Community and population dynamics in lakes under global change

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Abstract

Humans have been strongly impacting natural processes both directly (e.g. via land transformation, overfishing or introducing invasive species) and indirectly (e.g. via air, land and water pollution through agricultural and industrial emissions and waste products). Consequences of these influences - encompassed as global environmental change - include climatic alterations, biodiversity loss and shifts in disease spread. In lake ecosystems, environmental alterations can affect for instance the thermal stratification, light regime, as well as loadings of humic dissolved organic carbon (DOC) and phosphorus (P). Increases in concentrations of humic DOC have been reported to reduce primary production due to darkening of the surface water, and to lead to changes in microbial activity. Input of P generally increases lake productivity, while differently affecting certain organismal groups. All these mechanisms can induce cascading effects, leading to changes in food web interactions and community composition. In extreme cases, P input can also result in the occurrence of algal blooms which disrupt ecosystem functioning and decouple nutrient transfer from primary to secondary producers. In this thesis I focused on two main topics: first, how increases in humic DOC, with or without simultaneous P addition, affect a key zooplankter in lakes - the cladoceran *Daphnia* - at the organismal, population and lake community level. Second, I investigated the role of parasites in the nutrient transfer between phytoplankton and zooplankton during algal blooms. I exposed different *Daphnia* species and genotypes to several concentrations of humic DOC in two laboratory experiments. Direct effects of humic DOC on *Daphnia* physiology strongly differed between species and genotypes, potentially affecting *Daphnia* community and population dynamics (i.e. inter- and intraspecific competition) in natural settings. Simultaneous additions of humic DOC and P in a large-scale mesocosm experiment revealed that negative effects of humic DOC on *Daphnia* fitness observed in the laboratory do not necessarily translate into natural conditions due to additional interactions. Furthermore, no significant effects of humic DOC on intraspecific genetic diversity or parasitic infection in *Daphnia* were found in the mesocosm experiment. To investigate how parasites can affect food web interactions, a laboratory experiment

was carried out in which *Daphnia* were fed with cyanobacteria that were either uninfected or infected with a chytrid fungus. This study demonstrated the existence of a parasite-mediated trophic transfer between phytoplankton and zooplankton, as *Daphnia* fitness was improved under infected cyanobacteria diet. Overall, this thesis shows that environmental alterations can have complex effects on organisms, populations and communities in lakes, whereas the specific consequences are system-dependent. Thus, different systems need to be investigated in detail to better understand complex dynamics and their underlying mechanisms under varying environmental conditions to possibly predict responses of lake ecosystems to long-term global change.

Deutsche Zusammenfassung

Die menschliche Zivilisation hat einen starken Einfluss auf natürliche Prozesse, sowohl direkt, z.B. durch Veränderung von Landnutzung, Überfischung oder der Einfuhr von invasiven Spezies, als auch indirekt, z.B. durch Luft-, Land- und Wasserverschmutzung durch landwirtschaftliche und industrielle Emissionen und Abfallprodukte. Die Auswirkungen dieser Einflüsse – zusammengefasst unter dem Begriff Globaler Umweltwandel – umfassen klimatische Veränderungen, Verlust von Biodiversität und Verschiebungen in der Ausbreitung von Krankheiten. In Seeökosystemen können Umweltveränderungen unter anderem die Temperaturschichtung, den Lichteinfall sowie den Eintrag von Huminstoffen und Phosphor beeinflussen. Die Zunahme von Huminstoffkonzentrationen kann die Primärproduktion durch Verdunkelung des Oberflächenwassers verringern, und zu Veränderungen in der mikrobiellen Aktivität führen. Der Eintrag von Phosphor erhöht generell die Produktivität von Seen, wobei bestimmte Organismengruppen unterschiedlich darauf reagieren. All diese Mechanismen können Dominoeffekte auslösen, die zu Veränderungen in Nahrungsnetzinteraktionen und der Zusammensetzung von Taxongemeinschaften führen. In schwerwiegenden Fällen kann der Eintrag von Phosphor zum Auftreten von Algenblüten führen, die die Funktionsfähigkeit von Ökosystemen stören sowie die Nährstoffübertragung zwischen Primär- und Sekundärproduktion unterbrechen. In der vorliegenden Arbeit habe ich mich zwei Hauptthematiken gewidmet: erstens, wie die Zunahme von Huminstoffkonzentrationen, mit oder ohne gleichzeitigem Phosphor-Eintrag, eine Schlüssel-Zooplanktonspezies in Seen, den Cladoceren *Daphnia*, auf Organismen-, Populations- und Taxonebene beeinflusst. Zweitens, habe ich die Bedeutung von Parasiten in der Nährstoffübertragung zwischen Phytoplankton und Zooplankton während Algenblüten untersucht. Ich habe verschiedene *Daphnien* Spezies und Genotypen mehreren Konzentrationen eines Huminstoffs in zwei Laborexperimenten ausgesetzt. Es gab deutliche Unterschiede in den direkten Effekten des Huminstoffs auf die Physiologie der *Daphnien* Spezies und Genotypen. Unter natürlichen Bedingungen könnte dies zu Auswirkungen auf Taxon- und Populationsdynamiken von *Daphnia* führen (d.h. inter- und intraspezifische

Konkurrenz). Die gleichzeitige Zugabe des Huminstoffs und Phosphor in einem groß angelegten Mesokosmen-Experiment zeigte, dass negative Effekte des Huminstoffs auf die Fitness von *Daphnia* durch zusätzliche Einflüsse nicht notwendigerweise unter natürlichen Bedingungen zutage kommen. Zudem wurden keine signifikanten Effekte des Huminstoffs auf die intraspezifische genetische Diversität oder auf Infektionen von *Daphnia* in dem Mesokosmen-Experiment festgestellt. Um zu untersuchen, wie Parasiten Nahrungsnetzinteraktionen beeinflussen, wurde ein Laborexperiment durchgeführt. Darin wurde *Daphnia* mit Cyanobakterien gefüttert, die entweder mit einem Chytridpilz infiziert wurden oder uninfiziert blieben. Diese Studie demonstrierte die Existenz einer Parasiten-vermittelten Nährstoffübertragung zwischen Phytoplankton und Zooplankton, da die Fitness von *Daphnia* unter infizierter Nahrung verbessert war. Im Großen und Ganzen zeigt die vorliegende Arbeit, dass Umweltveränderungen komplexe Effekte auf Organismen, Populationen und Taxongemeinschaften in Seen haben können, wobei die spezifischen Auswirkungen System-abhängig sind. Daher sollten verschiedene Systeme detailliert untersucht werden, um komplexe Dynamiken und deren zugrunde liegenden Mechanismen unter sich verändernden Umweltbedingungen besser zu verstehen. Dadurch könnten Reaktionen von Seeökosystemen auf einen langzeitigen Globalen Umweltwandel vorhergesagt werden.

Thesis outline

This doctoral thesis consists of five Chapters, where Chapter 1 and 5 provide the broader context of the thesis by a general introduction and discussion, respectively. Chapters 2 to 4 comprise two published manuscripts and one manuscript in preparation - each of them constitutes a separate thesis chapter, including its own introduction, methodology, results and discussion section. The layouts of Chapters 2 to 4 are following the journal regulations where the manuscripts have been published in, or will be submitted to. Supporting material and cited literature are presented at the end of each chapter. A list of references cited in the general introduction and discussion can be found at the end of the thesis.

Chapter 1 – General introduction

Chapter 2

Manja Saebelfeld, Laëtitia Minguez, Johanna Griebel, Mark O. Gessner, Justyna Wolinska (2017) Humic dissolved organic carbon drives oxidative stress and severe fitness impairments in *Daphnia*. *Aquatic Toxicology* 182: 31-38

All authors conceived the study. MS and LM conducted the experiment and processed the samples. MS, LM and JG analysed the data. MS and LM wrote the manuscript with help from JW and MOG.
MS and LM contributed equally to this work.

Chapter 3

Manja Saebelfeld, Yari A. Osenberg, Stella A. Berger, Jens C. Nejstgaard, Justyna Wolinska. Combined mesocosm and laboratory experiments reveal complex effects of humic DOC, with or without simultaneous phosphorus enrichment, on *Daphnia* fitness. In preparation.

SAB and JCN were members of the steering group contributing to the scientific design of the overall LakeLab mesocosm experiment, and also organised and supervised its conduct. MS and JW conceived the current study (i.e. zooplankton sampling, analysis

of the *Daphnia* community and conduct of the laboratory experiment). MS collected the zooplankton samples. MS and YAO conducted the laboratory experiment. MS, YAO and JW processed the samples. MS and YAO analysed the data. MS wrote the manuscript with help from JW, SAB and JCN.

Chapter 4

Ramsy Agha, Manja Saebelfeld, Christin Manthey, Thomas Rohrlack, Justyna Wolinska (2016) Chytrid parasitism facilitates trophic transfer between bloom-forming cyanobacteria and zooplankton (*Daphnia*). *Scientific Reports* 6

MS, RA and JW conceived the study. MS, RA and CM prepared the experiment. MS and RA conducted the experiment and analysed the data. MS and RA wrote the manuscript with help from JW.
MS and RA contributed equally to this work.

Chapter 5 – General discussion

Chapter 1

General Introduction

General Introduction

A global change

One of the most discussed and controversial topics in science, politics, media and the public since the late 1980s has been the issue of climate change or global warming (Boykoff and Boykoff, 2007; Doran and Zimmerman, 2009; Lewandowsky et al., 2013). The controversy comprises arguments of gradually rising temperatures caused by anthropogenic emissions on the one side, and on the other side different facets of so-called "climate change denial", such as claims that global warming is a hoax or a result of naturally occurring climatic fluctuations (Farmer and Cook, 2013; Washington and Cook, 2013). Within the scientific community the former case is widely accepted (scientific consensus) (Oreskes, 2004). Increased emissions of the greenhouse gases carbon dioxide, methane and nitrous oxide, in combination with other ozone-forming chemicals or aerosols (e.g. sulphate, nitrate, carbon monoxide, hydrocarbons) are now seen as the main drivers for climate change. This phenomenon involves not only rising air and ocean temperatures, but also increasing rainfall, as well as more severe and frequent extreme events such as storms and droughts (Solomon, 2007).

Changes in climatic conditions, however, are not the only symptoms of the "Anthropocene"- a proposed new geological epoch, starting in the late 18th century, which is human-dominated and characterised by rising global concentrations of greenhouse gases (Crutzen, 2002). In various ways, humans have left their footprint on this planet (Sanderson et al., 2002). Human alterations can be direct in nature, including land transformation (e.g. dam constructions, deforestation, agriculture, urbanisation), hunting, and overfishing as well as the introduction of invasive species, or through more indirect effects such as air, land and water pollution due to industrial and agricultural waste and by-products (e.g. pesticides, photochemicals, nutrients) (Vitousek et al., 1997; Tilman et al., 2001). All these anthropogenic influences are encompassed by the term "global environmental change".

One of the most prominent consequences of environmental change is the loss of biodiversity, which is mainly driven by habitat fragmentation due to land-use change

(Chapin et al., 2000; Sala et al., 2000; Brashares et al., 2001). There has been some success in reducing the rate of biodiversity loss in some local areas, and habitat quality has improved due to management and policy responses since the early 2000s. Despite that, biodiversity decline as well as indicators of pressure on biodiversity (e.g. deposition of reactive nitrogen, number of invasive species, human consumption) show increasing trends (Butchart et al., 2010). Extinction rates strongly exceed those of pre-industrial times, and the loss of species can have functional consequences on ecosystems through cascading effects (Chapin et al., 2000). The specific prospects for ecosystems in this changing world are difficult to assess and depend on various factors such as the type of habitat, local or regional climate, latitude and/or altitude, and the intensity of land use (Redford and Richter, 1999).

A sicker world?

Another reported consequence of environmental change is shifts in patterns of disease spread. Host susceptibility and parasite infectivity are often sensitive to environmental variation (Thomas and Blanford, 2003; Wolinska and King, 2009). Rising annual temperatures, for instance, have been identified to cause higher infection risks due to decreased pathogen mortality during mild winters in temperate regions, higher pathogen fitness through elevated development or transmission rates, weakened host fitness through thermal stress, and changes in host or pathogen geographic ranges. All of these may lead to severe disease outbreaks (Harvell et al., 2002; Altizer et al., 2013). Other anthropogenic influences, such as human settlement, landscape changes, or introduction of pathogens and domestic animals, also increase disease risk (Patz et al., 2000; Daszak et al., 2001). Emerging infectious diseases in humans, their domestic animals, and in wildlife have been associated with the spread of pathogens into new systems via increased contact with new host populations or species, as well as altered selection pressures (Daszak et al., 2001). Increases in epidemics over recent decades contribute further to the above mentioned worldwide decreases in biodiversity, as they can wipe out populations or even species (Daszak et al., 2001; Harvell et al., 2002).

For certain systems, however, disease prevalence in a population could decrease or even be reversed, depending on the particular requirements of the antagonists involved. Some diseases, such as the amphibian chytridiomycosis or the coldwater disease of fish, require rather cold conditions to spread. Epidemics of such diseases are hampered by increasing temperatures (Harvell et al., 2002). Further, host immune response can be increased by higher temperatures, enabling better pathogen defence (Harvell et al., 2002; Altizer et al., 2013). It has also been proposed that shifts in the geographic ranges of species need not lead to disease spread, due to the so-called "dilution effect". This mechanism applies when species richness is higher in the new habitat. In this scenario, vector species face a more diverse host community where some species have a low reservoir competence, i.e. the ability to transmit a specific pathogen to a feeding vector. The lower proportion of competent host species then "dilutes" disease risk by decreasing the density of infected animals within populations and thus lowering transmission rates (Schmidt and Ostfeld, 2001). Other community effects may also play a role in disease prevalence in populations. Predators can strongly influence pathogen infection rates, i.e. number of infected hosts in populations, either by feeding on free-living stages of the parasite (Searle et al., 2013) or by selectively feeding on infected hosts (Duffy et al., 2005; Hall et al., 2006). The latter has been shown for fish, feeding on infected individuals of the freshwater cladoceran *Daphnia* and thus preventing epidemics of bacterial (Duffy et al., 2005) and fungal parasites (Hall et al., 2006). The decline in infected individuals can be further enhanced by warm conditions, as productivity (i.e. higher metabolic demands and increased movement) of fish, and thus their feeding rate, increases with rising temperatures (Duffy et al., 2005). Non-selective predation may also decrease disease risk, either by reducing life-span of infected individuals and thus their ability to infect healthy hosts (Packer et al., 2003), or if predation is strong enough to keep the host population below the density threshold needed for transmission of the parasite (Lafferty, 2004).

Effects of environmental change on lake ecosystems

Human settlement concentrates disproportionately along both freshwater and marine waterways, which are intensively used for consumption, recreation, agriculture, industry, as well as for transportation and as disposal sites for pollutants (Postel et al., 1996; McGranahan et al., 2007). This strongly impacts the hydrology of aquatic systems, and damages the ecosystem in many ways (Sala et al., 2000; McGranahan et al., 2007; Crook et al., 2015). In lake ecosystems, for instance, human influences in interaction with climate change are predicted to affect thermal stratification (e.g. deepening of the upper mixed layer), nutrient input and availability, and also to cause shifts in the light regime and timing of onset of phyto- and zooplankton growth (Hondzo and Stefan, 1993; Mooij et al., 2007). These changes can massively affect food web interactions as well as the diversity and composition of aquatic communities (Sala et al., 2000; Mooij et al., 2005). Two specific effects of environmental change on lakes are alterations in the concentration of humic dissolved organic carbon (DOC) and nutrients, both of which are further explained in the following two paragraphs.

Trends in humic DOC concentrations

A major component of aquatic systems is organic matter. Most dissolved organic matter in lakes consists of humic substances (HS). HS result from decomposition of plant and animal residues and contain subfractions of humic acids, fulvic acids and humin (Thurman, 1985; Suffet and MacCarthy, 1989). They do not have a discrete definition and in the environment are often associated with other classes of materials, such as amino acids or sugars. HS are very reactive and participate in numerous metabolic pathways. They can indirectly affect aquatic organisms through different mechanisms such as binding to xenobiotics, metals and other chemicals (i.e. forming complexes of higher molecular size). This leads to changes in their bioconcentration, permeability or toxicity, potentially resulting in less chemical stress on aquatic organisms (Steinberg, 2003). Low concentrations of HS have also been shown to increase activity of organisms, resulting in higher active or passive uptake of particulate or dissolved substances (Steinberg, 2003).

Due to the difficulty of characterising HS, the amount of material is commonly indicated by the concentration of dissolved organic carbon (DOC) (Suffet and MacCarthy, 1989). In lakes, humic DOC originates from two different sources: (i) input from land (e.g. discharge from soil), called allochthonous carbon, and (ii) autochthonous carbon that emerges within the lake (e.g. algal or littoral DOC) (Thurman, 1985). The concentration of humic DOC in lakes depends on a number of conditions such as precipitation patterns, latitude and altitude, algal growth, runoff, and the composition of the catchment area (Thurman, 1985; Sobek et al., 2007). In most natural fresh waters, concentrations of humic DOC range from 0.5 to 50 mg L^{-1} (Steinberg, 2003), and lakes typically experience strong seasonal changes in DOC concentrations (Thurman, 1985; Sobek et al., 2007). Fluctuations in humic DOC concentrations are positively related to water colour and negatively to UV-B penetration (Schindler et al., 1996).

Besides the common seasonal changes in humic DOC concentrations in lakes, long-term trends of both decreasing and increasing concentrations have been reported. Both patterns have been related to different aspects of environmental change. Decreases in humic DOC have been associated with climate warming (reduced export from catchments during droughts) and lake acidification (Schindler et al., 1996). Underlying mechanisms for the latter are unclear, but have been proposed to result either from lower solubility of DOC at low pH values, decreased leaching of DOC from soil (Krug and Frink, 1983), or alternatively due to greater microbial utilisation of DOC under increasing nitrogen availability (Driscoll and Van Dreason, 1993). Consequences of decreases in humic DOC include increased UV-B penetration into deeper water layers, which can damage organisms through exposure to harmful wavelengths (Schindler et al., 1996). Most studies on long-term DOC trends, however, show increasing concentrations in humic DOC in many lakes worldwide, up to 0.5 mg DOC L^{-1} per year (e.g. Worrall et al., 2004; Evans et al., 2005; Monteith et al., 2007). Potential drivers which have been identified include increased temperature, atmospheric CO_2, precipitation, and runoff (reviewed in Solomon et al., 2015). Reported consequences of this pattern include lowered primary production via alterations in the light regime

(i.e. shading effect through so-called “brownification”), changes in microbial activity and in phyto- and zooplankton community composition (Arvola et al., 1996; Strecker et al., 2008; Shurin et al., 2010; Nicolle et al., 2012). Apart from gradually increasing annual mean concentrations, input of humic DOC can be sudden and extreme in rare cases. After a long period of strongly increased precipitation, 5-fold increases in humic DOC concentrations were observed in a lake in northeastern Germany. Together with the discharge of nutrients this caused a chain of events and feedback loops, which resulted in the almost complete loss of microinvertebrate and fish populations in this lake (Brothers et al., 2014).

Humic DOC has long been considered to interact only indirectly with organisms at the cellular level (Steinberg, 2003). In the last two decades various laboratory studies have changed this view, showing that functional groups or parts of building blocks of humic DOC can cross cell membranes, and have the capacity to bind enzymes, toxins or DNA, interact with signaling pathways, and induce internal ROS production (reviewed in Steinberg et al., 2006). Furthermore, humic DOC can be used as a nutrient source. Microbes incorporate DOC via the microbial loop, and particles can even be directly consumed by zooplankton and fish (Karlsson et al., 2012). Effects of humic DOC on fitness parameters of aquatic organisms show varying, partially contradictory results, depending on the source and concentration of humic DOC, the studied system and additional stressors. Growth of freshwater oomycetes has been found to be either inhibited or supported by different types of natural, commercial or synthetic humic substances (Meinelt et al., 2007). In fish, humic DOC has been shown to contribute to a multiple stress resistance, probably due to the stimulation of metabolism and defence mechanisms (Meinelt et al., 2004). Other studies have reported increases in life-span of different zooplankton species, coupled with either increased (Engert et al., 2013) or decreased (Bouchnak and Steinberg, 2013) offspring numbers. Another way in which humic DOC may affect populations is by influencing disease spread. Studies of the impacts of humic DOC on infections are scarce and have focused mainly on parasite fitness traits. Specifically, medical surveys have shown that synthetic humic acids inhibit in vitro infection or replication of human pathogens such as HIV and influenza (Schneider et al., 1996; Lu et al., 2002; van Rensburg et al., 2002). Field studies in lakes

have found humic DOC to either increase (Lymer et al., 2008) or decrease (Anesio et al., 2004) viral abundances and thus virus-induced mortality of bacteria. Mobility of oocysts of the protozoan *Toxoplasma gondii* was increased under humic DOC enrichment, potentially facilitating widespread contamination of waterways with this multi-host parasite (Shapiro et al., 2009). Studies on how humic DOC affects dynamics between hosts and their parasites are missing.

