PARASITISM IN A CHANGING WORLD:

INVESTIGATING THE OUTCOME OF INFECTION IN FRESHWATER ZOOPLANKTON (DAPHNIA) UNDER THE INFLUENCE OF ANTHROPOGENICALLY-DERIVED ENVIRONMENTAL SHIFTS

Inaugural-Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry, Pharmacy

of Freie Universität Berlin

by

Florent Manzi

2022

This thesis was conducted under the supervision of Professor Justyna Wolinska from January 2018 to December 2021 at the Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin.

1st Reviewer: Prof. Dr. Justyna Wolinska

2nd Reviewer: Prof. Dr. Dino McMahon

Date of defence: 13.05.2022

We cannot change the past

But we can start today

To make a better tomorrow.

DECLARATION OF INDEPENDENCE

I, Florent Manzi, certify that I have prepared and written this thesis independently and that I have not used any sources and aids other than those indicated in the present document.

TABLE OF CONTENTS

SUMMARY

Human activity is generating environmental shifts i) at a global scale and ii) at an unprecedented pace. In addition to the release of greenhouse gases, largely responsible for the ongoing elevation of the Earth's average surface temperature, other sources of anthropic disturbances have been associated with abrupt changes in the abiotic parameters of natural ecosystems. In particular, freshwater bodies around the globe are facing a combination of warming, eutrophication and pollution by a variety of man-made contaminants. Because the influence of the external environment can strongly dictate the outcome of host-parasite interactions, the scientific literature has expressed concern that the occurrence and severity of diseases may be favoured under the influence of such disturbances. Using a commonly occurring system of a zooplanktonic host (*Daphnia* sp.) and its fungal parasite (*Metschnikowia bicuspidata*), the present work examines four possible sources of interference between anthropogenically-derived environmental shifts and the performance of a highly virulent parasite in controlled infection assays. In **Chapter 1**, we examined the conjoint effects of elevated temperature and host diet quality on distinct parameters of host and parasite fitness. We showed that a 4°C elevation in water temperature could greatly affect the success of infection, though the direction of these effects varied widely across specific associations of host genotype and diet quality. When incorporated as major components of the host's diet, cyanobacterial species generally resulted in a sharp decrease of the parasite's reproduction. To follow up on these observations, **Chapter 2** asked whether the putative antifungal effects of cyanobacteria against *Metschnikowia* could also apply to free-living stages of the parasite, independently of their consumption by the host. Here, prior incubation of infective stages to high concentrations of cyanobacterial extracts did not reduce the success of infection, as we suspected. However, we found similar occurrences of genotype-by-environment interactions, supporting that phytoplankton composition and host genotypic diversity are important determinants of infection success in this system. In **Chapter 3**, we explored a contemporary source of environmental pollution affecting freshwater bodies. We provide the first experimental evidence that polystyrene nanoplastic particles (≤ 100 nm) can modulate the outcome of infection in a zooplankton-microparasite system, showing that high concentrations of nanoplastics can strongly reduce the parasite's ability to produce spores within the host. Finally, to determine how climate-associated shifts in the phenology of co-occurring parasites could influence the dynamics of infection, **Chapter 4** used sequential infections between our focal parasite and a competing, less virulent microsporidium (*Ordospora colligata*). We found evidence for priority effects at the within-host level, suggesting that prior emergence of the microsporidium in natural populations may be detrimental to the transmission of both parasites. Overall, these results provide only few examples of enhanced parasite transmission under the influence of anthropic disturbances, rather supporting that future environmental shifts will exert strong pressure on fitness traits of both hosts and parasites in this commonly occurring freshwater assemblage.

ZUSAMMENFASSUNG

Menschliche Aktivitäten verursachen Umweltveränderungen i) auf globaler Ebene und ii) in einem noch nie dagewesenen Tempo. Neben der Freisetzung von Treibhausgasen, die weitgehend für den anhaltenden Anstieg der durchschnittlichen Oberflächentemperatur der Erde verantwortlich ist, wurden auch andere Quellen anthropogener Störungen mit abrupten Veränderungen der abiotischen Parameter natürlicher Ökosysteme in Verbindung gebracht. Vor allem Süßwasserkörper auf der ganzen Welt sind mit einer Kombination aus Erwärmung, Eutrophierung und Verschmutzung durch eine Vielzahl von anthropogenen Schadstoffen konfrontiert. Da die äußere Umgebung das Ergebnis von Wirt-Parasit-Interaktionen stark beeinflussen kann, wurde in der wissenschaftlichen Literatur die Sorge geäußert, dass das Auftreten und die Schwere von Krankheiten unter dem Einfluss solcher Störungen begünstigt werden könnten. Anhand eines häufig vorkommenden Systems aus einem zooplanktonischen Wirt (*Daphnia* sp.) und seinem Pilzparasiten (*Metschnikowia bicuspidata*) werden in der vorliegenden Arbeit vier mögliche Störquellen zwischen anthropogen bedingten Umweltveränderungen und der Leistung eines hochvirulenten Parasiten in kontrollierten Infektionstests untersucht. In **Kapitel 1** untersuchten wir die gemeinsamen Auswirkungen einer erhöhten Temperatur und der Qualität der Wirtsnahrung auf verschiedene Parameter der Fitness von Wirt und Parasit. Wir konnten zeigen, dass eine Erhöhung der Wassertemperatur um 4 °C den Erfolg der Infektion stark beeinflussen kann, obwohl die Richtung dieser Auswirkungen je nach Wirtsgenotyp und Nahrungsqualität stark variiert. Wenn Cyanobakterienarten als Hauptbestandteile der Nahrung des Wirts aufgenommen wurden, führten sie im Allgemeinen zu einem starken Rückgang der Reproduktion des Parasiten. Um diese Beobachtungen weiterzuverfolgen, wurde in **Kapitel 2** die Frage gestellt, ob die mutmaßliche antimykotische Wirkung von Cyanobakterien gegen *Metschnikowia* auch für freilebende Stadien des Parasiten gelten könnte, unabhängig von ihrem Verzehr durch den Wirt. In diesem Fall führte die vorherige Inkubation der infektiösen Stadien mit hohen Konzentrationen von Cyanobakterienextrakten nicht zu einer Verringerung des Infektionserfolgs, wie wir vermutet hatten. Wir fanden jedoch ähnliche Interaktionen zwischen Genotyp und Umgebung, was darauf hindeutet, dass die Zusammensetzung des Phytoplanktons und die Vielfalt der Wirtsgenotypen wichtige Faktoren für den Infektionserfolg in diesem System sind. In **Kapitel 3** untersuchten wir eine zeitgenössische Quelle der Umweltverschmutzung, die sich auf Süßwasserkörper auswirkt. Wir erbrachten den ersten experimentellen Nachweis, dass Polystyrol-Nanoplastikpartikel (≤ 100 nm) das Ergebnis der Infektion in einem Zooplankton-Mikroparasiten-System beeinflussen können. Wir konnten zeigen, dass hohe Konzentrationen von Nanoplastik die Fähigkeit des Parasiten, Sporen im Wirt zu produzieren, stark verringern können. Um schließlich festzustellen, wie klimabedingte Verschiebungen in der Phänologie von gemeinsam auftretenden Parasiten die Infektionsdynamik beeinflussen könnten, wurden in **Kapitel 4** sequenzielle Infektionen zwischen unserem Hauptparasiten und einem konkurrierenden, weniger virulenten Mikrosporidium (*Ordospora colligata*) durchgeführt. Wir fanden Belege für Prioritätseffekte innerhalb des Wirts, was darauf hindeutet, dass ein früheres Auftreten des Mikrosporidiums in natürlichen Populationen für die Übertragung beider Parasiten nachteilig sein könnte. Insgesamt liefern diese Ergebnisse nur wenige Beispiele für eine verstärkte Parasitenübertragung unter dem Einfluss anthropogener Störungen, was eher dafür spricht, dass künftige Umweltveränderungen einen starken Druck auf die Fitnessmerkmale sowohl der Wirte als auch der Parasiten in dieser häufig vorkommenden Süßwassergemeinschaft ausüben werden.

AUTHOR CONTRIBUTIONS

Chapter 1: FM, JW, RA and YL conceptualized the study, with input from FBA. **FM** performed the experiment, with help from JW, RA and YL. **FM** performed data analysis and results visualization. **FM** wrote the manuscript, with input from JW, RA, YL and FBA. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Chapter 2: FM, MM, JW and RA conceptualized the study. **FM** and MM performed the experiment, data analysis and results visualization. **FM** wrote the manuscript, with help from RA, JW and MM. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Chapter 3: JW conceived the presented idea. All authors designed the study. JW, **FM**, RA and CS coordinated and supervised the study. SM and NA equally carried out the experimental procedure. SM carried out the data visualization and formal analysis, **FM** critically directed it and all authors validated the results. SM drafted the manuscript and JW, **FM**, RA and CS critically revised every version. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Chapter 4: All authors conceived and designed the study. **FM**, LS and JW performed the experiment. **FM** performed data analysis and results visualization. **FM** wrote the article, with input from JW, FBA and SH. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

FM wrote the general sections (i.e. introduction and discussion) of the doctoral dissertation.

GENERAL INTRODUCTION

1. Parasitism in a changing world

1.1 Human activity is generating environmental shifts at a global scale

Ever since the emergence of templated information and functionality from non-living matter (i.e. short peptides and proto-RNA, Lahav et al., 2001), potentially dating back to 4.28 Gya (Dodd et al., 2017), the primordial environmental conditions laying down the evolution of life on Earth have changed dramatically. While early environmental shifts initially reflected global changes of abiotic nature (e.g. due to the Earth's intense volcanism and stratospheric activity), the following proliferation and diversification of cell-based life forms allowed for early organisms to exert their own influence on the surrounding environment, by implementing themselves into complex biogeochemical cycles (Trevors, 2001; Sánchez-Baracaldo et al., 2021). One famous example – and perhaps the most commonly cited in this regard – is the emergence of oxygenic photosynthesis (i.e. the process of cleaving water molecules into molecular oxygen) in cyanobacteria and their progenitors, thus enabling the initial oxygenation of the atmosphere and surface oceans ca. 2.4 billion years ago (Des Marais, 2000; Dismukes et al., 2001; Kasting & Siefert, 2002). As the Earth's atmosphere progressively became suited to the more energy-efficient process of aerobic metabolism, this biotically-driven phenomenon known as the Great Oxygenation Event (Lyons et al., 2014) ultimately paved the way for the emergence of more complex, eukaryotic life-forms (Dismukes et al., 2001).

Throughout the remainder of its geological history and to the present day, the Earth has continued to experience multiple cyclical shifts in its abiotic conditions (Khairullina et al., 2019). These include important variations in its surface temperature and sea levels, punctuated by episodes of denitrification and oceanic anoxia (Melchin et al., 2013). While mostly driven by external forces, such as solar radiation (Budyko, 1969), stratospheric volcanism (Rohde et al., 2013; Hu et al., 2020), or glaciations (Delabroye & Vecoli, 2010), these environmental shifts were sometimes enhanced by the contribution of living species to global biogeochemical cycles. For instance, the ecological imbalance between the processes of carbon fixation by phototrophs and respiration by the heterotrophs led to a global drawdown of carbon and the burial of organic carbon within marine sediments (Holland, 2006) and contributed to a second major oxygenation event during the Neoproterozoic (Scott et al., 2008). In a similar manner, increasing levels of human activity coinciding with the first industrial revolution in the 1750s (Mohajan, 2019) and the resulting modification of its environment have been largely associated with an ongoing global climate crisis. While the aforementioned climactic events consisted in gradual processes, taking place over the course of several hundred Ma (Holland, 2006; Melchin et

al., 2013), global average temperatures have already increased by more than 1 °C since pre-industrial times (Ritchie & Roser, 2020) and are predicted to rise by an additional 2.8°C by the end of the twenty-first century, with some hotspots of warming (e.g. the Arctic and Southern Africa) potentially reaching up to 4°C increases (IEA, 2021; Fan et al., 2021).

The main contributing factor to this phenomenon is the global increase in emissions of greenhouse gases, including carbon dioxide (CO2), methan (CH4), ozone (O3) and nitrous oxide (N2O), with minor contributions from chlorofluorocarbons and volatile organic compounds (Dalal & Allen, 2008). However, temperature is far from the only abiotic variable expected to shift under the influence of human activity. In fact, long-term variations in abiotic factors such as temperature, precipitations and wind patterns over the globe are collectively referred to as 'climatic changes' (Bernstein et al., 2008; Sattar et al., 2021). Aside from the much topical and heavily publicized elevation of the average 'global surface temperature' (GST), the increasing influence of anthropic activity on Earth has been attributed to a grand ensemble of global shifts in abiotic conditions affecting virtually all ecosystems, which include: increasing frequency and duration of heatwaves (Perkins et al., 2012; Sharma & Mujumdar, 2017), modification of the Earth's atmospheric composition (Lamb et al., 2021), disruption of the nitrogen (N) and phospohorus (P) intakes in the biosphere (Glibert, 2017), resulting in the eutrophication of both marine and freshwater ecosystems (Callisto et al., 2014; Malone & Newton, 2020), acidification of the oceans (Wallace et al., 2014), landscape fragmentation and loss of habitat (Jaeger et al., 2016; Lawrence et al., 2021), increased salinization of freshwater bodies (Castillo et al., 2018), rising ocean and sea levels attributed to the melting of polar ice caps (Mimura, 2013; Zurbenko & Potrzeba-Macrina, 2021) and changes in surface humidity (Zurbenko & Luo, 2015), along with the concurrent occurrence of natural disasters, such as heavy rainfall, hurricanes, and forest fires (Banholzer et al., 2014; Wotton et al., 2010; Zurbenko & Potrzeba, 2013). In addition, human activity – in particular the sectors of industry and waste management – are responsible for worldwide environmental pollution, due to the release and gradual accumulation of pollutants such as: plastic microparticles (Karbalaei et al., 2018), heavy metals (Mohammed et al., 2011), endocrine disruptors (Graca et al., 2021; Ojha et al., 2021) and pesticides (Tang et al., 2021) in both in the soil and several aquatic compartments.

Evidently, such profound modifications of the Earth's abiotic conditions are not without consequences for the biotic agents of natural ecosystems (i.e. the biosphere). The above-listed phenomenon – all shown to be either enhanced, accelerated or induced by human activity – have been responsible for shifting or reducing the ecological niche of several species (Wiens et al., 2009; Rödder et al., 2021; Navarro et al., 2021), generating intense selection pressure leading to rapid evolution or adaptations (Colautti & Barrett, 2013; Hoberg & Brooks, 2015) and favouring the introduction and range expansion of some invasive species (King et al., 2021; McDowell et al., 2014), all of which contribute to species extinctions occurring at a yet unprecedented rate (Ceballos et al., 2015). Whereas the previous mass extinction events recorded on Earth were attributed to natural catastrophes, this global

loss of biodiversity has been compared to a 'Sixth Mass Extinction crisis', bearing the distinction of its sole anthropic origin (Saupe et al., 2020; Shivanna, 2020). Thus, it is evident that despite its relatively recent evolutionary emergence (≤ 400 kya; Stringer, 2016) and the overall short timespan represented by the industrial era (1750-present) on a geological time scale, the ecological imprints of a single vertebrate species (*Homo sapiens*) are occurring both at a scale – and pace – consequent enough to drastically influence the ecology and evolution of virtually all other organisms on Earth.

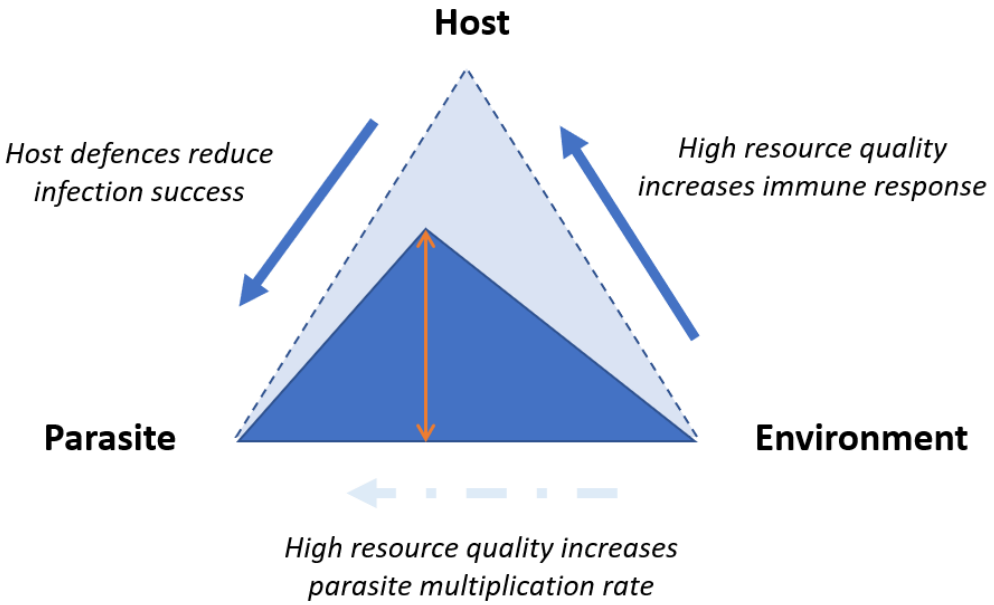
1.2 Environmental dependency of species interactions: a focus on host-parasite dynamics

Beyond the concerning impacts of anthropogenic disturbances on the phenology, ecology or life-history traits of individual species, future changes in the environment are also predicted to affect species interactions (Gilman et al., 2010; Lord et al., 2017, Abrego et al., 2021). For instance, habitat disturbance due to land-use intensification can modify the overlap of temporal activity between predators and preys (Gálvez et al., 2021), the introduction of permanent sources of light at night disrupts intra-specific communication and plant-pollinator interactions in insect communities (Grubisic & van Grunsven, 2021), climate-induced changes in phenology enhances the competition between native and newly migrating species of plant (Caplat et al., 2008), while acidification of the oceans disrupts the perception of chemical cues and the expression of anti-predator traits in mussel populations (Jahnsen-Guzmán et al., 2021).

One category of species interactions that has been particularly dissected through the lens of climate change is parasitism (Marcogliese, 2001; Harvell et al., 2002). Indeed, a multitude of traits relating to the occurrence, severity and transmission of parasitic diseases have been shown to vary depending on environmental factors. However, in any host-pathogen interaction, the outcome of infection does not simply rely on the influence of external abiotic factors, but instead results from complex interactions between a host (serving as a living substrate or microhabitat), its parasite, and their shared external environment (Scholthof, 2007; Duneau et al., 2011). This concept was notably popularized by plant pathologist George McNew (1960), who used the analogy of a 'disease triangle', the height of which determined the amount of damage inflicted on the host by its pathogen (Box 1). For instance, changes in temperature can reflect on the metabolism of ectothermic host species and affect the efficiency of their immune responses, including processes such as phagocytosis, lymphocyte distribution and the production of antibodies (Rijkers et al., 1980; Truscott & White, 1990; Ainsworth et al., 1991; Murdock et al., 2012). Meanwhile, the survivability, infectivity and multiplication rate of many parasites have also been shown to fluctuate with temperature (King & Monis, 2007; Fels & Kaltz, 2006; Ward et al., 2007; Studer et al., 2010), often following a unimodal response where infection is maximized at intermediate temperatures and constrained under extreme values (Shocket et al., 2019). Concurrently, the geographical incidence of diseases is also predicted to increase under global warming,

either resulting from newly suitable climate emerging in previously inaccessible areas (Kutz et al., 2013) or pathogens benefitting from the concurrent range expansion of their vectors (Rochlin et al., 2013; Sonenshine, 2018). This environmental-dependency of infection traits and disease risks has notably driven the emergence of an entire subset of the literature in epidemiology over the past two decades, informally referred to as the 'warmer is sicker' hypothesis (Martens et al., 1995; Lafferty et al., 2004; Lafferty & Mordecai, 2016). Using both experimental and modelling approaches, several studies have since attempted to determine whether a warmer world would see both the prevalence and severity of diseases increase under the direct influence of global warming (although these failed to reach a unidirectional, formal consensus; see Hall et al., 2006; Ibelings et al., 2011; Rohr & Cohen, 2020).

Box 1. The disease triangle. The outcome of infection is determined by three components: the host's inherent susceptibility, the parasite's transmission potential, and the influence of their shared environment. Direct and indirect interactions between these components will determine the outcome of infection and overall amount of damage inflicted on the host, represented by the triangle's height. In this example, high resource availability indirectly impairs the parasite by increasing the strength of its host's immune response. However, high resource availability may also increase the parasite's growth rate. The relative strength of each arrow will determine the overall outcome of infection. Here, the beneficial effect of resources reduce the host's susceptibility more than it increases the parasite's growth, thus resulting in a lower triangle (i.e. lower virulence and transmission success).



Similar to the influence of temperature on parasitic infections, complex within-host processes can be derived from the nutritional adequacy of the host's diet: whereas a high-quality resource may improve the host's immune defences (Navarro-Gonzalez et al., 2011; Sanchez-Thirion et al., 2019), many endoparasites derive their growth from hijacking mechanisms of resource allocation, often reducing host fecundity at the benefit of their own multiplication. Thus, parasites may simultaneously benefit from increased resource uptake, allowing them to produce a higher number of infective propagules (Hall et al., 2009; Schlotz et al., 2013). Several other examples of anthropogenic disturbances have been considered as potential drivers of diseases and suggested to increase epidemic risks in a variety of host-parasite assemblages, with a surprisingly high representation of aquatic biota in the literature: sewage wastes, metabolic products from fish cultures and pesticides have been associated with disease outbreaks in fishes (Snieszko, 1974; Khan & Thulin, 1991; Poulin, 1992), metal contamination (cadmium) was shown to reduce phagocytosis in crabs (Truscott & White, 1990), overfishing of predatory fish can indirectly increase epidemics of parasites that reproduce in a density-dependent manner (Lafferty, 2004), while the development of agricultural landscapes around streams and ponds was positively correlated with helminthic diversity in fish populations (Hernandez et al., 2007) and the occurrence of echinostome parasites in amphibian populations (King et al., 2010; Koprivnikar & Redfern, 2012).

2. Freshwater zooplankton as a model for epidemiology

2.1 Freshwater ecosystems: hotspots of diversity under direct threats

Due to the historical tendency of human populations to aggregate upon large bodies of water (Yevjevich, 1992) and the multitude of services provided by such environments (e.g. trade routes, drinking water supply, fisheries, wastewater disposal and recreational activities), freshwater bodies present themselves as especially vulnerable to contemporary anthropic disturbances (Søndergaard & Jeppesen, 2007; Geist, 2011; Reid et al., 2019). Some examples include excessive nutrient loading into surface waters, favoured by the discharge of domestic wastes, forest clearance, urban development and agricultural practices (Schelske et al., 1983; Mainstone & Parr, 2002; Callisto et al., 2014), the increasing frequency of harmful algal blooms throughout the past century (Hallegraef, 1993; Ho et al., 2017; Wurtsbaugh et al., 2019) or the accumulation of micro- and nanoplastics in freshwater food chains, driven by a combination of sewage discharge and atmospheric transportation (Chae et al., 2018; Meng et al., 2020; Wang et al., 2021). In addition, freshwater lakes have been subject to an abrupt elevation of their surface temperature over the past few decades (Schindler, 1997; Burgmer et al., 2007), driven by a combination of local and climate-induced phenomenon, from increases in solar radiations and air temperature to diminishing cloud covers (O'Reilly et al., 2015).

Current threats to the stability of freshwater environments are concerning from a conservation perspective, given the multitude of wider ecosystem and human-related services provided by these hotspots of diversity (Covich et al., 2004). Moreover, there is growing concern about losing some of these services, as a result of declining species diversity (Albert et al., 2021): despite representing less than 1% of the Earth's surface, freshwater ecosystems are home to a wide diversity of organisms (close to 10% of all known species; Strayer & Dudgeon, 2010) involved in a complex network of biogeochemical cycles (Kuehn & Suberkropp, 2006; Anderson, 2018) and multi-layered species interactions (Bronmark et al., 1992; Kagami et al., 2014; Agha et al., 2016). In particular, phyto- and zooplanktonic taxa of high scientific relevance have been identified as excellent bioindicators for the management of aquatic pollution, eutrophication, and ecosystem health, due to their sensitivity to environmental stress and basal position in freshwater food chains (Jakhar, 2013; Gazonato et al., 2014).

In freshwater ecosystems, heterotrophy (i.e. the consumption of organic sources of carbon by species unable to synthesize their own) is primarily ensured by an assemblage of functionally similar taxa of protists and small-sized animals, collectively referred to as 'zooplankton'. Occupying a central position in freshwater food chains, zooplankters feed upon primary producers (i.e. autotrophs, such as cyanobacteria and green algae) and serve as important prey items for secondary consumers, mainly planktivorous fish and insect larvae (Sterner, 1989; Weider & Pijanowska, 1993). While zooplankton community assemblages can vary between sites (Stemberger & Lazorchak, 1994), they are primarily dominated by ciliates, rotifers and small crustaceans in freshwater environments (Pace & Orcutt, 1981). Common representatives include members of the class Hexanauplia (e.g. copepods) and the superorder Cladocera (commonly referred to as waterfleas), both of which can dominate community composition in the mesoplankton (0.2 - 20mm) (Cyr & Curtis, 1999). While cladocerans alone include more than 700 extant species (Van Damme & Kotov, 2016), over 150 of those belong to the genus *Daphnia*, which frequently dominate cladoceran communities in freshwater lakes and ponds (Perrow et al., 1999).

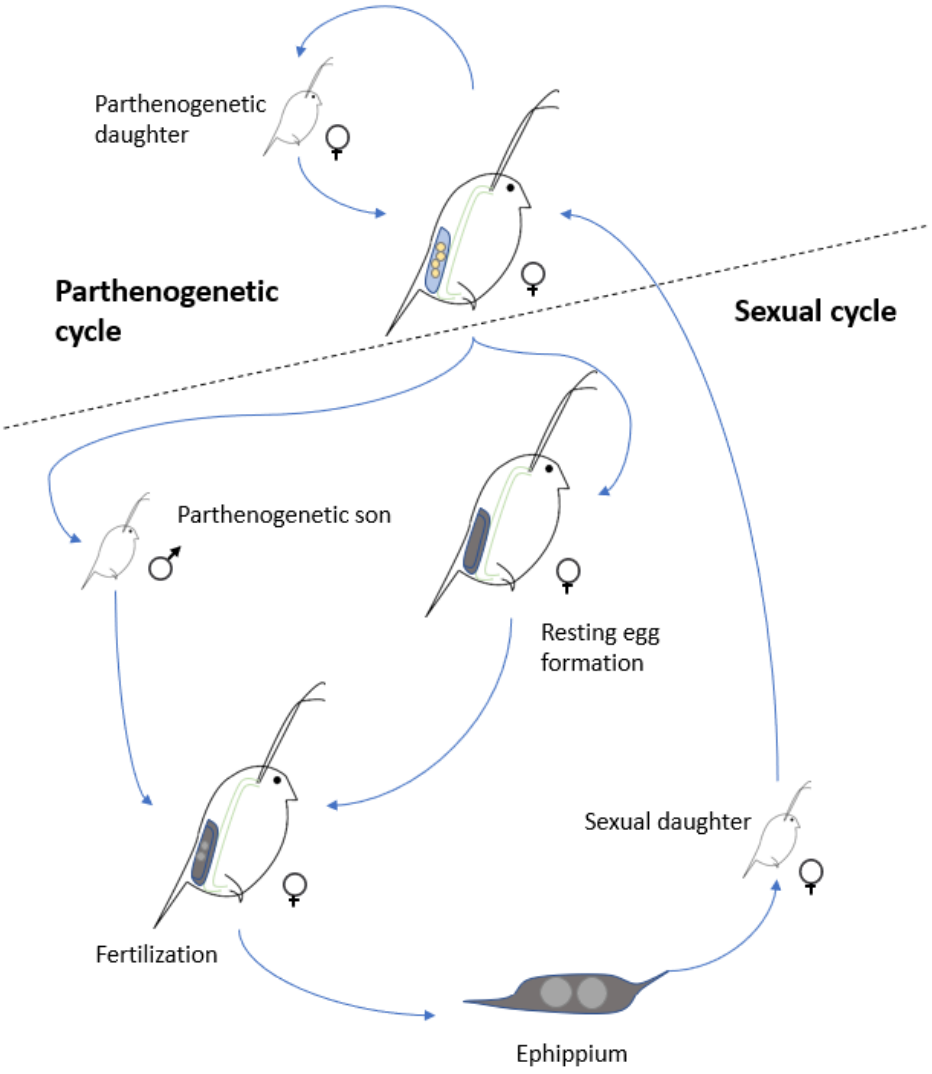
Daphnia are extremely widespread around the globe (Benzie, 1987; Adamowicz et al., 2009) and can be found across most types of standing water bodies, from ephemeral ponds susceptible to complete dry-outs (Altermatt et al., 2009) to holomictic lakes and water reservoirs spanning several km^2 in superficy (Wolinska et al., 2011). Species distribution and habitat selection in *Daphnia* is partly constrained by body size: due to being more visually detectable as prey items, larger species face strong predation pressure in lakes, where predators are plenty (Zaret, 1980). As such, larger species have remained mostly confined to ponds, rock pools and small lakes where fish predation is limited (e.g. *Daphnia magna*, $\leq$ 5 mm), while larger water bodies are rather dominated by a variety of smaller *Daphnia* species (e.g. *D. longispina*, *D. pulex*, $\leq$ 2.5 mm). Due to their central position in freshwater food chains, *Daphnia* are considered keystone species, which disproportionately affect the relative abundance and distribution of other species in their environment (Collinge et al., 2008). Notably, predation by *Daphnia* grazers was shown to exert strong effects on the species composition of

freshwater phytoplankton (Sarnelle, 2005), with the potential to reduce the biomass and control the dominance of bloom-forming cyanobacteria (Matveev et al., 1994; Gerasimova et al., 2018). Conversely, sedimental records of *Daphnia*'s resting eggs can be used to retrace historical changes in the distribution as well as the contemporary abundance of planktivorous fish (Jeppesen et al., 2002).

Besides their ecological relevance in freshwater environments, several characteristics inherent to the biology of *Daphnia* have contributed to their emergence as popular model systems in the fields of limnology and aquatic ecology (Reynolds, 2011; Seda & Petrusek, 2011). The propensity of *Daphnia* to reproduce asexually (Box 2) and rare occurrence of sexual reproduction under non-limiting laboratory conditions allows for the maintenance of clonal, iso-female lines. This is useful for simulating host diversity and has served to highlight strong genotypic variability in a wide array of life-history traits (De Meester, 1991; Soares et al., 1992; Dudycha & Tessier, 1999) and physiological responses to environmental stress (Hietala et al., 1997; Pauwels et al., 2005; Fitzsimmons & Innes, 2006). In addition, their small size (up to 5mm for the largest species) and short generation time (10-20 days at 20°C) allow for the evaluation of precise, individual-based responses (e.g. Frost et al., 2010; Cuco et al., 2016; Ogonowski et al., 2016) and population or community-level assays, using experimental mesocosms (e.g. Paterson et al., 2002; Van Doorslaer, 2010; Aljaibachi et al., 2020).

Abiotic variables such as light intensity, photoperiod, temperature, nutrient availability and pH can be easily manipulated in vitro, which is ideal for simulating environmental disturbances experimentally (Korpelainen, 1986; Hanazato, 1996; McKee et al., 2002; Bergman Filho et al., 2011). As ectothermic invertebrates, their entire metabolism slows down or speeds up relative to temperature or oxygen concentration, which directly impacts key life-traits such as longevity, body size, offspring production, age at maturity, and oxygen consumption (MacArthur & Baillie, 1929; Chopelet et al., 2008). Moreover, non-trivial interactions between some of these factors have been shown to further influence the life-history parameters of experimental *Daphnia* (Orcutt & Porter,1984; Giebelhausen & Lampert, 2001). Because many of these variables can be directly affected by climate change, *Daphnia* already represent a model of choice when trying to simulate and interpret possible climate-driven changes experimentally (e.g. McKee et al., 2002; Müller et al., 2018). In addition, they are also very sensitive to pollutants: a direct consequence of their role as mostly non-selective filter-feeders, *Daphnia* are especially prone to acquire and bioaccumulate contaminants released by human activity in freshwater ecosystems, granting them an equally important role in the fields of ecotoxicology over the past two decades (Stark & Vargas, 2005; Han et al., 2006; Lee et al., 2019; Yuan et al., 2020). But perhaps even more preponderant than their role as bioindicators and school introductory models, *Daphnia* have progressively earned their place as one of the most extensively studied organisms in the context of host-parasite interactions, both in the field and the laboratory (Ebert, 2005; Ebert, 2008).

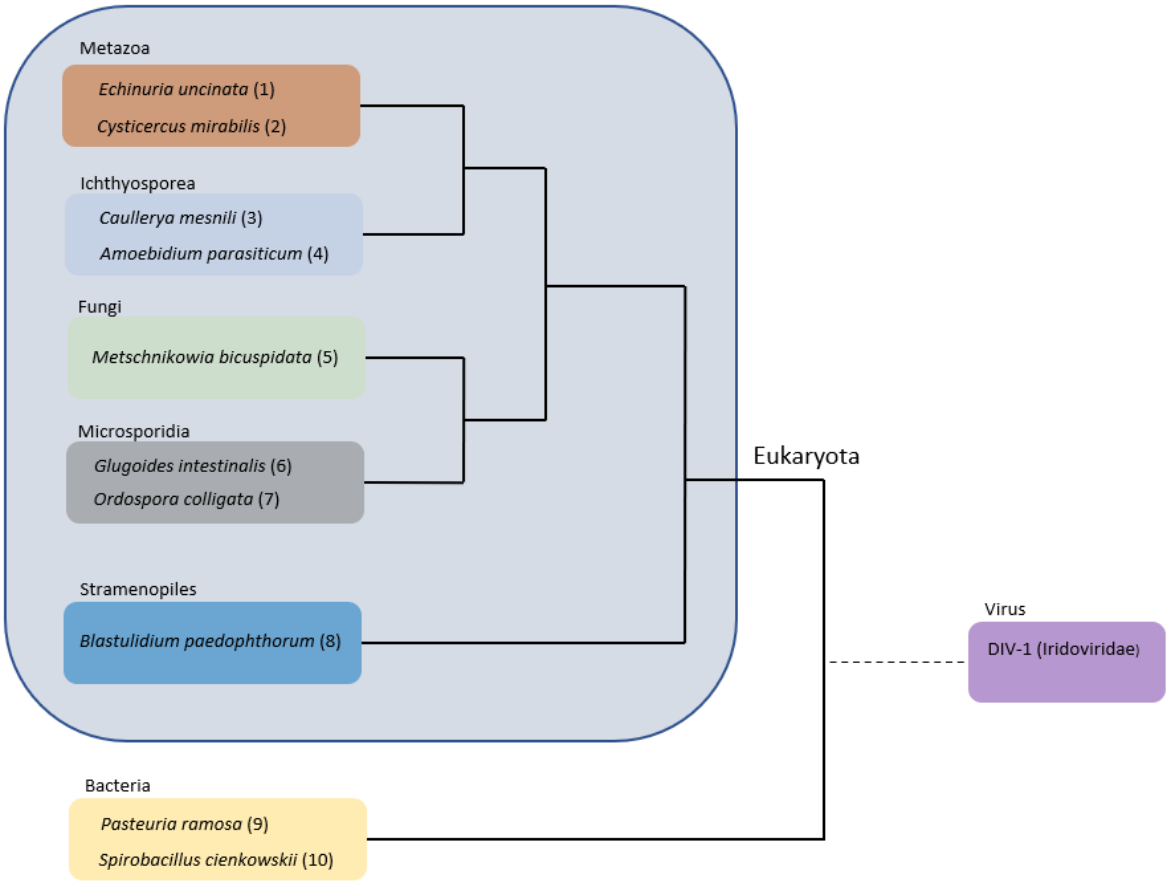
Box 2. The reproductive cycle of *Daphnia*. Most species of *Daphnia* reproduce through cyclical parthenogenesis: populations are dominated by diploid females, which are capable of reproducing asexually. Diploid eggs are produced by parthenogenesis (without fertilization) and develop in the dorsal brood chamber, until they are released as 'clutches' of up to several dozens of juveniles, which are genotypically identical to their mother (parthenogenetic daughters). When environmental conditions are unfavourable (i.e. detection of predator cues, high population density of low food availability), adult female *Daphnia* can produce diploid males (parthenogenetic sons) and haploid resting eggs, which are encased in an ephippium (protective structure in the brood chamber). Following internal fertilization by males, detachment of the ephippium upon moulting allows for fertilized eggs to be released into the environment. After a period of diapause, generally interrupted by specific environmental cues (e.g. desiccation followed by re-watering), these will then hatch into diploid females (sexual daughters). This process allows for genetic mixing in *Daphnia* populations and the emergence of hybrid genotypes, which can coexist with their parental lines, notably among the *D. longispina* species complex, which is widely distributed across lakes in Europe (Spaak, 1997).



2.2. Natural enemies of *Daphnia*: a variety of microparasites

In nature, *Daphnia* are commonly infected by a wide variety of parasites (Ebert, 2005; Wolinska et al., 2009). An overview of the taxonomic diversity of *Daphnia* parasites is represented in Box 3. Screenings of freshwater bodies frequently report the co-occurrence of several species of parasites in natural populations of *Daphnia* (Stirnadel & Ebert, 1997; Decaestecker et al., 2004; Wolinska et al., 2011; Weigl et al., 2012; Goren & Ben-Ami, 2013), and individual hosts are commonly observed to bear multiple infections (Decaestecker et al., 2005). Some of these parasites may have conflicting strategies of host exploitation and transmission (Ben-Ami et al., 2011). For instance, conflicts for optimal host exploitation can occur between related strains of a same parasite with varying degrees of virulence; in which case, faster replicating strains are usually favoured (Ben-Ami et al., 2008). By contrast, distant taxa of parasites may differ in more phylogenetically-constrained traits, such as their overall transmission mode (horizontal vs. vertical), their onset of spore release (while the host is still alive vs. after host death) or even colonize different niches inside the host (the body cavity vs. the gut epithelium) (Ebert, 2005). Endoparasites of *Daphnia* usually exert high virulence upon their host, often reducing the lifespan and fecundity of infected individuals (Ebert et al., 2000a; Haag et al., 2003; Decaestecker et al., 2003). However, functionally similar parasites can still vary widely in their level of virulence, such as intracellular parasites of the gut epithelium ranging from the relatively low-damage of microsporidia (*Ordospora colligata*, *Glugoides intestinalis*; Ebert et al., 2000a) to the castrating strategy of the ichthyosporean *Caullerya mesnili* (Lohr et al., 2010a). While most parasites of *Daphnia* are horizontally-transmitted, usually through the accidental consumption of spores found in the water column or sediment, vertical transmission from mothers to their offspring has been documented in microsporidia of the genus *Hamiltosporidium* (Haag et al., 2020). By contrast, oomycete parasites of *Daphnia* only appear to interfere with the development of embryos in the brood chamber, with no apparent detriment to the longevity of infected adults (*Blastulidium paedophthorum*, Duffy et al., 2015). Finally, representing opposite branches on the phylogenetic tree of life, the body cavity of *Daphnia* can be colonized by parasitic worms (Cestoda and Nematoda, Ebert, 2005) and bacterial pathogens alike (*Spirobacillus cienkowskii*, Bresciani et al., 2018; *Pasteuria ramosa*, Ebert et al., 1996).

Box 3. Cladogram representing the phylogenetic diversity of parasites infecting the genus *Daphnia*. Parasites of *Daphnia* can be found across a variety of Eukaryotic and Prokaryotic taxa, including Nematoda (1), Cestoda (2), Ichthyosporea (3-4), Ascomycota (5), Microsporidia (6-7) and Oomycota (8), as well as the Firmicutes (9) and Deltaproteobacteria (10). Only one representative of a viral pathogen has been identified so far in *Daphnia*: DIV-1, responsible for White Fat Cell Disease (Toenshoff et al., 2018). Phylogenetic relationships were reconstructed from Torruella et al., 2015.



Adding to an already vast spectrum of endoparasites, characterized by an obligatory phase of within-host reproduction, *Daphnia* are also frequently colonized by various taxa of epibionts, such as sessile ciliates (*Vorticella sp.*, Kankaala & Eloranta, 1987), epizoic rotifers (*Brachionus rubens*, Iyer & Rao, 1993) or the ichthyosporean *Amoebidium parasiticum* (Whisler, 1968; Benny & O'Donnell, 2000). While these usually exert a lesser impact on host fitness, ectoparasites and epibionts may also generate fitness reductions by impairing mobility, competing with their host for resource and influencing other characteristics of life history, such as population growth rates (Allen et al., 1993; Angell, 2016).

Perhaps due to their small size and the concealed nature of their life cycle, the contribution of parasites to overall biochemical cycles and food chains is often undervalued; yet, they tend to play very

important roles at the community level (Poulin, 1999; Marcogliese, 2004). Because parasites of *Daphnia* can reach very high prevalence in natural populations (Decaestecker et al., 2005; Wolinska et al., 2007), they are capable of generating profound changes in the ecology of their hosts. By producing opaque symptoms of infection in the transparent body cavity of their hosts, infected *Daphnia* are often more easily detectable by predators (Duffy et al., 2005; Duffy, 2007). Parasites may also reduce the mobility of their hosts, to the point of disrupting habitat selection (such as behaviour of vertical migration in the water column; Fels et al., 2004). Moreover, large outbreaks of parasites have been shown to drive *Daphnia* populations to extinction (Ebert et al., 2000a; Decaestecker et al., 2005).

Adding to the concern of environmental disturbances in freshwater ecosystems, parasitic infections may also interact with abiotic changes to further reduce the reproductive ability of *Daphnia*, such as epibionts delaying population growth in *D. magna* under high temperatures (Angell, 2016). Conversely, changes in temperature may favour parasite encounters by affecting body size and foraging rates (Shocket et al., 2018a), as most horizontally-transmitted parasites of *Daphnia* are recruited by non-selective filtering of spores in the environment (Ebert, 2005). Because pathogens have the potential to change the abundance of keystone species (such as *Daphnia*), such interactive effects between environmental disturbances and parasitic infections have the potential to cause major shifts in community composition and wider ecosystem functions (Duffy, 2007; Collinge et al., 2008). Therefore, understanding how climate change and other environmental disturbances of anthropic origin may disrupt host-parasite interactions and traits of epidemiology in this system is crucial, with possibly large ecological consequences within freshwater ecosystems.

3. Experimental approach

3.1 Our specific host-parasite system

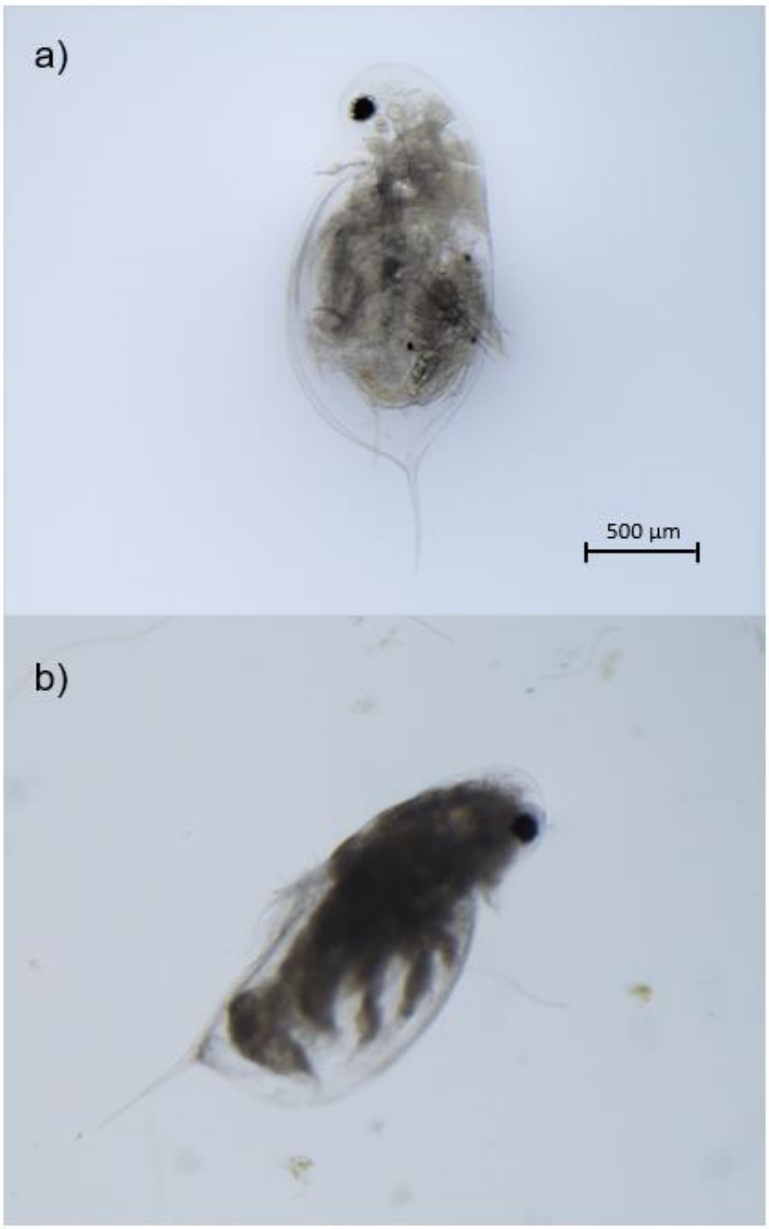
In order to simulate environmental disturbances under controlled experimental conditions, the present work will focus on a single assemblage of host and parasite, which will be used as a recurring system throughout the following chapters. The freshwater-dwelling species *Daphnia galeata* and *Daphnia longispina* are widely distributed across lakes and reservoirs in Central Europe (Giessler, 1997; Seda et al., 2007; Brzeziński et al., 2012) and belong to a taxonomic group or subgenus referred to as the *Daphnia longispina* complex (alternatively, “Hyalodaphnia”), which contains over 12 recognized distinct taxa (Adamowicz et al., 2009). These lineages differ in a number of morphological and physiological traits, as well as their geographical range and niche tolerance: for instance, *D. galeata* tends to inhabit warmer lakes than *D. longispina*, while the latter generally dominates in conditions of low phosphorus loads (Keller et al., 2008). The focal genotypes AMME_12 and AMME_51 (F1-hybrids

of *D. galeata* × *longispina*) were isolated from Ammersee, Germany (48°00'0.00" N 11°06'60.00" E) during two separate sampling events in the fall of 2008. These were produced sexually (see Box 2) and have since been maintained as parthenogenetic, iso-female lines under non-limiting laboratory conditions. In order to maximize the comparability of results across our experimental assays, the same two genotypes were used throughout most of the present work (with the notable exception of Chapter 4, where parasite specificity constrained our choice of host species). Given the very good water quality of this lake, both clones are expected to have little to no historical exposure to toxicants and environmental contaminants (Cuco et al., 2016). However, both genotypes most likely share an ancient evolutionary history with a common fungal parasite of *Daphnia* isolated throughout the same series of sampling: *Metschnikowia bicuspidata*.

The parasitic yeast *Metschnikowia bicuspidata* (hereafter referred to as *Metschnikowia*) will be used as the primary focal parasite throughout the present work. As a generalist pathogen, widely distributed around the globe and capable of infecting most *Daphnia* species, it is perhaps the best-described fungal parasite of *Daphnia* (Ebert, 2005). Its reproductive cycle is typical of the Ascomycota, which involves the production of reproductive conidia and elongated ascospores. As a parasitic yeast, *Metschnikowia* belongs to the order Saccharomycetales, better known for the commercial and medicinal use of the yeast *Saccharomyces cerevisiae*. Other strains of *M. bicuspidata* were also found to infect brine shrimps (Codreanu & Codreanu-Balcescu, 1981), prawns (Chen et al., 2003), crabs (Bao et al., 2021) and fish (Moore & Strom, 2003), though it appears that a complex of similar species was described instead (Ebert, 2005). The parasite is characterized by an obligatory-killing lifestyle: because it only produces one yield of spores that are simultaneously released into the environment (upon host death), it is defined as a semelparous parasite (i.e. limited to a single event of reproduction). This reproductive strategy is not unique among parasites of *Daphnia*, as it is notably shared by the bacterium *Pasteuria ramosa*; however, it distinguishes *Metschnikowia* from parasites of the digestive tract, which are capable of continually releasing spores throughout the lifespan of their hosts (Ebert, 2005).

Infective stages of the parasite (asci) present a characteristic, needle-shaped appearance; as such, they are easily identifiable within infected individuals and can be quantified using basic microscopy. This morphology is inherently tied to the parasite's transmission strategy: spores are immobile, left to drift along the water currents and often accumulating as spore banks in the sediment (Duffy & Hunsberger, 2019). Encounters with the host are entirely passive, resulting from the accidental ingestion of spores by water filtration. The needle-like morphology allows infective stages to puncture and move past the epithelium inside the digestive tract. Infective stages initiate their reproductive cycle in the haemolymph, the circulatory fluid flowing through the body cavity of *Daphnia*. Late stages of infection can be observed with the naked eye, forming opaque material in the body cavity. Typical symptoms of infection in the *Daphnia-Metschnikowia* system are depicted in Box 4.

Box 4. a) Adult female of the hybrid *Daphnia galeata* × *longispina* (genotype AMME_51). Three fully-developed juveniles can be observed in the dorsal brood chamber. **b)** Adult female (genotype AMME_12) heavily infected with the parasitic yeast *Metschnikowia bicuspidata* (strain METS_AMME_2008). This individual was retrieved and fixed in formaldehyde upon its death, 17 days following its exposure to the parasite. The dark-coloured material observed throughout the entire body cavity corresponds to the final reproductive stages of the fungus: elongated asci. Parasite spore loads generally range up to 100 000 spores per adult female in *D. galeata* × *longispina* (this specific individual was estimated to yield a total of 55 000 spores). Upon decomposition of the host's dead body, the needle-shaped spores are released into the water column, allowing for new infections to develop. Photography: Florent Manzi



3.2 Assessing the outcome of infection under simulated environmental disturbances

Metschnikowia is a highly virulent parasite: while not preventing reproduction completely, it usually reduces the number of juveniles produced per clutch (Ebert et al., 2000a) as well as reducing the lifespan of infected hosts (Ebert et al., 2000b), both effects contributing to a large decline in fecundity. These variables can be easily measured in experimental infection assays, where the complete life history of distinct individual *Daphnia* can be followed closely (i.e. life table response experiments, LTRE). The principle of LTRE consists of decomposing the effects of a given treatment on a dependent variable (for instance, growth rate) into contributions from differences in the parameters that determine this variable (Caswell, 2010). Thus, a combination of fixed and random effects, along with linear or quadratic regressions between continuous variables can be applied to assess the effects of simulated environmental stress (e.g. elevated temperature) on individual infection outcomes, while controlling the exact timing and dosage of parasite encounters. From here onward, the present work makes use of the term *outcome of infection* when referring to both i) the overall transmission success of the parasite, following its encounter with a susceptible host and ii) the amount of damage inflicted on individual hosts, measured as a reduction of host fitness traits. Thus, infection dynamics are considered from the point of view of both antagonists.

3.2.1 Determinants of parasite fitness

A parasite (i.e. consisting of one pool of infective spores, sharing a single genotype) may experience diverging success in its overall transmission depending on the realization of distinct infection steps, each of which can be differentially influenced by the environment (Duneau et al., 2011). For *Metschnikowia*, these include: i) encountering susceptible hosts in the environment; in nature, this process can be influenced by host filtering rates and habitat selection (e.g. diel vertical migration), both of which can be modulated by environmental factors (temperature, diet, light exposure) and intra-specific variability (Chow-Fraser & Sprules, 1986; De Meester, 1989). Because experimental inoculations of *Daphnia* can be performed at low volume and using non-limiting spore doses, the successful ingestion of spores will generally be ensured in our assays. From then, ii) only a limited number of needle-shaped spores will successfully cross over to the target host compartment (i.e. entering the haemolymph via the physical barrier of the gut epithelium; Stewart Merrill & Cáceres, 2018). This process can be influenced by clonal variation in the penetrability of the gut epithelium, which is further modulated by host age and body size at the time of infection (Stewart Merrill et al., 2019). Upon germination of infective stages in the haemolymph, successful transmission of the parasite still requires iii) the ability to overcome the host's immune response and iv) survival of the host until successful completion of the yeast's reproductive cycle.

Because the production of mature spores is a relatively slow process in the *Daphnia-Metschnikowia* system, hosts that end up dying prematurely may serve as 'infectious sinks'. Ingestion of spores by a host that is particularly vulnerable to environmental sources of mortality has a high probability of resulting in a wasted encounter, where spores are taken up from the environmental pool of the parasite but will not contribute to the next generation of infective stages. Thus, external sources of host mortality may play an important role in preventing the spread of *Metschnikowia* in the environment (Pulkkinen & Ebert, 2004). Because infections are difficult to determine before day 8-to-10 post-infection in this system, 'early deaths' are often disregarded in experimental assays (e.g. Lohr et al., 2010b). However, beyond a purely stochastic process, early host mortality in *Daphnia* can result from harsh environmental conditions influencing host lifespan (Korpelainen, 1986; DeMott et al., 1991; Eltemsah & Bøhn, 2019), genetic incompatibilities between the host and parasite (Ebert et al., 2016), or a combination of pollution- and parasite-induced stress (Buser et al., 2012). Because multiple infections are frequent in *Daphnia*, coinfection by two virulent parasites may also result in a greater reduction of the host's longevity, which could be maladaptive for our focal parasite and favour pathogens with shorter generation times (Lohr et al., 2010b). Thus, we propose to incorporate 'early deaths' into a measure of *host viability*, as a way to express non-random variations in the chances of the host dying prematurely, which are either exerted or mitigated by distinct environmental factors and contribute to the parasite's overall transmission success. Complementarily, we measure *parasite infectivity* (defined here as the probability of producing mature spores) among experimental hosts that are considered 'viable' for infection, that is, those which survive until the earliest possible observation of mature spores in the *Daphnia-Metschnikowia* system.

Host viability and parasite infectivity are important determinants of transmission success; together, they reflect the probability of the parasite successfully completing its reproductive cycle, following its ingestion by a potential host. Once reproduction is ensured, however, further contributions to the parasite's transmission may result from quantitative variations in its spore yield (i.e. the total amount of infective propagules carried by an infected host at the time of its death). This variable can be easily determined in the *Daphnia-Metschnikowia* system using standard counting chambers (Ebert et al., 2000b) and is usually referred to as the *intensity of infection.* Because the totality of spores produced from a single infection event remain sequestrated in the host's body cavity until its eventual demise, the number of mature spores found in a host at death is usually proportional to the number of secondary infections produced by obligate-killing parasites (Izhar & Ben-Ami, 2015; Clay et al., 2019b).

3.2.2 Determinants of host fitness

In the context of experimental infection assays, the fitness of a particular host genotype (e.g. 'AMME_12'; 'AMME_51') or group of individuals assigned to the same experimental treatment (e.g. 'high temperature', 'low temperature') can be decomposed into parameters of *longevity* (e.g. number of days survived by individual *Daphnia*, following their exposure to parasite spores) and *fecundity* (e.g. total number of parthenogenetic offspring produced throughout the lifespan of a single female). In addition, cumulative measures deriving from these variables can provide information about population growth, such as the *per capita intrinsic rate of increase* (Cuco et al., 2018). Evaluating the degree by which these variables differ between infected and control individuals provides a suitable proxy for determining a parasite's *virulence* (i.e. the amount of damage incurred by the host as a result of a pathogenic infection). Moreover, assessing the outcome of parasitic infections on the life expectancy and reproductive ability of *Daphnia* allows us to estimate the potential impacts of disease outbreaks (as well as potential interactions between parasites and environmental disturbances) on the dynamics of host populations. In the context of increasingly frequent environmental disturbances and changing climate, these features present valuable interest from an economic and conservation point of view, relative to *Daphnia*'s status as freshwater keystone species. Here, we do not only aim to explore how likely host populations are to resist and recover from disease outbreaks; instead, we attribute equal importance to how suitable of an environment they can provide for parasite reproduction, depending on such environmental variations. Host availability is a necessary requirement for the spread of their parasites; thus, negative effects on zooplankton abundance – resulting from a combination of environmentally and parasite-induced stress – may constrain the spread of parasites in freshwater ecosystems, especially those that rely on density-dependent mechanisms of horizontal transmission.

4. Aims and overview of the thesis

Following its initial description by Elie Metschnikoff in 1884, the *Daphnia-Metschnikowia* system has since benefitted from nearly 150 years of cumulative research, including descriptive studies (Green, 1974; Codreanu & Codreanu-Balcescu, 1981; Stewart-Merrill & Cáceres, 2018), molecular sequencing (Mendonça-Hagler et al., 1993), field work (Cáceres et al., 2006; Hall et al., 2009; Yin et al., 2012) and experimental infection trials in controlled conditions (Ebert, 2000b; Duffy, 2009; Hesse et al., 2012; Searle et al., 2015). While many of the features presented throughout this thesis will not revolutionize our general understanding of the parasite's life cycle, the idea of investigating the environmental-dependency of fungal infection traits in *Daphnia* remains a relatively recent topic, mostly emerging within the last two decades (e.g. Hall et al., 2009; Duffy & Hunsberger, 2019). Moreover, studies specifically tying the influence of the environment with concerns of climate change (Hall et al., 2006;

Overholt et al., 2012) and environmental pollution (Civitello et al., 2012; Cuco et al., 2020) in this system are still relatively scarce in the literature. Using the approach described above, this thesis will aim to uncover the potential impacts of temperature elevation (both direct and indirect effects) and environmental pollution on infections of freshwater zooplankton, focusing on four aspects of anthropic disturbances that had not been previously addressed in the *Daphnia*-microparasite system.

Given the documented sensitivity of *Daphnia* to fluctuating temperatures (McMahon ,1965; Burns, 1969) and the importance of host diet on a multitude of infection-related traits (Hall et al., 2009), prior studies have already constituted a considerable body of work regarding the influence of both factors on infections of zooplankton (the current state of research on independent effects of host diet and temperature in the *Daphnia*-microparasite system is summarized in Table 1 and Table 2). However, only one study so far attempted to cross these two factors in experimental infections of *Daphnia*, showing that maternal conditions of high temperature and low food availability could increase the offspring's resistance to a bacterial parasite (Garbutt et al., 2014). Because cyanobacteria respond positively to rising temperatures, CO2 concentrations and eutrophication (Visser et al., 2016; Bartosiewicz et al., 2019) and constitute a resource of poor nutritional value to zooplanktonic grazers (DeMott et al., 1991), global environmental shifts may indirectly reduce the availability of high-quality algal food in *Daphnia*. Moreover, cyanobacterial blooms display a temporal overlap with epidemics of *Metschnikowia,* which typically break out in the late-summer to early-autumn in temperate lakes (Duffy et al., 2009; Wolinska et al., 2011). For this reason, **Chapter 1** attempts to reconcile the direct effects of elevated temperature with temporary shifts in the quality of zooplankton diets, investigating the potential for interactive effects between temperature, phytoplankton composition and fungal epidemics in our system of interest. Genotypes AMME_12 and AMME_51 were exposed to the parasite in a full-factorial design, including one eukaryotic alga of high nutritional value and two species of toxic cyanobacteria with contrasting morphologies; two levels of temperature were tested, corresponding to a standard rearing temperature (19°C) against a predicted surface temperature elevation of 4°C. Experimental assays showed complex interactions between host diet, temperature and clonal identity of the host, demonstrating the importance of identifying concurrent sources of environmental stress in freshwater ecosystems. Low-quality diets strongly impaired transmission of the parasite, indicating that increased temperature and cyanobacterial dominance could constrain epidemics of *Metschnikowia* under predicted environmental changes.

