-MM) using the cardidates package for peak curve fitting (Rolinski et al. 2007) of the statistical data analyses system R(R-Core-Team 2013). This package is typically used for estimating changes in peak heights and timing of, for instance, phytoplankton and zooplankton (Feuchtmayr et al. 2012; Hardenbicker et al. 2014; Hülsmann et al. 2012; Rolinski et al. 2007). For our analyses, the following six parameters Weibull function was used:

$$f(x) = p_4 + e^{-\left(\frac{x}{p_5}\right)^{p_6}} \cdot \left(1 - p_1 \cdot e^{-\left(\frac{x}{p_2}\right)^{p_3}}\right)$$

For $x \geq 0$

This function yields distribution parameters (p1 - p6) which are used to calculate various descriptive parameters, including the timing of inflection points of increase (II) and decrease (ID), maximum of the peak (Max), total area under the curve (A), area to the left hand-side of maximum (LHS) and area to the right hand-side of maximum (RHS). Furthermore, also the slope at the inflection was calculated using a time step of 5 days in total, resulting in a steepness of increase (SI) or decrease (SD). These descriptive parameters were statistically compared between treatments using a t-test in the statistical package SigmaPlot for Windows version 12.5. (Systat Software Inc, London, U.K.). Variables were tested for normality and equal variance using the Shapiro-Wilk and Levene's test, respectively.

Results

The phytoplankton spring bloom took place from March to June, reaching maximum chlorophyll-a concentrations of 33.2±4.2 µg L^{-1} (mean±SE) and 29.8±1.8 µg L^{-1} in the control and warm treatments, respectively (see online supplementary). Chlorophyll-a concentrations were significantly different between the warm and control treatment (treatment effect; $p<0.001$; Table 3.1), and this warming effect did depend on time (time x treatment effect; $p<0.001$; Table 3.1). Generally, the chlorophyll-a concentrations were lower in the warm treatments, and the difference seemed to be strongest during the termination of the phytoplankton spring bloom. However, the Weibull distribution parameters did not indicate a clear temperature effect on chlorophyll-a dynamics (Table 3.2).

During the first two weeks of the experiment, the phytoplankton community mainly consisted of chlorophytes (*Desmodesmus* and *Tetraedon*), but became subsequently rapidly dominated by the diatom *Synedra*. At the peak of the bloom, *Synedra* contributed to ≥65% of the nano- and microphytoplankton (2-85 µm) counts, while the remaining part of the community was dominated by small chlorophytes (*Scenedesmus*, *Desmodesmus*, *Coelastrum* and *Sphaerocystis*). Because of their large cells size compared to the small chlorophytes, the relative contribution of *Synedra* to total biovolume was presumably much larger. *Synedra* cell counts in the warm treatments were generally lower as compared to the control (treatment effect; $p=0.005$; Fig. 1a; Table 3.1), and this difference also depended on time (time x treatment effect; $p<0.001$; Table 3.1). Furthermore, the Weibull distribution parameters showed that warming led to a faster decline in *Synedra*

Table 3.1: Output of the repeated measures ANOVA, with the degrees of freedom (df), the F-value, and the p-value.

Data	Unit	Effect	df	F	P
Chlorophyll-*a*	µg L^{-1}	Treatment	1	10.06	**0.000**
		Time	26	39.47	**0.019**
		Time x treatment	26	2.41	**0.000**
Synedra cell count	Cells mL^{-1}	Treatment	1	18.28	**0.005**
		Time	18	46.84	**0.000**
		Time x treatment	18	3.43	**0.000**
Synedra biovolume	μm^3 mL^{-1}	Treatment	1	5.67	0.055
		Time	7	26.94	**0.000**
		Time x treatment	7	2.31	**0.044**
Prevalence of infection	%	Treatment	1	3.51	0.110
		Time	19	16.84	**0.000**
		Time x treatment	19	6.08	**0.000**
Seston C:P	Molar	Treatment	1	4.56	0.077
		Time	12	28.63	**0.000**
		Time x treatment	12	2.10	**0.027**
Rotifer number	Individual L^{-1}	Treatment	1	6.54	**0.043**
		Time	11	81.60	**0.000**
		Time x treatment	11	13.49	**0.000**
Bacteria number	Individual L^{-1}	Treatment	1	1.08	0.338
		Time	15	13.51	**0.000**
		Time x treatment	15	4.93	**0.000**

population densities after the peak of the bloom, as indicated by the smaller surface area on the right side of the maximum (i.e. a lower RHS; p=0.049; Fig. 1a; Table 3.2). Also *Synedra* biovolumes showed a time-dependent effect of warming (time x treatment effect; p=0.044), while this effect was less pronounced with warming alone (treatment effect; p=0.055; Table 1). Specifically, *Synedra* biovolume was lower in the warm treatments during the termination of the bloom (see online supplementary).

The chytrid species in our experiments most closely matched the description of *Zygorhizidium planktonicum* Canter by Canter (1967), which is a parasitic chytrid known to be highly host specific (Canter and Jaworski 1986; Doggett and Porter 1995). Sporangia were spherical and formed by direct enlargement of the zoospore. Immature sporangia had a diameter of 2.2±0.3 µm (mean±SE), while mature sporangia were larger with a diameter of 4.2±0.5 µm (see online supplementary). The increase in prevalence of infection closely followed the decline in *Synedra* population densities. Prevalence of infection was not significantly affected by warming alone (treatment effect; p=0.110; Table 3.1), but

the warming effect did depend on time (time x treatment effect; p<0.001; Fig. 3.1b; Table 3.1). More specifically, the prevalence of infection was initially higher in the warm treatments, yet became lower in course of the infection. Interestingly, the Weibull fits showed that warming caused an acceleration of infection, indicated by a lower surface area before the peak of infection (i.e. a lower LHS; p=0.044) and an earlier decline of the infection (i.e. a lower ID; p=0.001; Table 3.2).

During the emergence of the phytoplankton spring bloom, concentrations of dissolved inorganic nutrients decreased until maximum phytoplankton population densities were reached. At the peak of the bloom, dissolved concentrations of silicon were 14.1±10.8 (mean±SE) and 8.6±2.2 µmol L^{-1} for the control and warm treatments respectively, while PO_4^{3-} concentrations were below the level

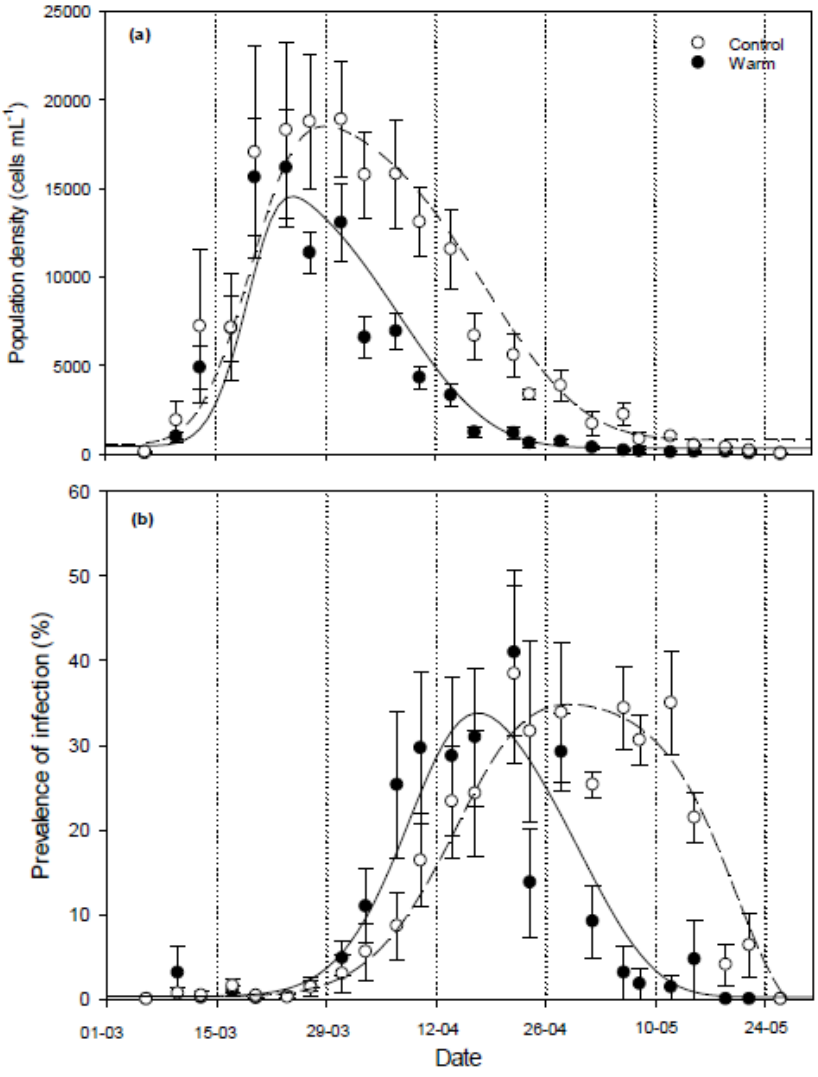

Figure 3.1: Synedra population densities (a) and prevalence of the chytrid infection (b) during the phytoplankton spring bloom (mean +SE). Lines represent the 6 parameter Weibull fits.

of detection in all limnotrons (<0.01 µmol L^{-1}). This depletion of phosphorus is also reflected in the seston C:P ratios, which increased during the course of the bloom development (Fig. 3.2a). The warm treatments showed lower C:P ratios as compared to the control, but this effect depended on time and was most pronounced after the peak of the bloom with maximum molar C:P ratios of 509±17 and 436±41 (mean ±SE) in the control and warm treatments, respectively (effect time x treatment; p=0.027; Table 3.1).

Zooplankton numbers gradually increased when the phytoplankton spring bloom was declining, and chytrid prevalence was around the maximum (Fig. 3.2b). The zooplankton community during the period of the spring bloom was dominated in numbers by rotifers (*Keratella*), succeeded by a small share of copepods (nauplii larvae, not identified) and cladocera (Bosmina). Specifically, rotifers contributed to 83±5% (mean±SE) of the total zooplankton counts until 19-5 in the control treatments, and to 84±2% until 5-5 in the warm treatments. Rotifer numbers were higher in the warm treatments (treatment effect; p=0.043), and this effect also depended on time (time x treatment effect; p<0.001; Fig. 2b; Table 3.1). The effect of warming on rotifers is supported by the Weibull fits, which indicate a higher abundance of rotifers in the warm treatments, represented by a larger area under the graph (i.e. a higher A; p=0.027; Table 3.2). Additionally, Weibull fits show that warming caused an earlier increase (i.e. a lower II; p=0.029) and decrease (i.e. a lower ID; p=0.013; Table 3.2) in rotifer numbers. Bacteria numbers showed dynamic changes in the course of the experiment (see online supplementary), with higher numbers in the warm treatments during the mid-phase of the experiment, and higher numbers in the control treatments at the end of the experiment (time x treatment effect; p<0.001; Table 3.1).

The plankton community showed a clear successional pattern in all treatments, with an initial bloom of *Synedra*, followed by a chytrid infection and the development of a *Keratella* population (Fig. 3.3). The timing of the strongest increase in infection (i.e. II of prevalence of infection) largely coincided with the timing of the strongest decrease in *Synedra* population densities (i.e. ID of *Synedra*), in both the warm and control treatments. More specifically, the strongest increase in prevalence of infection occurred only about 3-4 days after the strongest decline in *Synedra* population densities.

Table 3.3: Output of t-tests (p-values) for the Weibull fit parameters. Symbols between brackets indicate a positive (+) or negative (-) response to warming. Max: Maximum of peak, A: Total area under curve, RHS: Area under the curve to the right of maximum, LHS: Area under the curve to the left of maximum, II: Timing inflection point of increase, ID: Timing inflection point of decrease, SI: Steepness of increase, SD: Steepness of decrease. Values in bold denote a significant difference between warm and control treatments (α<0.05).

Data	Unit	Max	A	RHS	LHS	II	ID	SI	SD
Chlorophyll-*a*	µg L^{-1}	0.481 (-)	0.189 (-)	0.617 (-)	0.129 (-)	0.292 (-)	0.107 (-)	0.879 (+)	0.763 (-)
Synedra cell count	Cells mL^{-1}	0.166 (-)	0.122 (-)	**0.049 (-)**	0.390 (-)	0.257 (-)	0.135 (-)	0.242 (+)	0.213 (+)
Prevalence of infection	%	0.565 (-)	0.253 (-)	0.596 (-)	**0.044 (-)**	0.363 (-)	**0.001 (-)**	0.364 (+)	0.770 (+)
Rotifer number	Individual L^{-1}	0.082 (+)	**0.027 (+)**	0.552 (-)	0.143 (-)	**0.029 (-)**	**0.013 (-)**	0.521 (+)	0.262 (+)

Discussion

Warming resulted in a general decrease in phytoplankton densities, particularly after the peak of the bloom. The maximum of the bloom tended to be lower in the warm treatments as well, though this effect was not significant. The earlier termination of the bloom in the warm treatments was associated with a more rapid increase in chytrid prevalence, suggesting a warming-induced acceleration of infection. Because chytrids depend on their host for nutrition, the rapidly declining host numbers were accompanied by a decrease in chytrid prevalence that was more rapid in the warm as compared to the control treatments. After the collapse of the bloom in the warm treatments, the phytoplankton bloom together with the chytrid prevalence, persisted for a period of two weeks in the control treatments.

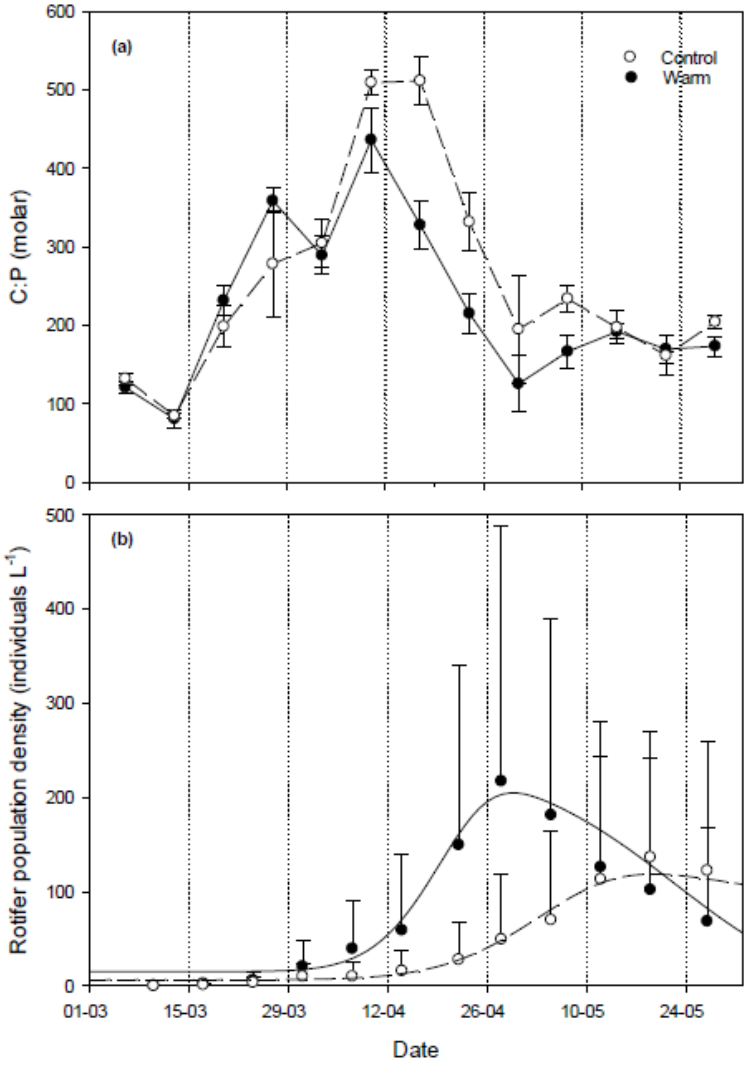

Fig. 3.3: Seston C:P ratios (a) and rotifer counts (b) during the phytoplankton spring bloom (mean +SE). In 2b, lines represent the 6 parameter Weibull fits, and for clarity SE values are only shown to one direction.

Various direct and indirect responses of phytoplankton biomass to warming have been reported, including a lack of effect (Kosten et al. 2012; Özen et al. 2013), an increase (De Senerpont Domis et al. 2014; Lewandowska et al. 2014; Mooij et al. 2005) or a decrease (George et al. 2015; Shurin et al. 2012; Sommer and Lengfellner 2008). Warming has been observed to change phytoplankton cell physiological processes, community composition and trophic structure of the food web (Kratina et al. 2012; Shurin et al. 2012; Strecker et al. 2004; Toseland et al. 2013). Based on the temperature dependency of diatom growth, we would expect a higher biomass in the warm treatments (Bruning 1991b; Butterwick et al. 2005; Van Donk and Kilham 1990). In our experiments, however, phytoplankton biomass after the peak was lower in the warm treatments. This lower biomass is presumably caused by a temperature-driven acceleration of chytrid infections. Earlier studies have suggested a threshold temperature for infection (Bruning 1991b; Ibelings et al. 2004). We did not observe a temperature-dependent shift in the start of the epidemic, but the chytrids in the warm treatments seemed to have a faster increase in abundance. This may be due to a shorter development time of the chytrids with a higher temperature, as was shown for *Rhizophydium planktonicum* (Bruning and Ringelberg 1987). Yet, whether the chytrid in our experiment, *Zyghorhizidium planktonicum* Canter cf., will respond similarly to a higher temperature still requires further investigation.

Besides temperature-sensitivity of the chytrids, infections may also be altered by changes in the host. Host cell size, elemental composition, and its susceptibility to disease were found to depend on the environment (Aalto and Pulkkinen 2013; Coopman et al. 2014; Frost et al. 2010; Frost et al. 2008). Cells of the *Synedra* population in the warm treatments were on average 11.3±1.8% (mean±SE) larger as compared to the control treatments across the entire duration of the experiment (see online supplementary). This is in contrast to temperature dependent cell size changes reported earlier by Peter and Sommer (2013) and Sommer and Lengfellner (2008). All mesocosms started with the same phytoplankton inoculum, suggesting that the observed differences in cell size occurred during the first week of the experiment. This may be a result of direct temperature effects, or a temperature dependent selection for larger genotypes. Experiments with multiple *Asterionella* genotypes yielded a comparable variation in the cell size (Gsell et al. 2013b). The observed cell size differences may contribute to the faster termination of the spring bloom. Larger host cells, for instance, may facilitate larger sporangia, which in turn may be associated to a higher zoospore production (Gsell et al. 2013b). Furthermore, larger cells may be more susceptible to infection, as the encounter rate with parasites increases. This has been observed in zooplankton, where larger sized Daphnia species were more affected by the presence of parasites compared to smaller species (Stirnadel and Ebert 1997).

Phytoplankton C:P ratios increased in both the control and warm treatments, indicating that P became limiting. Such a P-limitation may have constrained phytoplankton growth, and thereby directly contribute to the bloom decline. During bloom termination, C:P ratios in the control treatments were higher than in the warm treatments (Fig. 3.3a), suggesting a stronger P limitation. These higher phytoplankton C:P ratios may also have constrained growth of chytrids, which possibly explains the delayed termination in the control treatment. Indeed, a study by Bruning (1991a) showed that under P limitation host cells were less susceptible to infections as compared to non-limited conditions, and that chytrids developed less zoospores. Thus, chytrids seem to be more susceptible to P limitation as compared to their hosts, which might indicate higher P requirements. Comparably, P limitation caused a decrease in the bacterial infection of Daphnia that was also associated to higher P requirements of the parasites (Coopman et al. 2014; Frost et al. 2008). A study by Kagami et al. (2007b), however, showed an overlap in C:P ratios of the host and the chytrid, suggesting comparable P requirements. In other words, P limitation would stress the host and parasite to the same degree. It seems that stoichiometric requirements of the host and the parasite, and differences therein, may play an important role in the development of an epidemic (Aalto et al. 2015). It is thus not only important to

study the stoichiometry of hosts, but also that of their parasites. Zooplankton numbers showed a clear response to warming. Especially the rotifer genus *Keratella* responded very strong to the temperature increase, with an earlier timing of population development as well as higher population densities. Rotifers are capable of feeding on a broad range of different food sources including bacteria, heterotrophic flagellates, organic detritus and small phytoplankton cells (Arndt 1993; Bogdan and Gilbert 1982; Starkweather and Bogdan 1980; Starkweather et al. 1979). Bacteria numbers were generally higher in the warm treatments after the peak of the *Synedra* bloom, while during the development of *Keratella*, bacteria numbers were higher in the control treatment (see online supplementary). *Keratella* presumably also used bacteria as a food source in our experiment, though no clear relationship was observed. *Keratella* has been found to preferentially feed on living algae smaller than 15 µm, and possibly also detritus (Pourriot 1977; Stemberger 1981). *Synedra* cells in our experiments were much larger, ranging between 75-115 µm, and thus are presumably not an ideal food source for *Keratella* that have a typical size range of 83-117 µm (Bogdan and Gilbert 1984;

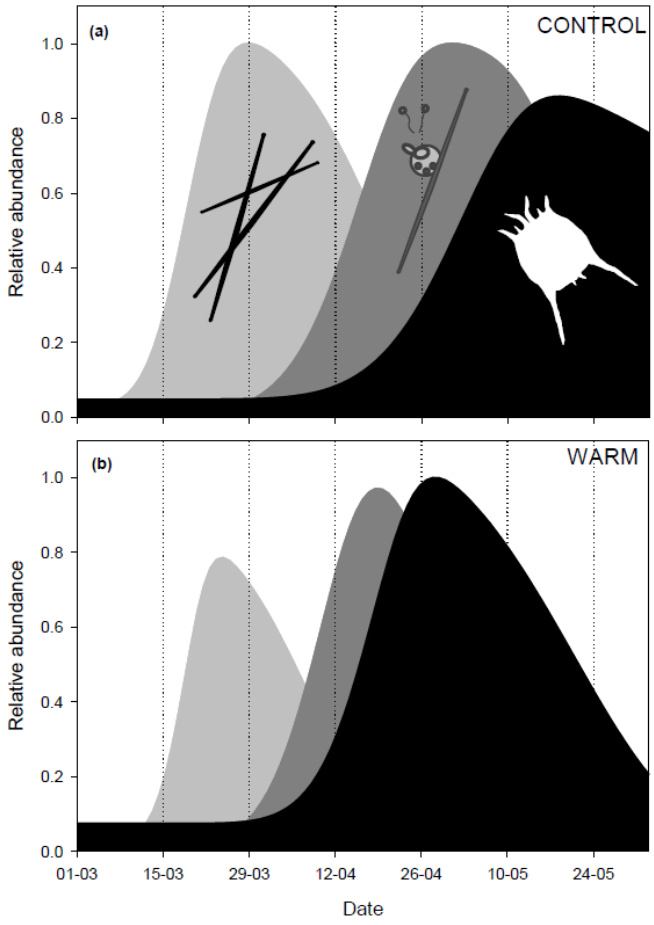

Figure 3.3: Population dynamics of Synedra, prevalence of chytrid infection, and Keratella in the control (a) and warm (b) treatments. Plots describe the average Weibull fits. Data is scaled to the maximum across treatment and within a group. Specifically, Synedra and chytrids are scaled to the maximum population density in the control treatment, while Keratella is scaled to the warm treatment.

Stemberger and Gilbert 1984; Stemberger and Gilbert 1985). It is likely that, in our experiments, the rotifers used chytrids as an alternative food source. Feeding on fungal zoospores by zooplankton has for instance been shown for copepods and cladocerans (Kagami et al. 2011; Kagami et al. 2004; Kagami et al. 2007b; Rasconi et al. 2014), and zoospores may constitute an important part of the diet of microzooplankton (Grami et al. 2011). In fact, various rotifer species showed the ability to lower the abundances of viable zoospores, indicating that they could feed on zoospores (Schmeller et al. 2014). Thus, our data seem to support the notion that rotifers are capable of feeding on chytrid zoospores, yielding a potential trophic link between *Synedra* and the rotifer *Keratella*. Warming caused an earlier development of the rotifer population. This may be a direct effect of temperature, and has been reported for *Keratella* as well as other zooplankton species including cladocerans and copepods (Feuchtmayr et al. 2010; Hansson et al. 2013; Winder and Schindler 2004). These studies showed that the timing of the zooplankton community significantly advanced, possibly due to a high thermal sensitivity of heterotrophs. Depending on the impacts of warming on autotrophs, such shifts in timing may cause a mismatch between a grazer and its food (Walther 2010). Hansson et al. (2013) showed that both phytoplankton and zooplankton communities advanced in response to future climate scenarios, thereby preventing a possible mismatch. Our results suggest that the timing of the *Keratella* population may also have advanced as a result of the faster development of a chytrid epidemic (Fig. 3). This indirect provision of food by fungal parasites may buffer a potential temperature driven mismatch between producers and consumers.

The succession of the chytrid infections and their *Synedra* host followed a typical disease pattern observed for natural chytrid epidemics (e.g. Van Donk and Ringelberg (1983), Holfeld (1998), Ibelings et al. (2011)), while the succession of the rotifer population and the chytrid infections matches a classical predator-prey cycle (e.g. Krebs (1972)). The rotifers thus seems to more closely follow the dynamics of the chytrids, than the chytrids follow the dynamics of *Synedra*. This 'delay' is likely caused by the density dependence of infections, as a fungal disease outbreak depends on a certain threshold density host population, especially if the parasite is host specific (e.g. Ibelings et al. (2004)). Thus, the observed dynamics do suggest a close coupling between the parasite and its host, as well as between the grazer and its food.

In conclusion, our findings suggest that warming accelerated the termination of a phytoplankton spring bloom by parasite infections. P limitation likely played an important role as well, as it may not only have constrained the host but also the parasite. Furthermore, our results suggest that the provision of zoospores due to fungal infections may facilitate higher trophic levels by providing them with an alternative food source. Our study thus emphasizes the importance of incorporating not only nutrient limitation and grazing, but also parasitism in understanding the response of plankton communities towards global warming.

Acknowledgements

We are grateful to Nico Helmsing for assistance during the experiment and analysing the seston elemental composition and dissolved nutrients, and to Erik Reichman for performing the chlorophyll-a analyses. We also thank Suzanne Naus-Wiezer for operating the flowcytometer, assisting during the experiment and help with microscopic work. We thank the editor and two anonymous reviewers for helpful comments. The work of MV was supported by the Gieskes-Strijbis Foundation, and SK was supported by Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) Veni grant 86312012.