Nutrient enrichment of lakes

Many aquatic ecosystems have experienced strong increases in external nutrient loading, i.e. eutrophication processes, since the 1950s. These nutrients are mostly phosphorus and nitrogen, and are discharged into the surface water through urban activities, agricultural and industrial runoff, and atmospheric deposition (Carpenter et al., 1998; Anderson et al., 2002). In lakes, the main cause of eutrophication is phosphorus (P) enrichment (Carpenter et al., 1998). As P is essential for many biological processes and is also the main limiting nutrient in freshwater systems, lakes often respond with increased productivity. This can positively affect all trophic levels (Watson et al., 1997; Noe et al., 2001). Additions of P have been shown to support phytoplankton growth, and also P-content of edible algae, resulting in higher food quantity and quality for zooplankton (Urabe et al., 1997; Boersma, 2000). Nevertheless, as organisms within communities respond differently to increases in nutrients, P enrichment is usually accompanied by changes in phyto- and zooplankton community composition in lakes (Watson et al., 1997; Noe et al., 2001).

In extreme cases, excess of P, coupled with climatic changes, can lead to disruption of lake ecosystem functioning. The most prominent example is the increased frequency of harmful algae blooms. These blooms are characterised by excessive growth of photosynthetic algae, cyanobacteria, or protozoans. They can occur in the form of nontoxic or toxic blooms (Anderson et al., 2002). The former case causes foul odors and reduces water clarity and quality. This interferes with water usage by humans for fisheries, recreation, industry, agriculture, and drinking. Furthermore, dense algal mats destroy habitats by shading, causing reduced growth of several trophic levels and leading to loss of biodiversity. During the decay of blooms at the end of the growing

season, microbial activity leads to anoxia, which can result in extensive fish kills. In the case of toxic cyanobacteria blooms, harmful metabolites, such as neuro- and hepatotoxins, have additional direct negative effects by killing livestock and wildlife, as well as presenting a threat to human health (Carpenter et al., 1998; Anderson et al., 2002; Chislock et al., 2013).

In lakes, the most common type of bloom is caused by cyanobacteria, where few or a single species often dominate the phytoplankton community (Anderson et al., 2002; Chislock et al., 2013). In addition to the harmful toxicity effects of some species, cyanobacterial blooms are believed to disrupt nutrient transfer from primary to secondary producers. This is attributed to their low nutritional value and their large cells, which cannot be grazed efficiently by most zooplankton species as they interfere with the filter apparatus (Tillmanns et al., 2008; Chislock et al., 2013; Ger et al., 2016). These assumptions contrast with the findings of some field studies, which have reported high biomasses of zooplankton during algal blooms (Bouvy et al., 2001; Sousa et al., 2008; Davis et al., 2012). Thus, alternative nutrition sources support zooplankton growth during bloom conditions, one of which is explained by the microbial loop (Azam et al., 1983). Recently, it has been proposed that another link in nutrient transfer between primary and secondary producers is provided by aquatic fungi that infect diverse phytoplankton species. This concept, called “Mycoloop”, suggests that zooplankton growth can be supported under algal bloom conditions by zooplankton feeding on chytrid zoospores (Kagami et al., 2007a). Though already recognised in the 1960s, these parasitic fungi of the phylum Chytridiomycota, commonly called chytrids, have only recently caught the attention of researchers investigating their role in food web interactions. Many questions on chytrids are still unclear, for instance regarding their general role in the ecosystem, habitat requirements, taxonomy, and host specificity (Ibelings et al., 2004; Gleason et al., 2008; Grossart et al., 2016). Chytrids infect their phytoplankton hosts via free living zoospores. Zoospores attach to the host cell and penetrate it with a fine thread, followed by the release of their contents into the host. This results in the formation of a prosporangium, from which tubular rhizoids expand within the host cell. Finally, an epi-endophytic bud emerges, which develops into a flask-shaped sporangium in which new zoospores are produced asexually. These

zoospores are then released into the environment after lethal lysis of the host cell (Ibelings et al., 2004; Gerphagnon et al., 2013). Chytrid zoospores contain high amounts of polyunsaturated fatty acids (PUFAs) and cholesterol, which are essential for zooplankton growth (Müller-Navarra et al., 2000; Kagami et al., 2007b). Moreover, they lie within the food size range of zooplankton. Indeed, laboratory studies have shown that zooplankton crustaceans do feed on chytrid zoospores (Ibelings et al., 2004; Kagami et al., 2011).

The waterflea *Daphnia* as model system

The cladoceran *Daphnia* is a key organism in lake ecosystems as it is both an important phytoplankton grazer and a major food source for planktivorous fish (Lampert and Sommer, 2007). This genus is widely used as a model system in many biological fields as it has various important features (Lampert, 2011; Seda and Petrusek, 2011). The worldwide abundance of *Daphnia* (Popova and Kotov, 2013), together with its short generation time and easy maintenance in laboratory cultures, make it an attractive experimental organism. For evolutionary studies or investigations of speciation and hybridisation, the reproductive mode of *Daphnia* is of interest. *Daphnia* has a facultative parthenogenic reproductive cycle, where females produce clonal offspring in their brood pouch under favourable environmental conditions. When conditions become unfavourable (e.g. in autumn), females produce haploid eggs and genetically identical diploid male offspring. After sexual fertilisation by males, the eggs are released to the sediment as resting stages (ephippia), from which females hatch after conditions have improved (e.g. in spring) (Ebert, 2005). *Daphnia* further represents an ideal organism to study the ecological consequences of different environmental stressors, as it possesses a great phenotypic plasticity towards various abiotic and biotic factors including predation (Wolinska et al., 2007), temperature (Altermatt et al., 2008), oxygen deficiency (Sell, 1998) and food quality (Jeyasingh and Weider, 2005). The availability of genetic and genomic tools for *Daphnia* further extends its usefulness in research (Miner et al., 2012). In the field of so-called "resurrection ecology", resting eggs can be used to reconstruct the history and evolutionary dynamics of natural

populations. Moreover, these tools facilitate studies of the molecular basis of phenotypic plasticity, changes in gene expression in response environmental conditions and genotype-by-environment interactions. Here, the ability to raise large numbers of genetically identical offspring provides the opportunity to control for developmental noise (Miner et al., 2012). In addition, the effects of environmental change on host-parasite interactions can be studied in this system. Various parasites of *Daphnia* are well described (Ebert, 2005). It has been shown that changes in infections rates of *Daphnia* can be related to several environmental factors, such as temperature (Schoebel et al., 2011), food concentration (Fels, 2005), cyanobacteria density (Tellenbach et al., 2016), and predation (Duffy et al., 2005).

Aim of this thesis

The main aim of this thesis was to address how several aspects of environmental change impact community interactions in lakes. Specifically, I focused on two main topics: first, the effects of humic DOC, with or without simultaneous phosphorous addition, on the physiology, and population and community dynamics of *Daphnia*, as an indicator of potential consequences for biodiversity. Second, I investigated food web interactions between *Daphnia* and parasitised cyanobacteria to better understand trophic links between primary and secondary production during harmful algae blooms.

In Chapter 2, impacts of high, but environmentally relevant, concentrations of humic DOC on *Daphnia* were studied. To investigate how humic DOC affects *Daphnia* from the organismal to the population level, two *Daphnia* species were exposed to two concentrations of a commercial humic substance under controlled laboratory conditions. This study aimed to understand how sudden and strong inputs of humic DOC, as expected under extreme weather conditions, directly affect *Daphnia* stress physiology and life-history traits apart from cascading effects within the lake community.

The combined effects of moderate concentrations of humic DOC and phosphorus (P) enrichment on *Daphnia* populations within the lake community were investigated in Chapter 3. A large-scale mesocosm field experiment was carried out to study impacts

on *Daphnia* fitness, parasite infection, and inter- and intraspecific genetic composition. To disentangle indirect effects within the lake community from direct effects of humic DOC, a controlled laboratory experiment was conducted to assess humic DOC effects on *Daphnia* population density and fitness. By using these two approaches, their advantages are combined, leading to a better understanding of the complex interactions in natural systems. This study aimed to contribute to a deeper mechanistic conception of how future discharge of humic DOC in combination with nutrient enrichment might affect interactions at the organismal, population and community level in lakes.

The “Mycoloop” mechanism has been proposed for large inedible algal cells, and has rarely been studied experimentally. In Chapter 4, the assumptions of the “Mycoloop” were tested for the first time under experimental bloom conditions, using an edible, but suboptimal, cyanobacteria diet to investigate fungi-mediated trophic transfer between phytoplankton and zooplankton. This study aimed to provide new insights into the natural coexistence of zooplankton and cyanobacteria, and the importance of parasites in food web interactions.

Chapter 2

Humic dissolved organic carbon drives oxidative stress and severe fitness impairments in *Daphnia*

Manja Saebelfeld, Laëtitia Minguez, Johanna Griebel, Mark O. Gessner, Justyna Wolinska

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Abstract

Increases in dissolved organic carbon (DOC) in the form of humic substances, causing browning of surface water, have been reported worldwide. Field surveys indicate that higher DOC levels can influence primary production and thus plankton composition. Experimental studies on the direct effects of humic DOC on aquatic organisms have shown varying results depending on concentration and additional environmental factors. Moreover, changes in life-histories and stress responses have usually been tested separately, rather than in combination. We experimentally tested the impact of a sudden increase in humic DOC on two species of the zooplankton cladoceran *Daphnia*, across several levels of biological organisation, from cellular to population responses. In *D. magna*, strong impacts on reproduction (delayed maturity and reduced number of offspring) were coupled with overall stress induction (increases in antioxidant capacity and oxidative damage, combined with a reduced amount of available energy). In *D. longispina*, increased mortality and lowered fecundity were observed. We conclude that a strong input of humic DOC into aquatic systems can have severe negative impacts on zooplankton species, and has the potential to alter zooplankton community structures.

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Appendix A. Supplementary data

Appendix A.1 - Synthetic *Daphnia* medium (SSS-medium)

Stock solutions

a) 0.00012 M SeO_2

b) 10 % sea salt solution (hw-Marinemix® professional, Wiegandt GmbH, Krefeld, Germany)

c) SMB buffer

1. Salt stock solution

   0.3 M NaCl
   0.01 M KCl
   0.08 M $CaCl_2$ x 2 H_2O
   0.01 M $MgCl_2$ x 6 H_2O
   0.01 M $MgSO_4$ x 7 H_2O

2. Phosphate buffer stock solution

   Solution 2a: 0.18 M NaH_2PO_4 x H_2O
   Solution 2b: 0.2 M Na_2HPO_4 x 2 H_2O

- mix both solutions to pH 6,8

for 1 L SMB buffer:

600 mL ultrapure water
add 5 mM of solution 1 and 10 mL of solution 2

- fill up to 1000 mL with DI water

SSS-Medium (for 20 L)

8 L ultrapure water

add 240 mL SMB buffer
36 mL sea salt solution
2.4 mL SeO_2

fill up to 20 L with tap water (from copper-free pipeline)

Appendix A.2 - Biochemical and physiological analyses

A.2.1 Total antioxidant capacity

The total antioxidant capacity was measured as oxygen radical absorbance capacity (ORAC), using the modified $ORAC_{FL}$ method. Fluorescein was used as a fluorescent probe (135 nM), AAPH (2,2'- azobis (2-amidinopropane) dihydrochloride; 22 mM $well^{-1}$) as a peroxyl radical source, and Trolox as a standard. All reagents were purchased from Sigma Aldrich. For each assay, 25 µL of the homogenate was added to each well and mixed with 120 µL of fluorescein and 55 µL of AAPH. Fluorescence was measured every minute during 80 minutes with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Levels of antioxidant defenses were expressed in trolox-equivalents, nmol mg $proteins^{-1}$ (see below the method used to quantify protein contents).

A.2.2 Oxidative damages

The amount of oxidative damages was measured using the thiobarbituric acid reactive substances assay (TBARS). Subsamples of 50 µL homogenate (in duplicate) were treated with 20 µL of 8.1 % sodium dodecyl sulfate, 150 µL of 20 % acetic acid (pH 3.5) and 150 µL of thiobarbituric acid. The mixture was made up to 400 µL with distilled water, and heated for 30 min in boiling water. After cooling to room temperature, the mixture was centrifuged at 5000 × g for 5 min. The fluorescence of the supernatant was measured in the organic phase at excitation/emission wavelengths of 530/590 nm. Concentrations were derived from a standard curve of 1,1,3,3-tetramethoxypropane. The results are reported in pmol TBARS mg $proteins^{-1}$.

A.2.3 Energy reserves

Total protein was measured according to the method of Bradford (1976) at 592 nm using bovine serum albumin as standard. Total lipids and carbohydrates were extracted from tissue homogenate using chloroform:methanol 2:1 (v/v). After 30 min on ice, the samples were centrifuged at 4000 g for 5 min at 4°C. The top phase and the bottom phase were used to determine the total carbohydrate and the total lipid contents, respectively. Samples of 100 µL of the bottom phase were transferred to culture tubes and placed in a water bath at 95°C to evaporate the solvent. Then, 200 µL of sulphuric acid (95%) were added in each tube and left for 10 min. The tubes were then cooled on ice and 300 µL of vanillin-phosphoric acid reagent were added (0.6 % of vanillin in 68 % phosphoric acid, w/v). After a 25-min reaction time, the optical density was measured at 535 nm. Commercial cholesterol was used as a standard. Samples of 200 µL of the top phase were transferred to culture tubes and 600 µL of anthrone reagent (0.1 % in sulfuric acid 75 %) were added. The mixture was placed in a water bath at 95°C for 17 min and then cooled on ice. The optical density was measured at 630 nm. Glucose was used as a standard.

Appendix A.3 - Figures

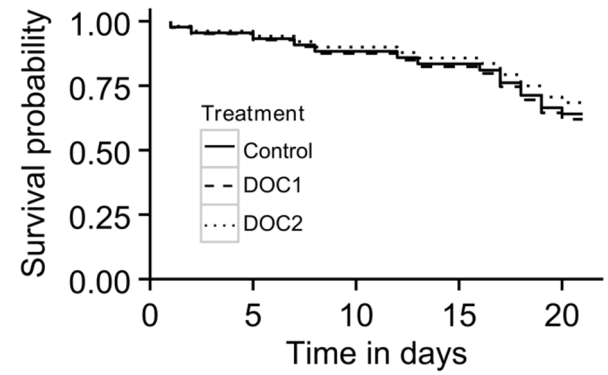

Figure S1. Survival of *D. magna*. Survival probability over 21 days as estimated in the Cox regression model, n was always 15.

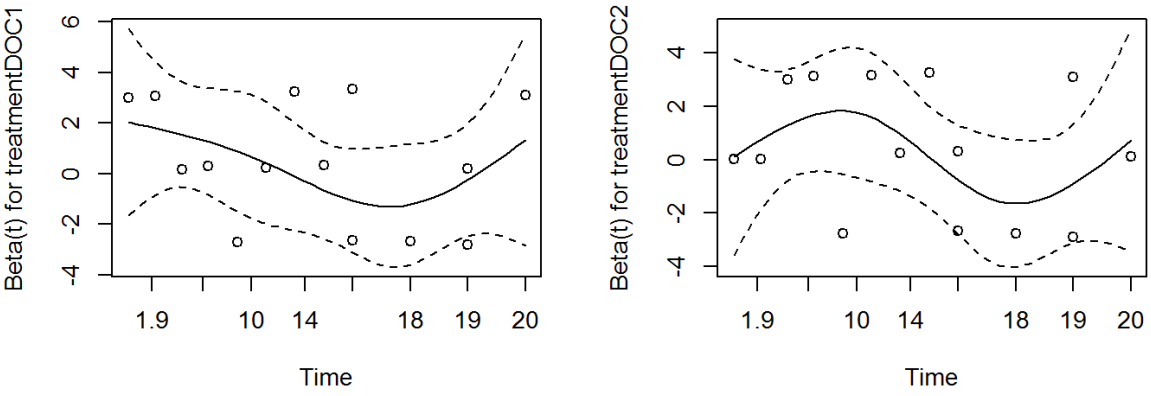

Figure S2. Scaled Schoenfeld residuals of the Cox regression model for *D. magna*. Solid lines (Beta(t)) give the estimated effect of the predictors through time in the experiment (with 95% confidence interval).

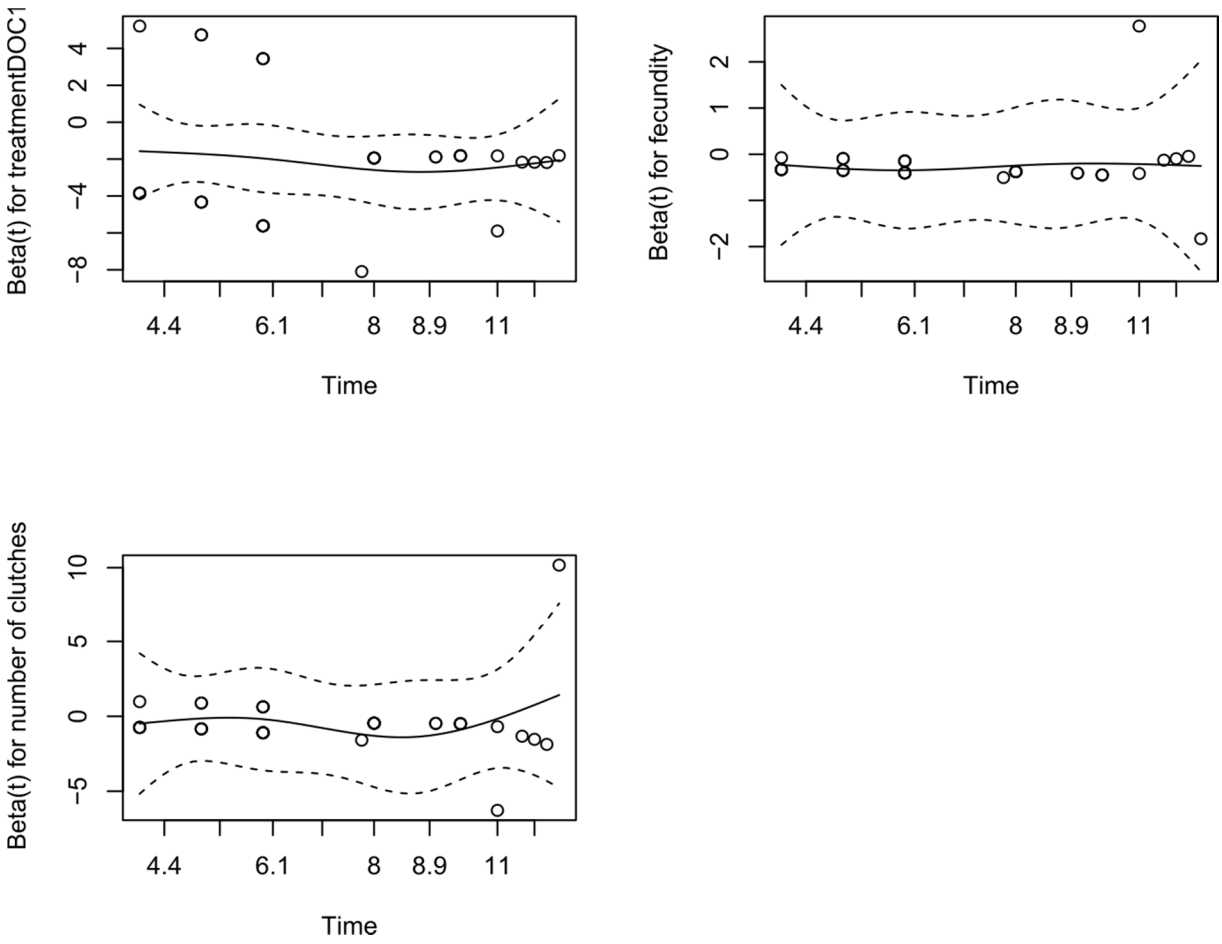

Figure S3. Scaled Schoenfeld residuals of the Cox regression model for *D. longispina*. In addition, fecundity and number of clutches versus time is shown for the Cox proportion hazard model fit. Solid lines (Beta(t)) give the estimated effect of the predictors through time in the experiment (with 95% confidence interval).

Chapter 3

Combined mesocosm and laboratory experiments reveal complex effects of humic DOC, with or without simultaneous phosphorus enrichment, on *Daphnia* fitness

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In preparation

Combined mesocosm and laboratory experiments reveal complex effects of humic DOC, with or without simultaneous phosphorus enrichment, on *Daphnia* fitness.