In a direct continuation of this thematic, **Chapter 2** examines whether the release of potent cyanobacterial toxins during the termination of harmful algal blooms could interfere with free-living stages of the parasite. Here, the same two host genotypes were inoculated with spores of *Metschnikowia*, that had received prior prolonged exposure to high concentrations of cyanobacterial toxins (extracted from *Microcystis aeruginosa*) or a placebo solution. Both infection treatments were crossed with two levels of diet quality, returning from the previous experiment. We predicted that exposing the parasite

to dissolved cell content of cyanobacteria would reduce its infectivity upon later encounter with the host and could interact synergistically with a toxic diet, further reducing the parasite's transmission. While the former hypothesis was not supported, our results restate a differential performance of *Metschnikowia* under specific associations of host diet and clonal identity. Overall, this section corroborates the findings of the previous chapter, while clarifying that increased cyanobacterial dominance may only interfere with the parasite *via* their consumption by zooplankton hosts, showing no evidence of direct interference with free-living stages.

While experimental assays had previously examined the effects of environmental pollution on *Metschnikowia* infections, including the influence of copper (Civitello et al., 2012) and the fungicide tebuconazole (Cuco et al., 2017), the implications of plastic pollution on host-parasite interactions remains mostly unexplored in the literature (but see Hernandez-Milian et al., 2019; Buss et al., 2021). This apparent lack of research stands in stark contrast with a recent surge of studies examining the single-species implications of nanoplastics in aquatic organisms, including *Daphnia* (Bergami et al., 2017; Lin et al., 2019; Auguste et al., 2020; Kelpsiene et al., 2020; Liu et al., 2021). However, besides recent reports on fungal infections of nematodes (Li et al., 2020) and phytoplankton (Schampera et al., 2021), potential effects of nanoplastics on the outcome of infection were not previously investigated in the *Daphnia*-microparasite system. In **Chapter 3**, control and infected individuals of genotype AMME_51 were exposed to three possible concentrations of polystyrene nanoplastics in the culture medium (0 mg/L, 5 mg/L and 20 mg/L). We found evidence for a hormetic dose-response (i.e. beneficial effects of low contaminant concentrations) on fitness parameters of the host. While the parasite displayed increased infectivity at low concentrations of nanoplastics, spore production was strongly reduced in the high concentration treatment. This experiment provides preliminary evidence that plastic pollution can strongly influence the outcome of host-parasite interactions, leading the way for future research to consider different assemblages of host and parasite species.

In natural populations of *Daphnia*, epidemics often display seasonal patterns of emergence (Duffy et al., 2009; Hall et al., 2011). Researchers have attempted to identify which environmental factors control the onset and termination of such epidemics, citing periodic variations in temperature and host density as potential factors controlling the seasonal emergence of fungal and microsporidian parasites of *Daphnia* (Duffy & Hunsberger, 2019; Kirk et al., 2020). While many studies have already investigated climate-induced shifts in the spatial distribution of parasites around the globe (Messina et al., 2019; Morales-Castilla et al., 2021), future climate conditions may also influence the temporal overlap of parasite species that are already sympatric (Clay et al., 2020). Furthermore, the order in which distinct parasite species emerge in the environment influences the sequence in which they encounter their hosts in multiple infections, generating within-host priority effects that can have strong implications on parasitic infections (Halliday et al., 2017; Clay et al., 2019b). While sequential infections involving *Metschnikowia* were previously carried out in *Daphnia*, these few studies opposed

Metschnikowia to parasites of comparably high virulence (see Table 3). In **Chapter 4**, we ask how changes in the sequence of infection between a relatively avirulent parasite of the digestive tract (*Ordospora colligata*) and our focal parasite (*Metschnikowia bicuspidata*) can influence parasite transmission and the overall amount of damage inflicted on the host. To accommodate for the narrow host range of *Ordospora*, this experiment was carried out on a single genotype of *Daphnia magna* Straus (genotype NO-V-7). Individual *D. magna* were inoculated with either one or both parasites, with two possible orders of arrival in sequential coinfections, to simulate prior residency of either parasite in *Daphnia* populations. We found that prior residency of the gut microsporidium – a pattern that is supported by the natural phenology of this parasite – strongly reduced the viability of sequentially-infected hosts, compromising the transmission success of both species. By contrast, we found no differences between single and sequential infections in the treatment where *Metschnikowia* was introduced early. This final chapter denotes the importance of species succession patterns in nature, suggesting that future shifts in the temporality of epidemics could modulate the within-host processes that govern competition among parasites.

The present work provides an overview of the many ways in which a changing world can influence complex processes of species interactions in freshwater communities, addressing environmental shifts beyond the scope of rising temperatures. Chapters 1, 2 and 4 provide specific examples of indirect, yet potentially impactful repercussions of climactic shifts on the spread of disease. Meanwhile, Chapter 3 evaluates the importance of an anthropogenically-derived source of freshwater pollution. Overall, we provide overarching support for constrained epidemics and suboptimal performance of a fungal parasite of zooplankton under an increasingly anthropic world, challenging the view that environmental disturbances will unanimously favour parasites over their host. The potential for interactive effects among global environmental shifts is discussed, with some insights into future conservation methods of the economically-relevant freshwater ecosystems.

Table 1. Summary of previous publications investigating the effects of food quantity and food quality on infection traits in *Daphnia*-parasite systems.

Host	Parasite	Traits	Effect	Reference
D. magna	*Glugoides intestinalis* (microsporidium)	Parasite growth	LF (/)	Ebert, 1995
		Infection rate	LF (↓)	
D. galeata	*Caullerya mesnili* (ichthyosporean)	Parasite virulence	HF (↑)	Bittner et al., 2002
D. magna	*Pasteuria ramosa* (bacterium)	Parasite growth	Maternal LF (↓)	Mitchell & Read, 2005
D. magna	*Pasteuria ramosa* (bacterium)	Infection rate	LF (↓)	Frost et al., 2008
		Parasite growth	LF (↓)	
		Host fecundity	LF (↓)	
D. dentifera	*Metschnikowia bicuspidata* (yeast)	Spore production	HQ (↑)	Hall et al., 2009
		Transmission	HQ (↓)	
D. magna	*Pasteuria ramosa* (bacterium)	Host susceptibility	Maternal LF (↓)	Ben-Ami et al., 2010
D. magna	*Pasteuria ramosa* (bacterium)	Parasite establishment	Maternal LF (↓)	Stjernman & Little, 2011
		Parasite growth	Maternal LF (↓)	
D. magna	*Glugoides intestinalis* (microsporidium)	Spore production	LQ (/)	Aalto & Pulkkinen, 2013
D. magna	*Pasteuria ramosa* (bacterium)	Offspring production	Current HQ (↑)	Schlotz et al., 2013
			Maternal HQ (↑)	
		Infection rate	Maternal HQ (↑)	
			Current HQ (↓)	

D. magna	DIV-1 (virus)	Infection rate	LQ (↓)	Coopman et al., 2014
		Host survival	LQ (↑)	
		Offpsring production	LQ (↑)	
		Clutch size	LQ (↑)	
D. magna	Unicellular Gut Parasite (Micro1)	Spore production in coinfection with DIV-1	LQ (↓)	Lange et al., 2014
D. dentifera	*Metschnikowia bicuspidata* (yeast)	Encounter rate (feeding behaviour)	LQ (↓)	Penczykowski et al., 2014
D. dentifera	*Metschnikowia bicuspidata* (yeast)	Prevalence	HF (↑)	Civitello et al., 2015
		Parasite load	HF (↑)	
		Fecundity	HF (↑)	
		Host density	HF (↑)	
D. longispina	*Caullerya mesnili* (ichthyosporean)	Host susceptibility	LQ (↑)	Tellenbach et al., 2016
D. dentifera	*Metschnikowia bicuspidata* (yeast)	Infection rate	LQ (↓)	Sánchez et al., 2019
		Spore yield	LQ (/)	
		Host lifespan	LQ (/)	
		Host fecundity	LQ (/)	
	Pasteuria ramosa (bacterium)	Infection rate	LQ (/)	
		Spore yield	LQ (↑)	
		Host lifespan	LQ (↑)	
		Host fecundity	LQ (↓)	

Abbreviations: ***HQ: High quality*** ***LQ: Low quality*** ***HF: High food*** ***LF: Low food***

Arrows in the 'Effect' column indicate that a given experimental condition (for instance, 'High Food') either decreased (↓), increased (↑), or did not have any significant effect (/) on the value of a given infection 'Trait'. Maternal treatments (e.g. Mitchell & Read, 2005) indicate that different conditions of food quantity or diet quality were applied to the previous generation (i.e. mothers) of parthenogenetically reproducing daphnids.

Table 2. Summary of previous publications investigating the effects of temperature on infection traits in *Daphnia*-parasite systems.

Host	Parasite	Traits	Effect	Reference
D. magna	*Glugoides intestinalis* (microsporidium)	Transmission	LT (↓)	Ebert, 1995
D. magna	*Pasteuria ramosa* (bacterium)	Virulence	LT (↓)	Mitchell et al., 2005
D. magna	*Pasteuria ramosa* (bacterium)	Spore production	LT (↓) HT (↓)	Vale et al., 2008
		Infection rate	LT (↓)	
		Fecundity	LT (↓)	
D. longispina	*Caullerya mesnili* (microsporidium)	Infection rate	LT (↑)	Schoebel et al., 2011
D. longispina	*Metschnikowia bicuspidata* (yeast)	Time to infection	HT (↓)	Cuco et al., 2018
		Host fecundity	HT (↓)	
		Life expectancy	HT (↓)	
D. dentifera	*Metschnikowia bicuspidata* (yeast)	Size of epidemics (due to increased foraging rate)	HT (↑)	Shocket et al., 2018a
D. dentifera	*Metschnikowia bicuspidata* (yeast)	Transmission rate	HT (↑)	Shocket et al., 2018b
		Foraging rate	HT (↑)	
D. magna	*Ordospora colligata* (microsporidium)	Infection rate	LT (↓) HT (↓)	Kirk et al., 2018
		Infection intensity	HT (↓)	
		Life expectancy	HT (↓)	
D. dentifera	*Metschnikowia bicuspidata* (yeast)	Exposure to spores	HT (↑)	Shocket et al., 2019
		Host susceptibility	HT (/)	
		Spore production	HT (↓)	
		Infectivity of free-living stages	HT (↓)	

Abbreviations: ***HT: High Temperature*** ***LT: Low Temperature***

Table 3. Summary of previous publications investigating the effects of sequential infections (i.e. prior or later residency of the focal parasite) between distinct species of parasites in *Daphnia*-parasite systems.

Host	Parasite	Traits	Effect	Reference
D. galeata	*Metschnikowia bicuspidata* (yeast)	Time to infection	PR (↑)	Lohr et al., 2010b
		Spore production	PR (↓)	
	Caullerya mesnili (ichthyosporean)	Time to infection	LR (/)	
		Spore production	LR (↓)	
D. dentifera	*Metschnikowia bicuspidata* (yeast)	Spore production	LR (↑)	Clay et al., 2019b
	Pasteuria ramosa (bacterium)	Spore production	LR (/)	

Abbreviations: ***PR: Prior residency*** ***LR: Late residency***

CHAPTER 1:

TEMPERATURE AND HOST DIET JOINTLY INFLUENCE THE OUTCOME OF INFECTION IN A DAPHNIA-FUNGAL PARASITE SYSTEM

Temperature and host diet jointly influence the outcome of infection in a *Daphnia*-fungal parasite system

Florent Manzi[1,2*], Ramsy Agha[1], Yameng Lu[1,3], Frida Ben-Ami[4], Justyna Wolinska[1,2]

[1] *Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2] *Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany*

[3] *Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland*

[4] *School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel*

Corresponding author:* **florent.vmanzi@gmail.com

Author contributions: FM, JW, RA and YL. conceptualized the study, with input from FBA. **FM** performed the experiment, with help from JW, RA and YL. **FM** performed data analysis and results visualization. **FM** wrote the article, with input from JW, RA, YL and FBA. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Manuscript published in *Freshwater Biology* (22.12.2019):

https://doi.org/10.1111/fwb.13464

Received: 1 April 2019 | Revised: 5 November 2019 | Accepted: 22 November 2019

DOI: 10.1111/fwb.13464

ORIGINAL ARTICLE

Freshwater Biology WILEY

Temperature and host diet jointly influence the outcome of infection in a *Daphnia*-fungal parasite system

Florent Manzi[1,2] | Ramsy Agha[1] | Yameng Lu[1,3] | Frida Ben-Ami[4] | Justyna Wolinska[1,2]

[1]Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

[2]Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany

[3]Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

[4]School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

Correspondence
Florent Manzi, Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany.
Email: manzi@igb-berlin.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: WO 1587/6-1 and WO 1587/8-1

Abstract

1. Climate change has the potential to shape the future of infectious diseases, both directly and indirectly. In aquatic systems, for example, elevated temperatures can modulate the infectivity of waterborne parasites and affect the immune response of zooplanktonic hosts. Moreover, lake warming causes shifts in the communities of primary producers towards cyanobacterial dominance, thus lowering the quality of zooplankton diet. This may further affect host fitness, resulting in suboptimal resources available for parasite growth.
2. Previous experimental studies have demonstrated the respective effects of temperature and host diet on infection outcomes, using the zooplankter *Daphnia* and its microparasites as model systems. Although cyanobacteria blooms and heat waves are concurrent events in nature, few attempts have been made to combine both stressors in experimental settings.
3. Here, we raised the zooplankter *Daphnia* (two genotypes) under a full factorial design with varying levels of temperature (the standard 19°C and elevated 23°C), food quality (*Scenedesmus obliquus* as high-quality green algae, *Microcystis aeruginosa* and *Planktothrix agardhii* as low-quality cyanobacteria) and exposed them to the parasitic yeast *Metschnikowia bicuspidata*. We recorded life history parameters of the host as well as parasite traits related to transmission.
4. The combination of low-quality cyanobacterial diets and elevated temperature resulted in additive detrimental effects on host fecundity. Low-quality diets reduced parasite output, while temperature effects were context dependent. Overall, we argue that the combined effects of elevated water temperature and poor-quality diets may decrease epidemics of a common fungal parasite under a climate change scenario.

KEYWORDS
climate change, cyanobacteria, disease spread, *Metschnikowia*, zooplankton

1 | INTRODUCTION

Climate change may have important repercussions for the spread and severity of pathogenic diseases. Direct effects of temperature have been studied in a variety of host–parasite assemblages (reviewed in Altizer, Ostfeld, Johnson, Kutz, & Harvell, 2013; Lafferty & Mordecai, 2016), with general concern that a warmer world would also become a sicker world (Brooks & Hoberg, 2007; de La Rocque,

Rioux, & Slingenbergh, 2008). Elevated temperatures have been shown to modulate the intensity, timing, and transmission of infectious diseases, including bacterial pathogens of nematodes (Stirling, 1981), microsporidia infecting honeybees (Martín-Hernández et al., 2009) and human malaria (Paaijmans et al., 2010). Further signs of a rapidly changing world are shifts in food-web structures: climate change may alter the community composition of key trophic groups (Petchey, McPhearson, Casey, & Morin, 1999), induce trophic mismatch through changes in phenology (Edwards & Richardson, 2004), alter the primary productivity, and even reduce the total biomass in a food web (O'Connor, Piehler, Leech, Anton, & Bruno, 2009). However, in addition to temperature, diet quality and nutrient uptake are equally important drivers of the metabolic processes governing host immunity (Landolt, 1989; Las Heras et al., 2019) and parasite performance (Arostegui, Hovel, & Quinn, 2018; Crompton, 1987). For instance, protein supplementation of ruminants has been shown to increase resistance to gastrointestinal nematodes (Coop & Holmes, 1996), while experimental increases in nutrient concentrations enhanced the severity of different coral pathogens (Bruno, Petes, Drew Harvell, & Hettinger, 2003). Although hosts may benefit from high-quality diets in the form of enhanced immune response or increased fecundity, higher host densities may in turn provide parasites with a larger pool of potential hosts, each serving as a nutritive resource to their own parasite (Pike, Lythgoe, & King, 2019). Thus, in the context of epidemiological studies, temperature increase and food-web alterations are two aspects of climate change that should be regarded as related phenomena.

In freshwater environments, there is a tight interplay between climate change and food composition. By extending the duration of lake stratification periods, elevated water temperatures promote blooms of cyanobacteria (Paerl & Paul, 2012; Paul, 2008). Compared to green algae, cyanobacteria constitute a resource of poor nutritional quality to primary consumers (Ahlgren, Lundstedt, Brett, & Forsberg, 1990). Indeed, cyanobacteria do not contain sterols and lack many of the polyunsaturated fatty acids essential to zooplankton, which must be acquired through their diet (Brett & Muller-Navarra, 1997; Elert, Martin-Creuzburg, & Coz, 2003). Moreover, cyanobacteria often display filamentous morphologies, which can cause clogging of the zooplankton's filtering apparatus and hamper nutrition (Gliwicz, 1977; Lampert, 1987). Finally, a number of commonly occurring cyanobacteria species are known to produce potent toxins, such as microcystins, compromising the sanitary status of water bodies (Falconer, Burch, Steffensen, Choice, & Coverdale, 1994; Gholami, Mortazavi, & Karbassi, 2019; Lampert, 1987). As typically dominant zooplankton and key herbivores in freshwater food webs (Lampert & Kinne, 2011), waterfleas (*Daphnia*) are likely to be affected by these harmful blooms (Rohrlack, Dittmann, Henning, Börner, & Kohl, 1999).

Considering the central role of *Daphnia* in the trophic structure of aquatic food webs, any factor modulating the abundance and composition of zooplankton populations might lead to detrimental effects on the functioning of freshwater ecosystems. In addition to the negative effects associated with the dominance of cyanobacteria, a wide range of microparasites such as microsporidia, fungi and bacteria represent another threat to *Daphnia* hosts (Ebert, 2005). Most of these parasites negatively affect *Daphnia* survival and reproduction (Ebert, 2005; Green, 1974) and can reduce their abundance to such levels that control of phytoplankton by grazing is inhibited (Duffy, 2007). Predicting the overall direction of *Daphnia* parasitism under a climate change scenario is challenging, as warming may trigger cascading effects that modulate disease outcomes in complex and intricate ways. First, rising temperatures could directly alter zooplankton susceptibility to infection (Mitchell, Rogers, Little, & Read, 2005; Schoebel, Tellenbach, Spaak, & Wolinska, 2011), as well as the physiology of their parasites (Shocket et al., 2018; Vale, Stjernman, & Little, 2008). Second, the resulting proliferation and dominance of cyanobacteria might weaken host defences due to reduced nutrient uptake or cyanotoxin-induced stress. This has been suggested for *Daphnia* populations infected by the gut parasite *Caullerya mesnili*, as cyanobacterial density positively correlated with the occurrence of epidemics (Tellenbach et al., 2016). However, by producing antibiotic or antifungal effects, cyanobacteria may also interfere with pathogens (Abed, Dobretsov & Sudesh, 2009; Singh, Tiwari, Rai, & Mohapatra, 2011). Such medicinal properties have been suggested for the common cyanobacterium *Microcystis aeruginosa* against two parasites of *Daphnia*: the viral agent of white fat cell disease (Coopman, Muylaert, Lange, Reyserhove, & Decaestecker, 2014) and the yeast *Metschnikowia bicuspidata* (Sánchez, Huntley, Duffy, & Hunter, 2019). Overall, despite the substantial effort to relate the fitness of *Daphnia* parasites to single factors, such as food quality (Hall, Knight, et al., 2009a; Sánchez et al., 2019), nutrient availability (Frost, Ebert, & Smith, 2008; Narr, Ebert, Bastille-Rousseau, & Frost, 2019) and water temperature (Cuco, Castro, Gonçalves, Wolinska, & Abrantes, 2018; Vale et al., 2008), the combined effects of these stressors remain relatively unexplored in this system (but see Garbutt, Scholefield, Vale, & Little, 2014). As cyanobacteria blooms and heat waves are concurrent phenomena in nature (Joehnk et al., 2008), a comprehensive approach is required to make better epidemiological predictions in freshwater ecosystems.

To explore how elevated water temperature and decreased food quality interact at the host-parasite interface, we used two *Daphnia* genotypes in a fully factorial design including three food sources of varying quality (*Scenedesmus obliquus* as high-quality green algae/*M. aeruginosa* or *Planktothrix agardhii* as morphologically distinct, low-quality cyanobacteria), two levels of temperature (standard/elevated) and infection by the parasitic yeast *M. bicuspidata* (control/exposed). We recorded the proportion of successful infections following exposure (parasite infectivity) and the number of spores produced at host death (parasite reproduction). We combined those metrics into an estimate of parasite fitness (*net parasite output*, which conveys the expected number of transmission stages contributing to the next generation of parasites). Fitness parameters (average lifespan, fecundity, and body size) were measured to quantify the effects of environmental conditions and infection on *Daphnia* hosts. We predicted a generally enhancing effect of elevated temperature, but a detrimental effect of low food quality on net parasite output, which might result in a potential equilibrium when both stressors are combined.

2 | METHODS

2.1 | Study system

The zooplankter *Daphnia* (Crustacea: Cladocera) was used as the focal host. *Daphnia* reproduce through cyclical parthenogenesis, allowing for the inclusion of distinct clonal lines in the experimental design (Ebert, 2005). Two genotypes of *Daphnia longispina* × *galeata* hybrids (AMME_12 and AMME_51) were selected randomly from a wider collection of clonal lines isolated from Ammersee, Germany. Hybrids belonging to the *D. longispina* species complex are common and sometimes dominant inhabitants of permanent water bodies across the world (Griebel et al., 2015; Keller, Wolinska, Manca, & Spaak, 2008), being also able to colonise intermediate habitats that are not shared by their respective progenitor species (Ma, Hu, Smilauer, Yin, & Wolinska, 2018). *Daphnia* were maintained in synthetic culture medium (Saebelfeld, Minguez, Griebel, Gessner, & Wolinska, 2017) at 19°C, under a 12:12 light-dark photoperiod and fed three times per week with 1 mg C/L of green alga *S. obliquus*.

The yeast *M. bicuspidata* (Ascomycota: Saccharomycetales) is a generalist parasite infecting several *Daphnia* species (Dallas, Holtackers, & Drake, 2016; Ebert, 2005). Infections of *Daphnia* hosts by *Metschnikowia* are common in nature, typically starting in late summer/early autumn (Wolinska, Seda, Koerner, Smilauer, & Petrusek, 2011) and can reach prevalence up to 60% in some lakes (Cáceres et al., 2006; Penczykowski, Hall, Civitello, & Duffy, 2014). Infection takes place upon ingestion of spores by water-filtering hosts. Mature, needle-shaped spores pierce the gut wall before reaching the haemolymph (Codreanu & Codreanu-Balcescu, 1981). Infection symptoms become clearly visible after 9–10 days, when the host's body cavity starts to fill with the ascus stage (Stewart Merrill & Cáceres, 2018). Spore release occurs after host death, once the cuticle starts to decompose, allowing for parasite spores to be ingested by new hosts. A single *M. bicuspidata* strain was used, also isolated from Ammersee. This strain was later propagated on a laboratory-reared *Daphnia magna* clone (Hesse, Engelbrecht, Laforsch, & Wolinska, 2012). Due to its low host specificity, the parasite can be raised on *D. magna*—a larger host species which conveniently provides high spore output upon death—and later used to infect other *Daphnia* species (Cuco et al., 2018; Hesse et al., 2012).

Three phytoplankton species were used as different food sources for the host: the unicellular green alga *S. obliquus* (long-standing laboratory culture used as standard food for *Daphnia*), the coccoid cyanobacterium *M. aeruginosa* (MaGr01, isolated from Greifensee in Switzerland; Tellenbach et al., 2016) and the filamentous cyanobacterium *P. agardhii* (NIVA-CYA 630, isolated from Lake Lyseren in Norway; https://niva-cca.no). Both cyanobacteria species were selected as common bloom-forming taxa (Reynolds & Wakby, 1975; WHO 2009). Laboratory cultures of MaGr01 lost their colonial morphology, and single cells display an optimal size range for *Daphnia* ingestion. While both MaGr01 and NIVA-CYA 630 have been confirmed to produce microcystin (Rohrlack et al., 2008; Tellenbach et al., 2016), *Planktothrix* also displays a filamentous morphology, which reduces its susceptibility to grazing (Gliwicz, 1977; Lampert, 1987). *Scenedesmus* cultures were maintained in WC algal medium at 19°C, while *Microcystis* and *Planktothrix* cultures were maintained in Z8 medium at 19°C and 16°C, respectively. All cultures were maintained under constant light.

2.2 | Experimental setup

Prior to the start of the experiment, the two *D. longispina* × *galeata* genotypes were maintained for three generations under standard conditions (12:12 light–dark photoperiod, fed daily with 1 mg C/L of *S. obliquus)* and kept in separate incubators at 19°C (standard temperature) or 23°C (elevated temperature); 19°C is the standard rearing temperature of stock cultures in the laboratory and matches the typical August/September epilimnion temperature in Ammersee, when infection by *Metschnikowia* is usually first observed (J. Wolinska, personal observation). In contrast, 23°C was chosen based on climate change scenarios predicting a 4°C increase by the end of the century (Betts et al., 2011; New, Liverman, Schroder, & Anderson, 2011), with recent evidence suggesting that summer surface temperatures in lakes have already experienced an average 0.34°C increase per decade since the 1980s (O'Reilly et al., 2015). Individual *Daphnia* were used in a fully factorial design including two *Daphnia* genotypes (AMME_12/AMME_51), three food sources of varying quality (*Scenedesmus/Microcystis/Planktothrix)*, two temperatures (standard/elevated) and two infection treatments (control/exposed to *Metschnikowia*). Ten replicates were set up for unexposed *Daphnia* and 20 replicates for exposed ones, accounting for a total of 360 experimental units. To establish similar exposure conditions across temperature treatments, a unit of physiological time was employed, namely degree-days (calculated as the product of real-time in days and temperature in °C). This was used to account for relatively faster growth at 23°C, which leads to higher filtration rate due to larger body sizes, and thus higher spore uptake (Burns, 1969; Hall et al., 2007).

Experimental *Daphnia* were born within a 48-hr time span, after which mothers were removed from the common jars. At degree-day 95 (day 5 at 19°C/day 4 at 23°C), experimental subjects were transferred to individual jars containing 5 ml of fresh culture medium. At degree-day 115 (day 6 at 19°C/day 5 at 23°C), all jars were checked for early mortality and *Daphnia* were replaced if needed. Experimental jars were then inoculated with a suspension obtained by crushing the same amount of tissue from either infected or uninfected *D. magna* in the exposed and control solutions, respectively. To maximise infection success, this exposure protocol was repeated after 2 days, as in Yin, Laforsch, Lohr, and Wolinska (2011) (applied concentrations: 700 spores/ml and 550 spores/ml for the first and second exposure events, respectively). To determine spore concentrations, the homogenised suspension was loaded twice (2 × 10 µl) on an Improved Neubauer counting chamber. Total spore yield was

estimated from the mean number of mature spores counted in four squares of 1 µl capacity, across two independent loads.

During the first few days following the onset of the experiment, *Daphnia* were fed with 1 mg C/L of *S. obliquus*. Food quantity was reduced to 0.5 mg C/L once *Daphnia* were transferred into individual jars. To maximise infection success, animals were not fed during the first day of exposure (low food density was shown to promote spore uptake, Hall et al., 2007). *Daphnia* were separated into their respective food treatments the day following the first exposure event, i.e. at degree-day 135 (day 7 at 19°C/day 6 at 23°C). In the *Scenedesmus* food treatment, animals were fed daily with 0.5 mg C/L of *S. obliquus*. In the other two treatments, a food mixture was used in which either *Microcystis* or *Planktothrix* contributed 75% of the total amount of carbon, with *Scenedesmus* contributing the remaining 25%. The correlation between optical density and carbon content for each phytoplankton taxon was established and used to prepare food suspensions accordingly. Following the second exposure event, the experimental volume was raised to 15 ml (day 9 at 19°C/day 8 at 23°C). From this point onward, individuals were transferred to fresh medium every 4 days. Neonates were counted and removed daily, with those from the second clutch kept frozen for body size determination (–20°C). All exposed individuals which died after 8 days post-exposure (earliest observation of infection) were fixed in 3% formaldehyde. As no further deaths were observed after 27 days into the experiment, it was terminated soon after. All surviving individuals were fixed in 3% formaldehyde.

2.3 | Recorded parameters

2.3.1 | Parasite fitness

Parasite infectivity (calculated as the proportion of successfully infected individuals) was assessed by checking fixed animals for the presence of parasite spores under a dissecting microscope (30× magnification). Parasite reproduction (the number of spores produced until host death, calculated individually per infected host) was estimated from a suspension of crushed infected *Daphnia* using a counting chamber (see *Experimental setup*). Conveniently, parasite reproduction was shown to be a good estimate of transmission rates in *Daphnia* (Izhar & Ben-Ami, 2015). To combine these intermediate fitness components into a single metric that encompasses parasite success, we devised the *net parasite output*. For the parasite to contribute to the next generation, two conditions need to be met. First, the host has to survive long enough for the parasite to complete its infection cycle (defined here as host survival probability). Second, the surviving host has to become terminally infected (this probability was conveyed as parasite infectivity). Consequently, net parasite output is defined as the product of host survival probability, parasite infectivity and parasite reproduction. Host survival and parasite infectivity were computed for each combination of food quality, temperature and host genotype (12 treatments), out of 20 *Daphnia* which were exposed to the parasite in each treatment (Table S1).

2.3.2 | Host fitness

Age at death was recorded for each individual *Daphnia* that died starting from day 7 at 19°C and day 6 at 23°C (after the initial replacement of early deaths due to background mortality). Animals that were fixed in formaldehyde on the last experimental day were considered to have died at that time (none of these individuals were found to be infected). Body size was recorded for juveniles from the second clutch and for adult *Daphnia* which were retrieved on the last experimental day, including those that were *exposed but not infected* (see Figure S1) and those from the control treatment (due to age differences, body size was otherwise not recorded for animals that died from infection). *Daphnia* were measured under a dissecting microscope using Nikon NIS Elements Basic Research software (v4.50). Body size was recorded by drawing a straight line from the top of the eye to the base of the spine. The number of offspring and timing of each clutch were recorded for all individuals. Per capita intrinsic rates of increase (r) were computed for each combination of food quality, temperature, and host genotype (12 treatments), following Euler–Lotka's equation:

$$\sum_{x=0}^{n} e^{-rx} l_x m_x$$

with r as the rate of population increase (/day), x the age class in days, l_x the probability of surviving to age x, and m_x the fecundity at age x (Cuco et al., 2018; McCallum, 2000). Pseudovalues were generated by jackknifing and reassigned as individual values for each replicate in a given treatment (Meyer, Ingersoll, McDonald, & Boyce, 1986). Prior to inspection of infection status, spore yield, body size of adults and juveniles, all samples were assigned random numbers and relabelled to ensure blind assessment.

2.4 | Data analysis

Data were analysed using R version 3.6.0 (R Core Team, 2019). Graphical outputs were produced using the ggplot2 (Wickham, 2016) and Hmisc (Harrell & Harrell, 2019) packages. Analysis of variance (F-test or χ^2 test) was performed with the car package (Fox et al., 2012) using type III sums-of-squares. Whenever no significant interaction was recorded or missing values led to aliased coefficients in a model, type II sums-of-squares were used instead. Model selection was then performed by a stepwise regression approach based on Akaike information criterion.

2.4.1 | Parasite fitness

Host survival until day 8 post-exposure (0 = died early, 1 = survived) and parasite infectivity (0 = no infection, 1 = infection) were analysed by performing a binary logistic regression with *Food*, *Temperature*, and *Clone* as explanatory variables. Parasite reproduction and net parasite output were analysed using a linear model with *Food*, *Temperature*, and

Clone as explanatory variables. Normal distribution and homoscedasticity of the residuals were verified by visual inspection of quantile-quantile plots and residuals against fitted values, respectively.

2.4.2 | Host fitness

Age at death, fecundity (the total number of offspring) and growth rate (the per capita intrinsic rate of increase, *r*) were analysed using generalised linear models with *Food*, *Temperature*, *Infection*, and *Clone* as explanatory variables, assuming a negative binomial distribution (package *MASS*, function *glm.nb*) or γ-distribution of the residuals. Body size of adults and body size of juveniles (averaged per mother) were analysed using linear models with *Food*, *Temperature*, *Infection*, and *Clone* as explanatory variables. Preliminary analyses were run with all four factors (*Food*, *Temperature*, *Infection*, and *Clone*) as main effects only. If no significant effect of *Clone* was detected, this factor was deleted from the subsequent analysis and a three-way ANOVA was performed instead, including all interactions between the remaining factors. Since exposed *Daphnia* could only be confirmed as infected after surviving at least 8 days after exposure, early deaths were pooled together with terminally infected individuals in order to be compared with the control treatment (Figure S1).

3 | RESULTS

3.1 | Parasite fitness

Out of 240 *Daphnia* exposed to *Metschnikowia* spores, seven individuals were lost due to handling error and 78 individuals died before day 8 post-exposure (categorised as *early death*, see Figure S1). Among the 155 remaining individuals, 98 were confirmed as infected and 57 remained uninfected (categorised as *infected* and *exposed but not infected*, respectively). Host survival until day 8 post-exposure was lowest under a *Planktothrix* diet (significant *Food* effect, Table 1, Figure 1a), especially under elevated temperature (significant *Food* × *Temperature* interaction). Parasite infectivity was generally higher on clone AMME_51 (significant *Clone* effect, Figure 1b), whereas temperature increased infectivity for

TABLE 1 Three-way ANOVA (*F*-test or χ^2 test) testing for fixed effects of food quality, temperature, host genotype, and their interactions on life history parameters of the parasite. Model selection was performed by stepwise regression based on Akaike information criterion, and only the final model is reported here

Response variable	Distribution (link function)	Explanatory variables	Statistic (degrees of freedom)	*p*-value
Host survival until day 8 post-exposure	Binomial (link: logit)	Food	$\chi^2_{(2, 228)} = 41.140$	**<0.001**
		Temperature	$\chi^2_{(1,228)} = 8.363$	**0.004**
		Clone	$\chi^2_{(1, 228)} = 1.639$	0.201
		Food × Temperature	$\chi^2_{(2, 228)} = 9.293$	**0.01**
		Food × Clone	$\chi^2_{(2, 228)} = 8.972$	**0.011**
		Temperature × Clone	$\chi^2_{(1, 228)} = 2.570$	0.109
Parasite infectivity	Binomial (link: logit)	Food	$\chi^2_{(2, 147)} = 0.296$	0.863
		Temperature	$\chi^2_{(1, 147)} = 0.185$	0.668
		Clone	$\chi^2_{(1, 147)} = 12.130$	**<0.001**
		Food × Clone	$\chi^2_{(2, 147)} = 4.295$	0.117
		Temperature × Clone	$\chi^2_{(1, 147)} = 8.251$	**0.004**
Parasite reproduction	Normal	Food	$F_{(2, 88)} = 28.519$	**<0.001**
		Temperature	$F_{(1, 88)} = 2.337$	0.13
		Clone	$F_{(1, 88)} = 0.002$	0.963
		Food × Temperature	$F_{(2, 88)} = 3.188$	**0.046**
		Food × Clone	$F_{(2, 88)} = 5.141$	**0.008**
		Temperature × Clone	$F_{(1, 88)} = 6.194$	**0.015**
Net parasite output	Normal	Food	$F_{(2, 88)} = 3.268$	**0.043**
		Temperature	$F_{(1, 88)} = 2.044$	0.156
		Clone	$F_{(1, 88)} = 14.992$	**<0.001**
		Food × Temperature	$F_{(2, 88)} = 2.689$	0.074
		Food × Clone	$F_{(2, 88)} = 4.650$	**0.012**
		Temperature × Clone	$F_{(1, 88)} = 3.406$	0.068
		Food × Temperature ×Clone	$F_{(2, 88)} = 3.344$	**0.04**

Significant *p*-values (≤ 0.05) are highlighted in bold.

clone AMME_12 only (significant *Temperature* × *Clone* interaction). Parasite reproduction within infected hosts was highest in the *Scenedesmus* treatment (significant *Food* effect, Figure 1c). However, this effect of host diet was clone dependent. For instance, under a *Microcystis* diet, parasite reproduction was higher on clone AMME_51 (significant *Food* × *Clone* interaction). Net parasite output was generally higher when the host was maintained on the high-quality diet, *Scenedesmus* (significant *Food* effect, Figure 1d). However, when clone AMME_51 was exposed to elevated temperature under a *Scenedesmus* diet, net parasite output was greatly reduced, being surpassed by the low-quality *Microcystis* diet (significant *Food* × *Temperature* × Clone interaction). Moreover, parasite output on clone AMME_51 was higher than on clone AMME_12 under a *Microcystis* diet (significant *Food* × *Clone* interaction).

3.2 | Host fitness

Preliminary analyses revealed no significant effect of *Daphnia* genotype on any of the variables related to host fitness. Consequently, this factor was removed from the analyses. Host lifespan was greatly reduced by infection (significant *Infection* effect, Table 2, Figure 2a). Higher temperature caused earlier death except for *Daphnia* kept on a *Microcystis* diet (significant *Food* × *Temperature* interaction). Infected *Daphnia* from the *Planktothrix* × 23°C treatment died earliest. Host fecundity was reduced by infection, under elevated temperature, as well as under both cyanobacterial diets (significant main effects, Figure 2b). Non-exposed *Daphnia* produced up to five times more offspring under a *Scenedesmus* diet, compared to *Microcystis* or *Planktothrix* diets. The infection-induced reduction in fecundity was particularly strong under a *Scenedesmus* diet: infected *Daphnia* produced three to four times fewer offspring than unexposed conspecifics. While elevated temperature reduced host lifespan and fecundity, host growth rate was only influenced by food quality and infection (Figure 2c). Adult *Daphnia* grew largest under a *Scenedesmus* diet (significant *Food* effect, Table S2, Figure S2a). However, exposed hosts maintained on a *Scenedesmus* diet, which did not become infected (*exposed but not infected*) reached smaller adult sizes than their control conspecifics (significant *Food* × *Infection* interaction). As opposed to adult *Daphnia*, the body size of juveniles from the second clutch was highest under a *Microcystis* diet (significant *Food* effect, Figure S2b). Neither temperature nor infection influenced the size of offspring (Table S2).

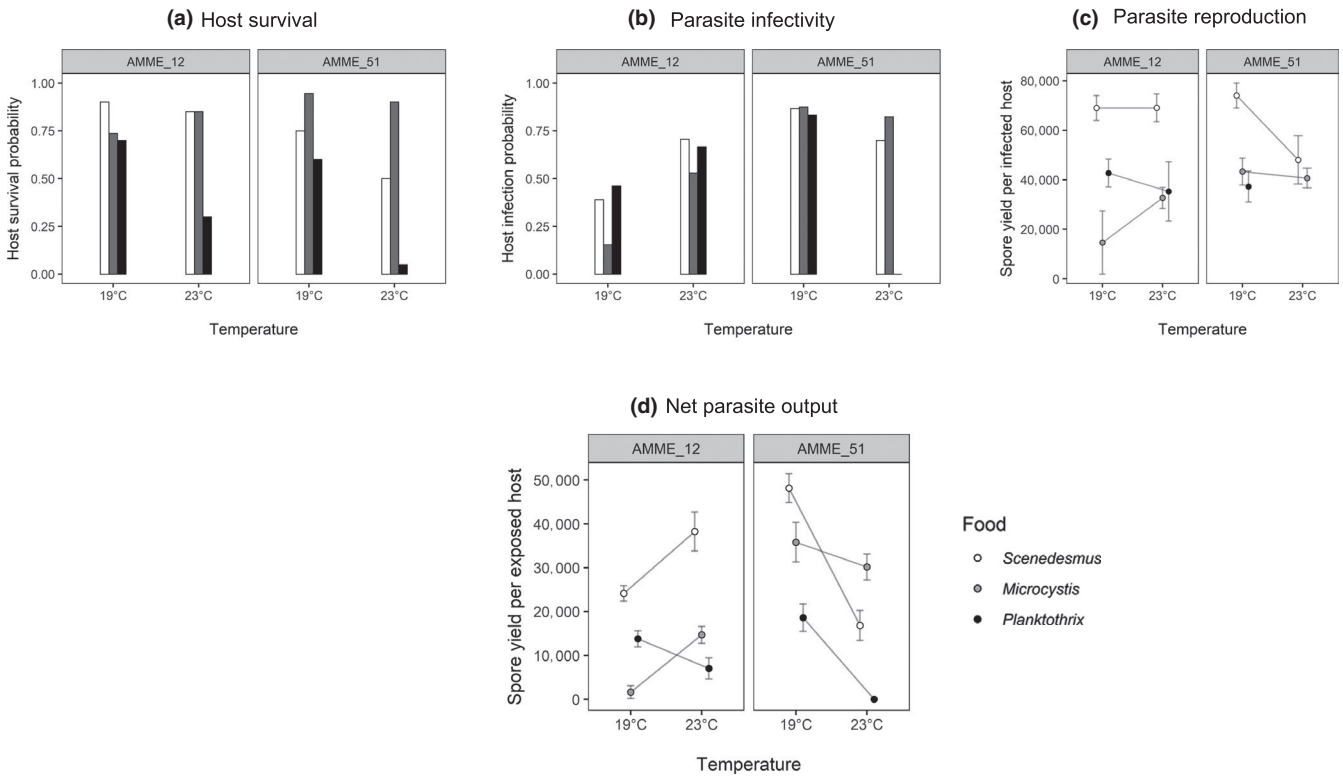


FIGURE 1 Comparison of traits relating to infection success of the yeast parasite, *Metschnikowia bicuspidata*. Two *Daphnia* genotypes (AMME_12, AMME_51) were exposed to the parasite under two temperatures (19°C, 23°C) and three food treatments (*Scenedesmus*, *Microcystis*, *Planktothrix*). (a) Host survival (proportion of hosts which survived until day 8 post-exposure); (b) parasite infectivity (proportion of successful infections); (c) parasite reproduction (number of spores produced); (d) net parasite output (product of the previous three variables). Error bars represent the standard error of the mean. Due to high mortality of AMME_51 in the *Planktothrix* × 23°C treatment, parasite reproduction could not be estimated for this combination: only one individual survived until parasite inspection, but was not infected (Table S1)

TABLE 2 Three-way ANOVA testing for fixed effects of food quality, temperature, infection, and their interactions on life history parameters of the host (two *Daphnia* genotypes). The *dataset* entries describe which subsets of data were compared as levels of the *Infection* factor (see also Figure S1). Model selection was performed by stepwise regression based on Akaike information criterion, and only the final model is reported here

Response variable (dataset)	Distribution (link function)	Explanatory variables	Statistic (degrees of freedom)	*p*-value
Host lifespan (control/infected and early death)	Negative binomial (link: log)	Food	$\chi^2_{(2, 288)} = 3.393$	0.183
		Temperature	$\chi^2_{(1, 288)} = 0.367$	0.544
		Infection	$\chi^2_{(1, 288)} = 94.612$	**<0.001**
		Food × Temperature	$\chi^2_{(2, 283)} = 25.148$	**<0.001**
Host fecundity (control/infected and early death)	Negative binomial (link: log)	Food	$\chi^2_{(2, 291)} = 86.358$	**<0.001**
		Temperature	$\chi^2_{(1, 291)} = 9.953$	**0.002**
		Infection	$\chi^2_{(1, 291)} = 56.941$	**<0.001**
Host growth rate (*r*) (control/infected and early death)	Gamma (link: log)	Food	$\chi^2_{(2, 281)} = 14.284$	**<0.001**
		Temperature	$\chi^2_{(1, 281)} = 1.370$	0.242
		Infection	$\chi^2_{(1, 281)} = 7.064$	**0.008**

Significant *p*-values (≤ 0.05) are highlighted in bold.

4 | DISCUSSION

By exposing *Daphnia* hosts to the common waterborne parasite *Metschnikowia*, our aim was to gain insight into how specific combinations of temperature and diets (representing future environmental disturbances in warmed lakes) may affect key traits of this host–parasite system. To enable ecologically relevant predictions regarding the potential for disease spread in future environments, we chose to focus on two synthetic variables: the *net parasite output* per exposed host, as well as the population growth rate of the host (*r*), which ensures the renewal of new hosts for the parasite to infect.

4.1 | Parasite fitness

Food quality appeared to be the main driver of net parasite output, contributing to each of the intermediate conditions for transmission (most notably host survival and parasite reproduction). Indeed,

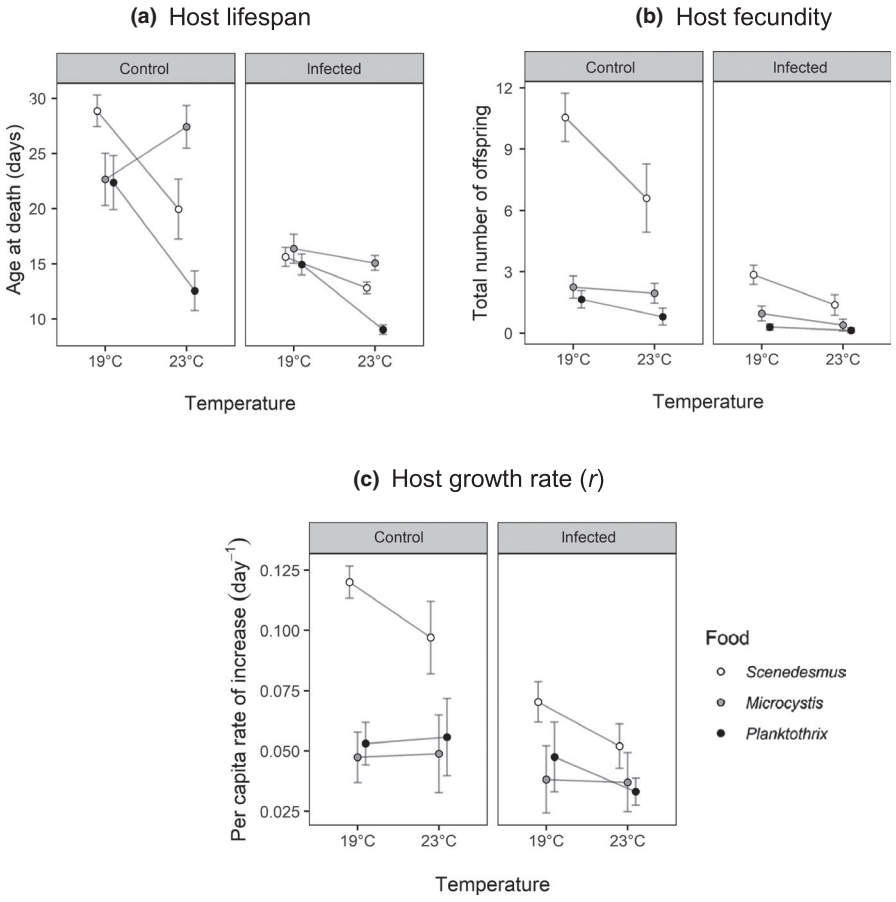


FIGURE 2 Comparison of *Daphnia* fitness components. *Daphnia* were exposed to the parasite *Metschnikowia bicuspidata* under two temperatures (19°C, 23°C) and three food treatments (*Scenedesmus*, *Microcystis*, *Planktothrix*). (a) Host lifespan (age of *Daphnia* at death); (b) fecundity (total number of offspring); (c) per capita intrinsic rate of increase (*r*). Results for both host genotypes are pooled together. Error bars represent the standard error of the mean. Since exposed *Daphnia* could only be confirmed as infected after surviving at least 8 days post-exposure, early deaths were pooled together with terminally infected individuals as one level of *Infection*, which was compared to the control treatment

Scenedesmus led to significantly higher parasite output in all but one treatment. By contrast, the *Planktothrix* diet was consistently deleterious for the parasite, reflecting a combination of impaired parasite reproduction and unreliable host survival (few hosts survived long enough to enable completion of the parasite cycle). Especially high levels of mortality were observed at 23°C, both in infected and uninfected hosts. This phenomenon might be attributed to increased filtering rates at high temperatures (Shocket et al., 2018), which aggravate clogging of the host's filtering apparatus by the filamentous cyanobacterium, thereby limiting proper nutrition. Interestingly, the *Microcystis* diet supported parasite growth in one of two tested clones; under elevated temperature, this diet even resulted in highest parasite output. *Microcystis* appeared to edge out the other food sources, most likely because it allowed the host to maintain high survivability under conditions of elevated temperature, as opposed to the other diets. This advantage of *Microcystis* over *Scenedesmus* was seemingly large enough to compensate for the moderate spore yield associated with a supposedly *low-quality* cyanobacterial diet.

The significance of food quality in our results is attributable to the low nutritional value of *Microcystis* and *Planktothrix*, compared to the green algae. Indeed, hosts feeding on a suboptimal diet are expected to provide fewer resources to exploiting endoparasites, resulting in slower development and less efficient multiplication within the host (Crompton, 1987; Hall, Simonis, Nisbet, Tessier, & Cáceres, 2009b). Similarly, spore production of *Metschnikowia* was hampered when its host was fed with field-collected, poor-quality algae as opposed to *Ankistrodesmus falcatus* (Hall, Knight, et al., 2009a), and was also found to be lower in lakes with high C:P ratios (Civitello et al., 2015). In addition to food quality, restricted quantities of a standard resource were also found to reduce growth of another *Daphnia* parasite, the bacterium *Pasteuria ramosa* (Frost et al., 2008; Mitchell & Read, 2005; Stjernman & Little, 2011). Arguably, rather than a consequence of low food quality per se (i.e. lack of sterols and long-chain poly-unsaturated fatty acids in cyanobacteria; Gerphagnon et al., 2018), our results could also be partially explained by the reported antifungal properties of *M. aeruginosa* (Sánchez et al., 2019). While the genus *Planktothrix* has not been tested for its antifungal properties against *Daphnia* parasites, it produces a wide array of bioactive secondary metabolites (Kurmayer, Deng, & Entfellner, 2016), that are likely to be involved in the defence against fungal chytrid parasites (Rohrlack, Christiansen, & Kurmayer, 2013; Sønstebø & Rohrlack, 2011).

While host diet turned out to be a preponderant driver of parasite fitness, the effects of temperature were less straightforward, manifesting mostly as complex interactions with host genotype or food quality, rather than as main effects. The absence of a general effect of temperature was surprising, as elevated temperatures are associated with an increase in metabolic rates (O'Connor & Bernhardt, 2018). Thus, high temperatures may increase the filtration rate of zooplankton, thereby facilitating the uptake of fungal spores (Shocket et al., 2018). Based on such findings, we expected both *Daphnia* genotypes to display increased susceptibility to the fungal parasite at 23°C. Instead, one host genotype became more easily infected when exposed to elevated temperature, while the other experienced compromised survival and reduced spore yield, leading to inferior parasite success. Such host genotype-specific responses to elevated temperature have also been discovered for other pathogens of *Daphnia* (Garbutt et al., 2014; Schoebel et al., 2011), as well as across many other host-parasite systems (reviewed in Wolinska & King, 2009). Such clonal effects further suggest that high genetic diversity in host populations might be crucial to resist disease at the population level (Agha, Gross, Rohrlack, & Wolinska, 2018; King & Lively, 2012; O'Brien & Evermann, 1988; Spielman, Brook, Briscoe, & Frankham, 2004).

In light of these results, we hypothesise a potential protective effect of cyanobacteria against infection outbreaks. Impaired parasite output under these suboptimal diets might reduce the risk of infection, slowing down the spread of the parasite in the environment. Although some specific scenarios relevant to climate change, such as *Microcystis* dominance under elevated temperature, would appear to favour parasite fitness, the net parasite output defined here only gives an estimation of how many transmission stages are expected to contribute to the next parasite generation. If we are to make predictions about the general epidemiology of the parasite, it is necessary to examine how this metric compares to the reproductive output of the host. Virulent effects such as increased host mortality or reduced fecundity (reinforced under harsh environmental conditions) also represent a risk for the parasite, in so far as they limit the pool of available hosts in the environment.

4.2 | Host fitness

In the absence of the parasite, cyanobacterial diets severely reduced host growth, due to a combination of impaired offspring production (both cyanobacterial species) and compromised survival (*Planktothrix*). The combination of high levels of host fecundity and efficient net parasite output under a *Scenedesmus* diet suggest that high-quality, green algal diets are more likely to promote epidemic outbreaks than the typical cyanobacteria occurring under bloom conditions. Furthermore, hosts exposed to a combination of elevated temperatures and high densities of toxic cyanobacteria, such as *Planktothrix*, might not live long enough to ensure transmission of the parasite. If such conditions became more prevalent as a result of climate change (Paerl & Paul, 2012; Paul, 2008), selection for faster replicating parasite strains might arguably occur in the wild. While the fungal parasite used in this experiment displays limited genetic diversity in natural populations (Duffy & Sivars-Becker, 2007; Searle et al., 2015; Wolinska, Giessler, & Koerner, 2009) and did not respond to a selection experiment (Auld, Hall, Housley Ochs, Sebastian, & Duffy, 2014), such evolutionary responses could still apply to other parasites of *Daphnia* with higher evolutionary potentials, such as the bacterium *P. ramosa* (Ebert et al., 2016).

5 | CONCLUSION

By investigating the main and interactive effects of temperature and host diet in the *Daphnia–Metschnikowia* system, we conclude that elevated temperature does not universally enhance parasite fitness. Instead, climate change is expected to promote the dominance of poor-quality algae and favour conditions of suboptimal nutrition in zooplanktonic hosts. This implies a reduction of exploitable resources for the parasite, resulting in decreased output, as an indirect effect of climate change. Distinct food sources appear to modulate host and parasite fitness in diverging ways, depending on host genotype and temperature. Such discrepancies suggest that toxic blooms might have different consequences depending on which cyanobacterial taxa become dominant when outbreaks occur. However, none of the tested cyanobacteria seem to enhance parasite epidemics, as they reduced host growth rates to negligible levels. Given the complex interactions that can arise between specific host diets and temperature conditions, the inclusion of both environmental factors in future experimental or modelling work on zooplankton pathologies seems pertinent and necessary.

ACKNOWLEDGEMENTS

This work was supported by a joint German–Israeli project (WO 1587/8-1 to J.W., 0604317501 to F.B.A.) and one other project (WO 1587/6-1 to J.W.), both funded by the German Science Foundation. We would like to thank Ursula Newen for the maintenance of *Daphnia* cultures, Francesco Pomatti for providing us with the *Microcystis* strain and Bruno B. Castro for his help with calculation methods of *r* values. We also thank Mark Phillipo for linguistic help.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Florent Manzi https://orcid.org/0000-0002-2826-7933

REFERENCES

Abed, R. M., Dobretsov, S., & Sudesh, K. (2009). Applications of cyanobacteria in biotechnology. *Journal of Applied Microbiology*, *106*(1), 1–12.

Agha, R., Gross, A., Rohrlack, T., & Wolinska, J. (2018). Adaptation of a chytrid parasite to its cyanobacterial host is hampered by host intraspecific diversity. *Frontiers in Microbiology*, *9*, 921. https://doi.org/10.3389/fmicb.2018.00921

Ahlgren, G., Lundstedt, L., Brett, M., & Forsberg, C. (1990). Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *Journal of Plankton Research*, *12*(4), 809–818. https://doi.org/10.1093/plankt/12.4.809

Altizer, S., Ostfeld, R. S., Johnson, P. T., Kutz, S., & Harvell, C. D. (2013). Climate change and infectious diseases: From evidence to a predictive framework. *Science*, *341*(6145), 514–519. https://doi.org/10.1126/science.1239401

Arostegui, M. C., Hovel, R. A., & Quinn, T. P. (2018). *Schistocephalus solidus* parasite prevalence and biomass intensity in threespine stickleback vary by habitat and diet in boreal lakes. *Environmental Biology of Fishes*, *101*(3), 501–514. https://doi.org/10.1007/s10641-018-0719-1

Auld, S. K., Hall, S. R., Housley Ochs, J., Sebastian, M., & Duffy, M. A. (2014). Predators and patterns of within-host growth can mediate both among-host competition and evolution of transmission potential of parasites. *The American Naturalist*, *184*(S1), S77–S90. https://doi.org/10.1086/676927

Betts, R. A., Collins, M., Hemming, D. L., Jones, C. D., Lowe, J. A., & Sanderson, M. G. (2011). When could global warming reach 4°C? *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *369*(1934), 67–84. https://doi.org/10.1098/rsta.2010.0292

Brett, M., & Muller-Navarra, D. (1997). The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology*, *38*(3), 483–499. https://doi.org/10.1046/j.1365-2427.1997.00220.x

Brooks, D. R., & Hoberg, E. P. (2007). How will global climate change affect parasite–host assemblages? *Trends in Parasitology*, *23*(12), 571–574. https://doi.org/10.1016/j.pt.2007.08.016

Bruno, J. F., Petes, L. E., Drew Harvell, C., & Hettinger, A. (2003). Nutrient enrichment can increase the severity of coral diseases. *Ecology Letters*, *6*(12), 1056–1061. https://doi.org/10.1046/j.1461-0248.2003.00544.x

Burns, C. W. (1969). Relation between filtering rate, temperature, and body size in four species of *Daphnia*. *Limnology and Oceanography*, *14*(5), 693–700.

Cáceres, C. E., Hall, S. R., Duffy, M. A., Tessier, A. J., Helmle, C., & MacIntyre, S. (2006). Physical structure of lakes constrains epidemics in *Daphnia* populations. *Ecology*, *87*(6), 1438–1444.

Civitello, D. J., Penczykowski, R. M., Smith, A. N., Shocket, M. S., Duffy, M. A., & Hall, S. R. (2015). Resources, key traits and the size of fungal epidemics in *Daphnia* populations. *Journal of Animal Ecology*, *84*(4), 1010–1017.

Codreanu, R., & Codreanu-Balcescu, D. (1981). On two *Metschnikowia* yeast species producing hemocoelic infections in *Daphnia magna* and *Artemia salina* (Crustacea, Phyllopoda) from Romania. *Journal of Invertebrate Pathology*, *37*(1), 22–27. https://doi.org/10.1016/0022-2011(81)90049-5

Coop, R. L., & Holmes, P. H. (1996). Nutrition and parasite interaction. *International Journal for Parasitology*, *26*(8–9), 951–962. https://doi.org/10.1016/S0020-7519(96)80070-1

Coopman, M., Muylaert, K., Lange, B., Reyserhove, L., & Decaestecker, E. (2014). Context dependency of infectious disease: The cyanobacterium *Microcystis aeruginosa* decreases white bacterial disease in *Daphnia magna*. *Freshwater Biology*, *59*(4), 714–723.

Crompton, D. W. T. (1987). Host diet as a determinant of parasite growth, reproduction and survival. *Mammal Review*, *17*(2–3), 117–126. https://doi.org/10.1111/j.1365-2907.1987.tb00057.x

Cuco, A. P., Castro, B. B., Gonçalves, F., Wolinska, J., & Abrantes, N. (2018). Temperature modulates the interaction between fungicide pollution and disease: Evidence from a *Daphnia*-microparasitic yeast model. *Parasitology*, *145*(7), 939–947.

Dallas, T., Holtackers, M., & Drake, J. M. (2016). Costs of resistance and infection by a generalist pathogen. *Ecology and Evolution*, *6*(6), 1737–1744. https://doi.org/10.1002/ece3.1889

de La Rocque, S., Rioux, J. A., & Slingenbergh, J. (2008). Climate change: Effects on animal disease systems and implications for surveillance and control. *Revue Scientifique Et Technique De L'office International Des Epizooties*, *27*(2), 339–354.