Chapter 4

Changes in N:P supply ratios affect the ecological stoichiometry of a toxic cyanobacterium and its fungal parasite

Published as

Frenken T, Wierenga J, Gsell A.S., van Donk E, Rohrlack T and Van de Waal D.B. (2017), 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

Abstract

Human activities have dramatically altered nutrient fluxes from the landscape into receiving waters. As a result, not only the concentration of nutrients in surface waters has increased, but also their elemental ratios have changed. Such shifts in resource supply ratios will alter autotroph stoichiometry, which may in turn have consequences for higher trophic levels, including parasites. Here, we hypothesize that parasite elemental composition will follow changes in the stoichiometry of its host, and that its reproductive success will decrease with host nutrient limitation. We tested this hypothesis by following the response of a host-parasite system to changes in nitrogen (N) and phosphorus (P) supply in a controlled laboratory experiment. To this end, we exposed a fungal parasite (the chytrid *Rhizophydium megarrhizum*) to its host (the freshwater cyanobacterium *Planktothrix rubescens*) under control, low N:P and high N:P conditions. Host N:P followed treatment conditions, with a decreased N:P ratio under low N:P supply, and an increased N:P ratio under high N:P supply, as compared to the control. Shifts in host N:P stoichiometry were reflected in the parasite stoichiometry. Furthermore, at low N:P supply, host intracellular microcystin concentration was lowered as compared to high N:P supply. In contrast to our hypothesis, zoospore production decreased at low N:P and increased at high N:P ratio as compared to the control. These findings suggest that fungal parasites have a relatively high N, but low P requirement. Furthermore, zoospore elemental content, and thereby presumably their size, decreased at high N:P ratios. From these results we hypothesize that fungal parasites may exhibit a trade-off between zoospore size and production. Since zooplankton can graze on chytrid zoospores, changes in parasite production, stoichiometry and cell size may have implications for aquatic food web dynamics.

Introduction

Human activities have substantially increased the flux of nutrients from land into receiving waters (Smith 2003). This nutrient enrichment enhances aquatic primary production, and may lead to dramatic changes in the composition and structure of aquatic food webs (Schindler and Fee 1974; Smith et al. 2006). Specifically, an increased nutrient supply might promote development of harmful cyanobacterial blooms (Paerl et al. 2001; Paerl et al. 2011; Smith and Schindler 2009). Although nutrient loading has increased, primary production in aquatic ecosystems is often still limited by nitrogen (N) and/or phosphorus (P) (Bracken et al. 2014; Elser et al. 2007). This may be a result of an imbalanced nutrient supply (Carpenter et al. 1996; Sterner et al. 2007), as well as an increased nutrient demand associated to high phytoplankton densities (Carpenter et al. 1996). Nutrient limitation will alter the elemental composition of phytoplankton, and may specifically increase carbon:nutrient ratios (Sterner and Elser 2002). As a consequence, nutritional quality of the phytoplankton decreases, thereby possibly constraining higher trophic levels (Hessen et al. 2013; Sterner and Elser 2002). This may particularly apply to parasites that solely depend on their host as a food source (Smith 2007).

Fungal parasites are very common pathogens infecting phytoplankton (Gerphagnon et al. 2015), which represent an important but yet overlooked ecological driving force in aquatic food web dynamics (Sime-Ngando 2012). These parasites, belonging to the phylum Chytridiomycota and often referred to as chytrids, are host specific zoosporic fungi that can parasitize on phytoplankton and completely rely on their host to obtain energy and nutrients leading to death of the host (Barr 2001; Sparrow 1960; Sparrow 1968). Thereby, they play an important role in natural aquatic ecosystems, in which chytrids can significantly change phytoplankton abundance and seasonal succession (Reynolds 1973; Van Donk 1984; Van Donk 1989). Additionally, the free swimming stage of the chytrids (i.e. zoospores) may provide higher trophic levels with an alternative food source during blooms of large inedible diatoms (Frenken et al. 2016; Kagami et al. 2007b) or cyanobacteria (Agha et al. 2016). Earlier work indicates that zoospores might find their host by chemotaxis (Muehlstein et al. 1988), and penetrate host cells using a rhizoidal system through which nourishment is conveyed to the zoospore (Van Donk and Ringelberg 1983). After infection, the spore forms a sessile stage (i.e. sporangium) in which new zoospores (up to 60) are produced (Canter and Lund 1951; Sparrow 1960).

Chytrid zoospores generally contain a relatively high amount of nucleic acids that are particularly rich in P, but also contain substantial amounts of lipids, including fatty acids and sterols, which are rich in carbon (Barr and Hadland-Hartmann 1978; Beakes et al. 1988; Beakes et al. 1993; Elser et al. 1996; Kagami et al. 2007b). Chytrids thus seem to have high P demands, as has been indicated by their low C:P as compared to their host (Kagami et al. 2007b). As a consequence, limitation by P may affect a chytrid more than its host. Chytrid infections were indeed shown to be affected by host P limitation. More specifically, chytrid growth rate and the number of zoospores per sporangium decreased, and, as a function of lower host growth rate, zoospore loss as well as searching time increased, as compared to non-limited conditions (Bruning 1991a). If, however, P limitation impedes algal growth to a greater extent than that of the chytrid, epidemics may still occur (Bruning 1991a; Bruning and Ringelberg 1987).

Nutrient limitation not only alters growth and reproduction of the parasite, it may also affect host defense. Freshwater cyanobacteria produce a wide range of oligopeptides including toxic microcystins (MC) (Welker et al. 2004), which have been associated to chytrid defense (Rohrlack et al. 2013). These oligopeptides are N rich compounds, and their synthesis is typically constrained under N limitation (Van de Waal et al. 2010a; Van de Waal et al. 2014). Thus, during low N:P conditions, host defense may be reduced and thereby facilitate chytrid infections. In contrast, cellular N may

accumulate under high N:P conditions and thereby enhance host defense. Limitation by N and P may thus have contrasting effects on chytrid infections of cyanobacteria. We hypothesized that parasite elemental composition will follow changes in the stoichiometry of its host, and that its reproductive success will decrease with host nutrient limitation. To test this hypothesis, we exposed the cyanobacterium *Planktothrix rubescens* to its chytrid Rhizophydium megarrhizum under control, low N and low P conditions, leading to a range of host N:P ratios. We predict that infections will decrease with increasing host N:P, as the availability of P for chytrid nutrition will decrease and the host defense by oligopeptides will increase.

Materials and methods

Description of test organisms

In this study the filamentous cyanobacterial host Planktothrix *rubescens* NIVA-CYA97/1 was used in combination with one of its parasites, the chytrid Chy-Lys2009 (photo provided in the Supplementary Material). This chytrid possesses identical morphological characteristics and infection patterns in agreement with *Rhizophydium megarrhizum* described earlier by Canter and Lund (1951). More information on host specificity and virulence of the chytrid can be found in Sønstebø and Rohrlack (2011) and Rohrlack et al. (2013). All cultures used in this study were monoclonal and non-axenic.

Culture maintenance

The *Planktothrix* and the chytrid 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. The applied low light levels resemble the conditions where the tested *Planktothrix* species was isolated, i.e. in the vicinity of the thermocline. All cultures were maintained in exponential growth in batch using 100 mL Erlenmeyer flasks with 50 mL suspension. Every other week, *Planktothrix* cultures were diluted using WC-medium (Guillard and Lorenzen 1972) and chytrid cultures were diluted using host culture and WC-medium to 1/10 (v/v). Additionally, Erlenmeyer flasks were shaken every other day to prevent aggregation. The chytrid cultures were visually inspected for infection patterns and contaminations at least once a week.

Description of the experiment

Culture acclimatization and inoculation

Prior to the experiment, *Planktothrix* was grown at 16°C on WC-medium at three distinct N:P supply ratios by modifying standard NO_3^- and PO_4^{3-} concentrations of 1000 and 50 µmol L^{-1} (N:P=20) as control, to 200 and 50 µmol L^{-1} (N:P=4) as the low N:P treatment, and 1000 and 10 µmol L^{-1} (N:P=100) as the high N:P treatment. Cultures were acclimatized for about 18 generations to the distinct nutrient conditions by three consecutive transfers at late exponential phase. During each transfer, i.e. after each 7 day period, cultures were diluted back to half of maximum biovolume reached in order to maintain nutrient limited conditions. After acclimatization, *Planktothrix* was first grown without chytrids (unexposed treatment, 4 replicates per nutrient treatment, 12 experimental units) to late stationary phase to obtain uninfected host growth rates, stoichiometry and toxin composition. Subsequently, the host cultures were then pooled by nutrient treatment and used to inoculate the chytrid exposed treatments (4 replicates per nutrient treatment, 12 experimental units). At the start of the chytrid exposed treatment, *Planktothrix* cultures were inoculated together with a zoospore suspension that was obtained from a highly infected *Planktothrix* culture (with 58% Chy-Lys2009 infected filaments) by sieving gently over a 30 µm and a subsequent 5 µm nylon mesh to remove host cells, while collecting zoospores that have a typical size range of 2.5-3.5 µm (Sparrow 1960). This

zoospore suspension was washed with N and P free WC-medium and concentrated on a 1.2 µm cellulose acetate membrane filter (Whatmann, Maidstone, U.K.), and used to inoculate to a final density of 18 zoospores mL^{-1}. The chytrid exposed cultures were grown for 7 days to obtain host and parasite growth rates, host and parasite stoichiometry, parasite zoospore production and toxin composition of parasite exposed host. Each treatment was performed in 500 mL Erlenmeyer flasks with 300 mL of culture.

Host and parasite quantification

During the experiment, cultures were sampled daily to determine biovolume using a CASY Cell Counter (Schärfe System GmbH, Reutlingen, Germany). Next, at least 5 mL of culture suspension was fixed with alkaline Lugol's iodine solution to a final concentration of 1.2% (v/v) and stored in the dark at room temperature. Prevalence of infected filaments was counted in duplicate (technical replicate) for each biological replicate within two weeks after the experiment by inspecting at least 50 filaments. Additionally, during the infection treatment, the number of free swimming zoospores was counted daily in duplicate, also for each biological replicate, in at least 15 fields of view (FOV) in fresh cultures. All microscopic counting was performed using a magnification of 200x on an inverted microscope (DMI 4000B, Leica Microsystems CMS GmbH, Mannheim, Germany). Cultures were harvested at the early stationary phase for the analyses of dissolved inorganic nutrients, elemental composition of the host and parasite, and the MC contents and composition of the host.

Elemental analyses

Particulate organic carbon (C), N and P were determined in duplicate by collecting 5-15 mL of seston on a prewashed GF/F filter (Whatman, Maidstone, U.K.) applying gentle filtration (< 1-2 psi). Filters were dried overnight at 60˚C, and stored in a desiccator in the dark. For C and N analyses, a subsample (22%) of every filter was taken by a hole puncher, folded into a tin cup and analyzed on a FLASH 2000 organic elemental analyzer (Brechbueler Incorporated, Interscience B.V., Breda, The Netherlands). Particulate organic P was analyzed (Eaton 2005) by first combusting the remainder of the filter (78%) for 30 min at 550°C in Pyrex glass tubes, followed by a digestion step with 2.5 mL persulfate (2.5%) for 30 min at 120˚C. This digested solution was measured for PO_4^{3-} on the QuAAtro39 AutoAnalyzer (SEAL Analytical Ltd., Southampton, U.K.) following Armstrong et al. (1967). During the infection treatment, particulate organic C, N and P were determined for the seston fraction as well as for the zoospores. For this purpose, 90-160 mL infected culture suspension was gently filtered twice over a 30 µm nylon mesh filter to remove the larger cyanobacterial filaments. Subsequently, the smaller filaments were removed by an additional filtration over a 5 µm nylon mesh, and zoospores in the filtrate were collected on a prewashed GF/F filter (Whatman GF/F, Maidstone, U.K.). Organic C, N and P on the filters were analyzed as described above.

Microcystin analyses

Extractions

Samples for MC analyses were collected in duplicate by filtering 5-15 mL of culture over a GF/C filter (Whatman, Maidstone, U.K.) applying low pressure after which the filters were stored at -20˚C. Filters were lyophilized overnight before performing three rounds of extractions at 60 °C using 2.5 mL 75% methanol-25% Millipore water (v/v) in 8 mL Pyrex glass tubes. After drying the samples with N_2, extracts were reconstituted in 900 µl methanol, filtered and centrifuged (Corning© Costar© Spin-X© polypropylene centrifuge tube filters with a 0.22 µm cellulose-acetate filter (Corning Inc., Corning, U.S.) for 5 min at 16,000 × g (Sigma 1-15P, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Filtrates were transferred to amber glass vials and analysed by LC-MS/MS.

Analyses

Samples were analysed for eight MC variants (dm-7-MC-RR, MC-RR, MC-YR, dm-7-MC-LR, MC-LR, MC-LY, MC-LW and MC-LF). Calibration standards were obtained from the National Research Council (Ottawa, Canada) for dm-7-MC-LR, and from Enzo Life Sciences Inc. (Farmingdale, U.S.) for the other variants. Measurements were performed on an Agilent 1260 LC and an Agilent 6460A QQQ (Agilent Technologies, Santa Clara, U.S.). The compounds were separated on an Agilent Zorbax Eclipse XDB-C18 4.6 × 150 mm, 5 µm column using Millipore water with 0.1% formic acid (v/v, eluent A) and acetonitrile with 0.1% formic acid (v/v, eluent B). The elution program was set at 0–2 min 30% B, 6–12 min 90% B, with a linear increase of B between 2 and 6 min and a 5 min post run at 30% B. Sample injection volume was set at 10 µL, with a flow of 0.5 mL min^{-1} at a column temperature of 40°C. The LC-MS/MS was operated in positive mode with an ESI source, nitrogen was used as a drying, sheath and collision gas. For each compound, two transitions were monitored in MRM mode: m/z 491.3 to m/z 135.1 and m/z 981.5 to m/z 135.2 (dm-7-MC-LR, ratio between product ions 17%), m/z 498.3 to m/z 135.1 and m/z 995.6 to m/z 135.1 (MC-LR, ratio between product ions 16%). This protocol is based on the protocol earlier described by Faassen and Lürling (2013).

Data analyses

Host population growth and zoospore production rates were calculated according to $\mu = \ln\left(B_{n+t}/B_n\right)/t$. In which µ is the maximum specific growth rate, B_n is the initial population density of non-infected or infected host (biovolume), or zoospores (counts), B_{n+t} is the final population density of these variables over time t in the exponential growth phase. Infected biomass was calculated by multiplying the proportion of infected filaments with biovolume. Zoospore production efficiency was calculated as the number of zoospores produced per infected host biovolume.

Host maximum specific growth rate, zoospore production rate, seston stoichiometry of the host and parasite and MC content of the host were tested for normality and equal variance using the Shapiro–Wilk and Brown-Forsythe tests, respectively. Data were transformed, log or reciprocal, if this improved normality. Host growth rates, zoospore production rate, seston and zoospore stoichiometry and host MC content were analyzed to test for effects of nutrient supply by performing a one-way ANOVA. Pairwise comparisons were conducted using the Holm-Sidak test (Sidak 1967). The strength and direction of associations between variables were assessed by Pearson product-moment correlations. All analyses were performed using SigmaPlot version 13 (Systat Software Inc, London, UK). Detailed output of the different statistical tests can be found in the supplementary.

Results

Host growth and biovolume build-up

In the absence of the parasite, *Planktothrix* population growth rates were comparable in all treatments (Table 4.1), with replicates ranging between 0.30-0.59 d^{-1}. In the presence of the parasite, nutrient supply also had no clear effect on net population growth rate of the total biovolume (Table 4.1). After four days of infection, infected host biomass increased at the expense of susceptible host biomass (Fig. 4.1). The total *Planktothrix* biomass build-up after 4-7 days was lower in the chytrid exposed cultures than in the unexposed cultures. The rate at which the infected biomass increased was highest under a high N:P supply, while it did not differ between the control and low N:P treatment (Table 4.1).

Table 4.1: Planktothrix maximum net population growth rates of the different biomass fractions in the unexposed and chytrid exposed cultures. Superscript letters denote significant differences between nutrient treatments based on One-way ANOVA and post-hoc comparison of the means (α<0.05).

	Unexposed	*Exposed*		
Treatment	Total	Total	Susceptible	Infected
Low N:P	0.42±0.05[a]	0.06±0.05[a]	-0.05±0.02[a]	0.94±0.09[a]
Control	0.40±0.04[a]	0.11±0.04[a]	0.10±0.03[b]	0.92±0.09[a]
High N:P	0.40±0.06[a]	0.11±0.03[a]	0.10±0.03[b]	1.34±0.09[b]

Elemental composition

Host N:P ratios followed N:P supply (r=0.99, P<0.001; Table 4.2), and were lowest with 7.8 ±0.1 (mean±SE) under low N:P conditions, intermediate with 11.4 ±0.4 in the control, and highest with 46.2 ±2.7 under high N:P conditions (Fig. 4.2A). This was also largely resembled in the overall N:P ratios of the cultures when the parasite was present (i.e. infected host + chytrids). The N:P ratio in the high N:P treatments was also highest with 44.2±1.7, while the low N:P treatment and the control were not statistically different with an N:P ratio of 8.6±0.22 and 10.3±0.14, respectively. Similarly, N:P ratios of the chytrid zoospores increased with host N:P ratios (r=0.96, P<0.001). Specifically, chytrid N:P ratios increased from 12.6±0.4 in the control to 25.9 ±1.0 under high N:P, while remained largely unaltered in the low N:P treatment as compared to the control (Fig. 4.2B; Table 4.3).

The observed shifts in host N:P ratios were mainly caused by changes in P contents (r=-0.97, P<0.001), which decreased from 107.8±2.5 pmol mm^{-3} at low N:P conditions down to 82.7±4.3 pmol mm^{-3} in the control and 17.2±1.0 pmol mm-3 under high N:P conditions, while C and N contents remained largely unaltered across all treatments (Fig. 4.2C; Table 4.2). Zoospore C, N as well as P contents decreased with increasing host N:P (r=-0.77, P=0.003, r=-0.77, P=0.003 and r=-0.84, P<0.001, respectively), with highest values under low N:P conditions, intermediate values in the control, and lowest values under high N:P conditions (Fig. 4.2D; Table 4.3). The observed difference in treatment (Fig. 4.5). N:P stoichiometry between high N:P conditions and the other nutrient supply treatments resulted from a stronger decline in P contents relative to N.

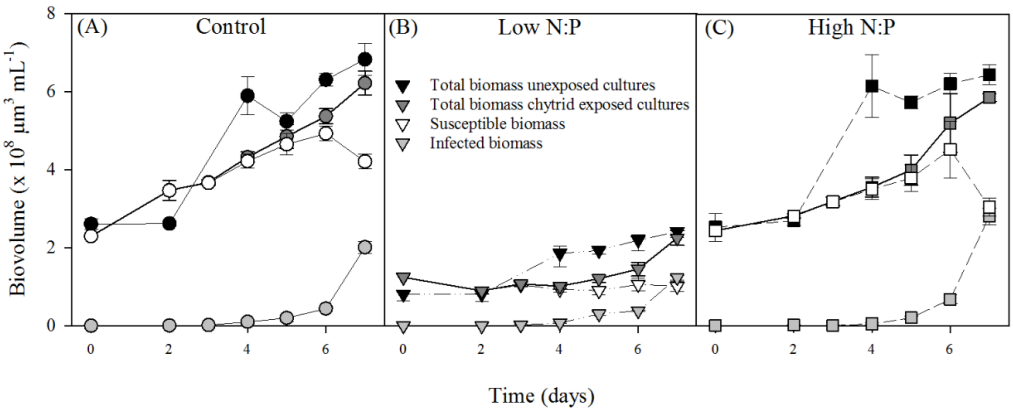

Figure 4 1: Biovolumes of Planktothrix in the in the cultures with and without parasite exposure in the control (A), low N:P (B) and high N:P (C) treatments. Symbols represent mean ± standard error (n = 4).

Table 4.2: Host nutrient content and stoichiometry (mean±SE) in unexposed cultures. Superscript letters denote significant differences between nutrient treatments based on One-way ANOVA and post-hoc comparison of the means (α<0.05).

	Nutrient content (µmol mm^3)			Stoichiometry (molar)		
Treatment	C	N	P	C:P	C:N	N:P
Low N:P	5148±268^{a}	837±22^{ab}	108±3^{a}	47.7±1.9^{a}	6.1±0.2^{a}	7.8±0.1^{a}
Control	4098±120^{b}	935±26^{a}	83±4^{b}	49.9±1.9^{a}	4.4±0.0^{b}	11.4±0.4^{b}
High N:P	3705±242^{b}	791±48^{b}	17±1^{c}	216.4±11.7^{b}	4.7±0.1^{b}	46.2± 2.7^{c}

Table 4.3: Zoospore nutrient content and stoichiometry (mean±SE). Superscript letters denote significant differences between nutrient treatments based on One-way ANOVA and post-hoc comparison of the means (α<0.05).

	Nutrient content (×10-4 µmol per spore)			Stoichiometry (molar)		
Treatment	C	N	P	C:P	C:N	N:P
Low N:P	10.71±0.77^{a}	2.41±0.18^{a}	0.19±0.01^{a}	57.8±0.7^{a}	4.4±0.0^{a}	13.0±0.2^{a}
Control	5.27±0.55^{b}	1.11±0.11^{b}	0.09±0.01^{b}	59.9±1.7^{a}	4.7±0.0^{b}	12.6±0.4^{a}
High N:P	2.63±0.49^{c}	0.45±0.08^{c}	0.02±0.00^{c}	149.9±6.5^{b}	5.8±0.1^{c}	25.9±1.0^{b}

Parasite prevalence and production

Prevalence of infection on the last day of the experiment ranged between 30 ±1.4% (mean±SE) in the control up to 48 ±2.1% and 56 ±3.6% in high N:P and low N:P treatments, respectively (Fig. 3A). Growth rates of the infection were highest in the high N:P cultures and lowest, although not significantly, in the low N:P cultures (Table 4.1). Comparably, zoospore concentrations, zoospore production rate and the amount of zoospores produced per unit of infected host biomass, i.e. the zoospore production efficiency, were all highest in the high N:P cultures and lowest in low N:P cultures (Fig. 4.3B, 4.4A,B; Table 4.4). Zoospore production rate and efficiency increased with host N:P ratio (r=0.61, P=0.035, and r=0.85, P<0.001, respectively), while production efficiency furthermore decreased with zoospore C contents (r=-0.87, P<0.001; Fig. 4.4C).

Microcystin

Four MC variants were detected, including dm-7-MC-RR, MC-YR, dm-7-MC-LR and MC-LR. On average, dm-7-MC-RR was the dominant MC variant present, representing 56.6±0.5% (mean±SE) of the total amount of MC. The total cellular MC contents ranged between 60 and 250 µg mm^{-3}. MC concentrations were highest in the high N:P, lowest in the low N:P, and intermediate in the control treatment (Fig. 4.5)Furthermore, in the chytrid exposed cultures, the total amount of intracellular MC seemed to be lower.

Table 4.4: Zoospore production rates and production efficiencies (mean±SE). Superscript letters denote significant differences between nutrient treatments based on One-way ANOVA and post-hoc comparison of the means (α<0.05).

Treatment	Rate	Efficiency
Low N:P	1.14±0.07[a]	2.17±0.04[a]
Control	1.27±0.18[ab]	3.14±0.42[b]
High N:P	1.86±0.21[b]	5.02±0.53[c]

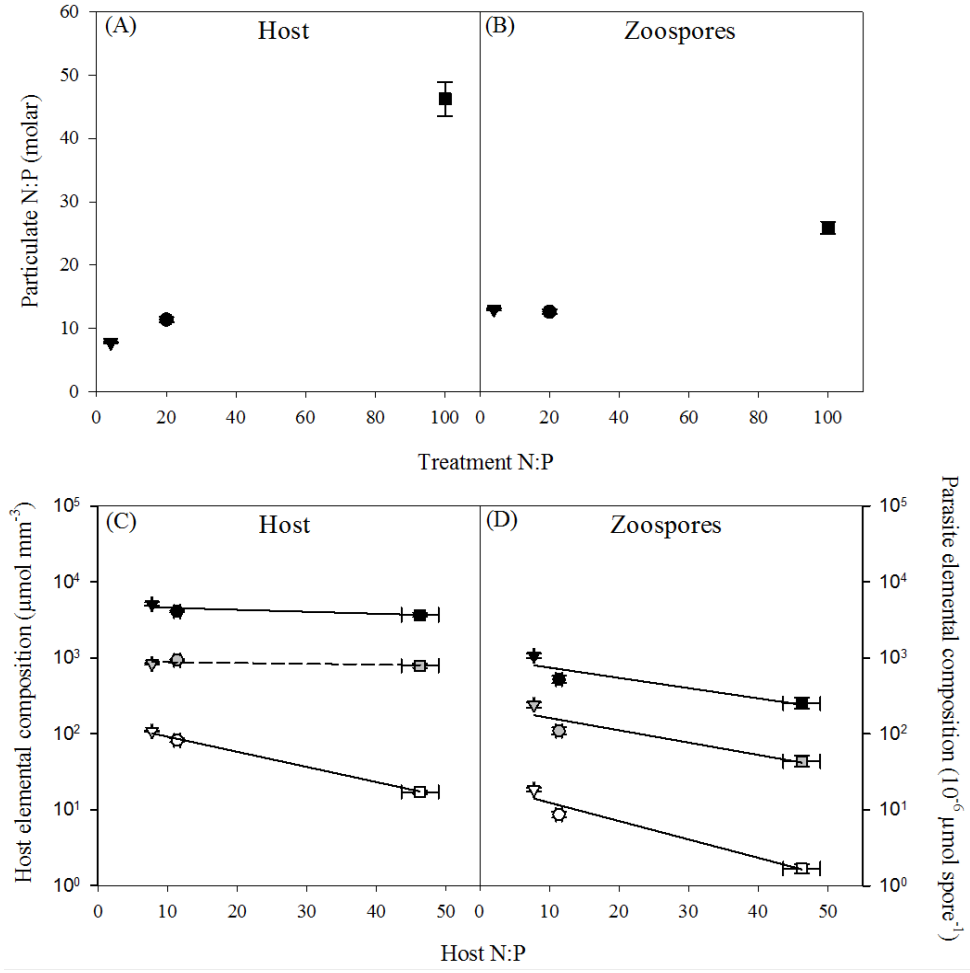

Figure 4.2: N:P ratios of uninfected host (A) and zoospores (B), and elemental content of the host (C) and zoospores (D) in the control (circle), low N:P (triangle) and high N:P (square) treatments. Symbols represent mean ± standard error (n = 4). In (C,D) black, gray, and white symbols indicate carbon, nitrogen and phosphorus content, respectively. Solid lines indicate significant correlations (P < 0.05).