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Abstract

Environmental change has caused increased loading of phosphorus (P) and humic dissolved organic carbon (DOC) into freshwater systems. While P discharge is often accompanied by increased lake productivity, the shading effect of humic DOC reduces primary production, which can negatively affect higher trophic levels. Field and mesocosm studies have mostly focused on the effects of humic DOC, with or without simultaneous P addition, on abundance or richness of zooplankton at the taxon level. As relative abundances within taxa, both at the species and genotype level, can strongly affect food web dynamics in lakes, it is important to study the impact of environmental factors on the inter- and intraspecific composition of certain organismal groups. In a large-scale mesocosm experiment, we investigated the combined effects of humic DOC and P on fitness, parasite infection and genetic composition of the key zooplankter *Daphnia*. To disentangle cascading effects within the lake community from direct effects, we conducted an additional laboratory experiment, in which we investigated the impact of humic DOC on fitness of several *Daphnia longispina* genotypes. Results of the laboratory experiment showed that humic DOC can potentially impact intraspecific competition by differently affecting the relative fitness of *Daphnia* genotypes. These results did not translate into effects within the lake community, as we did not detect impacts on *Daphnia* fitness or intraspecific composition under humic DOC exposure in the mesocosm experiment. We showed that direct effects of stressors on organisms can be mask in the field by community interactions.

Introduction

Many freshwater systems have been experiencing enrichment with nutrients like phosphorus (P), due to agricultural and industrial activities. P-enriched lakes often respond with higher productivity and partially positive effects on higher trophic levels (Watson et al., 1997; Noe et al., 2001). Another major component in lakes that has been experiencing alterations in concentration is organic matter. The main sources of dissolved organic matter in lakes are humic substances (HS) - decomposed plant and animal residues. Due to the difficulty of characterising HS, the concentration of dissolved organic carbon (DOC) is used as an operational measure (Thurman, 1985; Suffet and MacCarthy, 1989). For many lakes worldwide, increasing concentrations of humic DOC have been reported in the last few decades, and various drivers for this pattern related to environmental change have been discussed (reviewed in Evans et al., 2005; Solomon et al., 2015). Increases in concentrations of humic DOC are accompanied by darkening of the water. This shading effect has been reported to reduce primary production, resulting in negative consequences for higher trophic levels, such as zooplankton and fish (Shurin et al., 2010; Brothers et al., 2014). The impact of humic DOC on zooplankton has been investigated in lake and mesocosm studies, focusing on total (Nicolle et al., 2012) or relative abundance (Strecker et al., 2008), as well as richness (Shurin et al., 2010) of zooplankton taxa (classified down to order or genus). Effects of humic DOC on genetic composition within single zooplankton species or between closely related species have not been investigated. Studies on the combined effects of P and humic DOC are scarce and have focused on overall effects on primary production or growth of the whole zooplankton community,

rather than on specific zooplankton groups (Arvola et al., 1996; Christensen et al., 1996). Thus, the question of how humic DOC, with or without simultaneous P addition, may impact relative abundances of closely related species and genotypes within lake communities have not been investigated in detail.

One key zooplankton group in lake food webs is the cladoceran *Daphnia*, as it can exert both top-down effects as a phytoplankton grazer, and bottom-up effects as a food source for predatory invertebrates and planktivorous fish (Kitchell and Carpenter, 1993; Brett et al., 1994; Lampert, 2011). *Daphnia* has among the highest grazing rates (i.e. the ability to suppress phytoplankton growth) of freshwater zooplankton groups (Brett et al., 1994), although the strength of grazing is species dependent (Kreutzer and Lampert, 1999). The composition of *Daphnia* communities, i.e. relative abundances of distinct species, has been shown to differently impact the biomass, size structure, taxonomic composition and richness of the phytoplankton community (Tessier et al., 2001), as well as to affect timing and duration of the clear-water phase (Hairston, 2005). These effects have been attributed to differences in body size or in seasonal phenology between *Daphnia* species. Furthermore, grazing efficiency in *Daphnia* (i.e. maximising food uptake under varying food quality) can be regulated by changes in the mesh size of the filter apparatus. This plastic response is not only species but also genotype-dependent (Bednarska and Dawidowicz, 2007).

In Europe, the most abundant *Daphnia* species in lakes belong to the *Daphnia longispina* complex. This species complex mainly includes *D. cucullata*, *D. galeata, D. longispina*, and their interspecific hybrids. Different taxa (i.e. parental species or hybrids) of this species complex usually co-occur within the same lake (Schwenk and

Spaak, 1995; Keller et al., 2008; Petrusek et al., 2008), while intraspecific (genotype) genetic diversity can be high (Keller and Spaak, 2004; Yin et al., 2010). Natural communities of the *D. longispina* complex have been shown to undergo rapid structural changes, for example replacement of taxa between years (Yin et al., 2010) or changes of relative species and genotype abundance within a single season (Yin et al., 2012). Underlying reasons for the changes were not identified in these studies, but the relationship to environmental variation, such as changes in temperature, was discussed. In another study, continuous shifts in species composition of the *D. longispina* complex community over several decades were associated with ongoing eutrophication (Brede et al., 2009).

Laboratory studies have shown that growth rates of *Daphnia* can be improved by feeding on P-rich algae (Boersma, 2000). Accordingly, P enrichment of whole lakes has resulted in increased zooplankton biomass including *Daphnia*, but this effect is also dependent on strength of predation (Carpenter et al., 2001). Exposure to humic DOC in laboratory studies has mostly been associated with negative effects on *Daphnia* such as induction of oxidative stress (Meems et al., 2004; Steinberg et al., 2010a), as well as lower survival probability and reduced offspring numbers (Steinberg et al., 2010a; Saebelfeld et al., 2017). Laboratory studies have focused on humic DOC effects on single genotypes of the tested *Daphnia* species. It is thus unclear whether the previously observed strong genotype × environment interactions in *Daphnia* toward various environmental conditions (Jeyasingh and Weider, 2005; Weider et al., 2005; Wolinska et al., 2007) are also detectable when exposed to humic DOC. Another potential effect of humic DOC on *Daphnia* is displayed by its impact on parasite spread.

It has been proposed that humic DOC can indirectly support infections in *Daphnia* populations by eliminating selective predation on infected individuals by visual predators, which would otherwise decrease disease prevalence (Johnson et al., 2006). Various biotic and abiotic factors have been shown to elevate infection rates in *Daphnia*, partially associated with increased host susceptibility under stressful conditions (Ebert, 2008; Schoebel et al., 2011; Yin et al., 2011; Tellenbach et al., 2016). Considering the negative impact of humic DOC on *Daphnia* fitness (Meems et al., 2004; Steinberg et al., 2010a; Saebelfeld et al., 2017), it is conceivable that increases in humic DOC concentrations affect host-parasite interactions in *Daphnia*. Infection spread within *Daphnia* populations has been proposed to affect trophic interactions, for example by reducing the population density of *Daphnia* to a level where top-down control of phytoplankton is inhibited (Duffy, 2007), or by lowering the food quality of *Daphnia* itself for predators (Forshay et al., 2008). Overall, *Daphnia* is a major player in lake food web stability. As impacts on inter- and intraspecific composition, as well as on parasite spread within *Daphnia* communities will likely affect the whole lake ecosystem (Miner et al., 2012), it is important to take these parameters into account when studying effects of environmental alterations on *Daphnia*.

Here, we investigated the combined effect of increased inputs of humic DOC and phosphorus on *Daphnia longispina* species complex communities, regarding: (i) life-history traits (fecundity and body size), (ii) parasitic infection and (iii) inter- and intraspecific composition, in a large-scale mesocosm experiment. To disentangle cascading effects within the food web (e.g. decreased primary production) from direct effects of humic DOC at the organismal level, we conducted a laboratory experiment

to study impacts of humic DOC on the carrying capacity and life-history (body size) of three *D. longispina* genotypes. In the mesocosm experiment, we expected to observe (i) increased *Daphnia* fitness with rising P concentrations, (ii) decreased *Daphnia* fitness with rising humic DOC concentrations, (iii) increased parasitic infection of *Daphnia* under humic DOC exposure, and (iv) alterations in relative abundances of *Daphnia* species and genotypes under humic DOC exposure. For the laboratory experiment, we hypothesised direct negative effects of humic DOC on *Daphnia* carrying capacity and body size, with variation in susceptibility (i.e. strength of impact on the measured parameters) among genotypes.

Material and Methods

Mesocosm experiment

Experimental setup

The mesocosm experiment was conducted over seven weeks from June 2nd to July 21st 2015. We used the IGB LakeLab, a large-scale mesocosm facility located in Lake Stechlin, a deep oligo-mesotrophic lake in northeastern Germany. The LakeLab consists of 24 experimental units (each 9 m in diameter) stretching from 0.5 m above the water surface down into the lake sediment at a depth of ca. 17-20 m. With a total volume of >2000 m^3 per mesocosm, permanently installed with a high degree of automated instrumentation, it is the only facility of its kind (http://www.lake-lab.de). A more detailed description of the general setup of the LakeLab is given in Giling et al. (2016) for an experiment performed one year earlier. In short, the experimental design of the current study differed mainly by using 21 mesocosms that were filled with the

surrounding natural lake water. For this, two pumps (DOMO10T/B, Xylem Water Solutions, Germany) per mesocosm were used, pumping water out and in simultaneously, replacing first the hypolimnic and then the epilimnic water of the mesocosms. First, eleven mesocosms and afterwards the remaining ten mesocosms were filled over two days each. To minimise disturbance of live organisms we used carefully selected and tested pumps (Nejstgaard et al., unpublished). Before starting the experiment, the mesocosms were cleared of fish by gillnet fishing, and the water pumped into the mesocosms was screened through a mesh size of 3 mm to exclude fish and larger organisms.

Average concentrations of DOC and total phosphorus in the lake during the study period were around 5 mg L^{-1} and 13 µg L^{-1}, respectively. Humic DOC was added to the mesocosms in the form of the commercially available humic substance HuminFeed® (HuminTech GmbH, Grevenbroich, Germany) at the beginning of the experiment (8-10th June). Details of the elemental composition and physical characteristics of HuminFeed® (HF) can be found in Meinelt et al. (2007). The applied DOC levels were: control (no addition of HF), low humic DOC (5 mg HF L^{-1}), and high humic DOC (10 mg HF L^{-1}). The added HF concentrations correspond to DOC concentrations of about 3 and 5 mg L^{-1}, respectively (as measured when dissolved in deionised water). Within each of the three DOC levels, a gradient of seven phosphorus (P) concentrations was applied, where the first P concentration (18 µg L^{-1}) served as a control (i.e. no addition of P). For the remaining six concentrations, 85 % orthophosphoric acid was added shortly before the humic DOC application, to the following final concentrations: 19, 22, 27, 34, 43, 54 µg L^{-1} (cf. Appendix S1: Figure S1).

Sampling

For this study, one integrated zooplankton sample per mesocosm was taken on two occasions: at the beginning of the experiment (June 2nd, i.e. a several days before the addition of humic DOC and P), and after six weeks of exposure (July 21st). Samples were collected using a 90 µm mesh size zooplankton net, hauled through the whole water column in the middle of the mesocosm. The sampling depth depended on the depth of the particular mesocosm and varied between 15 and 18.5 m. Samples were concentrated through a 180 µm sieve, fixated in pure ethanol to a final concentration of 70 %, and blinded for subsequent morphological and genotypic analyses.

Morphological parameters

All analysed *Daphnia* were morphologically assigned to their species, categorised into two groups: *D. cucullata*, or *D. longispina* and putative hybrids (i.e. *D. cucullata* × *D. longispina* hybrids). *D. longispina* and hybrids were pooled into one group as they could not be distinguished unambiguously. Body size (length from top of eye to base of tail spine) was measured for 30-35 randomly chosen adult *Daphnia* females per sample at the end point of the experiment (= 21 samples), using a Nikon SMZ 25 stereomicroscope and NIS-Element BR 4.5 software. Only individuals that were morphologically assigned to *D. longispina* or hybrids were used for this analysis (*D. cucullata*, a species much smaller than *D. longispina*, was excluded as this would bias the results according to species ratios). For a further 46 adult female *Daphnia* per sample (regardless of species), the following fitness estimates were assessed, using the stereomicroscope: ratio of gravid females, fecundity (number of asexual eggs/embryos

in the brood pouch per gravid female), and type of microparasitic infection (according to Wolinska et al., 2009). These measures were taken at both the start and end point of the experiment (= 2 × 21 samples). In addition, three samples from the long-term monthly lake monitoring program of Lake Stechlin were assessed for morphological species assignment and microparasitic infection (as above), for 46 randomly chosen adult female *Daphnia* per sample. These samples were taken using a 90 µm zooplankton net, hauled through the whole water column at the deepest point of Lake Stechlin (about 65 m) on June 9th, July 9th and August 11th, 2015.

Genotyping

For the assessment of species and genotype composition of the *Daphnia* community, four out of the seven samples per DOC level were chosen from both the start and the end point of the experiment, representing phosphorus levels 2, 3, 5 and 7 (= 2 × 12 samples). 46 *Daphnia* per sample (same individuals as used for fecundity and infection analyses) were genotyped at ten microsatellite loci (Dgm109, Dp196, Dp281, Dp512, SwiD1, SwiD2, SwiD10, SwiD12, SwiD14, SwiD15; Brede et al., 2006) in a multiplex polymerase chain reaction using a Multiplex PCR Kit (Qiagen). Details of DNA extraction and PCR reaction protocols can be found in Yin et al., 2010. Fragment analyses were performed by the company Services in Molecular Biology GmbH (SMB; Berlin, Germany). Fragment lengths at each locus were assessed using the software GeneMapper 4.1 (Applied Biosystems). The consistency of alleles was checked against loci-specific patterns of a reference clone used in each run, allowing for distinct scoring of alleles with small differences in fragment lengths.

Laboratory experiment

Daphnia cultures

Three *Daphnia longispina* genotypes (clones: Stech33, Stech52, Stech56), isolated from Lake Stechlin in June/July 2015, were exposed to experimental conditions for three weeks prior to the experiment. Individuals were kept in the respective experimental medium (see below), at a constant temperature of 20 ± 1 °C, a 12:12h dark:light cycle and fed three times per week *ad libitum* with the green unicellular algae *Scenedesmus obliquus* (1.5 mg L^{-1} C).

Preparation of experimental media

HuminFeed (HF) was applied in the same concentrations as used in the mesocosm experiment: 5 mg L^{-1} (low humic DOC treatment) and 10 mg L^{-1} (high humic DOC treatment). No HF was added to the control treatment. The day before usage, HF was dissolved in ultrapure water to a concentration of 200 mg L^{-1}, heated to 60°C for an hour to prevent quick precipitation in the experimental media, and refrigerated. Depending on the treatment, different amounts of ultrapure water and/or HF solution were added to an artificial *Daphnia* medium (SSS-medium, Saebelfeld et al., 2017); control: ultrapure water to 5 % of the final volume, low DOC: 2.5 % ultrapure water + 2.5 % HF solution, high DOC: 5 % HF solution. DOC and pH analyses were performed on one batch each of freshly prepared experimental solutions.

Experimental setup

To start the experiment, 10 adult *Daphnia* females were transferred by pipetting into a 250-mL jar containing 200 mL of the respective experimental medium (3 treatments × 3 clones × 6 replicates = 54 experimental units). *Daphnia* were fed three times per week with 1.5 mg C L^{-1} *S. obliquus*. The position of the jars was randomised twice a week. Half of the medium was refreshed weekly and all *Daphnia* were counted by pipetting them individually into a new jar. The experiment was terminated after six weeks; *Daphnia* from each jar were concentrated through a 250 µm sieve, fixated with denatured ethanol to a final concentration of 70 %, and blinded for subsequent body size measurements. Body size of 15 to 20 adult *Daphnia* females per replicate was measured as described above.

Statistical analyses

Mesocosm experiment

Morphological parameters. For analyses of body size and fecundity, the average value of each parameter was calculated per mesocosm. The ratio of gravid females was determined per mesocosm by dividing the number of gravid females by the number of all adult females (excluding those carrying resting eggs or infected by the sterilising parasite *Caullerya mesnili*). Regarding the analysis of infection, number of infected individuals was calculated per mesocosm. First, the effect of phosphorus concentration on each parameter was tested within each DOC treatment, with a simple linear regression analysis. As the applied P concentrations were not evenly distributed along the gradient (i.e. exponential rather than linear increase from level 1 to 7; cf. Appendix

S1: Figure S1), missing response values along the entire P concentration range (in steps of 1 µg L^{-1}) were replaced by "NA" for the regression analysis. In case a given parameter yielded significant results, differences between DOC levels were assessed using a Friedman test, which takes dependency of samples into account. If no regression was significant (per measured parameter), differences between DOC levels were assessed using a Kruskal-Wallis test. All analyses were performed in Rstudio 1.0.44 (R Core Team, 2016).

Taxon assignment. To describe in detail the *Daphnia* community present in the experimental mesocosms, all samples from the start and end point (= 2 × 12 samples) were pooled. Two microsatellite loci (SwiD2, SwiD15) were excluded from the analyses as they failed to amplify in more than half of the samples. Among individuals with complete genotypes at all remaining eight loci (n = 1028), unique multilocus genotypes (MLGs) were identified using GenAlEx 6.502 (Peakall and Smouse, 2006, 2012). For species identification of the MLGs, a factorial correspondence analysis (FCA), in which each different MLG was represented once, was performed in GENETIX 4.05 (Belkhir et al., 1996) by comparison with 48 reference genotypes. These genotypes comprised three parental species (*D. cucullata*, *D. galeata, D. longispina*) and two hybrid taxa of the European *Daphnia longispina* species complex (for details on the reference clones see Yin et al., 2010, whereas reference genotype D9 was excluded from the present study). All individuals with scored alleles for at least six loci (n=1060) were further assigned to one of six predefined taxa (two parental species and four hybrid groups: F1, F2 and both backcrosses) using the program NewHybrids 1.1 (Anderson and Thompson, 2002), which applies a Bayesian model-based clustering method.

NewHybrids was run for 10^{6x6} iterations after a burn-in length of 10^6. For taxon identification a threshold of 95 % posterior probability was applied. The results of NewHybrids were compared with the output of the program STRUCTURE 2.3.4 (Pritchard et al., 2000), which estimates the most likely number of taxonomic units K (Evanno et al., 2005). In STRUCTURE, the same data set as used for NewHybrids analysis was run for values of K from 1 to 15, for 10^5 iterations after a burn-in length of 10^6, using the *admixture model* and *correlated allele frequency model* (Pritchard et al., 2000). From the output, the correct K was determined using the online version of STRUCTURE HARVESTER 0.6.94 (Earl and von Holdt, 2012). For the assignment of individuals to their respective taxonomic groups, a threshold of 95 % was applied. All following calculations of taxon-specific parameters are based on the taxon assignment by NewHybrids.

Genetic diversity within taxa. Genetic diversity was assessed separately for each taxon, in pooled samples from the start point of the mesocosm experiment (= 12 samples). Per taxon, observed heterozygosity (H_o), expected heterozygosity (H_e), exact tests for significant deviation from Hardy-Weinberg equilibrium (HWE; 10^6 Markov chain length, 10^5 dememorisation steps), and the inbreeding coefficient F_{IS} (10^4 permutations), which measures the extent of nonrandom mating, were calculated in Arlequin 3.5.2.2 (Excoffier and Lischer, 2010); for all individuals with scored alleles for at least six loci (n = 506). The number of alleles (N_a) among the 506 individuals was determined in MLGsim 2.0 (Stenberg et al., 2003, http://www.rug.nl/research/gelifes/tres/software). Unique MLGs were identified using GenAlEx 6.502, for individuals with complete genotypes at all eight loci (n = 474). Clonal richness R was determined per taxon by

dividing the number of unique MLGs by the number of individuals (MLG/N). To evaluate the distribution of MLGs in each taxon, the evenness index E_{var} (Smith and Wilson, 1996) was calculated, which measures the relative abundance of genotypes and takes values between 0 (= uneven distribution) and 1 (= even distribution).