Duffy, M. A. (2007). Selective predation, parasitism, and trophic cascades in a bluegill–*Daphnia*–parasite system. *Oecologia*, *153*(2), 453–460. https://doi.org/10.1007/s00442-007-0742-y

Duffy, M. A., & Sivars-Becker, L. (2007). Rapid evolution and ecological host-parasite dynamics. *Ecology Letters*, *10*(1), 44–53. https://doi.org/10.1111/j.1461-0248.2006.00995.x

Ebert, D. (2005). *Ecology, Epidemiology, and Evolution of Parasitism in Daphnia*.National Library of Medicine.

Ebert, D., Duneau, D., Hall, M. D., Luijckx, P., Andras, J. P., Du Pasquier, L., & Ben-Ami, F. (2016). A population biology perspective on the stepwise infection process of the bacterial pathogen *Pasteuria ramosa* in *Daphnia*. *Advances in Parasitology*, *91*, 265–310.

Edwards, M., & Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*(7002), 881. https://doi.org/10.1038/nature02808

Elert, E. V., Martin-Creuzburg, D., & Le Coz, J. R. (2003). Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (*Daphnia galeata*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1520), 1209–1214.

Falconer, I. R., Burch, M. D., Steffensen, D. A., Choice, M., & Coverdale, O. R. (1994). Toxicity of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. *Environmental Toxicology and Water Quality*, *9*(2), 131–139. https://doi.org/10.1002/tox.2530090209

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., …Heiberger, R. (2012). Package 'car'. *Vienna: R Foundation for Statistical Computing*.

Frost, P. C., Ebert, D., & Smith, V. H. (2008). Responses of a bacterial pathogen to phosphorus limitation of its aquatic invertebrate host. *Ecology*, *89*(2), 313–318. https://doi.org/10.1890/07-0389.1

Garbutt, J. S., Scholefield, J. A., Vale, P. F., & Little, T. J. (2014). Elevated maternal temperature enhances offspring disease resistance in *Daphnia magna*. *Functional Ecology*, *28*(2), 424–431.

Gerphagnon, M., Agha, R., Martin-Creuzburg, D., Bec, A., Perriere, F., Rad-Menéndez, C., … Wolinska, J. (2018). Comparison of sterol and fatty acid profiles of chytrids and their hosts reveals trophic upgrading of nutritionally inadequate phytoplankton by fungal parasites. *Environmental Microbiology*, *21*(3), 949–958. https://doi.org/10.1111/1462-2920.14489

Gholami, Z., Mortazavi, M. S., & Karbassi, A. (2019). Environmental risk assessment of harmful algal blooms case study: Persian Gulf and Oman Sea located at Hormozgan Province, Iran. *Human and Ecological Risk Assessment: An International Journal*, *25*(1-2), 271–296. https://doi.org/10.1080/10807039.2018.1501660.

Gliwicz, Z. M. (1977). Food size selection and seasonal succession of filter feeding zooplankton in a eutrophic lake. *Ecologia Polska*, *25*, 179–225.

Green, J. (1974). Parasites and epibionts of Cladocera. *The Transactions of the Zoological Society of London*, *32*(6), 417–515. https://doi.org/10.1111/j.1096-3642.1974.tb00031.x

Griebel, J., Gießler, S., Poxleitner, M., Faria, A. N., Yin, M., & Wolinska, J. (2015). Extreme environments facilitate hybrid superiority–The story of a successful *Daphnia galeata* × *longispina* hybrid clone. *PLoS ONE*, *10*(10), e0140275. https://doi.org/10.1371/journal.pone.0140275

Hall, S. R., Knight, C. J., Becker, C. R., Duffy, M. A., Tessier, A. J., & Cáceres, C. E. (2009a). Quality matters: Resource quality for hosts and the timing of epidemics. *Ecology Letters*, *12*(2), 118–128. https://doi.org/10.1111/j.1461-0248.2008.01264.x

Hall, S. R., Simonis, J. L., Nisbet, R. M., Tessier, A. J., & Cáceres, C. E. (2009b). Resource ecology of virulence in a planktonic host-parasite system: An explanation using dynamic energy budgets. *The American Naturalist*, *174*(2), 149–162. https://doi.org/10.1086/600086

Hall, S. R., Sivars-Becker, L., Becker, C., Duffy, M. A., Tessier, A. J., & Cáceres, C. E. (2007). Eating yourself sick: Transmission of disease as a function of foraging ecology. *Ecology Letters*, *10*(3), 207–218. https://doi.org/10.1111/j.1461-0248.2007.01011.x

Harrell, F. E. Jr, & Harrell, M. F. E. Jr. (2019).Package 'Hmisc'. *CRAN2018*, 235-6.

Hesse, O., Engelbrecht, W., Laforsch, C., & Wolinska, J. (2012). Fighting parasites and predators: How to deal with multiple threats? *BMC Ecology*, *12*(1), 12. https://doi.org/10.1186/1472-6785-12-12

Izhar, R., & Ben-Ami, F. (2015). Host age modulates parasite infectivity, virulence and reproduction. *Journal of Animal Ecology*, *84*(4), 1018–1028. https://doi.org/10.1111/1365-2656.12352

Joehnk, K. D., Huisman, J. E. F., Sharples, J., Sommeijer, B. E. N., Visser, P. M., & Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*, *14*(3), 495–512. https://doi.org/10.1111/j.1365-2486.2007.01510.x

Keller, B., Wolinska, J., Manca, M., & Spaak, P. (2008). Spatial, environmental and anthropogenic effects on the taxon composition of hybridizing *Daphnia*. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1505), 2943–2952.

King, K. C., & Lively, C. M. (2012). Does genetic diversity limit disease spread in natural host populations? *Heredity*, *109*(4), 199. https://doi.org/10.1038/hdy.2012.33

Kurmayer, R., Deng, L., & Entfellner, E. (2016). Role of toxic and bioactive secondary metabolites in colonization and bloom formation by filamentous cyanobacteria *Planktothrix*. *Harmful Algae*, *54*, 69–86. https://doi.org/10.1016/j.hal.2016.01.004

Lafferty, K. D., & Mordecai, E. A. (2016). The rise and fall of infectious disease in a warmer world. *F1000Research*, *5*, 2040. https://doi.org/10.12688/f1000research.8766.1

Lampert, W. (1987). Laboratory studies on zooplankton-cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research*, *21*(3), 483–490. https://doi.org/10.1080/00288330.1987.9516244

Lampert, W., & Kinne, O. (2011). *Daphnia: Development of a model organism in ecology and evolution*, Vol. *21*. Oldendorf/Luhe: International Ecology Institute.

Landolt, M. L. (1989). The relationship between diet and the immune response of fish. *Aquaculture*, *79*(1–4), 193–206. https://doi.org/10.1016/0044-8486(89)90461-4

Las Heras, V., Clooney, A. G., Ryan, F. J., Cabrera-Rubio, R., Casey, P. G., Hueston, C. M., … Gahan, C. G. M. (2019). Short-term consumption of a high-fat diet increases host susceptibility to *Listeria monocytogenes* infection. *Microbiome*, *7*(1), 7. https://doi.org/10.1186/s40168-019-0621-x

Ma, X., Hu, W., Smilauer, P., Yin, M., & Wolinska, J. (2018). *Daphnia galeata* and *D. dentifera* are geographically and ecologically separated whereas their hybrids occur in intermediate habitats: Survey of 44 Chinese lakes. *Molecular Ecology*, *28*(4), 785–802.

Martin-Hernandez, R., Meana, A., Garcia-Palencia, P., Marin, P., Botias, C., Garrido-Bailon, E., … Higes, M. (2009). Effect of temperature on the biotic potential of honeybee microsporidia. *Applied and Environmental Microbiology*, *75*(8), 2554–2557. https://doi.org/10.1128/AEM.02908-08

McCallum, H. (2000). *Population parameters: Estimation for ecological models*. Oxford: Wiley-Blackwell.

Meyer, J. S., Ingersoll, C. G., McDonald, L. L., & Boyce, M. S. (1986). Estimating uncertainty in population growth rates: Jackknife vs. bootstrap techniques. *Ecology*, *67*(5), 1156–1166.

Mitchell, S. E., & Read, A. F. (2005). Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings of the Royal Society of London Series B: Biological Sciences*, *272*(1581), 2601–2607.

Mitchell, S. E., Rogers, E. S., Little, T. J., & Read, A. F. (2005). Host-parasite and genotype-by-environment interactions: Temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, *59*(1), 70–80.

Narr, C. F., Ebert, D., Bastille-Rousseau, G., & Frost, P. C. (2019). Nutrient availability affects the prevalence of a microsporidian parasite. *Journal of Animal Ecology*, *88*(4), 579–590. https://doi.org/10.1111/1365-2656.12945

New, M., Liverman, D., Schroder, H., & Anderson, K. (2011). Four degrees and beyond: the potential for a global temperature increase of four degrees and its implications. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *369*(1934), 6–19. https://doi.org/10.1098/rsta.2010.0303.

O'Connor, M. I., & Bernhardt, J. R. (2018). The metabolic theory of ecology and the cost of parasitism. *PLoS Biology*, *16*(4), e2005628. https://doi.org/10.1371/journal.pbio.2005628

O'Brien, S. J., & Evermann, J. F. (1988). Interactive influence of infectious disease and genetic diversity in natural populations. *Trends in Ecology & Evolution*, *3*(10), 254–259. https://doi.org/10.1016/0169-5347(88)90058-4

O'Connor, M. I., Piehler, M. F., Leech, D. M., Anton, A., & Bruno, J. F. (2009). Warming and resource availability shift food web structure and metabolism. *PLoS Biology*, *7*(8), e1000178.

O'Reilly, C. M., Sharma, S., Gray, D. K., Hampton, S. E., Read, J. S., Rowley, R. J., … Zhang, G. (2015). Rapid and highly variable warming of lake surface waters around the globe. *Geophysical Research Letters*, *42*(24), 10–773. https://doi.org/10.1002/2015GL066235

Paaijmans, K. P., Blanford, S., Bell, A. S., Blanford, J. I., Read, A. F., & Thomas, M. B. (2010). Influence of climate on malaria transmission depends on daily temperature variation. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(34), 15135–15139. https://doi.org/10.1073/pnas.1006422107

Paerl, H. W., & Paul, V. J. (2012). Climate change: Links to global expansion of harmful cyanobacteria. *Water Research*, *46*(5), 1349–1363. https://doi.org/10.1016/j.watres.2011.08.002

Paul, V. J. (2008). Global warming and cyanobacterial harmful algal blooms. In H. K. Hudnell, (Ed.) *Cyanobacterial harmful algal blooms: State of the science and research needs*. Advances in experimental medicine and biology (Vol. *619*, pp. 239-267. New York, NY: Springer.

Penczykowski, R. M., Hall, S. R., Civitello, D. J., & Duffy, M. A. (2014). Habitat structure and ecological drivers of disease. *Limnology and Oceanography*, *59*, 340–348. https://doi.org/10.4319/lo.2014.59.2.0340

Petchey, O. L., McPhearson, P. T., Casey, T. M., & Morin, P. J. (1999). Environmental warming alters food-web structure and ecosystem function. *Nature*, *402*(6757), 69. https://doi.org/10.1038/47023

Pike, V. L., Lythgoe, K. A., & King, K. C. (2019). On the diverse and opposing effects of nutrition on pathogen virulence. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *286*(1906), 20191220. https://doi.org/10.1098/rspb.2019.1220

R Core Team (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/.

Reynolds, C. S., & Wakby, A. E. (1975). Water blooms. *Biological Reviews*, *50*, 432–481. https://doi.org/10.1111/j.1469-185X.1975.tb01060.x

Rohrlack, T., Christiansen, G., & Kurmayer, R. (2013). A putative anti-parasite defensive system in the cyanobacterium *Planktothrix* involving ribosomal and non-ribosomal oligopeptides. *Applied and Environmental Microbiology*, *79*(8), 2642–2647.

Rohrlack, T., Dittmann, E., Henning, M., Börner, T., & Kohl, J. G. (1999). Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by the cyanobacterium *Microcystis aeruginosa*. *Applied and Environmental Microbiology*, *65*(2), 737–739.

Rohrlack, T., Edvardsen, B., Skulberg, R., Halstvedt, C. B., Utkilen, H. C., Ptacnik, R., & Skulberg, O. M. (2008). Oligopeptide chemotypes of the toxic freshwater cyanobacterium *Planktothrix* can form sub-populations with dissimilar ecological traits. *Limnology and Oceanography*, *53*(4), 1279–1293. https://doi.org/10.4319/lo.2008.53.4.1279

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

Sánchez, K. F., Huntley, N., Duffy, M. A., & Hunter, M. D. (2019). Toxins or medicines? Phytoplankton diets mediate host and parasite fitness in a freshwater system. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *286*(1894), 20182231. https://doi.org/10.1098/rspb.2018.2231

Schoebel, C. N., Tellenbach, C., Spaak, P., & Wolinska, J. (2011). Temperature effects on parasite prevalence in a natural hybrid complex. *Biology Letters*, *7*(1), 108–111. https://doi.org/10.1098/rsbl.2010.0616

Searle, C. L., Ochs, J. H., Caceres, C. E., Chiang, S. L., Gerardo, N. M., Hall, S. R., & Duffy, M. A. (2015). Plasticity, not genetic variation, drives infection success of a fungal parasite. *Parasitology*, *142*(6), 839–848. https://doi.org/10.1017/S0031182015000013

Shocket, M. S., Vergara, D., Sickbert, A. J., Walsman, J. M., Strauss, A. T., Hite, J. L., … Hall, S. R. (2018). Parasite rearing and infection temperatures jointly influence disease transmission and shape seasonality of epidemics. *Ecology*, *99*(9), 1975–1987. https://doi.org/10.1002/ecy.2430

Singh, R. K., Tiwari, S. P., Rai, A. K., & Mohapatra, T. M. (2011). Cyanobacteria: An emerging source for drug discovery. *Journal of Antibiotics*, *64*(6), 401–412. https://doi.org/10.1038/ja.2011.21

Sønstebø, J. H., & Rohrlack, T. (2011). Possible implications of chytrid parasitism for population subdivision in freshwater cyanobacteria of the genus *Planktothrix*. *Applied and Environmental Microbiology*, *77*(4), 1344–1351.

Spielman, D., Brook, B. W., Briscoe, D. A., & Frankham, R. (2004). Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genetics*, *5*(4), 439–448. https://doi.org/10.1023/B:COGE.0000041030.76598.cd

Stewart Merrill, T. E., & Cáceres, C. E. (2018). Within-host complexity of a plankton-parasite interaction. *Ecology*, *99*(12), 2864–2867.

Stirling, G. R. (1981). Effect of temperature on infection of *Meloidogyne javanica* by *Bacillus penetrans*. *Nematologica*, *27*(4), 458–462. https://doi.org/10.1163/187529281X00458

Stjernman, M., & Little, T. J. (2011). Genetic variation for maternal effects on parasite susceptibility. *Journal of Evolutionary Biology*, *24*(11), 2357–2363. https://doi.org/10.1111/j.1420-9101.2011.02363.x

Tellenbach, C., Tardent, N., Pomati, F., Keller, B., Hairston, N. G., Wolinska, J., & Spaak, P. (2016). Cyanobacteria facilitate parasite epidemics in *Daphnia*. *Ecology*, *97*(12), 3422–3432.

Vale, P. F., Stjernman, M., & Little, T. J. (2008). Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions. *Journal of Evolutionary Biology*, *21*(5), 1418–1427. https://doi.org/10.1111/j.1420-9101.2008.01555.x

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. New York, NY: Springer-Verlag.

Wolinska, J., Giessler, S., & Koerner, H. (2009). Molecular identification and hidden diversity of novel *Daphnia* parasites from European lakes. *Applied and Environmental Microbiology*, *75*(22), 7051–7059. https://doi.org/10.1128/AEM.01306-09

Wolinska, J., & King, K. C. (2009). Environment can alter selection in host–parasite interactions. *Trends in Parasitology*, *25*(5), 236–244. https://doi.org/10.1016/j.pt.2009.02.004

Wolinska, J., Seda, J., Koerner, H., Smilauer, P., & Petrusek, A. (2011). Spatial variation of *Daphnia* parasite load within individual water bodies. *Journal of Plankton Research*, *33*(8), 1284–1294. https://doi.org/10.1093/plankt/fbr016

Yin, M., Laforsch, C., Lohr, J. N., & Wolinska, J. (2011). Predator-induced defense makes *Daphnia* more vulnerable to parasites. *Evolution: International Journal of Organic. Evolution*, *65*(5), 1482–1488. https://doi.org/10.1111/j.1558-5646.2011.01240.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Manzi F, Agha R, Lu Y, Ben-Ami F, Wolinska J. Temperature and host diet jointly influence the outcome of infection in a *Daphnia*-fungal parasite system. *Freshwater Biology*. 2019;00:1–11. https://doi.org/10.1111/fwb.13464

Supporting Information for: Temperature and host diet jointly influence the outcome of infection in a *Daphnia*-fungal parasite system

Florent Manzi[1,2], Ramsy Agha[1], Yameng Lu[1,3], Frida Ben-Ami[4], Justyna Wolinska[1,2]

[1] *Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2] *Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany*

[3] *Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland*

[4] *School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel*

Supporting Information

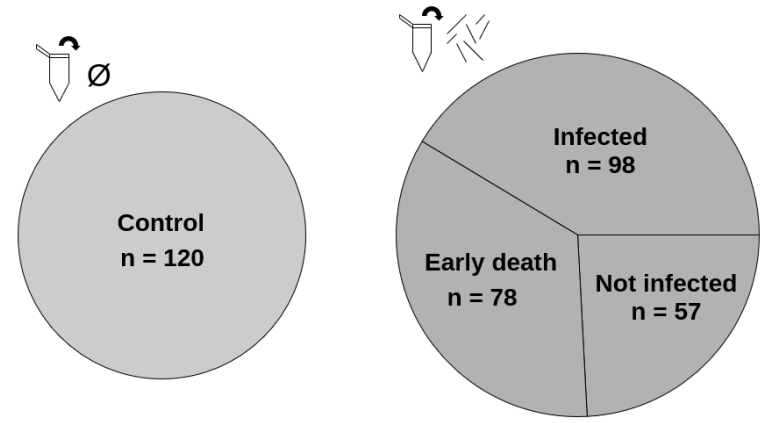


Figure S1. Diagram illustrating the different subsets of *Daphnia* used for statistical analyses: *control* = animals exposed to the control solution; *early death* = animals exposed to spores which died before infection could be assessed (before day 8 post-exposure); *infected* = animals exposed to spores whose infection symptoms were confirmed by microscopic examination after at least day 8 post-exposure; *exposed but not infected* = animals which were exposed to spores but did not show infection symptoms. *Daphnia* from the *early death* and *infected* categories were pooled together for analyses of host fitness parameters, before being compared with the *control* treatment.

Table S1. Number of *Daphnia* from each of the exposed treatments which i) survived until day 8 post-exposure (earliest time point where infection symptoms could be observed) and ii) became terminally infected, among those individuals which could be inspected under the microscope. Discrepancies between 'survived until day 8 post-exposure' and 'terminally infected' are attributed to missing individuals lost due to handling error.

Treatment (*Temperature* × *Food* × *Clone*)	**Survived until day 8 post-exposure**	**Terminally infected**
23°C × *Microcystis* × AMME_12	17 out of 20	9 out of 17
23°C × *Microcystis* × AMME_51	18 out of 20	14 out of 17
23°C × *Planktothrix* × AMME_12	6 out of 20	4 out of 6
23°C × *Planktothrix* × AMME_51	1 out of 20	0 out of 1
23°C × *Scenedesmus* × AMME_12	17 out of 20	12 out of 17
23°C × *Scenedesmus* × AMME_51	10 out of 20	7 out of 10
19°C × *Microcystis* × AMME_12	14 out of 19	2 out of 13
19°C × *Microcystis* × AMME_51	17 out of 18	14 out of 16
19°C × *Planktothrix* × AMME_12	14 out of 20	6 out of 13
19°C × *Planktothrix* × AMME_51	12 out of 20	10 out of 12
19°C × *Scenedesmus* × AMME_12	18 out of 20	7 out of 18
19°C × *Scenedesmus* × AMME_51	15 out of 20	13 out of 15

Supporting Information

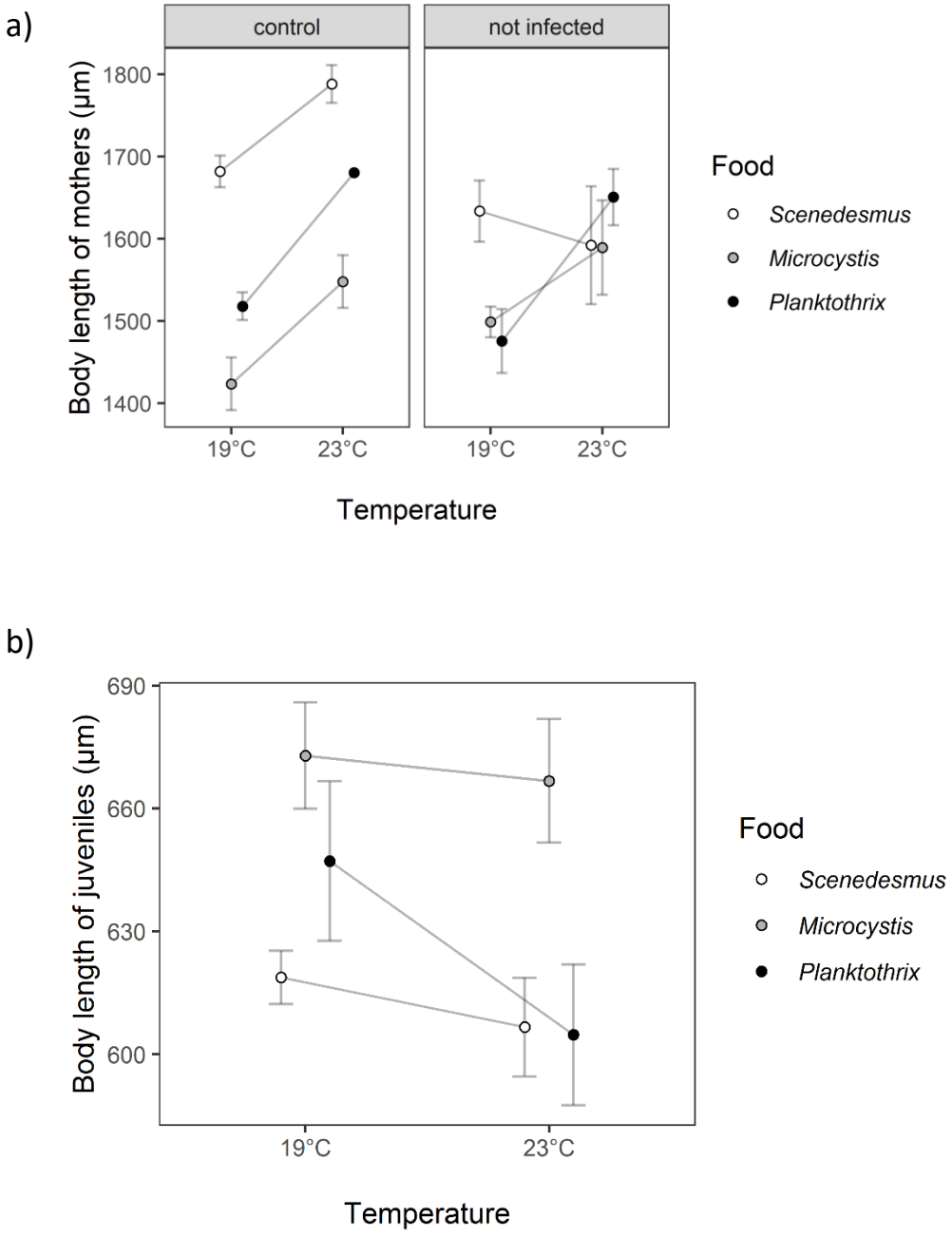


Figure S2. (a) Comparison of the body size of adult *Daphnia*. *Daphnia* were exposed to two temperatures (19°C, 23°C) and three food treatments (*Scenedesmus*, *Microcystis*, *Planktothrix*). Results for both host genotypes are pooled together. Error bars represent the standard error of the mean. (b) Comparison of the body size of *Daphnia* juveniles from the second clutch (averaged per mother). *Daphnia* were exposed to two different temperatures (19°C, 23°C) and three different food treatments (*Scenedesmus*, *Microcystis*, *Planktothrix*). Results for both host genotypes and infection status are pooled together. Error bars represent the standard error of the mean.

Supporting Information

Table S2. Three-way ANOVA (F-test or χ^2 test) testing for fixed effects of food quality, temperature, infection status and their interactions on the body size of adult *Daphnia* and the body size of juveniles from the second clutch (averaged per mother). The 'dataset' entries describe which subsets of data were compared as levels of the *Infection* factor. Model selection was performed by stepwise regression based on Akaike's Information Criterion (AIC), only the final model is reported here.

Response variable (dataset)	**Distribution (link function)**	**Explanatory variables**	**Statistic** (degree of freedom)	**p-value**
Body size (*control* / *exposed but not infected*)	**Normal**	Food	$F_{(2, 87)} = 6.776$	**< 0.001**
		Temperature	$F_{(1, 87)} = 3.063$	0.084
		Infection	$F_{(1, 87)} = 6.724$	**0.011**
		Food × Temperature	$F_{(2, 87)} = 2.537$	0.085
		Food × Infection	$F_{(2, 87)} = 7.028$	**0.001**
		Temperature × Infection	$F_{(1, 87)} = 2.826$	0.096
Body length of juveniles (*control* / *infected* / *exposed but not infected*)	**Normal**	Food	$F_{(2, 104)} = 9.621$	**< 0.001**
		Temperature	$F_{(1, 104)} = 1.790$	0.184
		Infection	$F_{(2, 104)} = 1.385$	0.255
		Food × Infection	$F_{(4, 104)} = 1.976$	0.104

CHAPTER 2:

PRIOR EXPOSURE OF A FUNGAL PARASITE TO CYANOBACTERIAL EXTRACTS DOES NOT IMPAIR INFECTION OF ITS DAPHNIA HOST

Prior exposure of a fungal parasite to cyanobacterial extracts does not impair infection of its *Daphnia* host

Florent Manzi[1,2 *], Ramsy Agha[1], Max Mühlenhaupt[1,2], Justyna Wolinska[1,2]

[1] *Department of Evolutionary and Integrative Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2] *Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany*

Corresponding author:* **florent.vmanzi@gmail.com

Author contributions: FM, MM, JW and RA conceptualized the study. **FM** and MM performed the experiment, data analysis and results visualization. **FM** wrote the manuscript, with help from RA, JW and MM. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Unpublished manuscript.

Abstract

Cyanobacteria periodically dominate phytoplankton composition in freshwater lakes, and produce a wide array of toxic secondary metabolites. Harmful blooms of cyanobacteria often coincide with late-summer infections of zooplankton by microparasites (such as *Metschnikowia bicuspidata*, a parasitic yeast of *Daphnia*), and prior research has shown that cyanobacteria-based diets could mitigate fungal infections of the host. However, direct detrimental effects of cyanotoxins against free-living stages of the parasite were not previously tested in this system. Here, we inoculated two genotypes of the host *Daphnia galeata* × *longispina* with fungal spores, which were previously exposed either to extracts of the common bloom-forming cyanobacterium *Microcystis aeruginosa* or to a placebo solution. Additionally, *Daphnia* hosts were fed on two levels of food quality, using either green algae (high quality) or the same cyanobacterium (low quality). Exposing parasite spores to cyanobacterial extracts did not reduce their infectivity, which was against our expectations. Instead, parasite infectivity was increased, but only on one host genotype. Then, neither parasite reproduction nor virulence were influenced by an exposure to cyanobacterial extract. The effect of host diet on parasite growth was also host-genotype dependent, with only one *Daphnia* genotype showing impaired spore production under a toxic diet. Our results suggest that dissolved cyanobacterial compounds released during blooms do not exert any detrimental effect on benthic spore banks, but may still influence transmission of the parasite, mostly when incorporated as part of the host diet.

1. Introduction

Cyanobacteria are one of the most ancient organisms on Earth (Garcia-Pichel, 1998; Sánchez-Baracaldo & Cardona, 2020). With an evolutionary origin dating back to at least 2000 Ma, their photosynthetic and nitrogen-fixing abilities have contributed to greatly affecting the Earth's atmospheric composition and carbon cycle at a global scale (Knoll, 2008). Acting as main primary producers throughout most of the Proterozoic, cyanobacteria have since remained important – and sometimes dominant – components of phytoplankton communities within both marine and freshwater ecosystems (Sergeev et al., 2002). The seasonal ability of cyanobacteria to proliferate and dominate phytoplankton species assemblages during short periods of time is commonly referred to as 'blooms', which have been recorded increasingly throughout the 20th century (Hallegraeff, 1993; Gobler, 2020) and elevated to a greater level of concern throughout the past three decades (Van Dolah et al., 2001; Moore et al., 2008; Grattan et al., 2016; Sukenik & Kaplan, 2021). In part thanks to their ancient evolutionary history, cyanobacteria exhibit a number of competitive traits, allowing them to outcompete other phytoplankton (such as green algae) when conditions are favourable. This includes their buoyancy regulation – allowing them to occupy superficial niches in the water column, thus optimizing light harvesting while shading competitors (Carey et al., 2012) – and faster growth under conditions of elevated temperatures, high concentrations of inorganic carbon (CO2) or replete nutrient availability (Mantzouki et al., 2016, Ji et al., 2017). Incidentally, such environmental conditions have been associated with contemporary ecological shifts of anthropogenic origin: human activity is causing harmful blooms to become more frequent, due to a combination of greenhouse gas effects and the eutrophication of aquatic ecosystems (Mantzouki et al., 2016), although increasing evidence show that cyanobacteria can also thrive in relatively low-nutrients environments (Reinl et al., 2021).

In addition to the inherent phenology and population dynamics of cyanobacteria, their interactions with primary consumers (i.e. zooplankton) are also expected to change in a warming, increasingly eutrophic world (Benndorf & Henning, 1989; Ger et al., 2014). Cyanobacteria act as a relatively poor food source for common zooplankton taxa, such as copepods (DeMott & Moxter, 1991) or various species of cladocerans (Hanazato & Yasuno, 1987; Lundstedt & Brett, 1991; Smith & Gilbert, 1995). This is commonly imputed to low nutritional value, due to a lack of poly-unsaturated fatty acids (PUFAs) and/or sterols (DeMott & Müller-Navarra 1997; Ravet et al., 2003), in addition to their toxicity (DeMott et al., 1991). A major concern regarding the increasing occurrence of harmful algal blooms, cyanobacteria are renowned for their exceptional bioactivity and toxin production (Namikoshi & Rinehart, 1996; Huang & Zimba, 2019). Over time, cyanobacteria have evolved a variety of secondary metabolites and bioactive compounds (Welker & Von Döhren, 2007; Agha & Quesada, 2014), some of which can cause adverse effects to other organisms (including humans) and have thus been referred to as cyanotoxins (Carmichael, 1992; Chorus, 2012). These are proposed to have evolved as bio-chemical defences against invertebrate grazers (Ghadouani et al., 2004; Rohrlack et al., 2004;

Czarnecki et al., 2006), following an evolutionary pathway akin to that of terrestrial plants (Kirk & Gilbert, 1992).

In addition to their anti-grazing properties, a wider allelopathic activity was also documented in several compounds produced by cyanobacteria, reportedly efficient against algae and other bacteria (Berry et al., 2008). Associating such properties with the ancient evolutionary origin of cyanobacteria (Knoll, 2008), Sánchez et al. (2019) hypothesized that cyanotoxins could have evolved under the selective pressure of more ancestral antagonists, predating the apparition of their zooplankton (i.e. metazoan) grazers. Seeing as some cyanobacteria are infected by highly specialized parasites, such as fungi of the order Chytridiomycota (Canter, 1950) and viruses (i.e. cyanophages; Suttle, 2000), it is not surprising that a number of cyanobacterial secondary metabolites also display strong antifungal and antiviral properties (Volk & Furkert, 2006; Shishido et al., 2015; Marrez & Sultan, 2016). While the maintenance of cyanotoxins and other bioactive compounds may be driven by the selective pressure of such parasites (Rohrlack et al., 2013), several other hypotheses have been put forward regarding the primary biological functions of these metabolites, including: nutrient (Fe) scavenging (Utkilen & Giølme, 1995), protection against oxidative stress (Zilliges et al., 2011), allelopathy (von Elert & Jüttner, 1997; Schagerl et al., 2002; Sukenik et al., 2002, Leão et al., 2010) and quorum sensing (Kehr et al., 2006; Schatz et al., 2007). Given a somewhat lax binding specificity and their ability to act as inhibitors for a wide spectrum of enzymes (Teta et al., 2015), cyanotoxins could have later gained an adaptive value when competitors (prokaryotes, algae) and grazers (metazoan) emerged, thus promoting their conservation and diversification in some extant taxa.

Among the many grazers involved in the consumption of cyanobacteria in freshwater ecosystems, cladocerans of the genus *Daphnia* have been suggested as a potential source of control for their proliferation in eutrophic ponds and lakes (Chislock et al., 2013; Urrutia-Cordero et al., 2016). As non-selective filter-feeders (DeMott, 1990), *Daphnia* are likely to feed on a mixed-diet seston, whose composition is expected to be dominated by cyanobacterial cells during blooms (Ferrão-Filho et al., 2000). Of particular interest, in this regard, is the temporal co-occurrence of cyanobacterial blooms, in temperate lakes of the Northern hemisphere, with seasonal epidemics of the yeast *Metschnikowia bicuspidata*, a generalist parasite of *Daphnia* causing typical outbreaks in the late summer to early autumn (Duffy et al., 2009; Hall et al., 2011; Wolinska et al., 2011). Thus far, information on how cyanobacteria-based diets could modulate fungal infection in *Daphnia* have provided fairly consistent observations, often resulting in impaired parasite transmission (Penczykowski et al., 2014; Sánchez et al., 2019; Manzi et al., 2020). However, it is not always clear whether such observations stem from cyanobacteria's poor nutritional value – leading to suboptimal parasite growth – or rather involve direct antagonistic effects, attributed to their putative antifungal properties.

Considering the effects of cyanobacterial diets reported thus far in the *Daphnia-Metschnikowia* system, three prospective mechanisms may be considered: i) cyanobacteria reduce the success of infection through indirect consequences of their low nutritional value on host growth (i.e. reduced body size and filtering rate), which later reflect on parasite growth and exposure; ii) cyanobacteria exert 'medicinal' effects when incorporated as part of the host's diet; for instance, digested cell content could affect functions of host immunity; or iii) by way of their suspected antifungal properties, secondary metabolites of cyanobacteria are capable of direct interference with the parasite. In the latter case, antagonistic processes which usually occur at the within-host level may also be replicated outside of the host's internal environment. This hypothesis was not previously tested in experimental infections of *Daphnia*. Yet, a number of environmental contaminants (e.g. heavy metals) and persistent organic pollutants have been shown to influence the longevity and infectivity of environmental stages of parasites, particularly in aquatic ecosystems (reviewed in Pietrock & Marcogliese, 2003; Morley et al., 2006). Moreover, other external factors were already shown to influence the infectivity and environmental survival of *Metschnikowia*, independently of later encounters with the host, such as solar radiation (Overholt et al., 2012) or extreme temperatures (Shocket et al., 2019; Duffy & Hunsberger, 2019).

To explore the possibility of direct antagonistic interactions between cyanobacteria and a fungal parasite of zooplankton, we inoculated two genotypes of the common lake hybrid *Daphnia galeata* × *longispina* with spore suspensions of the parasitic yeast *Metschnikowia bicuspidata*. Prior to their inoculation, fungal spores were either pre-exposed to i) cyanobacterial extracts or ii) to a placebo solution, consisting of cyanobacterial culture medium. *Daphnia* from each treatment were later maintained under two separate diets, one of which consisted in a high quality, green algae-based diet and the other in a mixture of green algae and toxic cyanobacteria. We measured three variables influencing the overall transmission success of the parasite. Based on the putative antifungal properties of the cyanobacterium, we hypothesized that i) prior exposure of the parasite to cyanobacterial toxins would impair the success of later infections and ii) the combined treatments of toxin-exposed parasite and toxic host diet would further reduce infection success.

2. Methods

2.1 Study system

Two genotypes of *Daphnia galeata* × *longispina* hybrids (AMME_12 and AMME_51) were selected from a wider collection of clones isolated from Lake Ammersee, Germany; the same two host lines were previously used in a life-table experiment involving mixed cyanobacterial diets, which revealed distinct clonal responses to such treatments (Manzi et al., 2020). Within the *D. longispina* species complex, hybrid genotypes commonly occur, and sometimes even dominate community composition

in permanent lakes (Keller et al., 2008). Hybrids may also occupy intermediate habitats beyond the ecological niche of their respective progenitor species (Griebel et al., 2015; Ma et al., 2018). *Daphnia* were maintained in synthetic SSS culture medium (Saebelfeld et al., 2017) at 19°C, under a 12:12 light-dark photoperiod and fed three times per week with 1 mg C/L of green algae *Scenedesmus obliquus.*

The yeast *Metschnikowia bicuspidata* (hereafter referred to as *Metschnikowia*) is a generalist parasite infecting several *Daphnia* species (Ebert, 2005; Dallas et al., 2016). Infections of *Daphnia* hosts by *Metschnikowia* are common in nature. Epidemics typically start in late summer/early autumn (Wolinska et al., 2011) and the parasite can reach high prevalence (i.e. up to 60%) in lake *Daphnia* populations (Cáceres et al., 2006). Infection takes place upon ingestion of spores by water-filtering hosts. Mature, needle-shaped spores pierce the gut wall before reaching the haemolymph (Metschnikoff, 1884). Infection symptoms become clearly visible after 9 to 10 days; at this point, the parasite's final developmental stage (elongated asci) can be seen throughout the entire body cavity (Stewart Merrill & Cáceres, 2018). As an obligate killer, damage to the cuticle or decomposition of the host's corpse is necessary for the parasite to be released into the environment; infective stages can then encounter new hosts, or build up as spore banks in the sediment. During this 'environmental' phase of the parasite's life-cycle, spores could potentially be exposed to dissolved cyanobacterial toxins. A single *M. bicuspidata* strain was used (METS_AMME_2008), isolated from the same lake as the host. This strain was later propagated on lab-reared *Daphnia magna* (clone E17:07) for long-term maintenance (Hesse et al., 2012). Due to its low host specificity, the parasite can be raised on *D. magna* – a larger host species, providing high spore outputs – and later used to infect other *Daphnia* species (Hesse et al., 2012; Manzi et al., 2020).

Two phytoplankton species were used as different food sources for the host: the unicellular green alga *Scenedesmus obliquus* (long standing laboratory culture, used as standard food for *Daphnia*) and the coccoid cyanobacterium *Microcystis aeruginosa* (MaGr01, isolated from Greifensee, Switzerland; Tellenbach et al., 2016), one of the most common bloom-forming taxa in freshwater lakes (Reynolds & Walsby, 1975). Laboratory cultures of MaGr01 lost their colonial morphology, thus displaying an optimal shape and size range for *Daphnia* ingestion. This strain was confirmed to produce microcystin, with a reported concentration of 696 fg per cell (Tellenbach et al., 2016): a family of heptapeptides, microcystins are the main group of cyanobacterial toxins associated with the toxicity to vertebrates and invertebrates (Carmichael, 1992). *Scenedesmus* cultures were maintained in modified Z-medium (Zehnder & Gorham, 1960), under constant light exposure at 19°C. *Microcystis* were maintained as long-standing batch cultures in Z8 medium (Kótai, 1972), exposed to constant light at 16°C. Prior to the experiment, a subset of *Microcystis* cultures were brought up to the same conditions as *Scenedesmus* (19°C) to accelerate growth and equate the rearing temperature of *Daphnia*.

2.2 Experimental setup

Prior to the start of the experiment, the two *D. galeata* × *longispina* genotypes were maintained for two generations under standard conditions (19°C, 12:12 light-dark photoperiod, fed daily with 1 mg C/L of *S. obliquus)*. 19°C is the standard rearing temperature of stock cultures in the laboratory and matches the typical August / September epilimnion temperature in Ammersee, when infection by *Metschnikowia* is usually first observed (Wolinska, personal observation). Individual *Daphnia* from two clonal lines (AMME_12 / AMME_51) were used in a full factorial design, including two food sources of varying quality (*Scenedesmus / Microcystis*), and two infection treatments ('METS': spores exposed to a placebo solution / 'METS + Extract': spores exposed to *Microcystis* extracts). Either 20 replicates (AMME_12) or 16 replicates (AMME_51) were set up for each combination of food and infection treatments, accounting for a total of 144 experimental units. All 144 jars containing individual *Daphnia* were inoculated with the parasite.

Experimental *Daphnia* were born within a 48-hour time span, after which mothers were removed from the common jars (day 1). On day 4, spore suspensions of the fungal parasite *Metschnikowia* were prepared; to determine spore concentrations, the suspension obtained from crushed infected *D. magna* was homogenized and loaded twice (2 × 10 µL) on an Improved Neubauer counting chamber. Total spore yield was estimated from the number of mature spores counted in four squares of 1×10^{-4} mL capacity, across two independent loads. Two Eppendorf tubes were prepared (each containing 500 000 spores in 1 mL medium), which were either completed with a *Microcystis* extract (1 mL) or with a placebo solution (Z8 medium, 1 mL). *Microcystis aeruginosa* (strain MaGr01) were taken from an exponentially-growing stock culture (OD at 750 nm = 0.181), which was then diluted (using Z8 medium) to reach an optical density of 0.09 in 5 mL. This whole volume was briefly submerged into a solution of liquid nitrogen, to induce cell lysis (Zheng et al., 2011). The solution was then incubated at 4°C for a period of 48 hours, to maximize extraction. Following this incubation period, the extracted cell content was left to thaw at room temperature for two hours and later filtrated using a GF/F glass fiber filter, to exclude any cell debris. From the resulting filtrate, 1 mL was added to the spore suspension used in the 'METS + Extract*'* treatment, while the *'*METS*'* treatment received 1 mL of sterile Z8 medium (placebo solution).

Based on the level of microcystin production for strain MaGr01 assessed by Tellenbach et al. (2016) and the average absorbance / biomass ratio of *Microcystis aeruginosa* (Kaebernick et al., 2000), we aimed for an incubation concentration approximating 1300 µg/L of microcystin. Assuming a 50% to 80% extraction success granted by the liquid nitrogen method (Zheng et al., 2011), however, we estimated a final incubation concentration in the range of 650-1050 µg/L in the 'METS + Extract' treatment. Such a concentration exceeds usual levels of microcystin observed during blooms in open water (≤ 240 µg/L, Francy et al., 2015), though higher concentrations are occasionally reported upon

termination of large blooms in enclosed sections of freshwater bodies (≤ 1800 µg/L, Jones & Orr, 1994). The two spore solutions were kept at 4°C for 24h, allowing for infective stages of the parasite to be directly exposed to cyanobacterial cell content, prior to their encounter with the host. On day 5, all experimental *Daphnia* were transferred to individual jars containing 5 mL of fresh culture medium. Experimental jars were then inoculated with a dose of 1000 spores/mL (i.e. 5000 spores per individual *Daphnia*), from either of these two suspensions. As the present protocol only allowed for a single inoculation event, this concentration was chosen to roughly equate the amount of spores previously introduced in Manzi et al., 2020 (i.e. 1250 spores/mL split across two separate events of parasite exposure). No food was provided on the day of inoculation, as low food supplies were shown to promote spore uptake (Hall et al., 2007).

Throughout the first six days of the experiment, all *Daphnia* were fed daily with 0.5 mg C/L of *S. obliquus* (with the exception of inoculation day, see above). On day 7, *Daphnia* were transferred to 15 mL of fresh medium and split into their respective diets (*Scenedesmus* or *Microcystis*). At this point in time (i.e. 48 hours after inoculation), the parasite should have been able to reach and settle into the haemolymph (Stewart Merrill & Caceres, 2018), and the further ingestion of infective stages would not be possible, due to the change in medium. In the *Microcystis* treatment, a food mixture was used in which *Microcystis* contributed 75% of the total amount of carbon, with *Scenedesmus* making up the remaining 25% (similarly as in Manzi et al., 2020). The correlation between optical density and carbon content for each phytoplankton taxon was established and used to prepare food suspensions accordingly. According to Ferrão-Filho et al. (2000), mixed diets are a suitable approach to estimate the toxicity of cyanobacteria, since *Daphnia* are likely to feed on seston of mixed origin, even in lakes temporally dominated by cyanobacteria.

Daphnia were transferred to fresh medium every four days. Neonates were counted and removed daily. Starting from day 12 (i.e. 7 days after parasite inoculation), all animals that died were fixed in 3.7% formaldehyde, to score them later for the presence of parasite spores. The earliest record of infection symptom in this system is day 8 (Manzi et al., 2020). As no further deaths were observed following 16 days after parasite inoculation, the experiment was terminated on day 21; all surviving individuals were then fixed for later inspection.

2.3 Recorded parameters

Parasite infectivity (calculated as the proportion of successfully infected *Daphnia*) was assessed by checking fixed animals for the presence of infection symptoms. The presence of mature spores was confirmed in suspensions obtained from crushed individuals (Nikon SMZ25 stereomicroscope, 200× magnification). Parasite reproduction (the total number of spores accumulated upon host death, calculated individually per infected host) was estimated from a suspension of crushed infected *Daphnia*

using a counting chamber (see *Experimental setup*). Age at death was recorded for each individual *Daphnia* that died starting from day 6 (one day after the introduction of the parasite); animals that were fixed in formaldehyde on the last experimental day were considered to have died at the age of 21 days (no natural death occurred on that day).

2.4 Data analysis

Data were analyzed using R version 4.1.0 (R Core Team, 2021). Graphical outputs were produced using the 'ggplot2' (Wickham, 2016) and 'Hmisc' (Harrell & Harrell, 2019) packages. Analysis of variance (F-test or χ^2 test) was performed with the 'car' package (Fox et al., 2012) using type II sums-of-squares. Model selection was then performed by a stepwise regression approach based on Akaike's Information Criterion (AIC).

Parasite infectivity was analyzed by performing a binomial logistic regression (0 = not infected, 1 = infected) with *Exposure*, *Diet* and *Clone* as explanatory variables. Only those individuals, which survived until at least day 8 post-inoculation (i.e. earliest observation of fungal asci in this experiment and one previous study, Manzi et al., 2020) were considered for infectivity, as reliable detection of infection symptoms was not possible prior to that day. To control for the potential influence of host lifespan on the cumulative number of spores produced by the parasite, parasite growth was estimated as the ratio of total spore yield over the number of days survived post-inoculation. Parasite growth and host lifespan post-inoculation were analyzed using generalized linear models with *Exposure*, *Diet* and *Clone* as explanatory variables. Normal distribution and homoscedasticity of the residuals were verified by visual inspection of quantile-quantile plots and residuals against fitted values, respectively. Only animals which became successfully infected by the parasite were included in these analyses.

3. Results

3.1 Parasite infectivity

Out of 144 *Daphnia* exposed to *Metschnikowia* spores, 63 individuals died before day 8 post-inoculation (categorized as *early death*). As infection could only be confirmed starting from day 8 post-inoculation, these individuals were not included in the determination of parasite infectivity. Two individuals were lost due to handling error, prior to their inspection under the microscope. Among the 79 remaining individuals, 53 were confirmed infected (67.08%) and 26 remained uninfected.

Spores which were pre-exposed to cyanobacterial toxins ('METS + Extract') had higher infectivity on clone AMME_51, as compared with the placebo treatment ('METS'). However, no such effect was found on AMME_12 (significant *Exposure* × *Clone* interaction, Table 1). Specifically, pre-

exposed spores led to 100% infection on clone AMME_51, while non-exposed spores infected less than 60% of this genotype (Figure 1a). Otherwise, there was a tendency towards higher proportion of infected hosts under a *Microcystis* diet (Table 1, Figure 1a).

3.2 Parasite growth

Pre-exposure of *Metschnikowia* did not influence the parasite's ability to produce spores (there were neither a main effect nor any significant interaction involving this factor, Table 1). Parasite growth was reduced by about two-fold when AMME_12 were fed with *Microcystis*, as compared with the *Scenedesmus* diet (Figure 1b). Within clone AMME_51, however, parasite reproduction was comparable under both diets (significant *Diet* × *Clone* interaction, Table 1). In addition, the parasite's ability to produce spores under a *Microcystis* diet was significantly higher on clone AMME_51 than AMME_12 (Tukey's HSD test, $P = 0.032$).

3.3 Host lifespan post-inoculation

The maximum lifespan recorded among successfully infected individuals was 15 days post-inoculation. Pre-exposure of the parasite did not influence the lifespan of infected hosts (Table 1). However, those maintained on a *Microcystis* diet survived on average 2 days longer, compared to the standard diet (Figure 1c).

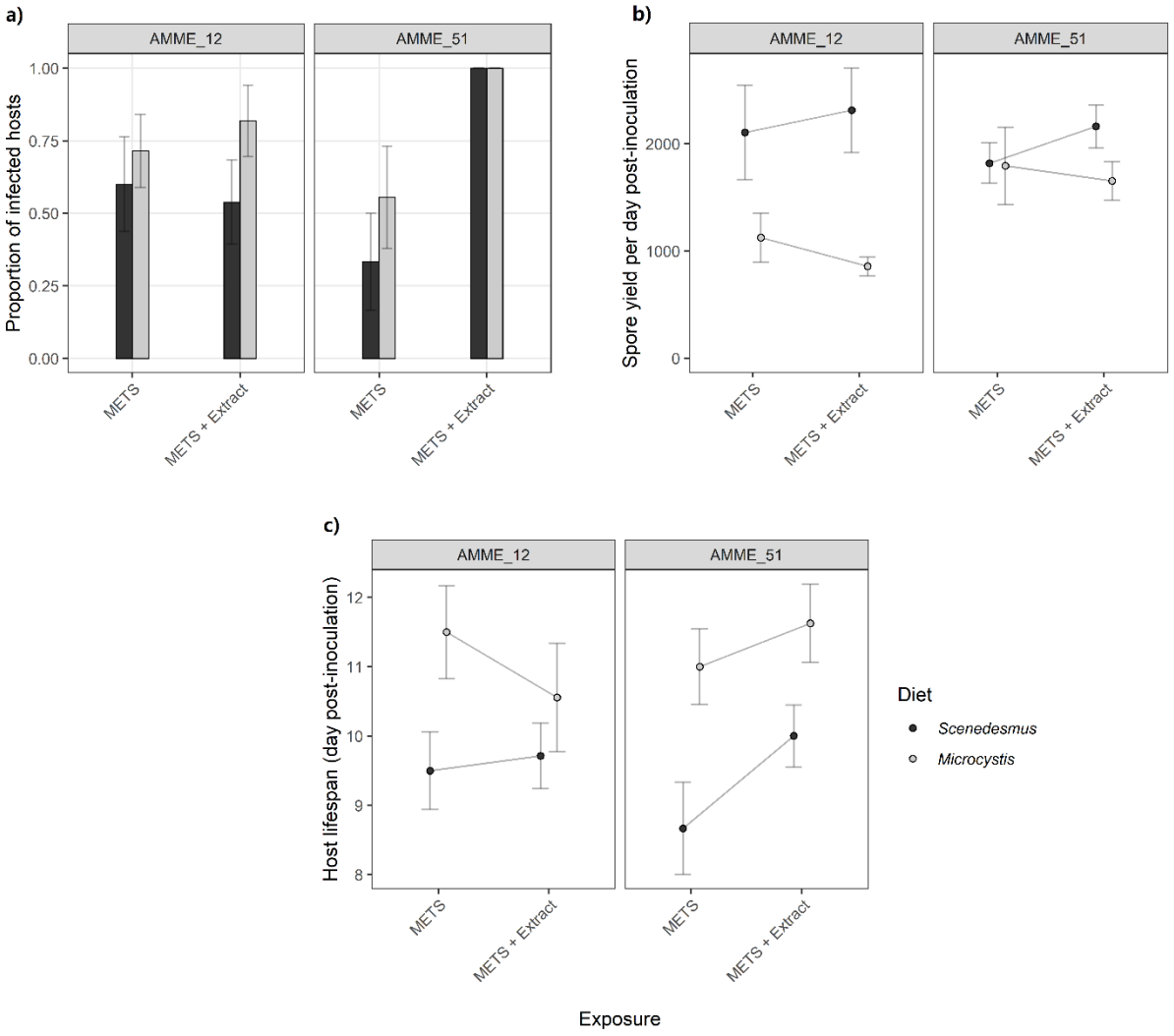


Figure 1. Graphical representation of (**a**) parasite infectivity, computed as the proportion of infected hosts among those which survived long enough to allow for completion of the parasite's life cycle; (**b**) parasite growth, computed as the ratio of total spore yield upon host death over the number of days survived after inoculation; and (**c**) host lifespan post-inoculation, computed as the number of days survived by the host, following introduction of the parasite on day 5. Two genotypes of *Daphnia galeata* × *longispina* hybrids (AMME_12; AMME_51) were inoculated with 1000 spores/mL of the parasitic yeast *Metschnikowia bicuspidata*. These spore solutions were previously exposed to an extract of *Microcystis aeruginosa* ('METS + Extract'), or to a placebo solution ('METS'). Following completion of the inoculation process, individual *Daphnia* were then fed either with a standard diet, consisting of the green alga *Scenedesmus obliquus* or with a mixed diet, where the cyanobacterium *Microcystis aeruginosa* contributed 75% of the total carbon content. Error bars depict the standard error of the mean.

Table 1. Three-way ANOVA (χ^2 test or F-test) testing for fixed effects of spore *Exposure*, host *Diet*, host *Clone* and their interactions on fitness components of the host and parasite. Model selection was performed by stepwise regression based on Akaike's Information Criterion (AIC); only the final model is reported here. Significant *P*-values (≤ 0.05) are highlighted in bold.

Response variable	**Distribution (link function)**	**Explanatory variables**	**Statistic (df)**	***P*-value**
Parasite infectivity	Binomial (link: logit)	Exposure	$\chi^2_{(1, 74)} = 4.649$	**0.031**
		Diet	$\chi^2_{(1,74)} = 3.001$	0.083
		Clone	$\chi^2_{(1, 74)} = 0.072$	0.789
		Exposure × Clone	$\chi^2_{(1, 74)} = 9.234$	**0.002**
Parasite growth	Normal	Exposure	$F_{(1, 48)} = 0.023$	0.881
		Diet	$F_{(1, 48)} = 19.40$	< **0.001**
		Clone	$F_{(1, 48)} = 3.333$	0.074
		Diet × Clone	$F_{(1, 48)} = 4.898$	**0.032**
Host lifespan post-inoculation	Normal	Exposure	$F_{(1, 49)} = 0.014$	0.908
		Diet	$F_{(1, 49)} = 11.24$	**0.002**
		Clone	$F_{(1, 49)} = 0.097$	0.757

4. Discussion

Given the complex evolutionary history and putative antifungal properties of cyanobacteria, we expected isolated cyanotoxins to exert direct antagonistic effects against a fungal parasite of *Daphnia*, possibly lowering its infection success upon later encounter with the host. While our experiment was not the first attempt to incorporate extracted cyanobacterial toxins into infection assays, using the *Daphnia-Metschnikowia* system (Sánchez et al., 2019; Penczykowski et al., 2014), our protocol differed from previous studies by one key aspect. Specifically, the inclusion of cyanobacterial toxins was performed in such a way that would guarantee prolonged exposure of the parasite (i.e. 24 hours), while limiting potential adverse effects to the host. By contrast, previous studies incorporated cyanobacterial compounds into the host's diet, thus exposing both antagonists at the same time. This specificity allowed us to test for possible negative effects of secondary metabolites against infective stages of a fungal parasite, independently of their consumption by infected *Daphnia*.

4.1 Effects of parasite pre-exposure

Contrary to our expectations, pre-exposure to cyanobacterial toxins did not impair the parasite's ability to infect in any treatment. Instead, we report a significant increase in infectivity for one of the tested genotypes (AMME_51). While the amount of cyanobacterial toxins introduced as part of the inoculate (i.e. about 1% of the initial jar's volume) is unlikely to have caused direct effects to *Daphnia* upon exposure, we cannot rule out that some toxic compounds extracted from *Microcystis* could have adhered to the surface of fungal spores. Upon passing through the gut's epithelium, lingering cyanotoxins may have thwarted the immune response of the host (i.e. mobilization of the haemocytes; Metschnikoff, 1884), thus directly acting against defence mechanisms of the host, rather than 'enhancing' the infectious potential of the parasite's propagules. Although previous studies did not report such an increase in host susceptibility, the apparent lack of detrimental effect on the parasite remains somewhat consistent with the results of Penczykowski et al. (2014): suspecting that cyanobacterial compounds involved in the inhibition of digestive proteases contributed to the low nutritional value of *Microcystis*, the authors artificially coated green algal cells with cyanobacterial extracts, showing no reduction in either the transmission rate or spore yield of *Metschnikowia*.

4.2 Effects of the Microcystis-based diet

Based on previous results using this system, we expected a two- to three-fold reduction in parasite reproduction, when the host is maintained under a sustained *Microcystis* diet (Manzi et al., 2020). Surprisingly, this effect only applied to infections of the AMME_12 genotype. In AMME_51, the *Microcystis* diet allowed the parasite to produce spores at a rate comparable to that of the high-quality diet. These types of host-genotype-by-environment ($G_H \times E$) interactions affecting parasitic infections are commonly reported in the *Daphnia* system, meaning that the outcome of infection can depend on environmental conditions, in a genotype-specific way (Wolinska & King, 2009). For instance, it was found that the infectivity and virulence of the bacterium *Pasteuria ramosa* would vary interactively between clonal identity of the host and either nutrition (Mitchell & Read, 2005; Little et al., 2007) or temperature (Mitchell et al., 2005; Little et al., 2007). Previously, we observed that the reduction in parasite reproduction induced by a *Microcystis* diet applied to both genotypes, but was stronger on AMME_12 than AMME_51 (Manzi et al., 2020). While the disparity was more pronounced here, it still reflects a similar interaction and thus remains consistent with our previous findings.

Additionally, we observed that a *Microcystis*-based diet allowed infected hosts to survive slightly longer than the *Scenedesmus* treatment (by about two days). A similar increase in the longevity of infected hosts was previously observed on a *Microcystis*-based diet, although this was found exclusively at 23°C (Manzi et al., 2020). Paradoxically, what could be interpreted as a protective effect for the host might in fact increase the parasite's transmission success: in spite of a less-nutritious diet,

the parasite can benefit from an extended infection time, allowing it to accumulate a higher spore yield upon host death; a paradox that has long been described as the virulence-transmission trade-off (Anderson & May, 1982; Acevedo et al., 2019). Because *Microcystis* strongly reduced the rate of spore production in AMME_12 hosts, the small increase in host longevity granted by this diet was not sufficient to outcompete spore outputs in the *Scenedesmus* treatment (Figure S1). By contrast, the mitigated effects of a toxic diet on AMME_51 allowed the parasite to perform equally under both levels of food quality (Figure S2).

Microcystis has been reported to induce feeding inhibition in cladocerans (even when integrated as low as 5% of a mixed diet), which may be imputed in part to behavioural avoidance of toxic cells (Ferrão-Filho et al., 2000) or diet-induced reduction of the host's body size, leading to lower filtering rates (Penczykowski et al., 2014). In the latter study, a drastic reduction in the infection rate of *Metschnikowia* was attributed to both behavioural and size-related effects. In our experimental assay, we found an opposite trend, with systematically higher proportion of infected hosts under a *Microcystis* diet, although this tendency was not statistically significant ($P = 0.08$). However, this was expected, due to a different layout in our experimental design: because the *Microcystis* diet was only introduced after parasite exposure, any potential effect of food quality on parasite encounter *per se* can be ruled out. Thus, within the context of our study, the *Microcystis* diet could only have influenced within-host processes involved in the parasite's infectivity, such as its ability to overcome the host's immune response. Our results thereby suggest that maintaining the host on a toxic diet throughout the progression of the parasite's development cycle does not impair the parasite's infectivity. Instead, the greatest consequence of a poor diet under natural conditions should consist of limited exposure to the parasite, due to behavioural and/or physiological decreases in feeding rates contributing to the overall success of infection (Ferrão-Filho et al., 2000; Penczykowski et al., 2014).