Discussion

Although the different nutrient supply ratios had only minor effects on *Planktothrix* growth rates after acclimatization (Table 4.1), there were clear changes in the elemental composition of the cyanobacteria (Fig. 4.2A). This indicates that nutrient depletion did affect host physiology, but not growth, at the time of sampling. Apparently, *Planktothrix* is able to maintain similar maximum growth rates as compared to the control at both a high and low N:P supply ratio (Table 4.1). The reduced host N:P under low N:P supply and increased host N:P under high N:P supply (Fig. 4.2A) indicates nutrient limitation at the end of the exponential phase and/or early stationary phase. Moreover, population densities in the low N:P treatment at the end of the experiment were lower as compared to the control and high N:P treatment (Fig. 4.1B). These lower population densities are mainly caused by a low N availability in the low N:P treatment, but may also result from the lower *Planktothrix* population densities at the start of the experiment. Differences in host population density may affect light availability in the cultures. A lowered population density, as observed in the low N:P treatment, may have resulted in an increased light availability due to reduced self-shading. Earlier studies have indicated that zoospores may find their host using chemical cues that are related to photosynthetic activity, since zoospores are generally attracted to carbohydrates, polysaccharides, proteins and amino acids (Donaldson and Deacon 1993; Moss et al. 2008; Muehlstein et al. 1988). Some studies, however, also reported attachment of zoospores to new hosts during dark conditions (Barr and Hickman 1967). Indirectly, the relative higher light availability in the low N:P treatment may thus have favoured parasite attraction. Moreover, with a comparable amount of zoospores added at the start of the experiment, the zoospore-to-host ratio was also higher in the N limited treatment, which may favour infection rates. Yet, both the zoospore production rate and production efficiency were lower in the low N:P treatment, and did not lead to a different infection rate as compared to the control (Table 4.1). This suggests that higher relative light availabilities as well as higher initial zoospore-to-host ratios did not stimulate, and possibly even impeded the infection dynamics in our low N:P treatment.

In response to an increased N:P supply in the medium, host N:P increased as well. This resulted in a consecutive increase in the N:P of the zoospores (Fig. 4.2B). Our results thus show that stoichiometry of a host can cascade to their parasites. In the host, changes in stoichiometry seem to be driven mainly by a change in P content, as host C and N remain constant with changing N:P supply (Fig. 4.2C). In the parasite, changes in zoospore stoichiometry are also mainly driven by P content, but C and N contents decrease as well. Yet, P content decreases faster, suggesting a higher flexibility of the chytrid with respect to P (Fig. 4.2D). These findings indicate that chytrid parasites can be stoichiometrically flexible, while maintaining their ability to infect along an N:P supply gradient. N:P and C:P ratios of the chytrid used in this experiment are relatively high as compared to two other studies using different chytrid species (Kagami et al. 2017; Kagami et al. 2007b), but fall well within the range of aquatic fungi reported before (Danger and Chauvet 2013; Danger et al. 2015). These data furthermore suggest that fungal elemental homeostasis is indeed limited (Danger et al. 2015; Persson et al. 2010). Zoospore N:P ratios largely resembled that of the host under control conditions, but were different from the host in the low and high N:P treatment. We could not separate zoospores from the heterotrophic bacteria, and our results may therefore have been confounded by shifts in bacterial numbers. To prevent high bacterial numbers fuelled by the lyses of *Planktothrix*, we ran the experiments over a relatively short time period. Consequently, the overall biomass of bacteria and thereby their contribution to the elemental composition at the time of sampling is likely to be small.

Increasing N:P supply ratios resulted in an increased zoospore production rate and production efficiency (Fig 4.4A, B). These results are in contrast to earlier findings of an experiment that showed that under P-limitation (and presumably high N:P) zoospore production decreased (Bruning 1991a). It

might be possible that the chytrid species (*Rhizophydium planktonicum* Canter emend.) used in the experiment by Bruning (1991a) has higher P-requirements, and therefore suffered more from P-limitation. Or, because the chytrid in our experiment can potentially infect and exploit multiple adjacent cells within one cyanobacterial filament (Canter and Lund 1951), it might be less vulnerable to nutrient limitation. In other words, the chytrid might continue infecting adjacent cells until it has consumed sufficient nutrients to complete an infection cycle. However, this is only profitable if the energetic costs of growing rhizoids and producing degrading enzymes to invade host cells balance the gains with respect to resource acquisition.

Under low N:P conditions, the parasite zoospores were fewer but contained more C, as well as N and P as compared to the control (Fig. 4.2D). Although we did not assess zoospore size in this experiment directly, increases in elemental contents do suggest that the chytrid produced larger zoospores. Zoospore size of the used chytrid was shown to vary from 2.84 µm to 5.36 µm under control growth conditions (see online supplementary), and variation in spore size was also shown in other studies describing shifts in spore size with climatic conditions (Kauserud et al. 2008; Kauserud et al. 2011). Presumably, larger zoospores facilitate zoospore survival time, since they can contain more lipids and fatty acids that might represent an energy source to fuel zoospore metabolism (Steinhoff et al. 2011). A longer spore survival time may be particularly favourable at lower host densities, and may explain the unaltered chytrid infections in the low N:P treatment. Conversely, in the high N:P conditions, more zoospores were produced per host biomass but contained less C, N and P per zoospore, suggesting that they were smaller. These findings are supported by earlier observations indicating that the efficiency of spore production by a parasitic dinoflagellate is increased under high N:P conditions, which might result in a higher transmission to new hosts under high host density conditions (Yih and Coats 2000).

At low N:P supply the chytrid seems to produce a low amount of large zoospores, while at high N:P supply it produced a higher amount of small zoospores. The chytrid thus possibly produced smaller spores with a higher production efficiency (Fig 4.4C), suggesting a trade-off between size and production rate as well as success of infection. In other words, larger spores may survive longer providing the chytrid more time to find a suitable host under low host density conditions, while smaller ones survive less long but due to their high numbers achieve a higher infection transmission in high host density conditions. A trade-off between organism size and growth rate has also been reported for various other organisms, including phytoplankton (Nielsen 2006) and zooplankton (Stemberger and Gilbert 1985). Moreover, a trade-off between zoospore survival time and production rate was observed in another chytrid, the amphibian killing fungus Batrachochytrium dendrobatidis (Woodhams et al. 2008). Thus, changes in host N:P stoichiometry may affect the growth strategy of the parasite, following a more general trade-off between cell size and production rates (Fig. 4.4C). Such changes can have consequences not only for the infection dynamics, but also for higher trophic levels that are provided with either many smaller zoospores, or fewer larger ones.

As expected, intracellular MC content closely followed the relative availability of N, and thus increased with cellular N:P ratios (Fig. 4.5). These results are in line with earlier work, showing a strong dependency of MC contents on N availability (Van de Waal et al. 2010a; Van de Waal et al. 2009). In the treatment with high N:P supply and high MC production, however, zoospore production (Fig. 4.4A) and infection rate (Table 4.1) were highest. Additionally, in the low N:P treatment, MC contents was lowest while zoospore production and infection rate were not different from the

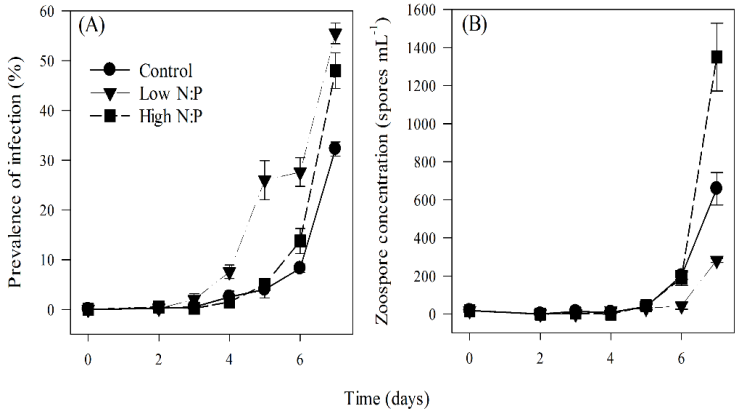

Figure 4.3: Prevalence of infection (A) and zoospore concentration (B) in the control, low N:P and high N:P treatments. Symbols represent mean ± standard error (n = 4).

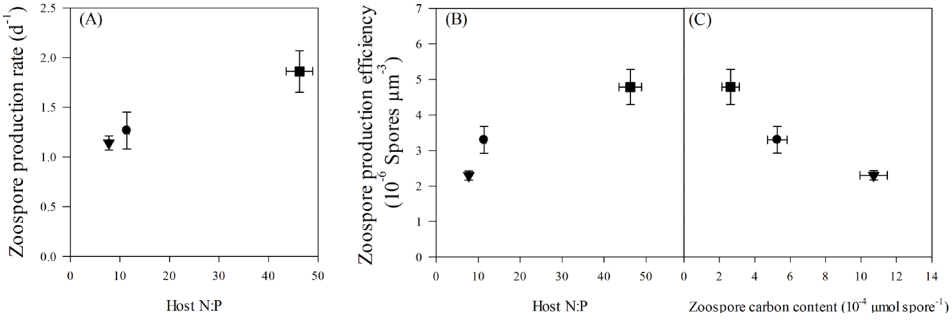

Figure 4.4: Zoospore production rate (A) and production efficiency (B) plotted against host N:P and zoospore carbon content (C) in the control (circle), low N:P (triangle) and high N:P (square) treatments. Symbols represent mean ± standard error (n = 4).

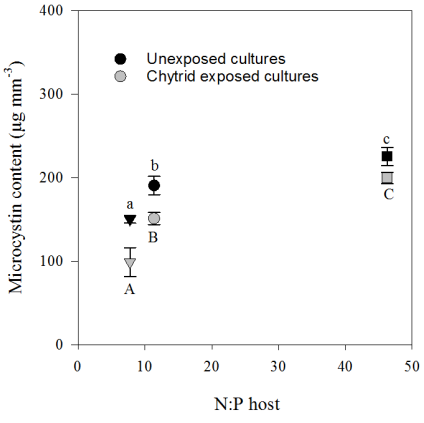

Figure 4.5: Seston microcystin content in the cultures with and without parasite exposure, in the control (circle), low N:P (triangle) and high N:P (square) treatments. Symbols represent mean ± standard error (n = 4). Letters denote significant differences between treatments of unexposed (lower case) and chytrid exposed (upper case) cultures based on One-way ANOVA and post hoc comparison of the means (a < 0.05).

control. So, there was no clear relation between intracellular MC content and chytrid proliferation. Possibly, a considerable fraction of the total MC might be bound to proteins of cyanobacterial cells (Zilliges et al. 2011), which were not included in our extraction processes. Furthermore, *Planktothrix* may produce other oligopeptides that play a role in parasite defence systems (Rohrlack et al. 2013; Sønstebø and Rohrlack 2011), which were not analysed here.

The intracellular MC seemed to be lower in the parasite exposed treatments as compared to the unexposed treatments. This may possibly result from leakage of MC from the cells into the liquid phase (Jones and Orr 1994). Moreover, chytrid rhizoids that invade the host cells might use enzymes that are able to digest MC. Indeed, fungi were shown to be capable of degrading MC (Jia et al. 2012). In our experiment, however, extracellular MC concentrations nor chytrid MC contents were analysed. If MC is released into the water column from cyanobacterial cells, it can have consequences for other organisms present (Carmichael 1992; Zurawell et al. 2005). For instance, high MC concentrations in the water can accumulate in Daphnia (Chen et al. 2005) and have adverse effects on growth and development of fish (Jacquet et al. 2004). Yet, actual exposure of other organisms to MC in the water may be limited, as MCs can be rapidly biodegraded and detoxified by bacteria and adsorb to plants and sediments (Harada and Tsuji 1998; Kato et al. 2007; Pflugmacher et al. 2001). Whether MCs can bind to- or be transported into zoospores is unknown. But, if this would occur, zooplankton might be exposed to MCs via this indirect route, since zoospores can serve as a food source for copepods, cladocerans and possibly rotifers (Agha et al. 2016; Buck et al. 2011; Frenken et al. 2016; Kagami et al. 2011; Kagami et al. 2004; Kagami et al. 2007b).

Our results demonstrate an increase in infection rate with host N:P stoichiometry, thereby showing the opposite to what we hypothesized. Because chytrids seemed relatively more P rich as compared to their host, we initially predicted that host P content would constrain chytrid growth more than it would constrain the host (Bruning 1991a; Bruning and Ringelberg 1987). Our results suggest, however, that chytrid proliferation is much more sensitive to the relative availability of N. Specifically, if this increases (i.e. higher host N:P), infection rates increase, while if this decreases (i.e. lower host N:P), infection rates decrease. This is also shown by the lower flexibility of the parasite N content as compared to P, suggesting that spores are more likely to be constrained under low N conditions. It remains unclear why infection rates increase under P limitation and relative high N contents. Particularly as under these high N:P conditions, MC contents were highest as well. We initially expected that under such conditions, chytrid infections can be inhibited by MCs in its host. The increase in MC content with high N:P conditions, however, was relatively small and may therefore not have been sufficient to inhibit the chytrid infection. Possibly, regulation of other oligopeptides in response to N:P supply could have explained the observed responses, and should thus be included in future analyzes. Moreover, other metabolites synthesized by cyanobacteria under high N:P supply may have facilitated chytrid growth and reproduction. Further detailed biochemical analyses of chytrids and their distinct developmental stages would be required to fully understand the stoichiometric interactions with their hosts, and particularly the putative important role of N in controlling infections.

Our analysis revealed some still poorly understood effects of nutrient availability on the interaction of a host-parasite system. Shifts in nutrient supply ratios not only lead to a shift in host stoichiometry, but also to comparable changes in the parasite. Thereby, we show that elemental stoichiometry of a host can cascade to their parasites. We hypothesize that, in response to changes in nutrient supply, the parasite may exhibit a trade-off between size and zoospore production rate to optimize reproductive success. Therefore, nutrient limitation may indirectly affect parasite abundance and stoichiometry. Since chytrids can facilitate growth of zooplankton (Agha et al. 2016; Kagami et al.

2007b), changes in parasite production, stoichiometry and cell size may have implications for aquatic food web dynamics.

Author Contributions

TF, JW, DW designed the study; JW, TF performed the experiment; TF, JW, AG, DW analysed and interpreted the data. TF and DW wrote a first draft of the manuscript which was corrected, revised and approved by all authors.

Funding

AG is supported by a NWO-veni grant (016.Veni.171.063).

Acknowledgements

We are grateful to Nico Helmsing and Erik Reichman for assistance during the experiment and for analyses of MC and seston elemental composition. We also thank Suzanne Naus-Wiezer for help and advice with microscopic work and Els Faassen for advice on MC analyses.

Chapter 5

Fungal parasite infection of a toxic cyanobacterium provide consumers with an alternative food source

In review at Limnology & Oceanography as

Frenken, T., Wierenga, J., Van Donk, E., Declerck, S.A.J., de Senerpont Domis, L.N., Rohrlack, T., Van de Waal, D.B., Fungal parasites of a toxic inedible cyanobacterium provide food to zooplankton

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 equalled the growth observed for populations fed with a more suitable green algal diet (*Chlorella sorokiniana*). Feeding of *Keratella* on zoospores was furthermore confirmed by a reduction in the number of zoospores. These findings not only underline that rotifers can survive on zoospores, but also that they can maintain high population growth rates. We thus show that parasites can regulate host populations and thereby facilitate other trophic levels by providing them with alternative food sources, emphasizing the important role parasites may play in food webs.

Introduction

During the onset of spring, phytoplankton communities of 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 (De Senerpont Domis et al. 2013; Sommer et al. 1986). Since these taxa are generally less easily ingested by zooplankton, they may represent trophic bottlenecks (Havens 2008; Havens and East 1997). 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 amongst the most common pathogenic agents involved in the regulation of phytoplankton blooms (Brussaard et al. 1995; Brussaard 2004b; Canter and Lund 1951; Frenken et al. 2017a; Frenken et al. 2016; Gerphagnon et al. 2015; Ibelings et al. 2004; Sime-Ngando 2012; Tijdens et al. 2008). 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 (Frenken et al. 2016; Van Donk 1989; Van Donk and Ringelberg 1983) or filamentous cyanobacteria (Gerphagnon et al. 2013b; Gerphagnon et al. 2015; Rasconi et al. 2012). 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 (Agha et al. 2016; Kagami et al. 2007a; Kagami et al. 2004; Kagami et al. 2007b) and as such alleviate trophic bottlenecks associated to blooms of diatoms or filamentous cyanobacteria (Frenken et al. 2016; Rasconi et al. 2012). 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). Additionally, these zoospores can complement the zooplankton diet, since they are rich in polyunsaturated fatty acids and sterols (Kagami et al. 2007b). Chytrids were shown to support somatic growth of cladocerans and facilitate an increased survival of copepods and cladocerans (Agha et al. 2016; Kagami et al. 2011; Kagami et al. 2004; Kagami et al. 2007b). 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 (Meunier et al. 2016; Richman and Rogers 1969), the mechanisms behind improved survival in infected phytoplankton cultures remained unresolved (Kagami et al. 2011).

Previous work has suggested that chytrid zoospores may also serve as a food source to selectively feeding rotifers (Frenken et al. 2016; Schmeller et al. 2014). 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 non-infected *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 & methods

Test organisms and stock culture conditions

Keratella were isolated during spring (April 5, 2016) from a small pond in Wageningen, the Netherlands (coordinates in DMS: 51°59'16.3"N 5°40'06.0"E), five weeks prior to the start of the experiment. For culturing, 20 L of water was filtered over a 30 µm plankton mesh from which *Keratella* 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. (2017b). All cultures used in this study were monoclonal and non-axenic. The host and parasite cultures were prepared as described in Frenken et al. (2017b). 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*. Experiments were performed in six series of five 12-well plates (VWR, Amsterdam, The Netherlands). The first and last 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' samples). See supplementary files for a schematic overview of the experimental setup.

One day before the start of the experiment, *Keratella* were picked from the stock cultures and washed 3 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 plates 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 they were 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. (2013a), 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 (Brussaard (2004a)). 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, Ltd., Paisley, U.K.) for 10 min in the dark at 80ºC (final concentration of 5 x 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 (Fluoresbrite ® Polychromatic Red Microspheres 2.0µm, Polysciences Inc., Hirschberg an der Bergstraße, Germany). This suspension was analysed 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, Bethesda, Maryland, USA). 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 population size in time assuming exponential growth (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 Inc, 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 decrease, especially after 6 days 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* (Table 1, 2). Body length of *Keratella*, body width and volume showed no differences between treatments (Table 1).

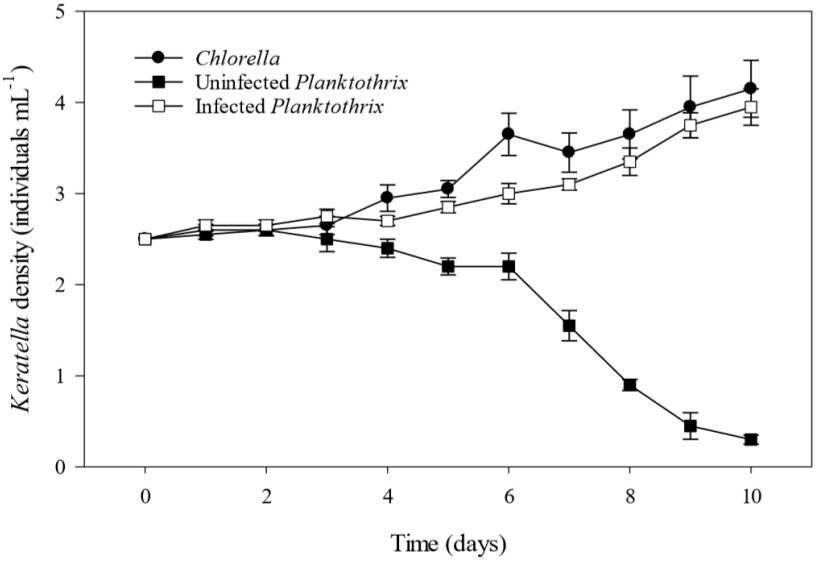

Figure 5.1: Keratella density in the treatments fed with either Chlorella, uninfected Planktothrix, or infected Planktothrix. Values denote mean ± SE (n=5)

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 days 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 1). 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 ($F(1,21)=7$, $p=0.015$) in the *Chlorella* treatment but not in the *Planktothrix* treatments.

Table 5.1: Keratella population growth rate (μ), body length (L), body width (W) and body volume (V). Values denote average with standard deviation between brackets. Superscript represents output of the pairwise comparison between treatments (Tukey test).

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

Discussion

Our results clearly demonstrate that chytrid infections supported *Keratella* population growth when the food source is entirely 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 and 0.09 d^{-1} when fed on *Chlorella*, and between 0.04 and 0.07 d^{-1} when fed with infected *Planktothrix* cultures. These growth rates are largely in line with earlier reported values for *Keratella* feeding on a good quality food source (Walz 1983; Walz 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 thereby provide evidence that a 'mycoloop' may play a considerable role in transferring nutrients from large inedible algae to zooplankton via zoospores of parasitic fungi (Kagami et al. 2007a). 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 to 5 days of exposure. This is comparable to earlier observations, where *Keratella* were fed with the filamentous cyanobacterium *Anabaena flos-aquae* (Gilbert and Durand 1990). Filamentous cyanobacteria generally cannot serve as food for rotifers, or at least poorly support rotifer population growth (Fey et al. 2010; Gilbert and Durand 1990; Rothhaupt 1991; Weithoff and Walz 1995). 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. 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 was starved in the uninfected *Planktothrix* treatment.

Secondly, 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 (De Bernardi and Giussani 1990; Haney 1987; Lampert 1987). 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 unsuitable to be ingested 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. 2017b). 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 (Nandini and Rao 1997; Porter and Orcutt 1980; 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.

Table5.2: Output of the RM ANOVA reporting the significance, degrees of freedom and the F-value of treatment effect on different variables.

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

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; Gobler et al. 1998; Middelboe et al. 1996). Rotifer growth can be supported by bacteria as a sole food source (Arndt 1993; Starkweather et al. 1979). 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. Also, infections might have affected community composition of bacteria. But, 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 is 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 (Freese and Martin-Creuzburg 2013; Martin-Creuzburg et al. 2011).

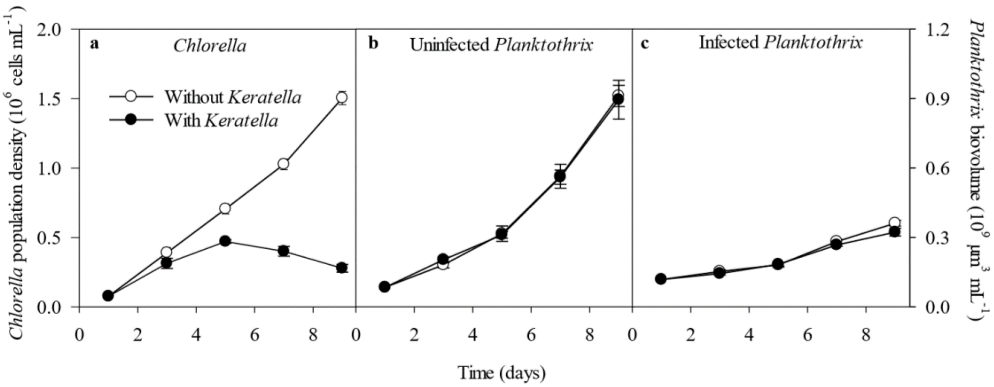

Figure 5.2: Population densities of Chlorella (a) and biovolume of uninfected (b) and infected Planktothrix (c), in treatments with and without Keratella. Values denote mean ±SE (n=5)

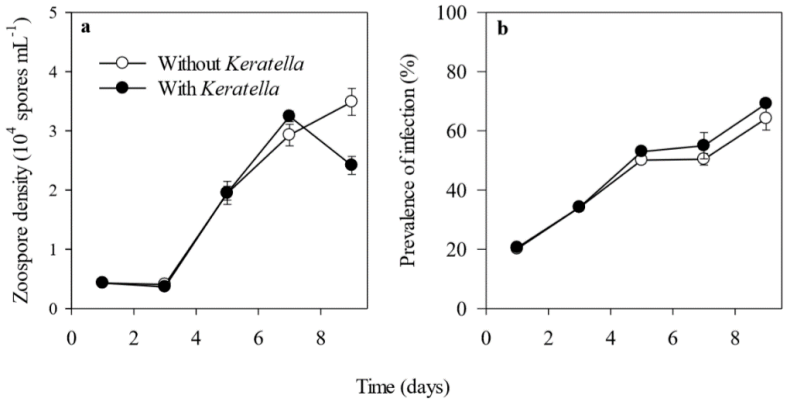

Figure 5.3: Zoospore densities (a) and prevalence of infection (b) in the treatments with and without Keratella. Values denote mean ± SE (n=5).

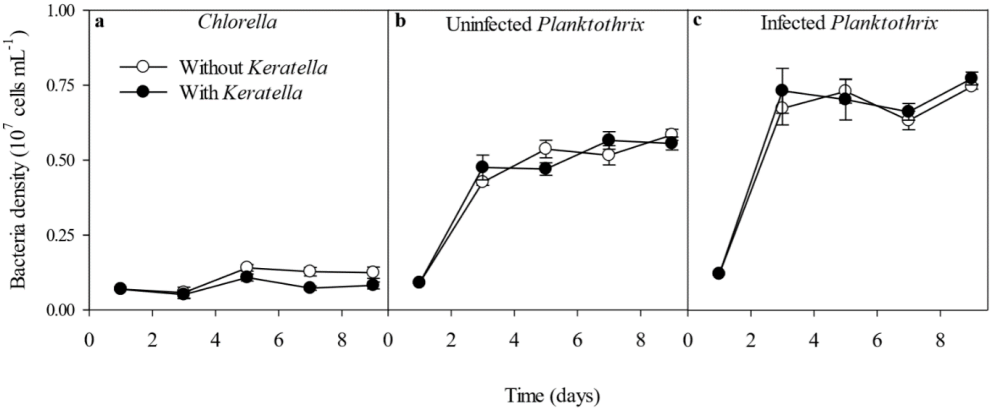

Figure 5.4: Bacteria densities in treatments with Chlorella (a), uninfected Planktothrix (b) and infected Planktothrix (c), with and without Keratella. Values denote mean ± SE (n=5).

Grazing on fungal zoospores by zooplankton has been reported before. Chytrid zoospores are presumed to be a high-quality food source for daphnids and copepods, both in terms of size, shape, and nutritional quality (Kagami et al. 2011; Kagami et al. 2017; Kagami et al. 2007b). Moreover, it has been demonstrated that zooplankton can lower the density of zoospores detected in the water (Schmeller et al. 2014; Searle et al. 2013). As hypothesised 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, 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.