Genetic composition over space and time. Differences in taxon distribution were assessed among mesocosms at the start point of the experiment (= 12 samples), applying a chi-squared test of independence to the counts of *D. cucullata*, *D. longispina*, and F1 hybrids (according to NewHybrids assignment). To test for changes in distribution of the three taxa over time, pairwise comparisons of counts within mesocosms (between start and end point) were carried out using Fisher's exact tests, followed by Holm's p-value adjustment. Genetic variation within taxa over space and over time was assessed for F1 hybrids only, between three mesocosms of the control (A3, A5, A7) and three mesocosms of the high humic DOC treatment (C2, C3, C5; cf. Appendix S1: Figure S1). For other taxa, and/or mesocosms, sample sizes were too small (Figure 1). First, for all possible pairwise combinations (i.e. six mesocosms, within and between time points), F_{ST} values were calculated in Arlequin 3.5.2.2 (10^3 permutations). Second, change over time in clonal richness (i.e. MLG/N start – MLG/N end) was compared between control and high DOC treatments using a *t*-test. Third, clonal turnover rate was compared between control and high DOC treatments, also using a *t*-test. For this, the Morisita-Horn index (Wolda, 1981) was computed, using the online version of the program SpadeR (Chao et al., 2016) and 100 bootstrap replications. As the number of different genotypes was too low to calculate the turnover rate for two mesocosms, "dummy data" were added to all input files: two

additional genotypes present once, at both time points. The Morisita-Horn index (MH) output from SpadeR (determined as *relative genotype abundance* from *estimated similarity measures*) was then used to calculate the clonal turnover rate (1-MH). A clonal turnover rate calculated in this way takes into account both changes in the presence of individual MLGs and changes in MLG frequencies, with values from 0 (complete similarity) to 1 (no similarity).

Laboratory experiment

Per capita growth rate (change in individual numbers) was calculated per experimental unit, per week, and plotted against population size. The carrying capacity (i.e. the maximum population size that the environment can sustain) was obtained for each experimental unit using a linear regression (assuming logistic growth, Pratt, 1943), by calculating the intercept point with the x-axis (Sibly et al., 2005; Remus-Emsermann et al., 2012). Body sizes, as measured at the end of the experiment, were averaged per experimental unit. To assess differences in carrying capacity or body size among *Daphnia* clones and DOC treatments, a two-way ANOVA was performed (after testing for normality and variance homogeneity of the residuals), including clone and DOC treatment as fixed factors. This was followed by a contrast test for the effect of treatment within clones (least-squares means test and Holm's p-value adjustment). All analyses were performed in Rstudio 1.0.44 (R Core Team, 2016).

Results

Mesocosm experiment

Morphological parameters

Two of the mesocosm samples (A4 and B1, cf. Appendix S1: Figure S1) were found to contain no *Daphnia* at the end of the experiment and were thus excluded from all further microscopic analyses. Body size of *Daphnia* was affected neither by phosphorus additions within DOC levels (Appendix S1: Table S1), nor by humic DOC levels (Kruskal-Wallis test, χ^2=1.544, df=2, p=0.462). For fecundity assessments, another mesocosm (B7) was excluded from the analysis as no gravid females were found in the sample. Fecundity was positively related to P concentration, but only within the high humic DOC treatment (Figure 2a, Appendix S1: Table S1). Humic DOC caused no significant effect on fecundity (Friedman test, χ^2=3.5, df=2, p=0.174). The ratio of gravid females was affected neither by P (Appendix S1: Table S1) nor by humic DOC levels (Kruskal-Wallis tests, χ^2=5.006, df=2, p=0.08).

The number of infected *Daphnia* was very low at the start of the experiment (before additions of P and humic DOC). In 6 out of 21 mesocosms, 1-4 *Daphnia* (2-9 %) were infected with an oomycete brood parasite (Tellenbach et al., 2007). At the end of the experiment, types of infection were diverse with five parasite taxa recorded. Three mesocosms within the high humic DOC treatment showed high numbers of infected *Daphnia* (50-63 %, Figure 2b), where most of the individuals were infected by the ichthyosporean *Caullerya mesnili* - a gut parasite that sterilises the host (Lohr et al., 2010). Other infections were: microsporidia, bacteria, oomycete brood parasite, oomycete head parasite (functional categories as in Wolinska et al., 2009). Neither

number of pooled infected individuals (regardless of infection type) nor the number of *C. mesnili* infected individuals was affected by P (Appendix S1. Table S1) or humic DOC levels (Kruskal-Wallis tests, χ^2=5.094, df=2, p=0.078 for pooled infections; χ^2=5.173, df=2, p=0.075 for *C. mesnili* infection). In the lake samples, no infected *Daphnia* individuals were recorded in June or July; in August, 15 % of individuals were infected with microsporidia. The distribution of *Daphnia* species differed strongly between lake and mesocosm samples, as assessed by microscopy. While the lake samples were dominated by *D. cucullata* (78-98 % across June to August), few individuals of this species were recorded in the mesocosm samples (on average, 17 % and 4 % across June and July samples respectively).

Taxon assignment

Among all individuals from the mesocosm experiment with unique MLGs (pooled samples from the start and end point), FCA analysis revealed three clusters: two clusters around each of the reference genotypes, *D. cucullata* and *D. longispina*; and one cluster between them. NewHybrids assigned the analysed individuals to three taxonomic groups: *D. cucullata*, *D. longispina* and F1 hybrids (Appendix S1: Figure S2); 16 individuals (1.5 %) could not be assigned to any of the groups at a 95 % posterior probability. STRUCTURE analysis divided the dataset into two taxonomic groups. Here, parental species assignment coincided with NewHybrids results (100 % and 95 % individuals as assigned by NewHybrids to *D. cucullata* and *D. longispina*, respectively, were assigned accordingly by STRUCTURE, while the remaining 5 % of *D. longispina* were left unidentified). F1 hybrids (as assigned by NewHybrids), were assigned by

STRUCTURE to either of the parental species (*D. cucullata* = 41 %, *D. longispina* = 35 %) or left unidentified (24 %) (Appendix S1: Figure S3a). Repeated STRUCTURE analysis, excluding individuals that were assigned to *D. cucullata* by NewHybrids, divided the dataset into five taxonomic groups: *D. longispina* individuals (as assigned by NewHybrids) were assigned to a single group by STRUCTURE or left unidentified (23 %), while F1 hybrids (as assigned by NewHybrids) were split into four subgroups by STRUCTURE (Appendix S1: Figure S3b).

Genetic diversity within taxa

Among 1028 individuals with complete MLG profiles (pooled mesocosms from the start and end point), 146 unique MLGs were detected. Clonal richness (i.e. MLG/N) was highest for *D. longispina*, followed by *D. cucullata* and F1 hybrids (Table 1). The distribution of genotypes differed between parental and hybrid taxa. While F1 hybrids showed a rather uneven distribution of genotypes (E_{var} = 0.34), genotypes of *D. cucullata* and *D. longispina* were equally distributed (E_{var} = 0.70 and 0.89, respectively), reflecting an excess of rare genotypes in both species. *D. longispina* showed no deviation from HWE and a F_{IS} close to zero, indicating a high rate of sexual reproduction. For *D. cucullata*, significant deviation from HWE and a F_{IS} value of 0.36 point to more frequent asexual reproduction. The F1 hybrids, being in HWE and with a negative F_{IS} value (Table 1), indicate an excess of heterozygotes, originating from sexual crosses of the two parental species.

Genetic composition over space and time

Mesocosms differed in taxon distribution at the start point of the experiment (χ^2=407.62, df=22, p<0.0001; Figure 1). Specifically, in three mesocosms the most abundant taxon was *D. cucullata*, in two *D. longispina*, and in the remaining seven mesocosms F1 hybrids. Five mesocosms (one in the control and two in each humic DOC treatment) showed significant differences in taxon distribution between start and end point. This change was mostly driven by the loss of *D. cucullata* individuals over time (Figure 1). For the most abundant taxon, F1 hybrids, pairwise F_{ST} values were assessed among populations, for all possible combinations of balanced design (i.e. three mesocosms of the control and high DOC treatment, within and between time points: = 2 × 6 samples). All comparisons between different mesocosms, both within and between time points, yielded significant genetic differentiation. In contrast, there was no significant differentiation within mesocosms between time points, except for mesocosm C5 (Appendix S1: Table S2). This differentiation was mostly driven by one genotype that was not detected at the start and was recorded 18 times at the end, replacing another genotype that was detected 8 times at the start and not found at the end. Assessment of within mesocosm changes in clonal richness and turnover rates between control and high DOC treatment yielded non-significant results (Appendix S1: Table S3). Again mesocosm C5 stands out, showing a much higher turnover rate (0.39) compared to other mesocosms (ranging from 0 – 0.15).

Laboratory experiment

DOC concentrations for the control, low and high DOC treatments were 2.9, 3.7, and 5.2 mg L^{-1}, respectively, and pH was not affected by HF addition (Appendix S1: Table S4). Three replicates of the control treatment for clone Stech33 were lost during the experiment (two in the first few days as transferred *Daphnia* died before reproducing, and one replicate did not contain any individuals in the last week after individual numbers were very low over the whole experimental phase), resulting in three remaining replicates. Two-way ANOVAs showed significant effects of treatment (i.e. DOC level) and clone on both carrying capacity and body size, and in addition a significant treatment-by-clone interaction effect on the body size of *Daphnia* (Appendix S1: Table S5). The carrying capacity decreased in clone Stech52 with rising humic DOC concentrations, by 10 % and 22 % at low and high humic DOC levels compared to controls (Figure 3a, Appendix S1: Table S5). Body size increased by 4 % in clone Stech56 under humic DOC exposure compared to controls, with no differences between the two humic DOC levels (Figure 3b, Appendix S1: Table S5).

Discussion

To disentangle cascading lake community effects of combined P and humic DOC additions from direct effects of humic DOC on *Daphnia*, we analysed responses of *Daphnia* in a large-scale mesocosm study as well as in a complementary laboratory experiment. We showed that while humic DOC has the potential to directly affect intraspecific competition of *Daphnia*, complex effects of humic DOC and P on

community interactions complicate the analysis of impacts on specific taxa, making predictions of ongoing trends difficult.

Effects of P and humic DOC on *Daphnia* fitness and parasite infections (mesocosm experiment)

Fecundity of *Daphnia* increased with rising initial P concentrations under high humic DOC exposure, but not in the control and low humic DOC treatments. It is known that P enrichment can improve zooplankton growth rates due to increased food quality and quantity (Watson et al., 1997; Boersma, 2000; Noe et al., 2001). The potential positive effects of P addition, however, depend on several other factors, such as UV radiation or the availability of other nutrients like nitrogen (reviewed in Gulati and DeMott, 1997). Increases in concentrations of humic DOC have been shown to directly diminish zooplankton reproductive performance, including *Daphnia* (Engert et al., 2013; Saebelfeld et al., 2017). Furthermore, the shading effect of humic DOC has been reported to reduce primary production, resulting in cascading effects on higher trophic levels (Shurin et al., 2010; Brothers et al., 2014). The addition of humic DOC in the mesocosm experiment resulted, as expected, in a decrease in primary production, phytoplankton biomass and subsequently in zooplankton biomass at both applied concentrations (Stephan et al., unpublished; Berger, personal communication). Conversely, in the control mesocosms, biomasses of phytoplankton and zooplankton increased toward the middle of the experiment, peaked, and dropped towards the end - a characteristic pattern described by the PEG models (Sommer et al., 1986, 2012). On the last sampling day, the declines of zooplankton biomasses in the controls were still

ongoing. If phytoplankton biomasses at this point were too low to sustain zooplankton growth, this could explain the lack of an observable effect of different P levels on *Daphnia* fitness. On the contrary, in the humic DOC treated mesocosms, photo degradation caused a bleaching effect over the course of the experiment, allowing phytoplankton populations to start recovering toward the end of the experiment. Here, different P concentrations could potentially support first phytoplankton growth, followed by zooplankton growth in a concentration dependent manner. Indeed, *Daphnia* abundances and biomasses showed increasing trends in both humic DOC concentrations along the P gradient at the end point of the experiment, though this was only significant for the lower humic DOC concentration (data not shown). Studies of mixed effects of P and humic DOC are scarce and show varying results. Additions of combined P and humic DOC in a mesocosm experiment have been shown to support primary production more than addition of P alone, though this has been explained by the extra P that was added with the humic material itself (Arvola et al., 1996). On the contrary, enrichment of a humic lake basin with P failed to support phytoplankton growth in comparison to the control basin (Christensen et al., 1996). Closer investigation of the combined effects of P and humic DOC on lake communities should be a task for future studies, as these components are often discharged together and can result in complex interactions (Dillon and Molot, 2005; Brothers et al., 2014).

In laboratory studies, humic DOC has been shown to affect reproduction and body size of cladocerans, including *Daphnia* (Engert et al., 2013; McMeans et al., 2015; Saebelfeld et al., 2017). In our mesocosm experiment, however, we could not find any effects of humic DOC on these two parameters in *Daphnia*. One reason for this finding

could be that effects of humic DOC on food quality have masked direct negative effects on *Daphnia* physiology compared to controls (Minguez et al., unpublished). In addition, the analysed individuals were a mixture of diverse taxa and genotypes, as well as of different ages. It is known that there is strong taxon and genotype variation in reactions to environmental stressors in *Daphnia* (Weider et al., 2005; Wolinska et al., 2007; Spaak et al., 2012; Griebel et al., 2015), and that reproductive output and body size also depend on the age of individuals (Green, 1956; Ebert, 1993). Thus, genetic and age variation among analysed individuals may have left average effects on life-history traits undetected.

There was further no significant effect of humic DOC on the number of infected *Daphnia* individuals, but variation between mesocosms was strong with three out of seven mesocosms in the high DOC treatment containing high numbers of infected *Daphnia*, mostly with the ichthyosporean gut parasite *Caullerya mesnili*. It is widely accepted that environmental fluctuations can enhance disease spread within populations (Thomas and Blanford, 2003; Wolinska and King, 2009). For *Daphnia*, increased *C. mesnili* infection rates have been associated with decreased temperature or increased cyanobacteria density (Schoebel et al., 2011; Tellenbach et al., 2016). The high number of *C. mesnili* infected individuals in three mesocosms of the high DOC treatment suggests that humic DOC may support infection spread. Studies on impacts of humic DOC on infections have so far focused mainly on fitness traits of human pathogens in medical surveys (Schneider et al., 1996; Lu et al., 2002; van Rensburg et al., 2002), and on parasite abundance (of bacteria-infecting viruses) (Anesio et al., 2004; Lymer et al., 2008) or mobility (of the zoonotic multihost protozoan *Toxoplasma*

gondii) (Shapiro et al., 2009) in lake studies. To our knowledge there are no studies on the effects of humic DOC on host-parasite interactions in aquatic invertebrate systems, or with a focus on impacts on host susceptibility. As parasites can strongly influence population and community dynamics (e.g. Wood et al., 2007 and references therein), consequences of increasing humic DOC concentrations on disease spread in freshwater organisms should be a focus of future studies.

Genetic diversity within and among taxa

Among all *Daphnia* individuals sampled during the mesocosm experiment, three taxa were identified: *D. cucullata, D. longispina*, and *Daphnia cucullata* × *longispina* hybrids. The *Daphnia* community composition was reconstructed from 12 pooled mesocosm samples at the start point of the experiment, i.e. before the introduction of treatments, and might thus be a relatively good representation of the natural community composition of lake *Daphnia*. Indeed, genetic diversity within taxa showed typical characteristics for communities of the *Daphnia longispina* species complex. As previously described for the *D. galeata* × *D. longispina* complex (Yin et al., 2010, 2012; Griebel et al., 2016), the *D. cucullata* × *D. longispina* hybrids studied here showed lower genetic diversity than their parental species. This has been attributed to parents investing more in sexual reproduction, while hybrids mainly reproduce asexually (Keller and Spaak, 2004; Keller et al., 2007; Yin et al., 2014). Indeed, we found higher clonal richness and uneven genotype distribution (E_{var}) due to excesses of unique genotypes in parents compared to hybrids, as well as *D. longispina* showing a low F_{IS} value (indicating absence of inbreeding) and being in HWE (indicating random mating).

We found low abundances of *D. cucullata* in the mesocosms compared to the lake. One explanation for this could be the different sampling locations, depth and times between mesocosm and lake samplings. It is well known that zooplankton species, including *Daphnia*, show different patterns of vertical and horizontal distribution within the same lake (Stich and Lampert, 1981; Masson et al., 2001; Pinel-Alloul et al., 2004). Alternatively, the differences in *Daphnia* species abundance between mesocosm and lake samples may be attributed to selection against *D. cucullata* within the mesocosms. It is conceivable that hybrids, which were the most abundant taxon in the mesocosm, outcompeted *D. cucullata*. The *temporal hybrid superiority hypothesis* suggests higher fitness of hybrids under certain environmental conditions (Moore, 1977). Hybrids of the *D. longispina* species complex have been found to perform better than their parentals under cold temperatures (Griebel et al., 2015) and fish predation pressure (Spaak and Hoekstra, 1995). The latter might have played a role in our study. *D. cucullata*, as the smallest *Daphnia* species, has a low predation risk by fish, which are visual predators (Schwenk and Spaak, 1995). This would act in favour of *D. cucullata* over their presumably larger hybrids (Schwenk and Spaak, 1995) in the lake. As fish were excluded from the mesocosm experiment, this advantage could have been lost in the mesocosms.

Treatment effect on genetic composition

Taxon composition changed significantly over time in five out of twelve mesocosms across all DOC treatments, due to loss of *D. cucullata* individuals. This further supports the idea of a negative mesocosm effect on this species, rather than a treatment effect.

Moreover, there was no effect of humic DOC on genetic composition (i.e. clonal turnover rate and clonal richness) of F1 hybrid populations. Though it is accepted that impacts of humic DOC on the light regime, and primary and bacterial production may affect higher trophic levels (Jansson et al., 2000; Karlsson et al., 2009; Jones and Lennon, 2015), only a few mesocosm or lake studies have addressed the effects of increasing humic DOC concentrations on zooplankton community structure and diversity. In a long-term study of 53 temperate North American and European lakes, richness of zooplankton taxa was negatively correlated with short-term humic DOC increases, but positively with long-term average concentrations (Shurin et al., 2010). Other studies did not find any response by zooplankton communities to humic DOC-induced lowered primary production (Nicolle et al., 2012) or changes in phytoplankton community composition (Arvola et al., 1996). In the latter case this was attributed to the short study period. In arctic lakes, humic DOC was positively correlated with zooplankton taxon richness, likely due to decreased stress from harmful UV radiation (Strecker et al., 2008). To the best of our knowledge, we investigated for the first time the impact of humic DOC on genetic composition at the species and genotype level within a single zooplankton genus. We did not find an effect of humic DOC on species distribution nor on intraspecific composition in *Daphnia* hybrid populations. However, changes in relative genotype abundances under increased humic DOC inputs are feasible as humic DOC can differently affect genotypes of the same species (see below).

Laboratory experiment

Results of the laboratory experiment indicate that humic DOC may have various direct effects on fitness estimates among genotypes within the same *Daphnia* species. Humic DOC has been shown to induce oxidative stress in several freshwater organisms - attributed to the internal production of reactive oxygen species (ROS) - resulting in increased metabolic costs from upregulated antioxidant defenses (Timofeyev et al., 2006; Saebelfeld et al., 2017). In *Daphnia* this has been shown to lead to reproductive impairment and/or higher mortality (Steinberg et al., 2010a; Saebelfeld et al., 2017). It is likely that oxidative stress-induced direct negative effects on reproductive success and/or survival led to the observed concentration-dependent decrease of the carrying capacity in one of the three *D. longispina* clones exposed to humic DOC. Conversely, another clone showed increases in body size under exposure to humic DOC, indicating a rather positive effect. Body size is an important fitness parameter in *Daphnia* as it is linked to higher offspring production (Green, 1956; Lampert, 1993). Increase in body size under exposure to humic DOC has been found in the cladoceran *Moina macrocopa* when exposed to additional stressors (Suhett et al., 2011; Engert et al., 2013). It has been proposed that humic DOC can be used as a direct energy source for zooplankton (Suhett et al., 2011; Karlsson et al., 2012). Additionally, increased bacterial biomass under higher humic DOC concentrations, acting as a food source and thus supporting *Daphnia* growth, has been found under food-limiting conditions (McMeans et al., 2015). Using potentially increased bacterial biomasses and/or humic DOC as a nutritional source may be the reason for the observed body size increase in one *D. longispina* clone in our experiment. However, as *Daphnia* were grown under sufficient

food conditions, this explanation is rather unlikely. Nevertheless, the observed clonal differences highlight the danger of extrapolating results from one genotype to the whole species. Humic DOC has been shown to differently affect the fitness of two co-occurring *Moina* species, potentially shaping zooplankton community structures in natural settings (Steinberg et al., 2010b). Our results indicate that humic DOC may not only affect zooplankton communities at the species, but also at the genotype level.

In summary, we showed that at the lake community level, combined inputs of humic DOC and P lead to complex interactions, potentially masking direct effects of humic DOC on life-history characteristics of specific taxa. Our data also suggest that humic DOC may increase parasite spread in populations. Further, though there was no detectable effect of humic DOC on the intraspecific genetic composition of *Daphnia* F1 hybrids in the mesocosm experiment, results from a complementary laboratory experiment showed that humic DOC has the potential to shape *Daphnia* populations at the genotype level.