4.3 Environmental relevance

When cyanobacteria are incorporated as part of *Daphnia*'s diet, endotoxins are delivered upon digestion of bacterial cells, during their passage through the gut lumen (Carmichael, 1992). Considering that *Microcystis* compounds are capable of binding to (thus inhibiting) digestive proteases within the gut lumen of *Daphnia* (Agrawal et al., 2005; von Elert et al., 2012), within the context of parasitic infections, the digested cell content of cyanobacteria may also get in contact with ingested spores. Therefore, cyanobacteria are likely to interact with spores of horizontally-transmitted parasites, when both are consumed by the host and concurrently passing through the host's gut.

Throughout the course of harmful algal blooms, cell lysis of dominant cyanobacteria results in the release of large amounts of (otherwise intracellular) bioactive compounds in the water column (Jones & Orr, 1994; Ha et al., 2009). The levels of cyanotoxins released during blooms have been

remarked to approximate half the LC50 for *Daphnia* (DeMott et al., 1991; Sánchez et al., 2019), with concentrations of microcystins reportedly ranging from < 0.10 to 240 µg/L in freshwater lakes and recreational sites (Francy et al., 2015). Upon termination of a large bloom within enclosed water bodies, particularly high concentrations of microcystins (1300-1800 µg/L) have been noted to persist for up to 9 days, prior to a rapid phase of degradation (Jones & Orr, 1994). Thus, such concentrations of dissolved compounds may be high enough for cyanobacteria to exert adverse effects on cladoceran hosts, independently of their consumption as live cells (Ibelings et al., 2005). As far as cyanobacterial toxins directly interfering with free-floating spores or spore banks in the sediment, however, the likelihood of such event is more difficult to infer. On the one hand, some waterborne parasites (e.g. fish helminths) are able to bioconcentrate chemicals and pollutants at a much higher rate than free-living species, even when these are present at very low concentrations in the environment (Sures, 2003; Nachev & Sures, 2016). However, it can be argued that cyanobacteria and parasite spore banks normally occupy different layers in the stratified water column. While cyanobacteria's high buoyancy allows them to maintain close proximity to the surface, spores of the fungal parasite *Metschnikowia* are commonly recruited from the sediment, as infected hosts tend to sink to the bottom upon their death (Cáceres et al., 2006; Duffy & Hunsberger, 2019). Thus, by filter-feeding onto higher parts of the water column, cladocerans may sequestrate large amounts of dissolved cyanobacterial toxins and prevent those from reaching the sediment.

While our results did not provide support for our starting hypothesis (i.e. that the putative antifungal effects of *Microcystis aeruginosa* may not be limited to 'medicinal effects' but could instead directly interfere with the parasite, prior to their ingestion by *Daphnia* hosts), the specificity of our experimental design allowed us to raise the question of the parasite's vulnerability to an environmental stress of its own. Considering that outbreaks of this parasite typically occur for the span of a few months (i.e. from late summer to early winter) in temperate lakes, infective propagules contributing to the next seasonal epidemic will remain for considerable amounts of time buried in the sediment (Decaestecker et al., 2004). In temporary ponds susceptible to draining, spore banks of the parasite can even survive extended periods of complete host absence, such as during the dry seasons (Ebert, 2005). This effectively means that most of the parasite's lifespan (i.e. from release into the environment, to completion of a new reproductive cycle) is effectively spent outside of the host's internal environment, where infective propagules of parasites may be prone to a number of environmental disturbances (Pietrock & Marcogliese, 2003; Sures et al., 2017). Specifically, resting stages of *Metschnikowia* were previously shown to degrade or decrease in infectivity, following their exposure to solar UV (Overholt et al., 2012), high temperatures (Shocket et al., 2019) or near-freezing conditions (Duffy & Hunsberger, 2019). Such studies demonstrate that the transmission potential of this parasite can indeed be compromised by external environmental factors acting prior to – and independently of – its encounter with the host. Our results suggest, however, that exposure to dissolved cyanobacterial toxins during

blooms does not constitute one such mechanism. While cyanobacterial dominance may not directly interfere with the infectivity of fungal spore banks, other parasites of cladocerans belonging to distant taxa, such as the endospore-forming bacterium *Pasteuria ramosa* (Ebert et al., 1996) or the viral agent for White Fat Cell Disease (Toenshoff et al., 2018) may be worthy of investigation, in light of the wider allelopathic properties of cyanobacteria.

Acknowledgements

We would like to thank Ursula Newen for the maintenance of *Daphnia* cultures and Francesco Pomati for providing us with the *Microcystis* strain.

Funding

This work was supported by a joint German–Israeli project (WO 1587/8-1, 0604317501) funded by the German Science Foundation.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

FM, MM, JW and RA conceptualized the study. FM and MM performed the experiment, data analysis and results visualization. FM wrote the article, with help from RA, JW and MM. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

References

Acevedo, M. A., Dillemuth, F. P., Flick, A. J., Faldyn, M. J., & Elderd, B. D. (2019). Virulence-driven trade-offs in disease transmission: A meta-analysis. *Evolution*, *73*(4), 636-647. https://doi.org/10.1111/evo.13724

Agha, R., & Quesada, A. (2014). Oligopeptides as biomarkers of cyanobacterial subpopulations. Toward an understanding of their biological role. *Toxins*, *6*(6), 1929-1950. https://doi.org/10.3390/toxins6061929

Agrawal, M. K., Zitt, A., Bagchi, D., Weckesser, J., Bagchi, S. N., & von Elert, E. (2005). Characterization of proteases in guts of *Daphnia magna* and their inhibition by *Microcystis aeruginosa* PCC 7806. *Environmental Toxicology: an International Journal*, *20*(3), 314-322. https://doi.org/10.1002/tox.20123

Anderson, R., & May, R. (1982) Coevolution of hosts and parasites. *Parasitology*, *85*, 411–426. https://doi.org/10.1017/S0031182000055360

Benndorf, J., & Henning, M. (1989). *Daphnia* and toxic blooms of *Microcystis aeruginosa* in Bautzen Reservoir (GDR). *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, *74*(3), 233-248.

Berry, J. P., Gantar, M., Perez, M. H., Berry, G., & Noriega, F. G. (2008). Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. *Marine Drugs*, *6*(2), 117-146. https://doi.org/10.3390/md6020117

Cáceres, C. E., Hall, S. R., Duffy, M. A., Tessier, A. J., Helmle, C., & MacIntyre, S. (2006). Physical structure of lakes constrains epidemics in *Daphnia* populations. *Ecology*, *87*(6), 1438-1444. https://doi.org/10.1890/0012-9658(2006)87[1438:PSOLCE]2.0.CO;2

Canter, H. M. (1950). Fungal parasites of the phytoplankton. I (Studies on British chytrids, X). Annals of Botany, 14(54), 263-289. http://www.jstor.org/stable/42906609.

Carey, C. C., Ibelings, B. W., Hoffmann, E. P., Hamilton, D. P., & Brookes, J. D. (2012). Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Research*, 46(5), 1394-1407. https://doi.org/10.1016/j.watres.2011.12.016

Carmichael W.W. (1992) Cyanobacteria secondary metabolites: the cyanotoxins. *Journal of Applied Bacteriology*, 72, 445–459. https://doi.org/10.1111/j.1365-2672.1992.tb01858.x

Chislock, M. F., Sarnelle, O., Jernigan, L. M., & Wilson, A. E. (2013). Do high concentrations of microcystin prevent *Daphnia* control of phytoplankton? *Water Research*, *47*(6), 1961-1970. https://doi.org/10.1016/j.watres.2012.12.038

Chorus, I. (2012). *Cyanotoxins: occurrence, causes, consequences*. Springer Science & Business Media.

Czarnecki, O., Henning, M., Lippert, I., & Welker, M. (2006). Identification of peptide metabolites of *Microcystis* (Cyanobacteria) that inhibit trypsin-like activity in planktonic herbivorous *Daphnia* (Cladocera). *Environmental Microbiology*, *8*(1), 77-87. https://doi.org/10.1111/j.1462-2920.2005.00870.x

Dallas, T., Holtackers, M., & Drake, J. M. (2016). Costs of resistance and infection by a generalist pathogen. *Ecology and Evolution*, *6*(6), 1737-1744. https://doi.org/10.1002/ece3.1889

Decaestecker, E., Lefever, C., De Meester, L., & Ebert, D. (2004). Haunted by the past: evidence for dormant stage banks of microparasites and epibionts of *Daphnia*. *Limnology and Oceanography*, 49(4), 1355-1364. https://doi.org/10.4319/lo.2004.49.4_part_2.1355

DeMott, W. R. (1990). Retention efficiency, perceptual bias, and active choice as mechanisms of food selection by suspension-feeding zooplankton. In *Behavioural Mechanisms of Food Selection* (pp. 569-594). Springer, Berlin, Heidelberg.

DeMott, W. R., & Moxter, F. (1991). Foraging cyanobacteria by copepods: responses to chemical defense and resource abundance. *Ecology*, *72*(5), 1820-1834. https://doi.org/10.2307/1940981

DeMott, W. R., Zhang, Q. X., & Carmichael, W. W. (1991). Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. *Limnology and Oceanography*, *36*(7), 1346-1357. https://doi.org/10.4319/lo.1991.36.7.1346

DeMott, W. R., & Müller-Navarra, D. C. (1997). The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. *Freshwater Biology*, *38*, 649– 664. https://doi.org/10.1046/j.1365-2427.1997.00222.x

Duffy, M. A., Hall, S. R., Cáceres, C. E., & Ives, A.R. (2009). Rapid evolution, seasonality, and the termination of parasite epidemics. *Ecology*, 90, 1441–1448. https://doi.org/10.1890/08-1130.1

Duffy M. A., & Hunsberger K. K. (2019). Infectivity is influenced by parasite spore age and exposure to freezing: do shallow waters provide *Daphnia* a refuge from some parasites? *Journal of Plankton Research*, 41, 12–16. https://doi.org/10.1093/plankt/fby046

Ebert, D. (2005). *Ecology, Epidemiology, and Evolution of Parasitism in Daphnia*. National Library of Medicine.

Ebert, D., Rainey, P., Embley, T. M., & Scholz, D. (1996). Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *351*(1348), 1689-1701. https://doi.org/10.1098/rstb.1996.0151

von Elert, E., Zitt, A., & Schwarzenberger, A. (2012). Inducible tolerance to dietary protease inhibitors in *Daphnia magna*. *Journal of Experimental Biology*, 215, 2051–2059. https://doi.org/10.1242/jeb.068742

von Elert, E., & Jüttner, F. (1997). Phosphorus limitation and not light controls the extracellular release of allelopathic compounds by *Trichormus doliolum* (Cyanobacteria). *Limnology and Oceanography*, *42*(8), 1796-1802. https://doi.org/10.4319/lo.1997.42.8.1796

Ferrão-Filho, A. S., Azevedo, S. M., & DeMott, W. R. (2000). Effects of toxic and non-toxic cyanobacteria on the life history of tropical and temperate cladocerans. *Freshwater Biology*, *45*(1), 1-19. https://doi.org/10.1046/j.1365-2427.2000.00613.x

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., … & Heiberger, R. (2012). Package 'car'. Vienna: R Foundation for Statistical Computing.

Francy, D. S., Graham, J. L., Stelzer, E. A., Ecker, C. D., Brady, A. M. G., Struffolino, P., & Loftin, K. A. (2015). Water quality, cyanobacteria, and environmental factors and their relations to microcystin concentrations for use in predictive models at Ohio Lake Erie and inland lake recreational sites, 2013–14: U.S. Geological Survey Scientific Investigations Report 2015–5120, 58 p. https://doi.org/10.3133/sir20155120

Ghadouani, A., Pinel-Alloul, B., Plath, K., Codd, G. A., & Lampert, W. (2004). Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulicaria*. *Limnology and Oceanography*, 49(3), 666-679. https://doi.org/10.4319/lo.2004.49.3.0666

Garcia-Pichel, F. (1998). Solar ultraviolet and the evolutionary history of cyanobacteria. *Origins of Life and Evolution of the Biosphere*, *28*(3), 321-347. https://doi.org/10.1023/A:1006545303412

Ger, K. A., Hansson, L. A., & Lürling, M. (2014). Understanding cyanobacteria-zooplankton interactions in a more eutrophic world. *Freshwater Biology*, *59*(9), 1783-1798. https://doi.org/10.1111/fwb.12393

Gobler, C. J. (2020). Climate change and harmful algal blooms: insights and perspective. *Harmful Algae*, *91*, 101731. https://doi.org/10.1016/j.hal.2019.101731

Grattan, L. M., Holobaugh, S., & Morris Jr, J. G. (2016). Harmful algal blooms and public health. *Harmful Algae*, 57, 2-8. https://doi.org/10.1016/j.hal.2016.05.003

Griebel, J., Gießler, S., Poxleitner, M., Faria, A. N., Yin, M., & Wolinska, J. (2015). Extreme environments facilitate hybrid superiority–The story of a successful *Daphnia galeata* × *longispina* hybrid clone. *PLoS ONE*, *10*(10), e0140275. https://doi.org/10.1371/journal.pone.0140275

Ha, J. H., Hidaka, T., & Tsuno, H. (2009). Quantification of toxic *Microcystis* and evaluation of its dominance ratio in blooms using real-time PCR. *Environmental Science & Technology*, *43*(3), 812-818. https://doi.org/10.1021/es801265f

Hall, S. R., Becker, C. R., Duffy, M. A., & Cáceres, C. E. (2011). Epidemic size determines population-level effects of fungal parasites on *Daphnia* hosts. *Oecologia*, *166*(3), 833–842. https://doi.org/10.1007/s00442-011-1905-4

Hall, S. R., Sivars-Becker, L., Becker, C., Duffy, M. A., Tessier, A. J., & Cáceres, C. E. (2007). Eating yourself sick: Transmission of disease as a function of foraging ecology. *Ecology Letters*, *10*(3), 207–218. https://doi.org/10.1111/j.1461-0248.2007.01011.x

Hallegraeff, G. M. (1993). A review of harmful algae blooms and their apparent global increase. *Phycologia*, *32*(2), 79-99. https://doi.org/10.2216/i0031-8884-32-2-79.1

Hanazato, T., & Yasuno, M. (1987). Evaluation of *Microcystis* as food for zooplankton in a eutrophic lake. *Hydrobiologia*, *144*(3), 251-259. https://doi.org/10.1007/BF00005559

Harrell, F. E. Jr, & Harrell, M. F. E. Jr. (2019). Package 'Hmisc'. CRAN2018, 235-6.

Hesse, O., Engelbrecht, W., Laforsch, C., & Wolinska, J. (2012). Fighting parasites and predators: How to deal with multiple threats? *BMC Ecology*, *12*(1), 12. https://doi.org/10.1186/1472-6785-12-12

Huang, I. S., & Zimba, P. V. (2019). Cyanobacterial bioactive metabolites—A review of their chemistry and biology. *Harmful Algae*, *83*, 42-94. https://doi.org/10.1016/j.hal.2018.11.008

Ibelings, B. W., Bruning, K., De Jonge, J., Wolfstein, K., Pires, L. D., Postma, J., & Burger, T. (2005). Distribution of microcystins in a lake foodweb: no evidence for biomagnification. *Microbial Ecology*, *49*(4), 487-500. https://doi.org/10.1007/s00248-004-0014-x

Ji, X., Verspagen, J. M., Stomp, M., & Huisman, J. (2017). Competition between cyanobacteria and green algae at low versus elevated CO2: who will win, and why? *Journal of Experimental Botany*, *68*(14), 3815-3828. https://doi.org/10.1093/jxb/erx027

Jones, G. J., & Orr, P. T. (1994). Release and degradation of microcystin following algicide treatment of a *Microcystis aeruginosa* bloom in a recreational lake, as determined by HPLC and protein phosphatase inhibition assay. *Water Research*, *28*(4), 871-876. https://doi.org/10.1016/0043-1354(94)90093-0

Kaebernick, M., Neilan, B. A., Börner, T., & Dittmann, E. (2000). Light and the transcriptional response of the microcystin biosynthesis gene cluster. *Applied and Environmental Microbiology*, *66*(8), 3387-3392. https://doi.org/10.1128/AEM.66.8.3387-3392.2000

Kehr, J. C., Zilliges, Y., Springer, A., Disney, M. D., Ratner, D. D., Bouchier, C., Seeberger, P. H., Tandeau De Marsac, N., & Dittmann, E. (2006). A mannan binding lectin is involved in cell–cell attachment in a toxic strain of *Microcystis aeruginosa*. *Molecular Microbiology*, *59*(3), 893-906. https://doi.org/10.1111/j.1365-2958.2005.05001.x

Keller, B., Wolinska, J., Manca, M., & Spaak, P. (2008). Spatial, environmental and anthropogenic effects on the taxon composition of hybridizing *Daphnia*. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1505), 2943–2952. https://doi.org/10.1098/rstb.2008.0044

Kirk, K. L., & Gilbert, J. J. (1992). Variation in herbivore response to chemical defenses: zooplankton foraging on toxic cyanobacteria. *Ecology*, *73*(6), 2208-2217. https://doi.org/10.2307/1941468

Knoll, A. H. (2008). Cyanobacteria and earth history. *The cyanobacteria: molecular biology, genomics, and evolution*, 484.

Kótai, J. (1972). Instructions for preparation of modified nutrient solution Z8 for algae. Norwegian Institute for Water Research, Oslo, *11*(69), 5.

Leão, P. N., Pereira, A. R., Liu, W. T., Ng, J., Pevzner, P. A., Dorrestein, P. C., König, G. M., Vasconselos, V. M., & Gerwick, W. H. (2010). Synergistic allelochemicals from a freshwater cyanobacterium. *Proceedings of the National Academy of Sciences*, *107*(25), 11183-11188. https://doi.org/10.1073/pnas.0914343107

Little, T., Birch, J., Vale, P., & Tseng, M. (2007). Parasite transgenerational effects on infection. *Evolutionary Ecology Research*, *9*(3), 459-469. https://www.evolutionary-ecology.com/abstracts/v09/2135.html

Lundstedt, L., & Brett, M. T. (1991). Differential growth rates of three cladoceran species in response to mono- and mixed-algal cultures. *Limnology and Oceanography*, *36*(1), 159-165. https://doi.org/10.4319/lo.1991.36.1.0159

Ma, X., Hu, W., Smilauer, P., Yin, M., & Wolinska, J. (2018). *Daphnia galeata* and *D. dentifera* are geographically and ecologically separated whereas their hybrids occur in intermediate habitats: survey of 44 Chinese lakes. *Molecular Ecology*, *28*(4), 785-802. https://doi.org/10.1111/mec.14991

Mantzouki, E., Visser, P. M., Bormans, M., & Ibelings, B. W. (2016). Understanding the key ecological traits of cyanobacteria as a basis for their management and control in changing lakes. *Aquatic Ecology*, *50*(3), 333-350. https://doi.org/10.1007/s10452-015-9526-3

Manzi, F., Agha, R., Lu, Y., Ben-Ami, F., & Wolinska, J. (2020). Temperature and host diet jointly influence the outcome of infection in a *Daphnia*-fungal parasite system. *Freshwater Biology 65*, 757–767. https://doi.org/10.1111/fwb.13464

Marrez, D. A., & Sultan, Y. Y. (2016). Antifungal activity of the cyanobacterium *Microcystis aeruginosa* against mycotoxigenic fungi. *Journal of Applied Pharmaceutical Science*, *6*(11), 191-198. https://doi.org/10.7324/JAPS.2016.601130

Metschnikoff, E. (1884). A disease of *Daphnia* caused by a yeast. A contribution to the theory of phagocytes as agents for attack on disease-causing organisms. *Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin*, *96*, 177-195. https://doi.org/10.1055/s-0029-1209659

Mitchell, S. E., & Read, A. F. (2005). Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1581), 2601–2607. https://doi.org/10.1098/rspb.2005.3253

Mitchell, S. E., Rogers, E. S., Little, T. J., & Read, A. F. (2005). Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, *59*(1), 70–80. https://doi.org/10.1111/j.0014-3820.2005.tb00895.x

Moore, S. K., Trainer, V. L., Mantua, N. J., Parker, M. S., Laws, E. A., Backer, L. C., & Fleming, L. E. (2008). Impacts of climate variability and future climate change on harmful algal blooms and human health. In Environmental Health (Vol. 7, No. 2, pp. 1-12). BioMed Central.

Morley, N. J., Lewis, J. W., & Hoole, D. (2006). Pollutant-induced effects on immunological and physiological interactions in aquatic host–trematode systems: implications for parasite transmission. *Journal of Helminthology*, *80*(2), 137-149. https://doi.org/10.1079/JOH2006345

Nachev, M., & Sures, B. (2016). Environmental parasitology: Parasites as accumulation bioindicators in the marine environment. *Journal of Sea Research*, *113*, 45-50. https://doi.org/10.1016/j.seares.2015.06.005

Namikoshi, M., & Rinehart, K. L. (1996). Bioactive compounds produced by cyanobacteria. *Journal of Industrial Microbiology*, *17*(5), 373-384. https://doi.org/10.1007/BF01574768

Overholt, E. P., Hall, S. R., Williamson, C. E., Meikle, C. K., Duffy, M. A., & Cáceres, C. E. (2012). Solar radiation decreases parasitism in *Daphnia*. *Ecology Letters*, *15*(1), 47-54. https://doi.org/10.1111/j.1461-0248.2011.01707.x

Penczykowski, R. M., Lemanski, B. C., Sieg, R. D., Hall, S. R., Housley Ochs, J., Kubanek, J., & Duffy, M. A. (2014). Poor resource quality lowers transmission potential by changing foraging behaviour. *Functional Ecology*, *28*(5), 1245-1255. https://doi.org/10.1111/1365-2435.12238

Pietrock, M., & Marcogliese, D. J. (2003). Free-living endohelminth stages: at the mercy of environmental conditions. *Trends in Parasitology*, *19*(7), 293-299. https://doi.org/10.1016/S1471-4922(03)00117-X

Ravet, J. L., Brett, M. T. & Müller-Navarra, D. C. (2003). A test of the role of polyunsaturated fatty acids in phytoplankton food quality for *Daphnia* using liposome supplementation. *Limnology and Oceanography*, *48*, 1938– 1947. https://doi.org/10.4319/lo.2003.48.5.1938

Reinl, K. L., Brookes, J. D., Carey, C. C., Harris, T. D., Ibelings, B. W., Morales-Williams, A. M., De Senerpont Domis, L. M., Atkins, K. S., Isles, P. D. F., Mesman, J. P., North, R. L., Rudstam, L. G., Stelzer, J. A. A., Venkiteswaran, J. J., Yokota, K., & Zhan, Q. (2021). Cyanobacterial blooms in oligotrophic lakes: Shifting the high-nutrient paradigm. *Freshwater Biology*, *66*(9), 1846-1859. https://doi.org/10.1111/fwb.13791

Reynolds, C. S., & Walsby, A. E. (1975). Water-blooms. *Biological Reviews*, *50*(4), 437-481. https://doi.org/10.1111/j.1469-185X.1975.tb01060.x

Rohrlack, T., Christoffersen, K., Kaebernick, M., & Neilan, B. A. (2004). Cyanobacterial protease inhibitor microviridin J causes a lethal molting disruption in *Daphnia pulicaria*. *Applied and Environmental Microbiology*, *70*(8), 5047-5050. https://doi.org/10.1128/AEM.70.8.5047-5050.2004

Rohrlack, T., Christiansen, G., & Kurmayer, R. (2013). A putative anti-parasite defensive system in the cyanobacterium *Planktothrix* involving ribosomal and non-ribosomal oligopeptides. *Applied and Environmental Microbiology*, *79*(8), 2642– 2647. https://doi.org/10.1128/AEM.03499-12

Saebelfeld, M., Minguez, L., Griebel, J., Gessner, M. O., & Wolinska, J. (2017). Humic dissolved organic carbon drives oxidative stress and severe fitness impairments in *Daphnia*. *Aquatic Toxicology*, *182*, 31-38. https://doi.org/10.1016/j.aquatox.2016.11.006

Sánchez, K. F., Huntley, N., Duffy, M. A., & Hunter, M. D. (2019). Toxins or medicines? Phytoplankton diets mediate host and parasite fitness in a freshwater system. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1894), 20182231. https://doi.org/10.1098/rspb.2018.2231

Sánchez-Baracaldo, P., & Cardona, T. (2020). On the origin of oxygenic photosynthesis and Cyanobacteria. *New Phytologist*, *225*(4), 1440-1446. https://doi.org/10.1111/nph.16249

Schagerl, M., Unterrieder, I., & Angeler, D. G. (2002). Allelopathy among cyanoprokaryota and other algae originating from Lake Neusiedlersee (Austria). *International Review of Hydrobiology: A Journal Covering all Aspects of Limnology and Marine Biology*, *87*(4), 365-374. https://doi.org/10.1002/1522-2632(200207)87:4<365::AID-IROH365>3.0.CO;2-B

Schatz, D., Keren, Y., Vardi, A., Sukenik, A., Carmeli, S., Börner, T., Dittmann, E., & Kaplan, A. (2007). Towards clarification of the biological role of microcystins, a family of cyanobacterial toxins. *Environmental Microbiology*, *9*(4), 965-970. https://doi.org/10.1111/j.1462-2920.2006.01218.x

Sergeev, V. N., Gerasimenko, L. M., & Zavarzin, G. A. (2002). The proterozoic history and present state of cyanobacteria. *Microbiology*, *71*(6), 623-637. https://doi.org/10.1023/A:1021415503436

Shishido, T. K., Humisto, A., Jokela, J., Liu, L., Wahlsten, M., Tamrakar, A., Fewer, D. P., Permi, P., Andreote, A. P. D., Fiore, M. F., & Sivonen, K. (2015). Antifungal compounds from cyanobacteria. *Marine Drugs*, *13*(4), 2124-2140. https://doi.org/10.3390/md13042124

Shocket, M. S., Magnante, A., Duffy, M. A., Cáceres, C. E., & Hall, S. R. (2019). Can hot temperatures limit disease transmission? A test of mechanisms in a zooplankton–fungus system. *Functional Ecology*, *33*(10), 2017-2029. https://doi.org/10.1111/1365-2435.13403

Smith, A. D., & Gilbert, J. J. (1995). Relative susceptibilities of rotifers and cladocerans to *Microcystis aeruginosa*. *Archiv für Hydrobiologie*, 309-336. https://doi.org/10.1127/archiv-hydrobiol/132/1995/309

Stewart Merrill, T. E., & Cáceres, C. E. (2018). Within-host complexity of a plankton-parasite interaction. *Ecology*, *99*(12), 2864-2867. https://www.jstor.org/stable/26627163

Sukenik, A., Eshkol, R., Livne, A., Hadas, O., Rom, M., Tchernov, D., Vardi, A., & Kaplan, A. (2002). Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp.(cyanobacteria): a novel allelopathic mechanism. *Limnology and Oceanography*, *47*(6), 1656-1663. https://doi.org/10.4319/lo.2002.47.6.1656

Sukenik, A., & Kaplan, A. (2021). Cyanobacterial harmful algal blooms in aquatic ecosystems: a comprehensive outlook on current and emerging mitigation and control approaches. *Microorganisms*, *9*(7), 1472. https://doi.org/10.3390/microorganisms9071472

Sures, B. (2003). Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. *Parasitology*, *126*(7), S53-S60. https://doi.org/10.1017/S003118200300372X

Sures, B., Nachev, M., Selbach, C., & Marcogliese, D. J. (2017). Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology'. *Parasites & Vectors*, *10*(1), 1-19. https://doi.org/10.1186/s13071-017-2001-3

Suttle, C. A. (2000). Cyanophages and their role in the ecology of cyanobacteria. *In The Ecology of Cyanobacteria* (pp. 563-589). Springer, Dordrecht.

Tellenbach, C., Tardent, N., Pomati, F., Keller, B., Hairston, N. G., Wolinska, J., & Spaak, P. (2016). Cyanobacteria facilitate parasite epidemics in *Daphnia*. *Ecology*, *97*(12), 3422–3432. https://doi.org/10.1002/ecy.1576

Teta, R., Della Sala, G., Glukhov, E., Gerwick, L., Gerwick, W. H., Mangoni, A., & Costantino, V. (2015). Combined LC–MS/MS and molecular networking approach reveals new cyanotoxins from the 2014 cyanobacterial bloom in Green Lake, Seattle. *Environmental Science & Technology*, *49*(24), 14301-14310. https://doi.org/10.1021/acs.est.5b04415

Toenshoff, E. R., Fields, P. D., Bourgeois, Y. X., & Ebert, D. (2018). The end of a 60-year riddle: Identification and genomic characterization of an iridovirus, the causative agent of white fat cell disease in zooplankton. *G3: Genes, Genomes, Genetic*s, *8*(4), 1259-1272. https://doi.org/10.1534/g3.117.300429

Urrutia-Cordero, P., Ekvall, M. K., & Hansson, L. A. (2016). Controlling harmful cyanobacteria: taxa-specific responses of cyanobacteria to grazing by large-bodied *Daphnia* in a biomanipulation scenario. *PLoS ONE*, *11*(4), e0153032. https://doi.org/10.1371/journal.pone.0153032

Utkilen, H., & Gjølme, N. (1995). Iron-stimulated toxin production in *Microcystis aeruginosa*. *Applied and Environmental Microbiology*, *61*(2), 797-800. https://doi.org/10.1128/aem.61.2.797-800.1995

Van Dolah, F. M., Roelke, D., & Greene, R. M. (2001). Health and ecological impacts of harmful algal blooms: risk assessment needs. *Human and Ecological Risk Assessment: An International Journal*, *7*(5), 1329-1345. https://doi.org/10.1080/20018091095032

Volk, R. B., & Furkert, F. H. (2006). Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. *Microbiological Research*, *161*(2), 180-186. https://doi.org/10.1534/g3.117.300429

Welker, M., & Von Döhren, H. (2006). Cyanobacterial peptides—nature's own combinatorial biosynthesis. *FEMS Microbiology Reviews*, *30*(4), 530-563. https://doi.org/10.1111/j.1574-6976.2006.00022.x

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. New York, NY: Springer-Verlag.

Wolinska, J., & King, K. C. (2009). Environment can alter selection in host–parasite interactions. *Trends in Parasitology*, *25*(5), 236-244. https://doi.org/10.1016/j.pt.2009.02.004

Wolinska, J., Seda, J., Koerner, H., Smilauer, P., & Petrusek, A. (2011). Spatial variation of *Daphnia* parasite load within individual water bodies. *Journal of Plankton Research*, *33*(8), 1284-1294. https://doi.org/10.1093/plankt/fbr016

Zehnder, A., & Gorham, P. R. (1960). Factors influencing the growth of *Microcystis aeruginosa* Kütz. Emend. Elenkin. *Canadian Journal of Microbiology*, *6*(6), 645-660. https://doi.org/10.1139/m60-077

Zheng, H., Yin, J., Gao, Z., Huang, H., Ji, X., & Dou, C. (2011). Disruption of *Chlorella vulgaris* cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. *Applied Biochemistry and Biotechnology*, *164*(7), 1215-1224. https://doi.org/10.1007/s12010-011-9207-1

Zilliges, Y., Kehr, J. C., Meissner, S., Ishida, K., Mikkat, S., Hagemann, M., Kaplan, A., Börner, T., & Dittmann, E. (2011). The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. *PLoS ONE*, *6*(3), e17615. https://doi.org/10.1371/journal.pone.0017615

Supporting Information for: Prior exposure of a fungal parasite to cyanobacterial extracts does not impair infection of its *Daphnia* host

Florent Manzi[1,2], Ramsy Agha[1], Max Mühlenhaupt[1,2], Justyna Wolinska[1,2]

[1] *Department of Evolutionary and Integrative Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2] *Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany*

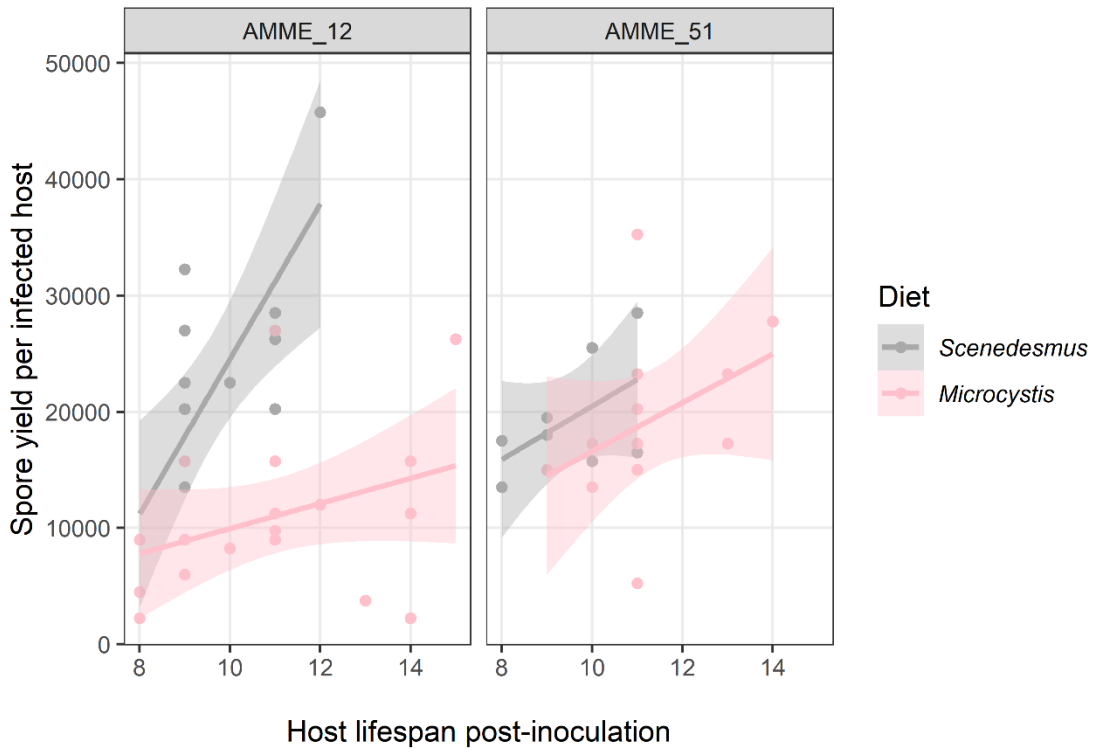


Figure S1. Linear regression between the total spore yield of *Metschnikowia bicuspidata* recorded upon host death, and the number of days survived by the host following inoculation of the parasite (days). Coloured bands depict 95% confidence intervals. Two genotypes of *Daphnia galeata* × *longispina* hybrids (AMME_12; AMME_51) were inoculated with 1000 spores/mL of the parasite. Individual *Daphnia* were fed either with a standard diet, consisting of the green alga *Scenedesmus obliquus* or with a mixed diet, where the cyanobacterium *Microcystis aeruginosa* contributed 75% of the total carbon content. The effect of diet on parasite reproduction differed between host genotypes: in infected individuals of the genotype AMME_12, the rate of spore production was greatly reduced under a *Microcystis* diet. However, individuals of the genotype AMME_51 displayed comparable rates of spore production under both levels of food quality.

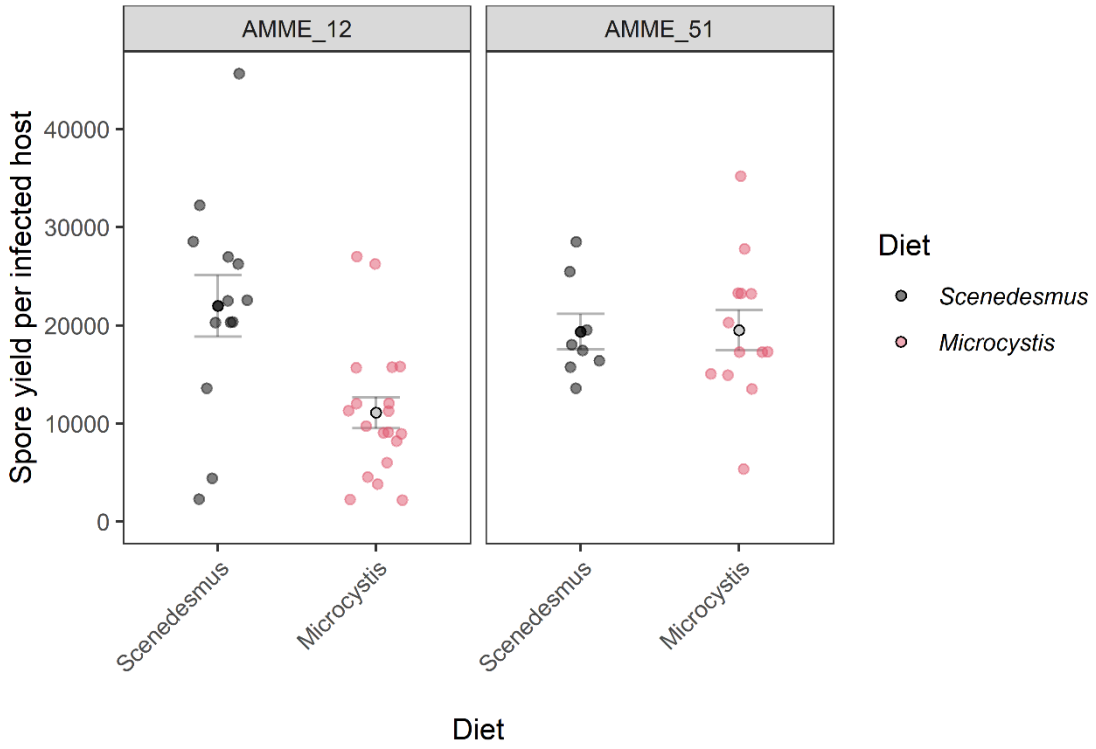


Figure S2. Total spore yield of *Metschnikowia bicuspidata* (recorded per individual host), as compared across two levels of food quality. Error bars depict the standard error of the mean. Two genotypes of *Daphnia galeata* × *longispina* hybrids (AMME_12; AMME_51) were inoculated with 1000 spores/mL of the parasite. Individual *Daphnia* were fed either with a standard diet, consisting of the green alga *Scenedesmus obliquus* or with a mixed diet, where the cyanobacterium *Microcystis aeruginosa* contributed 75% of the total carbon content. The effect of diet on total spore yield differed between host genotypes: in infected individuals of the genotype AMME_12, total spore yield was greatly reduced under a *Microcystis* diet. However, individuals of the genotype AMME_51 displayed comparable spore yields under both levels of food quality.

CHAPTER 3:

NANOPLASTICS MODULATE THE OUTCOME OF A DAPHNIA-MICROPARASITE INTERACTION

Nanoplastics modulate the outcome of a *Daphnia*-microparasite interaction

Stylianos Mavrianos[1,2], Florent Manzi[1,3 *], Ramsy Agha[1], Noemi Azoubib[1,3], Charlotte Schampera[1], Justyna Wolinska[1,3]

[1]*Department of Evolutionary and Integrative Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2]*Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*

[3]*Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany*

Corresponding author*: **florent.vmanzi@gmail.com

Author contributions: JW conceived the presented idea. All authors designed the study. JW, **FM**, RA and CS coordinated and supervised the study. SM and NA equally carried out the experimental procedure. SM carried out the data visualization and formal analysis, **FM** critically directed it and all authors validated the results. SM drafted the manuscript and JW, **FM**, RA and CS critically revised every version. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Unpublished manuscript.

Abstract

Widely abundant micro- and nanoplastics pose a threat for aquatic ecosystems. However, despite the extensive knowledge on their effects at the species level, the way they affect the interaction between species remains largely unexplored. We studied the effects of polystyrene nanoplastics on host-parasite interactions using the waterflea *Daphnia galeata* × *longispina* and its parasitic yeast *Metschnikowia bicuspidata* as a model system. Although nanoplastics increased parasite infectivity, higher rates of host mortality cancelled out parasite advantage. Moreover, under high nanoplastic concentrations, the parasite's reproductive output (i.e. spore yield measured per infected host) was strongly impaired. The host displayed characteristics of a hormetic effect, for instance, increased longevity and fecundity at low nanoplastic concentrations, supporting the idea of a dose-response model. Overall, our results highlight that the consequences of plastic pollution go beyond the effects on individual species, as they shape the outcome of host-parasite interactions.

1. Introduction

Plastic pollution is of global concern. A considerable proportion of plastic waste eventually ends up in aquatic ecosystems, either by direct disposal or by natural forces. Plastics in the environment break down into smaller pieces through mechanical processes, biological degradation, and/or UV radiation, leading to microplastics (with a size < 5 mm). Microplastics are also manufactured for a broad range of applications, such as cosmetics and medical research (Andrady, 2011). As a result, microplastics are widely distributed in water bodies worldwide (Andrady, 2003; Weinstein et al., 2016). Microplastics themselves can break down into even smaller particles of a size ≤ 100 nm, so-called nanoplastics (hereafter referred as NPs; Klaine et al., 2012; Koelmans et al., 2015). Microplastics, including NPs, can be directly ingested by aquatic biota, eliciting an array of ecotoxicological effects (Wang et al., 2019). For instance, NPs raise special toxicological concerns due to their unique ability to penetrate lipid cell membranes and their potential to alter cellular functions (Salvati et al., 2011), and cause inflammations (Brown et al., 2001). NPs have been shown to cause oxidative stress and inhibit photosynthetic growth of phytoplankton (Bhattacharya et al., 2010; Bergami et al., 2017; Wan et al., 2018). They can also reduce body size, fecundity, and survival of zooplankton (Lin et al., 2019; Kelpsiene et al., 2020). NPs are not biodegradable and, consequently, they transfer and accumulate throughout the trophic web (Setälä et al., 2014; Chae et al., 2018). For instance, bioaccumulation of NPs has been shown to affect metabolism in fish and molluscs (Casado-Gavalda et al., 2013; Mattson et al., 2016).

Most studies have investigated the effects of NPs at the species level (as reviewed in Mattson et al., 2018). However, the response of individual species is arguably a poor indicator of the ecological impact of NPs on ecosystem processes. Instead, the interactions between organisms (e.g. competition, predation, parasitism) that shape ecological processes at higher levels of biological complexity, such as trophic transfer and biogeochemical cycling (Lafferty et al., 2008; Valiente-Banuet et al., 2015), might arguably represent better endpoints when inferring the consequences of plastic pollution on ecosystem functioning (Segner, 2011). Nevertheless, only few studies tested so far how NPs modulate biotic interactions. For instance, exposure to NPs can cause behavioural changes affecting the outcome of predator-prey interactions (Mattson et al., 2017). Recent reports indicate that host-parasite interactions can also be affected; a parasitic chytrid fungus displayed lower success infecting their phytoplankton hosts when exposed to NPs (Schampera et al., 2021), and similar results were found for trematodes infecting amphibians (Buss et al., 2021). Then, the virulence of pathogenic yeast in nematode host was higher in the NP treatment (Li et al., 2020) whereas the virulence of the DC virus in fruit flies was not affected (Jimenez-Guri et al., 2021). This all suggests that effects of NPs on host-parasite interactions can vary from system to system.

Parasitism represents a ubiquitous ecological interaction and the most widespread consumer lifestyle in nature (Lafferty et al., 2008). Host-parasite interactions mediate a significant part of the trophic links in the food webs (Amundsen et al., 2009) and they act as important drivers of co-evolution and diversification (Combes, 2005). It is hence important to extend our knowledge on how emerging pollutants like NPs affect this ecological interaction. According to the classic concept of the disease triangle (Stevens, 1960), the outcome of an infection is determined by the reciprocal interaction between host, parasite, and also their common external environment, in a variety of direct and indirect ways. Firstly, environmental conditions, such as pollution, can suppress host immune defences and make the host more susceptible to infection. For example, unlike NPs, other pollutants such as pesticides are well studied in this respect; exposure to pesticides increases host susceptibility to infection in amphibians (e.g. Gendron et al., 2003), oysters (e.g. Chu & Hale, 1994), fish (e.g. Kreutz et al., 2010), and crustacean (e.g. Coors et al., 2008). Secondly, pollutants can also directly reduce parasite fitness via toxicity (Cuco et al., 2018; Ortiz-Cañavate et al., 2019). Lastly, suboptimal host conditions might result in decreased host densities, subsequently reducing parasite transmission (Lafferty & Holt, 2003).

The planktonic crustacean *Daphnia* plays a key role in the trophic structure of aquatic food webs, being the principal grazer of phytoplankton and serving as main prey for planktivorous fish (Lampert, 2011). Moreover, their high amenability to experimentation as well as rapid responses to environmental changes made *Daphnia* an iconic model organism in physiology, ecology, toxicology, and evolutionary biology (Reynolds, 2011; Seda & Petrusek, 2011; Miner et al., 2012). In their natural environment, *Daphnia* are constantly attacked by a number of microparasites (Green, 1974; Ebert, 2005; Wolinska et al., 2009) and within the last twenty years, *Daphnia* became additionally recognised as a model system for studying epidemiological questions (Ebert, 2005; Ebert, 2008). Still, while there have been dozens of experimental studies exploring the consequences of micro- and nanoplastic exposure on *Daphnia* fitness (e.g. Eltemsah & Bohn, 2019; Kelpsiene et al., 2020), the question of whether and how the exposure to NPs affects *Daphnia*'s susceptibility to infection is poorly investigated. One study has shown that *Daphnia* display elevated immune responses (upregulation of haemocytes) when exposed to microplastic particles (Sadler et al., 2019), although no exposure to parasites was applied. Triggered immune responses when exposed to either micro- or nanoplastics have also been reported in molluscs (Capolupo et al., 2018; Détrée & Gallardo-Escárate, 2018; Von Moos et al., 2012), fish (Veneman et al., 2017; Espinosa et al., 2017; Jin et al., 2018), sea urchin (Murano et al., 2021), and earthworms (Rodriguez-Seijo et al., 2017), suggesting that NPs might indirectly hamper or, alternatively, favour infection (Auguste et al., 2020), though direct experimental tests are lacking.

To gain more insights into the ecological consequences of plastic pollution on disease outcome and test the hypothesis that NP exposure will decrease infection success of the parasite, we performed an experimental study on water fleas (*Daphnia)*, as a model host species. Individual *Daphnia* were exposed to two different NP concentrations or a control medium and, simultaneously, to spores of the

parasitic yeast *Metschnikowia bicuspidata* or a placebo inoculum. We evaluated parasite infectivity and reproductive output as proxies of parasite fitness. We also monitored life history traits of the host to test the prediction that *Daphnia* fitness will decrease when simultaneously challenged by NPs and parasites as multiple stressors.

2. Methods

2.1 Study organisms

2.1.1 Parasite

The yeast *Metschnikowia bicuspidata* (hereafter referred to as *Metschnikowia*) is an obligate parasite commonly infecting lake and pond *Daphnia* populations (Duffy et al., 2010; Wolinska et al., 2011). It reduces both lifespan and fecundity of its host (Cáceres et al., 2006; Lohr et al., 2010; Hesse et al., 2012). The parasite strain (METS_AMME_2008) used in the experiment was isolated in 2008 from Ammersee, Germany, to be then routinely cultured on *Daphnia magna*, genotype E17:07 (*Metschnikowia* is a generalist parasite, which makes it possible to culture on various *Daphnia* species; *D. magna* was used for its large size, whose infection yields a high number of parasite spores). *Daphnia* become infected by ingesting *Metschnikowia* spores. The needle-shaped ascospores pierce through the gut epithelium, from which they can reach the haemolymph in the body cavity. Once there, the fungal development cycle triggers, and parasite multiplication leads to an accumulation of spores all throughout the body cavity, which leads to host death. Decomposition or mechanical damage to the carapace is necessary for mature ascospores to be released into the water and infect other hosts (Green, 1974; Metschnikoff, 1884).

2.1.2 Host

The *Daphnia longispina* × *galeata* hybrid (genotype AMME_51) was also isolated from Ammersee, in 2008. The clonal culture was kept in synthetic SSS-medium (Saebelfeld et al., 2017) at 19°C under a 12:12h light-dark photoperiod. *Daphnia* were fed three times a week (every second or third day) with the green algae *Scenedesmus obliquus* (maintained as continuous cultures at 19°C, under constant light). Before the experiment, *Daphnia* were scaled-up by daily feeding (1 mg C/L of *S. obliquus*; the correlation between optical density and carbon content was used to determine the appropriate feeding volumes). In order to obtain a large number of synchronised experimental juveniles, ten glasses with 200 mL medium, each containing fifteen to twenty adult *Daphnia*, were kept for two generations. Every second day, the juveniles were counted and removed until the onset of the experiment.

2.2 Nanoplastic media

Spherical polystyrene particles with a nominal diameter of 100 nm and tagged with fluorescent markers were purchased as a suspension in water with a concentration of 10 g/L (Micromod Partikeltechnologie GmbH, Germany, product code: 29-00-102, product name: micromer®-greenF). Two concentrations of nanoplastics were prepared in the SSS-medium as exposure treatments: 5 mg/L (i.e. "low") and 20 mg/L (i.e. "high"). The control (i.e. "zero") treatment was prepared without any addition of NPs. To allow for chemical equilibrium of the mixtures, the resulting media was incubated in the experimental jars at 19°C in darkness for 24 hours prior to transferring *Daphnia*.

2.3 Experimental design and procedures

Daphnia juveniles born within 48 hours were collected and used as experimental individuals. On experimental day 1, juveniles were individually distributed across 150 experimental jars (5 mL medium in 30 mL glass jars) following a full-factorial design: 2 parasite treatments (inoculated with the parasite *Metschnikowia* or with a placebo) × 3 NP treatments (0 mg/L, 5 mg/L, 20 mg/L) × 25 replicates.

On day 3 of the experiment, individual jars of the parasite treatments were inoculated with a concentration of 1000 spores/mL. To do so, pre-infected *D. magna* from the parasite stock cultures were crushed in SSS-medium to obtain a suspension of spores. The spore concentration was determined by loading 10 µl of the resulting suspension on an improved Neubauer counting chamber to estimate the spore concentration and calculate the inoculation volume to achieve the desired final spore concentration (Manzi et al., 2020). To account for any confounding effect of the added spore suspension (e.g. the presence of *Daphnia* tissue or bacteria), a placebo suspension was prepared using uninfected *D. magna* and distributed to the control treatments.

Throughout the experiment (except for the parasite inoculation day) *Daphnia* were fed daily with 0.5 mg C/L of *S. obliquus*. On day 4, *Daphnia* were transferred to a fresh medium, and the volume was increased from 5 mL to 10 mL per jar. From this point onwards, *Daphnia* were transferred to a new medium every four days. *Daphnia* were checked daily for mortality and for the counting and removal of the offspring from the experimental jars. Dead *Daphnia* from the parasite-inoculated treatments were preserved from day 11 onwards; i.e. starting from day 8 post-inoculation, as mature spores cannot be observed earlier (Stewart-Merrill & Cáceres, 2018). The samples were fixed in 3.7% formaldehyde and stored at 4°C, in order to later determine the proportion of successfully infected animals and the number of parasite spores produced per infected individual. The animals from the *Metschnikowia* treatment that survived until the end of the experiment were also preserved. The identities of all preserved samples were blinded before analysis. The experiment was terminated on day 29, as no further mortality was observed for 5 consecutive days in any of the treatments.

2.4 Data analysis

Data was analysed using R (version 4.0.3) (R Core Team, 2020). Graphical outputs were produced using the ggplot2 (Wickham, 2016) and ggpubr (Kassambaraype, 2017) package. Analysis of variance (ANOVAs) was performed with the car package (Fox et al., 2017) using type II sums-of-squares. ANOVA assumptions of normal distribution and homoscedasticity were verified by visual inspection of the residuals, using quantile-quantile plots. Effect sizes were measured as the proportion of variance explained by each factor. The animals that died before day 3 (parasite inoculation day) were removed from all analyses, as their death could not be attributed to the effect of the treatments (3 individuals). Individuals that were lost due to handling errors were also removed (5 individuals; Figure S1).

2.4.1 Parasite fitness

Host viability (the proportion of inoculated hosts that survived until at least day 9 post-inoculation, i.e. when ascospores are usually first observed), parasite infectivity (the proportion of inoculated hosts that became successfully infected), and parasite reproduction (number of spores upon host death per successfully infected host) were used to characterise parasite fitness. Host viability and parasite infectivity were analysed using binomial logistic regression with *NP concentration* as the explanatory variable. Parasite reproduction was analysed using a linear model with *NP concentration* as a fixed factor. All inoculated individuals were used for analysing host viability. Then, for analysing parasite infectivity, 'early deaths' (i.e. the individuals that died before day 9 post-exposure; before infection symptoms could be visually confirmed) were excluded, whereas for analysing parasite reproduction only the successfully infected individuals were selected (Figure S2).

2.4.2 Host fitness

Host lifespan (individuals which survived until the last experimental day were considered to have died on that day), the proportion of individuals that reached maturity, and host fecundity (i.e. total number of juveniles) were measured to characterise host fitness. Host lifespan and host fecundity were analysed using linear models with *NP concentration* and *Infection* as fixed factors. The proportion of individuals that reached maturity was analysed using a binomial logistic regression with both *NP concentration* and *Infection* as the explanatory variables. For host lifespan and maturity, individuals from the control treatment were compared with successfully infected ones from the infection treatments. The same applied for host fecundity, but only animals that reproduced at least once were included. In all analyses of host fitness parameters, 'early deaths' within the infection treatment were included with successfully infected *Daphnia* to ensure comparability of host fitness variables across the 'infected' and 'control'

treatments. Otherwise, excluding 'early deaths' from infection treatments would have resulted in an underestimation of parasite-related reduction of host fitness (for comparison, see Figure S3 & Figure 2).

3. Results

3.1 Parasite fitness

Initially, 75 *Daphnia* were inoculated with the parasite. Three of these individuals were lost due to handling errors or died before parasite inoculation was completed (day 3) and thus were excluded from every analysis. Eight out of 72 died before day 9 post-inoculation (and were therefore categorised as *early deaths*, Figure S2). Overall, host viability decreased with increasing NP concentrations; all individuals from the zero-NP treatment survived until day 9 post-inoculation whereas about 25% of the individuals died under high-NP concentration (Figure 1A, Figure S1; significant effect of *NP concentration*, Table 1). Parasite infectivity increased under NP exposure. Less than 50% of available hosts became successfully infected in the zero-NP treatment, whereas about 75% and 80% of available individuals in the low- and high-NP treatment did (Figure 1B; significant effect of *NP concentration*, Table 1). By contrast, parasite reproduction was three-to-four times lower under the high-NP treatment, in comparison to the zero-NP and low-NP groups (Figure 1C; significant effect of *NP concentration*, explaining 39% of the variance, Table 1).

Table 1. *ANOVA* (F-test or $\chi 2$ test) testing for fixed effects of NP concentration on life history parameters influencing parasite success (host viability, parasite infectivity, and reproduction). Significant *P*-values (< 0.05) are marked in bold.

Response variable (dataset)	*Distribution (link function)*	*Explanatory variables*	*Statistic (Degrees of freedom)*	*P-value*	*% Variance explained*
Host viability (0 = died before day 9 | 1 = survived)	Binomial (link: logit)	NP concentration	$\chi^2_{(2;69)} = 10.06$	< **0.001**	
Parasite infectivity (0 = no infection | 1 = infection)	Binomial (link: logit)	NP concentration	$\chi^2_{(2;61)} = 7.67$	< **0.05**	
Parasite reproduction (only non-zero value for spore yield were included)	Normal	NP concentration	$F_{(2;38)} = 12.14$	< **0.001**	38.98

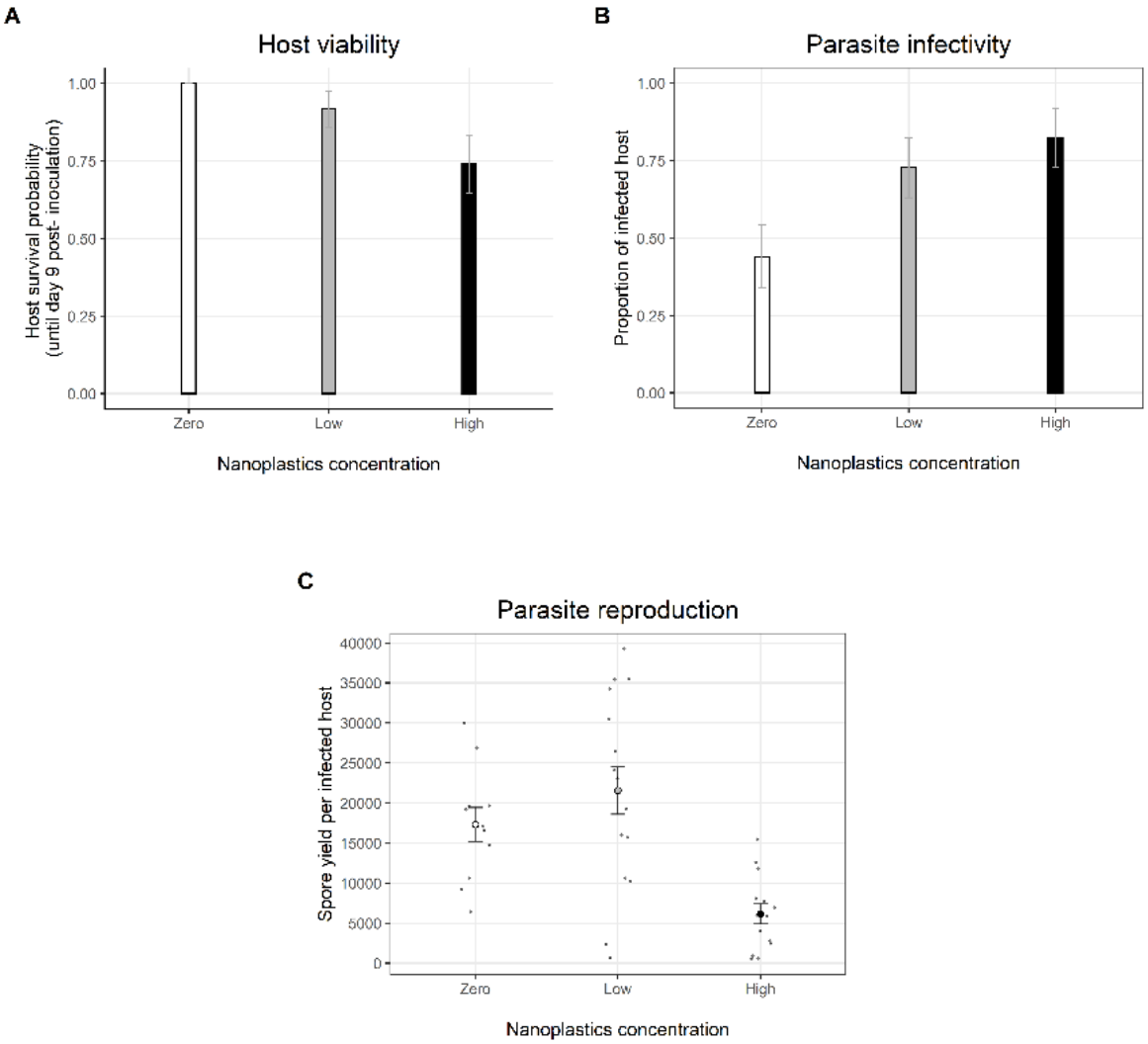


Figure 1. Parasite fitness traits under three NP concentrations: zero (0 mg/L), low (5 mg/L), and high (20 mg/L). **A)** The proportion of host *Daphnia* individuals that survived until day 9 post-inoculation (i.e. the earliest day when the parasite completed its life cycle). **B)** The proportion of host *Daphnia* individuals that were successfully infected by the parasite, and **C)** Spore yield per infected host. Error bars represent the standard error of the mean.