Until now, it was shown that grazing on zoospores can enhance body length of cladocerans (Kagami et al. 2007b) 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). Here, we report the first evidence that chytrids can support population growth of this 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.

Acknowledgements

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*.

Chapter 6

General discussion

Introduction

An ever growing body of evidence indicates that human activities have a major impact on ecosystems worldwide (Ripple et al. 2017). Although we are only at an early stage in the projected trends of global warming, ecological responses to recent climate change are already clearly visible (Walther et al. 2002). Climate change may also affect the occurrence of disease, as was shown in some systems, but not all (Karvonen et al. 2010). As an example, a large amount of research pointed to climate change as the main driver of the large amphibian decline, by promoting an epidemic chytrid disease due to loss of cold winter or night time temperature refuges for the host (Pounds et al. 2006; Skerratt et al. 2007). However, additional analyses found no evidence to support this climate-linked epidemic hypothesis but point towards a role for multiple introductions of the parasite in Central and South America (Lips et al. 2008). Thus, although it is clear that chytrids have contributed to the global decline of amphibians, it is not proven that climate change has been driving outbreaks of chytridiomycosis (Lips et al. 2008). Many factors, such as global warming, climatic anomalies, invasions, as well as ecological factors are interacting and together determine the spread of disease (Altizer et al. 2013; Daszak et al. 2001; Kilpatrick et al. 2010; Lips et al. 2008). This highlights the difficulties encountered in climate-change related disease ecological research. It is therefore important to first gain a better understanding of the environmental factors regulating disease, and afterwards investigate how these are affected by climate change.

The aim of this thesis was to investigate bottom-up and top-down factors that can regulate disease, and how these factors are affected by climate change. I have addressed this question by combining multiple approaches, including a literature review, a large-scale mesocosm experiment and a series of small-scale experiments with phytoplankton-chytrid cultures. In the second chapter I reviewed the current state of knowledge on phytoplankton chytrids ranging from taxonomy and physiology to ecology, highlighting solutions to practical challenges and indicating knowledge gaps. After that, I showed how warming may affect bottom-up and top-down effects in phytoplankton chytrid infections both directly and indirectly via shifts in phytoplankton stoichiometry and zooplankton grazing pressure (chapter 3). Thereafter, I investigated the ecological stoichiometry of a cyanobacterium-chytrid interaction in more detail (chapter 4) and demonstrated that chytrids can be a good food source to support rotifer population growth (chapter 5). Here, I will connect findings of the different chapters. Also, I will provide a short outlook on potential research directions that will contribute to a further understanding of chytrid infection dynamics, and hypothesize how in a future with global change chytrids might play a considerable role in shaping plankton ecology.

Does warmer mean sicker?

Surface water temperatures of lakes have increased rapidly as a result of global warming, both in Europe as in the rest of the world (Adrian et al. 2009). These increases are not distributed equally over the year. For instance, Central European lakes are warming most in spring (Woolway et al. 2017). During spring blooms, the phytoplankton community is often dominated by large celled diatoms, which are poorly edible to zooplankton, but the zoospores of their parasites can serve as food for this higher trophic level (Agha et al. 2016; Kagami et al. 2007b). So if during these periods faster warming of the water column promotes population growth of large diatoms and hence substrate for fungal infections, this may strongly stimulate fungal parasite population size as well. This is in line with earlier work that support the notion of increased top-down control in a warmed future environment (Hoekman 2010; O'Connor et al. 2009; Shurin et al. 2012). Not only the degree but also the timing of warming is important. If lakes, for instance, warm already more strongly in winter, population growth of large phytoplankton may be counteracted by prolonged activity of parasites, i.e. the frequency of 'disease

free' cold winters may decrease resulting in the absence of a large celled diatom spring bloom ((Ibelings et al. 2011), see also **chapter 1**).

Warming may also have other direct impacts on phytoplankton communities. For instance, it is generally expected that smaller cells or smaller species of phytoplankton are favoured under future global warming (Daufresne et al. 2009; Peter and Sommer 2013; Rasconi et al. 2015; Sommer and Lengfellner 2008). These findings are not in line with our experiment. In our warmed treatments, cells of the *Synedra* population were 11% longer as compared to the ambient treatment (**chapter 3**). If cells are spherical shaped, smaller cells are better in competition for nutrients than large ones are (Friebele et al. 1978; Smith and Kalff 1982). Nutrient uptake affinities, however, not only depend on cell size but specifically on the surface:volume ratio (Reynolds et al. 2006) in combination with the cellular requirements of the limiting nutrient (Thingstad et al. 2005). For elongated cells, as our dominant diatom *Synedra* sp., longer cells have a higher surface:volume ratio and therefore may be better competitors for P than small ones are (Grover 1989). Consequently, *Synedra* cells in the warm treatment might have been more efficient in acquiring P, as also indicated by their lower C:P ratio, which in turn might have increased their nutritional quality.

Increased cell sizes may also have several indirect effects. For instance the chytrid *Zygorhizidium* seemed to prefer larger host cells (within a population) as compared to small ones (Ibelings et al. 2004). Also, chytrids tend to be more common on larger sized phytoplankton species that are fairly resistant to grazing by zooplankton, such as diatoms and filamentous cyanobacteria (Holfeld 1998; Sime-Ngando 2012; Sommer 1987). Larger cells might facilitate larger sporangia, which in turn might be associated to a higher zoospore production (Gsell et al. 2013b). For viral infections, it has already been shown that host cell size is directly related to virus burst size (Parada et al. 2006). Consumer-producer interactions, in this case the chytrid-diatom interaction, depend strongly on the relative performance of both trophic levels. So, a host may perhaps increase its growth rate under warmer conditions, but if its parasite responds stronger it may result in a more rapid host population decline. Moreover, due to their larger size, they may also have a higher encounter probability with chytrid zoospores. Indeed, the mesocosm results demonstrated such a positive feedback in which warming resulted in longer, larger sized diatom cells with an improved physiological status (i.e. lower C:P). As a result, its susceptibility to disease, reproductive success of chytrids and encounter rates with zoospores may increase (see also **chapter 4**). Thus, warming does not only directly affect host growth rates and nutrient use efficiency, it also triggers indirect feedback loops that can alter host susceptibility to disease.

Elements of disease

Human activities have increased the amount of nutrients in freshwater bodies (Smith 2003; Smith et al. 2006). Increased inputs of N and P can affect the abundance of infectious diseases and can lead to epidemics (Johnson et al. 2007; Johnson et al. 2010; McKenzie and Townsend 2007; Smith et al. 2015). Indeed, in **chapter 4** I have shown that changes in the availability of nutrients and their ratio has a direct effect on the elemental balance of phytoplankton and its chytrid parasite. Contrary to our expectation, however, high N:P supply associated to P limitation resulted in an increased parasite reproduction and efficiency.

From a P perspective, I hypothesised that chytrids have higher P demands and thus a lower C:P ratio as compared to their hosts, assuming a higher need for P to support RNA production related to fast growth and reproduction (Elser et al. 1996; Sterner and Elser 2002). However, the C:P of chytrids under control conditions was slightly higher than that of their hosts, indicating lower P demands. Moreover, the tested chytrid was more homeostatic with respect to C:P stoichiometry as compared to

its host (table 4.2 and 4.3). From an N perspective, I hypothesised that N would play an important role in host defence mechanisms due to production of N-rich secondary (defence) metabolites (i.e. microcystins and other oligopeptides), thus I expected that high N:P supply would limit and not enhance parasite growth. Although production of microcystin by the cyanobacteria indeed increased under high N:P conditions, it did not result in less infections or zoospores. In fact, infections increased with increasing N:P ratios, and generally seemed to respond to the availability of N. The sensitivity of chytrid infections to N availability is furthermore emphasized by two additional findings. Firstly, during high N:P conditions chytrids can maintain a higher production rate and efficiency, while these decreased upon low N:P conditions. Secondly, zoospores were more flexible in their P-content, whilst they were more homeostatic regarding their N-content.

Luxury consumption, nutrient accumulation and storage are very common ecological mechanisms which occur at least in plants, phytoplankton and mycorrhiza (Bücking and Heyser 1999; Droop 1973; Hammer et al. 2011; Lehman 1976; Lipson et al. 1996; Olsson et al. 2008). The cyanobacterium used in our experiments, *Planktothrix*, possibly stores N in N-rich accessory pigments or storage polymers (Allen 1984; Allen and Smith 1969; Jacquet et al. 2005). Cyanobacteria can also accumulate P in P-rich polyphosphates (Kromkamp 1987; Thompson et al. 1994). Thereby, the amount of N or P in cyanobacteria can be relatively high, but also variable, depending on the growth limiting nutrient in its environment.

For aquatic fungi, much less is known about potential storage of nutrients. A recent paper shows that aquatic fungi can be homeostatic towards their C:N, but are more flexible in terms of C:P and N:P, which suggests that some of these fungi are able to store P as well (Gulis et al. 2017). This is in agreement with the findings in **chapter 4**, where I showed that the zoospores of our chytrid model system are more flexible in P than N. Homeostasis towards N suggests that the tested chytrid has a

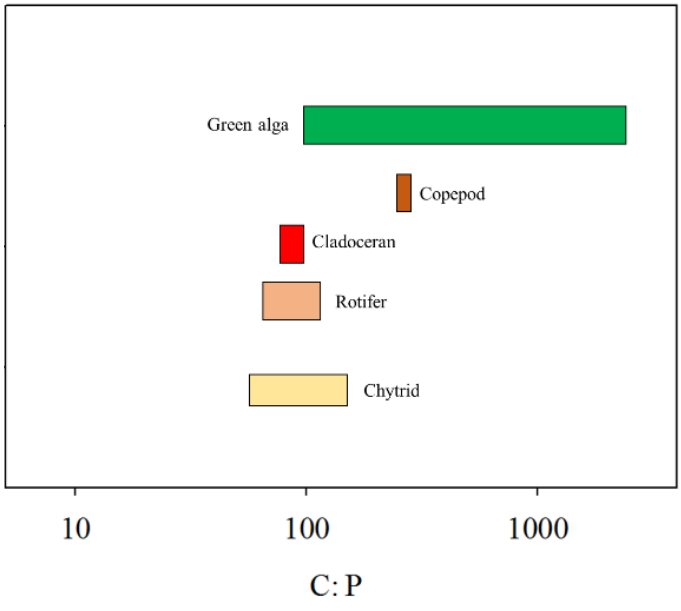

Figure 6.1: Carbon to phosphorus (C:P) stoichiometry of phytoplankton, zooplankton and chytrids. Different colours represent different species. Data extracted from Jensen et al. (2006) and Van de Waal et al. (2010b). Data on chytrids extracted from chapter 4.

relatively higher demand for N as compared to P. Possibly, N is needed for structural compounds and machinery for cell maintenance of zoospores, such as proteins used in the cell structure or enzymes needed for the digestion of phytoplankton host cells. Flexibility towards P suggests that chytrids perhaps accumulate P in their spores as well. This phenomenon was described already for the arbuscular mycorrhizal fungi (AMF). AMF form symbiotic associations with terrestrial plants to exchange mutual benefits in the form of C and nutrients. Although the spores of AMF are considered primarily a carbon reservoir, they can also accumulate mineral nutrients. As an example, during reduced carbon availability spores can accumulate over 7 times more P (Hammer et al. 2011). These nutrients could be important in supporting the growth of a new mycelium from a detached spore to find new host plants (Hammer et al. 2011; Olsson et al. 2008). Hereby, AMF potentially trade-off between growth with the current host plant and investment in spores for localizing new host plants. Similarly, I hypothesized that the used chytrid can also trade-off between production rate and zoospore size (see **chapter 4**). At low N:P ratios, the chytrid produced relatively fewer zoospores, but these contained over 4 times more carbon as compared to the spores at high N:P ratios. Possibly, this higher amount of carbon may fuel a longer survival, providing them with more time to find a suitable host.

Zooplankton can graze on chytrid zoospores ((Agha et al. 2016; Kagami et al. 2007a; Kagami et al. 2007b), see also next paragraph). So if chytrids can store nutrients in spores, this might have implication for their grazers as well. Feeding on stoichiometrically imbalanced food may lower zooplankton performance (Brett et al. 2000; Hessen et al. 2002; Urabe et al. 2003). Under these conditions, excess uptake of carbon needs to be respired, which is a costly process (Darchambeau et al. 2003; Hessen and Anderson 2008; Jensen and Hessen 2007). In nutrient limiting conditions with stoichiometrically imbalanced food, zooplankton may balance their diet by grazing on chytrid zoospores. Chytrid C:P is not only close to the Redfield ratio, but also close to the C:P ratio of some zooplankton, including rotifers (fig 6.1). Additionally, chytrids may also provide a biochemical supplement to the zooplankton diet, since they are rich in polyunsaturated fatty acids and sterols (Kagami et al. 2007b).

To understand and predict how climate change will affect stoichiometric interactions between hosts and parasites, more baseline data on the elemental and biochemical composition of grazers, parasites and hosts is required. As shown in this thesis, parasites may unlock nutrients stored in inedible fractions of the food web, as well as upgrade them from a stoichiometric and biochemical perspective. Thus, parasites can lift or even prevent stoichiometric mismatches in the food web.

Grazers as saviours

Activity of grazers can lead to cascading effects on the dynamics of ecosystem processes such as diseases, carbon sequestration, invasion success and biogeochemical cycles (Estes et al. 2011). Grazers can affect diseases generally in two different ways (see also **chapter 2**). Firstly, they can graze on the hosts, thereby reducing the encounter rate between host and parasite. Secondly, they can graze on the parasite, either on the free living stage or on infected hosts directly, thereby reducing parasite spread and reproduction. Examples of this phenomenon are active predation on infected mule deer by mountain lions (Krumm et al. 2010) or preferential grazing of molluscs on plants infected by rust fungi (Ramsell and Paul 1990). Preferential grazing of higher trophic levels on infected or unhealthy individuals can control disease dynamics in the population as a whole. This 'healthy herd' hypothesis was originally described as a rule of thumb: 'predation quite generally reduces the incidence of infection by specialist pathogens (hence, predators make for 'healthy herds') and in some circumstances predation can increase total prey population size'(Packer et al. 2003). Grazers can thereby safeguard their own food sources.

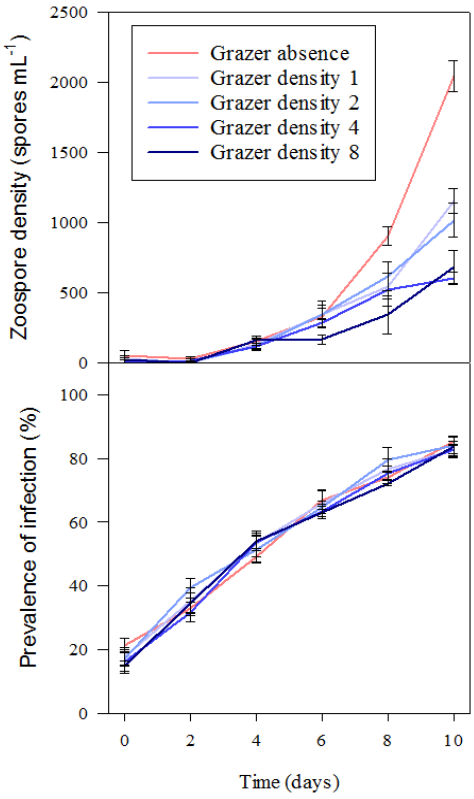

Figure 6.2: Zoospore densities (upper panel) and prevalence of infection (lower panel) in the control (=no grazer) and treatments with Keratella (numbers correspond to relative grazer densities, see table A1). *Values denote mean ± SE (n=5).*

Feeding on fungi is a commonly described phenomenon in terrestrial ecosystems. Some species even exclusively feed on fungi, referred to as mycophagy or fungivory (Leveau and Preston 2008). Fungi generally pose an easy digestible food source since they contain very low amounts of tannin as compared to the woods or plants the fungi grow on (Martin 1979). Also in soils fungi are known to be an important food source to microbes (Dighton and White 2017; Petz et al. 1986). For aquatic systems, feeding on fungi seems to be less commonly described. In the benthic zone, close to or in the top layers of the sediment, palatability of leaf litter to benthic invertebrates is often increased by presence of fungi (Bärlocher and Kendrick 1973; Danger et al. 2012). Although they are an integral part of the macroinvertebrate diet, it is not always clear how much of the fungus actually is consumed (Bärlocher and Kendrick 1975; Marcus and Willoughby 1978). Descriptions of grazers feeding on fungi in the pelagic are relatively limited. Some filter feeders, such as bivalves or zooplankton, in principle can ingest and thereby eliminate fungal spores from the water (Bärlocher and Brendelberger 2004; Kagami et al. 2007a; Schmeller et al. 2014; Searle et al. 2013).

In our grazing experiment (**chapter 5**), zoospores facilitated growth of zooplankton. But also, presence of zooplankton reduced the abundance of chytrid zoospores. This indicates that there might be a potential for top-down control of chytrid fungi by zooplankton. We expected that when zoospores are grazed, less spores are available to infect hosts, which will result in a lowered prevalence of infection. However, this experiment did not reveal direct effects of grazing on the prevalence of

infection. The sporangia possibly produced so many zoospores that, even though a portion of the zoospores was grazed, their density was sufficient to infect all phytoplankton filaments. Moreover, the filamentous cyanobacteria used in our experiments only provide a limited amount of sites for infection (at both ends of one filament). As a result, even a relatively low density of zoospores may be sufficient to occupy all available infection sites.

To investigate if grazing can actually affect prevalence of infection, I performed a preliminary experiment (fig 6.2 and appendix 1) with multiple densities of grazers (the rotifer *Keratella*) and a lower density of zoospores as compared to the experiment in chapter 5. Here, I hypothesized that an increased grazing intensity will lead to a lowered density of chytrid zoospores, resulting in decreased prevalence of infection. Indeed, with increasing grazing pressures the amount of zoospores was reduced (fig 6.2). However, despite reduction of zoospores, no decrease of prevalence of infection was observed. As a result, the potential of this grazer to control chytrid infection on this host might be limited. More work is required to evaluate the role of grazers in disease spread. Ideally, other types of grazers should be tested as well, such as non-selective grazers with a higher clearance rate like molluscs or sponges. Moreover, grazers able to graze on the (large) infected cells, such as copepods, amoeba and planktivorous fish may impose a strong control on disease spread. Besides differences in their grazing strategy, grazers may also differ in their nutritional demands. Copepods, for instance, tend to have higher C:P ratios and therefore lower P demands (Fig. 6.1). Zooplankton grazing on too low C:P food may become limited in energy, and thereby reduce growth or reproduction rates (Plath and Boersma 2001). Thus, the impacts of grazers on chytrid infections will depend on a range of factors, including the selectivity of grazers, their ability to feed on infected cells, as well as their elemental demands. Future studies on top-down control of chytrids by grazers should incorporate these factors when designing new experiments. In this way, novel biological control methods may be developed that can combat a variety of fungal diseases, such as chytrids of amphibians (Schmeller et al. 2014) and possibly even oomycetes of salmon egg in aquaculture.

Closing remarks

The research presented here shows how climate change factors may affect bottom-up and top-down control of phytoplankton disease. Herein, I focused on the effects of warming, nutrient availability and grazing on chytrid infection dynamics. During warmed conditions, chytrids may accelerate termination of diatom and cyanobacterial blooms. Moreover they provide alternative food to zooplankton in the form of chytrid zoospores. During N or P limited conditions (bottom-up), N:P of phytoplankton changes, which has consequences for reproduction of their parasites. During high N:P conditions chytrids can maintain a higher production rate and efficiency, while these decreased upon low N:P conditions. Furthermore, at high N:P conditions zoospore C content, and thereby presumably their size, decreased. From these results we hypothesize that fungal parasites may exhibit a trade-off between zoospore size and production rate to optimize reproductive success. Although our tested species of zooplankton could not control infection prevalence (top-down), chytrids still facilitated their survival and population growth. Fungal parasites thereby re-established trophic links between phytoplankton and zooplankton that are limited during presence of large inedible cyanobacteria or diatoms. This thesis contributes to the very limited body of experimental work that exists on the frontier of phytoplankton chytridiomycosis research, provides a further understanding of the role of chytrids in plankton food webs, and highlights the importance of integrating fungal parasites into plankton ecology.

Appendix

Appendix 1: Preliminary grazing experiment

Background

In chapter 5 I showed that the rotifer *Keratella* can graze and survive on a diet of zoospores. Despite grazing on zoopsores by *Keratella*, I did not find a reduction in the prevalence of infection. This was possibly related to the overall low grazer density. Here, we investigated the role of grazer density on the number of zoospores and success of infection. I hypothesized that an increased grazing pressure will lead to a lowered density of chytrid zoospores, which will lead to a decreased phytoplankton infection prevalence.

Materials & Methods

Test organisms and stock culture conditions

Test organisms were prepared as described in chapter 4 and 5. In short, *Keratella* were grown in sterile 12-well plates (VWR, Amsterdam, The Netherlands). Each well contained 15 randomly picked individuals with 4 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}. 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. *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. All *Planktothrix* and *Chlorella* cultures were grown in batch in 100 mL Erlenmeyers 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 a total of eight treatments (n=4, 32 experimental units) in which *Planktothrix* infected by chytrids were cultured in the presence of 4 different densities of *Keratella* (table A1). Additionally, 3 control treatments were run to control for *Keratella* survival without food or with *Planktothrix* only as well as for *Planktothrix* growth with and without parasite exposure. Experiments were performed in 500 mL Erlenmeyer's flasks with 240 mL culture suspension. The experiment lasted 10 days.

Table A1: Experimental design (0=absence, 1= presence, higher numbers indicate relative inoculation densities).

Treatment	*Planktothrix*	Chytrids	*Keratella*
1	1	0	0
2	1	1	0
3	1	1	1
4	1	1	2
5	1	1	4
6	1	1	8
7	1	0	2
8	0	0	2

To achieve a uniform age distribution in the *Keratella* populations, three days before the start of the experiment (t_{-3}), all individuals (~5,300) were collected in 1 Erlenmeyer and distributed into two 12- well plates. Two days before the experiment (t_{-2}), all individuals were mixed again and washed five times in sterile WC medium to remove *Chlorella*. Subsequently, all *Keratella* were inoculated again in sterile WC medium and left to starve overnight. One day before the experiment (t_{-1}) all individuals were mixed again and washed once more in sterile WC medium.

At the start of experiment (t_0) *Planktothrix* and infected *Planktothrix* cultures were added to the experimental units and diluted to a total biovolume of 1×10^7 $\mu m^{-3} mL^{-1}$. All prepared *Keratella* were collected in one bottle from which different volumes were added to the different grazing treatments. The highest grazing density was set at 2.5 *Keratella* mL^{-1}. All bottles were incubated randomly in a temperature and light controlled incubator at 16 ˚C and 10 µmol photons m^{-2} s^{-1} in a 16:8 light:dark cycle (Infors HT Multitron 2, Infors Benelux

B.V., Doetinchem, The Netherlands). All the bottles were gently shaken once a day and moved to a new random location within the incubator.

Measurements

Bottles were sampled (20 mL) every other day for *Keratella* counts, zoospore density and infection prevalence. Samples were preserved immediately after sampling using 25% glutaraldehyde (Merck, Darmstadt, Germany) to a final concentration of 0.5% (v/v). For zoospores, at least 250 cells were counted, or a minimum of 20 fields of view. Infections were counted as a categorical variable: at least 100 ends of filaments were inspected, which were either infected (i) or uninfected (ui). The prevalence of infection was subsequently calculated as $P = i/(i + ui)$. All counting was performed at a magnification of 400x on an inverted microscope (DMI 4000B, Leica Microsystems CMS GmbH, Mannheim, Germany).

Results

In the control treatment, without a grazer, the density of zoospores steadily increase to about 2,000 spores mL^{-1} within 10 days (fig A1). In the grazed treatments the spore concentration is much lower, with values of about 1200 and 500 spores mL^{-1} for densities 1-2 and 4-8, respectively. Prevalence of infection increases roughly from 20% to 80% in all treatments.

Conclusion

Increased grazing pressure resulted in less zoospores, but had no effect on the prevalence of infection.

List of references

Aalto, S. L., E. Decaestecker, and K. Pulkkinen. 2015. A three-way perspective of stoichiometric changes on host–parasite interactions. Trends in Parasitology **31:** 333-340.

Aalto, S. L., and K. Pulkkinen. 2013. Food stoichiometry affects the outcome of Daphnia–parasite interaction. Ecology and Evolution **3:** 1266-1275.

Adrian, R. and others 2009. Lakes as sentinels of climate change. Limnology and Oceanography **54:** 2283-2297.

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

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

Alacid, E., M. G. Park, M. Turon, K. Petrou, and E. Garcés. 2016. A game of Russian roulette for a generalist dinoflagellate parasitoid: host susceptibility is the key to success. Frontiers in microbiology **7**.

Allen, M. M. 1984. Cyanobacterial Cell Inclusions. Annual Review of Microbiology **38:** 1-25.

Allen, M. M., and A. J. Smith. 1969. Nitrogen chlorosis in blue-green algae. Archiv für Mikrobiologie **69:** 114-120.

Alster, A., and T. Zohary. 2007. Interactions between the bloom-forming dinoflagellate Peridinium gatunense and the chytrid fungus Phlyctochytrium sp. Hydrobiologia **578:** 131-139.

Altizer, S., R. Bartel, and B. A. Han. 2011. Animal Migration and Infectious Disease Risk. Science **331:** 296-302.

Altizer, S., R. S. Ostfeld, P. T. J. Johnson, S. Kutz, and C. D. Harvell. 2013. Climate change and infectious diseases: from evidence to a predictive framework. Science **341:** 514-519.

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. Journal of Animal Ecology **78:** 563-572.

Armstrong, F., C. Stearns, and J. Strickland. 1967. The measurement of upwelling and subsequent biological process by means of the Technicon Autoanalyzer® and associated equipment, p. 381-389. Deep Sea Research and Oceanographic Abstracts. Elsevier.

Arndt, H. 1993. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) — a review. Hydrobiologia **255-256:** 231-246.

Bärlocher, F., and H. Brendelberger. 2004. Clearance of aquatic hyphomycete spores by a benthic suspension feeder. Limnology and Oceanography **49:** 2292-2296.

Bärlocher, F., and B. Kendrick. 1973. Fungi and food preferences of *Gammarus pseudolimnaeus*. Arch. Hydrobiol **72:** 501-516.