Supplementary material

Table S1. Influence of phosphorus concentration on *Daphnia* body size, fecundity and parasite infections.

Table S2. Pairwise F_{ST} comparison of F1 hybrid populations.

Table S3. Analyses of changes in clonal richness and turnover rates of F1 hybrids over time, and differences between control and high DOC treatment.

Table S4. DOC and pH analyses of exposure medium for the laboratory experiment.

Table S5. Influence of DOC levels on *Daphnia* carrying capacity and body size.

Figure S1. Design of mesocosm experiment.

Figure S2. Factorial correspondence analysis (FCA) based on allelic variation at eight microsatellite loci.

Figure S3. Comparison between NewHybrids and STRUCTURE group assignments of *Daphnia* individuals from the mesocosm experiment.

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Author contributions

SAB and JCN were members of the steering group contributing to the scientific design of the overall LakeLab mesocosm experiment, and also organised and supervised its conduct. MS and JW conceived the current study (i.e. zooplankton sampling, analysis of the *Daphnia* community and conduct of the laboratory experiment). MS collected the zooplankton samples. MS and YAO conducted the laboratory experiment. MS, YAO and JW processed the samples. MS and YAO analysed the data. MS wrote the manuscript, with contributions from JW, SAB and JCN.

Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1. Genetic diversity of *Daphnia* taxa in the mesocosm experiment. Analyses were made on individuals pooled across 12 mesocosms, sampled at the start point of the experiment. Depending on the analysis, individuals with scored alleles at six or eight (grey shading) loci were used.

Taxon[a]	N[b]	N[c]	N_a	H_o	H_e	HWE	F_{IS}	MLG	R	E_{var}
D. cucullata	152	130	17	0.71	0.92	p<0.01	0.36	54	0.42	0.70
D. longispina	88	88	42	1.0	0.95	p=0.96	0.01	61	0.69	0.89
F1 hybrids	266	265	40	1.0	0.94	p=0.09	-0.08	31	0.12	0.34

[a]Taxon assignment as determined by NewHybrids software based on the allelic variation at six to eight microsatellite loci

[b]Number of individuals with at least six scored loci

[c]Number of individuals with eight scored loci

N_a: number of alleles, H_o: observed heterozygosity, H_e: expected heterozygosity/gene diversity, HWE: Hardy-Weinberg equilibrium, F_{IS}: inbreeding coefficient, MLG: number of unique multilocus genotypes, R: clonal richness (MLG/N[c]), E_{var}: evenness index (distribution of genotypes)

Figures

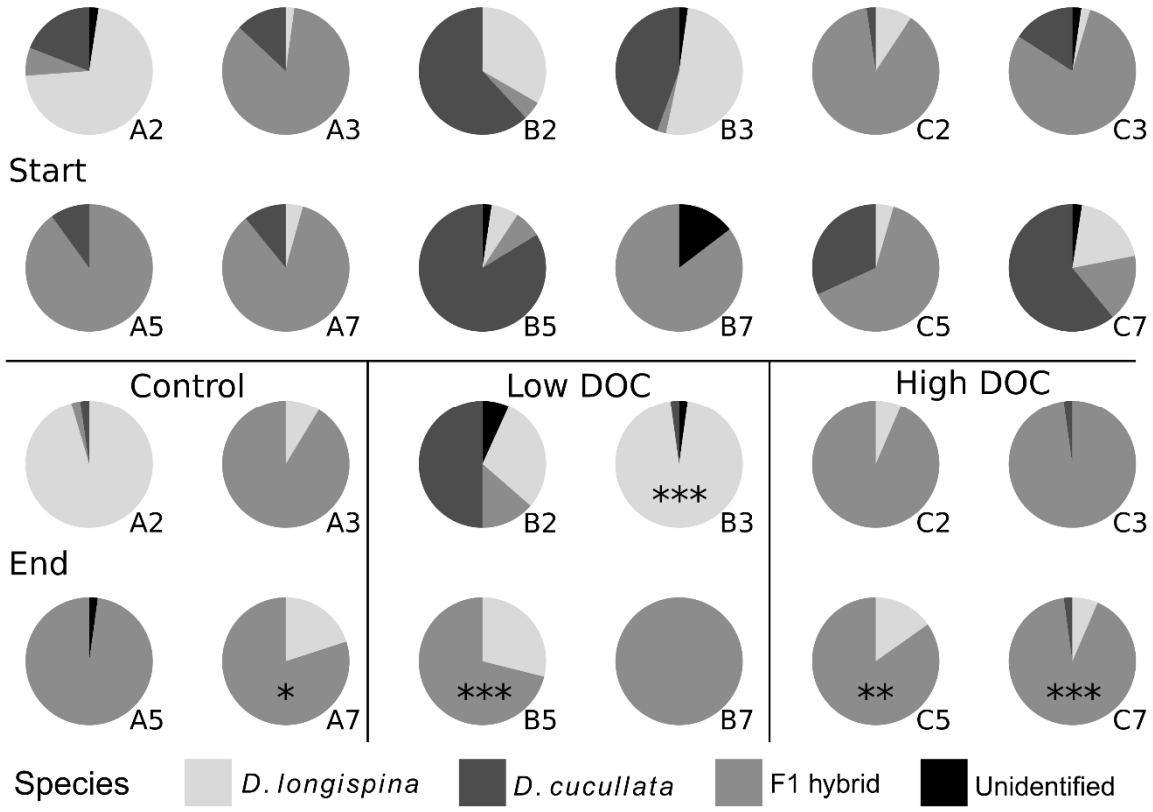

Figure 1. Taxon distribution in the mesocosm experiment. Taxon assignment for 41-46 individuals per mesocosm is based on NewHybrids analysis at a 95 % posterior probability. Taxon distributions are shown for 12 mesocosms, each at the start (upper two rows, before addition of humic DOC and P) and the end point (lower two rows) of the experiment. Letters and numbers at the lower right edge of each pie chart correspond to DOC and P levels (e.g. B2 = low humic DOC treatment and P level 2; cf. Appendix S1: Figure S1). Differences in taxon distribution over time are shown by asterisks in the respective mesocosm at the end point (* <0.05, ** <0.001, *** <0.0001).

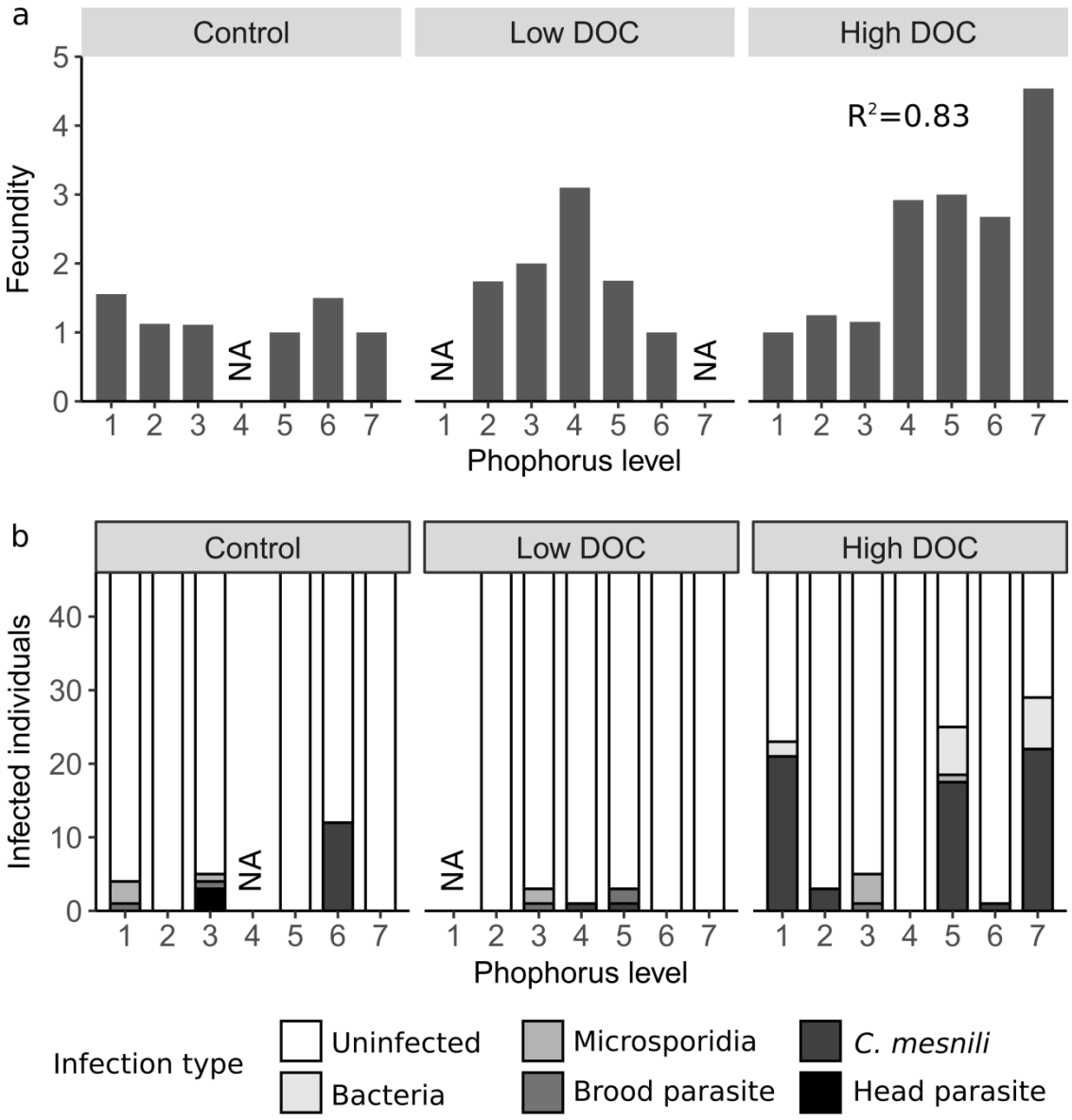

Figure 2. Fecundity and parasite infections of *Daphnia* at the end of the mesocosm experiment. (a) Average number of eggs per gravid female in each mesocosm along the P gradient (1-7, cf. Appendix S1: Figure S1), within DOC levels (control, low, high). Significant regression analysis among P levels, within DOC treatments is indicated by R^2 value. (b) Numbers of infected and uninfected *Daphnia* individuals along P levels (1-7). Infections were assessed for 46 randomly chosen adult females per sample.

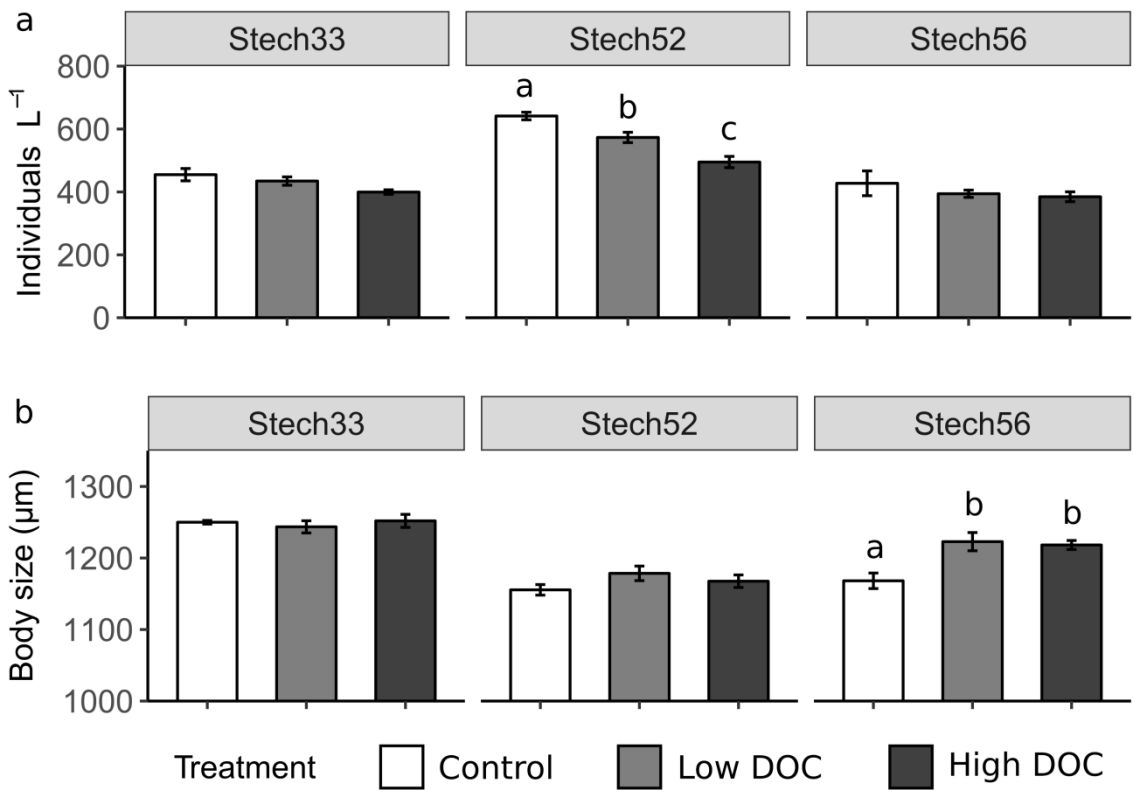

Figure 3. Carrying capacity (a) and body size (b) of *Daphnia* clones under different levels of DOC exposure. Data are shown as means (± SE). Significant differences between treatments within clones (Stech33, Stech52, Stech56) are indicated by different lowercase letters.

Appendix S1

Tables

Table S1. Influence of phosphorus concentration on *Daphnia* body size, fecundity and parasite infections. Significant differences are depicted in bold. Differences in degrees of freedom result from each one missing sample in the control and low DOC treatments, and, for the parameter fecundity, from one sample not containing any gravid females.

Parameter	DOC level	Linear regression	Standard error of the residuals	R^2	Adjusted R^2
Body size	Control	$F_{1,4}$=0.77, p=0.43	67.07	0.161	-0.05
	Low DOC	$F_{1,4}$=0.48, p=0.53	102.60	0.108	-0.12
	High DOC	$F_{1,5}$=2.68, p=0.16	101.20	0.349	0.22
Fecundity	Control	$F_{1,4}$=0.27, p=0.63	0.27	0.063	-0.17
	Low DOC	$F_{1,3}$=1.03, p=0.38	0.76	0.256	-0.01
	High DOC	$F_{1,5}$=23.64, **p=0.005**	0.59	0.825	0.79
Ratio of gravid females	Control	$F_{1,4}$=0.47, p=0.53	0.02	0.105	-0.12
	Low DOC	$F_{1,4}$=2.76, p=0.17	0.25	0.408	0.26
	High DOC	$F_{1,5}$=3.79, p=0.11	0.19	0.431	0.32
All infections	Control	$F_{1,4}$=0.04, p=0.86	5.26	0.009	-0.24
	Low DOC	$F_{1,4}$=0.66, p=0.46	1.52	0.143	-0.07
	High DOC	$F_{1,5}$=0.76, p=0.42	13.00	0.132	-0.04
C. mesnili infection	Control	$F_{1,4}$=0.67, p=0.46	5.07	0.144	-0.07
	Low DOC	$F_{1,4}$=0.10, p=0.77	0.57	0.024	-0.22
	High DOC	$F_{1,5}$=0.53, p=0.50	11.04	0.096	-0.08

Table S2. Pairwise F_{ST} comparison of F1 hybrid populations. Comparisons were made for each three mesocosm of the control (A) and high DOC treatment (C) between start (-s) and end (-e) point of the experiment. F_{ST} values are shown in the lower left corner, and respective significance levels of p-values in the upper right corner. Comparisons within enclosures between time points are shaded in grey.

	A3-s	A5-s	A7-s	C2-s	C3-s	C5-s	A3-e	A5-e	A7-e	C2-e	C3-e	C5-e
A3-s	/	***	***	***	***	***	n.s.	***	***	***	***	***
A5-s	0.23	/	***	***	***	***	***	n.s	***	***	***	***
A7-s	0.49	0.30	/	***	***	***	***	***	n.s.	***	***	***
C2-s	0.10	0.09	0.38	/	***	***	*	***	***	n.s.	***	***
C3-s	0.31	0.12	0.39	0.13	/	***	***	***	***	***	n.s.	***
C5-s	0.43	0.30	0.47	0.28	0.28	/	***	***	***	***	***	***
A3-e	< 0.01	0.27	0.52	0.12	0.34	0.46	/	***	***	**	***	***
A5-e	0.24	< 0.01	0.31	0.10	0.13	0.30	0.27	/	***	***	***	***
A7-e	0.48	0.28	< 0.01	0.36	0.37	0.45	0.51	0.29	/	***	***	***
C2-e	0.04	0.11	0.39	< 0.01	0.18	0.31	0.06	0.12	0.37	/	***	***
C3-e	0.31	0.16	0.42	0.15	< 0.01	0.28	0.34	0.17	0.40	0.19	/	***
C5-e	0.35	0.20	0.34	0.21	0.15	0.13	0.38	0.22	0.32	0.24	0.16	/

*** p<0.0001, ** p<0.001, * p<0.01, n.s. non-significant

Table S3. Analyses of changes in clonal richness and turnover rates of F1 hybrids over time, and differences between control and high DOC treatment. Per mesocosm differences in clonal richness (MLG/N) and clonal turnover rate were determined between start and end point of the mesocosm experiment. To assess differences between control (A mesocosms) and high DOC treatment (C mesocosms), P levels were pooled within DOC levels.

Mesocosm	Difference MLG/N	Turnover rate
A3	0.055	0
A5	0.025	0.1539
A7	-0.058	0
C2	0.065	0.0166
C3	0.063	0.0152
C5	0.009	0.3935
Results of *t*-test	t=-0.995, df=3.08, p=0.39	t=-0.666, df=2.65, p=0.56

Table S4. DOC and pH analyses of exposure medium for the laboratory experiment. Analyses were run on freshly prepared solutions. HF: HuminFeed; DOC: dissolved organic carbon.

Treatment	Added HF (mg L^{-1})	DOC (mg L^{-1})	pH
Control	0	2.88	7.65
Low DOC	5	3.72	7.67
High DOC	10	5.22	7.53

Table S5. Influence of DOC levels on *Daphnia* carrying capacity and body size. Results of two-way ANOVAs (for effects of *t* treatment, *c* clone and *t:c* treatment-by-clone interaction) followed by contrast tests (for comparisons between treatments within clones) are shown. Significant differences are depicted in bold.

Two-way ANOVA		Contrast test			
Parameter		Clone	Control vs. Low	Control vs. High	Low vs. High
Carrying capacity					
t	$F_{2,42}$=16.32, **p<0.0001**	Stech33	0.537	0.292	0.392
c	$F_{2,42}$=66.43, **p<0.0001**	Stech52	**0.014**	**<0.001**	**0.011**
t:c	$F_{4,42}$=2.24, p=0.0804	Stech56	0.438	0.348	0.722
Body size					
t	$F_{2,42}$=12.36, **p<0.0001**	Stech33	1.0	1.0	1.0
c	$F_{2,42}$=47.77, **p<0.0001**	Stech52	0.249	0.716	0.716
t:c	$F_{4,42}$=2.75, **p=0.0408**	Stech56	**<0.001**	**<0.001**	0.724

Figures

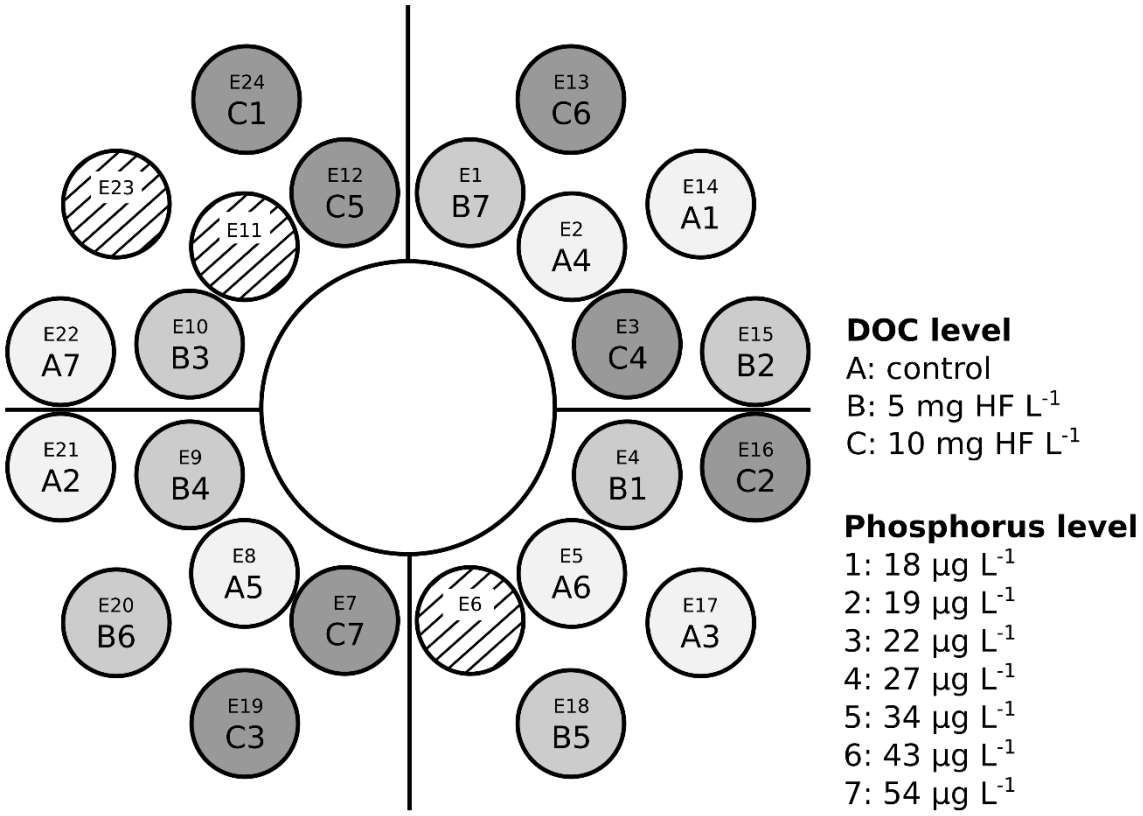

Figure S1. Design of mesocosm experiment. Circles represent mesocosms with consecutive numbering from E1 to E24; letters A-C and grey shading represent applied levels of DOC; numbers 1-7 represent applied levels of phosphorus.