3.2 Host fitness

Daphnia lifespan was reduced by about 10 days under the infection treatment, across all NP concentrations (Figure 2A; significant effect of *Infection*, explaining 52% of the variance, Table 2). Both control and infected *Daphnia* exposed to high-NP concentrations died about 4 to 5 days earlier than *Daphnia* exposed to low-NP concentrations (Figure 2A). The proportion of individuals that reached maturity was lower for infected *Daphnia*, but only under low- and high-NP treatments (Figure 2B; significant *NP concentration* × *Infection* interaction, Table 2). Specifically, 100% of control individuals, but only about 70% of infected individuals reached maturity under low-NP exposure. These proportions were reduced to about 80% and 55% under high-NP treatment, respectively. Under zero-NP condition, there were no differences in the proportion of animals reaching maturity between control and infected *Daphnia* (about 80% in both). Host fecundity was strongly reduced under the infection treatment across all NP concentrations; control individuals produced 8 to 12 offspring whereas infected ones produced 2 to 3 offspring on average (Figure 3A; *Infection* explained 61% of the variance, Table 2). Within control individuals, *Daphnia* under low-NP treatment produced 3 to 4 offspring more than *Daphnia* under zero-NP or high-NP treatments (significant *NP concentration* × *Infection* interaction, Table 2).

Table 2. ANOVA (F-test or $\chi 2$ test) testing for fixed effects of NP concentration, Infection, and their interaction on life history parameters of the host (host lifespan, maturity, and fecundity). Significant *P*-values (< 0.05) are marked in bold.

Response variable (Dataset)	*Distribution (Link function)*	*Explanatory variables*	*Statistic (Degrees of freedom)*	*P-value*	*% Variance explained*
Host lifespan (control/ successfully infected and early death)	Normal	NP concentration	$F_{(2;113)} = 7.63$	< **0.001**	5.65
		Infection	$F_{(1;113)} = 139.56$	< **0.001**	51.68
		NP × Infection	$F_{(2;113)} = 1.10$	0.335	0.82
Host maturity (control/ successfully infected and early death)	Binomial (link: logit)	NP concentration	$\chi^2_{(2;113)} = 3.77$	0.15	
		Infection	$\chi^2_{(1;113)} = 7.73$	< **0.01**	
		NP × Infection	$\chi^2_{(2;113)} = 6.14$	< **0.05**	
Host fecundity (control/ successfully infected and early death -only those that reproduced at least once-)	Normal	NP concentration	$F_{(2;87)} = 6.64$	< **0.01**	4.73
		Infection	$F_{(1;87)} = 171.14$	< **0.001**	61.02
		NP × Infection	$F_{(2;87)} = 4.52$	< **0.05**	3.23

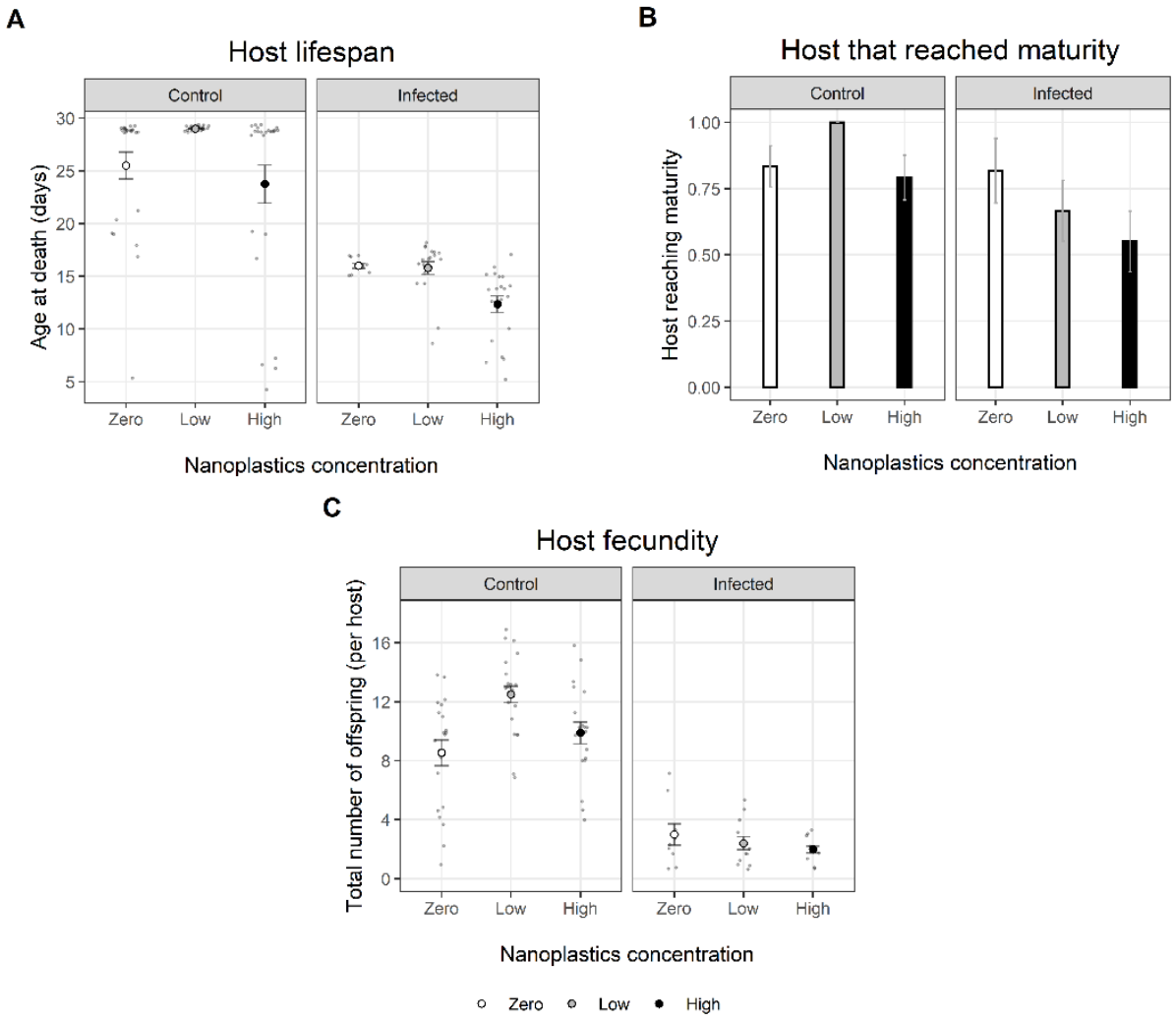


Figure 2. Host fitness traits in the parasite-inoculated (Infected) and Control groups under three NP concentrations: zero (0 mg/L), low (5 mg/L), and high (20 mg/L). **A)** Age at death of the individual *Daphnia*, **B)** Proportion of *Daphnia* that reached maturity, and **C)** Total number of offspring produced in the lifetime of a *Daphnia* individual. Infected group consists of successfully infected individuals and those that died before day 9 post-inoculation (as infection status could not be reliably assessed before that day). Error bars represent the standard error of the mean.

4. Discussion

In order to characterise the true impact of pollutants on ecological processes and ecosystem functioning, it is necessary to look beyond their effects on the fitness of individual species and additionally investigate how pollutants affect the way species interact with each other. Even though the effects of nanoplastics (NPs) on individual species have been thoroughly investigated, their effects on biotic interactions remain largely unknown. Considering the importance of host-parasite interactions and their role in ecosystem dynamics and structure (Amundsen et al., 2009), it is crucial to include parasitism when examining the ecological consequences of plastic pollution. Our study demonstrates profound negative effects of high concentrations of NPs (i.e. 20 mg/L) on disease outcome, including a 25% reduction in chances of hosts surviving until successful parasite reproduction, and a four-fold reduction in parasite spore yield under high NP concentrations. Moreover, infected hosts additionally exposed to NPs showed shorter lifespans and were less likely to reach maturity than those infected in the absence of NPs.

4.1 Parasite fitness

In the *Daphnia-Metschnikowia* system, three conditions need to be met to grant parasite transmission, upon encounter with a susceptible host: i) the parasite needs to enter and colonise the host (i.e. reach the haemolymph *via* the gut), ii) the host has to live long enough for the parasite to complete its infection cycle (production of mature asci generally takes > 8 days in this system; prior death of the host does not allow horizontal transmission), iii) host defences have to be overcome, and the parasite has to successfully establish and reproduce within the host, leading to possible transmission. These three conditions may be differently affected by exposure to NPs, thus modulating disease dynamics and shifting parasite transmission.

Firstly, significantly higher levels of early host mortality (i.e. host viability) were recorded in the high NP treatment. Decreased host lifespan directly correlates with the production of *Metschnikowia* spores. Specifically, if a host dies before the parasite cycle can be completed, this effectively leads to infection "failure" and a null spore output. If the host does survive beyond that point and the parasite starts to reproduce, then its reproductive output is positively correlated with host age (Ebert et al., 2004). Hence, negative effects of NPs on host lifespan may reflect on parasite performance, by decreasing its reproductive output. In other words, suboptimal conditions caused by particularly high levels of NP pollution might reduce the infection success and alter disease dynamics.

Secondly, entrance and colonisation of the host are crucial for a successful infection. We observed higher proportion of individuals becoming successfully infected in both NP treatments, in comparison to controls. Other investigated stressors, such as copper, are known to increase filtering rate

and, thus, parasite consumption rate in *Daphnia* (Civitello et al., 2012). Moreover, filtering rate of *Daphnia* is known to be boosted in nutritionally stressed individuals (Lampert & Brendelberger, 1996), resulting in enhanced parasite spore consumption (Dallas et al., 2016). *Daphnia*'s digestive tract can be blocked when exposed to micro- or nanoplastics (De Felice et al., 2019; An et al., 2021), which may indeed result in nutritional stress. Accumulation of particles in the gut is further known to cause inflammatory responses (Silva et al., 2021; Pirsaheb et al., 2020), possibly making it easier for the spores to penetrate the gut. However, while the host seemed to be more prone to get infected when exposed to NPs, this effect was counterbalanced by a decrease in host viability, which was especially pronounced under the high NP concentration (20 mg/L).

Lastly, provided that completion of the parasite's life cycle is ensured, environmental conditions such as suboptimal host diet or elevated temperature may still modulate the parasite's effective reproductive output (Manzi et al., 2020; Pulkkinen & Ebert, 2004). In our experiment, parasite spore yield was significantly reduced in the presence of the high NP concentration. A possible explanation could be that the parasite's reproductive cycle inside the host might be directly disrupted by NP particles. The NPs can potentially penetrate the fungal spores causing toxicity, as NPs' internalisation into living cells is well proven (Liu et al., 2021). Additionally, NPs in *Daphnia* haemolymph have been shown to be responsible for upregulation of haemocytes (Sadler et al., 2019), which is the primary mechanism of defence against *Metschnikowia* infections (Metschnikoff, 1884; Stewart Merrill & Cáceres, 2018). Assuming that NPs may enter the host haemolymph prior to parasite settlement, such earlier activated immune systems might affect subsequent infection in a similar manner to the phenomenon of immune priming caused by sequential infections (i.e. by other microparasites). Moreover, NPs may have promoted additional mechanisms of host immunity, which may not directly be involved in the defence against *Metschnikowia* (e.g. stimulation of phenoloxydase activity, Mucklow & Ebert, 2003), contributing to a dispersing of host resources that could otherwise be used by the parasite for its growth.

Either way, decreased total spore production within individual hosts should lead to reduced parasite transmission. However, it is necessary to consider how this finding relates to natural conditions. Indeed, negative effects of NPs on parasite growth only occurred in the high NP treatment (20 mg/L), while the lower dose tested in this experiment (5 mg/L) either resulted in positive (increased infectivity) or neutral (spore yield) effects on the parasite's fitness components.

4.2 Host fitness

As expected, *Metschnikowia* infection severely decreased host fitness (Cáceres et al., 2006; Hesse et al., 2012). A combination of high host mortality and strongly reduced reproduction was observed in the infected treatments, regardless of NP exposure. However, NPs alone did not reduce host fitness

components, characterised as host lifespan, the likelihood to reach maturity, or fecundity. This was inconsistent with our expectation that NPs alone would reduce host fitness (Rist et al., 2017; Lin et al., 2019).

In addition, a slight boost to all host fitness traits showed that low doses of NPs can appear beneficial, suggesting a hormetic effect, a commonly observed phenomenon characterised by a low-dose stimulation and a high-dose inhibition in dose-response models (Stanley et al., 2013). According to a recent review summarising the dose effect in micro- and nanoplastic studies, there are about 300 studies supporting the idea of a threshold or a hormetic dose-response model (Agathokleous et al., 2021). Evidence of a hormetic effect has also been recorded for *Daphnia;* for example, when *Daphnia pulex* was tested under environmentally relevant concentrations of microplastics (1 µg/L), toxic effects appeared only in the F2 generation, whereas NPs promoted fitness of F0 and F1 generations (Liu et al., 2020). A similar hormetic effect of plastic particles has been observed in crabs (Liu et al., 2019), algae (Gunasekaran et al., 2020), and oysters (Gardon et al., 2020). Even though in our study we could not identify the toxicological threshold, our results provide an initial estimation that the threshold dose should be between 5 mg/L and 20 mg/L in this system.

NPs and parasite infection seem to have a synergistic effect on *Daphnia*. The combination of both stressors harms the host especially in the high NP treatment, where almost half of the individuals did not reach maturity. Such a phenomenon could be relevant for these natural populations where NP concentrations are higher than usual (i.e. direct disposal sites), possibly leading to decreased host population densities. This raises the risk of extinction (Ebert et al., 2000), and changes in *Daphnia* densities might intensely affect trophic interactions. Moreover, increased mortality before reaching maturity may affect transmission of other types of parasites present in the ecosystem, particularly vertically transmitted ones. For instance, in a previous *Daphnia*-parasite study, other types of pollutants, such as the insecticide carbaryl, greatly reduced *Hamiltosporidium magnivora'*s fitness, a vertically transmitted microsporidium (Coors et al., 2008).

Considerably, to put our findings into an environmental context, it will be important to improve analytical methods that estimate environmentally relevant NP concentrations. Moreover, our findings were obtained from a single clone of *D. galeata* × *longispina*. Experiments testing different host genotypes (and species) are needed to generalise our results. Also, other parasites and types of parasite transmission (vertical vs horizontal) should be tested, to build upon these findings.

4.3 Conclusion

This study further demonstrates the need to consider biotic interactions when assessing potential risks of anthropogenic factors to the environment. As illustrated in the concept of the disease triangle, disease

severity is determined by the parasites' virulence, the hosts' susceptibility, and the environment. We have shown that altered environmental conditions caused by NP pollution may affect disease dynamics within *Daphnia* populations. Specifically, exposure to nanoplastics heavily affected parasite reproduction under the high-NP treatment. Even though parasite infectivity increased in the NP-exposed treatments, the parasite advantage was counter-balanced by increased host mortality. In this particular system, the effects of NPs seem to affect the parasite more negatively than the host itself, highlighting a need of including the species interaction perspective into the investigations of true effects of micro- and nanoplastics pollution on natural systems.

Data accessibility

The data supporting this study can be found at: https://doi.org/10.5281/zenodo.5512138

Author's contributions

JW conceived the presented idea. All authors designed the study. JW, FM, RA and CS coordinated and supervised the study. SM and NA equally carried out the experimental procedure. SM carried out the data visualization and formal analysis, FM critically directed it and all authors validated the results. SM drafted the manuscript and JW, FM, RA and CS critically revised every version. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests

We declare that we have no competing interests.

Acknowledgements

We would like to thank Ursula Newen for the maintenance of *Daphnia* cultures and Mareike Brehm for helping us during the experimental procedure. We also thank Lyn Westphal for linguistic help.

Funding

This work was supported by the Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany. S.M. received a grant from the Erasmus+ internship programme (Contract no.: UCY-SMP-2020-002).

References

Agathokleous, E., Iavicoli, I., Barceló, D., & Calabrese, E. J. (2021). Micro/nanoplastics effects on organisms: A review focusing on 'dose'. *Journal of Hazardous Materials*, 126084. https://doi.org/10.1016/j.jhazmat.2021.126084

Amundsen, P. A., Lafferty, K. D., Knudsen, R., Primicerio, R., Klemetsen, A., & Kuris, A. M. (2009). Food web topology and parasites in the pelagic zone of a subarctic lake. *Journal of Animal Ecology*, *78*(3), 563-572. https://doi.org/10.1111/j.1365-2656.2008.01518.x)

An, D., Na, J., Song, J., & Jung, J. (2021). Size-dependent chronic toxicity of fragmented polyethylene microplastics to *Daphnia magna*. *Chemosphere*, *271*, 129591. https://doi.org/10.1016/j.chemosphere.2021.129591)

Andrady, A. L. (2003). Plastics and the Environment. Hoboken New Jersey: John Wiley & sons inc. (ISBN: 0-471-09520-6)

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, *62*(8), 1596-1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Auguste, M., Lasa, A., Balbi, T., Pallavicini, A., Vezzulli, L., & Canesi, L. (2020). Impact of nanoplastics on hemolymph immune parameters and microbiota composition in *Mytilus galloprovincialis*. *Marine Environmental Research*, *159*, 105017. https://doi.org/10.1016/j.marenvres.2020.105017

Bergami, E., Pugnalini, S., Vannuccini, M. L., Manfra, L., Faleri, C., Savorelli, F., Dawson, K. A., & Corsi, I. (2017). Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*. *Aquatic Toxicology*, *189*, 159-169. https://doi.org/10.1016/j.aquatox.2017.06.008

Bhattacharya, P., Lin, S., Turner, J. P., & Ke, P. C. (2010). Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. *The Journal of Physical Chemistry C*, *114*(39), 16556-16561. https://doi.org/10.1021/jp1054759

Brown, D. M., Wilson, M. R., MacNee, W., Stone, V., & Donaldson, K. (2001). Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicology and Applied Pharmacology*, *175*(3), 191-199. https://doi.org/10.1006/taap.2001.9240

Buss, N., Sander, B., & Hua, J. (2021). Effects of Polyester Microplastic Fiber Contamination on Amphibian–Trematode Interactions. *Environmental Toxicology and Chemistry*, 1–11. https://doi.org/10.1002/etc.5035

Cáceres, C. E., Hall, S. R., Duffy, M. A., Tessier, A. J., Helmle, C., & MacIntyre, S. (2006). Physical structure of lakes constrains epidemics in *Daphnia* populations. *Ecology*, *87*(6), 1438-1444. https://doi.org/10.1890/0012-9658(2006)87[1438:PSOLCE]2.0.CO;2

Capolupo, M., Franzellitti, S., Valbonesi, P., Lanzas, C. S., & Fabbri, E. (2018). Uptake and transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean mussel *Mytilus galloprovincialis*. *Environmental Pollution*, *241*, 1038-1047. https://doi.org/10.1016/j.envpol.2018.06.035

Casado-Gavalda, M. P., Macken, A., & Byrne, H. J. (2013). Ecotoxicological assessment of silica and polystyrene nanoparticles assessed by a multitrophic test battery. *Environment International*, *51*, 97–105. https://doi.org/10.1016/j.envint.2012.11.001

Chae, Y., Kim, D., Kim, S. W., & An, Y. J. (2018). Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. *Scientific Reports*, *8*(1), 1-11. https://doi.org/10.1038/s41598-017-18849-y

Chu, F. L., & Hale, R. C. (1994). Relationship between pollution and susceptibility to infectious disease in the eastern oyster, *Crassostrea virginica*. *Marine Environmental Research*, *38*(4), 243-256. https://doi.org/10.1016/0141-1136(94)90026-4

Civitello, D. J., Forys, P., Johnson, A. P., & Hall, S. R. (2012). Chronic contamination decreases disease spread: a *Daphnia*–fungus–copper case study. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1741), 3146-3153. https://doi.org/10.1098/rspb.2012.0684

Combes, C. (2005). The Art of Being a Parasite Chicago: University of Chicago Press https://doi.org/10.7208/9780226778723

Coors, A., Decaestecker, E., Jansen, M., & De Meester, L. (2008). Pesticide exposure strongly enhances parasite virulence in an invertebrate host model. *Oikos*, *117*(12), 1840-1846. https://doi.org/10.1111/j.1600-0706.2008.17028.x

Cuco, A. P., Castro, B. B., Gonçalves, F., Wolinska, J., & Abrantes, N. (2018). Temperature modulates the interaction between fungicide pollution and disease: evidence from a *Daphnia*-microparasitic yeast model. *Parasitology*, *145*(7), 939-947. https://doi.org/10.1017/S0031182017002062

Détrée, C., & Gallardo-Escárate, C. (2018). Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel *Mytilus galloprovincialis*. *Fish & Shellfish Immunology*, *83*, 52-60. https://doi.org/10.1016/j.fsi.2018.09.018

Dallas, T., Hall, R. J., & Drake, J. M. (2016). Competition-mediated feedbacks in experimental multispecies epizootics. *Ecology*, *97*(3), 661-670. https://doi.org/10.1890/15-0305

De Felice, B., Sabatini, V., Antenucci, S., Gattoni, G., Santo, N., Bacchetta, R., Ortenzi, M. A., & Parolini, M. (2019). Polystyrene microplastics ingestion induced behavioral effects to the cladoceran *Daphnia magna*. *Chemosphere*, *231*, 423-431. https://doi.org/10.1016/j.chemosphere.2019.05.115

Duffy, M. A., Cáceres, C. E., Hall, S. R., Tessier, A. J., & Ives, A. R. (2010). Temporal, spatial, and between-host comparisons of patterns of parasitism in lake zooplankton. *Ecology*, *91*(11), 3322-3331. https://doi.org/10.1890/09-1611.1

Ebert, D., Joachim Carius, H., Little, T., & Decaestecker, E. (2004). The evolution of virulence when parasites cause host castration and gigantism. *The American Naturalist*, *164*(S5), S19-S32. https://doi.org/10.1086/424606

Ebert, D., Lipsitch, M., & Mangin, K. L. (2000). The effect of parasites on host population density and extinction: experimental epidemiology with *Daphnia* and six microparasites. *The American Naturalist*, *156*(5), 459-477. https://doi.org/10.2307/3079051

Ebert, D. (2005). Ecology, Epidemiology, and Evolution of Parasitism in *Daphnia*. Bethesda (MD): National Center for Biotechnology Information (US) (ISBN: 1932811060)

Ebert, D. (2008). Host–parasite coevolution: insights from the *Daphnia*–parasite model system. *Current Opinion in Microbiology*, *11*(3), 290-301. https://doi.org/10.1016/j.mib.2008.05.012

Eltemsah, Y. S., & Bøhn, T. (2019). Acute and chronic effects of polystyrene microplastics on juvenile and adult *Daphnia magna*. *Environmental Pollution*, *254*, 112919. https://doi.org/10.1016/j.envpol.2019.07.087

Espinosa, C., Cuesta, A., & Esteban, M. Á. (2017). Effects of dietary polyvinylchloride microparticles on general health, immune status and expression of several genes related to stress in gilthead seabream (*Sparus aurata* L.). *Fish & Shellfish Immunology*, *68*, 251-259. https://doi.org/10.1016/j.fsi.2017.07.006

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., …Heiberger, R. (2012). Package 'car'. Vienna: R Foundation for Statistical Computing.

Gardon, T., Morvan, L., Huvet, A., Quillien, V., Soyez, C., Le Moullac, G., & Le Luyer, J. (2020). Microplastics induce dose-specific transcriptomic disruptions in energy metabolism and immunity of the pearl oyster *Pinctada margaritifera*. *Environmental Pollution*, *266*, 115180. https://doi.org/10.1016/j.envpol.2020.115180

Gendron, A. D., Marcogliese, D. J., Barbeau, S., Christin, M. S., Brousseau, P., Ruby, S., Cyr, D., & Fournier, M. (2003). Exposure of leopard frogs to a pesticide mixture affects life history characteristics of the lungworm *Rhabdias ranae*. *Oecologia*, *135*(3), 469-476. https://doi.org/10.1007/s00442-003-1210-y

Green, J. (1974). Parasites and epibionts of Cladocera. *The Transactions of the Zoological Society of London*, *32*(6), 417-515. https://doi.org/10.1111/j.1096-3642.1974.tb00031.x

Gunasekaran, D., Chandrasekaran, N., Jenkins, D., & Mukherjee, A. (2020). Plain polystyrene microplastics reduce the toxic effects of ZnO particles on marine microalgae *Dunaliella salina*. *Journal of Environmental Chemical Engineering*, *8*(5), 104250. https://doi.org/10.1016/j.jece.2020.104250

Hesse, O., Engelbrecht, W., Laforsch, C., & Wolinska, J. (2012). Fighting parasites and predators: How to deal with multiple threats? *BMC Ecology*, *12*(1), 1-8. https://doi.org/10.1186/1472-6785-12-12

Jimenez-Guri, E., Roberts, K. E., García, F. C., Tourmente, M., Longdon, B., & Godley, B. J. (2021). Transgenerational effects on development following microplastic exposure in *Drosophila melanogaster*. *PeerJ*, *9*, e11369. https://doi.org/10.7717/peerj.11369

Jin, Y., Xia, J., Pan, Z., Yang, J., Wang, W., & Fu, Z. (2018). Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. *Environmental Pollution*, *235*, 322-329. https://doi.org/10.1016/j.envpol.2017.12.088

Kassambaraype A. (2017). ggplot2' Based Publication Ready Plots Version.

Kelpsiene, E., Torstensson, O., Ekvall, M. T., Hansson, L. A., & Cedervall, T. (2020). Long-term exposure to nanoplastics reduces life-time in *Daphnia magna*. *Scientific Reports*, *10*(1), 1-7. https://doi.org/10.1038/s41598-020-63028-1

Klaine, S. J., Koelmans, A. A., Horne, N., Carley, S., Handy, R. D., Kapustka, L., Nowack, B., & von der Kammer, F. (2012). Paradigms to assess the environmental impact of manufactured nanomaterials. Environmental Toxicology and Chemistry, *31*, 3–14. https://doi.org/10.1002/etc.733

Koelmans, A. A., Besseling, E., & Shim, W. J. (2015). Nanoplastics in the aquatic environment. Critical review. *Marine Anthropogenic Litter*, 325-340. https://doi.org/10.1007/978-3-319-16510-3

Kreutz, L. C., Barcellos, L. J. G., Marteninghe, A., Dos Santos, E. D., & Zanatta, R. (2010). Exposure to sublethal concentration of glyphosate or atrazine-based herbicides alters the phagocytic function and increases the susceptibility of silver catfish fingerlings (*Rhamdia quelen*) to *Aeromonas hydrophila* challenge. *Fish & Shellfish Immunology*, *29*(4), 694-697. https://doi.org/10.1016/j.fsi.2010.06.003

Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., De Leo, G., Dobson, A. P., Dunne, J. A., Johnson, P. T. J., Kuris, A. M., Marcogliese, D. J., Martinez, N. D., Memmott, J., Marquet, P. A., McLaughlin, J. P., Mordecai, E. A., Pascual, M., Poulin, R., & Thieltges, D. W. (2008). Parasites in food webs: The ultimate missing links. *Ecology Letters*, *11*, 533–546. https://doi.org/10.1111/j.1461-0248.2008.01174.x

Lampert, W., & Brendelberger, H. (1996). Strategies of phenotypic low-food adaptation in *Daphnia*: Filter screens, mesh sizes, and appendage beat rates. *Limnology and Oceanography*, *41*(2), 216-223. https://doi.org/10.4319/lo.1996.41.2.0216

Lin, W., Jiang, R., Hu, S., Xiao, X., Wu, J., Wei, S., Xiong, Y., & Ouyang, G. (2019). Investigating the toxicities of different functionalized polystyrene nanoplastics on *Daphnia magna*. *Ecotoxicology and Environmental Safety*, *180*, 509-516. https://doi.org/10.1016/j.ecoenv.2019.05.036

Li, D., Deng, Y., Wang, S., Du, H., Xiao, G., & Wang, D. (2020). Assessment of nanopolystyrene toxicity under fungal infection condition in *Caenorhabditis elegans*. *Ecotoxicology and Environmental Safety*, *197*, 110625. https://doi.org/10.1016/j.ecoenv.2020.110625

Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Chen, M., & Zhao, Y. (2019). Effects of microplastics on the innate immunity and intestinal microflora of juvenile *Eriocheir sinensis*. *Science of the Total Environment*, *685*, 836-846. https://doi.org/10.1016/j.scitotenv.2019.06.265

Liu, Z., Cai, M., Wu, D., Yu, P., Jiao, Y., Jiang, Q., & Zhao, Y. (2020). Effects of nanoplastics at predicted environmental concentration on *Daphnia pulex* after exposure through multiple generations. *Environmental Pollution*, *256*, 113506. https://doi.org/10.1016/j.envpol.2019.113506

Liu, L., Xu, K., Zhang, B., Ye, Y., Zhang, Q., & Jiang, W. (2021). Cellular internalization and release of polystyrene microplastics and nanoplastics. *Science of the Total Environment*, *779*, 146523. https://doi.org/10.1016/j.scitotenv.2021.146523

Lohr, J. N., Yin, M., & Wolinska, J. (2010). Prior residency does not always pay off–co-infections in *Daphnia*. *Parasitology*, *137*(10), 1493-1500. https://doi.org/10.1017/S0031182010000296

Mattsson, K., Johnson, E. V., Malmendal, A., Linse, S., Hansson, L. A., & Cedervall, T. (2017). Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. *Scientific Reports*, *7*(1), 1-7. https://doi.org/10.1038/s41598-017-10813-0

Mattsson, K., Jocic, S., Doverbratt, I., & Hansson, L. A. (2018). Nanoplastics in the aquatic environment. Microplastic Contamination in Aquatic Environments: An Emerging Matter of Environmental Urgency. Elsevier Inc. 379–399 p. https://doi.org/10.1016/B978-0-12-813747-5.00013-8

Mucklow, P. T., & Ebert, D. (2003). Physiology of immunity in the water flea *Daphnia magna*: environmental and genetic aspects of phenoloxidase activity. *Physiological and Biochemical Zoology*, *76*(6), 836-842. https://doi.org/10.1086/378917

Lafferty, K. D., & Holt, R. D. (2003). How should environmental stress affect the population dynamics of disease? *Ecology Letters*, *6*(7), 654-664. https://doi.org/10.1046/j.1461-0248.2003.00480.x

Lampert, W. (2011). *Daphnia*: Development of a model organism in ecology and evolution. Book 21. (Ed. by O. Kinne). International Ecology Institute. (ISBN: 9783946729211)

Manzi, F., Agha, R., Lu, Y., Ben-Ami, F., & Wolinska, J. (2020). Temperature and host diet jointly influence the outcome of infection in a *Daphnia*-fungal parasite system. *Freshwater Biology*, *65*(4), 757-767. https://doi.org/10.1111/fwb.13464

Metschnikoff, E. (1884). A disease of *Daphnia* caused by a yeast. A contribution to the theory of phagocytes as agents for attack on disease-causing organisms. *Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin*, *96*, 177-195. https://doi.org/10.1055/s-0029-1209659

Miner, B. E., De Meester, L., Pfrender, M. E., Lampert, W., & Hairston Jr, N. G. (2012). Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1735), 1873-1882. https://doi.org/10.1098/rspb.2011.2404

Murano, C., Bergami, E., Liberatori, G., Palumbo, A., & Corsi, I. (2021). Interplay between nanoplastics and the immune system of the Mediterranean sea urchin *Paracentrotus lividus. Frontiers in Marine Science*, *8*, 234. https://doi.org/10.3389/fmars.2021.647394

Ortiz-Cañavate, B. K., Wolinska, J., & Agha, R. (2019). Fungicides at environmentally relevant concentrations can promote the proliferation of toxic bloom-forming cyanobacteria by inhibiting natural fungal parasite epidemics. *Chemosphere*, *229*, 18-21. https://doi.org/10.1016/j.chemosphere.2019.04.203

Pirsaheb, M., Hossini, H., & Makhdoumi, P. (2020). Review of microplastic occurrence and toxicological effects in marine environment: Experimental evidence of inflammation. *Process Safety and Environmental Protection*, *142*, 1-14. https://doi.org/10.1016/j.psep.2020.05.050

Pulkkinen, K., & Ebert, D. (2004). Host starvation decreases parasite load and mean host size in experimental populations. *Ecology*, *85*(3), 823-833. https://doi.org/10.1890/03-0185

Reynolds, C. S. (2011). Daphnia: Development of Model Organism in Ecology and Evolution-2011. *Freshwater Reviews*, *4*(1), 85-87. https://doi.org/10.1608/frj-4.1.425

Rist, S., Baun, A., & Hartmann, N. B. (2017). Ingestion of micro-and nanoplastics in *Daphnia magna*–Quantification of body burdens and assessment of feeding rates and reproduction. *Environmental Pollution*, *228*, 398-407. https://doi.org/10.1016/j.envpol.2017.05.048

Rodriguez-Seijo, A., Lourenço, J., Rocha-Santos, T. A. P., Da Costa, J., Duarte, A. C., Vala, H., & Pereira, R. (2017). Histopathological and molecular effects of microplastics in *Eisenia andrei* Bouché. *Environmental Pollution*, *220*, 495-503. https://doi.org/10.1016/j.envpol.2016.09.092

Sadler, D. E., Brunner, F. S., & Plaistow, S. J. (2019). Temperature and clone-dependent effects of microplastics on immunity and life history in *Daphnia magna*. *Environmental Pollution*, *255*, 113178. https://doi.org/10.1016/j.envpol.2019.113178

Saebelfeld, M., Minguez, L., Griebel, J., Gessner, M. O., & Wolinska, J. (2017). Humic dissolved organic carbon drives oxidative stress and severe fitness impairments in *Daphnia*. *Aquatic Toxicology*, *182*, 31-38. https://doi.org/10.1016/j.aquatox.2016.11.006

Salvati, A., Åberg, C., dos Santos, T., Varela, J., Pinto, P., Lynch, I., & Dawson, K. A. (2011). Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(6), 818-826. https://doi.org/10.1016/j.nano.2011.03.005

Schampera, C., Wolinska, J., Bachelier, J. B., de Souza Machado, A. A., Rosal, R., González-Pleiter, M., & Agha, R. (2021). Exposure to nanoplastics affects the outcome of infectious disease in phytoplankton. *Environmental Pollution*, *277*, 116781. https://doi.org/10.1016/j.envpol.2021.116781

Seda, J., & Petrusek, A. (2011). *Daphnia* as a model organism in limnology and aquatic biology: introductory remarks. *Journal of Limnology*, *70*(2), 337-344. https://doi.org/10.3274/JL11-70-2-17

Segner, H. (2011). Moving beyond a descriptive aquatic toxicology: The value of biological process and trait information. *Aquatic Toxicology*, *105*, 50–55. https://doi.org/10.1016/j.aquatox.2011.06.016

Setälä, O., Fleming-Lehtinen, V., & Lehtiniemi, M. (2014). Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution*, *185*, 77-83. https://doi.org/10.1016/j.envpol.2013.10.013

Silva, C. J., Silva, A. L. P., Campos, D., Soares, A. M., Pestana, J. L., & Gravato, C. (2021). *Lumbriculus variegatus* (oligochaeta) exposed to polyethylene microplastics: biochemical, physiological and reproductive responses. *Ecotoxicology and Environmental Safety*, *207*, 111375. https://doi.org/10.1016/j.ecoenv.2020.111375

Stanley, J. K., Perkins, E. J., Habib, T., Sims, J. G., Chappell, P., Escalon, B. L., Wilbanks, M., & Garcia-Reyero, N. (2013). The good, the bad, and the toxic: approaching hormesis in *Daphnia magna* exposed to an energetic compound. *Environmental Science & Technology*, *47*(16), 9424-9433. https://doi.org/10.1021/es401115q

Stevens, R.B. (1960). Cultural Practices in Disease Control. In *Plant Pathology*, 357–429. Academic Press. https://doi.org/10.1016/B978-0-12-395678-1.50016-3

Stewart Merrill, T. E., & Cáceres, C. E. (2018). Within-host complexity of a plankton-parasite interaction. *Ecology*, *99*(12), 2864-2867. https://doi.org/10.1002/ecy.2483

Team R Core. (2020). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.r-project.org

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., García, M. B., García D., Gómez, J. M., Jordano, P., Medel, R., Navarro, L., Obeso, J. R., Oviedo, R., Ramírez, N., Rey, P. J., Traveset, A., Verdú, M., & Zamora, R. (2015). Beyond species loss: The extinction of ecological interactions in a changing world. *Functional Ecology*, *29*(3), 299-307. https://doi.org/10.1111/1365-2435.12356

Veneman, W. J., Spaink, H. P., Brun, N. R., Bosker, T., & Vijver, M. G. (2017). Pathway analysis of systemic transcriptome responses to injected polystyrene particles in zebrafish larvae. *Aquatic Toxicology*, *190*, 112-120. https://doi.org/10.1016/j.aquatox.2017.06.014

Von Moos, N., Burkhardt-Holm, P., & Köhler, A. (2012). Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environmental Science & Technology*, *46*(20), 11327-11335. https://doi.org/10.1016/j.envpol.2018.06.035

Wan, J. K., Chu, W. L., Kok, Y. Y., & Lee, C. S. (2018). Distribution of microplastics and nanoplastics in aquatic ecosystems and their impacts on aquatic organisms, with emphasis on microalgae. *Reviews of Environmental Contamination and Toxicology Volume 246*, 133-158. https://doi.org/10.1007/398_2018_14

Wang, W., Gao, H., Jin, S., Li, R., & Na, G. (2019). The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: A review. *Ecotoxicology and Environmental Safety*, *173*, 110-117. https://doi.org/10.1016/j.ecoenv.2019.01.113

Weinstein, J., Crocker, B.K., & Gray A.D. (2016). Plastic Debris in the Aquatic Environment: Degradation of high-density polyethylene, polypropylene, and polystyrene in a salt marsh habitat SETAC Press *35*, 1632–40. https://doi.org/10.1002/etc.3432

Wickham H. (2016). ggplot2-Elegant Graphics for Data Analysis. Springer International.

Wolinska, J., Seda, J., Koerner, H., Smilauer, P., & Petrusek, A. (2011). Spatial variation of *Daphnia* parasite load within individual water bodies. *Journal of Plankton Research*, *33*(8), 1284-1294. https://doi.org/10.1093/plankt/fbr016

Wolinska, J., Giessler, S., & Koerner, H. (2009). Molecular identification and hidden diversity of novel *Daphnia* parasites from European lakes. *Applied and Environmental Microbiology*, *75*(22), 7051-7059. https://doi.org/10.1128/AEM.01306-09

Supporting Information for: Nanoplastics modulate the outcome of a *Daphnia*-microparasite interaction

Stylianos Mavrianos[1,2], Florent Manzi[1,3], Ramsy Agha[1], Noemi Azoubib[1,3], Charlotte Schampera[1], Justyna Wolinska[1,3]

[1]*Department of Evolutionary and Integrative Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2]*Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*

[3]*Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany*

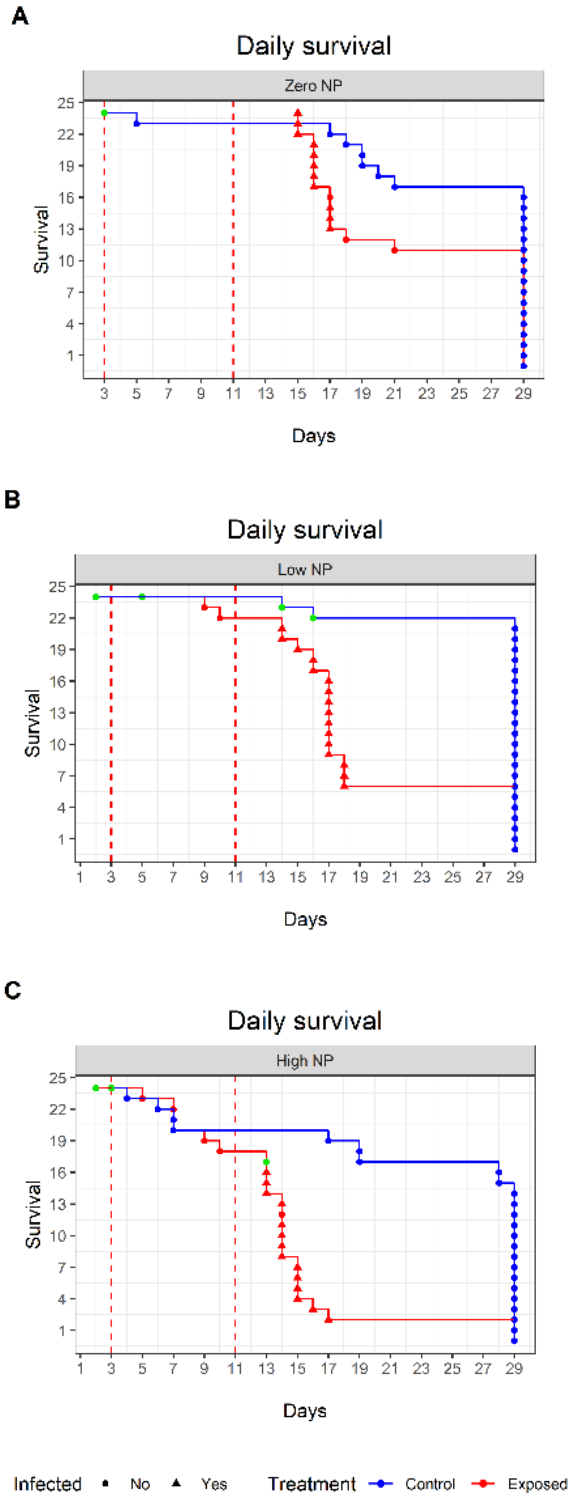


Figure S1. Comparison of daily survival of *Daphnia* individuals under three different NP concentrations: **A)** 0 mg/L, **B)** 5 mg/L, and **C)** 20 mg/L), either inoculated with a fungal parasite (red) or a control (blue). The left red vertical line marks the day of inoculation. The right red vertical line marks the day that the infection is observable and inspected. Each point represents the time of death of one individual. The shape reveals if the individual was successfully infected (triangle) or not (bullet point). The eight green bullet points represent the individuals that were lost due to background mortality and handling errors (excluded from every analysis).

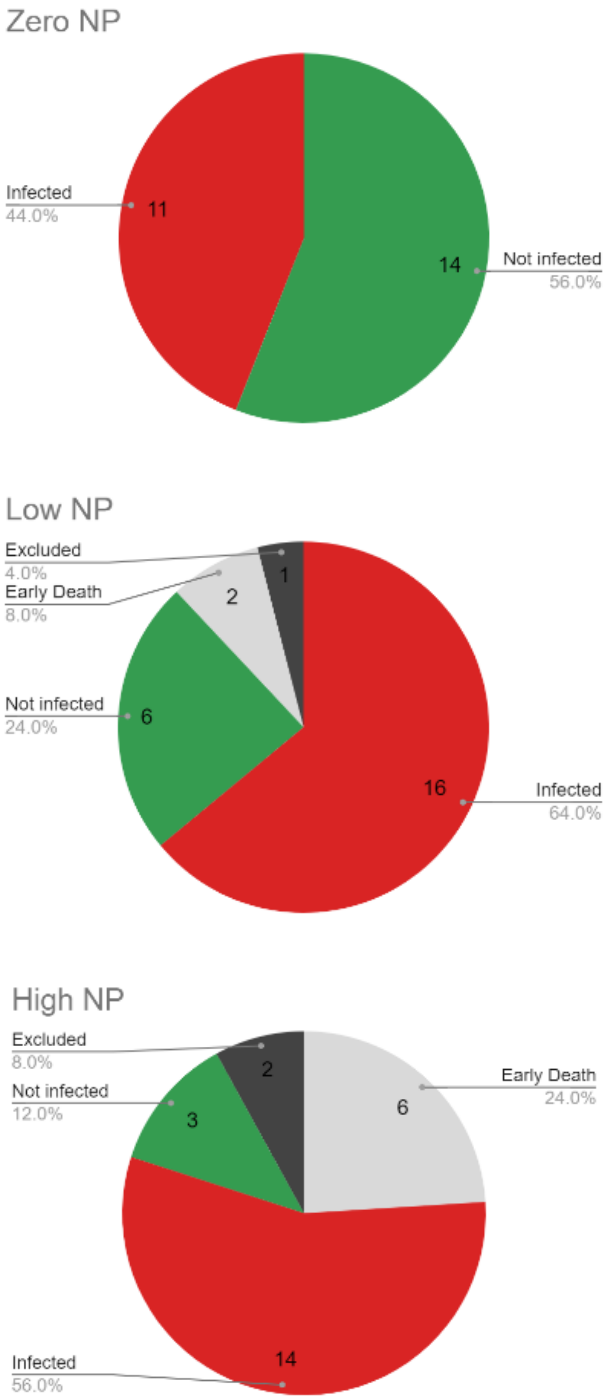


Figure S2. Pie charts illustrating the different subsets of *Daphnia* individuals under the three NP concentrations of the parasite-inoculated treatment. Red and green colours represent the individuals that were confirmed as infected and not infected after inspection, respectively. Individuals that died before infection and could not be assessed (day 9 post-inoculation) were categorised as "early death" (grey). For host fitness variables, "early death" individuals were added together with infected (green) individuals in order to compare them with the control (i.e. non-inoculated) treatment. Individuals which died before parasite inoculation (day 3) or due to handling error are shown with black colour and were excluded from every analysis.

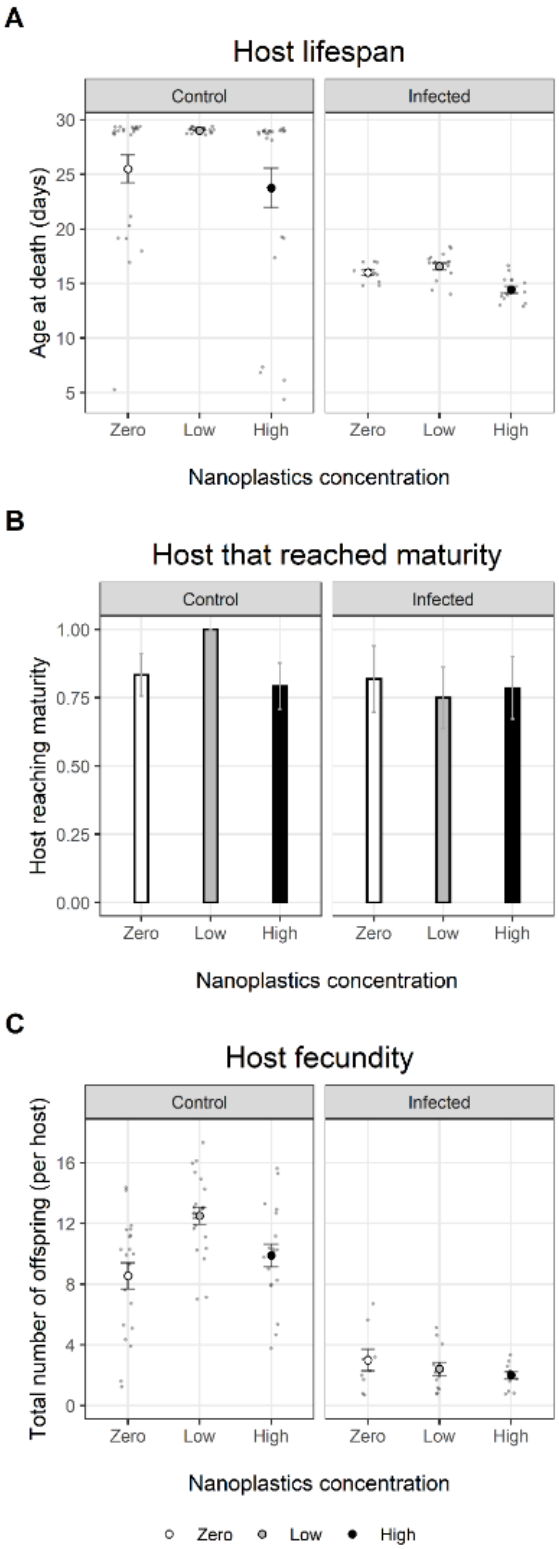


Figure S3. Host fitness traits in the parasite-inoculated (Infected) and Control groups under three NP concentrations: zero (0 mg/L), low (5 mg/L), and high (20 mg/L). **A)** Age at death of the individual *Daphnia*, **B)** Proportion of *Daphnia* that reached maturity, and **C)** Total number of offspring produced in a lifetime of a *Daphnia* individual. Infected group consists of strictly successfully infected individuals. Error bars represent the standard error of the mean.

CHAPTER 4:

SEQUENTIAL INFECTION OF DAPHNIA MAGNA BY A GUT MICROSPORIDIUM FOLLOWED BY A HAEMOLYMPH YEAST DECREASES TRANSMISSION OF BOTH PARASITES

Sequential infection of *Daphnia magna* by a gut microsporidium followed by a haemolymph yeast decreases transmission of both parasites

Florent Manzi[1,2], Snir Halle[3], Louise Seemann[1,2], Frida Ben-Ami[3], Justyna Wolinska[1,2]

[1] *Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2] *Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany*

[3] *School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 6997801, Israel*

Corresponding author:* **florent.vmanzi@gmail.com

Author contributions: All authors conceived and designed the study. **FM**, LS and JW performed the experiment. **FM** performed data analysis and results visualization. **FM** wrote the article, with input from JW, FBA and SH. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Manuscript published in *Parasitology* (10.08.2021):

https://doi.org/10.1017/S0031182021001384

Parasitology

cambridge.org/par

Sequential infection of *Daphnia magna* by a gut microsporidium followed by a haemolymph yeast decreases transmission of both parasites

Florent Manzi[1,2], Snir Halle[3], Louise Seemann[1,2], Frida Ben-Ami[3] and Justyna Wolinska[1,2]

[1]Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany; [2]Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany and [3]School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 6997801, Israel

Research Article

Cite this article: Manzi F, Halle S, Seemann L, Ben-Ami F, Wolinska J (2021). Sequential infection of *Daphnia magna* by a gut microsporidium followed by a haemolymph yeast decreases transmission of both parasites. *Parasitology* 1–12. https://doi.org/10.1017/S0031182021001384

Received: 13 April 2021
Revised: 14 July 2021
Accepted: 22 July 2021

Key words:
Coinfections; *Metschnikowia*; *Ordospora*; priority effects; zooplankton

Author for correspondence:
Florent Manzi,
E-mail: florent.vmanzi@gmail.com

Abstract

Over the course of seasonal epidemics, populations of susceptible hosts may encounter a wide variety of parasites. Parasite phenology affects the order in which these species encounter their hosts, leading to sequential infections, with potentially strong effects on within-host growth and host population dynamics. Here, the cladoceran *Daphnia magna* was exposed sequentially to a haemolymph-infecting yeast (*Metschnikowia bicuspidata*) and a gut microsporidium (*Ordospora colligata*), with experimental treatments reflecting two possible scenarios of parasite succession. The effects of single and co-exposure were compared on parasite infectivity, spore production and the overall virulence experienced by the host. We show that neither parasite benefited from coinfection; instead, when hosts encountered *Ordospora*, followed by *Metschnikowia*, higher levels of host mortality contributed to an overall decrease in the transmission of both parasites. These results showcase an example of sequential infections generating unilateral priority effects, in which antagonistic interactions between parasites can alleviate the intensity of infection and coincide with maladaptive levels of damage inflicted on the host.

Introduction

Over the course of their lifetime, most free-living organisms are bound to encounter parasites (Poulin and Morand, 2000). Realistically, individual hosts rarely encounter a single parasite, but rather progress through a series of events (exposure, infection and recovery) from a multitude of pathogens, some of which may coexist within the course of an infection. While some parasites may encounter their hosts simultaneously, such as several virus species being inoculated by a shared vector (Swanson *et al.*, 2006), the majority of multiple infections are thought to occur sequentially (Karvonen *et al.*, 2019). In a within-host framework, 'priority effects' occur when this sequence of infection alters the outcome of interactions among parasites (Halliday *et al.*, 2020). For instance, as different strains compete for a pool of susceptible hosts, faster replicating strains are generally favoured (Levin and Pimentel, 1981; Nowak and May, 1994). However, prior residency may allow 'weaker' strains to prevail in coinfection, by conferring protection against more competitive genotypes (Ben-Ami *et al.*, 2008; Seifi *et al.*, 2012). The biological mechanisms underlying such observations are likely the product of complex interactions between the defending host and coinfecting parasites (Alizon *et al.*, 2013), although common hypotheses have been proposed, which generally involve host immunity and competition for resources (Read and Taylor, 2001; de Roode *et al.*, 2005). For instance, prior exposure may weaken host immunity in such a way that secondary infections are facilitated (Graham, 2008) or trigger priming of the host's defences, so that subsequent infections are either alleviated (Rodrigues *et al.*, 2010) or prevented altogether (Ratcliff *et al.*, 1999; Syller and Grupa, 2016). Prior infection can also sequester within-host resources, which will then alter the developmental trajectory of late-arriving parasites (Graham, 2008). Although traditionally used in the context of species assemblages and community structures (Connell and Slatyer, 1977; Wilbur and Alford, 1985), this notion of priority effects has since been widely applied to the study of sequential infections (Hoverman *et al.*, 2013; Wuerthner *et al.*, 2017; Clay *et al.*, 2018; Carpenter *et al.*, 2021). Incidentally, a majority of studies have reported negative effects on later arriving parasites (reviewed in Karvonen *et al.*, 2019; but see also Ezenwa *et al.*, 2010, Lohr *et al.*, 2010*b*).

Over the past decade, water fleas of the genus *Daphnia* (Crustacea: Cladocera) and their microparasites have emerged as an ecologically relevant system for testing the outcome of interspecific coinfections (Ben-Ami *et al.*, 2011; Lange *et al.*, 2014; Sánchez *et al.*, 2019). As common inhabitants and crucial agents in the stability of freshwater ecosystems (Carpenter *et al.*, 1985; Lampert, 2006, 2011), *Daphnia* are known to harbour a functionally and taxonomically diverse range of parasite species, including microsporidia, fungi, ichthyosporea, bacteria (Ebert, 1995; Stirnadel and Ebert, 1997; Wolinska, *et al.*, 2009; Goren and Ben-Ami, 2013) and

viruses (Toenshoff *et al.*, 2018). For example, the gut microsporidium *Ordospora colligata* (Microsporidia: Ordosporidae, hereafter referred to as *Ordospora*) can be found in northern and western European ponds (Ebert, 2005), where high prevalences have been recorded in populations of its only host, *Daphnia magna* (Ebert *et al.*, 2001; Decaestecker *et al.*, 2005). In temperate ponds, the prevalence of microsporidian parasites increases from late spring to early summer, before waning back in the autumn and winter (Ebert, 1995; Larsson *et al.*, 1997). Epidemics usually start from infectious spore banks contained in the sediment, although transmission stages are also able to disperse in the water, where they can be encountered as free-floating spores (Mangin *et al.*, 1995; Kirk *et al.*, 2018). Microsporidian spores exhibit high survivability outside their hosts, allowing the parasite to overwinter and survive periods of host diapause (Ebert, 1995). Another common parasite of *Daphnia*, the waterborne yeast *Metschnikowia bicuspidata* (Ascomycota: Saccharomycetales, hereafter referred to as *Metschnikowia*) is a generalist capable of infecting several zooplankton species (Ebert, 2005; Dallas *et al.*, 2016). In temperate freshwater bodies of the Northern Hemisphere, epidemics of *Metschnikowia* typically peak in the late summer to early autumn (Duffy *et al.*, 2009; Hall *et al.*, 2011; Penczykowski *et al.*, 2014), although it has been found to overlap with gut microsporidia in the summer period (Ebert, 1995; Stirnadel and Ebert, 1997) or during the rainy season in Mediterranean to semi-arid climates of the Middle East (Goren and Ben-Ami, 2013). Transmission is also horizontal, although infective propagules are only released from dead hosts (i.e. obligate killer), and thus mostly restricted to the sediment (Duffy, 2009; Duffy and Hunsberger, 2019).

Due to their overlapping distribution, coinfections of *D. magna* involving both taxa are likely to occur. However, these phylogenetically distant species have been shown to differ greatly in their overall reproductive strategy: while infections by *Ordospora* typically reduce host lifespan by up to 20% (Ebert *et al.*, 2000), *Metschnikowia* is a highly virulent parasite, producing lethal infections under 2-to-3 weeks (Ebert, 2005). Because virulence in coinfection generally aligns with the amount of damage induced by the more virulent parasite (Ben-Ami *et al.*, 2008; Ben-Ami and Routtu, 2013), coinfection by an obligate killer may drastically reduce the timespan available to efficiently exploit host resources for growth (Lohr *et al.*, 2010*b*; Clay *et al.*, 2019). Furthermore, within-host competition for resources may be particularly relevant for parasites that colonize distinct niches within the host (Ben-Ami *et al.*, 2011). The intracellular *Ordospora* ensures reproduction by hijacking energy (i.e. ATP molecules) within the cytoplasm of epithelial cells (Tsaousis *et al.*, 2008), which serves both as a barrier and interface between the gut lumen and the haemolymph. Meanwhile, development of *Metschnikowia* takes place in the body cavity (Codreanu and Codreanu-Balcescu, 1981), which is in turn alimented by direct trophic exchanges along these compartments.

In addition to their contrasting reproductive strategies, the exact sequence in which parasites succeed each other within one host may further complicate such interactions (Hood, 2003; de Roode *et al.*, 2005; Jäger and Schjørring, 2006). The documented phenology of both parasites suggests that infections are likely to overlap in late summer, with a predicted prior presence of *Ordospora* in sympatric populations. Incidentally, some studies of priority effects have been conducted using *Metschnikowia*, along with ichtyhosporean (Lohr *et al.*, 2010*b*) and bacterial (Clay *et al.*, 2019) parasites of *Daphnia*, in which it was shown to consistently experience impaired transmission under prior residency. However, the literature is currently lacking such experimental assays for microsporidian parasites of *Daphnia*. In their exploratory study, Mangin *et al.* (1995) reported successful transmission of *Ordospora* to individuals previously infected with the microsporidium *Tuzetia* sp. (now referred to as *Hamiltosporidium magnivora*, Haag *et al.*, 2011). Nevertheless, systematic assays of sequential exposure using *Ordospora* have not been documented.

Here, we sequentially exposed the host *D. magna* to the parasites *Metschnikowia* and *Ordospora*. Experimental treatments were designed to reflect two possible scenarios of parasite succession: one in which a gut microsporidium (*Ordospora*) encounters the host after prior establishment of a fungal parasite in the haemolymph (*Metschnikowia*), and a second, opposite scenario in which the haemolymph-infecting yeast encounters the host after prior establishment of the gut parasite. We aimed to determine (i) whether sequential infections differ from single infections in terms of parasite transmission traits, specifically addressing the following questions: (a) how does *Metschnikowia* respond to later arrival of *Ordospora*; (b) how does *Metschnikowia* respond to prior infection by *Ordospora*; (c) how does *Ordospora* respond to later arrival of *Metschnikowia* and (d) how does *Ordospora* respond to prior infection by *Metschnikowia*; and (ii) whether opposite scenarios of parasite succession influence host fitness in diverging ways.