Bärlocher, F., and B. Kendrick. 1975. Assimilation efficiency of *Gammarus pseudolimnaeus* (Amphipoda) feeding on fungal mycelium or autumn-shed leaves. Oikos**:** 55-59.

Barr, D., and C. Hickman. 1967. Chytrids and algae: II. Factors influencing parasitism of *Rhizophydium Sphaeropcarpum* on *Spirogyra*. Canadian Journal of Botany **45:** 431-440.

Barr, D. J., and V. Hadland-Hartmann. 1978. Zoospore ultrastructure in the genus *Rhizophydium* (Chytridiales). Canadian Journal of Botany **56:** 2380-2404.

Barr, D. J. S. 1973. Six *Rhizophydium* species (Chytridiales) in culture. Canadian Journal of Botany **51:** 967-975.

---. 2001. Chytridiomycota, p. 93-112. *In* D. J. McLaughlin, E. G. McLaughlin and P. A. Lemke [eds.], Systematics and Evolution: Part A. Springer Berlin Heidelberg.

Beakes, G. W., H. M. Canter, and G. H. Jaworski. 1988. Zoospore ultrastructure of *Zygorhizidium affluens* and *Z. planktonicum*, two chytrids parasitizing the diatom *Asterionella formosa*. Canadian Journal of Botany **66:** 1054-1067.

---. 1992. Ultrastructural study of operculation (discharge apparatus) and zoospore discharge in zoosporangia of *Zygorhizidium affluens* and *Z. planktonicum*, chytrid parasites of the diatom *Asterionella formosa*. Mycological Research **96:** 1060-1067.

Beakes, G. W., H. M. Canter, and G. H. M. Jaworski. 1993. Sporangium differentiation and zoospore fine-structure of the chytrid *Rhizophydium planktonicum*, a fungal parasite of *Asterionella formosa*. Mycological Research **97:** 1059-1074.

Behrenfeld, M. J. and others 2006. Climate-driven trends in contemporary ocean productivity. Nature **444:** 752-755.

Berger, S. A., S. Diehl, T. J. Kunz, D. Albrecht, A. M. Oucible, and S. Ritzer. 2006. Light supply, plankton biomass, and seston stoichiometry in a gradient of lake mixing depths. Limnology and Oceanography **51:** 1898-1905.

Berger, S. A., S. Diehl, H. Stibor, P. Sebastian, and A. Scherz. 2014. Separating effects of climatic drivers and biotic feedbacks on seasonal plankton dynamics: no sign of trophic mismatch. Freshwater Biology **59:** 2204-2220.

Bergh, Ø., K. Y. Børsheim, G. Bratbak, and M. Heldal. 1989. High abundance of viruses found in aquatic environments. Nature **340:** 467-468.

Bogdan, K. G., and J. J. Gilbert. 1982. Seasonal patterns of feeding by natural populations of *Keratella*, *Polyarthra*, and *Bosmina*: Clearance rates, selectivities, and contributions to community grazing. Limnology and Oceanography **27:** 918-934.

---. 1984. Body size and food size in freshwater zooplankton. Proceedings of the National Academy of Sciences **81:** 6427-6431.

Bracken, M. E. S. and others 2014. Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. Oikos **124:** 113-121.

Bratbak, G., J. Egge, and M. Heldal. 1993. Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. Marine Ecology Progress Series **93**.

Bratbak, G., F. Thingstad, and M. Heldal. 1994. Viruses and the microbial loop. Microbial Ecology **28:** 209-221.

Brett, M. T., D. C. Müller-Navarra, and P. Sang-Kyu. 2000. Empirical analysis of the effect of phosphorus limitation on algal food quality for freshwater zooplankton. Limnology and Oceanography **45:** 1564-1575.

Bruning, K. 1991a. Effects of phosphorus limitation on the epidemiology of a chytrid phytoplankton parasite. Freshwater Biology **25:** 409-417.

---. 1991b. Effects of temperature and light on the population dynamics of the *Asterionella-Rhizophydium* association. Journal of plankton research **13:** 707-719.

---. 1991c. Infection of the diatom *Asterionella* by a chytrid. I. Effects of light on reproduction and infectivity of the parasite. Journal of plankton research **13:** 103-117.

---. 1991d. Infection of the diatom *Asterionella* by a chytrid. II. Effects of light on survival and epidemic development of the parasite. Journal of plankton research **13:** 119-129.

Bruning, K., and J. Ringelberg. 1987. The influence of phosphorus limitation of the diatom *Asterionella Formosa* on the zoospore production of its fungal parasite *Rhizophydium Planktonicum*. Hydrobiological Bulletin **21:** 49-54.

Brussaard, C. and others 1995. Effects of grazing, sedimentation and phytoplankton cell lysis on the structure of a coastal pelagic food web. Marine ecology progress series. Oldendorf **123:** 259-271.

Brussaard, C. P. D. 2004a. Optimization of procedures for counting viruses by flow cytometry. Applied and Environmental Microbiology **70:** 1506-1513.

---. 2004b. Viral control of phytoplankton populations—a review. Journal of Eukaryotic Microbiology **51:** 125-138.

Buck, J. C., L. Truong, and A. R. Blaustein. 2011. Predation by zooplankton on *Batrachochytrium dendrobatidis*: biological control of the deadly amphibian chytrid fungus? Biodiversity and Conservation **20:** 3549-3553.

Bücking, H., and W. Heyser. 1999. Elemental composition and function of polyphosphates in ectomycorrhizal fungi—an X-ray microanalytical study. Mycological Research **103:** 31-39.

Burdon, J. J., and T. Elmqvist. 1996. Selective sieves in the epidemiology of *Melampsora lini*. Plant Pathology **45:** 933-943.

Burson, A., M. Stomp, L. Akil, C. P. D. Brussaard, and J. Huisman. 2016. Unbalanced reduction of nutrient loads has created an offshore gradient from phosphorus to nitrogen limitation in the North Sea. Limnology and Oceanography **61:** 869-888.

Butterwick, C., S. I. Heaney, and J. F. Talling. 2005. Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. Freshwater Biology **50:** 291-300.

Canter-Lund, H., and J. W. G. Lund. 1995. Freshwater algae: their microscopic world explored. Biopress Limited.

Canter, H. M. 1946. Studies on British chytrids II. Some new monocentric chytrids. Transactions of the British Mycological Society **31:** 94-105.

Canter, H. M. 1950. Fungal parasites of the phytoplankton. I (Studies on British chytrids, X). Annals of Botany **14:** 263-289.

Canter, H. M. 1961. Studies on British chytrids: XVII. Species occurring on planktonic desmids. Transactions of the British Mycological Society **44:** 163-176.

---. 1967. Studies on British chytrids: XXVI. A critical examination of *Zygorhizidium melosirae* Canter and *Z. planktonicum* Canter. Journal of the Linnean Society of London, Botany **60:** 85-97.

---. 1969. Studies on British Chytrids. XXIX. A taxonomic revision of certain fungi found on the diatom *Asterionella*. Botanical Journal of the Linnean Society **62:** 267-278.

Canter, H. M., and G. Jaworski. 1980. Some General Observations on Zoospores of the Chytrid *Rhizophydium planktonicum* Canter Emend. New Phytologist **84:** 515-531.

Canter, H. M., and G. H. M. Jaworski. 1978. The Isolation, Maintenance and Host Range Studies of a Chytrid *Rhizophydium planktonicum* Canter emend., Parasitic on *Asterionella formosa* Hassall. Annals of Botany **42:** 967-979.

Canter, H. M., and G. H. M. Jaworski. 1979. The occurrence of a hypersensitive reaction in the planktonic diatom *Asterionella formosa hassal* parasitized by the chytrid *Rhizophydium planktonicum* Canter emend., In culture. New Phytologist **82:** 187-206.

Canter, H. M., and G. H. M. Jaworski. 1981. The Effect of Light and Darkness Upon Infection of *Asterionella formosa* Hassall by the Chytrid *Rhizophydium planktonicum* Canter emend. Annals of Botany **47:** 13-30.

Canter, H. M., and G. H. M. Jaworski. 1986. A study on the chytrid *Zygorhizidium planctonicum* Canter, a parasite of the diatoms *Asterionella* and *Synedra. Nova Hedw.* **43:** 269-298.

Canter, H. M., and J. Lund. 1948. Studies on plankton parasites. New Phytologist **47:** 238-261.

---. 1951. Studies on plankton parasites III. Examples of the interaction between parasitism and other factors determining the growth of diatoms. Annals of Botany **15:** 359-371.

Carmichael, W. 1992. Cyanobacteria secondary metabolites—the cyanotoxins. Journal of applied bacteriology **72:** 445-459.

Carney, L. T., and T. W. Lane. 2014. Parasites in algae mass culture. Frontiers in microbiology **5:** 8.

Carpenter, S. R. and others 1996. Chlorophyll Variability, Nutrient Input, and Grazing: Evidence from Whole- Lake Experiments. Ecology **77:** 725-735.

Chen, I.-C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid Range Shifts of Species Associated with High Levels of Climate Warming. Science **333:** 1024-1026.

Chen, W., L. Song, D. Ou, and N. Gan. 2005. Chronic toxicity and responses of several important enzymes in *Daphnia magna* on exposure to sublethal microcystin-LR. Environmental Toxicology **20:** 323-330.

Christoffersen, K., B. Riemann, L. R. Hansen, A. Klysner, and H. B. Sørensen. 1990. Qualitative importance of the microbial loop and plankton community structure in a eutrophic lake during a bloom of cyanobacteria. Microbial Ecology **20:** 253-272.

Cole, J. J. 1982. Interactions between bacteria and algae in aquatic ecosystems. Annual Review of Ecology and Systematics**:** 291-314.

Comeau, A. M., W. F. Vincent, L. Bernier, and C. Lovejoy. 2016. Novel chytrid lineages dominate fungal sequences in diverse marine and freshwater habitats. Scientific Reports **6:** 30120.

Coopman, M., K. Muylaert, B. Lange, L. Reyserhove, and E. Decaestecker. 2014. Context dependency of infectious disease: The cyanobacterium *Microcystis aeruginosa* decreases white bacterial disease in *Daphnia magna*. Freshwater Biology **59:** 714-723.

Danger, M., and E. Chauvet. 2013. Elemental composition and degree of homeostasis of fungi: are aquatic hyphomycetes more like metazoans, bacteria or plants? Fungal Ecology **6:** 453-457.

Danger, M., J. Cornut, A. Elger, and E. Chauvet. 2012. Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa. Freshwater Biology **57:** 1017-1030.

Danger, M., M. O. Gessner, and F. Bärlocher. 2015. Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. Fungal Ecology.

Danovaro, R. and others 2011. Marine viruses and global climate change. FEMS Microbiology Reviews **35:** 993-1034.

Darchambeau, F., P. J. Faerøvig, and D. O. Hessen. 2003. How *Daphnia* copes with excess carbon in its food. Oecologia **136:** 336-346.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica **78:** 103-116.

Daufresne, M., K. Lengfellner, and U. Sommer. 2009. Global warming benefits the small in aquatic ecosystems. Proceedings of the National Academy of Sciences **106:** 12788-12793.

Davis, W. J., P. M. Letcher, J. E. Longcore, and M. J. Powell. 2015. *Fayochytriomyces*, a new genus within Chytridiales. Mycologia **107:** 432-439.

De Bernardi, R. d., and G. Giussani. 1990. Are blue-green algae a suitable food for zooplankton? An overview, p. 29-41. Biomanipulation Tool for Water Management. Springer.

De Bruin, A., B. W. Ibelings, M. Kagami, W. M. Mooij, and E. Van Donk. 2008. Adaptation of the fungal parasite *Zygorhizidium planktonicum d*uring 200 generations of growth on homogeneous and heterogeneous populations of its host, the diatom *Asterionella formosa*. Journal of Eukaryotic Microbiology **55:** 69-74.

De Bruin, A., B. W. Ibelings, M. Rijkeboer, M. Brehm, and E. Van Donk. 2004. Genetic variation in *Asterionella formosa* (*Bacillariophyceae*): is it linked to frequent epidemics of host-specific parasitic fungi? Journal of Phycology **40:** 823-830.

De Senerpont Domis, L. N. and others 2013. Plankton dynamics under different climatic conditions in space and time. Freshwater Biology **58:** 463-482.

De Senerpont Domis, L. N., D. B. Van de Waal, N. R. Helmsing, E. Van Donk, and W. Mooij. 2014. Community stoichiometry in a changing world: Combined effects of warming and eutrophication on phytoplankton dynamics. Ecology **95:** 1485-1495.

De Vargas, C. and others 2015. Eukaryotic plankton diversity in the sunlit ocean. Science **348:** 1261605.

Demott, W. R., and D. Müller-Navarra. 1997. The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. Freshwater Biology **38:** 649-664.

Dighton, J., and J. F. White. 2017. The fungal community: its organization and role in the ecosystem. CRC Press.

Doggett, M. S., and D. Porter. 1995. Further Evidence for Host-Specific Variants in *Zygorhizidium planktonicum*. Mycologia **87:** 161-171.

Doggett, M. S., and D. Porter. 1996a. Fungal parasitism of *Synedra acus*(Bacillariophyceae) and the significance of parasite life history. European Journal of Protistology **32:** 490-497.

Doggett, M. S., and D. Porter. 1996b. Sexual Reproduction in the Fungal Parasite, *Zygorhizidium planktonicum*. Mycologia **88:** 720-732.

Donaldson, S. P., and J. W. Deacon. 1993. Effects of amino acids and sugars on zoospore taxis, encystment and cyst germination in *Pythium aphanidermatum* (Edson) Fitzp., *P. catenulatum* Matthews and *P. dissotocum* Drechs. New Phytologist **123:** 289-295.

Dorsey, J., C. M. Yentsch, S. Mayo, and C. McKenna. 1989. Rapid analytical technique for the assessment of cell metabolic activity in marine microalgae. Cytometry Part A **10:** 622-628.

Droop, M. R. 1973. Some thoughts on nutrient limitation in algae. Journal of Phycology **9:** 264-272.
Eaton, A. D. 2005. Standard methods for the examination of water and wastewater. APHA-AWWA-WEF.
Ebert, D. and others 2016. Chapter Five - A Population Biology Perspective on the Stepwise Infection Process of the Bacterial Pathogen *Pasteuria ramosa* in *Daphnia*, p. 265-310. *In* D. Rollinson and J. R. Stothard [eds.], Advances in Parasitology. Academic Press.
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*). Proceedings of the Royal Society B: Biological Sciences **270:** 1209-1214.
Elser, J. J. and others 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters **10:** 1135-1142.
Elser, J. J., D. R. Dobberfuhl, N. A. MacKay, and J. H. Schampel. 1996. Organism size, life history, and N: P stoichiometry toward a unified view of cellular and ecosystem processes. BioScience **46:** 674-684.
Elser, J. J. and others 2010. Atmospheric nitrogen deposition is associated with elevated phosphorus limitation of lake zooplankton. Ecology Letters **13:** 1256-1261.
Estes, J. A. and others 2011. Trophic downgrading of Planet Earth. Science **333:** 301-306.
Evison, S. E., G. Fazio, P. Chappell, K. Foley, A. B. Jensen, and W. O. Hughes. 2013. Host–parasite genotypic interactions in the honey bee: the dynamics of diversity. Ecology and Evolution **3:** 2214-2222.
Faassen, E., and M. Lürling. 2013. Occurrence of the Microcystins MC-LW and MC-LF in Dutch Surface Waters and Their Contribution to Total Microcystin Toxicity. Marine Drugs **11:** 2643-2654.
Falkowski, P. 2012. Ocean Science: The power of plankton. Nature **483:** S17-S20.
Feuchtmayr, H. and others 2010. Differential effects of warming and nutrient loading on the timing and size of the spring zooplankton peak: An experimental approach with hypertrophic freshwater mesocosms. Journal of plankton research **32:** 1715-1727.
Feuchtmayr, H. and others 2012. Spring phytoplankton phenology – are patterns and drivers of change consistent among lakes in the same climatological region? Freshwater Biology **57:** 331-344.
Fey, S. B., Z. A. Mayer, S. C. Davis, and K. L. Cottingham. 2010. Zooplankton grazing of *Gloeotrichia echinulata* and associated life history consequences. Journal of Plankton Research **32:** 1337-1347.
Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski. 1998. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. Science **281:** 237-240.
Frada, M., I. Probert, M. J. Allen, W. H. Wilson, and C. de Vargas. 2008. The "Cheshire Cat" escape strategy of the coccolithophore *Emiliania huxleyi* in response to viral infection. Proceedings of the National Academy of Sciences **105:** 15944-15949.
Frank, S. A. 1996. Models of Parasite Virulence. The Quarterly Review of Biology **71:** 37-78.
Franklin, D. J. and others 2012. Identification of senescence and death in *Emiliania huxleyi* and *Thalassiosira pseudonana:* cell staining, chlorophyll alterations, and dimethylsulfoniopropionate (DMSP) metabolism. Limnology and Oceanography **57:** 305-317.
Franklin, D. J., C. P. D. Brussaard, and J. A. Berges. 2006. What is the role and nature of programmed cell death in phytoplankton ecology? European Journal of Phycology **41:** 1-14.
Freese, H. M., and D. Martin-Creuzburg. 2013. Food quality of mixed bacteria–algae diets for *Daphnia magna*. Hydrobiologia **715:** 63-76.
Frenken, T. and others 2017a. Integrating chytrid fungal parasites into plankton ecology. Research gaps and needs. Environ. Microbiol. **19:** 3802-3822.
Frenken, T. and others 2016. Warming accelerates termination of a phytoplankton spring bloom by fungal parasites. Global Change Biology **22:** 299-309.
Frenken, T., J. Wierenga, A. S. Gsell, E. Van Donk, T. Rohrlack, and D. B. Van De Waal. 2017b. Changes in N:P Supply Ratios affect the Ecological Stoichiometry of a Toxic Cyanobacterium and its Fungal Parasite. Frontiers in microbiology.

Friebele, E. S., D. L. Correll, and M. A. Faust. 1978. Relationship between phytoplankton cell size and the rate of orthophosphate uptake: in situ observations of an estuarine population. Mar. Biol. **45:** 39-52.

Frost, P. C., D. Ebert, J. H. Larson, M. A. Marcus, N. D. Wagner, and A. Zalewski. 2010. Transgenerational effects of poor elemental food quality on *Daphnia magna*. Oecologia **162:** 865-872.

Frost, P. C., D. Ebert, and V. H. Smith. 2008. Responses of a bacterial pathogen to phosphorus limitation of its aquatic invertebrate host. Ecology **89:** 313-318.

Fuhrman, J., and C. Suttle. 1993. Viruses in marine planktonic systems. Oceanography **6:** 51-63.

Fuhrman, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature **399:** 541-548.

Fuller, M. S., and A. Jaworski. 1987. Zoosporic fungi in teaching & research. Southeastern Publishing Corporation.

Fulton, R. S., and H. W. Paerl. 1987. Toxic and inhibitory effects of the blue-green alga *Microcystis aeruginosa* on herbivorous zooplankton. Journal of Plankton Research **9:** 837-855.

Gachon, C. M. M., T. Sime-Ngando, M. Strittmatter, A. Chambouvet, and G. H. Kim. 2010. Algal diseases: spotlight on a black box. Trends in plant science **15:** 633-640.

Gawad, C., W. Koh, and S. R. Quake. 2016. Single-cell genome sequencing: current state of the science. Nature Reviews Genetics **17:** 175-188.

George, J. A., D. J. Lonsdale, L. R. Merlo, and C. J. Gobler. 2015. The interactive roles of temperature, nutrients, and zooplankton grazing in controlling the winter–spring phytoplankton bloom in a temperate, coastal ecosystem, Long Island Sound. Limnology and Oceanography **60:** 110-126.

Gerla, D. J., A. S. Gsell, B. W. Kooi, B. W. Ibelings, E. Van Donk, and W. M. Mooij. 2013. Alternative states and population crashes in a resource-susceptible-infected model for planktonic parasites and hosts. Freshwater Biology **58:** 538-551.

Gerphagnon, M., D. Latour, J. Colombet, and T. Sime-Ngando. 2013a. A Double Staining Method Using SYTOX Green and Calcofluor White for Studying Fungal Parasites of Phytoplankton. Applied and Environmental Microbiology **79:** 3943-3951.

---. 2013b. Fungal Parasitism: Life Cycle, Dynamics and Impact on Cyanobacterial Blooms. PLoS ONE **8:** e60894.

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.

Gilbert, J. J., and M. W. Durand. 1990. Effect of *Anabaena flos-aquae* on the abilities of *Daphnia* and *Keratella* to feed and reproduce on unicellular algae. Freshwater Biology **24:** 577-596.

Gilbert, J. J., and P. L. Starkweather. 1977. Feeding in the rotifer *Brachionus calyciflorus*. Oecologia **28:** 125-131.

Gillooly, J. F. 2000. Effect of body size and temperature on generation time in zooplankton. Journal of Plankton Research **22:** 241-251.

Gleason, F. H. and others 2015. Potential roles for recently discovered chytrid parasites in the dynamics of harmful algal blooms. Fungal Biology Reviews **29:** 20-33.

Gleason, F. H., M. Kagami, E. Lefevre, and T. Sime-Ngando. 2008. The ecology of chytrids in aquatic ecosystems: roles in food web dynamics. Fungal Biology Reviews **22:** 17-25.

Gleason, F. H. and others 2011. Zoosporic true fungi in marine ecosystems: a review. Marine and Freshwater Research **62:** 383-393.

Gleason, F. H. and others 2014. Ecological functions of zoosporic hyperparasites. Frontiers in microbiology **5**.

Gliwicz, Z. M. 1990. Why do cladocerans fail to control algal blooms? Hydrobiologia **200:** 83-97.

Gobler, C., D. Hutchins, and N. Fisher. 1998. Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. Oceanographic Literature Review **45:** 1523-1524.

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.

Greischar, M. A., and B. Koskella. 2007. A synthesis of experimental work on parasite local adaptation. Ecology Letters **10:** 418-434.

Gromov, B., A. Plujusch, and K. Mamkaeva. 1999. Morphology and possible host range of *Rhizophydium algavorum* sp. Nov..(Chytridiales) an obligate parasite of algae. Protistology **1:** 62-65.

Grossart, H.-P., C. Wurzbacher, T. Y. James, and M. Kagami. 2016. Discovery of dark matter fungi in aquatic ecosystems demands a reappraisal of the phylogeny and ecology of zoosporic fungi. Fungal Ecology **19:** 28-38.

Grover, J. P. 1989. Influence of cell shape and size on algal competitive ability. Journal of Phycology **25:** 402-405.

Gsell, A. S., L. N. de Senerpont Domis, S. M. Naus-Wiezer, N. R. Helmsing, E. Van Donk, and B. W. Ibelings. 2013a. Spatiotemporal variation in the distribution of chytrid parasites in diatom host populations. Freshwater Biology **58:** 523-537.

Gsell, A. S., L. N. De Senerpont Domis, E. Van Donk, and B. W. Ibelings. 2013b. Temperature alters host genotype-specific susceptibility to chytrid infection. PLoS ONE **8:** e71737.

Gsell, A. S., D. Özkundakci, M.-P. Hébert, and R. Adrian. 2016. Quantifying change in pelagic plankton network stability and topology based on empirical long-term data. Ecological Indicators **65:** 76-88.

Guillard, R. R. L., and C. J. Lorenzen. 1972. Yellow-green algae with chlorophyllide c. Journal of Phycology **8:** 10-14.

Gulis, V., K. A. Kuehn, L. N. Schoettle, D. Leach, J. P. Benstead, and A. D. Rosemond. 2017. Changes in nutrient stoichiometry, elemental homeostasis and growth rate of aquatic litter-associated fungi in response to inorganic nutrient supply. The Isme Journal **11:** 2729.

Gutiérrez, M. H., A. M. Jara, and S. Pantoja. 2016. Fungal parasites infect marine diatoms in the upwelling ecosystem of the Humboldt current system off central Chile. Environ. Microbiol.**:** 1646-1653.

Gutman, J., A. Zarka, and S. Boussiba. 2009. The host-range of *Paraphysoderma sedebokerensis*, a chytrid that infects *Haematococcus pluvialis*. European Journal of Phycology **44:** 509-514.

Halsall, D. 1976. Zoospore chemotaxis in Australian isolates of *Phytophthora* species. Canadian Journal of Microbiology **22:** 409-422.

Hamilton, W. 1982. Pathogens as causes of genetic diversity in their host populations. Population biology of infectious diseases**:** 269-296.

Hammer, E. C., J. Pallon, H. Wallander, and P. A. Olsson. 2011. Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. FEMS microbiology ecology **76:** 236-244.

Hampton, S. E. and others 2013. Quantifying effects of abiotic and biotic drivers on community dynamics with multivariate autoregressive (MAR) models. Ecology **94:** 2663-2669.

Haney, J. F. 1987. Field studies on zooplankton-cyanobacteria interactions. New Zealand Journal of Marine and Freshwater Research **21:** 467-475.

Hansson, L.-A. and others 2013. Food-chain length alters community responses to global change in aquatic systems. Nature Climate Change **3:** 228-233.

Harada, K.-I., and K. Tsuji. 1998. Persistence and Decomposition of Hepatotoxic Microcystins Produced by Cyanobacteria in Natural Environment. Journal of Toxicology: Toxin Reviews **17:** 385-403.

Hardenbicker, P., S. Rolinski, M. Weitere, and H. Fischer. 2014. Contrasting long-term trends and shifts in phytoplankton dynamics in two large rivers. International Review of Hydrobiology **99:** 287-299.

Harvell, C. D. and others 2002. Climate warming and disease risks for terrestrial and marine biota. Science **296:** 2158-2162.

Hassett, B. T., A.-L. L. Ducluzeau, R. E. Collins, and R. Gradinger. 2016. Spatial distribution of aquatic marine fungi across the western Arctic and sub-arctic. Environ. Microbiol.

Hassett, B. T., and R. Gradinger. 2016. Chytrids Dominate Arctic Marine Fungal Communities. Environ. Microbiol.**:18:** 2001-2009.

Havens, K. E. 2008. Cyanobacteria blooms: effects on aquatic ecosystems, p. 733-747. *In* H. K. Hudnell [ed.], Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer New York.

Havens, K. E., and T. L. East. 1997. Carbon dynamics in the 'grazing food chain'of a subtropical lake. Journal of Plankton Research **19:** 1687-1711.

Hessen, D. O., and T. Andersen. 1990. Bacteria as a source of phosphorus for zooplankton. Hydrobiologia **206:** 217-223.

Hessen, D. O., and T. R. Anderson. 2008. Excess carbon in aquatic organisms and ecosystems: Physiological, ecological, and evolutionary implications. Limnology and Oceanography **53:** 1685-1696.