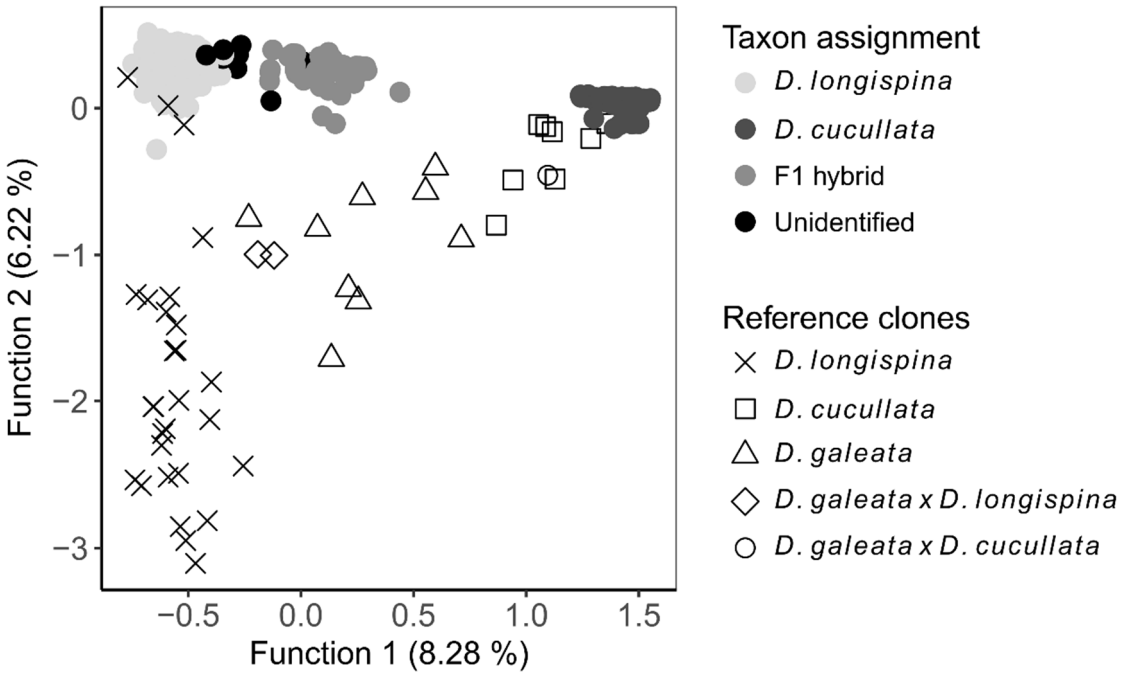

Figure S2. Factorial correspondence analysis (FCA) based on allelic variation at eight microsatellite loci. Analyses were made on individuals from the mesocosm experiment, pooled from each 12 mesocosms of the start and end point of the experiment. Taxon assignment is based on NewHybrids analysis at a 95 % posterior probability. 48 reference clones represent three parental species and two hybrid taxa of the European *Daphnia longispina* species complex.

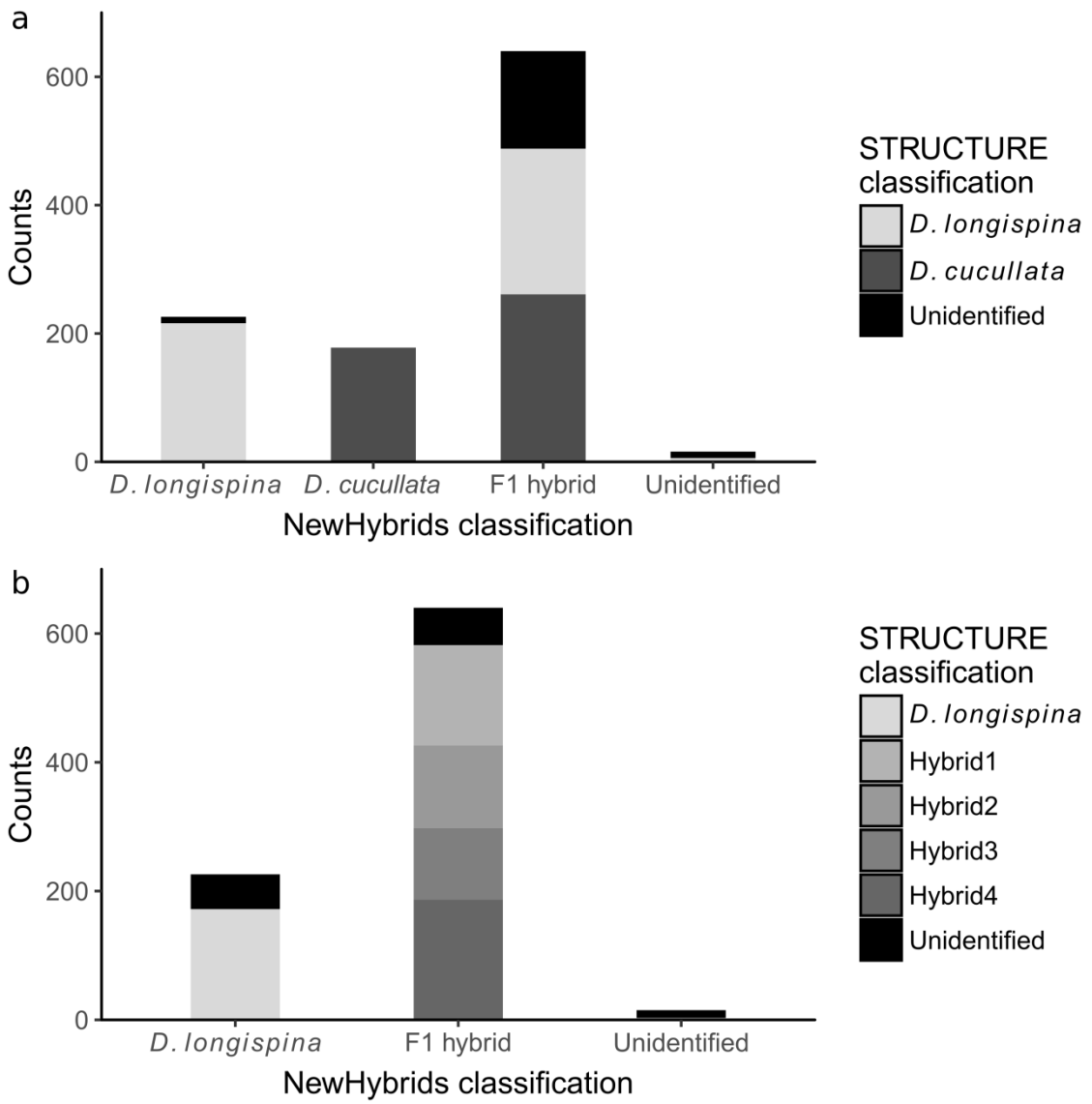

Figure S3. Comparison between NewHybrids and STRUCTURE group assignments of *Daphnia* individuals from the mesocosm experiment. Group/taxon assignments were made at a 95% posterior probability in both methods, based on allelic variation at 6-8 microsatellite loci. Individuals from all mesocosms and time points are pooled (= 2 × 12 samples). (a) Comparison of all 1060 individuals (b) Comparison of individuals, excluding the *D. cucullata* taxon (as classified by NewHybrids). The F1 hybrid taxon (as classified in NewHybrids) is split into four subgroups by STRUCTURE.

Chapter 4

Chytrid parasitism facilitates trophic transfer between bloomforming cyanobacteria and zooplankton (*Daphnia*)

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Chytrid parasitism facilitates trophic transfer between bloom-forming cyanobacteria and zooplankton (*Daphnia*)

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Parasites are rarely included in food web studies, although they can strongly alter trophic interactions. In aquatic ecosystems, poorly grazed cyanobacteria often dominate phytoplankton communities, leading to the decoupling of primary and secondary production. Here, we addressed the interface between predator-prey and host-parasite interactions by conducting a life-table experiment, in which four *Daphnia galeata* genotypes were maintained on quantitatively comparable diets consisting of healthy cyanobacteria or cyanobacteria infected by a fungal (chytrid) parasite. In four out of five fitness parameters, at least one *Daphnia* genotype performed better on parasitised cyanobacteria than in the absence of infection. Further treatments consisting of purified chytrid zoospores and heterotrophic bacteria suspensions established the causes of improved fitness. First, *Daphnia* feed on chytrid zoospores which trophically upgrade cyanobacterial carbon. Second, an increase in heterotrophic bacterial biomass, promoted by cyanobacterial decay, provides an additional food source for *Daphnia*. In addition, chytrid infection induces fragmentation of cyanobacterial filaments, which could render cyanobacteria more edible. Our results demonstrate that chytrid parasitism can sustain zooplankton under cyanobacterial bloom conditions, and exemplify the potential of parasites to alter interactions between trophic levels.

Trophic interactions govern the flow of material and energy in ecosystems and modulate many of their fundamental properties, such as productivity, regime shifts, or biogeochemical cycles[1–3]. Advances in food web theory and modelling have contributed to our picture of the network of feeding relationships in ecological communities. Still, they often fail to explain processes observed in natural systems[4]. One reason for this is that most food web studies do not incorporate what is perhaps the most common trophic interaction - parasitism[5]. Despite their ubiquity, parasites are usually overlooked because of their cryptic nature, the difficulties in quantifying their effects, and their assumed low biomass[6]. However, they can account for greater biomass than predators[7] and participate in the majority of trophic links[8]. Parasites can modulate trophic flows in a number of ways. They can drive reductions in host biomass, not only by increasing host mortality rates, but also by influencing growth, fecundity, nutritional status, susceptibility to predation, or behaviour[9]. While their role as consumers is better known, parasites can also be prey for other organisms. They can be consumed together with their host (i.e. concomitant predation) or as free living life stages. Given the enormous reproductive output of parasites, free living infecting stages potentially constitute a significant nutrient source and can account for a substantial transfer of material and energy to higher trophic levels[10,11].

The efficiency of energy and material entry into the food web is largely determined by the trophic coupling between primary and secondary production. In aquatic pelagic ecosystems, primary production is often dominated by cyanobacteria. Promoted by eutrophication and global warming[12,13], cyanobacteria often develop into blooms that severely disrupt ecosystem functioning and raise health concerns due to the production of diverse

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Treatment	Carbon (mg l^{-1})	Filaments (ml^{-1})	Zoospores (ml^{-1})	Bacteria (10^6 ml^{-1})
Cyn	1.01 ± 0.020	1,901 ± 100	—	0.75 ± 0.06
Inf	0.80 ± 0.090	1,582 ± 171	2,433 ± 285	4.42 ± 0.30
Zoo	0.09 ± 0.005	—	2,182 ± 263	4.45 ± 0.23
Bac	0.08 ± 0.004	—	—	4.24 ± 0.20

Table 1. Final carbon concentrations, and filament, zoospore and heterotrophic bacteria densities supplied to *Daphnia* in each feeding treatment. Cyn: uninfected cyanobacteria, Inf: infected cyanobacteria, Zoo: zoospores, Bac: heterotrophic bacteria. Data are shown as means (± s.e.m.) of all feeding occasions ($n = 11$).

toxins[14,15]. Cyanobacteria display high resistance to grazing, which often leads to the decoupling of primary and secondary production and inefficient carbon transfer to zooplankton[16]. The inability of zooplankton to exert effective top-down control on cyanobacterial populations has traditionally been linked to the poor edibility of cyanobacteria with colonial or filamentous morphologies, the production of toxic metabolites, and their low nutritional value[17]. Meta-analyses of experimental data have shown that, although grazing resistance cannot be generalised, cyanobacteria in fact constitute a poor food resource, as they lack essential nutritional compounds for zooplankton, such as sterols and polyunsaturated fatty acids (PUFAs)[18,19]. However, field observations often report a high biomass of grazers during bloom events[20–22], suggesting alternative sources of nutrition capable of sustaining zooplankton growth.

Besides grazing, parasitism can act as an additional top-down control on phytoplankton[23,24]. In addition to virus and prokaryotic parasites (e.g. lytic bacteria), a so-far hidden diversity of small microeukaryotes has been revealed by metagenomics surveys, many of which display parasitic lifestyles[25]. Among these eukaryotic parasites, phytoplankton is particularly affected by chytrids, a group of primitive fungi characterised by a free-swimming zoosporic life stage[26]. Chytrid infection is lethal and has the potential to act as a controlling agent on cyanobacterial populations, often reaching epidemic proportions[24]. Zoosporic fungi can be an important food source for zooplankton, as they provide sterols and long-chain PUFAs that are lacking in prokaryotic prey[27–29]. Arthropods cannot synthesise these compounds *de novo*, so that they need to obtain these essential lipids from their diet[30,31]. Experimental work has shown that the cladoceran *Daphnia*, a keystone crustacean herbivore that drives much of the secondary production in pelagic ecosystems[32], is able to feed on chytrid zoospores infecting the large inedible diatom *Asterionella formosa*. Thus, constituents may potentially be channeled from inedible algae to zooplankton *via* a trophic link termed the "mycoloop"[33,34]. In a similar way, chytrids may enable nutrient transfer from *A. formosa* to copepods[35]. However, due to its size, *A. formosa* cannot be grazed upon either by *Daphnia* or copepods, leaving open the question of whether chytrids can enhance the coupling between zooplankton and smaller, yet nutritionally suboptimal phytoplankton, like cyanobacteria.

Here, we experimentally address the interrelation between predator-prey and host-parasite interactions. To do so, we used a host-parasite system based on the filamentous, bloom-forming cyanobacterium *Planktothrix agardhii* and its obligate chytrid parasite *Rhizophydium megarrhizum*. A laboratory experiment was conducted in which four genotypes of *Daphnia galeata* were maintained under quantitatively comparable diets of infected and uninfected cyanobacteria, together with additional experimental treatments consisting of chytrid zoospores and heterotrophic bacteria suspensions. We hypothesise that chytrid infection on phytoplankton can sustain zooplankton growth and improve its fitness under cyanobacterial dominance.

Results

Table 1 shows particulate organic carbon (POC) concentrations and counts of cyanobacteria, zoospores and heterotrophic bacteria in the respective treatments. In both infected and uninfected cyanobacteria treatments, POC concentrations were well above *Daphnia* requirements (>0.6 mg C l^{-1}; refs 36,37), although about 20% lower in the infected compared to the uninfected treatment. Additional treatments consisting of zoospores or heterotrophic bacteria provided comparable food quantities, yet supplied only about 10% of POC relative to cyanobacteria treatments. The density of heterotrophic bacteria in the uninfected cyanobacteria treatment was about 80% lower compared to other treatments. Infected cyanobacterial filaments were halved in length relative to conditions of absence of infection (Fig. 1).

Two-way ANOVAs showed a significant effect of diet (i.e. treatment) on all measured fitness parameters. *Daphnia* genotype had an effect for all parameters except fecundity (Table 2). Significant treatment by genotype interactions were found for fecundity and body size of *Daphnia*, both of adults and offspring. For the parameters age at maturity and body size of adults (which presented non-normal distributions), additional two-way ANOVAs were performed on aligned rank transformed data resulting in the same significance levels for adults body size, and an additional significant treatment by genotype interaction for age at maturity ($p < 0.001$).

Within-genotype comparisons revealed that, for all fitness parameters except fecundity, at least one *Daphnia* genotype performed better on an infected compared to an uninfected cyanobacteria diet (Fig. 2, Table 2). Two genotypes matured significantly earlier (Fig. 2a) and larger body sizes in adults were found for one genotype (Fig. 2d). All genotypes displayed offspring with bigger size (Fig. 2e), whereas two genotypes showed higher growth rates when fed with infected cyanobacteria (Fig. 2c). There were no significant differences in total offspring number (Fig. 2b) or in the proportion of individuals that survived until the third reproductive cycle between infected and uninfected cyanobacteria treatments (data not shown).

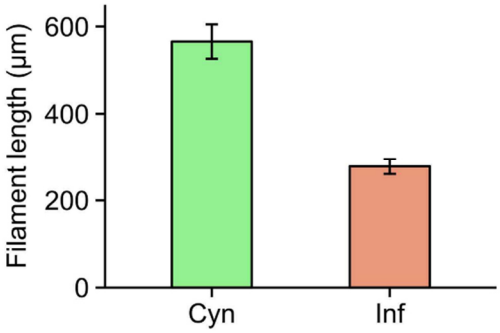

Figure 1. Mean length of cyanobacteria filaments. Data are shown as mean filament lengths (±s.e.m.) in each feeding suspension (n = 11). Kruskal-Wallis test revealed significant differences between treatments ($\chi^2 = 13.772$, p = 0.0002) Cyn: uninfected cyanobacteria, Inf: infected cyanobacteria.

Zoospore and heterotrophic bacteria diets induced a strong reduction in total offspring number and growth rates relative to treatments supplying cyanobacteria (Fig. 2b,c, Table 2). However, despite lower POC concentrations supplied (about 90% lower), *Daphnia* on a zoospore diet performed equal or better in all other fitness parameters compared to the uninfected cyanobacteria treatment. In all cases, *Daphnia* displayed bigger offspring on zoospore *vs.* bacteria (Fig. 2e). On a zoospore diet significant differences in size of adults, total offspring and growth rates were found for one out of four genotypes when compared with a heterotrophic bacteria diet. Also, a higher proportion of replicates survived until the third reproductive cycle when *Daphnia* were fed with zoospores (Fig. 3), although food quantity in both treatments was comparable (Table 1).

Discussion

In aquatic pelagic systems, primary and secondary production is subject to severe decoupling when poorly edible colonial or filamentous cyanobacteria dominate primary production[16]. Here, we addressed the question of whether parasites are able to prevent such decoupling by providing alternative trophic links between cyanobacteria and zooplankton. Chytrid parasites of cyanobacteria, although typically neglected, are ubiquitous and often burst into epidemics, reaching infection prevalence over 90%[38,39]. Our study shows that *Daphnia* fitness can be significantly improved when cyanobacteria are attacked by parasites. Upon chytrid infection, filamentous cyanobacteria get fragmented, potentially increasing their edibility, and alternative food sources are made available for grazers in the form of chytrid zoospores and increased abundances of heterotrophic bacteria.

In our experiment, we observed for at least one genotype a significant improvement in fitness in terms of age at maturity, growth rates, and body size of adults and offspring when *Daphnia* was fed with infected cyanobacteria diet compared to an uninfected cyanobacteria diet. We measured five different parameters to better characterize *Daphnia* fitness. Under natural conditions, earlier born offspring can imply strong contributions to the establishment of the population, especially under food limitation and in the presence of predators[40]. Body size is another important fitness trait in *Daphnia*. Bigger size at birth leads to higher resistance to starvation and larger body size of adults[41,42]. Adult size in turn is positively correlated with clutch size[42,43]; hence, larger animals potentially contribute to the population with higher amounts of offspring. In our study, no differences between infected and uninfected diets were detected concerning total number of offspring. However, in natural settings, the time needed to produce offspring can be another crucial factor. We thus compared growth rates between genotypes. Two genotypes displayed increased growth rates in the infected diet compared to healthy cyanobacteria, indicating that an infected cyanobacteria diet contributes to *Daphnia* fitness by accelerating reproduction, rather than increasing total offspring.