Materials and methods

Study system

Daphnia magna is commonly found in lakes and temporary freshwater bodies of the Northern Hemisphere (Ebert, 2005). Due to its large size (i.e. up to 5 mm) and efficient filtering rate, *D. magna* is particularly prone to multiple infections in general, as compared with smaller sympatric species (Stirnadel and Ebert, 1997). One clonal line of *D. magna* was used as the focal host for this experiment (clone NO-V-7, isolated from Norway; Haag *et al.*, 2020). This single genotype was selected on the basis of having the highest compatibility with both strains of parasites used in this study, as reported by preliminary infectivity assays.

A single strain of the yeast *Metschnikowia* was used, isolated from Ammersee, Germany and later propagated on lab-reared *D. magna* (clone E17:07). Spores are needle-shaped and puncture the gut epithelium to reach the haemolymph, where fungal development takes place (Codreanu and Codreanu-Balcescu, 1981; Stewart Merrill and Cáceres, 2018). Infection symptoms are clearly visible after 9–10 days, when the host's body cavity starts to fill with elongated asci (Stewart Merrill and Cáceres, 2018).

A single strain of *Ordospora* was used, isolated and maintained on lab-reared cultures of the experimental host (NO-V-7). Late stages of infection are characterized by the presence of several dozens of spore clusters in the gut epithelium, which are mostly confined to the upper half of the gut epithelium (Refardt and Ebert, 2006) and notably visible in the 'angular' sections of the digestive tract, such as the anterior diverticuli (Ebert, 2005). Spore release can occur from live host after 3 days (Mangin *et al.*, 1995; Refardt and Ebert, 2007), although reliable detection of infection is usually possible after 11 days, due to the exponential increase in parasite spore load throughout the infection (Kirk *et al.*, 2019).

Experimental setup

The experimental design included four single-exposure treatments ('METS early': exposed to spores of *Metschnikowia* on day 5; 'METS late': exposed to spores of *Metschnikowia* on day 7; 'ORDO early': exposed to spores of *Ordospora* on day 5; 'ORDO late': exposed to spores of *Ordospora* on day 7), two

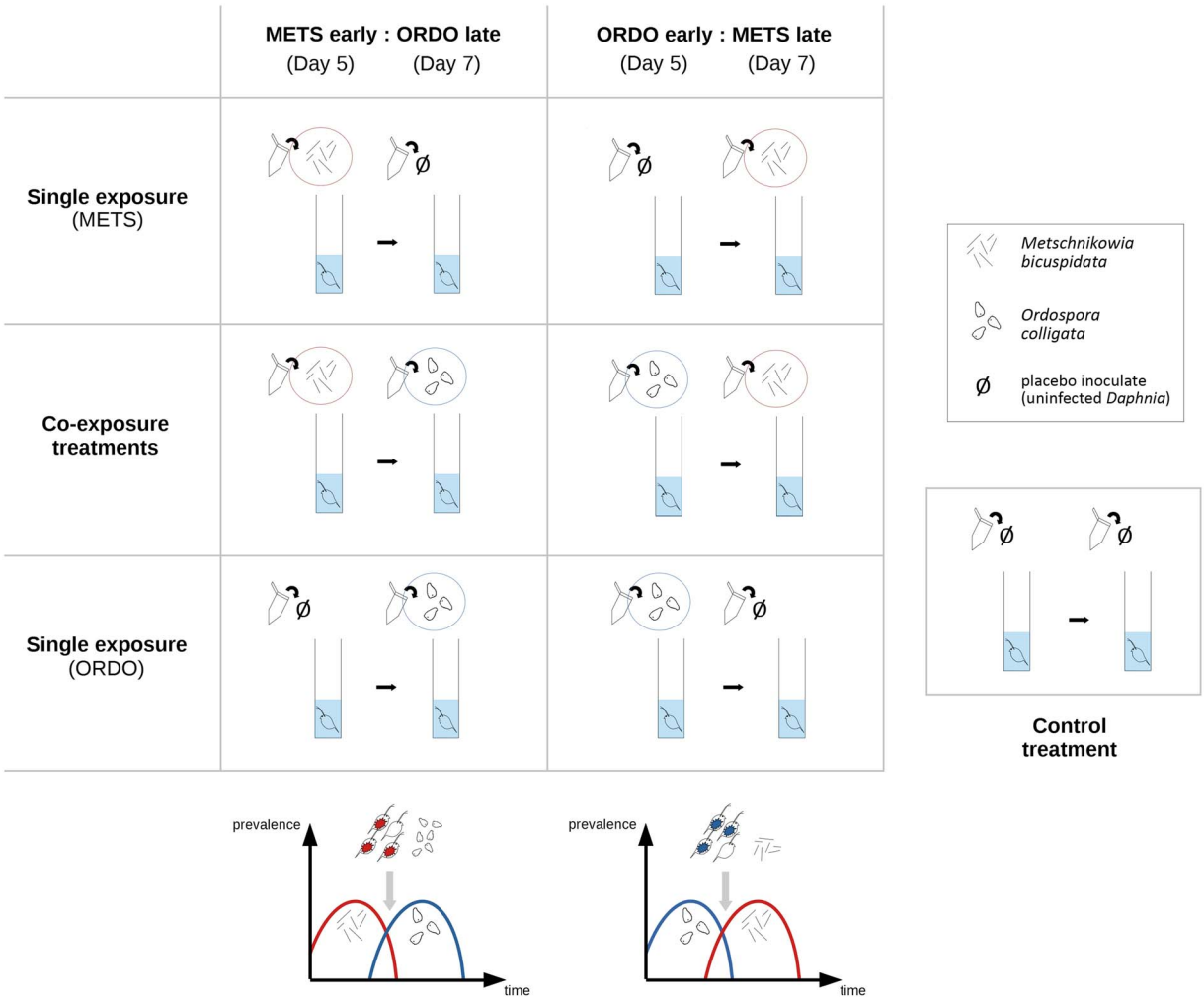


Fig. 1. Graphical representation of the six exposure treatments, corresponding to two possible scenarios of parasite succession. On the left, the haemolymph parasite *Metschnikowia bicuspidata* arrives 'early' and the gut parasite *Ordospora colligata* arrives 'late'. On the right, the gut parasite *O. colligata* arrives 'early' and the haemolymph parasite *M. bicuspidata* arrives 'late'. Single-exposure treatments within each scenario follow the same timing of infection as the co-exposure treatment, to allow proper comparison of parasite and host fitness parameters across single and co-exposure settings. The control treatment received the same placebo inoculate (obtained from crushed uninfected *Daphnia*) as single-exposure treatments, albeit on both inoculation days.

co-exposure treatments ('CO:METS early:ORDO late': exposed to spores of *Metschnikowia* on day 5 and *Ordospora* on day 7; 'CO:ORDO early:METS late': exposed to spores of *Ordospora* on day 5 and *Metschnikowia* on day 7) and one control treatment (exposed to crushed tissue of uninfected *D. magna* on both days). On the day which did not feature exposure to the parasite, all single infection treatments were exposed to the same placebo as the control. Forty replicates (individual *Daphnia*) were used for each treatment, yielding a total of 280 experimental units (Fig. 1).

Inoculation process

Juvenile *Daphnia* born within a 24-h time span (i.e. day 1) were transferred to individual jars containing 5 mL of synthetic culture medium (SSS-medium, Saebelfeld *et al.*, 2017). *Daphnia* were maintained at a constant temperature of 19°C, under a 12:12 light–dark photoperiod and fed three times per week with 1 mg C L^{-1} of *Scenedesmus obliquus* (green algae, maintained in WC algal medium). On day 5, spore solutions were prepared for both parasites. Infected individuals were gathered in Eppendorf tubes and crushed with a plastic pestle. The equivalent of ten adult *Daphnia* were crushed per 40 replicates, ensuring a balanced amount of host tissue was introduced in all seven treatments. To prepare the stock solution of *Metschnikowia*, the appropriate number of infected *Daphnia* (clone E17:07) was crushed to achieve a target dose of 17 500 spore per recipient *Daphnia* (3500 spores mL^{-1}) across 80 replicates in two treatments (METS early; CO:METS early:ORDO late). This dose was comparably higher than previous studies utilizing the same system (Hesse *et al.*, 2012), in order to maximize chances of successful coinfection in the co-exposure treatments. The solution was completed by crushing additional uninfected individuals up to a total of 20. To prepare the stock solution of *Ordospora*, 20 *Daphnia* (clone NO-V-7) presenting signs of late stage infection (large amount of spore clusters in the gut) were crushed to achieve a target dose of approximately 38 000 spores per recipient *Daphnia* (7600 spores mL^{-1}) across 80 replicates in two treatments (ORDO early; CO:ORDO early:METS late). Repeated counts from stock cultures were shown to provide the required number of spores from 20 individuals ($CI_{95\%}$ of average spore yield per inoculation dose: 38 100 ± 6.5%). These spore solutions were then distributed across all replicates of their respective treatments. Single infection treatments that did not receive spores on day 5 (METS late; ORDO late), as well as the control treatment were exposed to a placebo inoculate, prepared by crushing uninfected individuals (clone NO-V-7) using the same ratio of ten adult *Daphnia* for each treatment of 40 replicates.

After an exposure period of 2 days allocated to the first parasite, all *Daphnia* were transferred to 5 mL of clean medium, and the inoculation process was repeated on day 7. This delay was

chosen to ensure that either *Metschnikowia* (Stewart Merrill and Cáceres, 2018) or *Ordospora* (Mangin *et al.*, 1995; Refardt and Ebert, 2007) would reach their target compartment, before exposing the host to the second parasite (consistent with the definition of sequential infection as following establishment of the prior parasite; Marchetto and Power, 2018). Spore solutions were prepared anew, using the same methods as described for day 5, and inoculated into their respective treatments (METS late; CO:ORDO early:METS late; ORDO late; CO:METS early:ORDO late). *Daphnia* were not fed on either exposure day, in order to promote spore uptake (Hall *et al.*, 2007). On experimental day 9 (i.e. the end of the exposure period allocated to the second parasite), *Daphnia* were transferred to 20 mL of fresh, spore-free medium.

From day 9 onwards (both exposure periods having been completed), dead individuals were collected and fixed in 3.7% formaldehyde. Samples were kept at 4°C until the assessment of spore production (see below). Juveniles were removed and counted daily, and *Daphnia* were transferred to fresh medium (20 mL) every 4 days. The experiment was terminated on day 81, when the last surviving *Daphnia* in the control treatment had died.

Recorded parameters

Parasite fitness

Individual *Daphnia* from all treatments were assigned a binary value for host viability (0 = early death, 1 = viable host). Viable hosts were described as individual *Daphnia* having survived until the first possible detection of infection symptoms (i.e. presence of spores from crushed individuals), which was determined as 9 days post-exposure for *Metschnikowia* (Stewart Merrill and Cáceres, 2018) and 11 days post-exposure for *Ordospora* (Kirk *et al.*, 2019). Individuals from the six exposure treatments were assigned a separate value for parasite infectivity (0 = non infected, 1 = infected). Infected hosts were described as individual *Daphnia* in which spores of either parasite were detected (among those considered viable). Individuals which did not survive until at least both inoculation events had occurred (i.e. beyond experimental day 7) were excluded from both calculations, as these could not be properly attributed to their intended treatments (Appendix, Table S1). All retrieved samples (except for the control) were blinded to ensure reliable assessment of spore yield upon host death across single and co-exposure treatments. Samples were crushed in 0.3 mL, homogenized and loaded with 10 μL in a Neubauer Improved chamber. Samples were first screened for detection and quantification of needle-shaped *Metschnikowia* spores, under a Nikon SMZ25 stereomicroscope (200× magnification). For identification and quantification of *Ordospora*, samples were observed under a Nikon Ti Eclypse inverted microscope, using phase contrast and UV exposure (200× magnification); for each sample, 2 μL of Calcofluor-White (1 mg mL^{-1}) were added to the counting chamber to generate blue fluorescence, thereby staining the chitin-rich wall of pyriform spores (Krebs *et al.*, 2017).

Parasite growth (i.e. the rate of spore production) was computed as the ratio of spore yield over the number of days survived by the host post-exposure. A comprehensive measure of parasite fitness, the net spore output per exposed host, was computed as an estimation of overall transmission success. Here, in addition to individuals that produced a detectable spore yield, those that scored '0' for either host viability or parasite infectivity were also included, and recorded as a having a 'net' spore output of zero. This was done to reflect the probability of each encounter with an exposed host leading to subsequent reproduction of the parasite, which may differ across experimental treatments, independently of parasite growth (Manzi *et al.*, 2020).

Host fitness

Host fitness was recorded *via* three variables: host lifespan post-exposure was defined as the number of days survived by individual *Daphnia*, following the completion of both exposure events (i.e. beyond experimental day 7). Total offspring production per individual was used as a comprehensive measure of the host's reproductive success. Finally, the rate of offspring production was computed as the ratio of total offspring production over host lifespan post-exposure.

Data analysis

Data were analysed using R version 4.0.4 (R Core Team, 2021). Graphical outputs were produced using the 'ggplot2' (Wickham, 2016), 'Hmisc' (Harrell and Harrell, 2019) and 'patchwork' (Pedersen, 2020) packages. Analyses of variance (*F*-test or χ^2 test) were performed with the 'car' package (Fox and Weisberg, 2019).

Parasite fitness

Parasite fitness variables were analysed separately for each parasite and compared across single and co-exposure treatments with the same timing of infection. Host viability (0 = early death, 1 = viable host) and parasite infectivity (0 = non infected, 1 = infected) were analysed using a binary logistic regression with 'exposure' as explanatory variable (i.e. a factor with up to six possible levels). Additionally, host viability was compared to baseline mortality in the control treatment (Appendix, Table S2). In co-exposure treatments, infectivity of a given parasite included the total number of cases in which spores of that parasite were detected, either in single or coinfection. Parasite growth and the net spore output per exposed host were analysed with 'exposure' as explanatory variable in a linear model, assuming a normal distribution of residuals. Only successful infections (i.e. detection of a non-zero number of spores) were included in the analysis of parasite growth. All individuals which survived until at least both exposure events had occurred (i.e. beyond experimental day 7) were included in the analysis of net spore output. Normal distribution and homoscedasticity of the residuals were verified by visual inspection of quantile–quantile plots and residuals against fitted values.

Host fitness

Host fitness variables (namely lifespan post-exposure, rate of offspring production and total offspring production) were analysed using linear models, assuming a normal distribution of residuals, with 'exposure' as the explanatory variable (i.e. a factor with seven levels, including the control treatment). Only individuals successfully infected by either one (single exposure) or both parasites (co-exposure) were included in the non-control treatments. One individual from the control treatment was lost due to handling error and was thus excluded from these analyses. Post-hoc pairwise comparisons (Tukey's HSD test) were performed with the 'multcomp' package (Hothorn *et al.*, 2008).

Results

Parasite fitness

How does Metschnikowia respond to later arrival of Ordospora?

Under prior arrival of *Metschnikowia*, the viability of experimental *Daphnia* did not differ between the single and co-exposure treatments, with 94.7% (METS early) and 89.7% (CO:METS early:ORDO late) of hosts surviving until day 9 post-exposure (Fig. 2A, Table 1). Among hosts considered viable, the probability of successful infection did not differ significantly between single

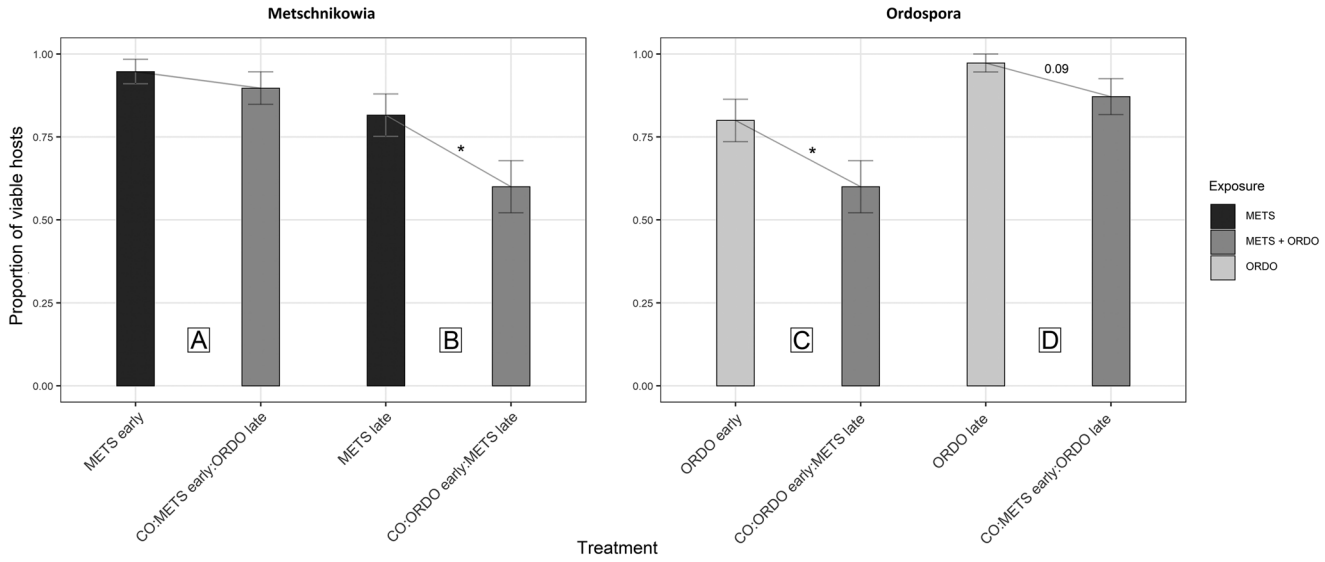


Fig. 2. Graphical representation of the proportion of *Daphnia* considered viable hosts, i.e. which survived until at least 9 days post-exposure (*Metschnikowia*) or 11 days post-exposure (*Ordospora*), allowing either parasite to produce detectable levels of infection (i.e. presence of spores in crushed individuals). Host viability was compared between single and co-exposure treatments, to answer the following: (A) How does *Metschnikowia* respond to later arrival of *Ordospora*? (B) How does *Metschnikowia* respond to prior infection by *Ordospora*? (C) How does *Ordospora* respond to later arrival of *Metschnikowia*? (D) How does *Ordospora* respond to prior infection by *Metschnikowia*? Individuals which did not survive until at least both inoculation events had occurred were excluded from these calculations. Error bars depict the standard error of the mean (calculated from binary values assigned to individual *Daphnia*: 0 = early death, 1 = viable host). Significance levels are provided by logistic regression performed across single and co-exposure treatments with shared timing of infection: $^{*}P \leqslant 0.05$.

(68.6%) and co-exposure (57.1%) treatments (Fig. 3A, Table 1). Parasite growth was comparable between single and co-exposure treatments (Fig. 4A, Table 1). Thus, the net output of *Metschnikowia* did not differ significantly across single and co-exposure treatments (Fig. 5A, Table 1).

How does Metschnikowia *respond to prior infection by* Ordospora*?*

Under late arrival of *Metschnikowia*, individuals which were first exposed to *Ordospora* suffered significant mortality during the early days of the experiment, with only 60.0% of hosts remaining viable (CO:ORDO early:METS late), as opposed to 81.6% in the single-exposure treatment (*METS late*) (Fig. 2B, Table 1). Infectivity did not differ significantly between the single (74.2%) and co-exposure (70.8%) treatments (Fig. 3B, Table 1). Parasite growth was significantly reduced in the co-exposure treatment (Fig. 4B, Table 1). Consequently, the net output of *Metschnikowia* in co-exposure was only half of that in the corresponding single-exposure treatment (Fig. 5B, Table 1).

How does Ordospora *respond to later arrival of* Metschnikowia*?*

Under prior arrival of *Ordospora*, the viability of experimental *Daphnia* was significantly reduced in the co-exposure treatment, with only 60.0% of hosts remaining viable (CO:ORDO early:METS late) compared to 80.0% in single exposure (ORDO early) (Fig. 2C, Table 1). There was a tendency towards higher infectivity in single exposure (43.8%) compared with the co-exposure treatment (29.2%) (Fig. 3C, Table 1). Parasite growth did not differ between the single and co-exposure treatments (Fig. 4C, Table 1). However, the net output of *Ordospora* was still 3-fold lower in co-exposure than in the single-exposure treatment (Fig. 5C, Table 1).

How does Ordospora *respond to prior infection by* Metschnikowia*?*

Under late arrival of *Ordospora*, there was a tendency towards higher viability in single exposure, with respectively 97.3% (ORDO late) and 87.2% (CO:METS early:ORDO late) of surviving hosts (Fig. 2D, Table 1). Infectivity did not differ between these treatments, with respectively 69.4% in single exposure and 61.8% in co-exposure (Fig. 3D, Table 1). Parasite growth did not differ either between those treatments (Fig. 4D, Table 1). Consequently, the net output of *Ordospora* did not differ significantly between single and co-exposure (Fig. 5D, Table 1).

Host fitness

Exposure had a significant effect on host lifespan ($F_{6,135} = 138.4$; $P < 0.001$) and total offspring production ($F_{6,135} = 74.46$; $P < 0.001$). On average, control *Daphnia* lived 56 days post-exposure ($CI_{95\%} \pm 2.19$; Fig. 6A) and produced 33 offspring ($CI_{95\%} \pm 2.28$; Fig. 6B). In comparison, hosts singly infected by *Ordospora* lived 38 days post-exposure (±2.89; Fig. 6A) and produced 23 offspring (±1.62; Fig. 6B), while those singly infected by *Metschnikowia* lived 17 days (±1.54; Fig. 6A) and produced only ten offspring (±1.56; Fig. 6B). Single-exposure treatments with opposite timing of infection did not differ significantly from each other (Appendix, Table S3). The reduction in host lifespan and total offspring production induced by coinfection was comparable to that of single infections by *Metschnikowia*, but much stronger overall than the effect of single infections by *Ordospora*. Post-hoc analyses of the rate of offspring production indicate that such differences in fecundity were mostly driven by lifespan (Fig. 6C). While exposure had a significant effect on the rate of offspring production ($F_{6,135} = 2.376$; $P = 0.033$), the only significant pairwise comparison occurred between the METS early and METS late treatments, with the former reducing host fecundity to a greater extent (Tukey's HSD: *t*-value: 3.315, $P = 0.018$; Appendix, Table S3).

Discussion

By exposing the host *D. magna* to sequential infections of the gut-dwelling microsporidium, *O. colligata* and the haemolymph-infecting yeast, *M. bicuspidata*, we investigated the potential for priority effects at the within-host level, in a system of sympatric

Table 1. Analysis of variance (*F*-test or χ^2 test) was performed across single and co-exposure treatments with shared timing of infection, to answer the following: (a) How does *Metschnikowia* respond to later arrival of *Ordospora*? (b) How does *Metschnikowia* respond to prior infection by *Ordospora*? (c) How does *Ordospora* respond to later arrival of *Metschnikowia*? (d) How does *Ordospora* respond to prior infection by *Metschnikowia*?

Response variable	Degree of freedom	χ^2/F value	*P* value
(a) METS early | CO:METS early:ORDO late			
Host viability	75	0.6807	0.4093
Parasite infectivity	68	0.9820	0.3217
Parasite growth	42	0.5758	04522
Net output per exposed host	75	0.8109	0.3708
(b) METS late | CO:ORDO early:METS late			
Host viability	76	4.4597	**0.0347**
Parasite infectivity	53	0.0768	0.7817
Parasite growth	38	5.7688	**0.0213**
Net output per exposed host	76	0.4945	**0.0291**
(c) ORDO early | CO:ORDO early:METS late			
Host viability	78	3.8652	**0.0493**
Parasite infectivity	58	2.9877	0.0839
Parasite growth	19	0.1618	0.6920
Net output per exposed host	78	6.0996	**0.0157**
(d) ORDO late | CO:METS early:ORDO late			
Host viability	74	2.9155	0.0877
Parasite infectivity	70	1.2013	0.2731
Parasite growth	43	1.2613	0.2676
Net output per exposed host	73	1.2907	0.2596

A generalized linear model was used, assuming a binomial distribution of residuals for host viability of individual *Daphnia* (0 = early death, 1 = viable host) and infection status of individual *Daphnia* (0 = non infected, 1 = infected). A general linear model was used, assuming a normal distribution of residuals for parasite growth (rate of spore production per infected host) and the net spore output per exposed host. Significant *P* values (⩽0.05) are highlighted in bold.

species. We simulated two orders of arrival, designed to reflect contrasting patterns of parasite succession. In sequential exposures where *Metschnikowia* arrived first (CO:METS early:ORDO late), parasite transmission traits (parasite infectivity, parasite growth) did not differ significantly from single exposures. However, in sequential exposures where *Ordospora* arrived first (CO:ORDO early:METS late), parasite growth was reduced for the fungal parasite. Though infectivity was not significantly impacted, there was also higher host mortality in this treatment, which contributed to a decrease in the net spore output of both parasites (i.e. a comprehensive measure of parasite fitness).

Performance of Metschnikowia *under single vs sequential infections*

Under prior residency of *Metschnikowia*, sequential exposures were not shown to influence its transmission potential, as none of the recorded parameters differed between single exposure (METS early) and co-exposure (CO:METS early:ORDO late). This apparent lack of effect was unexpected, as it somewhat contradicts previous findings involving this parasite. When pitting *Metschnikowia* against the ichthyosporean gut parasite *Caullerya mesnili*, Lohr *et al.* (2010*b*) found that given prior residency, *Metschnikowia* took longer to develop, and produced fewer spores in coinfection. Similarly, Clay *et al.* (2019) observed lower production of fungal spores in coinfected hosts, when *Metschnikowia* was first to arrive against the bacterium *Pasteuria ramosa*, as opposed to the treatment where it arrived second. Both studies suggest that *Metschnikowia* generally does not fare well under prior residency. However, the authors co-exposed *Daphnia* hosts to parasites that are considerably more virulent than *Ordospora*. Both *C. mesnili* and *P. ramosa* are known to induce complete castration of their hosts (Bittner *et al.*, 2002; Ebert *et al.*, 2004; Jensen *et al.*, 2006; Lohr *et al.*, 2010*a*). Parasites that shut down reproduction entirely (i.e. parasitic castration) are thought to redirect considerable amount or resources, that would normally support reproductive effort of the host, towards increased growth or survivorship instead (Baudoin, 1975). This difference in exploitation strategy may partly explain why *Metschnikowia* would experience strong priority effects against such virulent parasites, while demonstrating no apparent response to the later establishment of *Ordospora*.

By contrast, we found evidence for reduced transmission of *Metschnikowia*, when it was preceded by the gut parasite. Sequential exposure resulted in a 2-fold reduction of *Metschnikowia*'s net spore output, which was seemingly driven by two parameters of parasite fitness. First, parasite growth of *Metschnikowia* was slightly reduced in sequential exposure (CO:ORDO early:METS late), as opposed to the single-exposure treatment (METS late). This effect may be attributed to prior resource sequestration by the gut parasite. Intracellular microsporidian parasites ensure within-host growth by scavenging ATP molecules from host cells, through the activity of nucleotide transporters (Tsaousis *et al.*, 2008; Smith, 2009) and further interactions with host mitochondria (Terry *et al.*, 1997). Considering that infection by *Ordospora* takes place in the gut epithelium, prior sequestration of resources at the interface between the gut lumen and the haemolymph (i.e. where *Metschnikowia* completes its development and reproduction cycle) seems plausible. Second, a significant reduction of host viability was recorded in hosts that were first exposed to *Ordospora*, prior to *Metschnikowia* (CO:ORDO early:METS late), which resulted in a large proportion of co-exposed hosts not progressing towards successful reproduction of *Metschnikowia*.

While the mechanism responsible for such high mortality is difficult to infer from our results, this pattern is reminiscent of the ultrainfection phenomenon first described by Sofonea *et al.* (2015). Ultrainfection occurs when two parasites display adaptive levels of virulence and growth in single infection, while double infection triggers 'explosive' levels of host mortality, that are normally not found in each respective species. For this reason, coinfections are often hidden in the population, as cases that do occur only exist for a brief span of time, quickly interrupted by host death (Sofonea *et al.*, 2017). With regards to the present study, the CO:ORDO early:METS late treatment did result in excessive host mortality, which also contributed to a very low number of successfully coinfected hosts. A similar phenomenon has been described in nature, where interspecific coinfection of an insect host generates lethal levels of damage from a viral pathogen that is otherwise considered avirulent (Nazzi *et al.*, 2012).

Additionally, it has been observed that prior infection by a gut parasite can modify the structural integrity of the gut in *Daphnia*, which in turn modulates the probability of fungal spores successfully crossing into the haemolymph (T. Stewart Merrill, personal communication). Thus, we suspected prior colonization of epithelial cells by *Ordospora* could have altered susceptibility to *Metschnikowia*; however, parasite infectivity did not differ from single exposure in this treatment.

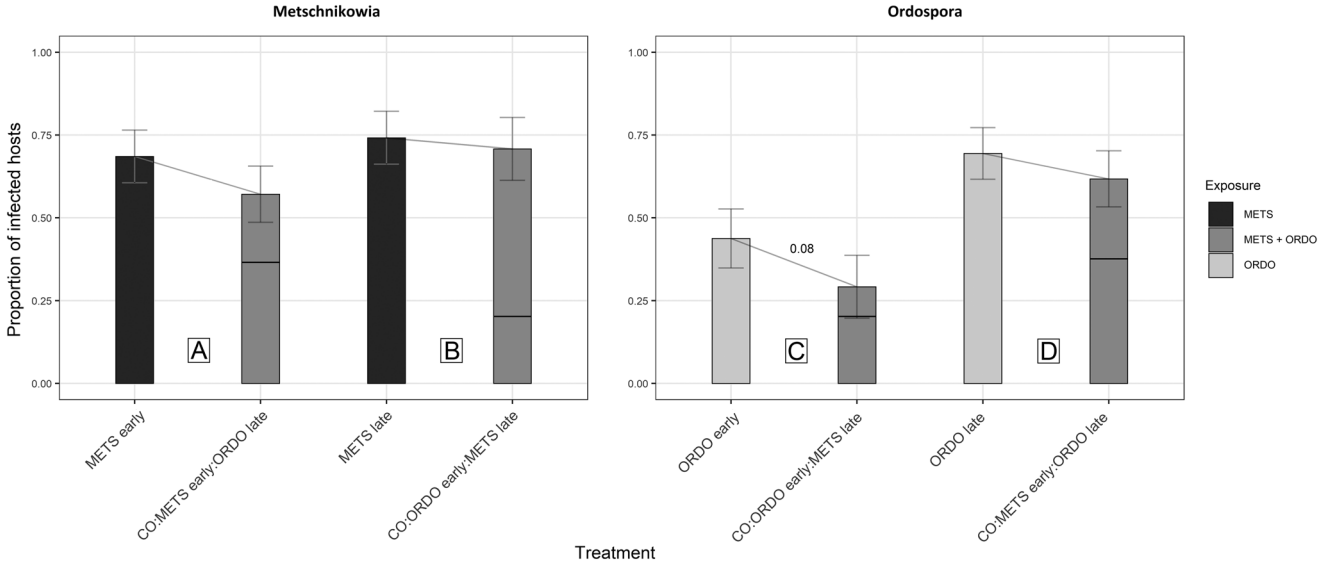


Fig. 3. Graphical representation of the proportion of *Daphnia* successfully infected by the parasites *Metschnikowia* and *Ordospora*. Parasite infectivity was compared between single and co-exposure treatments, to answer the following: (A) How does *Metschnikowia* respond to later arrival of *Ordospora*? (B) How does *Metschnikowia* respond to prior infection by *Ordospora*? (C) How does *Ordospora* respond to later arrival of *Metschnikowia*? (D) How does *Ordospora* respond to prior infection by *Metschnikowia*? The horizontal section of the bar in co-exposure treatments represents the contribution of coinfections to the overall number of successful infections by the focal parasite. Individuals which did not survive until the earliest possible observation of parasite symptoms were excluded from the analysis of infectivity; reported proportions are computed amongst the remaining number of individuals considered viable. Error bars depict the standard error of the mean (calculated from binary values assigned to individual *Daphnia*: 0 = non infected, 1 = infected). Significance levels are provided by logistic regression performed across single and co-exposure treatments with shared timing of infection; none of the pairwise comparisons were significant.

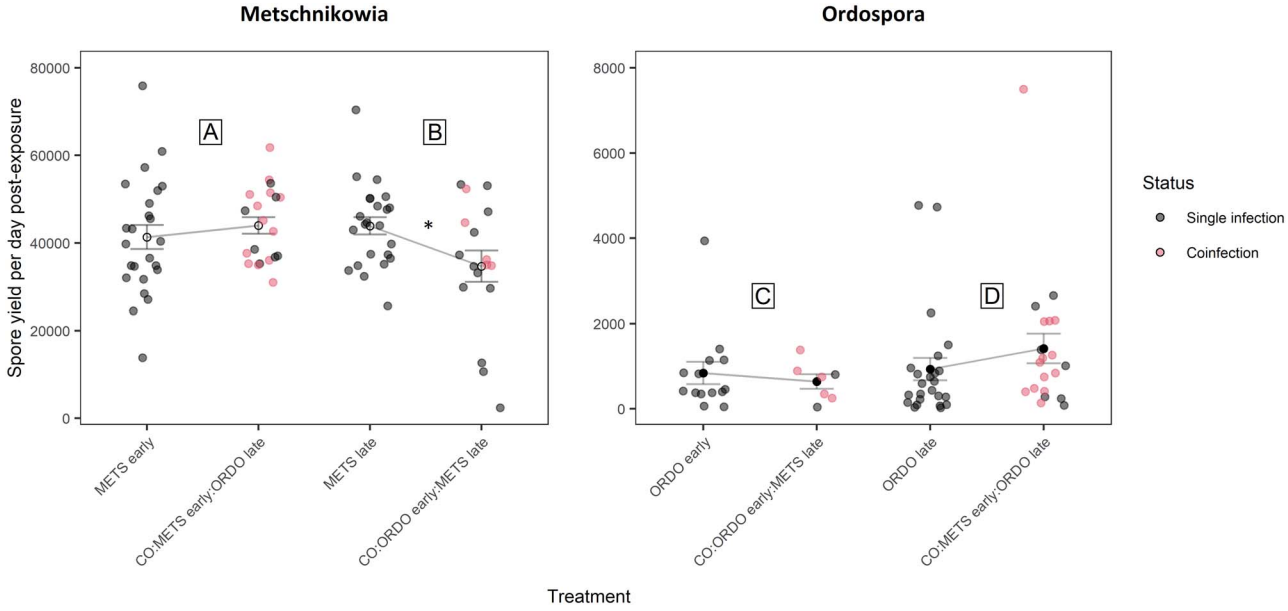


Fig. 4. Graphical representation of parasite growth (computed as the ratio of spore yield upon host death and the number of days survived by the host, post-second exposure event) for the parasites *Metschnikowia* and *Ordospora*. Parasite growth was compared between single and co-exposure treatments, to answer the following: (A) How does *Metschnikowia* respond to later arrival of *Ordospora*? (B) How does *Metschnikowia* respond to prior infection by *Ordospora*? (C) How does *Ordospora* respond to later arrival of *Metschnikowia*? (D) How does *Ordospora* respond to prior infection by *Metschnikowia*? Coloured dots depict individuals which were confirmed to be coinfected by *Metschnikowia* and *Ordospora*. Error bars depict the standard error of the mean, which was computed by pooling singly and coinfected individuals in the co-exposure treatments. Significance levels are provided by analysis of variance (*F*-test) across single and co-exposure treatments with shared timing of infection: $^{*}P \leqslant 0.05$.

Performance of Ordospora *under single vs sequential infections*

In single-exposure treatments, the overall infection success of *Ordospora* was lower when it was inoculated on day 5. Although we suspect possible heterogeneity between spore solutions may have contributed to this observation (as different parasite inoculates were used on days 5 and 7), age and body size-related effects could have further influenced infectivity (Izhar and Ben-Ami, 2015; Garbutt and Little, 2017). For instance, filtering rate and permeability of the gut epithelium (i.e. thickness of cell wall) in *Daphnia* have been shown to directly correlate with age and size class (Burns, 1969; Stewart Merrill

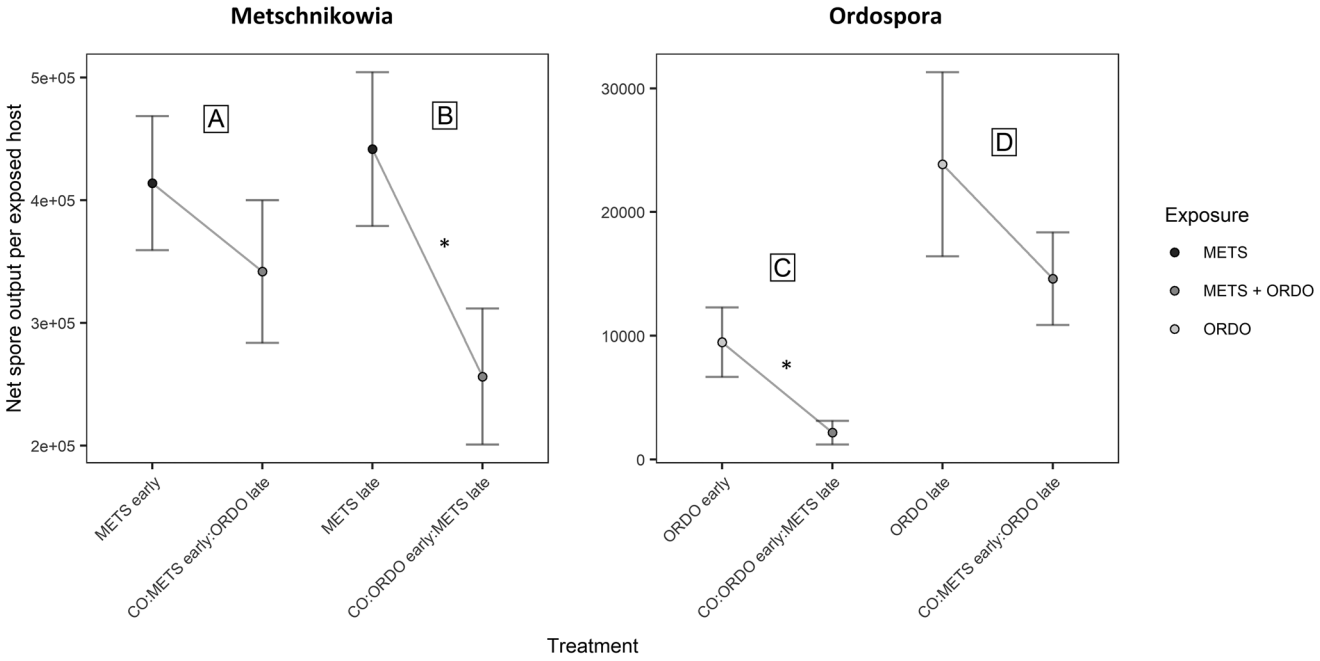


Fig. 5. Graphical representation of the net spore output (per exposed host) for the parasites *Metschnikowia* and *Ordospora*, as compared between single and co-exposure treatments, to answer the following: (A) How does *Metschnikowia* respond to later arrival of *Ordospora*? (B) How does *Metschnikowia* respond to prior infection by *Ordospora*? (C) How does *Ordospora* respond to later arrival of *Metschnikowia*? (D) How does *Ordospora* respond to prior infection by *Metschnikowia*? Error bars depict the standard error of the mean. Significance levels are provided by analysis of variance (*F*-test) across single and co-exposure treatments with shared timing of infection: $^{*}P \leqslant 0.05$.

et al., 2019). As *D. magna* can reach maturity starting from 7 days at 20°C (Lampert, 1993), the initial exposure of pre-adults *Daphnia* (i.e. from days 5–7) as opposed to potentially mature individuals (i.e. from days 7–9) may have influenced the parasite's infection success (Ben-Ami, 2019).

Independent of this observation, sequential exposure reduced transmission of *Ordospora*, when it was first to infect the host (CO:ORDO early:METS late). Contrary to our observations on *Metschnikowia*, these results seem to have been driven mostly by increased mortality of co-exposed hosts, as parasite growth did not differ between the single and co-exposure treatments. While our method for quantifying spores did not allow us to monitor the continuous shedding of propagules from live hosts, the number of spore clusters recorded in the gut of infected individuals increases exponentially throughout the course of infection (Mangin *et al.*, 1995; Kirk *et al.*, 2018), with each cluster bearing up to 60 infective stages (Kirk *et al.*, 2019). This suggests that spore yield recorded upon fixation of the host can be used to approximate the parasite's progression along the gut epithelium (i.e. infection intensity) and overall reproductive success. Although previous coinfection experiments using *Ordospora* were not available for comparison, *C. mesnili* benefited from an increase in spore production, when it was first to arrive in coinfection with *Metschnikowia* (Lohr *et al.*, 2010*b*). As mentioned above, the contrasting priority effects observed here may stem from distinct strategies of host exploitation and varying degrees of fitness impairment, as *Ordospora* is one of the least virulent endoparasites commonly found in *Daphnia* (Ebert, 2005).

Due to external factors, such as selective predation on infected individuals (Duffy *et al.*, 2005; Johnson *et al.*, 2006; Goren and Ben-Ami, 2017), *Daphnia* in their natural habitat may not experience such long lifespans as those observed in controlled conditions (instead, rarely surviving beyond 20 days; Lampert, 1993). In the present study, individuals which were successfully coinfected by both parasites experienced similar lifespan as those singly infected by *Metschnikowia*, but lived only half the span of those singly infected by *Ordospora* (Fig. 6A). Therefore, coinfections in nature may contribute fewer infective propagules to the overall transmission of *Ordospora*, especially when no benefit to coinfection was observed, that would help compensate this reduction in host lifespan.

From parasite phenology to sequential exposure

The phenology of symbionts often varies, causing them to emerge among a host population sequentially (Schmidt *et al.*, 2007; Dumbrell *et al.*, 2011). Because the probability of being the first to infect directly correlates with a parasite's prior prevalence in the population (Clay *et al.*, 2018), differences in species emergence patterns may in turn facilitate the occurrence of priority effects at the within-host level. While *Ordospora* may reach very high prevalence in natural populations of *D. magna* (Ebert, 2001), reportedly nearing 40% in shallow eutrophic ponds (Decaestecker *et al.*, 2005), much lower prevalences have been recorded for *Metschnikowia* in similar environments (<10%, Stirnadel and Ebert, 1997). Thus, co-occurrence of these two species could imply that a significant proportion of the host population may have already encountered *Ordospora*, around the time when *Metschnikowia* increases to peak prevalence (i.e. in the late summer).

Additionally, spores of these two parasites are likely to be found in separate locations of the water column. While epidemics of *Ordospora* typically start from infectious spore banks, following a period of inactivity from host populations (Mangin *et al.*, 1995), subsequent infections are likely to result in the continuous shedding of spores from live hosts. Because infective stages are able to disperse in the water (Mangin *et al.*, 1995; Kirk *et al.*, 2018), these may be encountered as free-floating spores across the upper parts of the water column. By contrast, spores of *Metschnikowia* gradually build up in the sediment, where infected hosts sink to and decompose after succumbing to infection (Duffy and Hunsberger, 2019). However, selective predation of spore-bearing individuals may contribute to the occasional resuspension of the parasite in the water column, as non-damaged asci can remain

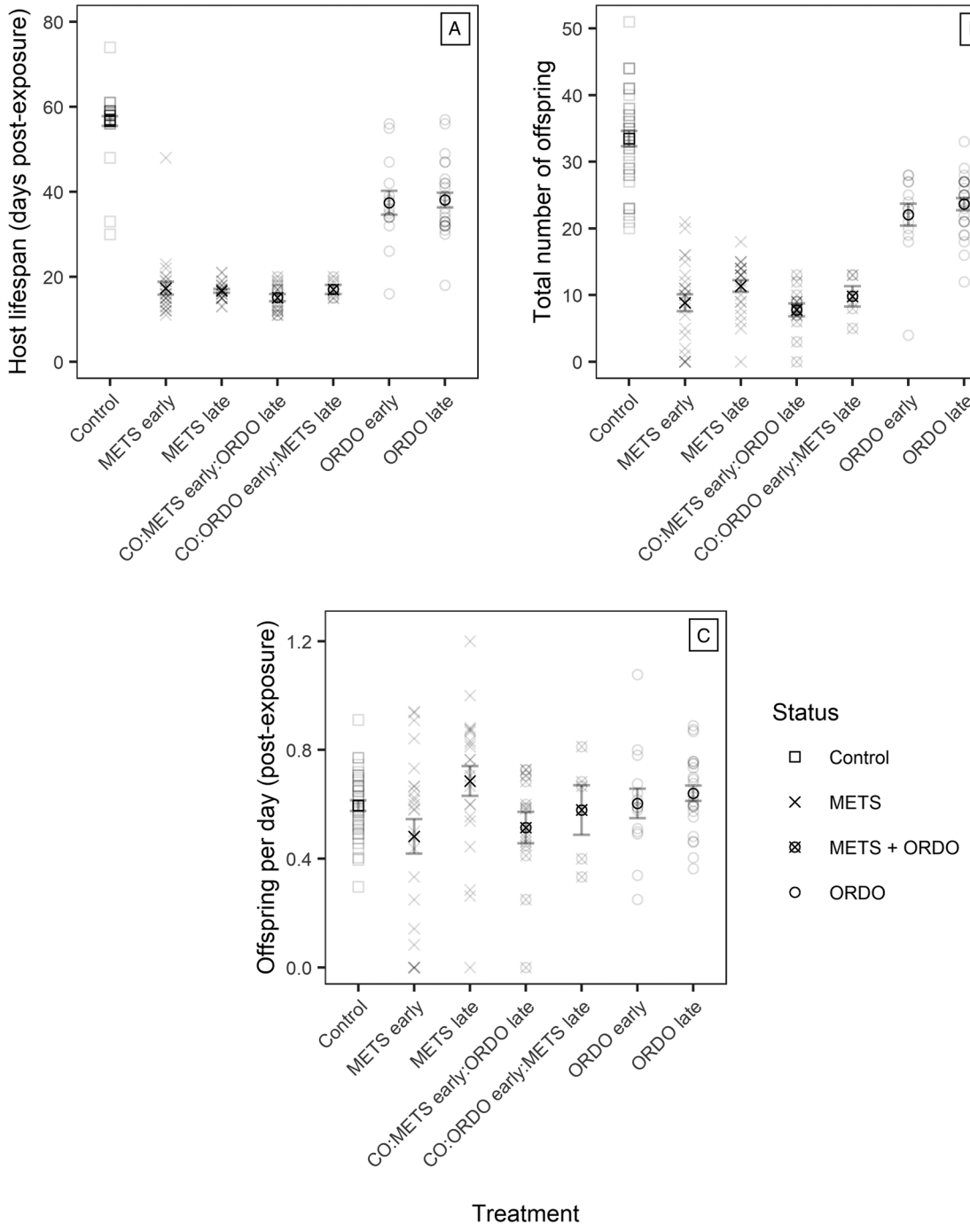


Fig. 6. Graphical representation of (A) lifespan post-exposure, (B) total offspring production and the resulting (C) rate of offspring production (number of offspring per day post-exposure) compared for individual *Daphnia* across the control and all six exposure treatments. Only individuals successfully infected by one (single exposure) or both parasites (co-exposure) were included in the non-control treatments. Error bars depict the standard error of the mean.

infectious following their passage through a fish's digestive tract (Duffy, 2009). Due to particularly strong diel vertical migration behaviour in *D. magna* (De Meester, 1992), this species is especially prone to contamination from infectious spore banks (Decaestecker *et al.*, 2002, 2004). However, differences in the likelihood of spore encounter may also be driven by individual variability in phototactic behaviour, which exhibits strong genotypic variation among clones of *D. magna* (De Meester, 1989; De Meester *et al.*, 1994). For instance, positively phototactic genotypes may recruit a higher proportion of free-floating microsporidian spores during the day, while being exposed to buried spore banks during the night. Finally, it has been shown that *D. magna* individuals infected with *Ordospora* exhibit much deeper position than uninfected ones in artificial mesocosms (Fels *et al.*, 2004). This suggests that prior infection by *Ordospora* may also influence host behaviour in such a way that secondary infections (e.g. by *Metschnikowia*) are facilitated in nature.

Within-host interactions between symbionts may scale up to influence host-parasite dynamics at the community level (Mordecai *et al.*, 2016; Marchetto and Power, 2018; Karvonen *et al.*, 2019), a phenomenon that has been demonstrated experimentally (Halliday *et al.*, 2017). For instance, mechanisms of positive or negative frequency dependence may arise from system-specific priority effects (Clay *et al.*, 2018). The unilateral priority effects highlighted in this study (i.e. reduced transmission under prior arrival of *Ordospora*) are likely to occur in

populations where both parasites are sympatric. These may be of particular importance during the early phase of parasite emergence, when every successful infection helps to kick-start a parasite's successful outbreak in the environment. For instance, species that usually emerge later in the season (e.g. *Metschnikowia*) are effectively starting in an environment where most – if not all – available hosts may have previously encountered a competing parasite species (e.g. *Ordospora*). Parasites that tend to suffer from late residency might face a 'critical early point' in their epidemic curve, during which most infections with previously infected hosts could result in a suboptimal outcome, potentially slowing – if not preventing – their successful establishment and emergence in the environment.

Concluding remarks

Our results suggest that specific patterns of parasite succession, with prior emergence of the microsporidium *Ordospora* over the yeast *Metschnikowia* (i.e. a plausible scenario in natural populations) may limit the transmission of both species, due to (i) impaired spore production of the yeast and (ii) maladaptive levels of host mortality that are not found in single infections. We also highlight the inherent specificity of priority effects among common parasites of *Daphnia*, showing that contrasting responses to sequential infections can be observed across a microsporidian gut parasite and functionally similar species. Thus, we encourage further research to consider other assemblages of ecologically relevant parasites, while monitoring temporal succession patterns that are observed in the field. Changes in parasite phenology could be especially relevant in light of climate change: distinct species may react differently to specific environmental triggers – such as light, temperature or nutrient availability – that are known to stimulate the emergence of resting stages, transmission and within-host reproduction (e.g. Overholt *et al.*, 2012; Kirk *et al.*, 2018). Elevated freshwater temperatures may cause asymmetric shifts between the overlapping epidemic curves of waterborne parasites, which could have implications for the likelihood of sequential infections at the within-host level.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182021001384

Data. The data supporting this study can be found at https://doi.org/10.5281/zenodo.5223097

Acknowledgements. We would like to thank two anonymous reviewers for their support and helpful comments. We also thank Ursula Newen for the maintenance of *Daphnia* cultures, Mark Phillipo for linguistic help, Sabrina Gattis for providing the *Ordospora* cultures and Florent Sylvestre for advice on statistical analyses.

Author contributions. All authors conceived and designed the study. FM, LS and JW conducted data gathering. FM analysed the data. FM wrote the article with input from JW, FBA and SH. All authors approved the final version of the manuscript.

Financial support. This work was supported by a joint German–Israeli project (WO 1587/8-1 to JW, 0604317501 to FBA) funded by the German Science Foundation (DFG).

Conflict of interest. None.

References

Alizon S, de Roode JC and Michalakis Y (2013) Multiple infections and the evolution of virulence. *Ecology Letters* **16**, 556–567.

Baudoin M (1975) Host castration as a parasitic strategy. *Evolution* **29**, 335–352.

Ben-Ami F (2019) Host age effects in invertebrates: epidemiological, ecological, and evolutionary implications. *Trends in Parasitology* **35**, 466–480.

Ben-Ami F and Routtu J (2013) The expression and evolution of virulence in multiple infections: the role of specificity, relative virulence and relative dose. *BMC Evolutionary Biology* **13**, 1–11.

Ben-Ami F, Mouton L and Ebert D (2008) The effects of multiple infections on the expression and evolution of virulence in a *Daphnia*-endoparasite system. *Evolution* **62**, 1700–1711.

Ben-Ami F, Rigaud T and Ebert D (2011) The expression of virulence during double infections by different parasites with conflicting host exploitation and transmission strategies. *Journal of Evolutionary Biology* **24**, 1307–1316.

Bittner K, Rothhaupt KO and Ebert D (2002) Ecological interactions of the microparasite *Caullerya mesnili* and its host *Daphnia galeata*. *Limnology and Oceanography* **47**, 300–305.

Burns CW (1969) Relation between filtering rate, temperature, and body size in four species of *Daphnia*. *Limnology and Oceanography* **14**, 693–700.

Carpenter SR, Kitchell JF and Hodgson JR (1985) Cascading trophic interactions and lake productivity. *BioScience* **35**, 635–639.

Carpenter SA, Vannatta JT and Minchella DJ (2021) Host exposure history and priority effects impact the development and reproduction of a dominant parasite. *International Journal for Parasitology*. doi: 10.1016/j.ijpara.2021.03.007

Clay PA, Cortez MH, Duffy MA and Rudolf VH (2018) Priority effects within coinfected hosts can drive unexpected population-scale patterns of parasite prevalence. *Oikos* **128**, 571–583.

Clay PA, Dhir K, Rudolf VH and Duffy MA (2019) Within-host priority effects systematically alter pathogen coexistence. *The American Naturalist* **193**, 187–199.

Codreanu R and Codreanu-Balcescu D (1981) On two *Metschnikowia* yeast species producing hemocoelic infections in *Daphnia magna* and *Artemia salina* (Crustacea, Phyllopoda) from Romania. *Journal of Invertebrate Pathology* **37**, 22–27.

Connell JH and Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. *The American Naturalist* **111**, 1119–1144.

Dallas T, Holtackers M and Drake JM (2016) Costs of resistance and infection by a generalist pathogen. *Ecology and Evolution* **6**, 1737–1744.

Decaestecker E, De Meester L and Ebert D (2002) In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proceedings of the National Academy of Sciences* **99**, 5481–5485.

Decaestecker E, Lefever C, De Meester L and Ebert D (2004) Haunted by the past: evidence for dormant stage banks of *Daphnia*. *Limnology & Oceanography* **49**, 1355–1364.

Decaestecker E, Declerck S, De Meester L and Ebert D (2005) Ecological implications of parasites in natural *Daphnia* populations. *Oecologia* **144**, 382–390.

De Meester L (1989) An estimation of the heritability of phototaxis in *Daphnia magna* Straus. *Oecologia* **78**, 142–144.

De Meester L (1992) The phototactic behaviour of male and female *Daphnia magna*. *Animal Behaviour* **43**, 696–698.

De Meester L, Vandenberghe J, Desender K and Dumont HJ (1994) Genotype-dependent daytime vertical distribution of *Daphnia magna* in a shallow pond. *Belgian Journal of Zoology* **124**, 3–9.

De Roode JC, Helinski ME, Anwar MA and Read AF (2005) Dynamics of multiple infection and within-host competition in genetically diverse malaria infections. *The American Naturalist* **166**, 531–542.

Duffy MA (2009) Staying alive: the post-consumption fate of parasite spores and its implications for disease dynamics. *Limnology and Oceanography* **54**, 770–773.

Duffy MA and Hunsberger KK (2019) Infectivity is influenced by parasite spore age and exposure to freezing: do shallow waters provide *Daphnia* a refuge from some parasites? *Journal of Plankton Research* **41**, 12–16.

Duffy MA, Hall SR, Tessier AJ and Huebner M (2005) Selective predators and their parasitized prey: are epidemics in zooplankton under top-down control? *Limnology and Oceanography* **50**, 412–420.

Duffy MA, Hall SR, Cáceres CE and Ives AR (2009) Rapid evolution, seasonality, and the termination of parasite epidemics. *Ecology* **90**, 1441–1448.

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH and Helgason T (2011) Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist* **190**, 794–804.

Ebert D (1995) The ecological interactions between a microsporidian parasite and its host *Daphnia magna*. *Journal of Animal Ecology* **64**, 361–369.

Ebert D (2005) *Ecology, Epidemiology, and Evolution of Parasitism in Daphnia*. Bethesda, MD: National Library of Medicine.

Ebert D, Lipsitch M and Mangin KL (2000) The effect of parasites on host population density and extinction: experimental epidemiology with *Daphnia* and six microparasites. *The American Naturalist* **156**, 459–477.

Ebert D, Hottinger JW and Pajunen VI (2001) Temporal and spatial dynamics of parasite richness in a *Daphnia* metapopulation. *Ecology* **82**, 3417–3434.

Ebert D, Joachim Carius H, Little T and Decaestecker E (2004) The evolution of virulence when parasites cause host castration and gigantism. *The American Naturalist* **164**, S19–S32.

Ezenwa VO, Etienne RS, Luikart G, Beja-Pereira A and Jolles AE (2010) Hidden consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African buffalo. *The American Naturalist* **176**, 613–624.

Fels D, Lee VA and Ebert D (2004) The impact of microparasites on the vertical distribution of *Daphnia magna*. *Archiv für Hydrobiologie* **161**, 65–80.

Fox J and Weisberg S (2019) *Package 'Car'*. Vienna: R Foundation for Statistical Computing.

Garbutt JS and Little TJ (2017) Bigger is better: changes in body size explain a maternal effect of food on offspring disease resistance. *Ecology and Evolution* **7**, 1403–1409.

Goren L and Ben-Ami F (2013) Ecological correlates between cladocerans and their endoparasites from permanent and rain pools: patterns in community composition and diversity. *Hydrobiologia* **701**, 13–23.

Goren L and Ben-Ami F (2017) To eat or not to eat infected food: a bug's dilemma. *Hydrobiologia* **798**, 25–32.

Graham AL (2008) Ecological rules governing helminth–microparasite coinfection. *Proceedings of the National Academy of Sciences* **105**, 566–570.

Haag KL, Larsson JI, Refardt D and Ebert D (2011) Cytological and molecular description of *Hamiltosporidium tvaerminnensis* gen. et sp nov., a microsporidian parasite of *Daphnia magna*, and establishment of *Hamiltosporidium magnivora* comb. nov. *Parasitology* **138**, 447–462.

Haag KL, Pombert JF, Sun Y, de Albuquerque NRM, Batliner B, Fields P, Lopes TF and Ebert D (2020) Microsporidia with vertical transmission were likely shaped by nonadaptive processes. *Genome Biology and Evolution* **12**, 3599–3614.

Hall SR, Sivars-Becker L, Becker C, Duffy MA, Tessier AJ and Cáceres CE (2007) Eating yourself sick: transmission of disease as a function of foraging ecology. *Ecology Letters* **10**, 207–218.

Hall SR, Becker CR, Duffy MA and Cáceres CE (2011) Epidemic size determines population-level effects of fungal parasites on *Daphnia* hosts. *Oecologia* **166**, 833–842.

Halliday FW, Umbanhowar J and Mitchell CE (2017) Interactions among symbionts operate across scales to influence parasite epidemics. *Ecology Letters* **20**, 1285–1294.

Halliday FW, Penczykowski RM, Barrès B, Eck JL, Numminen E and Laine AL (2020) Facilitative priority effects drive parasite assembly under coinfection. *Nature Ecology & Evolution* **4**, 1510–1521.

Harrell Jr FE and Harrell Jr MFE (2019) Package 'Hmisc'. CRAN R, 235-6.

Hesse O, Engelbrecht W, Laforsch C and Wolinska J (2012) Fighting parasites and predators: how to deal with multiple threats? *BMC Ecology* **12**, 1–8.

Hood ME (2003) Dynamics of multiple infection and within-host competition by the anther-smut pathogen. *The American Naturalist* **162**, 122–133.

Hothorn T, Bretz F and Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical Journal* **50**, 346–363.

Hoverman JT, Hoye BJ and Johnson PTJ (2013) Does timing matter? How priority effects influence the outcome of parasite interactions within hosts. *Oecologia* **173**, 1471–1480.

Izhar R and Ben-Ami F (2015) Host age modulates parasite infectivity, virulence and reproduction. *Journal of Animal Ecology* **84**, 1018–1028.

Jäger I and Schjørring S (2006) Multiple infections: relatedness and time between infections affect the establishment and growth of the cestode *Schistocephalus solidus* in its stickleback host. *Evolution* **60**, 616–622.