Hessen, D. O., J. J. Elser, R. W. Sterner, and J. Urabe. 2013. Ecological stoichiometry: an elementary approach using basic principles. Limnology and Oceanography **58:** 2219-2236.

Hessen, D. O., P. J. Færøvig, and T. Andersen. 2002. Light, nutrients and P:C ratios in algae: grazer performance related to food quality and quantity. Ecology **83:** 1886-1898.

Hibbett, D. and others 2016. Sequence-based classification and identification of Fungi. Mycologia**:** 16-130.

Hillebrand, H., C. D. Dürselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of phycology **35:** 403-424.

Hinch, J. M., and A. E. Clarke. 1980. Adhesion of fungal zoospores to root surfaces is mediated by carbohydrate determinants of the root slime. Physiological Plant Pathology **16:** 303-307.

Hoekman, D. 2010. Turning up the heat: Temperature influences the relative importance of top-down and bottom-up effects. Ecology **91:** 2819-2825.

Holfeld, H. 1998. Fungal infections of the phytoplankton: seasonality, minimal host density, and specificity in a mesotrophic lake. New Phytologist **138:** 507-517.

Howard, R. J., and M. A. Ferrari. 1989. Role of melanin in appressorium function. Experimental Mycology **13:** 403-418.

Hülsmann, S. and others 2012. Effects of winter conditions on *Daphnia* dynamics and genetic diversity in a dimictic temperate reservoir. Freshwater Biology **57:** 1458-1470.

Huntley, M. E., and M. D. G. Lopez. 1992. Temperature-dependent production of marine copepods: A global synthesis. The American Naturalist **140:** 201-242.

Ibelings, B. W., A. De Bruin, M. Kagami, M. Rijkeboer, M. Brehm, and E. Van Donk. 2004. Host parasite interactions between freshwater phytoplankton and chytrid fungi (Chytridiomycota). Journal of phycology **40:** 437-453.

Ibelings, B. W., A. S. Gsell, W. M. Mooij, E. Van Donk, S. Van den Wyngaert, and L. N. De Senerpont Domis. 2011. Chytrid infections and diatom spring blooms: Paradoxical effects of climate warming on fungal epidemics in lakes. Freshwater Biology **56:** 754-766.

Ishida, S., D. Nozaki, H. P. Grossart, and M. Kagami. 2015. Novel basal, fungal lineages from freshwater phytoplankton and lake samples. Environmental microbiology reports **7:** 435-441.

Ives, A. R., S. R. Carpenter, and B. Dennis. 1999. Community Interaction Webs and Zooplankton Responses to Planktivory Manipulations. Ecology **80:** 1405-1421.

Jacobson, R., and R. Doyle. 1996. Lectin-parasite interactions. Parasitology Today **12:** 55-61.

Jacquet, C. and others 2004. Effects of microcystin-LR on development of medaka fish embryos (*Oryzias latipes*). Toxicon **43:** 141-147.

Jacquet, S. and others 2005. The proliferation of the toxic cyanobacterium *Planktothrix rubescens* following restoration of the largest natural French lake (Lac du Bourget). Harmful Algae **4:** 651-672.

James, T. Y. and others 2006. A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia **98:** 860-871.

Jensen, T. C., T. R. Anderson, M. Daufresne, and D. O. Hessen. 2006. Does excess carbon affect respiration of the rotifer *Brachionus calyciflorus* Pallas? Freshwater Biology **51:** 2320-2333.

Jensen, T. C., and D. O. Hessen. 2007. Does excess dietary carbon affect respiration of *Daphnia*? Oecologia **152:** 191-200.

Jephcott, T. G. and others 2015. Ecological impacts of parasitic chytrids, syndiniales and perkinsids on populations of marine photosynthetic dinoflagellates. Fungal Ecology **19**: 47-58.

Jia, Y., J. Du, F. Song, G. Zhao, and X. Tian. 2012. A Fungus Capable of Degrading Microcystin-LR in the Algal Culture of *Microcystis aeruginosa* PCC7806. Applied Biochemistry and Biotechnology **166:** 987-996.

Jobard, M., S. Rasconi, L. Solinhac, H. M. Cauchie, and T. Sime-Ngando. 2012. Molecular and morphological diversity of fungi and the associated functions in three European nearby lakes. Environ. Microbiol. **14:** 2480-2494.

Johnson, P. T. J. and others 2007. Aquatic eutrophication promotes pathogenic infection in amphibians. Proceedings of the National Academy of Sciences **104:** 15781-15786.

Johnson, P. T. J. and others 2010. Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecological Applications **20:** 16-29.

Jones, G. J., and P. T. Orr. 1994. Release and degradation of microcystin following algicide treatment of a *Microcystis aeruginosa* bloom in a recreational lake, as determined by HPLC and protein phosphatase inhibition assay. Water research **28:** 871-876.

Jones, T. M., A. J. Anderson, and P. Albersheim. 1972. Host-pathogen interactions IV. Studies on the polysaccharide-degrading enzymes secreted by *Fusarium oxysporum* f. sp. lycopersici. Physiological Plant Pathology **2:** 153-166.

Joneson, S., J. E. Stajich, S.-H. Shiu, and E. B. Rosenblum. 2011. Genomic Transition to Pathogenicity in Chytrid Fungi. PLoS Pathog **7:** e1002338.

Kagami, M., A. de Bruin, B. W. Ibelings, and E. Van Donk. 2007a. Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. Hydrobiologia **578:** 113-129.

Kagami, M., N. Helmsing, and E. van Donk. 2011. Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms. Hydrobiologia **659:** 49-54.

Kagami, M., B. Ibelings, A. De Bruin, and E. Van Donk. 2005. Vulnerability of *Asterionella formosa* to *Daphnia* grazing: impact of a fungal parasite. Verh Int Verein Limno **29:** 350-354.

Kagami, M., T. Miki, and G. Takimoto. 2014. Mycoloop: chytrids in aquatic food webs. Frontiers in microbiology **5:** 166.

Kagami, M., Y. Motoki, H. Masclaux, and A. Bec. 2017. Carbon and nutrients of indigestible pollen are transferred to zooplankton by chytrid fungi. Freshwater Biology**:** 954-964.

Kagami, M., E. Van Donk, A. de Bruin, M. Rijkeboer, and B. W. Ibelings. 2004. *Daphnia* can protect diatoms from fungal parasitism. Limnology and Oceanography **49:** 680-685.

Kagami, M., E. von Elert, B. W. Ibelings, A. de Bruin, and E. Van Donk. 2007b. The parasitic chytrid, *Zygorhizidium*, facilitates the growth of the cladoceran zooplankter, *Daphnia*, in cultures of the inedible alga, *Asterionella*. Proceedings of the Royal Society B: Biological Sciences **274:** 1561-1566.

Karpov, S., P. Letcher, M. Mamkaeva, and M. KA. 2010. Phylogenetic position of the genus *Mesochytrium* (Chytridiomycota) based on zoospore ultrastructure and sequences from the 18S and 28S rRNA gene. Nova Hedwigia **90:** 81-94.

Karpov, S. A. and others 2014. *Gromochytrium mamkaevae* gen. & sp. nov. and two new orders: Gromochytriales and Mesochytriales (Chytridiomycetes). Persoonia - Molecular Phylogeny and Evolution of Fungi **32:** 115-126.

Karvonen, A., P. Rintamäki, J. Jokela, and E. T. Valtonen. 2010. Increasing water temperature and disease risks in aquatic systems: Climate change increases the risk of some, but not all, diseases. International Journal for Parasitology **40:** 1483-1488.

Kato, H., S. Y. Imanishi, K. Tsuji, and K.-i. Harada. 2007. Microbial degradation of cyanobacterial cyclic peptides. Water Research **41:** 1754-1762.

Kauserud, H., J. E. Colman, and L. Ryvarden. 2008. Relationship between basidiospore size, shape and life history characteristics: a comparison of polypores. Fungal Ecology **1:** 19-23.

Kauserud, H., E. Heegaard, R. Halvorsen, L. Boddy, K. Høiland, and N. C. Stenseth. 2011. Mushroom's spore size and time of fruiting are strongly related: is moisture important? Biology Letters **7:** 273-276.

Keesing, F. and others 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature **468:** 647-652.

Kilpatrick, A. M., C. J. Briggs, and P. Daszak. 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends in Ecology & Evolution **25:** 109-118.

King, K., and C. Lively. 2012. Does genetic diversity limit disease spread in natural host populations? Heredity **109:** 199-203.

Koehler, A. V., Y. P. Springer, H. S. Randhawa, T. L. F. Leung, D. B. Keeney, and R. Poulin. 2012. Genetic and phenotypic influences on clone-level success and host specialization in a generalist parasite. Journal of Evolutionary Biology **25:** 66-79.

Kosten, S. and others 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biology **18:** 118-126.

Kratina, P., H. S. Greig, P. L. Thompson, T. S. A. Carvalho-Pereira, and J. B. Shurin. 2012. Warming modifies trophic cascades and eutrophication in experimental freshwater communities. Ecology **93:** 1421-1430.

Krebs, C. J. 1972. The experimental analysis of distribution and abundance. Ecology. . Harper and Row.

Kromkamp, J. 1987. Formation and functional significance of storage products in cyanobacteria. New Zealand Journal of Marine and Freshwater Research **21:** 457-465.

Krumm, C. E., M. M. Conner, N. T. Hobbs, D. O. Hunter, and M. W. Miller. 2010. Mountain lions prey selectively on prion-infected mule deer. Biology letters **6:** 209-211.

Kudela, R., and R. Dugdale. 2000. Nutrient regulation of phytoplankton productivity in Monterey Bay, California. Deep Sea Research Part II: Topical Studies in Oceanography **47:** 1023-1053.

Kupper, F., I. Maier, D. Müller, S. L.-d. Goer, and L. Guillou. 2006. Phylogenetic affinities of two eukaryotic pathogens of marine macroalgae, E*urychasma dicksonii* (Wright) Magnus and *Chytridium polysiphoniae* Cohn. Cryptogamie-Algologie **27:** 165-184.

Kyle, M., T. Andersen, S. Haande, and T. Rohrlack. 2015. Historical Planktothrix diversity across seven Norwegian lakes implies environmentally driven niche differentiation. Frontiers in Ecology and Evolution **3:** 104.

Lafferty, K. D. 2009. The ecology of climate change and infectious diseases. Ecology **90:** 888-900.

Lafferty, K. D., A. P. Dobson, and A. M. Kuris. 2006. Parasites dominate food web links. Proc. Natl. Acad. Sci. U. S. A. **103:** 11211-11216.

Lafferty, K. D., and E. A. Mordecai. 2016. The rise and fall of infectious disease in a warmer world. F1000Research **5**.

Lampert, W. 1987. Laboratory studies on zooplankton-cyanobacteria interactions. New Zealand Journal of Marine and Freshwater Research **21:** 483-490.

Lampert, W., W. Fleckner, H. Rai, and B. E. Taylor. 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. Limnology and Oceanography **31:** 478-490.

Lefèvre, E. and others 2007. Unveiling fungal zooflagellates as members of freshwater picoeukaryotes: evidence from a molecular diversity study in a deep meromictic lake. Environ. Microbiol. **9:** 61-71.

Lefèvre, E., P. M. Letcher, and M. J. Powell. 2012. Temporal variation of the small eukaryotic community in two freshwater lakes: emphasis on zoosporic fungi. Aquat. Microb. Ecol. **67:** 91-105.

Lehman, J. T. 1976. Photosynthetic capacity and luxury uptake of carbon during phosphate limitation in *Pedriastrum duplex* (Chlorophyceae). Journal of Phycology **12:** 190-193.

Lepelletier, F. and others 2014. *Dinomyces arenysensis* gen. et sp. nov. (Rhizophydiales, Dinomycetaceae fam. nov.), a Chytrid Infecting Marine Dinoflagellates. Protist **165:** 230-244.

Lepère, C., I. Domaizon, and D. Debroas. 2008. Unexpected importance of potential parasites in the composition of the freshwater small-eukaryote community. Applied and Environmental Microbiology **74:** 2940-2949.

Leshem, T., P. M. Letcher, M. J. Powell, and A. Sukenik. 2016. Characterization of a new chytrid species parasitic on the dinoflagellate, *Peridinium gatunense*. Mycologia **108:** 731-743.

Letcher, P., and M. Powell. 2005. Phylogenetic position of *Phlyctochytrium planicorne* (Chytridiales, Chytridiomycota) based on zoospore ultrastructure and partial nuclear LSU rRNA gene sequence analysis. . Nova Hedwigia **80:** 135-146.

Letcher, P. M., M. J. Powell, P. F. Churchill, and J. G. Chambers. 2006. Ultrastructural and molecular phylogenetic delineation of a new order, the *Rhizophydiales* (Chytridiomycota). Mycological Research **110:** 898-915.

Letcher, P. M., M. J. Powell, and W. J. Davis. 2015a. A new family and four new genera in Rhizophydiales (Chytridiomycota). Mycologia **107:** 808-830.

Letcher, P. M., M. J. Powell, S. Lopez, P. A. Lee, and R. C. McBride. 2015b. A new isolate of *Amoeboaphelidium protococcarum*, and *Amoeboaphelidium occidentale*, a new species in phylum Aphelida (Opisthosporidia). Mycologia **107:** 522-531.

Letcher, P. M., M. J. Powell, and K. T. Picard. 2012. Zoospore ultrastructure and phylogenetic position of *Phlyctochytrium aureliae Ajello* is revealed (Chytridiaceae, Chytridiales, Chytridiomycota). Mycologia **104:** 410-418.

Letcher, P. M., C. G. Vélez, M. E. Barrantes, M. J. Powell, P. F. Churchill, and W. S. Wakefield. 2008. Ultrastructural and molecular analyses of Rhizophydiales (Chytridiomycota) isolates from North America and Argentina. Mycological Research **112:** 759-782.

Leung, T. L. F., K. C. King, and J. Wolinska. 2012. Escape from the Red Queen: an overlooked scenario in coevolutionary studies. Oikos **121:** 641-645.

Leveau, J. H. J., and G. M. Preston. 2008. Bacterial mycophagy: definition and diagnosis of a unique bacterial–fungal interaction. New Phytologist **177:** 859-876.

Levitz, S. M. 2010. Innate recognition of fungal cell walls. PLoS Pathog **6:** e1000758.

Lewandowska, A. M., D. G. Boyce, M. Hofmann, B. Matthiessen, U. Sommer, and B. Worm. 2014. Effects of sea surface warming on marine plankton. Ecology Letters **17:** 614-623.

Lips, K. R., J. Diffendorfer, J. R. Mendelson, III, and M. W. Sears. 2008. Riding the Wave: Reconciling the Roles of Disease and Climate Change in Amphibian Declines. PLOS Biology **6:** e72.

Lipson, D. A., W. D. Bowman, and R. K. Monson. 1996. Luxury Uptake and Storage of Nitrogen in the Rhizomatous Alpine Herb, *Bistorta Bistortoides*. Ecology **77:** 1277-1285.

Longcore, J. E., A. P. Pessier, and D. K. Nichols. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia **91**.

Longcore, J. E., and D. R. Simmons. 2012. The Polychytriales ord. nov. contains chitinophilic members of the rhizophlyctoid alliance. Mycologia **104:** 276-294.

Lorenzen, C. 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnology and Oceanography **12:** 343-346.

Maier, M. A., K. Uchii, T. D. Peterson, and M. Kagami. 2016. Evaluation of daphnid grazing on microscopic zoosporic fungi using comparative CT qPCR. Applied and Environmental Microbiology**:** 3868-3874.

Marantelli, G., L. Berger, R. Speare, and L. Keegan. 2004. Distribution of the amphibian chytrid *Batrachochytrium dendrobatidis* and keratin during tadpole development. Pacific Conservation Biology **10:** 173-179.

Marcogliese, D. J. 2008. The impact of climate change on the parasites and infectious diseases of aquatic animals. Rev Sci Tech **27:** 467-484.

Marcus, J. H., and L. G. Willoughby. 1978. Fungi as food for the aquatic invertebrate *Asellus aquaticus*. Transactions of the British Mycological Society **70:** 143-146.

Martin-Creuzburg, D., B. Beck, and H. M. Freese. 2011. Food quality of heterotrophic bacteria for *Daphnia magna*: evidence for a limitation by sterols. FEMS microbiology ecology **76:** 592-601.

Martin-Creuzburg, D., A. Wacker, and E. von Elert. 2005. Life history consequences of sterol availability in the aquatic keystone species *Daphnia*. Oecologia **144:** 362-372.

Martin, M. M. 1979. Biochemical implications of insect mycophagy. Biological reviews **54:** 1-21.

McKenzie, V., and A. Townsend. 2007. Parasitic and Infectious Disease Responses to Changing Global Nutrient Cycles. EcoHealth **4:** 384-396.

Meunier, C. L., M. Boersma, K. H. Wiltshire, and A. M. Malzahn. 2016. Zooplankton eat what they need: copepod selective feeding and potential consequences for marine systems. Oikos **125:** 50-58.

Meunier, C. L., A. M. Malzahn, and M. Boersma. 2014. A new approach to homeostatic regulation: towards a unified view of physiological and ecological concepts. PLOS ONE **9:** e107737.

Middelboe, M., N. Jorgensen, and N. Kroer. 1996. Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. Applied and Environmental Microbiology **62:** 1991-1997.

Middelboe, M., and P. G. Lyck. 2002. Regeneration of dissolved organic matter by viral lysis in marine microbial communities. Aquat. Microb. Ecol. **27:** 187-194.

Miki, T., G. Takimoto, and M. Kagami. 2011. Roles of parasitic fungi in aquatic food webs: a theoretical approach. Freshwater Biology **56:** 1173-1183.

Mitchell, R. T., and J. W. Deacon. 1986. Selective accumulation of zoospores of chytridiomycetes and oomycetes on cellulose and chitin. Transactions of the British Mycological Society **86:** 219-223.

Monchy, S. and others 2011. Exploring and quantifying fungal diversity in freshwater lake ecosystems using rDNA cloning/sequencing and SSU tag pyrosequencing. Environ. Microbiol. **13:** 1433-1453.

Mooij, W. and others 2005. The impact of climate change on lakes in the Netherlands: a review. Aquat Ecol **39:** 381-400.

Morgan, E. R., E. J. Milner-Gulland, P. R. Torgerson, and G. F. Medley. 2004. Ruminating on complexity: macroparasites of wildlife and livestock. Trends in Ecology & Evolution **19:** 181-188.

Moss, A. S., N. S. Reddy, J. I. M. Dorta, and M. J. S. Francisco. 2008. Chemotaxis of the amphibian pathogen *Batrachochytrium dendrobatidis* and its response to a variety of attractants. Mycologia **100:** 1-5.

Muehlstein, L. K., J. P. Amon, and D. L. Leffler. 1988. Chemotaxis in the Marine Fungus *Rhizophydium littoreum*. Applied and Environmental Microbiology **54:** 1668-1672.

Müller, D. G., F. C. Küpper, and H. Küpper. 1999. Infection experiments reveal broad host ranges of *Eurychasma dicksonii* (Oomycota) and *Chytridium polysiphoniae* (Chytridiomycota), two eukaryotic parasites in marine brown algae (Phaeophyceae). Phycological Research **47:** 217-223.

Nandini, S., and T. R. Rao. 1997. Somatic and population growth in selected cladoceran and rotifer species offered the cyanobacterium it *Microcystis aeruginosa* as food. Aquat Ecol **31:** 283-298.

Nielsen, S. L. 2006. Size-dependent growth rates in eukaryotic and prokaryotic algae exemplified by green algae and cyanobacteria: comparisons between unicells and colonial growth forms. Journal of Plankton Research **28:** 489-498.

Niquil, N. and others 2011. 9.07 - Inverse Modeling in Modern Ecology and Application to Coastal Ecosystems, p. 115-133. Treatise on Estuarine and Coastal Science. Academic Press.

Noble, R. T., and J. A. Fuhrman. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. **14:** 113-118.

Nusch, E. A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Archives of Hydrobiology Bulletin **14:** 14-36.

O'Connor, M. I., M. F. Piehler, D. M. Leech, A. Anton, and J. F. Bruno. 2009. Warming and Resource Availability Shift Food Web Structure and Metabolism. PLOS Biology **7:** e1000178.

O'Neil, J. M., T. W. Davis, M. A. Burford, and C. J. Gobler. 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae **14:** 313-334.

Ogden, N. H. and others 2006. Climate change and the potential for range expansion of the Lyme disease vector Ixodes scapularis in Canada. International Journal for Parasitology **36:** 63-70.

Olenina, I. and others 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea. HELCOM Balt, Baltic Sea Environment Proceedings 106 (2006) (ISSN 0357–2994).

Olsson, P. A., E. C. Hammer, H. Wallander, and J. Pallon. 2008. Phosphorus availability influences elemental uptake in the mycorrhizal fungus *Glomus intraradices*, as revealed by particle-induced X-ray emission analysis. Applied and environmental microbiology **74:** 4144-4148.

Orpin, C. G., and L. Bountiff. 1978. Zoospore chemotaxis in the rumen phycomycete *Neocallimastix frontalis*. Microbiology **104:** 113-122.

Özen, A. and others 2013. Long-term effects of warming and nutrients on microbes and other plankton in mesocosms. Freshwater Biology **58:** 483-493.

Pace, M. L., and J. D. Orcutt. 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnology and Oceanography **26:** 822-830.

Packer, C., R. D. Holt, P. J. Hudson, K. D. Lafferty, and A. P. Dobson. 2003. Keeping the herds healthy and alert: implications of predator control for infectious disease. Ecology Letters **6:** 797-802.

Paerl, H. W., R. S. Fulton, P. H. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. The Scientific World Journal **1:** 76-113.

Paerl, H. W., N. S. Hall, and E. S. Calandrino. 2011. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. Science of the Total Environment **409:** 1739-1745.

Paerl, H. W., and J. Huisman. 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environmental Microbiology Reports **1:** 27-37.

Paerl, H. W., and V. J. Paul. 2012. Climate change: Links to global expansion of harmful cyanobacteria. Water Research **46:** 1349-1363.

Parada, V., G. J. Herndl, and M. G. Weinbauer. 2006. Viral burst size of heterotrophic prokaryotes in aquatic systems. Journal of the Marine Biological Association of the United Kingdom **86:** 613-621.

Park, M. G., W. Wih, and D. W. Coats. 2004. Parasites and phytoplankton, with special emphasis on dinoflagellate infections. Journal of Eukaryotic Microbiology **51:** 145-155.

Parker, B. J., S. M. Barribeau, A. M. Laughton, J. C. de Roode, and N. M. Gerardo. 2011. Non-immunological defense in an evolutionary framework. Trends in Ecology & Evolution **26:** 242-248.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature **421:** 37-42.

Paterson, R. A. 1963. Observations on two species of *Rhizophydium* from Northern Michigan. Transactions of the British Mycological Society **46:** 530-536.

Paull, S. H., and P. T. J. Johnson. 2014. Experimental warming drives a seasonal shift in the timing of host-parasite dynamics with consequences for disease risk. Ecology Letters **17:** 445-453.

Persson, J., P. Fink, A. Goto, J. M. Hood, J. Jonas, and S. Kato. 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos **119:** 741-751.

Peter, K. H., and U. Sommer. 2013. Phytoplankton cell size reduction in response to warming mediated by nutrient limitation. PLoS ONE **8:** e71528.

Peters, R. H. 1984. Methods for the study of feeding, grazing and assimilation by zooplankton. A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. IBP Handbook **17:** 336-412.

Petre, B., and S. Kamoun. 2014. How do filamentous pathogens deliver effector proteins into plant cells? PLoS Biology **12:** e1001801.

Petz, W., W. Foissner, E. Wirnsberger, W. D. Krautgartner, and H. Adam. 1986. Mycophagy, a new feeding strategy in autochthonous soil ciliates. Naturwissenschaften **73:** 560-562.

Pflugmacher, S., C. Wiegand, K. A. Beattie, E. Krause, C. E. W. Steinberg, and G. A. Codd. 2001. Uptake, effects, and metabolism of cyanobacterial toxins in the emergent reed plant *Phragmites australis* (Cav.) Trin. ex steud. Environmental Toxicology and Chemistry **20:** 846-852.

Plath, K., and M. Boersma. 2001. Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. Ecology **82:** 1260-1269.

Pohnert, G. 2000. Wound-activated chemical defense in unicellular planktonic algae. Angewandte Chemie International Edition **39:** 4352-4354.

Polovina, J. J., E. A. Howell, and M. Abecassis. 2008. Ocean's least productive waters are expanding. Geophysical Research Letters **35**.

Porter, K. G., and J. Orcutt. 1980. Nutritional adequacy, manageability, and toxicity as factors that determine the food quality of green and blue-green algae for *Daphnia*. Evolution and ecology of zooplankton communities. University Press of New England**:** 268-281.

Poulin, R., B. R. Krasnov, and D. Mouillot. 2011. Host specificity in phylogenetic and geographic space. Trends in Parasitology **27:** 355-361.

Pounds, J. A. and others 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature **439:** 161.

Pourriot, R. 1977. Food and feeding habits of *Rotifera*. Arch. Hydrobiol. Beih **8:** 243-260.

Powell, M. J., P. M. Letcher, J. G. Chambers, and S. Roychoudhury. 2015. A new genus and family for the misclassified chytrid, *Rhizophlyctis harderi*. Mycologia **107:** 419-431.

Proctor, L. M., and J. A. Fuhrman. 1990. Viral mortality of marine-bacteria and cyanobacteria. Nature **343:** 60-62.

R-Core-Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsell, J., and N. D. Paul. 1990. Preferential grazing by molluscs of plants infected by rust fungi. Oikos **58:** 145-150.

Rasconi, S., A. Gall, K. Winter, and M. J. Kainz. 2015. Increasing water temperature triggers dominance of small freshwater plankton. PLoS ONE **10:** e0140449.

Rasconi, S., B. Grami, N. Niquil, M. Jobard, and T. Sime-Ngando. 2014. Parasitic chytrids sustain zooplankton growth during inedible algal bloom. Frontiers in microbiology **5**.

Rasconi, S., M. Jobard, L. Jouve, and T. Sime-Ngando. 2009. Use of calcofluor white for detection, identification, and quantification of phytoplanktonic fungal parasites. Applied and Environmental Microbiology **75:** 2545-2553.

Rasconi, S., M. Jobard, and T. Sime-Ngando. 2011. Parasitic fungi of phytoplankton: ecological roles and implications for microbial food webs. Aquat. Microb. Ecol. **62:** 123-137.

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.

Reynolds, C. 1973. The seasonal periodicity of planktonic diatoms in a shallow eutrophic lake. Freshwater Biology **3:** 89-110.