To disentangle the contribution of the aforementioned additional food sources to *Daphnia* fitness, zoospores and heterotrophic bacteria were separated from the infected cultures and used as food in two additional experimental treatments. The resulting reduction in POC in these treatments (about 90%) compared to cyanobacterial diets induced a strong reduction in the production of offspring in all genotypes. However, these adverse effects were not observed in other fitness parameters. Notably, the zoospore diet yielded higher or otherwise not significantly different fitness in other parameters compared to the uninfected cyanobacterial diet, despite a 90% reduction in supplied POC. This strongly suggests enhanced carbon transfer efficiency when conveyed to grazers *via* chytrid zoospores. Zoospores are within the optimal size range for ingestion by *Daphnia*, and display high cellular contents of PUFAs and sterols (in particular, cholesterol and its precursors sitosterol and stigmasterol[34,44]) that prevent the lipid limitation typically observed when cladoceran grazers feed on cyanobacteria[45]. By extracting, transforming and repacking nutrients from cyanobacteria into higher quality, more readily ingestible zoospores, chytrids upgrade cyanobacterial carbon for zooplankton, thereby increasing the coupling between primary and secondary production under cyanobacterial dominance or bloom conditions.

A chytrid-mediated trophic link between *Daphnia* and inedible diatoms has been proposed previously, giving rise to the concept of the "mycoloop". Kagami *et al.*[34] undertook a 6-day life-table experiment in which *Daphnia* were fed with chytrid-infected and uninfected cultures of the large inedible diatom *Asterionella formosa*. *Daphnia* displayed significantly larger body size when fed with infected diatoms, as a result of feeding on chytrid zoospores. However, in that study reproduction was not assessed, raising questions as to whether zoospores alone can sustain

Two-way ANOVA		Contrast test						*n*			
Parameter	Genotype	Cyn vs. Inf	Cyn vs. Zoo	Cyn vs. Bac	Inf vs. Zoo	Inf vs. Bac	Zoo vs. Bac	Cyn	Inf	Zoo	Bac
Age at maturity*											
t $F_{3,171}=9.73$, **$p<0.001$** g $F_{3,171}=4.36$, **$p<0.01$** *t:g* $F_{9,171}=1.44$, $p=0.175$	Mugg6b	**0.019**	**0.023**	0.051	1.0	1.0	1.0	13	13	14	7
	Mugg7a	0.658	1.0	1.0	0.139	0.658	1.0	13	12	15	13
	Mugg11c	1.0	1.0	1.0	0.166	0.695	1.0	12	12	10	9
	Mugg13c	**<0.01**	0.207	0.612	0.369	**0.012**	0.369	14	10	9	11
Fecundity[1]											
t $F_{3,147}=94.19$, **$p<0.001$** g $F_{3,147}=0.92$, $p=0.433$ *t:g* $F_{9,147}=2.67$, **$p<0.01$**	Mugg6b	0.869	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.01**	13	12	14	6
	Mugg7a	0.871	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.871	13	12	14	6
	Mugg11c	0.485	**0.017**	**<0.001**	**<0.01**	**<0.001**	0.085	12	12	7	3
	Mugg13c	0.306	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.306	14	10	8	7
Growth rate											
t $F_{3,147}=118.17$, **$p<0.001$** g $F_{3,147}=3.43$, **$p=0.019$** *t:g* $F_{9,147}=1.40$, $p=0.192$	Mugg6b	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.01**	13	12	14	6
	Mugg7a	0.213	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.396	13	12	14	6
	Mugg11c	**<0.01**	**<0.01**	**<0.001**	**<0.001**	**<0.001**	0.084	12	12	7	3
	Mugg13c	0.953	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.953	14	10	8	7
Body size adults*											
t $F_{3,146}=9.15$, **$p<0.001$** 4 g $F_{3,146}=38.72$, **$p<0.001$** *t:g* $F_{9,146}=2.09$, **$p<0.001$**	Mugg6b	1.0	1.0	0.288	1.0	0.250	0.288	13	12	14	6
	Mugg7a	**<0.001**	**0.014**	0.093	0.093	**<0.001**	**<0.001**	13	12	14	6
	Mugg11c	1.0	1.0	1.0	1.0	1.0	1.0	12	12	7	3
	Mugg13c	1.0	1.0	0.703	1.0	0.259	0.943	14	9	8	7
Body size offspring[2]											
t $F_{3,143}=88.28$, **$p<0.001$** g $F_{3,143}=14.52$, **$p<0.001$** *t:g* $F_{9,143}=2.37$, **$p=0.016$**	Mugg6b	**0.036**	0.451	**<0.001**	**<0.01**	**<0.001**	**<0.001**	12	13	14	4
	Mugg7a	**<0.001**	**0.030**	**<0.001**	0.115	**<0.001**	**<0.001**	12	11	10	9
	Mugg11c	**<0.001**	0.290	**<0.001**	**<0.01**	**<0.001**	**<0.001**	10	12	8	5
	Mugg13c	**<0.001**	**0.011**	**<0.01**	0.236	**<0.001**	**<0.001**	13	9	7	10

Table 2. Effect of different food treatments on *Daphnia* fitness parameters. Results of two-way ANOVAs (for effects of *t* treatment, *g* genotype and *t:g* treatment by genotype interaction) followed by contrast tests (comparisons between treatments within genotypes) are shown. The number of replicates (*n*) included in each comparison is given. Discrepancies in number of replicates between parameters resulted from unmeasurable animals (*see* Methods). Significant p-values are depicted in bold. Cyn: uninfected cyanobacteria, Inf: infected cyanobacteria, Zoo: zoospores, Bac: heterotrophic bacteria. *data are not normally distributed but show equal variances of the residuals (*see* Methods). [1]data$^{(1/2)}$ transformed. [2]data$^{(-4)}$ transformed.

Daphnia populations. The present experiment was conducted over three reproductive cycles, which not only allowed testing for population viability, but also minimised the potential effects of maternal nutrient reserves, which might mask nutrient limitations imposed by the different diets tested[46]. Moreover, in the former study, limitations in the experimental design (6 replicates per treatment with only one replicate left in the uninfected diet treatment) complicate the interpretation of the results. Whereas that study tested only a single *Daphnia* genotype, the present experiment included four different genotypes. Our results showed significant effects of genotype and/or genotype by diet interactions for all measured fitness parameters and support the importance of addressing inter-clonal variability in *Daphnia* populations[47,48]. Finally, whereas *Asterionella* represents an inaccessible carbon source that cannot be exploited by *Daphnia,* cyanobacteria constitute a suboptimal, yet edible food source. This difference uncovers a new facet of the mycoloop whereby chytrid parasites act as trophic upgraders of suboptimal prey, illustrating their potential to operate in ways other than making carbon available from inedible sources.

Our experiment shows that chytrid zoospores alone are sufficient to sustain not only *Daphnia* growth, but also reproduction, and can enhance fitness relative to parasite-free conditions. This demonstrates that natural zooplankton populations can, in principle, be sustained solely by mycoloop contributions. The extent of the mycoloop largely depends on the range of naturally-occurring zoospore densities in the water column. Direct quantifications of zoospores from environmental samples, although seldom undertaken, have shown maximal zoospore densities of up to 500 zoospores ml^{-1} treatments[49], which are about one quarter of those provided in our experimental treatments. However, much higher densities can potentially be reached. For example, Rasconi *et al.*[39] reported sporangia (i.e. sessile reproductive structures that release new zoospores upon maturation) densities of $3\times10^4\,ml^{-1}$ under epidemic conditions. Considering a mean release of 4-25 zoospores per sporangium[50], free-swimming zoospores could reach densities two or even three orders of magnitude higher than provided in our assay, suggesting that the contribution of zoospores to zooplankton diet under natural conditions may be greater than shown by this experiment.

In addition to chytrid zoospores, our results suggest that heterotrophic bacteria cannot be neglected as an extra food source for *Daphnia*. In contrast to the study of Kagami *et al.*[34], chytrid infection caused a 5-fold increase in bacterial densities compared to uninfected conditions. Such increase is attributable to the decay of

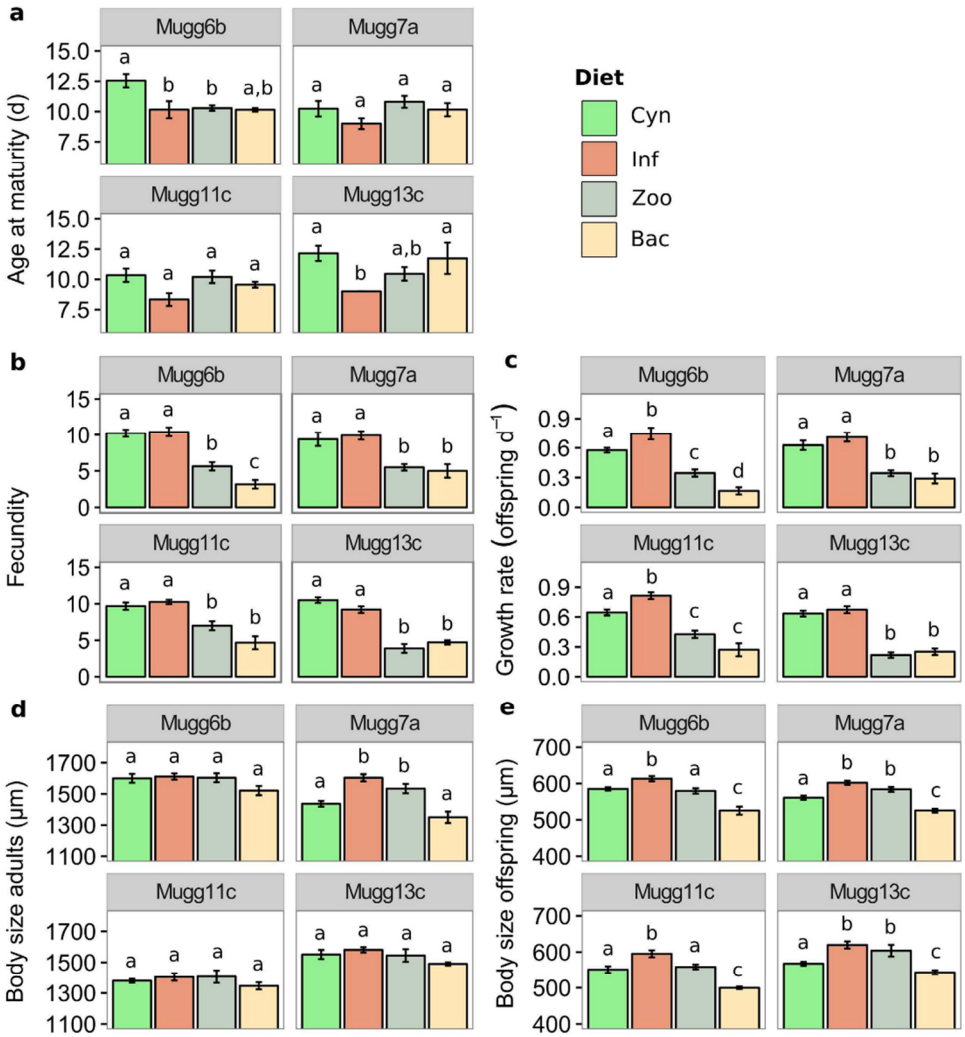

Figure 2. Fitness parameters of *Daphnia* genotypes fed with different diets. (**a**) Age at maturity, (**b**) fecundity, (**c**) growth rate, (**d**) body size of adults, (**e**) body size of offspring. Data are shown as means (±s.e.m.). Significant differences between treatments within genotypes (Mugg6b, Mugg7a, Mugg11c, Mugg13c) are indicated by different lowercase letters. Cyn: uninfected cyanobacteria, Inf: infected cyanobacteria, Zoo: zoospores, Bac: heterotrophic bacteria. The respective *n* are shown in Table 2.

infected cyanobacteria and the subsequent release of dissolved organic carbon, which can be readily used by heterotrophic bacteria for growth. Observed increase in bacterial biomass was enough to sustain *Daphnia* growth and reproduction. However, on a purified bacterial diet, fewer individuals reached the third reproductive cycle (Fig. 3), and their offspring were smaller relative to the zoospore diet in all cases (Fig. 2e). This indicates that bacteria constitute a food source of lower quality than chytrids, which is consistent with the general lack of essential lipids in prokaryotes[51–53]. Still, increased shares of bacterial food sources during the decay of inedible phytoplankton can act as a conveyor of dietary energy to *Daphnia*. Bacteria repack otherwise poorly ingestible cyanobacteria into smaller, easily ingestible particles and potentially detoxify cyanobacterial carbon. Moreover, bacterial proliferation under natural conditions activates the microbial loop[54], promoting the growth of heterotrophic nanoflagellates and ciliates, which in turn have the potential to upgrade the biochemical quality of prokaryotic carbon by *de novo* synthesis of essential lipids[55,56]. Thus, increased bacterial densities may have more profound effects under natural conditions than our experiment can show.

Beside the aforementioned effects of zoospores and increased densities of heterotrophic bacteria, cyanobacterial filaments underwent fragmentation upon chytrid infection. Although the present experimental design did not allow a direct comparison of *Daphnia* grazing rates on shorter (infected) and longer (uninfected) filaments, a halving in length arguably reduces mechanical feeding interference and could facilitate grazing[20]. Filament

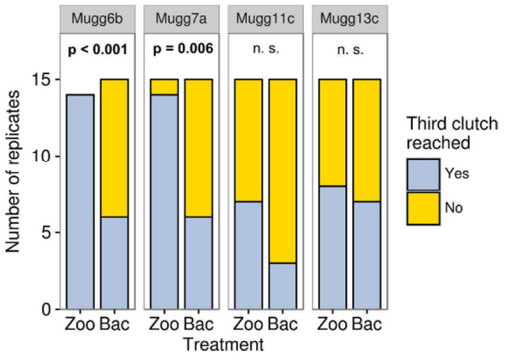

Figure 3. Number of replicates of each *Daphnia* genotype that reached the third reproductive cycle when fed with zoospores (Zoo) and heterotrophic bacteria (Bac) suspensions. Significant differences are highlighted by p-values in bold (Fisher's exact test) (n.s.: not significant). Comparisons of infected and uninfected cyanobacteria treatments yielded no significant differences (data not shown).

fragmentation upon infection by chytrids has been documented in nature[57], but its implications for zooplankton grazing remain to be assessed.

All in all, our experiment shows how the decoupling of primary and secondary production, typically assumed in cyanobacteria-dominated aquatic ecosystems, can be circumvented by the effect of parasites, which can establish new, alternative trophic links and enhance existing ones, facilitating the transfer of carbon up the food web.

Methods

Daphnia, cyanobacteria and chytrid cultures. Four *D. galeata* genotypes (Mugg6b, Mugg7a, Mugg11c, Mugg13c) were isolated from the eutrophic Lake Müggelsee in eastern Germany. Clonal lines were kept in jars containing 200 ml of medium (five individuals per jar) consisting of a mixture of 95% synthetic *Daphnia* medium (based on ultrapure water, trace elements and phosphate buffer) and 5% Z8 medium[58]. Pre-experimental conditions included: constant temperature of 20 ± 1 °C, 12:12 h light:dark cycle, and feeding every two days with 1 mg C l^{-1} of the green algae *Scenedesmus obliquus*. The filamentous *Planktothrix agardhii* strain NIVA CYA630, isolated from Lake Lyseren (Norway), was maintained in Z8 medium as non-axenic semi continuous cultures under 20 °C and 20 μmol photons m^{-2} s^{-1}. The obligate chytrid parasite strain Chy-Kol2008, isolated from Lake Kolbotnvatnet (Norway) and identified as *Rhizophydium megarrhizum*[59], was used to infect cultures of the cyanobacterial strain NIVA CYA630.

Preparation and characterisation of feeding treatments. *Daphnia* were fed with two diets consisting of uninfected or chytrid-infected cyanobacteria (*Planktothrix agardhii* NIVA CYA630), respectively, both providing similar POC concentrations above *Daphnia* requirements (Table 1). To determine feeding volumes, optical density at 750 nm (measured with 5 cm cuvettes) was correlated with POC concentrations. For the preparation of chytrid-infected cyanobacterial feeding suspensions, a standard infection protocol was used: seven days before each feeding occasion, chytrid zoospores (final conc. 2000 ml^{-1}) were added to exponentially growing cyanobacterial cultures (density 4×10^5 filaments ml^{-1}) and incubated at 20 °C and 20 μmol photons m^{-2} s^{-1}. After incubation, infected cultures were used to feed *Daphnia* providing a final POC concentration of ~1 μg C ml^{-1}. Adequate feeding volumes were estimated by independent POC determinations from five standard infected replicate cultures before the start of the experiment.

In order to disentangle the contribution of chytrid zoospores and heterotrophic bacteria to changes in *Daphnia* fitness under infected and uninfected cyanobacterial diets, two additional feeding treatments were included, consisting of chytrid zoospores (and heterotrophic bacteria) and heterotrophic bacteria only, respectively. At each feeding occasion, zoospore suspensions were obtained by sequential filtration of the cultures used for the infected cyanobacterial treatment through a 10 μm nylon mesh, followed by 5 and 3 μm polycarbonate filters. The filtrate was microscopically checked for the absence of cyanobacterial filaments and used as a zoospore feeding suspension. The remaining volume was filtered through a 1 μm filter. The zoospore-free filtrate was microscopically checked for the absence of zoospores and used as a heterotrophic bacteria feeding suspension. When feeding with zoospores and heterotrophic bacteria, the same feeding volume as for the infected cyanobacteria diet was used.

For all four treatments and feeding occasions, acid Lugol and formaldehyde-fixed (2% final concentration) samples were collected. Cyanobacteria and zoospore densities were determined by Utermöhl's technique[60]. Heterotrophic bacteria were counted in a haemocytometer under an epifluorescence microscope after staining with 4′, 6-diamidino-2-phenylindole (DAPI; 1 μg ml^{-1} final conc.). For each feeding occasion, the lengths of at least fifty *Planktothrix* filaments in both infected and uninfected treatments were measured under a Nikon Ti Eclypse inverted microscope using the NIS-Element BR 4.5 software. Actual POC concentrations of all treatments at each feeding occasion (Table 1) were determined by filtering aliquots of each feeding suspension through pre-combusted and pre-weighted GF/F filters (pore size approx. 0.7 μm), dried for at least 24 h, weighed and analysed using an Elementar Vario EL analyzer. POC concentrations in heterotrophic bacteria suspensions were estimated from bacterial counts, assuming an average carbon content of 20 fg $cell^{-1}$[61].

Experimental setup. *Daphnia* neonates of the 3rd generation, all born within 24 h, were transferred individually to 50 ml vials containing 40 ml synthetic *Daphnia* medium. 15 replicates per treatment and genotype were set up. Clonal line identities were blinded. *Daphnia* were fed every 2 days with the appropriate diet (cyanobacteria, infected cyanobacteria, zoospores, heterotrophic bacteria) and media was exchanged every 4 days. All replicates were checked daily for survival and offspring production. Offspring were counted and frozen at −20 °C for subsequent analysis. Offspring found to be dead were excluded from body size, fecundity and growth rate analyses. Adult *Daphnia* were taken out of the experiment after the release of the 3rd clutch and frozen. After 24 days, the experiment was stopped for all remaining replicates that had not reached the 3rd clutch (n = 12) or had not reproduced at all (n = 3); in the latter case *Daphnia* were checked microscopically for the presence of males. Body size (length from top of head to base of tail spine) of offspring from the first clutch and of adults that had released three clutches was measured using a Nikon SMZ 25 stereomicroscope and NIS-Element BR 4.5 software.