Jensen KH, Little TJ, Skorping A and Ebert D (2006) Empirical support for optimal virulence in a castrating parasite. *PLoS Biology* **4**, 1265–1269.

Johnson PT, Stanton DE, Preu ER, Forshay KJ and Carpenter SR (2006) Dining on disease: how interactions between infection and environment affect predation risk. *Ecology* **87**, 1973–1980.

Karvonen A, Jokela J and Laine AL (2019) Importance of sequence and timing in parasite coinfections. *Trends in Parasitology* **35**, 109–118.

Kirk D, Jones N, Peacock S, Phillips J, Molnár PK, Krkošek M and Luijckx P (2018) Empirical evidence that metabolic theory describes the temperature dependency of within-host parasite dynamics. *PLoS Biology* **16**, e2004608.

Kirk D, Luijckx P, Stanic A and Krkošek M (2019) Predicting the thermal and allometric dependencies of disease transmission *via* the metabolic theory of ecology. *The American Naturalist* **193**, 661–676.

Krebs M, Routtu J and Ebert D (2017) QTL mapping of a natural genetic polymorphism for long-term parasite persistence in *Daphnia* populations. *Parasitology* **144**, 1686–1694.

Lampert W (1993) Phenotypic plasticity of the size at first reproduction in *Daphnia*: the importance of maternal size. *Ecology* **74**, 1455–1466.

Lampert W (2006) *Daphnia*: model herbivore, predator and prey. *Polish Journal of Ecology* **54**, 607–620.

Lampert W (2011) *Daphnia: Development of a Model Organism in Ecology and Evolution*. Excellence in Ecology: Book 21. Oldendorf/Luhe, Germany: International Ecology Institute.

Lange B, Reuter M, Ebert D, Muylaert K and Decaestecker E (2014) Diet quality determines interspecific parasite interactions in host populations. *Ecology and Evolution* **4**, 3093–3102.

Larsson JR, Ebert D and Vavra J (1997) Ultrastructural study and description of *Ordospora colligata* gen. et sp. nov. (*Microspora*, *Ordosporidae* fam. nov.), a new microsporidian parasite of *Daphnia magna* (Crustacea, Cladocera). *European Journal of Protistology* **33**, 432–443.

Levin S and Pimentel D (1981) Selection of intermediate rates of increase in parasite-host systems. *The American Naturalist* **117**, 308–315.

Lohr JN, Laforsch C, Koerner H and Wolinska J (2010a) A *Daphnia* parasite (*Caullerya mesnili*) constitutes a new member of the ichthyosporea, a group of protists near the animal–fungi divergence. *Journal of Eukaryotic Microbiology* **57**, 328–336.

Lohr JN, Yin M and Wolinska J (2010b) Prior residency does not always pay off–co-infections in *Daphnia*. *Parasitology* **137**, 493–500.

Mangin KL, Lipsitch M and Ebert D (1995) Virulence and transmission modes of two microsporidia in *Daphnia magna*. *Parasitology* **111**, 133–142.

Manzi F, Agha R, Lu Y, Ben-Ami F and Wolinska J (2020) Temperature and host diet jointly influence the outcome of infection in a *Daphnia*-fungal parasite system. *Freshwater Biology* **65**, 757–767.

Marchetto KM and Power AG (2018) Coinfection timing drives host population dynamics through changes in virulence. *The American Naturalist* **191**, 173–183.

Mordecai EA, Gross K and Mitchell CE (2016) Within-host niche differences and fitness trade-offs promote coexistence of plant viruses. *The American Naturalist* **187**, E13–E26.

Nazzi F, Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, Della Vedova G, Cattonaro F, Caprio E and Pennacchio F (2012) Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathogens* **8**, e1002735.

Nowak MA and May RM (1994) Superinfection and the evolution of parasite virulence. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **255**, 81–89.

Overholt EP, Hall SR, Williamson CE, Meikle CK, Duffy MA and Cáceres CE (2012) Solar radiation decreases parasitism in *Daphnia*. *Ecology Letters* **15**, 47–54.

Pedersen TL (2020) Patchwork: The composer of plots (R package version 1.1.1). Available at https://CRAN.R-project.org/package=patchwork.

Penczykowski RM, Hall SR, Civitello DJ and Duffy MA (2014) Habitat structure and ecological drivers of disease. *Limnology and Oceanography* **59**, 340–348.

Poulin R and Morand S (2000) The diversity of parasites. *The Quarterly Review of Biology* **75**, 277–293.

R Core Team (2021) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0. Available at http://www.R-project.org/.

Ratcliff FG, MacFarlane SA and Baulcombe DC (1999) Gene silencing without DNA: RNA-mediated cross-protection between viruses. *The Plant Cell* **11**, 1207–1215.

Read AF and Taylor LH (2001) The ecology of genetically diverse infections. *Science (New York, N.Y.)* **292**, 1099–1102.

Refardt D and Ebert D (2006) Quantitative PCR to detect, discriminate and quantify intracellular parasites in their host: an example from three microsporidians in *Daphnia*. *Parasitology* **133**, 11–18.

Refardt D and Ebert D (2007) Inference of parasite local adaptation using two different fitness components. *Journal of Evolutionary Biology* **20**, 921–929.

Rodrigues J, Brayner FA, Alves LC, Dixit R and Barillas-Mury C (2010) Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science (New York, N.Y.)* **329**, 1353–1355.

Saebelfeld M, Minguez L, Griebel J, Gessner MO and Wolinska J (2017) Humic dissolved organic carbon drives oxidative stress and severe fitness impairments in *Daphnia*. *Aquatic Toxicology* **182**, 31–38.

Sánchez KF, Huntley N, Duffy MA and Hunter MD (2019) Toxins or medicines? Phytoplankton diets mediate host and parasite fitness in a freshwater system. *Proceedings of the Royal Society B: Biological Sciences* **286**, 20182231.

Schmidt SK, Costello EK, Nemergut DR, Cleveland CC, Reed SC, Weintraub MN, Meyer AF and Martin AM (2007) Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* **88**, 1379–1385.

Seifi A, Nonomura T, Matsuda Y, Toyoda H and Bai Y (2012) An avirulent tomato powdery mildew isolate induces localized acquired resistance to a virulent isolate in a spatiotemporal manner. *Molecular Plant-Microbe Interactions* **25**, 372–378.

Smith JE (2009) The ecology and evolution of microsporidian parasites. *Parasitology* **136**, 1901–1914.

Sofonea MT, Alizon S and Michalakis Y (2015) From within-host interactions to epidemiological competition: a general model for multiple infections. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**, 20140303.

Sofonea MT, Alizon S and Michalakis Y (2017) Exposing the diversity of multiple infection patterns. *Journal of Theoretical Biology* **419**, 278–289.

Stewart Merrill TE and Cáceres CE (2018) Within-host complexity of a plankton-parasite interaction. *Ecology* **99**, 2864–2867.

Stewart Merrill TE, Hall SR, Merrill L and Cáceres CE (2019) Variation in immune defense shapes disease outcomes in laboratory and wild *Daphnia*. *Integrative and Comparative Biology* **59**, 1203–1219.

Stirnadel HA and Ebert D (1997) Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. *Journal of Animal Ecology* **66**, 212–222.

Swanson SJ, Neitzel D, Reed KD and Belongia EA (2006) Coinfections acquired from *Ixodes* ticks. *Clinical Microbiology Reviews* **19**, 708–727.

Syller J and Grupa A (2016) Antagonistic within-host interactions between plant viruses: molecular basis and impact on viral and host fitness. *Molecular Plant Pathology* **17**, 769–782.

Terry RS, Dunn AM and Smith JE (1997) Cellular distribution of a feminizing microsporidian parasite: a strategy for transovarial transmission. *Parasitology* **115**, 157–163.

Toenshoff ER, Fields PD, Bourgeois YX and Ebert D (2018) The end of a 60-year riddle: identification and genomic characterization of an iridovirus, the causative agent of white fat cell disease in zooplankton. *G3: Genes, Genomes, Genetics* **8**, 1259–1272.

Tsaousis AD, Kunji ER, Goldberg AV, Lucocq JM, Hirt RP and Embley TM (2008) A novel route for ATP acquisition by the remnant mitochondria of *Encephalitozoon cuniculi*. *Nature* **453**, 553–556.

Wickham H (2016) *ggplot2-Elegant Graphics for Data Analysis*. Cham, Switzerland: Springer International Publishing.

Wilbur HM and Alford RA (1985) Priority effects in experimental pond communities: responses of *Hyla* to *Bufo* and *Rana*. *Ecology* **66**, 1106–1114.

Wolinska J, Giessler S and Koerner H (2009) Molecular identification and hidden diversity of novel *Daphnia* parasites from European lakes. *Applied and Environmental Microbiology* **75**, 7051–7059.

Wuerthner VP, Hua J and Hoverman JT (2017) The benefits of coinfection: trematodes alter disease outcomes associated with virus infection. *Journal of Animal Ecology* **86**, 921–931.

Supporting Information for: Sequential infection of *Daphnia magna* by a gut microsporidium followed by a haemolymph yeast decreases transmission of both parasites

Florent Manzi[1,2], Snir Halle[3], Louise Seemann[1,2], Frida Ben-Ami[3], Justyna Wolinska[1,2]

[1] *Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2] *Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany*

[3] *School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 6997801, Israel*

Appendix

Table S1. Proportion of *viable* hosts: experimental *Daphnia* which survived until at least day 9 post-exposure (i.e. earliest observation of infection symptoms for *Metschnikowia*) or day 11 post-exposure (i.e. earliest observation of infection symptoms for *Ordospora*); proportion of *infected* hosts: experimental *Daphnia* confirmed to have produced spores of the parasites (a) *Metschnikowia* or (b) *Ordospora*. Individuals which did not survive until at least both inoculation events had occurred (beyond experimental day 7) were excluded; reported proportions are computed from ≤ 40 surviving hosts per exposure treatment.

a) Successful infections by *Metschnikowia*

Exposure	Proportion of viable hosts		Proportion of infected hosts	
METS early	36/38	0.947	24/36	0.686
CO:METS early:ORDO late	35/39	0.897	20/35	0.571
METS late	31/38	0.816	23/31	0.742
CO:ORDO early:METS late	24/40	0.600	17/24	0.708
Total (≤ 160 replicates)	126/155	0.813	84/126	0.666

b) Successful infections by *Ordospora*

Exposure	Proportion of viable hosts		Proportion of infected hosts	
ORDO early	32/40	0.800	14/32	0.438
CO:ORDO early:METS late	24/40	0.600	7/24	0.292
ORDO late	36/37	0.973	25/36	0.694
CO:METS early:ORDO late	34/39	0.872	21/34	0.618
Total (≤ 160 replicates)	126/156	0.808	67/126	0.532

Table S2. Logistic regression performed across combinations of single exposure and co-exposure treatments with the same timing of infection. The number of individuals surviving until the first possible detection of the parasites *Metschnikowia* (i.e. from day 9 onward) or *Ordospora* (i.e. from day 11 onward) was compared to baseline mortality in the control treatment. A generalized linear model was used, assuming a binomial distribution of residuals. Significant *P*-values (≤ 0.05) are highlighted in bold.

Response variable	**Degree of freedom**	χ^2	***P*-value**
Control | METS early | CO:METS early:ORDO late			
Host viability (≥ day 9)	2; 113	5.762	0.056
Control | METS late | CO:ORDO early:METS late			
Host viability (≥ day 9)	2; 114	25.829	**< 0.001**
Control | ORDO early | CO:ORDO early:METS late			
Host viability (≥ day 11)	2; 116	27.921	**< 0.001**
Control | ORDO late | CO:METS early:ORDO late			
Host viability (≥ day 11)	2; 112	8.9352	**0.0115**

Table S3. Results of Tukey's HSD test applied to a one-way analysis of variance (ANOVA), with (a) host lifespan, (b) total offspring production and (c) the rate of offspring production as response variables, compared across the control and all exposure treatments. Only significant *P*-values (≤ 0.05) are reported and highlighted in bold.

a) Host lifespan

Compared levels		Estimate	Standard error	t value	*P*-value
Control	METS early	+39.33	1.80	21.835	**< 0.0001**
Control	METS late	+39.97	1.83	21.900	**< 0.0001**
Control	ORDO late	+18.59	1.78	10.448	**< 0.0001**
Control	ORDO early	+19.20	2.16	8.889	**< 0.0001**
Control	CO:METS early:ORDO late	+41.56	2.22	18.716	**< 0.0001**
Control	CO:ORDO early:METS late	+39.63	3.29	12.056	**< 0.0001**
ORDO early	METS early	+20.14	2.32	8.665	**< 0.0001**
ORDO early	METS late	+20.78	2.34	8.870	**< 0.0001**
ORDO early	CO:METS early:ORDO late	+22.35	2.66	8.398	**< 0.0001**
ORDO early	CO:ORDO early:METS late	+20.43	3.60	5.674	**< 0.0001**
ORDO late	METS early	+20.75	1.97	10.507	**< 0.0001**
ORDO late	METS late	+21.39	2.00	10.712	**< 0.0001**
ORDO late	CO:METS early:ORDO late	+22.96	2.36	9.718	**< 0.0001**
ORDO late	CO:ORDO early:METS late	+21.04	3.39	6.215	**< 0.0001**

b) Total offspring production

Compared levels		Estimate	Standard error	t value	*P*-value
Control	METS early	+24.64	1.48	16.614	**< 0.001**
Control	METS late	+22.13	1.50	14.724	**< 0.001**
Control	ORDO early	+11.40	1.79	6.412	**< 0.001**
Control	ORDO late	+9.83	1.46	6.713	**< 0.001**
Control	CO:METS early:ORDO late	+25.70	1.82	14.064	**< 0.001**
Control	CO:ORDO early:METS late	+23.67	2.71	8.749	**< 0.001**
ORDO early	METS early	+13.24	1.91	6.921	**< 0.001**
ORDO early	METS late	+10.72	1.92	5.562	**< 0.001**
ORDO early	CO:METS early:ORDO late	+14.302	2.19	6.528	**< 0.001**
ORDO early	CO:ORDO early:METS late	+12.271	2.96	4.141	**0.001**
ORDO late	METS early	+14.807	1.63	9.109	**< 0.001**
ORDO late	METS late	+12.292	1.64	7.480	**< 0.001**
ORDO late	CO:METS early:ORDO late	+15.871	1.95	8.160	**< 0.001**
ORDO late	CO:ORDO early:METS late	+13.840	2.786	4.967	**< 0.001**

c) Rate of offspring production

Compared levels		Estimate	Standard error	t value	*P*-value
METS late	METS early	+0.20	0.06	3.315	**0.0185**

GENERAL DISCUSSION

Abiotic and biotic parameters of the environment can modulate the outcome of parasitic infections, and many of these factors are expected to shift under the influence of anthropogenic disturbances. Understanding whether and how future environmental shifts will modify the occurrence and severity of diseases is of high interest in the *Daphnia*-microparasite system, due to the host's ecological relevance as a keystone organism and its contribution to ecosystem-level processes. Throughout the four chapters of this thesis, we aimed to identify both direct (i.e. rising water temperature, plastic pollution) and indirect (i.e. cyanobacterial dominance, temporality of epidemics) sources of interference between human activity and host-parasite interactions in a commonly occurring freshwater system.

In **Chapter 1**, we identified a glaring gap in the literature by considering water temperature and phytoplankton community composition (a major determinant of zooplankton diet quality) as deeply interconnected factors, rather than separate sources of environmental stress. We found that these can have complex, associative effects on parameters of host and parasite fitness. In **Chapter 2**, we examined a potential source of environmental interference between toxin-producing cyanobacteria and free-living stages of the parasite. Although parameters associated with climate change were previously shown to decrease the infectious potential of parasite transmission stages, high concentrations of dissolved cyanobacterial toxins were not identified as one such mechanism. In **Chapter 3**, we provided the first experimental evidence of direct interactions between plastic pollution and infections of zooplankton, adding our contribution to a relatively new – yet promising – body of work. Finally, **Chapter 4** highlighted the importance of considering within-host priority effects, resulting from the spatial and temporal overlap of distinct species of parasites competing for host resources.

1. How will future environmental shifts affect the performance of *Metschnikowia* in natural *Daphnia* populations?

1.1 Effects of lake warming and increased cyanobacterial dominance

Among the four scenarios that were investigated throughout this thesis, none of the tested factors had a straightforward, net positive effect on parasite transmission. For instance, we found that elevated temperature increased the net spore output of *Metschnikowia* when genotype AMME_12 was fed either *Scenedesmus* or *Microcystis* (Chapter 1, Section 3.1); however, we found the opposite effect when AMME_12 was fed with *Planktothrix*. Furthermore, elevated temperature consistently reduced parasite transmission in genotype AMME_51 (i.e. across all three diets). Thus, out of six possible combinations

of host diet and genotype tested in this chapter, only two benefitted from increased parasite transmission under a predicted temperature elevation of 4°C. Our results suggest that the interactive effects of high temperature and *Planktothrix*-based diets could be highly unfavourable to the parasite in communities that are dominated by this common genus, or possibly other filamentous species of similar nutritional value and toxicity (e.g. genus *Limnothrix* and *Pseudanabaena*), which were found to dominate phytoplankton composition in some European eutrophic lakes during the summer/autumn period (Nixdorf et al., 2003). Moreover, given the particularly low growth rates found for *D. galeata* × *longispina* under conditions reflective of future lake warming, we suspect that increasing occurrences of heat-waves and cyanobacterial dominance in summer could limit the spread of horizontally-transmitted parasites that rely on high host population densities for their efficient transmission (particularly if such conditions occur at an early phase of their epidemic curve).

Overall, the findings of Chapters 1-2 are consistent with previous studies supporting negative effects of either low food quality or quantity on transmission traits of *Metschnikowia* (Penczykowski et al., 2014; Sánchez et al., 2019) and other parasites of *Daphnia* (Mitchell & Read, 2005; Frost et al., 2008; Ben-Ami et al., 2010; Stjernman & Little, 2011; Coopman et al., 2014; Lange et al., 2014). Our results also support non-straightforward effects of temperature elevation on parasite transmission. While previous reports suggested that *Metschnikowia* benefits from higher transmission at high temperatures, due to increased foraging rates of the host (Cuco et al., 2018; Shocket et al., 2018a, 2018b), a later study found that high temperature did not alter post-exposure infectivity and even reduced spore production of the parasite (Shocket et al., 2019). Finally, elevated temperature could also influence freshwater dynamics at higher trophic levels, generating top-down control on *Daphnia*-parasite interactions: as the thermal physiology of fish increases more steeply than that of the host, an epidemiological model suggested that high temperatures could inhibit epidemics, by way of increased predation risk in the *Daphnia-Metschnikowia* system (Hall et al., 2006).

1.2 Effects of the external environment on parasite resting stages

In freshwater environments, spores of environmentally-transmitted parasites often display high survivability outside their hosts (Ebert, 2005). For instance, endospores of the bacterium *P. ramosa* can remain infectious for over 20 years (Decaestecker et al., 2004). Spores of horizontally-transmitted parasites that are not immediately transmitted to susceptible hosts after release often build up as spore banks in the sediment. Because they exhibit such high lifespan in the water column, these resting stages are potentially vulnerable to environmental sources of degradation (Duffy & Hunsberger, 2019). Here, we hypothesized that high concentrations of secondary metabolites produced by cyanobacteria could reduce the infectivity and/or reproductive ability of resting stages of *Metschnikowia*, should they get in contact prior to their ingestion by a susceptible host. However, we found no evidence for negative

effects of dissolved cyanobacterial content against fungal spores (Chapter 2; Section 3.1). Instead, the proportion of successfully infected hosts even increased in one of two tested genotypes (though we suspected this could be due to variable sensitivity to cyanobacterial toxins at the host level, rather than a direct alteration of the parasite itself).

Although our initial hypothesis was not supported by these results, prior studies addressing more direct consequences of global warming on abiotic parameters of freshwater bodies promote the idea of constrained epidemics under future climactic changes. For instance, degradation of the atmospheric ozone layer influences the amount of ultraviolet radiations entering the environment, with potentially harmful effects on live organisms (Jankowski & Cader, 1997). Following this premise, Overholt et al. (2012) found that relatively low levels of UV radiations could sharply decrease the infectivity of *Metschnikowia* spores, but did not affect the host's inherent susceptibility to the parasite. In a later study, increasing intensities of UVs and visible light were also shown to reduce the infectivity of *P. ramosa* (Overholt et al., 2020). Finally, Shocket et al. (2019) found that prolonged exposure to temperatures above 20°C could reduce the infectivity of free-living stages in *Metschnikowia*. Therefore, results currently available in the literature point towards the existence of direct mechanisms of interference between climate change-induced parameters of freshwater environments and the resting stages of fungal and bacterial parasites of *Daphnia*.

1.3 Effects of freshwater contamination by nanoplastics

Throughout Chapters 1-2, we found that infected *Daphnia* of genotype AMME_51 generally supported poor parasite transmission under the effects of environmental disturbances. Using the same assemblage of host and parasite, we showed that a different source of environmental stress (high concentrations of nanoplastics in the culture medium) also had diverging effects on distinct parameters contributing to the parasite's success (Chapter 3, Section 3.1). For instance, both concentrations of nanoplastics (5 mg/L and 20 mg/L) increased host susceptibility, as compared to the control (0 mg/L) treatment. However, when taking into account an offsetting effect of nanoplastics on host viability (i.e. early mortality of the host increased as NP concentrations went higher), we found that the overall success of infection did not differ across all NPs treatments. Furthermore, the highest concentration of nanoplastics tested in our study strongly reduced the parasite's ability to produce spores. As pointed out by Duneau et al. (2011), distinct phases of the parasite's infection cycle may be influenced differentially by the environment. Here, we showed that a seemingly positive increase in the parasite's infectivity could be offset by a higher proportion of hosts dying before the parasite could complete its infectious cycle, resulting in either neutral effects ("low" NP concentration) or negative effects on the parasite's overall transmission success ("high" NP concentration).

Although studies investigating the direct consequences of micro- and nanoplastic pollution on parasitic infections are still in their infancy, our findings in the *Daphnia-Metschnikowia* system remain consistent with a limited number of experimental reports available thus far. Notably, Schampera et al. (2021) found that polystyrene nanoplastics could form hetero-aggregates around filaments of *Planktothrix agardhii*, which reduced their availability to zoospores and contributed to an overall reduction of infection severity by a chytrid (i.e. fungal) parasite. In their study investigating trematode parasites of amphibians, Buss et al. (2021) found that polyester microfibers did not affect the survival of cercarian stages or host susceptibility when applied independently; however, simultaneous exposure of both antagonists reduced infection success by about 33% at concentrations of 10 and 20 mg/L. In a complementary field survey, the authors also found evidence for ubiquitous microfiber contamination across all of nine sampled ponds (USA, Pennsylvania). These few pioneer studies suggest that plastic contaminants of different size and shape can exhibit strong inhibitive effects on common parasites of freshwater biota. Still, besides cladoceran and cyanobacteria, important actors of freshwater ecosystems remain to be explored in this context. Examining the effects of micro- and nanoplastics on waterborne diseases should be relatively easy to implement for those host-parasite systems that already benefit from well-defined protocols of experimental infection, such as fish (Bracamonte et al., 2019), crayfish (Mojžišová et al., 2020) and freshwater snails (Babaran et al., 2021).

1.4 Effects of parasite phenology and sequential infections

In Chapter 4, we showed that sequential infections between *Metschnikowia* and *Ordospora* had diverging effects on the fitness of both parasites, depending on which species encountered the host first. The direction of these effects ranged from neutral to negative on host viability and spore production, as compared with single infections; therefore, none of the two tested scenarios involved an increase in the fitness of either parasite. While we did not test for simultaneous infection in our study, it is unlikely that these would result in any positive gain to the parasites, as can be inferred from the results of simultaneous infections in Lohr et al., 2010b (i.e. simultaneous infection of *Metschnikowia* and a castrating parasite of the gut, *Caullerya mesnili* induced a strong reduction in the number of mature spores produced by *Metschnikowia* and spore clusters of *Caullerya*). Whether environmental shifts associated with climate change will influence the distribution of both parasites is not clear; however, it is likely that abiotic parameters play an important part in controlling the seasonal emergence and withdrawal of either parasite. Green (1974) hypothesized that the distribution of certain parasites of *Daphnia* was influenced by the severity of winter and spring temperatures. For instance, maximally hot temperatures in summer and lake cooling in winter were suggested to constrain transmission of *Metschnikowia* in temperate lakes, thus confining seasonal outbreaks to the autumn period (Hall et al., 2006; Shocket et al., 2019; Duffy & Hunsberger, 2019). While factors governing the seasonal patterns

of microsporidia are not fully understood, several hypotheses have been proposed, including mechanisms of density-dependent transmission (Green, 1974; Brambilla, 1983; Ebert, 1995) and limited success of infection at low temperatures (Ruttner-Kolisko, 1977; Vidtmann, 1993; Ebert, 1995). The latter received further support from recent experimental studies, as no successful infection by *Ordospora* could be detected below 11.8°C, which could explain their absence in winter and later re-emergence in the spring (Kirk et al., 2018; 2020).

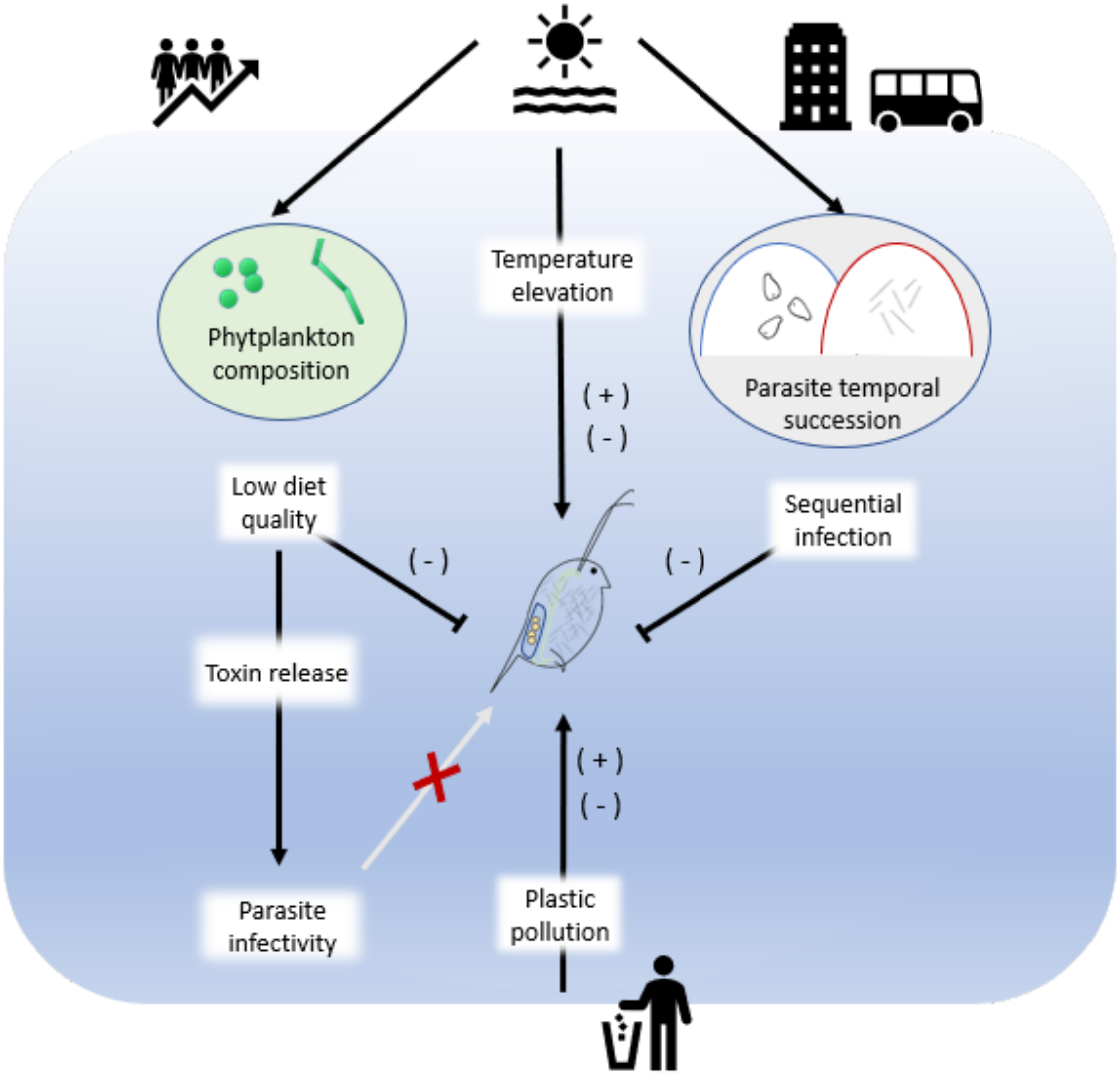
Due to *Ordospora* being a specialist parasite of *D. magna* only, coinfections by these two parasites are most likely to occur in rock pools and pond populations, where *Metschnikowia* generally exhibits much lower prevalence than in lakes (Stirnadel & Ebert, 1997; Goren & Ben-Ami, 2013). Because the probability of infecting the host first correlates with a parasite's prior prevalence in the population (Clay et al., 2019a), field data suggests that most sequential infections between these two parasite should occur with prior establishment of *Ordospora* in the gut epithelium (unless transmission stages are picked up from mixed spore banks residing in the sediment). Thus, the current pattern of infection expected in natural populations seems slightly unfavourable to both parasites, as compared with single infections by either species (Chapter 4). Future changes in the climate may shift the emergence of either parasite in ways that either reinforce or minimize the temporal overlap of their respective epidemic curves. For instance, the minimal temperature threshold compatible with *Ordospora* infections could be reached earlier in the spring, thus pushing microsporidian outbreaks earlier in the year. Such a pattern could reinforce prior dominance of microsporidia in *D. magna* populations and impair the later emergence of *Metschnikowia*. Using epidemiological models implementing within-host priority effects, parameterized from sequential infection assays between *Metschnikowia* and the bacterium *Pasteuria ramosa*, Clay et al. (2020) predicted that advancing the start date of bacterial epidemics relative to that of the fungus would decrease the mean prevalence of *Pasteuria*, supporting the idea that future climactic changes could affect the timing and outcome of co-occurring epidemics.

Overall, it appears that most anthropogenically-derived shifts explored throughout this thesis resulted in either neutral or negative effects on the transmission of our focal parasite, with only limited evidence for net positive gains in transmission-related traits. The few positive effects of anthropic disturbances on parameters of parasite fitness were either highly context-dependent (Chapter 1) or applied to discrete parameters of parasite fitness that were not representative of the parasite's overall transmission success (Chapter 3). Meanwhile, evidence for negative effects were found in all four chapters, when comparing the performance of the parasite across disturbed and control conditions. The respective effects of each factor explored throughout this thesis, as well as the different pathways connecting these anthropic disturbances in freshwater ecosystems are represented graphically in Box 5.

In this regard, our combined findings provide further nuance to the highly topical 'warmer is sicker' hypothesis, thus converging with the opinions of Hall et al. (2006), who concluded that parasite infection traits would mostly respond negatively to temperature changes in the *Daphnia-Metschnikowia* system. Moreover, it introduces the possibility of shifting the question slightly: rather than focusing on rising temperatures alone, one would legitimately ask whether the spread of parasitic diseases should increase or decrease in an increasingly 'disturbed' or 'anthropized' world (as was previously proposed by Lafferty & Kuris, 2005). In the present work, we highlight that most disturbances introduced by human activity appear to converge towards lower parasite success – rather than the opposite – with the potential to constrain epidemics in freshwater ecosystems, as evidenced by a commonly distributed and ecologically relevant host-parasite assemblage.

However, one should bear in mind that the sensitivity of distinct infection traits to environmental variables may be highly system-specific, and thus not necessarily generalizable to the wider diversity of parasites encountered in the *Daphnia* genus. For instance, direct counterexamples were found by Sánchez et al. (2019), who showed that the overall transmission success of *P. ramosa* was favoured by low-quality diets. The authors suggested that high-quality, non-toxic diets may help *Daphnia* suppress the pathogen's replication; by contrast, low-quality diets would prevent the successful display of host defences, while no medicinal effect of cyanobacteria was found against this bacterial parasite. In other freshwater host-parasites systems, contrasting effects of environmental disturbances were also found, which seemed to depend on the taxonomic identity (e.g. trematode vs. echinostome) and levels of host-specificity (e.g. generalist vs. specialist) displayed by such parasites (Koprivnikar & Redfern, 2012). Overall, we argue that the spread of waterborne parasites should not be inferred from discrete parameters of infection that do not reflect, on their own, the overall transmission success of a pathogen in the environment. Moreover, future modelling and experimental studies should not fail to consider the interdependency of environmental disturbances, instead aiming for more integrative approaches to epidemiological predictions in a changing world.

Box 5. Graphical summary of our findings. Environmental disturbances associated with climate change and plastic pollution of freshwater environments promote neutral to negative effects on the transmission of *Metschnikowia*. Regular arrows represent context-dependent effects that either enhanced (+) or reduced (-) parameters of parasite transmission in our studies. Flat-ended arrows represent strictly inhibitive effects (-) of environmental disturbances on parasite transmission. The greyed out arrow represents the absence of a predicted effect.



2. Interactive effects of environmental stressors: the missing links

In their review summarizing the effects of micro- and nanoplastics on freshwater biota, Agathokleous et al. (2021) remind their audience that "*no single stress occurs in the environment*". This point was made to highlight the recent discovery of interactive effects between micro- and nanoplastics and other environmental factors, including copper contamination (Zhu et al., 2020), pH levels (Piccardo et al., 2020), growing media (Ustabasi & Baysal, 2020; Ziajahromi et al., 2019) and natural food availability (Ogonowski et al., 2016). More importantly, Yang et al. (2020) found that biological responses to nanoplastics could be altered by environmental shifts representative of climate change, showing that elevated levels of CO2 and warmer temperatures could attenuate the toxicity of nanoplastics towards *Scenedesmus obliquus*. The implications of such findings are compelling, and highlight the importance of using 'big picture' approaches when making predictions about the spread of disease in freshwater ecosystems. For instance, most freshwater bodies threatened by plastic pollution are likely to be concurrently affected by warming. While each independent factor was shown to modulate the outcome of infection in our focal system (Chapters 1 & 3), complex interactions may arise that change the directional effects that would be expected under the single threat of nanoplastics and temperature. Chapter 1 represented such an attempt to account for the interdependency of environmental challenges at play in freshwater ecosystems, and allowed us to observe this precise phenomenon across different assemblages of temperature and host diet quality.

Nevertheless, experimental studies focusing on simpler designs are still required, especially when trying to identify biological processes that are still poorly understood in the literature. For instance, Chapter 3 focused on the effects of nanoplastics on host-parasite interactions in a *Daphnia*-yeast system. Because such effects were previously unaccounted for in the literature, we deliberately limited the scope of this study, prioritizing the distribution of our replicative and statistical power towards the analysis of a single explanatory variable (i.e. nanoplastic concentration). By addressing discrete biological processes that lack prior documentation, such experiments can then serve as stepping stone for future research to improve upon. For instance, we were able to identify negative effects of nanoplastics on parasite reproduction, which did not occur until concentrations > 5 mg/L. Building upon such preliminary findings, future research could then focus on i) refining the determination of a dose-response relationship, using a greater selection of nanoplastic concentrations or ii) attempt to simulate more realistic conditions in the laboratory, by accounting for the interactive effects of nanoplastics and other environmental stressors. Similarly, epidemiological modelling approaches may benefit from the establishment of prior experimental data (e.g. spore yield, host lifespan), which can then be used to parameterize predictive models (e.g. Clay et al., 2019b, 2020).

Based on these premises, future research could focus on investigating the joint effects of elevated temperature and plastic pollution in experimental infections of *Daphnia*. As water temperature increases, the metabolic rate of *Daphnia* is elevated, promoting higher rates of particle uptake (McMahon, 1965). This phenomenon was previously suggested to promote the recruitment of parasite spores, including those of *Metschnikowia* under conditions of high temperature (Cuco et al., 2018; Shocket al., 2019). The increased filtering rate of *Daphnia* under elevated temperature may create contradictory effects on infection success, favouring the acquisition of parasite spores, while simultaneously increasing the intake of nanoplastic particles, which were found to negatively affect the parasite's ability to produce spores at high concentrations (Chapter 3; Section 3.1). Moreover, it was previously shown that nanoplastic uptake could reinforce the host's haemocyte response, particularly at high temperature (Sadler et al., 2019). Such negative effects of nanoplastics on parasite growth may balance out the beneficial effects of temperature on encounter rates, suggesting that lowest parasite success could be attained under high NP × high temperature conditions. On a related side note, Feng et al. (2020) showed that nanoplastics could promote the production and later release of microcystin in *Microcystis aeruginosa*. Although results from Chapter 2 suggest that this should not interfere with the parasite in its free-living stage, concurrent exposure to nanoplastics and *Microcystis*-based diet could exert stronger effects of *Metschnikowia* when ingested as part of the host diet, as was observed in Chapters 1 & 2. Finally, Besseling et al. (2014) found that nanoplastics could impair the growth rate of *Scenedesmus obliquus*, by reducing the chlorophyll concentration of algal cells. Such a phenomenon could further contribute to a reduction of zooplankton diet quality, which begs the question of interactive effects between nanoplastics and food levels in the context of parasitic infections, and may motivate yet another link to explore throughout future studies.

Besides the gradual accumulation of plastic particles in the food chain, sediments and the water column, freshwater ecosystems are additionally threatened by a variety of chemical contaminants, many of which are derived from industrial and agricultural purposes. Adding to our understanding of interactive environmental stressors, Cuco et al. (2018) already demonstrated that a temperature elevation of 4°C (equivalent to the one tested in Chapter 1) could reinforce the anti-parasitic actions of an agricultural fungicide towards *Metschnikowia*, suggesting that temperature can act as a modulator of interactions between pollution and disease in freshwater ecosystems. Similar interactions between temperature and contaminants were previously identified in *Daphnia;* however, these studies were conceived as single-species ecotoxicological assays, and did not address the possible implications for parasites of *Daphnia*. For instance, a combination of high temperature (26°C) and high concentration of nitrate (>250 mg NO3/L) reduced the mortality of *D. magna* by 60%, which was three times stronger than the effect of either stressor applied independently (Maceda-Veiga et al., 2015). Similarly, Bae et al. (2016) found that multi-generational exposure to elevated temperature aggravated the toxicity of Cu particles, in the form of stronger inducement of *D. magna*'s oxidative stress response, which could

translate to the next generation. Such interactive effects of temperature and environmental pollutants (other than nanoplastics) represent other promising leads that could be explored in future experimental assays using the *Daphnia*-microparasite system.

3. Understanding the complexity of within-host processes

Experimental studies on sequential infections have determined that complex within-host processes – resulting from the competition between sequentially arriving parasites – can scale up to alter epidemic dynamics at the community level (Ezenwa et al., 2010; Bushman et al., 2018; Clay et al., 2020). Whether these imply modifications of the host's immune system, metabolic rate or mechanisms of resource allocation, the influence of external environmental factors on parameters of parasite transmission often find an explanation in complex processes that occur at the intra-host level; however, these may be difficult to observe *in situ*. While some of the factors contributing to infectious cycles are easy to identify and can readily be measured in experimental settings, such as foraging rates (Penczykowski et al., 2014) or the gut residence time for parasite spores (Kirk et al., 2019) and contaminants (Ogonowski et al., 2018), identifying the exact sub-cellular processes tying environmental disturbances with variable infection outcomes may require advanced techniques of visualization. For instance, we hypothesized that direct interference between nanoplastics and the expression of immune defences could explain the higher susceptibility of hosts exposed to NPs in Chapter 3; however, the experimental setup of our study did not allow us to confirm this conjecture by direct observations. For this purpose, one may consider combining experimental infection assays with fluorescent markings of nanoplastic particles (e.g. a similar method was previously used by Chae et al., 2018 to observe the distribution of NPs in live *Daphnia*). Alternatively, the staining technique that we used to facilitate quantification of microsporidian spores in Chapter 4 may be adapted to monitor the movements of *Ordospora* in live *Daphnia*, provided that staining agents do not bear toxicity to either the parasite or the host itself. Such complementary methods may be used to shed light on the sub-cellular processes underlying infections in conditions of environmental stress.

Ultimately, some of the environmental stressors that were investigated throughout this thesis may have influenced infection outcomes via mechanically similar processes. For instance, Chapter 4 supported the existence of within-host priority effects resulting from the sequential infection order of *Ordospora* and *Metschnikowia*. Among the antagonistic processes thought to derive from sequential infections, one can cite mechanisms of priming of the host's immune system (Rodrigues et al., 2010; Syller & Grupa, 2016) or the phenomenon of prior resource sequestration by early arriving parasites (Graham, 2008). Moreover, some highly virulent parasites of *Daphnia* are capable of castrating their hosts (e.g. *Caullerya mesnili;* Wolinska et al., 2004), a parasitic strategy that is often accompanied by

an atrophy or malformation of the host's gonadal tissues (Baudoin, 1975). While we showed that *Daphnia* fed with *Microcystis* and *Planktothrix* diets grew smaller and produced fewer offspring than control individuals fed with green algae (Chapter 1, Supporting Information), Allan (1976) additionally reported that the ovaries of *Microcystis*-fed individuals also appeared to be less developed. In other words, whether prior atrophy of the ovaries and depletion of the host's resource that would normally be allocated towards reproduction were caused by i) the ingestion of a low-quality diet or ii) prior infection by a parasite competing for resource exploitation, two distinct sources of environmental stress may result in the same physiological end point, which could have been responsible for the lower growth rate of *Metschnikowia* reported across Chapters 1, 2 and 4. To further this analogy, it was previously shown that nanoplastics could inhibit reproduction, induce abnormal embryonic development in *Daphnia galeata* (Cui et al., 2017) and that the uptake of polystyrene nanoparticles could be mediated through the brood chamber in *D. magna* (Brun et al., 2017). Finally, the possible recognition of nanoplastic particles as 'non-self' by the host's immune system may result in similar mechanisms of immune priming to those attributed to sequential infections: in fact, short-term exposure to polystyrene nanoplastics was shown to induce the expression of stress defence mechanisms in *Daphnia*, such as the production of reactive oxygen species (Liu et al., 2018, 2019, 2020).

The inherent life mode and nature of the host tissue targeted by distinct endoparasites may also influence how much of an impact nanoplastics could have on their respective development. Because cell membranes are permeable to polystyrene nanoplastics (Bojic et al., 2020; Jung et al., 2020), plastic pollution may be more detrimental to intracellular parasites of *Daphnia* (e.g. *Ordospora*, which colonizes cells of the gut epithelium) as opposed to parasites reproducing in the host's body cavity (e.g. *Metschnikowia*). Thus, in the presence of nanoplastics, intra-host competition may be swayed in favour of the fungal parasite. This hypothesis is currently undergoing testing in our research group, using the same assemblage of host and parasites used in Chapter 4 (*Daphnia magna* NO-V-7, singly or coinfected with *Metschnikowia bicuspidata* or *Ordospora colligata*). Using the experience and knowledge gained from working with nanoplastics (Chapter 3) and the *Metschnikowia-Ordospora* coinfection system (Chapter 4), a follow-up experiment was recently performed in our laboratory. Although full disclosure of the data is not possible at the time of writing this dissertation, preliminary analyses suggest a similar threshold for hormesis in uninfected *Daphnia magna* (i.e. slightly positive effects of NPs on parameters of host fitness up to 5 mg/L), while the virulence of *Metschnikowia* in both single and co-infections seemed to increase even at the lowest NP concentration (Schlösser et al., in prep). Moreover, the upcoming results will allow us to estimate the impact that these two parasites exert under simultaneous infection, which was not previously tested in Chapter 4. One possible mechanism that could interfere with the establishment and later multiplication of *Ordospora* in the gut epithelium involves the formation of an intracellular parasitophorous vacuole, which is believed to be produced by the host at the time of infection (Larsson et al., 1997). Prior or later intrusion of nanoplastics into epithelial cells

may disrupt the host-mediated processes involved in the formation of this structure, thus preventing the development of sporogonial stages.

Finally, one should bear in mind the limits of extrapolating individual-based results to natural populations. The same experimental dose (i.e. number of spores / volume) applied to an experimental population of *Daphnia*, as opposed to individually-monitored *Daphnia* in reduced volume, may result in different transmission dynamics, based on factors such as between-host competition for resource acquisition or simply individual stochasticity. Even within clonally-reproducing hosts, *Daphnia* of the same genotype may still differ in size, due to stochastic differences in their early life-history or the clutch order they originated from (Ebert, 1991). As such, larger or healthier individuals are expected to exert more efficient filtering rates, thus promoting the ingestion of a larger proportion of dissolved particles (whether it be food, pollutants, or infective propagules), which could simultaneously reduce the encounter rate of smaller or younger *Daphnia*. In natural populations, distinct age classes can coexist at a given time, and all *Daphnia* do not encounter parasite spores at the exact same age. Thus, there could be an interesting equilibrium between more resistant adults depleting the pool of parasite propagules towards an infectious sink (i.e. failed infection, that will not result in the production of new parasite propagules) while juvenile *Daphnia,* shown to have weaker immune systems and gut barriers (Stewart Merrill et al., 2019), would benefit from a diminished risk of ingesting parasite spores. Whereas simulating realistic age structure at the population level would more accurately represent the conditions for parasite transmission in nature, individual-based experiments that provide such knowledge in the first place (e.g. Izhar & Ben-Ami, 2015; Stewart Merrill et al., 2019 for age- and size-related susceptibility to infection) represent equally important contributions to the literature. According to Kirk et al. (2021), while processes observed among vector-borne parasites tend to translate from individual to the population level, environmentally-transmitted parasitism may operate by a different set of rules, suggesting the need for trait-based studies at both the individual and population levels in these systems. As *Metschnikowia* belongs to the second category, both approaches should contribute equally to the literature, each providing different – yet complementary – sets of information towards our understanding of infection dynamics. Ideally, epidemiological models should aim at parameterizing their equations, using values determined from individual-based data, while confirming that simulated transmission patterns do indeed match with those observed in the field as well as population-level experiments.

4. Looking forward : conservation and restoration of freshwater ecosystems

Although global environmental shifts may result in detrimental effects towards a common fungal parasite of *Daphnia*, the growth rate of host populations should also respond negatively to future environmental disturbances. Indeed, Chapter 1 and Chapter 3 both showed strong combinatory effects of parasite-induced virulence and environmental stress (i.e. elevated temperature, low-quality diets, nanoplastics) on fitness parameters of *Daphnia*, which could be even more exacerbated, considering that all three of those environmental stressors may occur concurrently with parasite epidemics in nature. The ecological implications of parasites, however, may extend beyond negative effects towards their hosts: with the potential to establish alternative trophic links and the ability to modulate existing ones (Valois & Burns, 2016; Frenken et al., 2020), parasites may even create positive feedback loops at the community level, with parasite diversity being sometimes considered as an indicator of ecosystem health (Hudson et al., 2006; Hatcher et al., 2012). Thus, aiming towards a reduced occurrence of parasitic infections in freshwater environments does not represent a conceivable scenario. Instead, future conservation measures should focus on i) understanding how climactic changes and anthropic disturbances will affect disease dynamics in the future and ii) developing and applying methods to either limit – or revert – the ecological impacts of human activity on the status of freshwater bodies.

Overall, the experimental data presented throughout the present work should provide incremental support towards the former objective. While this overarching topic has been promoted since the end of the 20th century (Martens et al., 1995), we were able to identify a number of grey areas – particularly relating to freshwater ecosystems – that have only recently started to gain traction in the scientific literature. Beyond the contribution of experimental infection assays, field-based science and environmental monitoring may also capitalize on sampling sites of interest. For instance, using a set of lakes artificially heated by adjacent power-plant cooling activity, researchers have the possibility to observe the effects of future lake warming in 'live-action' settings (Dziuba et al., 2020, 2021). By comparing these sites of interest with neighbouring, 'control' lakes (that were not affected by the same anthropization pressure), such studies have and will continue to provide valuable insights into the response of *Daphnia* communities and their parasites towards a predicted temperature elevation of 4°C.

Although anthropic pressures have already altered the trophic state and abiotic conditions of freshwater bodies, there is increasing support in the literature for innovative methods that would enable a gradual restoration and possible de-contamination of said environments. One of the first measure to consider – and perhaps the most relevant to our system of interest – consists in the preservation of *Daphnia* populations themselves. Indeed, filter-feeding cladocerans have been regularly considered for conservation purposes, and may contribute to the control of bloom-forming cyanobacteria (Gerasimova

et al., 2018) and de-eutrophication of water bodies (Gerasimova & Pogozhev, 2002; Pogozhev & Gerasimova, 2005). Using similar approaches, recent studies have provided support for the biological control (i.e. 'bioremediation') of various contaminants. Such options that have been considered include the biodegradation of plastics (via enzymatic digestion) by specialized bacteria (Yoshida et al., 2016; Hiraga et al., 2019) and aquatic fungi (Paço et al., 2017), or the biodegradation and phytoremediation of pesticides using freshwater macrophytes and algae (Vandermaesen et al., 2016; Riaz et al., 2017). Recent technological innovations may also contribute to this purpose, including advanced technologies of wastewater treatments to remove plastic pollution (Lares et al., 2018), endocrine disruptors (Kim et al., 2021) or the removal of pesticides using multi-walled carbon nanotubes (Dehghani et al., 2019). However, it must be noted that such restoration methods may not be sufficient to compensate the rate at which excess nutrients and contaminants enter the environment; as such, conservation policies should concentrate their efforts towards reducing their discharge in the first place (Zamparas & Zacharias, 2014). Alternatively, this may be achieved by encouraging the production of biodegradable plastic matters, which can be derived from cellulose or lignin (Silva et al., 2018).

Not unlike the aforementioned process of environmental remediation, which can be achieved by localized efforts to restore the chemical properties of contaminated sites, a similar course of action may be taken at a global scale in order to limit our future contribution to climate change. According to the most recent report from the International Panel for Climate Change (IPCC 2021), predicted scenarios implying very low (SSP1-1.9) or low (SSP1-2.6) emissions of greenhouse gases could have rapid and sustainable effects to limit the impact of human activity on climate change. Provided a strict and globalized commitment to a lowest-emission scenario, it may be possible to limit the rising occurrence of climate-induced disasters (such as extreme sea levels) and avoid the crossing of dangerous heat thresholds by the end of the century. With regards to global surface temperature elevation, noticeable differences to the predicted trends may even emerge under shorter terms. Finally, provided the successful implementation of anthropogenic CO2 removal to a scale that would exceed residual emissions, it may not be too late to envision a future reversal and possible shift towards a net cooling scenario (Allan et al., 2021). Though many efforts and long-term promises are still required in order to revert – or at the very least hinder – our impact on the environment, understanding in which way future environmental conditions will affect host-parasite dynamics in threatened ecosystems constitutes a necessary and commendable first step towards applying possible conservation and restoration measures.

Conclusion: Human activity translates into a wide array of environmental shifts, from rising temperatures at a global scale to the localized pollution of freshwater bodies. Disturbances of anthropogenic origin may act independently or interactively to affect the growth of zooplankton, as well as modulate their interactions with environmentally-transmitted parasites. Convergent evidence suggests that the transmission of a common fungal parasite of *Daphnia* may be constrained under increasingly anthropic conditions. Overall, future predictions of disease dynamics should consider that no single stress occurs in the environment, and that distinct phases of a parasite's infectious cycle may be influenced differentially by such disturbances.