Reynolds, C. S. and others 2006. Ecology of phytoplankton. Cambridge University Press Cambridge.

Richman, S., and J. N. Rogers. 1969. The feeding of *Calanus helgolandicus* on synchronously growing population of the marine diatom *Ditylum brightwellii*. Limnology and Oceanography **14:** 701-709.

Ripple, W. J. and others 2017. World Scientists' Warning to Humanity: A Second Notice. BioScience **67:** 1026-1028.

Rohrlack, T., G. Christiansen, and R. Kurmayer. 2013. Putative antiparasite defensive system involving ribosomal and nonribosomal oligopeptides in cyanobacteria of the genus *Planktothrix*. Applied and Environmental Microbiology **79:** 2642-2647.

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*. Applied and Environmental Microbiology **65:** 737-739.

Rohrlack, T., S. Haande, Å. Molversmyr, and M. Kyle. 2015. Environmental conditions determine the course and outcome of phytoplankton Chytridiomycosis. PLoS ONE **10:** e0145559.

Rolinski, S., H. Horn, T. Petzoldt, and L. Paul. 2007. Identifying cardinal dates in phytoplankton time series to enable the analysis of long-term trends. Oecologia **153:** 997-1008.

Ronneberger, D. 1998. Uptake of latex beads as size-model for food of planktonic rotifers. Hydrobiologia **387:** 445-449.

Rothhaupt, K. O. 1991. The influence of toxic and filamentous blue-green algae on feeding and population growth of the rotifer *Brachionus rubens*. International Review of Hydrobiology **76:** 67-72.

Rusconi, R., M. Garren, and R. Stocker. 2014. Microfluidics expanding the frontiers of microbial ecology. Annual Review of Biophysics **43:** 65–91.

Rutschmann, S., H. Detering, S. Simon, J. Fredslund, and M. T. Monaghan. 2016. Data from: DISCOMARK: nuclear marker discovery from orthologous sequences using draft genome data. 257-266.

Schindler, D., and E. Fee. 1974. Experimental lakes area: whole-lake experiments in eutrophication. Journal of the Fisheries Board of Canada **31:** 937-953.

Schmeller, D. S. and others 2014. Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. Current Biology **24:** 176-180.

Schmid-Hempel, P. 2008. Parasite immune evasion: a momentous molecular war. Trends in Ecology & Evolution **23:** 318-326.

Schmidt, K., and S. Jonasdottir. 1997. Nutritional quality of two cyanobacteria: How rich is' poor'food? Marine Ecology-Progress Series **151:** 1-10.

Schoch, C. L. and others 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences **109:** 6241-6246.

Scholz, B., C. F. Küpper, W. Vyverman, G. H. Ólafsson, and U. Karsten. 2017. Chytridiomycosis of marine diatoms—The role of stress physiology and resistance in parasite-host recognition and accumulation of defense molecules. Marine Drugs **15:** 26.

Schulte, R. D., C. Makus, B. Hasert, N. K. Michiels, and H. Schulenburg. 2010. Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. Proceedings of the National Academy of Sciences **107:** 7359-7364.

Searle, C. L., J. R. Mendelson, L. E. Green, and M. A. Duffy. 2013. *Daphnia* predation on the amphibian chytrid fungus and its impacts on disease risk in tadpoles. Ecology and evolution **3:** 4129-4138.

Seto, K., M. Kagami, and Y. Degawa. 2016. Phylogenetic position of parasitic chytrids on diatoms: characterization of a novel clade in Chytridiomycota. Journal of Eukaryotic Microbiology.

Shin, W., S. M. Boo, and J. E. Longcore. 2001. *Entophlyctis apiculata*, a chytrid parasite of *Chlamydomonas* sp. (Chlorophyceae). Canadian Journal of Botany **79:** 1083-1089.

Shurin, J. B., J. L. Clasen, H. S. Greig, P. Kratina, and P. L. Thompson. 2012. Warming shifts top-down and bottom-up control of pond food web structure and function. Philosophical Transactions of the Royal Society B: Biological Sciences **367:** 3008-3017.

Sidak, Z. 1967. Rectangular confidence regions for the means of multivariate normal distributions. Journal of the American Statistical Association **62:** 626-633.

Sikora, S., A. Strongin, and A. Godzik. 2005. Convergent evolution as a mechanism for pathogenic adaptation. Trends in Microbiology **13:** 522-527.

Sime-Ngando , T. 2012. Phytoplankton chytridiomycosis: fungal parasites of phytoplankton and their imprints on the food web dynamics. Frontiers in microbiology **3**.

Simmons, D. R. 2011. Phylogeny of *Powellomycetaceae* fam. nov. and description of *Geranomyces variabilis* gen. et comb. nov. Mycologia **103:** 1411-1420.

Skerratt, L. F. and others 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth **4:** 125.

Skjånes, K., C. Rebours, and P. Lindblad. 2013. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Critical reviews in biotechnology **33:** 172-215.

Smith, R. E. H., and J. Kalff. 1982. Size-dependent phosphorus uptake kinetics and cell quota in phytoplankton. Journal of Phycology **18:** 275-284.

Smith, V. H. 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environmental Science and Pollution Research **10:** 126-139.

---. 2007. Host resource supplies influence the dynamics and outcome of infectious disease. Integrative and Comparative Biology **47:** 310-316.

Smith, V. H., R. D. Holt, M. S. Smith, Y. Niu, and M. Barfield. 2015. Resources, mortality, and disease ecology: importance of positive feedbacks between host growth rate and pathogen dynamics. Israel Journal of Ecology & Evolution **61:** 37-49.

Smith, V. H., S. B. Joye, and R. W. Howarth. 2006. Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography **51:** 351-355.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends in Ecology & Evolution **24:** 201-207.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution **100:** 179-196.

Soares, M. C. S., M. Lürling, and V. L. Huszar. 2010. Responses of the rotifer *Brachionus calyciflorus* to two tropical toxic cyanobacteria (*Cylindrospermopsis raciborskii* and *Microcystis aeruginosa*) in pure and mixed diets with green algae. Journal of Plankton Research **32:** 999-1008.

Sommer, U. 1987. Factors controlling the seasonal variation in phytoplankton species composition—a case study for a deep, nutrient rich lake. Progr. Phycol. Res. **5:** 124-178.

Sommer, U. and others 2012. Beyond the plankton ecology group (PEG) model: mechanisms driving plankton succession. Annu. Rev. Ecol. Evol. Syst. **43:** 429-448.

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.

Sommer, U., and K. Lengfellner. 2008. Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. Global Change Biology **14:** 1199-1208.

Sommer, U., and A. Lewandowska. 2011. Climate change and the phytoplankton spring bloom: warming and overwintering zooplankton have similar effects on phytoplankton. Global Change Biology **17:** 154-162.

Sønstebø, J. H., and T. Rohrlack. 2011. Possible implications of chytrid parasitism for population subdivision in freshwater cyanobacteria of the genus *Planktothrix*. Applied and Environmental Microbiology **77:** 1344-1351.

Sparrow, F. K. 1960. Aquatic Phycomycetes. University of Michigan Press.

---. 1968. Ecology of freshwater fungi, p. 41-93. *In* G. Ainsworth and A. Sussman [eds.], The fungi - an advanced treatise. The fungal population. Academia Press.

Stam, R. and others 2013. Identification and characterisation CRN effectors in *Phytophthora capsici* shows modularity and functional diversity. PLoS One **8:** e59517.

Starkweather, P., and K. Bogdan. 1980. Detrital feeding in natural zooplankton communities: discrimination between live and dead algal foods, p. 83-85. *In* H. J. Dumont and J. Green [eds.], Rotatoria. Developments in Hydrobiology. Springer Netherlands.

Starkweather, P., J. Gilbert, and T. Frost. 1979. Bacterial feeding by the rotifer *Brachionus calyciflorus*: Clearance and ingestion rates, behavior and population dynamics. Oecologia **44:** 26-30.

Steinhoff, F. S., M. Graeve, C. Wiencke, A. Wulff, and K. Bischof. 2011. Lipid content and fatty acid consumption in zoospores/developing gametophytes of *Saccharina latissima* (Laminariales, Phaeophyceae) as potential precursors for secondary metabolites as phlorotannins. Polar Biology **34:** 1011-1018.

Stemberger, R. S. 1981. A general approach to the culture of planktonic rotifers. Canadian Journal of Fisheries and Aquatic Sciences **38:** 721-724.

Stemberger, R. S., and J. J. Gilbert. 1984. Spine development in the rotifer *Keratella cochlearis*: Induction by cyclopoid copepods and *Asplanchna*. Freshwater Biology **14:** 639-647.

Stemberger, R. S., and J. J. Gilbert. 1985. Body size, food concentration, and population growth in planktonic rotifers. Ecology **66:** 1151-1159.

Sterner, R. W. and others 2007. Increasing stoichiometric imbalance in North America's largest lake: Nitrification in Lake Superior. Geophysical Research Letters **34:** 1-5.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press.

Stevens, S. S. 1960. The psychophysics of sensory function. American Scientist **48:** 226-253.

Stielow, J. B. and others 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia - Molecular Phylogeny and Evolution of Fungi **35:** 242-263.

Stirnadel, H. A., and D. Ebert. 1997. Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. Journal of Animal Ecology **66:** 212-222.

Stocker, T. F. and others 2013. Climate change 2013: The physical science basis. Cambridge University Press.

Strecker, A. L., T. P. Cobb, and R. D. Vinebrooke. 2004. Effects of experimental greenhouse warming on phytoplankton and zooplankton communities in fishless alpine ponds. Limnology and Oceanography **49:** 1182-1190.

Sugihara, G. and others 2012. Detecting causality in complex ecosystems. Science **338:** 496-500.

Suttle, C. A., A. M. Chan, and M. T. Cottrell. 1990. Infection of phytoplankton by viruses and reduction of primary productivity. Nature **347:** 467-469.

Thingstad, T., and R. Lignell. 1997. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquat. Microb. Ecol. **13:** 19-27.

Thingstad, T. F., L. Øvreås, J. K. Egge, T. Løvdal, and M. Heldal. 2005. Use of non-limiting substrates to increase size; a generic strategy to simultaneously optimize uptake and minimize predation in pelagic osmotrophs? Ecology Letters **8:** 675-682.

Thompson, P.-A., H.-M. Oh, and G. Y. Rhee. 1994. Storage of phosphorus in nitrogen-fixing *Anabaena flos-aquae* (cyanophyceae). Journal of Phycology **30:** 267-273.

Tijdens, M., D. B. Van De Waal, H. Slovackova, H. L. Hoogveld, and H. J. Gons. 2008. Estimates of bacterial and phytoplankton mortality caused by viral lysis and microzooplankton grazing in a shallow eutrophic lake. Freshwater Biology **53:** 1126-1141.

Toseland, A. and others 2013. The impact of temperature on marine phytoplankton resource allocation and metabolism. Nature Climate Change **3:** 979-984.

Turner, J. T., and P. A. Tester. 1989. Zooplankton feeding ecology: nonselective grazing by the copepods *Acartia tonsa* Dana, *Centropages velificatus* De Oliveira, and *Eucalanus pileatus* Giesbrecht in the plume of the Mississippi River. Journal of Experimental Marine Biology and Ecology **126:** 21-43.

Urabe, J., J. U. N. Togari, and J. J. Elser. 2003. Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. Global Change Biology **9:** 818-825.

Van de Waal, D. B. and others 2010a. Pulsed nitrogen supply induces dynamic changes in the amino acid composition and microcystin production of the harmful cyanobacterium Planktothrix agardhii. FEMS microbiology ecology **74:** 430-438.

Van de Waal, D. B., V. H. Smith, S. A. J. Declerck, E. C. M. Stam, and J. J. Elser. 2014. Stoichiometric regulation of phytoplankton toxins. Ecology Letters **17:** 736-742.

Van de Waal, D. B., A. M. Verschoor, J. M. Verspagen, E. van Donk, and J. Huisman. 2010b. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Frontiers in Ecology and the Environment **8:** 145-152.

Van de Waal, D. B., J. M. Verspagen, M. Lürling, E. Van Donk, P. M. Visser, and J. Huisman. 2009. The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis. Ecology Letters **12:** 1326-1335.

Van den Wyngaert, S., M. Möst, R. Freimann, B. W. Ibelings, and P. Spaak. 2015. Hidden diversity in the freshwater planktonic diatom *Asterionella formosa*. Molecular Ecology**:** n/a-n/a.

Van den Wyngaert, S., K. Seto, K. Rojas-Jimenez, M. Kagami, and H.-P. Grossart. 2017. A New Parasitic Chytrid, *Staurastromyces oculus* (Rhizophydiales, Staurastromycetaceae fam. nov.), Infecting the Freshwater Desmid *Staurastrum* sp. Protist.

Van den Wyngaert, S., O. Vanholsbeeck, P. Spaak, and B. W. Ibelings. 2014. Parasite fitness traits under environmental variation: Disentangling the roles of a chytrid's immediate host and external environment. Microbial Ecology **68:** 645-656.

Van Donk, E. 1984. Factors influencing phytoplankton growth and succession in Lake Maarsseveen. Hydrobiological Bulletin **18:** 69-71.
---. 1989. The role of fungal parasites in phytoplankton succession, p. 171-194. Plankton Ecology. Springer Berlin Heidelberg.
Van Donk, E., and K. Bruning. 1992. Ecology of aquatic fungi in and on algae. Algal Symbiosis-Plants, Animals, Fungi, Interactions Explored.
Van Donk, E., and S. S. Kilham. 1990. Temperature effects on silicon- and phosphorus- limited growth and competitive interactions among three diatoms. Journal of Phycology **26:** 40-50.
Van Donk, E., and J. Ringelberg. 1983. The effect of fungal parasitism on the succession of diatoms in Lake Maarsseveen (The Netherlands). Freshwater Biology **13:** 241-251.
van Oevelen, D., K. Van den Meersche, F. J. Meysman, K. Soetaert, J. J. Middelburg, and A. F. Vézina. 2010. Quantifying food web flows using linear inverse models. Ecosystems **13:** 32-45.
Van Rooij, P. and others 2012. Germ Tube Mediated Invasion of *Batrachochytrium dendrobatidis* in Amphibian Skin Is Host Dependent. PLoS One **7:** e41481.
Vélez, C. G., P. M. Letcher, S. Schultz, G. Mataloni, E. Lefèvre, and M. J. Powell. 2013. Three new genera in Chytridiales from aquatic habitats in Argentina. Mycologia **105:** 1251-1265.
Vélez, C. G., P. M. Letcher, S. Schultz, M. J. Powell, and P. F. Churchill. 2011. Molecular phylogenetic and zoospore ultrastructural analyses of *Chytridium olla* establish the limits of a monophyletic Chytridiales. Mycologia **103:** 118-130.
Velthuis, M. and others 2017. Warming advances top-down control and reduces producer biomass in a freshwater plankton community. Ecosphere **8:** e01651.
Verschoor, A. M., J. Takken, B. Massieux, and J. Vijverberg. 2003. The Limnotrons: A facility for experimental community and food web research. Hydrobiologia **491:** 357-377.
Větrovský, T., M. Kolařík, L. Žifčáková, T. Zelenka, and P. Baldrian. 2016. The rpb2 gene represents a viable alternative molecular marker for the analysis of environmental fungal communities. Molecular Ecology Resources **16:** 388-401.
Vézina, A. F. 1989. Construction of flow networks using inverse methods, p. 62-81. Network Analysis in Marine Ecology. Springer.
Vézina, A. F., F. Berreville, and S. Loza. 2004. Inverse reconstructions of ecosystem flows in investigating regime shifts: impact of the choice of objective function. Progress in Oceanography **60:** 321-341.
Vidal, J. 1980. Physioecology of zooplankton. I. Effects of phytoplankton concentration, temperature, and body size on the growth rate of *Calanus pacificus* and *Pseudocalanus sp*. Mar. Biol. **56:** 111-134.
Vogels, C. B. F., G. P. Göertz, G. P. Pijlman, and C. J. M. Koenraadt. 2017a. Vector competence of European mosquitoes for West Nile virus. Emerging Microbes &Amp; Infections **6:** e96.
Vogels, C. B. F., G. P. Göertz, G. P. Pijlman, and C. J. M. Koenraadt. 2017b. Vector competence of northern and southern European *Culex pipiens* pipiens mosquitoes for West Nile virus across a gradient of temperatures. Medical and Veterinary Entomology **31:** 358-364.
Vrede, T., T. Andersen, and D. O. Hessen. 1999. Phosphorus distribution in three crustacean zooplankton species. Limnology and Oceanography **44:** 225-229.
Wacker, A., and D. Martin-Creuzburg. 2012. Biochemical nutrient requirements of the rotifer *Brachionus calyciflorus*: co-limitation by sterols and amino acids. Functional Ecology **26:** 1135-1143.
Wakefield, W. S. and others 2010. A molecular phylogenetic evaluation of the Spizellomycetales. Mycologia **102:** 596-604.
Walther, G.-R. 2010. Community and ecosystem responses to recent climate change. Philosophical Transactions of the Royal Society B: Biological Sciences **365:** 2019-2024.
Walther, G.-R. and others 2002. Ecological responses to recent climate change. Nature **416:** 389-395.
Walz, N. 1983. Individual culture and experimental population dynamics of *Keratella cochlearis* (Rotatoria). Hydrobiologia **107:** 35-45.

---. 1987. Comparative population dynamics of the rotifers *Brachionus angularis* and *Keratella cochlearis*, p. 209-213. Rotifer Symposium IV. Springer.

Weinbauer, M. G., and F. Rassoulzadegan. 2004. Are viruses driving microbial diversification and diversity? Environ. Microbiol. **6:** 1-11.

Weisse, T., and A. Frahm. 2002. Direct and indirect impact of two common rotifer species (*Keratella spp.*) on two abundant ciliate species (*Urotricha furcata, Balanion planctonicum*). Freshwater Biology **47:** 53-64.

Weithoff, G., and N. Walz. 1995. Influence of the filamentous cyanobacterium *Planktothrix agardhii* on population growth and reproductive pattern of the rotifer *Brachionus calyciflorus*. Hydrobiologia **313:** 381-386.

Welker, M., G. Christiansen, and H. von Döhren. 2004. Diversity of coexisting *Planktothrix* (Cyanobacteria) chemotypes deduced by mass spectral analysis of microystins and other oligopeptides. Arch Microbiol **182:** 288-298.

Wilhelm, S. W., and C. A. Suttle. 1999. Viruses and Nutrient Cycles in the Sea Viruses play critical roles in the structure and function of aquatic food webs. Bioscience **49:** 781-788.

Winder, M., and D. E. Schindler. 2004. Climatic effects on the phenology of lake processes. Global Change Biology **10:** 1844-1856.

Wolinska, J., and K. C. King. 2009. Environment can alter selection in host–parasite interactions. Trends in Parasitology **25:** 236-244.

Woodhams, D. C., R. A. Alford, C. J. Briggs, M. Johnson, and L. A. Rollins-Smith. 2008. Life-history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. Ecology **89:** 1627-1639.

Woodward, G., D. M. Perkins, and L. E. Brown. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philosophical Transactions of the Royal Society B: Biological Sciences **365:** 2093-2106.

Woolhouse, M. E., J. P. Webster, E. Domingo, B. Charlesworth, and B. R. Levin. 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nature Genetics **32:** 569-577.

Woolway, R. I., M. T. Dokulil, W. Marszelewski, M. Schmid, D. Bouffard, and C. J. Merchant. 2017. Warming of Central European lakes and their response to the 1980s climate regime shift. Climatic Change **142:** 505-520.

Wurzbacher, C., S. Rösel, A. Rychła, and H.-P. Grossart. 2014. Importance of saprotrophic freshwater fungi for pollen degradation. PLOS ONE **9:** e94643.

Yih, W., and D. W. Coats. 2000. Infection of *Gymnodinium sanguineum* by the Dinoflagellate *Amoebophrya* sp.: Effect of nutrient environment on parasite generation time, reproduction, and infectivity. Journal of Eukaryotic Microbiology **47:** 504-510.

Zilliges, Y. and others 2011. The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. PloS one **6:** e17615.

Zurawell, R. W., H. Chen, J. M. Burke, and E. E. Prepas. 2005. Hepatotoxic Cyanobacteria: A Review of the Biological Importance of Microcystins in Freshwater Environments. Journal of Toxicology and Environmental Health, Part B **8:** 1-37.

English summary

Human activities have a major impact on ecosystems worldwide. Although we are only at an early stage in the projected trends of global warming, we do not really know and understand its consequences on aquatic ecosystems yet. Emission of greenhouse gasses, such as carbon dioxide, methane and nitrous oxide has resulted in global warming. Warming and nutrient rich conditions can stimulate mass development of phytoplankton. Understanding these impacts of global change on phytoplankton bloom development is complex, since biomass accumulation is a net process of many growth and mortality related factors. Indeed, blooms are not only a result of enhanced growth, they can also be a product of reduced mortality caused by a lack of top down control. Algal blooms often consist of relatively large and inedible species, which are generally less easily ingested by zooplankton. This may benefit bloom development and result in trophic bottlenecks. In these cases, other top down factors, such as parasites, may become more important in regulating phytoplankton densities. Common parasites of phytoplankton include viruses, bacteria and parasitic fungi.

Since the relatively recent discovery of viruses as ecologically important drivers of phytoplankton succession and biomass, interest in other parasites has been gaining momentum (**chapter 2**). Chytridiomycota, often referred to as chytrids, can be virulent fungal parasites which kill their host and thereby delay or suppress phytoplankton blooms. Chytrids occur across all climatic regions in both freshwater and marine systems. They are characterized by their motile flagellated zoospores that can swim through the water to find suitable hosts. After settling on the host they penetrate the cell and develop a rhizoidal system that extracts nutrients. In a period of one to a few days the zoospore enlarges and forms a sporangium in which new zoospores are produced. Then, the infection matures and the zoospores (up to 60) are released into the water, ready to find new hosts. These zoospores have been found to constitute a highly nutritional food source to zooplankton. Consequently, carbon and nutrients from inedible host cells can be transferred to zooplankton via the zoospores of chytrids, thereby forming the so-called 'mycoloop'.

Despite an increasing amount of studies showcasing the widespread occurrence of chytrids parasitizing a wide range of phytoplankton species, these parasites have not yet been integrated in plankton ecology theory. In **chapter 2**, I have identified three main research areas for future work to better integrate parasitism by chytrids into plankton ecology. Firstly, elucidating the mechanisms of chytrid infections. Secondly, understanding the ecophysiology of chytrids and thirdly, characterisation of nutritional value to higher trophic levels and the stoichiometric interactions with their host. In this thesis, I contribute to the last two, namely the effects of rising temperatures and changes in nutrient availability. Specifically, I assessed their role in steering chytrid infection dynamics, and subsequent effects on zooplankton growth.

Temperature affects host growth and physiology as well as that of their parasites and grazers. I have shown that warming (+4°C) leads to a general decrease in phytoplankton biomass, particularly after the peak of a spring bloom (**chapter 3**). The diatom *Synedra* dominated during the bloom and was over 40% infected at the peak of infection. Furthermore, in the warm treatments the bloom terminated earlier, which was associated to a faster increase in chytrid prevalence. Phytoplankton in the warm treatment seemed less limited by phosphorus, as indicated by a lower carbon: phosphorus (C:P) stoichiometry as compared to the control. This may have benefitted chytrid infections. Timing of the zooplankton community also advanced in the warmed treatment. Possibly the faster development of the chytrid epidemic sustained the mycoloop, which provisioned zoospores to this grazer. Fungal parasites thus seem to have facilitated growth of zooplankton, when phytoplankton abundances were low. These findings emphasize the importance of incorporating not only nutrient limitation and grazing, but also parasitism in understanding the response of plankton communities towards global warming.

Changes in nutrient concentrations and supply ratios can lead to limitation of phytoplankton by N and P. In turn, N or P limitation will alter the N:P ratio of phytoplankton, which has consequences for higher trophic levels, including parasites. Indeed, I showed that cyanobacteria (*Planktothrix rubescens*) and their chytrid (*Rhizophydium megarrhizum*) follow changes in the N:P supply ratio of the growth medium (**chapter 4**). Zoospore production decreased at low N:P and increased at a high N:P ratio, as compared to the control. Consequently, the chytrid seemed to have a relatively high demand for N as compared to P. Furthermore, zoospore carbon content, and thereby presumably their size, decreased at a high N:P ratio. From these results we hypothesize that fungal parasites may exhibit a trade-off between zoospore size and production to optimize reproductive success. Presumably, larges zoospores contain more energy to fuel metabolism and thereby facilitate survival time. Since zooplankton can graze on chytrid zoospores, changes in parasite production, stoichiometry and cell size may have implications for the mycoloop and thereby aquatic food web dynamics.

Chytrids infecting inedible phytoplankton may support zooplankton populations by providing them with an alternative food source in the form of fungal zoospores. Indeed, I have shown that chytrid fungal parasites can support population growth of the rotifer *Keratella* (**chapter 5, 6**)**.** More specifically, in cultures of an inedible filamentous cyanobacterium (*Planktothrix rubescens*), *Keratella* population density rapidly declined, while in *Planktothrix* cultures infected with chytrids, *Keratella* population growth rate equalled the growth observed for populations fed with a suitable green algal diet (*Chlorella sorokiniana*). These 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, and that consumption of fungal zoospores not only allows survival but even population growth.

The overall aim of this thesis was to investigate how climate change factors affect bottom-up and top-down control of phytoplankton disease. Specifically, I focused on direct and interactive effects of warming, nutrient availability and grazing on chytrid infections. With my experiments, I showed that warming promoted chytrid infections and thereby accelerated the termination of a spring bloom. Furthermore, chytrid zoospores may support zooplankton growth by providing them with an alternative food source. Fungal parasites thereby can re-establish trophic links between phytoplankton and zooplankton, if the latter are food limited during presence of large inedible cyanobacteria or diatoms. Changes in the nutrient supply will alter phytoplankton elemental composition, and I have shown that this has consequences for reproduction and stoichiometry of their parasites. Specifically, during low P conditions chytrids can maintain a higher production rate and efficiency, while these decreased upon low N conditions. Furthermore, the chytrids revealed a putative trade-off between zoospore size, and thereby possibly survival, and production rate. The observed changes in chytrid production and stoichiometry may have consequences for higher trophic levels, and thereby alter food web functioning. In conclusion, this thesis contributes to the work existing on the frontier of phytoplankton chytridiomycosis research, providing a better understanding on the role of parasites in plankton food webs and the potential impacts of global change on parasite infection dynamics. With this work, I highlight the importance of integrating fungal parasites into plankton ecology.