Statistical analysis. Three replicates were excluded from the overall data set: two that were found to be males and one that lay at the bottom of the vial for several days in the third week, barely moving, before recovering and reproducing toward the end of the experiment. The following *Daphnia* fitness parameters were assessed: maturity (age at first reproduction), fecundity (total number of living offspring), growth rate (number of living offspring per day), body size of adults and body size of offspring from the first clutch. To assess differences in age at maturity and offspring size, replicates that reproduced at least once were included in the analyses. For fecundity and growth rates, only replicates that released a third clutch were included in the analyses. If multiple offspring were born, body size of all living individuals was measured and averaged for the analysis. Occasionally, *Daphnia* individuals showed deformed bodies upon thawing; these individuals were excluded from body size analyses. Two-way ANOVAs were performed for each fitness parameter, including diet (i.e. treatment) and genotype as fixed factors, followed by a contrast test for the effect of diet within genotypes (least-squares means test with Holm's p-value adjustment). Data of the parameters fecundity and body size of offspring were transformed (see Table 2). For two parameters (maturity and body size of adults), data were not normally distributed, even after transformation. Results shown in the main text stem from parametric ANOVAs, given that both parameters showed equal variances of the residuals and that ANOVA is reasonably robust to non-normality. However, in these two cases, a non-parametric two-way ANOVA on aligned rank transformed data[62] was performed in parallel. The number of replicates per genotype that reached the third reproductive cycle (within the 24-day experiment) under each treatment was compared using Fisher´s exact test (uninfected *vs.* infected and zoospores *vs.* bacteria). All statistical analyses were performed in Rstudio (v.0.99.903).

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Author Contributions

R.A. and M.S. conceived the study. R.A., M.S., T.R. and J.W. designed the experiment. R.A., M.S. and C.M. prepared the experiment. R.A. and M.S. conducted the experiment, analysed the data and wrote the manuscript. All authors commented on the manuscript.

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Chapter 5

General Discussion

Discussion

This doctoral thesis aimed to investigate how increased loadings of humic dissolved organic carbon (DOC) and nutrients, especially phosphorus (P), affect lake plankton communities and food web interactions. To better understand the complex mechanisms involved in such scenarios, different organismal systems were used, and methods of several biological disciplines (e.g. ecotoxicology, ecology, population genetics) as well as both laboratory and field experiments were applied. Thereby, this study contributes to a deeper insight into the diverse responses of lake ecosystems to specific aspects of future environmental change.

Extrapolation of results from the laboratory to the field

Laboratory experiments have the advantage of investigating specific mechanisms under controlled conditions, but their simplified design makes extrapolations of results to complex natural conditions difficult. Often, results obtained from one species are generalised to closely related species (Dietert, 1995). In Chapter 2, the two *Daphnia* species *D. magna* and *D. longispina* were exposed to the same experimental conditions of high levels of humic DOC compared to controls, but the effects on *D. longispina* were much more severe than on *D. magna*. Thus, in natural settings, the tested DOC concentrations may have significantly more drastic consequences on small lake-inhabiting species like *D. longispina* than on the bigger pond-inhabiting *D. magna*. These results support previous findings that *D. longipina* is generally more sensitive than *D. magna* to a number of chemicals and xenobiotics (Marques et al., 2004; Gonçalves et al., 2007). *D. magna* is widely used as a standard test organism in ecotoxicological studies, investigating effects of toxic chemicals and other pollutants to predict their ecological consequences (Martins et al., 2007). Results obtained for *D. magna* are then generalised to other *Daphnia* and zooplankton species. It has already been suggested that *D. magna* does not represent a suitable model organism to extrapolate results of toxicity tests to species in other aquatic habitats such as lakes; the habitat characteristics and thus the life-history strategies of *D. magna* and lake-

inhabiting zooplankton species differ strongly from each other (Koivisto, 1995). Still, *D. magna* is the only cladoceran species recommended for use in standard toxicity tests (OECD, 2004, 2012). Future studies should consider the ecological characteristics of the test organism, and ideally use several species (e.g. different *Daphnia* and/or cladoceran species) to better demonstrate possible differences in responses to test conditions between organismal groups.

Extrapolations from the laboratory to natural settings are not only made at the interspecific but also at the intraspecific level. Many studies use one single genotype of a species and then relate results to the whole species (Dietert, 1995). In Chapter 3, humic DOC differently affected three *D. longispina* genotypes, as assessed in a laboratory experiment. Effects on life-history traits were either negative, neutral or rather positive. Likewise, in Chapter 4, several fitness traits of four *D. galeata* genotypes were either affected in one direction or remained unaffected by different diets. This highlights the great intraspecific variability of *Daphnia* in response to different environmental factors, which has already been found in previous studies in response to, for example, food quality (Weider et al., 2005), predation pressure (Wolinska et al., 2007), or combined stressors of cyanobacteria diet and a heavy metal (De Coninck et al., 2013). Similar observations have been made in other systems, for instance in wheat cultivars toward salinity stress (Sairam et al., 2002), in *Rana sphenocephala* tadpoles under pesticide exposure (Bridges and Semlitsch, 2000), in the nematode *Caenorhabditis elegans* toward different bacterial food sources (Petersen et al., 2015), and in humans regarding the eventual development of depression after experiencing stressful life events (e.g. financial, health or relationship problems) (Caspi et al., 2003). Thus, not only species but also genotype variation in response to changing environmental conditions should be taken into account when extrapolating laboratory results to the field.

Another problem with transferring results from laboratory experiments to natural conditions is that direct effects on organisms found in the laboratory can be masked in the field by complex community interactions and/or by abiotic responses (e.g. effects on trophic cascades or on nutrient availability) (Carpenter et al., 2001; Kelly et al.,

2016). In Chapter 3, contrary to expectations, no adverse effects of humic DOC on *Daphnia* life-history traits were found at the end of the mesocosm experiment. Though this might be attributed to the samples being a mix of different *Daphnia* species and genotypes, as well as different individual ages which could diminish treatment effects, community interactions likely played a role in this result. Specifically, population density and food quality interactions may have been the reason for the absence of expected negative effects on *Daphnia* fitness at this time point of the mesocosm experiment (Minguez et al., unpublished). Carpenter (1996) stated that microcosm or laboratory experiments are not able to capture the complexity of natural systems and are thus not suitable for studying community interactions and ecosystem ecology. In his synthesis of the relevance of microcosm experiments for freshwater community studies, he pointed out that problems primarily with the scale and duration of such experiments can result in misleading interpretations. In the case of growing concerns over eutrophication during the 1960s, for example, management strategies for phosphorus loading were delayed because the importance of inorganic carbon limitation in this process was overstated based on small-scale, short-term experiments (Schindler et al., 1972; Schindler, 1977; Edmondson, 1996). In summary, interpreting results from microcosm or laboratory experiments in ecological contexts should be done with caution and taking restrictions of the experimental design into account, as "we must face the fact that misinterpretation of results can cost us precious populations, species, or entire ecosystems" (Murphy, 1989).

Humic DOC and biodiversity

Different aspects of environmental change have been associated with biodiversity loss (Chapin et al., 2000; Pereira et al., 2010; Urban, 2015). In the case of humic DOC, only a few studies have focused on the consequences of increased inputs on zooplankton biomass and taxon community composition, showing varying results (Arvola et al., 1996; Strecker et al., 2008; Shurin et al., 2010; Nicolle et al., 2012). Biodiversity, however, might not only be affected at the species but also at the genotype level. As mentioned above, many species have been shown to have great intraspecific

variability toward different environmental factors. Under natural conditions, this variability could lead to changes in diversity at the genotype level, even if species diversity and biomasses stay stable. In the mesocosm experiment presented in Chapter 3, no effect of humic DOC on genetic composition of F1 hybrid populations was detected. It is feasible that the short study period of six weeks was not long enough to result in treatment-related genetic changes, especially as hybrids of the *D. longispina* species complex generally show low genetic diversity due to asexual reproduction (see discussion of Chapter 3). Genetic changes of *Daphnia* populations in relation to environmental factors have been shown previously. For example, within 1.5 decades the *D. galeata* population in Lake Constance, Germany, evolved resistance to a cyanobacteria diet coupled with reduced phenotypic plasticity. This phenotypic change was detectable at the level of genetic structure within *D. galeata*, i.e. different genotype composition (Hairston et al., 2001). Thus, effects of altered environmental conditions on intraspecific genetic diversity should be taken into account in studies on biodiversity.

Environmental change and parasitism

Many studies have raised concerns that environmental change, e.g. temperature increase or human demography, supports disease spread in various aquatic and terrestrial populations (Patz et al., 2000; Daszak et al., 2001; Harvell et al., 2002; Altizer et al., 2013). In Chapter 3, the potential of humic DOC to increase parasitic infection in *Daphnia* is discussed. Previous studies, as well as the current study (Chapter 2 and 3), have shown that humic DOC imposes stress on *Daphnia*, coupled with negative effects on the fitness of this organism (Meems et al., 2004; Steinberg et al., 2010). In the light of host-parasite interactions, this increased stress could lead to higher susceptibility toward parasites. In *D. magna*, simultaneous exposure to sublethal concentrations of a pesticide and each one of two different parasites reduced fecundity and/or survival of the host compared to pesticide or parasite exposure alone (Coors et al., 2008). The underlying mechanisms were not revealed in that study, but several potential causes were discussed. These included impairment of the host's immune system by the pesticide, tradeoffs in energy allocation between immune defence responses and

toxicant-induced response, or toxicant-induced reduction in food uptake resulting in impaired host condition. The potential of pollutants to affect host-parasite interactions has been investigated in various other studies, both in aquatic systems (e.g. protozoan or worm parasites and their fish or freshwater snail and amphipod hosts; reviewed in Lafferty and Kuris, 1999) and in terrestrial systems (e.g. nematode or bacterial parasites and various insect hosts; reviewed in Holmstrup et al., 2010). The ways in which pollutants and other anthropogenic stressors can influence host-parasite interactions are diverse and the impact of the stressor on the spread of infectious diseases depends on the system. Several mechanisms can hamper or support infection rates in natural populations by positively or negatively affecting the host and/or the parasite. Decreases in host density due to the stressor, for instance, can keep host abundance below levels needed for infection (Lafferty and Holt, 2003). Moreover, parasite fitness can be affected by the stressor, either directly by lethal or sublethal effects on free-living stages of the parasite, or indirectly by negative effects on the host. Some stressors can even improve conditions for certain hosts and thereby strengthen their immune response (Lafferty and Kuris, 1999; Lafferty and Holt, 2003; Budria, 2017). All these examples would likely result in reduced disease spread in populations. Mechanisms that are expected to support disease spread include positive effects on parasite growth and transmission rates, altered migration patterns of the host, and shifts in transmission pathways of trophically transmitted parasites due to changes in community composition (Budria, 2017).

Another way in which anthropogenic stressors can enhance the occurrence of epidemics is by positive effects on host abundance. Eutrophication processes, for instance, increase the productivity of lakes which can lead to higher host densities (Lafferty and Kuris, 1999). In combination with climatic factors, eutrophication has resulted in frequent and more severe occurrences of harmful algal blooms which pose a threat to ecosystems (Carpenter et al., 1998; Anderson et al., 2002; Chislock et al., 2013). Some studies have reported sudden declines of blooms during the growing season, which have been associated with direct grazing by fish (Xie and Liu, 2001), as well as lysis by viruses or heterotrophic bacteria (reviewed in Gerphagnon et al., 2015). Another factor that has been proposed to contribute to such declines is chytrid

parasitism which leads to a breakdown of the bloom when epidemics of the parasite arise (Ibelings et al., 2011; Gerphagnon et al., 2015). The short life span of chytrid free-living zoospores, together with their low swimming capacity, requires a high host density for this parasite to spread (reviewed in Ibelings et al., 2004). Thus, epidemics are most likely to occur under phytoplankton bloom conditions. In this specific case, an epidemic can result in positive effects on ecosystems as it hampers harmful algal blooms. This highlights the complex and partially paradoxical effects of environmental change on ecosystems: algal blooms, which are induced by environmental alterations and disrupt ecosystem functioning, are counteracted by an epidemic that is supported by the bloom itself (Ibelings et al., 2011).

The role of parasites in food webs

The aforementioned chytrid epidemics during harmful algal blooms can have another positive effect on lake communities. As shown in Chapter 4, growth of zooplankton grazers like *Daphnia* can be supported by feeding on chytrid zoospores. Thus, chytrids can link primary and secondary production, which are otherwise decoupled under bloom conditions due to reduced zooplankton grazing efficiency on large algal cells. This shows what an important role parasites play in food web interactions. Though food webs in aquatic systems are well studied, parasites have historically not been considered in such investigations (Marcogliese and Cone, 1997). It is only during the last two decades that the importance of parasites in food web structure and functioning has been acknowledged. These studies have found parasites to increase the number of links between organisms, linkage density, food chain length and the number of trophic levels within the food web. Moreover, parasites can affect the nestedness of food webs, i.e. the asymmetry of interactions (Huxham et al., 1995; Lafferty et al., 2006; Amundsen et al., 2009; Thieltges et al., 2013). Thus, parasites are important not only for the stability of food webs, but also for the functioning of the whole ecosystem as they can shape host population dynamics, alter interspecific competition, influence energy flow and increase biodiversity (Hudson et al., 2006).

The potential positive effect on the food web by zooplankton grazing of chytrid zoospores, shown in Chapter 4, could in turn affect parasitism. The chytrid *Batrachochytrium dendrobatidis* (Bd) has caused worldwide declines and loss of amphibian populations and species (Skerratt et al., 2007). It has been shown that infections of tadpoles by this chytrid can be reduced by *Daphnia* feeding on Bd zoospores, and chytrid epidemics in amphibian populations could thereby be hampered (Searle et al., 2013). In the same way, *Daphnia* and other zooplankton grazers could prevent or slow down chytrid epidemics in phytoplankton (Kagami et al., 2004). In this case, grazing on chytrid zoospores could instead cause negative effects on the functioning of the whole ecosystem, if the reduction in chytrid abundance by zooplankton grazing was strong enough to hinder harmful algal blooms from declining.

Consequences of global change for lake ecosystems

In summary, this thesis has shown that different aspects of environmental change can have complex and partially contradictory effects on organisms, populations and communities in lakes. The specific consequences for lake ecosystems are thus difficult to foresee and will depend on several factors, such as intensity of change (e.g. concentration of elemental discharge) and involved antagonists (e.g. parasite vs. host, predator vs. prey). In the latter case, not only species composition but also genotype variability will likely affect the outcome of altered environmental conditions (Cable et al., 2017). Moreover it has been shown that differences between lake types (e.g. in trophic state or lake size) can lead to distinct, even contrasting, effects on ecological processes under the same climatic scenario, such as differences in seasonal water temperature, onset of stratification, and water loss due to evaporation processes (Hondzo and Stefan, 1993). It is thus important to investigate systems in detail, to better understand dynamics between biotic and abiotic factors under changing environmental conditions. A combination of laboratory and field experiments, as described in this thesis, provides a good approach to gain insight into such complex interactions and their underlying mechanisms at the organismal scale. In this manner,

it might be possible to predict how different lake ecosystems will respond to long-term global environmental change.

Future directions

Humic substances are very diverse and contain a great variation of functional groups that can indirectly and directly interact with aquatic organisms in various ways (Steinberg, 2003; Steinberg et al., 2006). It is thus not surprising that studies on humic DOC have found somewhat contradictory effects on organisms, depending on the source and concentration of humic DOC (Meinelt et al., 2007). The laboratory experiments carried out in Chapters 2 and 3 could be repeated or extended by including different sources of humic DOC, e.g. natural or synthetic humic substances, to analyse whether the effects on *Daphnia* life-history traits can be generalised for humic DOC at the concentrations used. Alternatively, different concentrations of the same substance as used in this study could be tested, for instance to investigate long-term effects of slowly increasing humic DOC concentrations (i.e. as observed in natural systems).

Three out of seven mesocosms within the high DOC treatment of the mesocosm experiment in Chapter 3 showed high numbers of infected *Daphnia* individuals. The strong variance in numbers of infected *Daphnia* among mesocosms, as well as different types of parasitic and *Daphnia* taxa, resulted in the lack of a statistically significant effect of humic DOC on parasitic infection. The potential negative effect of humic DOC on disease prevalence in *Daphnia* could be tested in controlled laboratory experiments. To account for intra- and interspecific variations, different *Daphnia* species and/or genotypes should be included in this experiment, as well as one or several parasite taxa. The controlled conditions of a laboratory experiment could reveal the underlying mechanism of potentially increased infection rates, e.g. decreased host fitness due to the additional stressor. Further, the relationship between humic DOC and parasite spread in *Daphnia* populations could be investigated in field studies. Humic DOC has been related to increased disease prevalence in *Daphnia* populations in natural humic-rich lakes (Johnson et al., 2006). This was

explained by the elimination of selective predation on infected individuals by visual predators, which would otherwise decrease disease prevalence (i.e. the disadvantage of a darker contrast of infected *Daphnia* ceased due to the shading effect of humic DOC, leading to equal predation by fish on infected and uninfected individuals). It would be interesting to compare these findings with studies either on humic-rich lakes with low fish predation, and/or on lakes that recently experienced or are experiencing increases in humic DOC concentrations.

The mesocosm experiment in Chapter 3 was carried out in an oligotrophic clear-water lake. Organisms in that lake are adapted to low concentrations of humic DOC, and addition of humic matter could affect this system differently than natural humic-rich lakes. Humic-rich lakes have been shown to display very distinct habitats in comparison to humic-poor lakes, as they differ substantially in their nutrient and carbon cycling, bacterial production, as well as the vertical distribution of light, heat and oxygen (Jansson et al., 2007; Solomon et al., 2015). The effects of combined inputs of humic DOC and phosphorus that were found in the mesocosm experiment of Chapter 3 might thus not apply to humic-rich lakes. Mesocosm or pond experiments could help in understanding how systems that differ in their natural humic content react to increased concentrations of humic DOC and/or phosphorus. Alternatively, *Daphnia* from natural humic-rich and humic-poor lakes could be exposed to the same increased DOC concentrations (i.e. same change in absolute or percentage increase compared to their natural environment), similarly to the laboratory experiments in Chapter 2 and 3 to investigate potential differences in their susceptibility. However, as different *Daphnia* species might inhibit lakes of varying humic DOC concentrations, this comparison could only be carried out if the same species occur in these differing habitats.

In Chapter 3 no changes in genetic diversity over time were observed in *Daphnia* hybrid populations under humic DOC exposure in the mesocosm experiment. The laboratory experiment in this chapter, however, showed differences in genotype susceptibility of *D. longispina* when exposed to humic DOC. This leaves open the question of whether humic DOC can affect the intraspecific diversity of *Daphnia*

populations in natural settings. To investigate this, field studies could be carried out in lakes that recently experienced or are experiencing increases in humic DOC concentrations. *Daphnia* could be sampled over several seasons or years to analyse the within-species genetic diversity in relation to humic DOC increases. Further, sediment samples could reveal long-term genetic changes of *Daphnia* populations by analysing ephippia from previous years or even decades (Miner et al., 2012), if long-term DOC concentration data are available for the respective lakes.

The role of chytrid parasites in the food web has only been acknowledged recently and many questions regarding their ecology are still open. More emphasis should be made on investigating this parasitic group in detail, with respect to both their ecological characteristics and their interactions with hosts (e.g. host specificity, attraction of free-living chytrid zoospores by hosts via chemotaxis). Additionally, the hypothesis that chytrid epidemics support zooplankton growth under algal bloom conditions should be investigated further. In Chapter 4, an experimental approach is presented that chytrid zoospores can increase *Daphnia* fitness under a cyanobacterial diet. However, the implications of this finding for natural settings are unclear. Field studies could link high biomasses of zooplankton during bloom conditions with the occurrence of chytrid epidemics. Another interesting direction would be to follow up previous experimental studies on the extent to which grazing of zoospores by zooplankton can potentially affect chytrid epidemics in phytoplankton. Though these studies have shown that zooplankton grazing can reduce chytrid zoospore abundance under controlled conditions (Kagami et al., 2004; Searle et al., 2013), the consequences for algal bloom events have not been investigated. One way to do this could be by monitoring lakes with annually recurring algal blooms, followed by chytrid epidemics, and then relating the strength of the epidemic with abundances of *Daphnia* and/or other zooplankters. However, many factors would be uncontrolled in such a study, for example predation as well as host, parasite and grazer densities. Thus, mesocosm or pond experiments would be a good alternative or additional way of investigating this question. In these setups, initial zooplankton densities (e.g. of *Daphnia*) could be manipulated while keeping phytoplankton host and chytrid parasite densities stable.

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"´Cause what's left to lose?
I've done enough.
And if I fail, well, then I fail but I gave it a shot.
And these last three years - I know they've been hard.
But now it's time to get out of the desert and into the sun;
Even if it's alone."

On your porch, The Format

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Erklärung

Declaration

Hiermit versichere ich, dass ich die vorliegende Dissertation selbstständig, ohne unerlaubte Hilfe und nur mit den angegebenen Hilfsmitteln verfasst habe. Das Promotionsverfahren wurde zu keinem früheren Zeitpunkt an einer anderen Hochschule oder bei einem anderen Fachbereich beantragt.

I hereby declare that I have written the present thesis independently, without enlisting any external assistance, and only using the specified aids. It has not been previously submitted, in part or whole, to any university of institution for any degree, diploma, or other qualification.

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