Nederlandse samenvatting

Activiteiten van de mens hebben een grote impact op ecosystemen wereldwijd. Hoewel we ons nog maar in een vroeg stadium van de geprojecteerde trends van de opwarming van de aarde bevinden, we kennen en begrijpen de gevolgen ervan voor aquatische ecosystemen nog niet echt. De uitstoot van broeikasgassen, zoals kooldioxide, methaan en stikstofoxide heeft geleid tot het broeikaseffect. Opwarmende en voedingsrijke omstandigheden kunnen de massale ontwikkeling van fytoplankton stimuleren. Het begrijpen van de mondiale effecten van klimaatsverandering op de ontwikkeling van fytoplankton bloeien is complex, omdat deze ophoping van biomassa een netto-proces is van vele groei en sterfte gerelateerde factoren. Bloei is inderdaad niet alleen een gevolg van versterkte groei, het is ook een product van verminderde sterfte door een gebrek aan controle van bovenaf (top-down). Algen bloeien bestaan vaak uit relatief grote en oneetbare soorten, die over het algemeen minder gemakkelijk opgenomen kunnen worden door zoöplankton. Dit kan de bloeifase stimuleren en resulteren in trofische knelpunten. In deze gevallen kunnen andere top-down factoren, zoals parasieten, belangrijker worden bij het reguleren van de fytoplankton biomassa. Veelvoorkomende parasieten op fytoplankton omvatten virussen, bacteriën en parasitaire schimmels.

Sinds de relatief recente ontdekking van virussen als ecologisch belangrijke drijvende krachten achter fytoplankton successie en -biomassa, is de belangstelling voor andere parasieten in een stroomversnelling terecht gekomen (**hoofdstuk 2**). Chytridiomycota, vaak chytriden genoemd, kunnen virulente schimmelparasieten zijn die hun gastheer doden en daardoor fytoplanktonbloei kunnen vertragen of onderdrukken. Chytriden komen voor in alle klimatologische gebieden in zowel zoetwater- als mariene systemen. Ze worden gekenmerkt door hun beweeglijke zoösporen die met behulp van hun zweepstaartje door het water kunnen zwemmen om geschikte gastheren te vinden. Na bevestiging op hun gastheer dringen ze de cel binnen en ontwikkelen een systeem van schimmeldraden dat voedingsstoffen extraheert. In een periode van één tot enkele dagen groeit de zoöspore uit tot een sporangium waarin nieuwe zoösporen worden geproduceerd. Vervolgens rijpt deze infectie en komen de zoösporen (tot wel 60) vrij in het water, klaar om nieuwe gastheren te vinden. Deze zoösporen blijken een zeer voedzame voedingsbron te zijn voor zoöplankton. Zodoende kunnen koolstof en voedingsstoffen van niet-eetbare gastheercellen worden overgebracht naar zoöplankton via de zoösporen van chytriden, waardoor de zogenaamde 'mycoloop' wordt gevormd.

Ondanks een toenemend aantal studies die het wijdverspreide voorkomen van chytriden demonstreren, die een breed scala van fytoplankton-soorten parasiteren, zijn deze parasieten nog niet geïntegreerd in de ecologische plankton-theorie. In **hoofdstuk 2** heb ik drie hoofd onderzoeksgebieden voor toekomstig werk geïdentificeerd om parasitisme door chytriden beter te integreren in de ecologie van plankton. Allereerst het ophelderen van de mechanismen van chytride infecties. Ten tweede, inzicht in de ecofysiologie van chytriden en ten derde, karakterisering van de voedingswaarde voor hogere trofische niveaus en de stoichiometrische interacties met hun gastheer. In dit proefschrift draag ik bij aan de laatste twee, namelijk de effecten van stijgende temperaturen en veranderingen in de beschikbaarheid van voedingsstoffen. Specifiek heb ik hun rol in het sturen van de chytride infectiedynamiek en de daaropvolgende effecten voor de groei van zoöplankton onderzocht.

Temperatuur beïnvloedt de groei en fysiologie van de gastheer evenals die van hun parasieten en grazers. Ik heb aangetoond dat opwarming (+4 °C) leidt tot een algemene afname van biomassa van fytoplankton, vooral na de piek van een voorjaarsbloei (**hoofdstuk 3**). De diatomee *Synedra* domineerde tijdens de bloei en was meer dan 40% geïnfecteerd op het hoogtepunt van de infectie. Bovendien eindigde de bloei bij de warme behandelingen eerder, wat geassocieerd was met een eerdere toename in chytride-prevalentie. Groei van fytoplankton in de warme behandeling leek minder beperkt door de voedingsstof fosfor (P), zoals aangegeven door een lagere koolstof: fosfor (C:P) stoichiometrie in vergelijking met die van de controle. De chytride-infecties kunnen hier baat bij

hebben gehad. De timing van de zoöplankton-gemeenschap vervroegde ook in de verwarmde behandeling. Mogelijk heeft de snellere ontwikkeling van de chytride-epidemie de mycoloop ondersteund, die zoösporen voor deze grazer bevoorraadde. Schimmelparasieten lijken dus de groei van zoöplankton te hebben bevorderd, toen de dichtheid van fytoplankton laag was. Deze bevindingen benadrukken het belang van het bestuderen van niet alleen voedingsstoffen en begrazing, maar ook parasitisme in het begrijpen van de respons van planktongemeenschappen naar aanleiding van een opwarmende aarde.

Veranderingen in nutriëntenconcentraties en toevoerverhoudingen kunnen leiden tot beperking van fytoplanktongroei door stikstof (N) of P. Op zijn beurt zal N- of P-beperking de N:P-verhouding van fytoplankton veranderen, wat gevolgen heeft voor hogere trofische niveaus, inclusief parasieten. Ik heb inderdaad laten zien dat cyanobacteriën (*Planktothrix rubescens*) en hun chytride (*Rhizophydium megarrhizum*) de veranderingen volgen in de N:P-toevoerverhouding van het groeimedium (**hoofdstuk 4**). De productie van zoösporen nam af bij een lage N:P en nam toe bij een hoge N:P-verhouding, in vergelijking met een controle. Zodoende leek deze chytride een relatief grote behoefte aan N te hebben in vergelijking tot P. Verder daalde het koolstofgehalte van de zoösporen, en daardoor waarschijnlijk hun grootte, bij een hoge N:P-verhouding. Uit deze resultaten veronderstellen we dat schimmelparasieten een afweging kunnen maken tussen de grootte en de productie van zoösporen om hun reproductieve succes te optimaliseren. Vermoedelijk bevatten grotere zoösporen meer energie om het metabolisme aan te sturen en daardoor de overlevingstijd te verlengen. Omdat zoöplankton kan grazen op chytride zoösporen, kunnen veranderingen in de productie van parasieten, stoichiometrie en celgrootte gevolgen hebben voor de mycoloop en daarmee de algehele dynamiek van het aquatische voedsel web.

Chytriden die oneetbaar fytoplankton infecteren kunnen zoöplanktonpopulaties ondersteunen door hen met een alternatieve voedselbron in de vorm van zoösporen te voorzien. Ik heb inderdaad aangetoond dat chytride-schimmelparasieten de populatiegroei van het radardiertje *Keratella* kunnen ondersteunen (**hoofdstuk 5, 6**). Meer in het bijzonder, in culturen van oneetbare filamenteuze cyanobacteriën (*Planktothrix rubescens*) daalde de populatiedichtheid van *Keratella* snel, terwijl in chytride geïnfecteerde *Planktothrix* culturen de groeisnelheid van *Keratella*-populaties gelijk was aan die gevoed met een geschikter dieet van groenalgen (*Chlorella sorokiniana*). Deze resultaten tonen aan dat trofische verbanden tussen primaire producenten en secundaire consumenten, die beperkt zijn tijdens de aanwezigheid van grote niet-eetbare cyanobacteriën, kunnen worden hersteld door schimmelparasieten, en dat consumptie van zoösporen niet alleen overleving maar zelfs populatiegroei mogelijk maakt.

Het algemene doel van dit proefschrift was om te onderzoeken hoe verschillende factoren van klimaatsverandering van invloed zijn op bottom-up en top-down controle van fytoplankton-ziekten. Specifiek richtte ik me op directe en interactieve effecten van opwarming, beschikbaarheid van voedingsstoffen en begrazing op chytride-infecties. Met mijn experimenten toonde ik aan dat opwarming chytride-infecties bevorderde en daardoor de beëindiging van een voorjaarsbloei versnelde. Bovendien kunnen chytride zoösporen de groei van zoöplankton ondersteunen door hen een alternatieve voedselbron te bieden. Schimmelparasieten kunnen daardoor trofische verbanden herstellen tussen fytoplankton en zoöplankton, als deze laatste last hebben van voedselschaarste tijdens de aanwezigheid van grote niet-eetbare cyanobacteriën of diatomeeën. Veranderingen in de toevoer van voedingsstoffen zal de elementaire samenstelling van fytoplankton veranderen, wat gevolgen heeft voor de voortplanting en stoichiometrie van hun parasieten. In het bijzonder, chytriden kunnen tijdens lage P-omstandigheden een hogere productiesnelheid en efficiëntie handhaven, terwijl deze afnamen bij lage N-omstandigheden. Hierbij lijken de chytriden een afweging te maken tussen de

grootte van de zoösporen, daarmee eventueel de overlevingskansen, en hun productiesnelheid. De waargenomen veranderingen in de chytride-productie en stoichiometrie kunnen gevolgen hebben voor hogere trofische niveaus en daarmee het functioneren van het voedsel web veranderen. Concluderend, dit proefschrift draagt bij aan het werk dat bestaat op de voorste linies van fytoplankton chytridiomycose onderzoek, wat een beter inzicht geeft in de rol van parasieten in plankton voedselwebben en de mogelijke impact van een veranderende wereld op de dynamiek van parasitaire infecties. Hierbij benadruk ik het belang van het integreren van schimmelparasieten in de ecologie van plankton.

Acknowledgements

Kan het me nog herinneren als de dag van gisteren. 'het' telefoontje: "Hoi met Ellen. Ik wilde je graag laten weten dat we je de baan als promovendus aan willen bieden". Euforie, maar ook stress. Ik was namelijk nog bezig met mijn afstudeerstage. Hierdoor moest ik de laatste loodjes snel afhandelen, anders zou ik mijn MSc diploma niet op tijd binnenhalen om mijn nieuwe uitdaging te kunnen starten.

Ellen, duizend maal dank voor het bieden van deze mogelijkheid. Ik ben trots dat ik aan 'jouw' onderwerp heb mogen werken. Jij hebt dit onderzoek namelijk op de kaart gezet. Bedankt voor wie je bent hoe je mij alle vrijheid hebt gegeven tijdens mijn promotietraject. Ik vind het heerlijk om gesprekken met je te voeren en over alles eerlijk en open te kunnen zijn.

Dedmer, bedankt voor hoe je me altijd weer hebt weten te motiveren tijdens onze eindeloze discussies. Als ik weer eens met mijn veel te wilde ideeën binnenkwam wist jij me toch altijd weer de goede kant op te sturen en te helpen inzien waar ik nu daadwerkelijk in geïnteresseerd was. Ik denk dat we een heel erg goede match waren! Ik hoop dat we in de toekomst nog vaak samen mogen werken.

Alena, ik was erg verheugd toen je weer bij ons terug kwam op het NIOO. Ik was zo blij dat ik iemand had om mee te sparren over onze grote liefde chytriden. Je staat altijd voor iedereen klaar en denkt altijd aan wat het beste is voor anderen. Je hebt me dan ook graag advies gegeven over werk gerelateerde zaken, maar ook over zaken daarbuiten. Vele malen dank hiervoor!

Mandy and the limnonauts. Almost sounds like a 70s or 80s girl band. Super bedankt Mandy voor onze tijd samen op het NIOO, jij was de drijvende kracht achter de limnotron experimenten. Naast kantoorgenoot was je ook veel meer voor me. Van koekjes bakken, speciaalbier drinken en mij compleet van de baan slaan met tennis heb ik ontzetten genoten. Susanne, thanks for our fun during the dilution experiments. Thanks to you I now always remember safety regulations regarding mixing acid fluids: "Erst das wasser, dann die säure - sonst geschieht das ungeheure". Ralf, ook al ben je officieel net geen Limburger, ik had toch altijd het gevoel dat je er een was. Bedankt voor de fijne gesprekken tijdens het monsteren. Maar ook jouw muzieksmaak heeft velen weten te ondersteunen in de limnotron ruimte. Garabet, thanks for the fun and laughter. When you were around there was always something to laugh about. Also many thanks to the other limnonauts (Sarian, Sabine, Dedmer Edwin, Ellen, Lisette).

Ook voor mijn promotie, Mike en Els, bedankt voor alle hulp tijdens mijn MSc thesis. Mike, ik denk nog steeds dat ik mijn huidige baan aan jou te danken heb. Ik weet nog goed dat je zei dat Ellen op zoek was naar een geschikte kandidaat en mij een mobiel nummer in mijn handen drukte. Bedankt voor alle lol in en rondom het lab. Heb ontzettend veel van je geleerd. Ook Els, bedankt voor de ondersteuning en je eerlijkheid. Sorry dat ik je vaak lastigviel met vragen waarvan het beantwoorden langer dan een koffiepauze duurde. Ik was ontzettend blij met de manier waarop jullie mij begeleid hebben. Dit vormde immers de basis voor het hele promotietraject erna.

But also without many other people my PhD time would have looked completely different. Kai, thanks so much for teaching me how to work with viruses. I loved your sense of humour, which kept us going during our long days of bioassays. Joren, zonder jouw zouden 2 van mijn hoofdstukken er compleet anders uitzien als nu (dat bedoel ik als compliment). Je hebt een ontzettend groot aandeel gehad in ons werk en ik hoop dat je hier ook veel geleerd hebt dat bruikbaar is voor je eigen promotie bij Bas. Corina, ook al staan de data die we gemeten hebben niet in dit proefschrift, ik wil je ontzettend danken voor je hulp en advies. Ook nu na het inleveren van mijn thesis kan ik toch weer altijd bij je aankloppen. Anna, bedankt voor de ondersteuning met de FCM. Je stond toch altijd weer voor me klaar, ook in de pauzes om het gezellig te maken.

My office buddy's. Karen, bedankt voor de vele gesprekken tijdens de koffiepauzes. Je nuchterheid over wat er nu wel of niet belangrijk is in een mensenleven heeft me vaak aan het denken gezet. Antonella, thanks for being a great friend. Thanks for often taking the lead in initiating social activities and bbq parties. With you around there is always something happening. Peiyu, thanks for your infectious smile and Libin, thanks for your optimisms and energy. Kim, although we shared our office only for a short while, thanks for the good time. You were always in for some fun. Onze technici, vele malen dank voor het helpen met experimenten en analyseren van monsters. Ook kwam ik vaak bij jullie spuien over wat ik wel en niet goed vond in het lab: bijv. klagen over pipetten, de afwas of het bijvullen van papierrollen. Nico, bedankt voor het 'ochtendgesprekje'. Vaak was ik, net als jou, al wat vroeger op het NIOO. Hierdoor konden we vaak even samen de eerste koffie van de dag pakken. Suzanne, bedankt voor alle uren die we samen achter de FCM hebben gezeten, en de eindeloze gesprekken die we toen gevoerd hebben. Ook heb je me geleerd hoe om te gaan met de algen en chytriden culturen, bedankt hiervoor. Dennis, bedankt voor je humor, zangtalent en positiviteit. Fijn dat we onze muzieksmaak zo vaak konden delen. Ik zou me geen leven meer kunnen voorstellen zonder radio 10. Telkens als ik 'liever te vroeg in de kist dan een feestje gemist' hoor moet ik aan jou denken. Erik, bedankt voor het plagen en pesten. Je kreeg me telkens toch weer boven op de kast. Zal het missen jouw naam op een hoge toon door de gang te roepen. Michaela, alhoewel we niet vaak hebben samengewerkt stond je wel altijd klaar om met alles te helpen wat ik nodig had.

Also, many thanks to my other AqE PhD and postdoc colleagues (Annette, Bart, Casper, Jan, Jing, Laura, Lukas, Luuk, Manqi, Marika, Michiel, Sven, Tania, Wei and others). Without you the lunch- and coffee breaks, outings and conferences would not have been so much fun. Steven, bedankt voor je hulp en advies met het rotiferen werk. Also the other senior researches Liesbeth, Lisette and Wolf, many thanks. Also my other colleagues from different departments at NIOO. To many to name. Thanks so much for all the fun we had in the last years, think about the Christmas dinners, PhD weekends and random drinking occasions on the terraces at the main square or at the zaaier. In special I want to thank Peter, Ed and the other people from the Christmas choir. I will miss our rehearsals so much.

A special thanks to my best friends and family. Mam, veel te lief en te zorgzaam. Denk ook eens aan jezelf. Pap, bedankt voor je kijk op het leven. Ik denk dat veel mensen jouw nuchterheid kunnen waarderen. Bedankt voor het helpen met moeilijke beslissingen. Tim en Stef, bedankt dat jullie mijn paranimfen wilden zijn. Zonder jullie was ik nooit zover gekomen. Kon en Lillian, bedankt voor alle ondersteuning, ook tijdens moeilijkere tijden. Ben blij dat jullie me gewoon nemen voor wie ik ben. Galina, thanks for our many evenings together and the great dinners. Paolo, thanks for helping me with my mental problems and being this good of a friend. You really helped me a lot. Ook Jori, de vele late avonden bij de zaaier functioneerden vaak als een soort van reset-knop. Jij bent dan ook de grondlegger van het fenomeen 'P.N. in de keuken'. Lijkt me beter daar hier niet verder op in te gaan. Susan en Giel, vakantieouders, ik mis het makkelijk voorbij kunnen komen. Lekker koffie drinken en blijven slapen als pap en mam op vakantie waren. Ook de rest van Neer, opa & oma, Noud en Guus, bedankt voor de vele familiefeestjes. Ik ben blij dat ik een familie heb die van het leven durft te genieten. Dan rest mij nog alle andere vrienden, veel te veel om op te noemen. KOV & CO ,Straffe mannen, Anker, Aars, de Hiltje-clan, Meloeke, Wursel en iedereen van de schutterij. Ontzettend bedankt voor alle feestjes en sociale activiteiten van de afgelopen jaren. Ben er echt van overtuigd dat een mens niet kan functioneren zonder goede ontspanning met vrienden. Viet, thanks for being around the last years. I know I'm not the easiest person to be with. Thanks for the many hours of listening to my complaints and giving me solutions to them. I cannot thank you enough.

Curriculum vitae
PE&RC certificate

Thijs Frenken was born on the 5th of April 1990 in Roermond, the Netherlands. In 2008 he graduated from Atheneum at the Bouwens van der Boijecollege Panningen and started his BSc studies 'Soil, Water & Atmosphere' at Wageningen University. Thereafter he continued his MSc 'Earth & Environment' at the same university with a specialisation in water quality. During these studies he performed research projects at Wageningen University and at B-WARE Research Centre in which he worked on multiple aspects of aquatic plants and phytoplankton. Since 2014, Thijs has worked on the role of pathogens in structuring phytoplankton communities which has resulted in this thesis.

Peer-reviewed publications

1. **Frenken, T**., M. Velthuis, L. N. de Senerpont Domis, S. Stephan, R. Aben, S. Kosten, E. van Donk, and D. B. Van de Waal. 2016. Warming accelerates termination of a phytoplankton spring bloom by fungal parasites. Global Change Biology 22.
2. Velthuis, M., L. N. de Senerpont Domis, **T. Frenken**, S. Stephan, G. Kazanjian, R. Aben, S. Hilt, S. Kosten, E. van Donk, and D. B. Van de Waal. 2017. Warming advances top-down control and reduces producer biomass in a freshwater plankton community. Ecosphere 8.
3. **Frenken, T**., J. Wierenga, A. S. Gsell, E. Van Donk, T. Rohrlack, and D. B. Van De Waal. 2017. Changes in N:P Supply Ratios affect the Ecological Stoichiometry of a Toxic Cyanobacterium and its Fungal Parasite. Frontiers in Microbiology 8.
4. **Frenken, T**., E. Alacid, S. A. Berger, E. C. Bourne, M. Gerphagnon, H.-P. Grossart, A. S. Gsell, B. W. Ibelings, M. Kagami, F. C. Küpper, P. M. Letcher, A. Loyau, T. Miki, J. C. Nejstgaard, S. Rasconi, A. Reñé, T. Rohrlack, K. Rojas-Jimenez, D. S. Schmeller, B. Scholz, K. Seto, T. Sime-Ngando, A. Sukenik, D. B. V. d. Waal, S. V. d. Wyngaert, E. Van Donk, J. Wolinska, C. Wurzbacher, and R. Agha. 2017. Integrating chytrid fungal parasites into plankton ecology. Research gaps and needs. Environmental Microbiology 19.
5. Aben, Ralf C.H., N. Barros, E. Van Donk, **T. Frenken**, S. Hilt, G. Kazanjian, L.P.M. Lamers, E.T.H.M. Peeters, J.G.M. Roelofs, L.N. de Senerpont Domis, S. Stephan, M. Velthuis, D.B. Van de Waal, M. Wik, B.F. Thornton, J. Wilkinson, T. DelSontro, S. Kosten. 2017. Cross continental increase in methane ebullition under climate change, Nature Communications 8.

In Review:

1. **Frenken, T.**, J. Wierenga, E. Van Donk, S.A.J. Declerck, L.N. de Senerpont Domis, T. Rohrlack, D.B. Van de Waal. Fungal parasites of a toxic inedible cyanobacterium provide food to zooplankton. Limnology & Oceanography.
2. Kazanjian, G. M. Velthuis, R.C.H. Aben, S. Stephan, E.T.H.M Peeters, **T. Frenken**, J. Touwen, F. Xue, S. Kosten, D.B. Van de Waal, L.N. de Senerpont Domis, E. Van Donk, S. Hilt. Dynamic response of aquatic primary production to warming due to enhanced bottom-up and top-down control. Scientific Reports.

Non peer-reviewed publications

1. **Frenken, T**. 2017. Top-down regulation of algal blooms: pathogens as a potential whole lake biological control measure? in: *LakeLine.* (Madison).

2. **Frenken, T.** & A.S. Gsell. Waterschimmels teffen algen. Chapter in the cahier schimmels published by 'stichting biowetenschappen en maatschappij'. http://www.biomaatschappij.nl/

Presentations at international conferences

1. Netherlands Annual Ecology Meeting 2015 (Lunteren, The Netherlands). Warming enhances termination of a phytoplankton spring bloom: Impacts of fungal parasites.

2. Association for the sciences of aquatic ecology and limnology (ASLO), aquatic sciences meeting 2015 (Granada, Spain). Impact of global warming on the fungal infection of a phytoplankton spring bloom.

3. The International Society of Limnology (SIL) meeting 2016 (Torino, Italy). The ecological stoichiometry of a fungal parasite infection.

4. Netherlands Annual Ecology Meeting 2017 (Lunteren, The Netherlands). Virus dynamics in freshwater ecosystems advances due to global warming.

Participation in international workshops

1. 1st Plankton Chytridiomycosis Workshop 2015 (Berlin, Germany)

2. 2nd Plankton Chytridiomycosis Workshop 2016 (Skagaströnd, Iceland)

3. 10th International GAP (the Group for Aquatic Primary productivity) Workshop 2017 (Třeboň, Czech Republic)

Invited talks

1. "The impact of warming and nutrients on phytoplankton diseases". Presentation at the IGB Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Neuglobsow, Germany. Invited by prof. dr. Hans-Peter Grossart (9th May 2017).

2. "Zieke blauwalgen en hun effecten op het aquatisch voedsel web". Presentation during the Dutch national blue-green algae meeting 2017 (Blauwalgenplatformdag), Netherlands Insitute of Ecology, Wageningen, The Netherlands. Invited by dr. ir. Dedmer B. van de Waal (19th April 2017).

Session / workshop organisation

1. Organised session titled "Disease interactions with communities and food webs". Netherlands Annual Ecology Meeting 2016 (Lunteren, The Netherlands). Co-organised with Kevin Matson (WUR) and Anouk Goedknegt (NIOZ).

2. Organise session titled "Parasites, Pathogens Everywhere: It's time for a closer look". ASLO summer meeting 2018 (Victoria, Canada). Co-organised with Alena S. Gsell (NIOO) and Corina Brussaard (NIOZ).

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)
- Integrating chytrid fungal parasites into plankton ecology; research gaps and needs (2017)

Writing of project proposal (4.5 ECTS)
- The role of pathogens in structuring phytoplankton communities (2014)

Post-graduate courses (6 ECTS)
- Consumer Resource Interactions; PE&RC (2014)
- 1st Plankton Chytridiomycosis workshop; IGB Berlin (2015)
- 2nd Plankton Chytridiomycosis workshop; University of Akureyri and Biopol (2016, 2017)
- 10th GAP Workshop; Institute of Microbiology CAS (2017)

Laboratory training and working visits (1.5 ECTS)
- SEM X-ray analyses, EDX analyses; IGB Berlin (2017)

Invited review of (unpublished) journal manuscript (9 ECTS)
- Scientific reports (2x): Host-parasite interaction (2016)
- Global Change Biology (2x): Climate change, disease risk (2016)
- Marine Biology Research (1x): Phytoplankton-parasite interactions (2016)
- Hydrobiologia (3x): Plant-phytoplankton competition (2016, 2017)
- Limnology and Oceanography Letters (1): Impact of pollution on phytoplankton (2017)

Deficiency, refresh, brush-up courses (1.5 ECTS)
- Basic statistics; PE&RC (2015)

Competence strengthening / skills courses (2.9 ECTS)
- WGS workshop carousel; PE&RC (2016)
- Brain Training; PE&RC (2016)
- WGS workshop carousel; PE&RC (2016)
- Writing Grant Proposals; Wageningen in'to Languages (2017)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)
- PE&RC First years weekend (2014)
- PE&RC Day (2016)
- PE&RC Last years weekend (2016)
- PE&RC Day (2017)

Discussion groups / local seminars / other scientific meetings (9.2 ECTS)
- Marine parasites conference
- NIOO seminar & scientific meetings
- Ecological Theory and Application' discussion group meetings

- NAEM (2014)
- NAEM (2018)

International symposia, workshops and conferences (10.4 ECTS)

- NAEM; oral presentation (2015)
- ASLO Conference Grenada oral presentation
- NAEM poster presentation (2016)
- SIL Conference Torino; oral presentation
- NAEM; oral presentation (2017)

Lecturing / Supervision of practicals / tutorials (6 ECTS)

- Aquatic ecology (2014, 2015, 2016, 2017)

Supervision of MSc students (5 ECTS)

- Stoichiometry in phytoplankton parasite interactions

Notes