BIBLIOGRAPHY

(Introduction & Discussion)

Aalto, S. L., & Pulkkinen, K. (2013). Food stoichiometry affects the outcome of *Daphnia*–parasite interaction. *Ecology and Evolution*, *3*(5), 1266–1275. https://doi.org/10.1002/ece3.545

Abrego, N., Roslin, T., Huotari, T., Ji, Y., Schmidt, N. M., Wang, J., Yu, D. W., & Ovaskainen, O. (2021). Accounting for species interactions is necessary for predicting how arctic arthropod communities respond to climate change. *Ecography*, *44*(6), 885-896. https://doi.org/10.1111/ecog.05547

Adamowicz, S. J., Petrusek, A., Colbourne, J. K., Hebert, P. D., & Witt, J. D. (2009). The scale of divergence: a phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus. *Molecular Phylogenetics and Evolution*, *50*(3), 423-436. https://doi.org/10.1016/j.ympev.2008.11.026

Agathokleous, E., Iavicoli, I., Barceló, D., & Calabrese, E. J. (2021). Micro/nanoplastics effects on organisms: A review focusing on 'dose'. *Journal of Hazardous Materials*, 126084. https://doi.org/10.1016/j.jhazmat.2021.126084

Agha, R., Saebelfeld, M., Manthey, C., Rohrlack, T., & Wolinska, J. (2016). Chytrid parasitism facilitates trophic transfer between bloom-forming cyanobacteria and zooplankton (*Daphnia*). *Scientific Reports*, *6*(1), 1-9. https://doi.org/10.1038/srep35039

Ainsworth, A. J., Dexiang, C., Waterstrat, P. R., & Greenway, T. (1991). Effect of temperature on the immune system of channel catfish (*Ictalurus punctatus*)—I. Leucocyte distribution and phagocyte function in the anterior kidney at 10° C. *Comparative Biochemistry and Physiology Part A: Physiology*, *100*(4), 907-912. https://doi.org/10.1016/0300-9629(91)90313-2

Albert, J. S., Destouni, G., Duke-Sylvester, S. M., Magurran, A. E., Oberdorff, T., Reis, R. E., Winemiller, K. O., & Ripple, W. J. (2021). Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio*, *50*(1), 85-94. https://doi.org/10.1007/s13280-020-01318-8

Aljaibachi, R., Laird, W. B., Stevens, F., & Callaghan, A. (2020). Impacts of polystyrene microplastics on *Daphnia magna*: a laboratory and a mesocosm study. *Science of The Total Environment*, *705*, 135800. https://doi.org/10.1016/j.scitotenv.2019.135800

Allan, J. D. (1976). Life history patterns in zooplankton. *The American Naturalist*, *110*(971), 165-180. https://doi.org/10.1086/283056

Allan, R. P. , Hawkins, E. , Bellouin, N. and Collins, B. (2021). IPCC, 2021: Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. https://www.ipcc.ch/report/ar6/wg1/#SPM

Allen, Y. C., De Stasio, B. T., & Ramcharan, C. W. (1993). Individual and population level consequences of an algal epibiont on *Daphnia*. *Limnology and Oceanography*, *38*(3), 592-601. https://doi.org/10.4319/lo.1993.38.3.0592

Altermatt, F., Pajunen, V. I., & Ebert, D. (2009). Desiccation of rock pool habitats and its influence on population persistence in a *Daphnia* metacommunity. *PLoS ONE*, *4*(3), e4703. https://doi.org/10.1371/journal.pone.0004703

Anderson, O. R. (2018). Evidence for coupling of the carbon and phosphorus biogeochemical cycles in freshwater microbial communities. *Frontiers in Marine Science*, *5*, 20. https://doi.org/10.3389/fmars.2018.00020

Angell, E. A. (2016). How biotic interactions between *Daphnia magna* and *Vorticella* alter the ability of *Daphnia magna* to respond to abiotic changes (Doctoral dissertation, University of Colorado Boulder). https://scholar.colorado.edu/concern/undergraduate_honors_theses/h702q6934

Auguste, M., Lasa, A., Balbi, T., Pallavicini, A., Vezzulli, L., & Canesi, L. (2020). Impact of nanoplastics on hemolymph immune parameters and microbiota composition in *Mytilus galloprovincialis*. *Marine Environmental Research*, *159*, 105017. https://doi.org/10.1016/j.marenvres.2020.105017

Babaran, D., Koprivnikar, J., Parzanini, C., & Arts, M. T. (2021). Parasites and their freshwater snail hosts maintain their nutritional value for essential fatty acids despite altered algal diets. *Oecologia*, 1-12. https://doi.org/10.1007/s00442-021-04944-5

Bae, E., Samanta, P., Yoo, J., & Jung, J. (2016). Effects of multigenerational exposure to elevated temperature on reproduction, oxidative stress, and Cu toxicity in *Daphnia magna*. *Ecotoxicology and Environmental Safety*, *132*, 366-371. https://doi.org/10.1016/j.ecoenv.2016.06.034

Banholzer, S., Kossin, J., & Donner, S. (2014). The impact of climate change on natural disasters. In *Reducing disaster: Early warning systems for climate change* (pp. 21-49). Springer, Dordrecht. https://doi.org/10.1007/978-94-017-8598-3_2

Bao, J., Jiang, H., Shen, H., Xing, Y., Feng, C., Li, X., & Chen, Q. (2021). First description of milky disease in the Chinese mitten crab *Eriocheir sinensis* caused by the yeast *Metschnikowia bicuspidata*. *Aquaculture*, *532*, 735984. https://doi.org/10.1016/j.aquaculture.2020.735984

Bartosiewicz, M., Przytulska, A., Deshpande, B. N., Antoniades, D., Cortes, A., MacIntyre, S., Lehmann, M. F., & Laurion, I. (2019). Effects of climate change and episodic heat events on cyanobacteria in a eutrophic polymictic lake. *Science of the Total Environment*, *693*, 133414. https://doi.org/10.1016/j.scitotenv.2019.07.220

Baudoin, M. (1975). Host castration as a parasitic strategy. *Evolution*, 335-352. https://doi.org/10.2307/2407221

Ben-Ami, F., Mouton, L., & Ebert, D. (2008). The effects of multiple infections on the expression and evolution of virulence in a *Daphnia*-endoparasite system. *Evolution*, *62*, 1700–1711. https://doi.org/10.1111/j.1558-5646.2008.00391.x

Ben-Ami, F., Ebert, D., & Regoes, R. R. (2010). Pathogen dose infectivity curves as a method to analyze the distribution of host susceptibility: a quantitative assessment of maternal effects after food stress and pathogen exposure. *The American Naturalist*, *175*(1), 106–115. https://doi.org/10.1086/648672

Ben-Ami, F., Rigaud, T., & Ebert, D. (2011). The expression of virulence during double infections by different parasites with conflicting host exploitation and transmission strategies. *Journal of Evolutionary Biology*, *24*(6), 1307-1316. https://doi.org/10.1111/j.1420-9101.2011.02264.x

Benny, G. L., & O'Donnell, K. (2000). *Amoebidium parasiticum* is a protozoan, not a Trichomycete. *Mycologia*, *92*(6), 1133-1137. https://doi.org/10.1080/00275514.2000.12061260

Benzie, J. A. (1987). The biogeography of Australian *Daphnia*: clues of an ancient (> 70 my) origin for the genus. *Hydrobiologia*, *145*(1), 51-65. https://doi.org/10.1007/BF02530265

Bergami, E., Pugnalini, S., Vannuccini, M. L., Manfra, L., Faleri, C., Savorelli, F., Dawson, K. A., & Corsi, I. (2017). Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*. *Aquatic Toxicology*, *189*, 159-169. https://doi.org/10.1016/j.aquatox.2017.06.008

Bergman Filho, T. U., Soares, A. M., & Loureiro, S. (2011). Energy budget in *Daphnia magna* exposed to natural stressors. *Environmental Science and Pollution Research*, *18*(4), 655-662. https://doi.org/10.1007/s11356-010-0413-0

Bernstein, L., Bosch, P., Canziani, O., Chen, Z., Christ, R., & Riahi, K. (2008). IPCC, 2007: Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and Reisinger, A. (eds.)]. IPCC, Geneva, Switzerland, 104 pp. https://www.ipcc.ch/report/ar4/syr/

Besseling, E., Wang, B., Lürling, M., & Koelmans, A. A. (2014). Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. *Environmental Science & Technology*, *48*(20), 12336-12343. https://doi.org/10.1021/es503001d

Bittner, K., Rothhaupt, K. O., & Ebert, D. (2002). Ecological interactions of the microparasite *Caullerya mesnili* and its host *Daphnia galeata*. *Limnology and Oceanography*, *47*(1), 300–305. https://doi.org/10.4319/lo.2002.47.1.0300

Bojic, S., Falco, M. M., Stojkovic, P., Ljujic, B., Gazdic Jankovic, M., Armstrong, L., Markovic, N., Dopazo, J., Lako, M., Bauer, R., & Stojkovic, M. (2020). Platform to study intracellular polystyrene nanoplastic pollution and clinical outcomes. *Stem Cells*, *38*(10), 1321-1325. https://doi.org/10.1002/stem.3244

Bracamonte, S. E., Johnston, P. R., Monaghan, M. T., & Knopf, K. (2019). Gene expression response to a nematode parasite in novel and native eel hosts. *Ecology and Evolution*, *9*(23), 13069-13084. https://doi.org/10.1002/ece3.5728

Brambilla, D. J. (1983). Microsporidiosis in a *Daphnia pulex* population. *Hydrobiologia*, *99*(3), 175-188. https://doi.org/10.1007/BF00008769

Bresciani, L., Lemos, L. N., Wale, N., Lin, J. Y., Strauss, A. T., Duffy, M. A., & Rodrigues, J. L. (2018). Draft Genome Sequence of "Candidatus *Spirobacillus cienkowskii*," a Pathogen of Freshwater *Daphnia* Species, Reconstructed from Hemolymph Metagenomic Reads. *Microbiology Resource Announcements*, *7*(22), e01175-18. https://doi.org.10.1128/MRA.01175-18

Bronmark, C., Klosiewski, S. P., & Stein, R. A. (1992). Indirect effects of predation in a freshwater, benthic food chain. *Ecology*, *73*(5), 1662-1674. https://doi.org/10.2307/1940018

Brun, N. R., Beenakker, M. M., Hunting, E. R., Ebert, D., & Vijver, M. G. (2017). Brood pouch-mediated polystyrene nanoparticle uptake during *Daphnia magna* embryogenesis. *Nanotoxicology*, *11*(8), 1059-1069. https://doi.org/10.1080/17435390.2017.1391344

Brzeziński, T., Fronk, J., Trzcińska-Danielewicz, J., & Dawidowicz, P. (2012). Interspecific hybridization in sympatric species of *Daphnia* inhabiting lakes in northeastern Poland. *Oceanological and Hydrobiological Studies*, *41*(2), 1-6. https://doi.org/10.2478/s13545-012-0011-5

Budyko, M. I. (1969). The effect of solar radiation variations on the climate of the Earth. *Tellus*, *21*(5), 611-619. https://doi.org/10.3402/tellusa.v21i5.10109

Burgmer, T., Hillebrand, H., & Pfenninger, M. (2007). Effects of climate-driven temperature changes on the diversity of freshwater macroinvertebrates. *Oecologia*, *151*(1), 93-103. https://doi.org/10.1007/s00442-006-0542-9

Burns, C. W. (1969). Relation between filtering rate, temperature, and body size in four species of *Daphnia*. *Limnology and Oceanography*, *14*(5), 693-700. https://doi.org/10.4319/lo.1969.14.5.0693

Buser, C. C., Jansen, M., Pauwels, K., De Meester, L., & Spaak, P. (2012). Combined exposure to parasite and pesticide causes increased mortality in the water flea *Daphnia*. *Aquatic Ecology*, *46*(2), 261-268. https://doi.org/10.1007/s10452-012-9397-9

Bushman, M., Antia, R., Udhayakumar, V., & de Roode, J. C. (2018). Within-host competition can delay evolution of drug resistance in malaria. *PLoS Biology*, *16*(8), e2005712. https://doi.org/10.1371/journal.pbio.2005712

Buss, N., Sander, B., & Hua, J. (2021). Effects of Polyester Microplastic Fiber Contamination on Amphibian–Trematode Interactions. *Environmental Toxicology and Chemistry*, 1–11. https://doi.org/10.1002/etc.5035

Cáceres, C. E., Hall, S. R., Duffy, M. A., Tessier, A. J., Helmle, C., & MacIntyre, S. (2006). Physical structure of lakes constrains epidemics in *Daphnia* populations. *Ecology*, *87*(6), 1438-1444. https://doi.org/10.1890/0012-9658(2006)87[1438:PSOLCE]2.0.CO;2

Callisto, M., Molozzi, J., & Barbosa, J. L. E. (2014). Eutrophication of lakes. In *Eutrophication: causes, consequences and control* (pp. 55-71). Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7814-6_5

Caplat, P., Anand, M., & Bauch, C. (2008). Interactions between climate change, competition, dispersal, and disturbances in a tree migration model. *Theoretical Ecology*, *1*(4), 209-220. https://doi.org/10.1007/s12080-008-0021-5

Castillo, A. M., Sharpe, D. M., Ghalambor, C. K., & De León, L. F. (2018). Exploring the effects of salinization on trophic diversity in freshwater ecosystems: a quantitative review. *Hydrobiologia*, *807*(1), 1-17. https://doi.org/10.1007/s10750-017-3403-0

Caswell, H. (2010). Life table response experiment analysis of the stochastic growth rate. *Journal of Ecology*, *98*(2), 324-333. https://doi.org/10.1111/j.1365-2745.2009.01627.x

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M., & Palmer, T. M. (2015). Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science Advances*, *1*(5), e1400253. https://doi.org/10.1126/sciadv.1400253

Chae, Y., Kim, D., Kim, S. W., & An, Y. J. (2018). Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. *Scientific Reports*, *8*(1), 1-11. https://doi.org/10.1038/s41598-017-18849-y

Chen, S. C., Chen, T. H., Wang, P. C., Chen, Y. C., Huang, J. P., Lin, Y. D., Chaung, H. C., & Liaw, L. L. (2003). *Metschnikowia bicuspidata* and *Enterococcus faecium* co-infection in the giant freshwater prawn *Macrobrachium rosenbergii*. *Diseases of Aquatic Organisms*, *55*(2), 161-167. https://doi.org/10.3354/dao055161

Chopelet, J., Blier, P. U., & Dufresne, F. (2008). Plasticity of growth rate and metabolism in *Daphnia magna* populations from different thermal habitats. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *309*(9), 553-562. https://doi.org/10.1002/jez.488

Chow-Fraser, P., & Sprules, W. G. (1986). Inhibitory effect of *Anabaena* sp. on in situ filtering rate of *Daphnia*. *Canadian Journal of Zoology*, *64*(9), 1831-1834. https://doi.org/10.1139/z86-273

Clay, P. A., Cortez, M. H., Duffy, M. A., & Rudolf, V. H. (2019a). Priority effects within coinfected hosts can drive unexpected population-scale patterns of parasite prevalence. *Oikos*, *128*(4), 571-583. https://doi.org/10.1111/oik.05937

Clay, P. A., Dhir, K., Rudolf, V. H., & Duffy, M. A. (2019b). Within-host priority effects systematically alter pathogen coexistence. *The American Naturalist*, *193*(2), 187-199. https://doi.org/10.1086/701126

Clay, P. A., Duffy, M. A., & Rudolf, V. H. (2020). Within-host priority effects and epidemic timing determine outbreak severity in co-infected populations. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1922), 20200046. https://doi.org/10.1098/rspb.2020.0046

Civitello, D. J., Forys, P., Johnson, A. P., & Hall, S. R. (2012). Chronic contamination decreases disease spread: a *Daphnia*–fungus–copper case study. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1741), 3146-3153. https://doi.org/10.1098/rspb.2012.0684

Civitello, D. J., Penczykowski, R. M., Smith, A. N., Shocket, M. S., Duffy, M. A., & Hall, S. R. (2015). Resources, key traits and the size of fungal epidemics in *Daphnia* populations. *Journal of Animal Ecology*, *84*(4), 1010–1017. https://doi.org/10.1111/1365-2656.12363

Codreanu, R., & Codreanu-Balcescu, D. (1981). On two *Metschnikowia* yeast species producing hemocoelic infections in *Daphnia magna* and *Artemia salina* (Crustacea, Phyllopoda) from Romania. *Journal of Invertebrate Pathology*, *37*(1), 22-27. https://doi.org/10.1016/0022-2011(81)90049-5

Colautti, R. I., & Barrett, S. C. (2013). Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science*, *342*(6156), 364-366. https://doi.org/10.1126/science.1242121

Collinge, S. K., Ray, C., Cully Jr, J. F., Ostfeld, R., Keesing, F., & Eviner, V. (2008). Effects of disease on keystone species, dominant species, and their communities. *Infectious Disease Ecology*, 129-144. https://doi.org/10.1515/9781400837885

Coopman, M., Muylaert, K., Lange, B., Reyserhove, L., & Decaestecker, E. (2014). Context dependency of infectious disease: the cyanobacterium *Microcystis aeruginosa* decreases white bacterial disease in *Daphnia magna*. *Freshwater Biology*, *59*(4), 714–723. https://doi.org/10.1111/fwb.12298

Covich, A. P., Ewel, K. C., Hall, R. O., Giller, P. E., Goedkoop, W., & Merritt, D. M. (2004). Ecosystem services provided by freshwater benthos. *Sustaining Biodiversity and Ecosystem Services in Soils and Sediments*, SCOPE *64*, 45-72. Edited by Diana H. Wall, Island Press.

Cuco, A. P., Abrantes, N., Gonçalves, F., Wolinska, J., & Castro, B. B. (2016). Toxicity of two fungicides in *Daphnia*: is it always temperature-dependent? *Ecotoxicology*, *25*(7), 1376-1389. https://doi.org/10.1007/s10646-016-1689-8

Cuco, A. P., Abrantes, N., Gonçalves, F., Wolinska, J., & Castro, B. B. (2017). Interplay between fungicides and parasites: Tebuconazole, but not copper, suppresses infection in a *Daphnia-Metschnikowia* experimental model. *PLoS ONE*, *12*(2), e0172589. https://doi.org/10.1371/journal.pone.0172589

Cuco, A. P., Castro, B. B., Gonçalves, F., Wolinska, J., & Abrantes, N. (2018). Temperature modulates the interaction between fungicide pollution and disease: evidence from a *Daphnia*-microparasitic yeast model. *Parasitology*, *145*(7), 939-947. https://doi.org/10.1017/S0031182017002062

Cuco, A. P., Wolinska, J., Santos, J. I., Abrantes, N., Gonçalves, F. J., & Castro, B. B. (2020). Can parasites adapt to pollutants? A multigenerational experiment with a *Daphnia* × *Metschnikowia* model system exposed to the fungicide tebuconazole. *Aquatic Toxicology*, *226*, 105584. https://doi.org/10.1016/j.aquatox.2020.105584

Cui, R., Kim, S. W., & An, Y. J. (2017). Polystyrene nanoplastics inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean *Daphnia galeata*. *Scientific Reports*, *7*(1), 1-10. https://doi.org/10.1038/s41598-017-12299-2

Cyr, H., & Curtis, J. M. (1999). Zooplankton community size structure and taxonomic composition affects size-selective grazing in natural communities. *Oecologia*, *118*(3), 306-315. https://www.jstor.org/stable/4222243

Dalal, R. C., & Allen, D. E. (2008). Greenhouse gas fluxes from natural ecosystems. *Australian Journal of Botany*, *56*(5), 369-407. https://doi.org/10.1071/BT07128

De Meester, L. (1989). An estimation of the heritability of phototaxis in *Daphnia magna* Straus. *Oecologia*, *78*(1), 142-144. https://doi.org/10.1007/BF00377210

De Meester, L. (1991). An analysis of the phototactic behaviour of *Daphnia magna* clones and their sexual descendants. In *Biology of Cladocera* (pp. 217-227). Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0918-7_21

Decaestecker, E., Vergote, A., Ebert, D., & Meester, L. D. (2003). Evidence for strong host clone-parasite species interactions in the *Daphnia* microparasite system. *Evolution*, *57*(4), 784-792. https://doi.org/10.1111/j.0014-3820.2003.tb00290.x

Decaestecker, E., Lefever, C., De Meester, L., & Ebert, D. (2004). Haunted by the past: evidence for dormant stage banks of microparasites and epibionts of *Daphnia*. *Limnology and Oceanography*, 49(4), 1355-1364. https://doi.org/10.4319/lo.2004.49.4_part_2.1355

Decaestecker, E., Declerck, S., De Meester, L., & Ebert, D. (2005). Ecological implications of parasites in natural *Daphnia populations*. *Oecologia*, *144*(3), 382-390. https://doi.org/10.1007/s00442-005-0083-7

Dehghani, M. H., Kamalian, S., Shayeghi, M., Yousefi, M., Heidarinejad, Z., Agarwal, S., & Gupta, V. K. (2019). High-performance removal of diazinon pesticide from water using multi-walled carbon nanotubes. *Microchemical Journal*, 145, 486-491. https://doi.org/10.1016/j.microc.2018.10.053

Delabroye, A., & Vecoli, M. (2010). The end-Ordovician glaciation and the Hirnantian Stage: a global review and questions about Late Ordovician event stratigraphy. *Earth-Science Reviews*, *98*(3-4), 269-282. https://doi.org/10.1016/j.earscirev.2009.10.010

DeMott, W. R., Zhang, Q. X., & Carmichael, W. W. (1991). Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. *Limnology and Oceanography*, *36*(7), 1346-1357. https://doi.org/10.4319/lo.1991.36.7.1346

Des Marais, D. J. (2000). When did photosynthesis emerge on Earth? *Science*, *289*(5485), 1703-1705. https://doi.org/10.1126/science.289.5485.1703

Dismukes, G. C., Klimov, V. V., Baranov, S. V., Kozlov, Y. N., DasGupta, J., & Tyryshkin, A. (2001). The origin of atmospheric oxygen on Earth: the innovation of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences*, *98*(5), 2170 2175. https://doi.org/10.1073/pnas.061514798

Dodd, M. S., Papineau, D., Grenne, T., Slack, J. F., Rittner, M., Pirajno, F., O'Neil, J., & Little, C. T. (2017). Evidence for early life in Earth's oldest hydrothermal vent precipitates. *Nature*, *543*(7643), 60-64. https://doi.org/10.1038/nature21377

Dudycha, J. L., & Tessier, A. J. (1999). Natural genetic variation of life span, reproduction, and juvenile growth in *Daphnia*. *Evolution*, *53*(6), 1744-1756. https://doi.org/10.1111/j.1558-5646.1999.tb04559.x

Duffy, M. A. (2007). Selective predation, parasitism, and trophic cascades in a bluegill–*Daphnia*–parasite system. *Oecologia*, *153*(2), 453-460. https://doi/org/10.1007/s00442-007-0742-y

Duffy, M. A. (2009). Staying alive: The post-consumption fate of parasite spores and its implications for disease dynamics. *Limnology and Oceanography*, *54*(3), 770-773. https://doi.org/10.4319/lo.2009.54.3.0770

Duffy, M. A., Hall, S. R., Cáceres, C. E., & Ives, A. R. (2009). Rapid evolution, seasonality, and the termination of parasite epidemics. *Ecology*, *90*(6), 1441-1448. https://doi.org/10.1890/08-1130.1

Duffy, M. A., Hall, S. R., Tessier, A. J., & Huebner, M. (2005). Selective predators and their parasitized prey: are epidemics in zooplankton under top-down control? *Limnology and Oceanography*, *50*(2), 412-420. https://doi.org/10.4319/lo.2005.50.2.0412

Duffy M. A., & Hunsberger K. K. (2019). Infectivity is influenced by parasite spore age and exposure to freezing: do shallow waters provide *Daphnia* a refuge from some parasites? *Journal of Plankton Research* 41, 12–16. https://doi.org/10.1093/plankt/fby046

Duffy, M. A., James, T. Y., & Longworth, A. (2015). Ecology, virulence, and phylogeny of *Blastulidium paedophthorum*, a widespread brood parasite of *Daphnia* spp. *Applied and Environmental Microbiology*, *81*(16), 5486-5496. https://doi.org/10.1128/AEM.01369-15

Duneau, D., Luijckx, P., Ben-Ami, F., Laforsch, C., & Ebert, D. (2011). Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host-parasite interactions. *BMC Biology*, *9*(1), 1-12. https://doi.org/10.1186/1741-7007-9-11

Dziuba, M. K., Herdegen-Radwan, M., Pluta, E., Wejnerowski, Ł., Szczuciński, W., & Cerbin, S. (2020). Temperature increase altered *Daphnia* community structure in artificially heated lakes: A potential scenario for a warmer future. *Scientific Reports*, *10*(1), 1-13. https://doi.org/10.1038/s41598-020-70294-6

Dziuba, M. K., Kuczyński, L., Wejnerowski, Ł., Cerbin, S., & Wolinska, J. (2021). Countergradient variation concealed adaptive responses to temperature increase in *Daphnia* from heated lakes. *Limnology and Oceanography*, *66*(4), 1268-1280. https://doi.org/10.1002/lno.11680

Ebert, D. (1991). The effect of size at birth, maturation threshold and genetic differences on the life-history of *Daphnia magna*. *Oecologia*, *86*(2), 243-250. https://doi.org/10.1007/BF00317537

Ebert, D. (1995). The ecological interactions between a microsporidian parasite and its host *Daphnia magna*. *Journal of Animal Ecology*, *64*(3), 361–369. https://doi.org/10.2307/5897

Ebert, D., Rainey, P., Embley, T. M., & Scholz, D. (1996). Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *351*(1348), 1689-1701. https://doi.org/10.1098/rstb.1996.0151

Ebert, D., Lipsitch, M., & Mangin, K. L. (2000a). The effect of parasites on host population density and extinction: experimental epidemiology with *Daphnia* and six microparasites. *The American Naturalist*, *156*(5), 459-477. https://doi.org/10.1086/303404

Ebert, D., Zschokke-Rohringer, C. D., & Carius, H. J. (2000b). Dose effects and density-dependent regulation of two microparasites of *Daphnia magna*. *Oecologia*, *122*(2), 200-209. https://doi.org/10.1007/PL00008847

Ebert, D. (2005). Ecology, Epidemiology, and Evolution of Parasitism in *Daphnia*. National Library of Medicine.

Ebert, D. (2008). Host–parasite coevolution: insights from the *Daphnia*–parasite model system. *Current Opinion in Microbiology*, *11*(3), 290-301. https://doi.org/10.1016/j.mib.2008.05.012

Eltemsah, Y. S., & Bøhn, T. (2019). Acute and chronic effects of polystyrene microplastics on juvenile and adult *Daphnia magna*. *Environmental Pollution*, *254*, 112919. https://doi.org/10.1016/j.envpol.2019.07.087

Ezenwa, V. O., Etienne, R. S., Luikart, G., Beja-Pereira, A., & Jolles, A. E. (2010). Hidden consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African buffalo. *The American Naturalist*, *176*(5), 613-624. https://doi.org/10.1086/656496

Fan, X., Miao, C., Duan, Q., Shen, C., & Wu, Y. (2021). Future climate change hotspots under different 21st century warming scenarios. *Earth's Future*, *9*(6), e2021EF002027. https://doi.org/10.1029/2021EF002027

Fels, D., Lee, V. A., & Ebert, D. (2004). The impact of microparasites on the vertical distribution of *Daphnia magna*. *Archiv für Hydrobiologie*, 65-80. https://doi.org/10.1127/0003-9136/2004/0161-0065

Fels, D., & Kaltz, O. (2006). Temperature-dependent transmission and latency of *Holospora undulata*, a micronucleus-specific parasite of the ciliate *Paramecium caudatum*. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1589), 1031-1038. https://doi.org/10.1098/rspb.2005.3404

Feng, L. J., Sun, X. D., Zhu, F. P., Feng, Y., Duan, J. L., Xiao, F., Li, X. Y., Shi, Y., Wang, W., Sun, J. W., Liu, X. Y., Liu, J. Q., Zhou, L. L., Wang, S. G., Ding, Z., Tian, H., Galloway, T. S., & Yuan, X. Z. (2020). Nanoplastics promote microcystin synthesis and release from cyanobacterial *Microcystis aeruginosa*. *Environmental Science & Technology*, *54*(6), 3386-3394. https://doi.org/10.1021/acs.est.9b06085

Fitzsimmons, J. M., & Innes, D. J. (2006). Inter-genotype variation in reproductive response to crowding among *Daphnia pulex*. *Hydrobiologia*, *568*(1), 187-205. https://doi.org/10.1007/s10750-006-0104-5

Frenken, T., Wolinska, J., Tao, Y., Rohrlack, T., & Agha, R. (2020). Infection of filamentous phytoplankton by fungal parasites enhances herbivory in pelagic food webs. *Limnology and Oceanography*, *65*(11), 2618-2626. https://doi.org/10.1002/lno.11474

Frost, P. C., Ebert, D., & Smith, V. H. (2008). Responses of a bacterial pathogen to phosphorus limitation of its aquatic invertebrate host. *Ecology*, *89*(2), 313–318. https://doi.org/10.1890/07-0389.1

Frost, P. C., Ebert, D., Larson, J. H., Marcus, M. A., Wagner, N. D., & Zalewski, A. (2010). Transgenerational effects of poor elemental food quality on *Daphnia magna*. *Oecologia*, *162*(4), 865-872. https://doi.org/10.1007/s00442-009-1517-4

Gálvez, N., Meniconi, P., Infante, J., & Bonacic, C. (2021). Response of mesocarnivores to anthropogenic landscape intensification: activity patterns and guild temporal interactions. *Journal of Mammalogy*, *102*(4), 1149-1164. https://doi.org/10.1093/jmammal/gyab074

Garbutt, J. S., Scholefield, J. A., Vale, P. F., & Little, T. J. (2014). Elevated maternal temperature enhances offspring disease resistance in *Daphnia magna*. *Functional Ecology*, *28*(2), 424–431. https://doi.org/10.1111/1365-2435.12197

Gazonato, A. J., Silva, L. C. D., Saggio, A. A., & Rocha, O. (2014). Zooplankton communities as eutrophication bioindicators in tropical reservoirs. *Biota Neotropica*, *14*(4), e20140018. https://doi.org/10.1590/1676-06032014001814

Geist, J. (2011). Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators*, *11*(6), 1507-1516. https://doi.org/10.1016/j.ecolind.2011.04.002

Gerasimova, T. N., & Pogozhev, P. I. (2002). Reduction of the trophic status of a water body with the use of large-size zooplankton. *Water Resources*, *29*(4), 412-421. https://doi.org/10.1023/A:1019626408011

Gerasimova, T. N., Pogozhev, P. I., & Sadchikov, A. P. (2018). Suppression of alga blooming by zooplankton filter feeders in small water bodies. *Water Resources*, *45*(2), 199-204. https://doi.org/10.1134/S0097807818020070

Giebelhausen, B., & Lampert, W. (2001). Temperature reaction norms of *Daphnia magna*: the effect of food concentration. *Freshwater Biology*, *46*(3), 281-289. https://doi.org/10.1046/j.1365-2427.2001.00630.x

Giessler, S. (1997). Gene flow in the *Daphnia longispina* hybrid complex (Crustacea, Cladocera) inhabiting large lakes. *Heredity*, *79*(3), 231-241. https://doi.org/10.1038/hdy.1997.150

Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in Ecology & Evolution*, *25*(6), 325-331. https://doi.org/10.1016/j.tree.2010.03.002

Glibert, P. M. (2017). Eutrophication, harmful algae and biodiversity—Challenging paradigms in a world of complex nutrient changes. *Marine Pollution Bulletin*, *124*(2), 591– 606. https://doi.org/10.1016/j.marpolbul.2017.04.027

Goren, L., & Ben-Ami, F. (2013). Ecological correlates between cladocerans and their endoparasites from permanent and rain pools: patterns in community composition and diversity. *Hydrobiologia*, *701*(1), 13-23. https://doi.org/10.1007/s10750-012-1243-5

Graca, B., Rychter, A., Staniszewska, M., Smolarz, K., Sokołowski, A., & Bodziach, K. (2021). Bioaccumulation of phenolic endocrine disruptors in the clam *Rangia cuneata*: Storage in shells and influence of size and sex. *Environmental Research*, *197*, 111181. https://doi.org/10.1016/j.envres.2021.111181

Graham, A. L. (2008). Ecological rules governing helminth–microparasite coinfection. *Proceedings of the National Academy of Sciences*, *105*(2), 566-570. https://doi.org/10.1073/pnas.0707221105

Green, J. (1974). Parasites and epibionts of Cladocera. *The Transactions of the Zoological Society of London*, *32*(6), 417-515. https://doi.org/10.1111/j.1096-3642.1974.tb00031.x

Grubisic, M., & van Grunsven, R. H. (2021). Artificial light at night disrupts species interactions and changes insect communities. *Current Opinion in Insect Science*. https://doi.org/10.1016/j.cois.2021.06.007

Haag, C. R., Sakwiska, O., & Ebert, D. (2003). Test of synergistic interaction between infection and inbreeding in *Daphnia magna*. *Evolution*, *57*(4), 777-783. https://doi.org/10.1111/j.0014-3820.2003.tb00289.x

Haag, K. L., Pombert, J. F., Sun, Y., de Albuquerque, N. R. M., Batliner, B., Fields, P., Lopes, T. F., & Ebert, D. (2020). Microsporidia with vertical transmission were likely shaped by nonadaptive processes. *Genome Biology and Evolution*, *12*(1), 3599-3614. https://doi.org/10.1093/gbe/evz270

Hall, S. R., Tessier, A. J., Duffy, M. A., Huebner, M., & Cáceres, C. E. (2006). Warmer does not have to mean sicker: temperature and predators can jointly drive timing of epidemics. *Ecology*, *87*(7), 1684-1695. https://doi.org/10.1890/0012-9658(2006)87[1684:WDNHTM]2.0.CO;2

Hall, S. R., Knight, C. J., Becker, C. R., Duffy, M. A., Tessier, A. J., & Cáceres, C. E. (2009). Quality matters: resource quality for hosts and the timing of epidemics. *Ecology Letters*, *12*(2), 118-128. https://doi.org/10.1111/j.1461-0248.2008.01264.x

Hall, S. R., Becker, C. R., Duffy, M. A., & Cáceres, C. E. (2011). Epidemic size determines population-level effects of fungal parasites on *Daphnia* hosts. *Oecologia*, *166*(3), 833-842. https://doi.org/10.1007/s00442-011-1905-4

Hallegraeff, G. M. (1993) A review of harmful algae blooms and their apparent global increase. *Phycologia*, *32*(2), 79-99. https://doi.org/10.2216/i0031-8884-32-2-79.1

Halliday, F. W., Umbanhowar, J., & Mitchell, C. E. (2017). Interactions among symbionts operate across scales to influence parasite epidemics. *Ecology Letters*, *20*(10), 1285-1294. https://doi.org/10.1111/ele.12825

Han, G. H., Hur, H. G., & Kim, S. D. (2006). Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: occurrence and toxicity to *Daphnia magna*. *Environmental Toxicology and Chemistry: An International Journal*, *25*(1), 265-271. https://doi.org/10.1897/05-193R.1

Hanazato, T. (1996). Combined effects of food shortage and oxygen deficiency on life history characteristics and filter screens of *Daphnia*. *Journal of Plankton Research*, *18*(5), 757-765. https://doi.org/10.1093/plankt/18.5.757

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science*, *296*(5576), 2158-2162. https://doi.org/10.1126/science.1063699

Hatcher, M. J., Dick, J. T., & Dunn, A. M. (2012). Diverse effects of parasites in ecosystems: linking interdependent processes. *Frontiers in Ecology and the Environment*, *10*(4), 186-194. https://doi.org/10.1890/110016

Hernandez, A. D., Bunnell, J. F., & Sukhdeo, M. V. K. (2007). Composition and diversity patterns in metazoan parasite communities and anthropogenic disturbance in stream ecosystems. *Parasitology*, *134*(1), 91-102. https://doi.org/10.1017/S0031182006001247

Hernandez-Milian, G., Lusher, A., MacGabban, S., & Rogan, E. (2019). Microplastics in grey seal (*Halichoerus grypus*) intestines: Are they associated with parasite aggregations? *Marine Pollution Bulletin*, *146*, 349-354. https://doi.org/10.1016/j.marpolbul.2019.06.014

Hesse, O., Engelbrecht, W., Laforsch, C., & Wolinska, J. (2012). Fighting parasites and predators: How to deal with multiple threats? *BMC Ecology*, *12*(1), 12. https://doi.org/10.1186/1472-6785-12-12

Hietala, J., Laurén-Määttä, C., & Walls, M. (1997). Sensitivity of *Daphnia* to toxic cyanobacteria: effects of genotype and temperature. *Freshwater Biology*, *37*(2), 299-306. https://doi.org/10.1046/j.1365-2427.1997.d01-555.x

Hiraga, K., Taniguchi, I., Yoshida, S., Kimura, Y., & Oda, K. (2019). Biodegradation of waste PET: A sustainable solution for dealing with plastic pollution. *EMBO Reports*, *20*(11), e49365. https://doi.org/10.15252/embr.201949365

Ho, J. C., Stumpf, R. P., Bridgeman, T. B., & Michalak, A. M. (2017). Using Landsat to extend the historical record of lacustrine phytoplankton blooms: A Lake Erie case study. *Remote Sensing of Environment*, *191*, 273– 285. https://doi.org/10.1016/j.rse.2016.12.013

Hoberg, E. P., & Brooks, D. R. (2015). Evolution in action: climate change, biodiversity dynamics and emerging infectious disease. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1665), 20130553. https://doi.org/10.1098/rstb.2013.0553

Holland, H. D. (2006). The oxygenation of the atmosphere and oceans. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1470), 903-915. https://doi.org/10.1098/rstb.2006.1838

Hu, D., Li, M., Zhang, X., Turchyn, A. V., Gong, Y., & Shen, Y. (2020). Large mass-independent sulphur isotope anomalies link stratospheric volcanism to the Late Ordovician mass extinction. *Nature Communications*, *11*(1), 1-8. https://doi.org/10.1038/s41467-020-16228-2

Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution*, *21*(7), 381-385. https://doi.org/10.1016/j.tree.2006.04.007

Ibelings, B. W., Gsell, A. S., Mooij, W. M., Van Donk, E., Van Den Wyngaert, S., & De Senerpont Domis, L. N. (2011). Chytrid infections and diatom spring blooms: paradoxical effects of climate warming on fungal epidemics in lakes. *Freshwater Biology*, *56*(4), 754-766. https://doi.org/10.1111/j.1365-2427.2010.02565.x

IEA (2021), World Energy Outlook 2021, IEA, Paris https://www.iea.org/reports/world-energy-outlook-2021

Iyer, N., & Rao, T. R. (1993). Effect of the epizoic rotifer *Brachionus rubens* on the population growth of three cladoceran species. In *Rotifer Symposium VI* (pp. 325-332). Springer, Dordrecht.

Izhar, R., & Ben-Ami, F. (2015). Host age modulates parasite infectivity, virulence and reproduction. *Journal of Animal Ecology*, *84*(4), 1018-1028. https://doi.org/10.1111/1365-2656.12352

Jaeger, J. A., Soukup, T., Schwick, C., Madriñán, L. F., & Kienast, F. (2016). Landscape fragmentation in Europe. In *European Landscape Dynamics* (pp. 187-228). CRC Press.

Jahnsen-Guzmán, N., Lagos, N. A., Quijón, P. A., Manríquez, P. H., Lardies, M. A., Fernández, C., Reyes, M., Zapata, J., García-Huidobro, M. R., Labra, F. A., & Duarte, C. (2021). Ocean acidification alters anti-predator responses in a competitive dominant intertidal mussel. *Chemosphere*, 132410. https://doi.org/10.1016/j.chemosphere.2021.132410

Jakhar, P. (2013). Role of phytoplankton and zooplankton as health indicators of aquatic ecosystem: A review. *International Journal of Innovation Research Study*, *2*(12), 489-500.

Jankowski, J., & Cader, A. B. (1997). The effect of depletion of the earth ozone layer on the human health condition. *International Journal of Occupational Medicine and Environmental Health*, *10*(4), 349-364. https://europepmc.org/article/med/9575662

Jeppesen, E., Jensen, J. P., Amsinck, S., Landkildehus, F., Lauridsen, T., & Mitchell, S. F. (2002). Reconstructing the historical changes in *Daphnia* mean size and planktivorous fish abundance in lakes from the size of *Daphnia* ephippia in the sediment. *Journal of Paleolimnology*, *27*(1), 133-143. https://doi.org/10.1023/A:1013561208488

Jung, B. K., Han, S. W., Park, S. H., Bae, J. S., Choi, J., & Ryu, K. Y. (2020). Neurotoxic potential of polystyrene nanoplastics in primary cells originating from mouse brain. *NeuroToxicology*, *81*, 189-196.https://doi.org/10.1016/j.neuro.2020.10.008

Kagami, M., Miki, T., & Takimoto, G. (2014). Mycoloop: chytrids in aquatic food webs. *Frontiers in Microbiology*, *5*, 166. https://doi.org/10.3389/fmicb.2014.00166

Kankaala, P., & Eloranta, P. (1987). Epizooic ciliates (*Vorticella* sp.) compete for food with their host *Daphnia longispina* in a small polyhumic lake. *Oecologia*, *73*(2), 203-206. https://www.jstor.org/stable/4218352

Karbalaei, S., Hanachi, P., Walker, T. R., & Cole, M. (2018). Occurrence, sources, human health impacts and mitigation of microplastic pollution. *Environmental Science and Pollution Research*, *25*(36), 36046-36063. https://doi.org/10.1007/s11356-018-3508-7

Kasting, J. F., & Siefert, J. L. (2002). Life and the evolution of Earth's atmosphere. *Science*, *296*(5570), 1066-1068. https://doi.org/10.1126/science.1071184

Kelpsiene, E., Torstensson, O., Ekvall, M. T., Hansson, L. A., & Cedervall, T. (2020). Long-term exposure to nanoplastics reduces life-time in *Daphnia magna*. *Scientific Reports*, *10*(1), 1-7. https://doi.org/10.1038/s41598-020-63028-1

Khairullina, E. R., Bogdanova, V. I., Slepneva, E. V., Nizamutdinova, G. F., Fatkhullina, L. R., Kovalenko, Y. A., & Skutelnik, O. A. (2019). Global climate change: cyclical nature of natural and permanent nature of man-made processes. *EurAsian Journal of BioSciences*, *13*(2). http://www.ejobios.org/article/global-climate-change-cyclical-nature-of-natural-and-permanent-nature-of-man-made-processes-7407

Khan, R. A., & Thulin, J. (1991). Influence of pollution on parasites of aquatic animals. *Advances in Parasitology*, *30*, 201-238. https://doi.org/10.1016/S0065-308X(08)60309-7

Kim, M., Song, Y. E., Xiong, J. Q., Kim, K. Y., Jang, M., Jeon, B. H., & Kim, J. R. (2021). Electrochemical detection and simultaneous removal of endocrine disruptor, bisphenol A using a carbon felt electrode. *Journal of Electroanalytical Chemistry*, *880*, 114907. https://doi.org/10.1016/j.jelechem.2020.114907

King, B. J., & Monis, P. T. (2007). Critical processes affecting *Cryptosporidium oocyst* survival in the environment. *Parasitology*, *134*(3), 309-323. https://doi.org/10.1017/S0031182006001491

King, K. C., Mclaughlin, J. D., Boily, M., & Marcogliese, D. J. (2010). Effects of agricultural landscape and pesticides on parasitism in native bullfrogs. *Biological Conservation*, *143*(2), 302-310. https://doi.org/10.1016/j.biocon.2009.10.011

King, N. G., Wilmes, S. B., Smyth, D., Tinker, J., Robins, P. E., Thorpe, J., Jones, L., & Malham, S. K. (2021). Climate change accelerates range expansion of the invasive non-native species, the Pacific oyster, *Crassostrea gigas*. *ICES Journal of Marine Science*, *78*(1), 70-81. https://doi.org/10.1093/icesjms/fsaa189

Kirk, D., Jones, N., Peacock, S., Phillips, J., Molnár, P. K., Krkošek, M., & Luijckx, P. (2018). Empirical evidence that metabolic theory describes the temperature dependency of within-host parasite dynamics. *PLoS Biology*, *16*(2), e2004608. https://doi.org/10.1371/journal.pbio.2004608

Kirk, D., Luijckx, P., Stanic, A., & Krkošek, M. (2019). Predicting the thermal and allometric dependencies of disease transmission via the metabolic theory of ecology. *The American Naturalist*, *193*(5), 661-676. https://doi.org/10.1086/702846

Kirk, D., Luijckx, P., Jones, N., Krichel, L., Pencer, C., Molnár, P., & Krkošek, M. (2020). Experimental evidence of warming-induced disease emergence and its prediction by a trait-based mechanistic model. *Proceedings of the Royal Society B*, *287*(1936), 20201526. https://doi.org/10.1098/rspb.2020.1526

Kirk, D., O'Connor, M. I., & Mordecai, E. A. (2021). Temperature effects on individual-level parasitism translate into predictable effects on parasitism in populations. *bioRxiv*. https://doi.org/10.1101/2020.12.01.406884

Koprivnikar, J., & Redfern, J. C. (2012). Agricultural effects on amphibian parasitism: Importance of general habitat perturbations and parasite life cycles. *Journal of Wildlife Diseases*, *48*(4), 925-936. https://doi.org/10.7589/2011-09-258

Korpelainen, H. (1986). The effects of temperature and photoperiod on life history parameters of *Daphnia magna* (Crustacea: Cladocera). *Freshwater Biology*, *16*(5), 615-620. https://doi.org/10.1111/j.1365-2427.1986.tb01004.x

Kuehn, V. G. K., & Suberkropp, K. (2006). The role of fungi in carbon and nitrogen cycles in freshwater ecosystems. *Fungi in Biogeochemical Cycles*, *24*, 404. Cambridge University Press.

Kutz, S. J., Checkley, S., Verocai, G. G., Dumond, M., Hoberg, E. P., Peacock, R., Wu, J. P., Orsel, K., Seegers, K., Warren, A. L., & Abrams, A. (2013). Invasion, establishment, and range expansion of two parasitic nematodes in the Canadian Arctic. *Global Change Biology*, *19*(11), 3254-3262. https://doi.org/10.1111/gcb.12315

Lafferty, K. D. (2004). Fishing for lobsters indirectly increases epidemics in sea urchins. *Ecological Applications*, *14*(5), 1566-1573. https://doi.org/10.1890/03-5088

Lafferty, K. D., Porter, J. W., & Ford, S. E. (2004). Are diseases increasing in the ocean? *Annual Review of Ecology, Evolution, and Systematics*, *35*, 31-54. https://doi.org/10.1146/annurev.ecolsys.35.021103.105704

Lafferty, K. D., & Kuris, A. M. (2005). Parasitism and environmental disturbances. In *Parasitism and Ecosystems*, 113-123. Oxford University Press.

Lafferty, K. D., & Mordecai, E. A. (2016). The rise and fall of infectious disease in a warmer world. *F1000Research*, *5*. https://doi.org/10.12688/f1000research.8766.1

Lahav, N., Nir, S., & Elitzur, A. C. (2001). The emergence of life on Earth. *Progress in Biophysics and Molecular Biology*, *75*(1-2), 75-120. https://doi.org/10.1016/S0079-6107(01)00003-7

Lamb, W. F., Wiedmann, T., Pongratz, J., Andrew, R., Crippa, M., Olivier, J. G., ... & Minx, J. C. (2021). A review of trends and drivers of greenhouse gas emissions by sector from 1990 to 2018. *Environmental Research Letters*, *16*, 073005. https://doi.org/10.1088/1748-9326/abee4e

Lange, B., Reuter, M., Ebert, D., Muylaert, K., & Decaestecker, E. (2014). Diet quality determines interspecific parasite interactions in host populations. *Ecology and Evolution*, *4*(15), 3093-3102. https://doi.org/10.1002/ece3.1167

Lares, M., Ncibi, M. C., Sillanpää, M., & Sillanpää, M. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. *Water Research*, *133*, 236-246. https://doi.org/10.1016/j.watres.2018.01.049

Larsson, J. R., Ebert, D., & Vavra, J. (1997). Ultrastructural study and description of *Ordospora colligata* gen. et sp. nov. (Microspora, Ordosporidae fam. nov.), a new microsporidian parasite of *Daphnia magna* (Crustacea, Cladocera). *European Journal of Protistology*, *33*(4), 432-443. https://doi.org/10.1016/S0932-4739(97)80055-7

Lawrence, A., Friedrich, F., & Beierkuhnlein, C. (2021). Landscape fragmentation of the Natura 2000 network and its surrounding areas. *PLoS ONE*, *16*(10), e0258615. https://doi.org/10.1371/journal.pone.0258615

Lee, B. Y., Choi, B. S., Kim, M. S., Park, J. C., Jeong, C. B., Han, J., & Lee, J. S. (2019). The genome of the freshwater water flea *Daphnia magna*: A potential use for freshwater molecular ecotoxicology. *Aquatic Toxicology*, *210*, 69-84. https://doi.org/10.1016/j.aquatox.2019.02.009

Li, D., Deng, Y., Wang, S., Du, H., Xiao, G., & Wang, D. (2020). Assessment of nanopolystyrene toxicity under fungal infection condition in *Caenorhabditis elegans*. *Ecotoxicology and Environmental Safety*, *197*, 110625. https://doi.org/10.1016/j.ecoenv.2020.110625

Lin, W., Jiang, R., Hu, S., Xiao, X., Wu, J., Wei, S., Xiong, Y., & Ouyang, G. (2019). Investigating the toxicities of different functionalized polystyrene nanoplastics on *Daphnia magna*. *Ecotoxicology and Environmental Safety*, *180*, 509-516. https://doi.org/10.1016/j.ecoenv.2019.05.036

Liu, Z., Cai, M., Yu, P., Chen, M., Wu, D., Zhang, M., & Zhao, Y. (2018). Age-dependent survival, stress defense, and AMPK in *Daphnia pulex* after short-term exposure to a polystyrene nanoplastic. *Aquatic Toxicology*, *204*, 1-8. https://doi.org/10.1016/j.aquatox.2018.08.017

Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Huang, Y., & Zhao, Y. (2019). Polystyrene nanoplastic exposure induces immobilization, reproduction, and stress defense in the freshwater cladoceran *Daphnia pulex*. *Chemosphere*, *215*, 74-81. https://doi.org/10.1016/j.chemosphere.2018.09.176

Liu, Z., Huang, Y., Jiao, Y., Chen, Q., Wu, D., Yu, P., Li, Y., Cai, M., & Zhao, Y. (2020). Polystyrene nanoplastic induces ROS production and affects the MAPK-HIF-1/NFkB-mediated antioxidant system in *Daphnia pulex*. *Aquatic Toxicology*, *220*, 105420. https://doi.org/10.1016/j.aquatox.2020.105420

Liu, Z., Malinowski, C. R., & Sepúlveda, M. S. (2021). Emerging trends in nanoparticle toxicity and the significance of using *Daphnia* as a model organism. *Chemosphere*, 132941. https://doi.org/10.1016/j.chemosphere.2021.132941

Lohr, J. N., Laforsch, C., Koerner, H., & Wolinska, J. (2010a). A *Daphnia* parasite (*Caullerya mesnili*) constitutes a new member of the Ichthyosporea, a group of protists near the animal–fungi divergence. *Journal of Eukaryotic Microbiology*, *57*(4), 328-336. https://doi.org/10.1111/j.1550-7408.2010.00479.x

Lohr, J. N., Yin, M., & Wolinska, J. (2010b). Prior residency does not always pay off–co-infections in *Daphnia*. *Parasitology*, *137*(10), 1493-1500. https://doi.org/10.1017/S0031182010000296

Lord, J. P., Barry, J. P., & Graves, D. (2017). Impact of climate change on direct and indirect species interactions. *Marine Ecology Progress Series*, *571*, 1-11. https://doi.org/10.3354/meps12148

Lyons, T. W., Reinhard, C. T., & Planavsky, N. J. (2014). The rise of oxygen in Earth's early ocean and atmosphere. *Nature*, *506*(7488), 307-315. https://doi.org/10.1038/nature13068

MacArthur, J. W., & Baillie, W. H. T. (1929). Metabolic activity and duration of life. I. Influence of temperature on longevity in *Daphnia magna*. *Journal of Experimental Zoology*, *53*(2), 221-242. https://doi.org/10.1002/jez.1400530205

Maceda-Veiga, A., Webster, G., Canals, O., Salvadó, H., Weightman, A. J., & Cable, J. (2015). Chronic effects of temperature and nitrate pollution on *Daphnia magna*: Is this cladoceran suitable for widespread use as a tertiary treatment? *Water Research*, *83*, 141-152. https://doi.org/10.1016/j.watres.2015.06.036

Mainstone, C. P., & Parr, W. (2002). Phosphorus in rivers—ecology and management. *Science of the Total Environment*, *282*, 25-47. https://doi.org/10.1016/S0048-9697(01)00937-8

Malone, T. C., & Newton, A. (2020). The globalization of cultural eutrophication in the coastal ocean: causes and consequences. *Frontiers in Marine Science*, *7*, 670. https://doi.org/10.3389/fmars.2020.00670

Marcogliese, D. J. (2004). Parasites: small players with crucial roles in the ecological theater. *EcoHealth*, *1*(2), 151-164. https://doi.org/10.1007/s10393-004-0028-3

Marcogliese, D. J. (2001). Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology*, *79*(8), 1331-1352. https://doi.org/10.1139/z01-067

Martens, W. J., Niessen, L. W., Rotmans, J., Jetten, T. H., & McMichael, A. J. (1995). Potential impact of global climate change on malaria risk. *Environmental Health Perspectives*, *103*(5), 458-464. https://doi.org/10.1289/ehp.95103458

Matveev, V., Matveeva, L., & Jones, G. J. (1994). Study of the ability of *Daphnia carinata* King to control phytoplankton and resist cyanobacterial toxicity: implications for biomanipulation in Australia. *Marine and Freshwater Research*, *45*(5), 889-904. https://doi.org/10.1071/MF9940889

McDowell, W. G., Benson, A. J., & Byers, J. E. (2014). Climate controls the distribution of a widespread invasive species: implications for future range expansion. *Freshwater Biology*, *59*(4), 847-857. https://doi.org/10.1111/fwb.12308

McKee, D., Atkinson, D., Collings, S., Eaton, J., Harvey, I., Heyes, T., Hatton, K., Wilson, D., & Moss, B. (2002). Macro-zooplankter responses to simulated climate warming in experimental freshwater microcosms. *Freshwater Biology*, *47*(8), 1557-1570. https://doi.org/10.1046/j.1365-2427.2002.00878.x

McMahon, J. W. (1965). Some physical factors influencing the feeding behavior of *Daphnia magna* Straus. *Canadian Journal of Zoology*, *43*(4), 603-611.

McNew, G. L. (1960). The nature, origin, and evolution of parasitism In: *Plant Pathology: An Advanced Treatise*. Edited by Horsfall J.G. and Dimond A.E.

Melchin, M. J., Mitchell, C. E., Holmden, C., & Štorch, P. (2013). Environmental changes in the Late Ordovician–early Silurian: Review and new insights from black shales and nitrogen isotopes. *Bulletin*, *125*(11-12), 1635-1670. https://doi.org/10.1130/B30812.1

Mendonça-Hagler, L. C., Hagler, A. N., & Kurtzman, C. P. (1993). Phylogeny of *Metschnikowia* species estimated from partial rRNA sequences. *International Journal of Systematic and Evolutionary Microbiology*, *43*(2), 368-373. https://doi.org/10.1099/00207713-43-2-368

Meng, Y., Kelly, F. J., & Wright, S. L. (2020). Advances and challenges of microplastic pollution in freshwater ecosystems: A UK perspective. *Environmental Pollution*, *256*, 113445. https://doi.org/10.1016/j.envpol.2019.113445

Metschnikoff, E. (1884). A disease of *Daphnia* caused by a yeast. A contribution to the theory of phagocytes as agents for attack on disease-causing organisms. *Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin*, *96*, 177-195. https://doi.org/10.1055/s-0029-1209659

Messina, J. P., Brady, O. J., Golding, N., Kraemer, M. U., Wint, G. W., Ray, S. E., ... & Hay, S. I. (2019). The current and future global distribution and population at risk of dengue. *Nature Microbiology*, *4*(9), 1508-1515. https://doi.org/10.1038/s41564-019-0476-8

Mimura, N. (2013). Sea-level rise caused by climate change and its implications for society. *Proceedings of the Japan Academy, Series B, 89*(7), 281-301. https://doi.org/10.2183/pjab.89.281

Mitchell, S. E., & Read, A. F. (2005). Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1581), 2601–2607. https://doi.org/10.1098/rspb.2005.3253

Mitchell, S. E., Rogers, E. S., Little, T. J., & Read, A. F. (2005). Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, *59*(1), 70–80. https://doi.org/10.1111/j.0014-3820.2005.tb00895.x

Mohajan, H. (2019). The first industrial revolution: creation of a new global human era. *Journal of Social Sciences and Humanities*, *5*(4), 377-387. https://mpra.ub.uni-muenchen.de/id/eprint/96644

Mohammed, A. S., Kapri, A., & Goel, R. (2011). Heavy metal pollution: source, impact, and remedies. In *Biomanagement of Metal-Contaminated Soils* (pp. 1-28). Springer, Dordrecht. https://doi.org/10.1007/978-94-007-1914-9

Mojžišová, M., Mrugała, A., Kozubíková-Balcarová, E., Vlach, P., Svobodová, J., Kouba, A., & Petrusek, A. (2020). Crayfish plague in Czechia: Outbreaks from novel sources and testing for chronic infections. *Journal of Invertebrate Pathology*, *173*, 107390. https://doi.org/10.1016/j.jip.2020.107390

Moore, M. M., & Strom, M. S. (2003). Infection and mortality by the yeast *Metschnikowia bicuspidata* var. *bicuspidata* in chinook salmon fed live adult brine shrimp (*Artemia franciscana*). *Aquaculture*, *220*(1-4), 43-57. https://doi.org/10.1016/S0044-8486(02)00271-5

Morales-Castilla, I., Pappalardo, P., Farrell, M. J., Aguirre, A. A., Huang, S., Gehman, A. L. M., Dallas, T., Gravel, D., & Davies, T. J. (2021). Forecasting parasite sharing under climate change. *Philosophical Transactions of the Royal Society B*, *376*(1837), 20200360. https://doi.org/10.1098/rstb.2020.0360

Müller, M. F., Colomer, J., & Serra, T. (2018). Temperature-driven response reversibility and short-term quasi-acclimation of *Daphnia magna*. *PLoS ONE*, *13*(12), e0209705. https://doi.org/10.1371/journal.pone.0209705

Murdock, C. C., Paaijmans, K. P., Bell, A. S., King, J. G., Hillyer, J. F., Read, A. F., & Thomas, M. B. (2012). Complex effects of temperature on mosquito immune function. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1741), 3357-3366. https://doi.org/10.1098/rspb.2012.0638

Navarro, A. B., Magioli, M., Bogoni, J. A., Silveira, L. F., Moreira, M. Z., Alexandrino, E. R., da Luz, D. T. A., Silva, W. R., Pizo, M. A., de Oliveira, V. C., & Ferraz, K. M. P. M. D. B. (2021). Isotopic niches of tropical birds reduced by anthropogenic impacts: a 100-year perspective. *Oikos*, *130*(11), 18092-1904. https://doi.org/10.1111/oik.08386

Navarro-Gonzalez, N., Verheyden, H., Hoste, H., Cargnelutti, B., Lourtet, B., Merlet, J., Daufresne, T., Lavín, S., Mark Hewison, A. J., Morand, S., & Serrano, E. (2011). Diet quality and immunocompetence influence parasite load of roe deer in a fragmented landscape. *European Journal of Wildlife Research*, *57*(3), 639-645. https://doi.org/10.1007/s10344-010-0474-x

Nixdorf, B., Mischke, U., & Rücker, J. (2003). Phytoplankton assemblages and steady state in deep and shallow eutrophic lakes—an approach to differentiate the habitat properties of Oscillatoriales. In *Phytoplankton and Equilibrium Concept: The Ecology of Steady-State Assemblages* (pp. 111-121). Springer, Dordrecht. https://doi.org/10.1007/978-94-017-2666-5_10

Ogonowski, M., Schür, C., Jarsén, Å., & Gorokhova, E. (2016). The effects of natural and anthropogenic microparticles on individual fitness in *Daphnia magna*. *PLoS ONE*, *11*(5), e0155063. https://doi.org/10.1371/journal.pone.0155063

Ogonowski, M., Edlund, U., Gorokhova, E., Linde, M., Ek, K., Liewenborg, B., Könnecke, O., Navarro, J. R. G., & Breitholtz, M. (2018). Multi-level toxicity assessment of engineered cellulose nanofibrils in *Daphnia magna*. *Nanotoxicology*, *12*(6), 509-521. https://doi.org/10.1080/17435390.2018.1464229

Ojha, A., Tiwary, D., Oraon, R., & Singh, P. (2021). Degradations of endocrine-disrupting chemicals and pharmaceutical compounds in wastewater with carbon-based nanomaterials: a critical review. *Environmental Science and Pollution Research*, 1-22. https://doi.org/10.1007/s11356-021-13939-x

O'Reilly, C. M., Sharma, S., Gray, D. K., Hampton, S. E., Read, J. S., Rowley, R. J., ... & Zhang, G. (2015). Rapid and highly variable warming of lake surface waters around the globe. *Geophysical Research Letters*, *42*(24), 10-773. https://doi.org/10.1002/2015GL066235

Orcutt, J. D., & Porter, K. G. (1984). The synergistic effects of temperature and food concentration on life history parameters of *Daphnia*. *Oecologia*, *63*(3), 300-306. https://doi.org/10.1007/bf00390657

Overholt, E. P., Hall, S. R., Williamson, C. E., Meikle, C. K., Duffy, M. A., & Cáceres, C. E. (2012). Solar radiation decreases parasitism in *Daphnia*. *Ecology Letters*, *15*(1), 47-54. https://doi.org/10.1111/j.1461-0248.2011.01707.x

Overholt, E. P., Duffy, M. A., Meeks, M. P., Leach, T. H., & Williamson, C. E. (2020). Light exposure decreases infectivity of the *Daphnia* parasite *Pasteuria ramosa*. *Journal of Plankton Research*, *42*(1), 41-44. https://doi.org/10.1093/plankt/fbz070

Pace, M. L., & Orcutt Jr, J. D. (1981). The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. *Limnology and Oceanography*, *26*(5), 822-830. https://doi.org/10.4319/lo.1981.26.5.0822

Paço, A., Duarte, K., da Costa, J. P., Santos, P. S., Pereira, R., Pereira, M. E., Freitas, A. C., Duarte, A. C., & Rocha-Santos, T. A. (2017). Biodegradation of polyethylene microplastics by the marine fungus *Zalerion maritimum*. *Science of the Total Environment*, *586*, 10-15. https://doi.org/10.1016/j.scitotenv.2017.02.017

Paterson, M. J., Findlay, D. L., Salki, A. G., Hendzel, L. L., & Hesslein, R. H. (2002). The effects of *Daphnia* on nutrient stoichiometry and filamentous cyanobacteria: a mesocosm experiment in a eutrophic lake. *Freshwater Biology*, *47*(7), 1217-1233. https://doi.org/10.1046/j.1365-2427.2002.00842.x

Pauwels, K., Stoks, R., & De Meester, L. (2005). Coping with predator stress: Interclonal differences in induction of heat-shock proteins in the water flea *Daphnia magna*. *Journal of Evolutionary Biology*, *18*(4), 867-872. https://doi.org/10.1111/j.1420-9101.2005.00890.x

Penczykowski, R. M., Lemanski, B. C. P., Sieg, R. D., Hall, S. R., Housley Ochs, J., Kubanek, J., & Duffy, M. A. (2014). Poor resource quality lowers transmission potential by changing foraging behaviour. *Functional Ecology*, *28*(5), 1245–1255. https://doi.org/10.1111/1365-2435.12238

Perkins, S. E., Alexander, L. V., & Nairn, J. R. (2012). Increasing frequency, intensity and duration of observed global heatwaves and warm spells. *Geophysical Research Letters*, *39*(20). https://doi.org/10.1029/2012GL053361

Perrow, M. R., Jowitt, A. J., Stansfield, J. H., & Phillips, G. L. (1999). The practical importance of the interactions between fish, zooplankton and macrophytes in shallow lake restoration. *Hydrobiologia*, *395*, 199-210. https://doi.org/10.1023/A:1017005803941

Piccardo, M., Provenza, F., Grazioli, E., Cavallo, A., Terlizzi, A., & Renzi, M. (2020). PET microplastics toxicity on marine key species is influenced by pH, particle size and food variations. *Science of the Total Environment*, *715*, 136947. https://doi.org/10.1016/j.scitotenv.2020.136947

Pogozhev, P. I., & Gerasimova, T. N. (2005). The role of filtering zooplankton in de-eutrophication of water bodies. *Water Resources*, *32*(3), 337-345. https://doi.org/10.1007/s11268-005-0043-y

Poulin, R. (1992). Toxic pollution and parasitism in freshwater fish. *Parasitology Today*, *8*(2), 58-61. https://doi.org/10.1016/0169-4758(92)90090-O

Poulin, R. (1999). The functional importance of parasites in animal communities: many roles at many levels? *International Journal for Parasitology*, *29*(6), 903-914. https://doi.org/10.1016/S0020-7519(99)00045-4

Pulkkinen, K., & Ebert, D. (2004). Host starvation decreases parasite load and mean host size in experimental populations. *Ecology*, *85*(3), 823-833. https://doi/org/doi:10.1890/03-0185

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849-873. https://doi.org/10.1111/brv.12480

Reynolds, C. S. (2011). *Daphnia*: Development of Model Organism in Ecology and Evolution-2011. *Freshwater Reviews*, *4*(1), 85-87. https://doi.org/10.1608/FRJ-4.1.425

Riaz, G., Tabinda, A. B., Iqbal, S., Yasar, A., Abbas, M., Khan, A. M., Mahfooz, Y., & Baqar, M. (2017). Phytoremediation of organochlorine and pyrethroid pesticides by aquatic macrophytes and algae in freshwater systems. *International Journal of Phytoremediation*, *19*(10), 894-898. https://doi.org/10.1080/15226514.2017.1303808

Rijkers, G. T., Frederix-Wolters, E. M., & Van Muiswinkel, W. B. (1980). The immune system of cyprinid fish. Kinetics and temperature dependence of antibody-producing cells in carp (*Cyprinus carpio*). *Immunology*, *41*(1), 91. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1458243/

Ritchie, H., & Roser, M. (2020). CO_2 and greenhouse gas emissions. Retrieved from: https://ourworldindata.org/co2-and-other-greenhouse-gas-emissions

Rochlin, I., Ninivaggi, D. V., Hutchinson, M. L., & Farajollahi, A. (2013). Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in Northeastern USA: implications for public health practitioners. *PLoS ONE*, *8*(4), e60874. https://doi.org/10.1371/journal.pone.0060874

Rödder, D., Schmitt, T., Gros, P., Ulrich, W., & Habel, J. C. (2021). Climate change drives mountain butterflies towards the summits. *Scientific Reports*, *11*(1), 1-12. https://doi.org/10.1038/s41598-021-93826-0

Rodrigues, J., Brayner, F. A., Alves, L. C., Dixit, R., & Barillas-Mury, C. (2010). Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science*, *329*(5997), 1353-1355. https://doi.org/10.1126/science.1190689

Rohde, R., Muller, R. A., Jacobsen, R., Muller, E., Perlmutter, S., Rosenfeld, A., Wurtele, J., Groom, D., & Wickham, C. (2013). A New Estimate of the Average Earth Surface Land Temperature Spanning 1753 to 2011, Geoinformatics & Geostatistics: An Overview, 1:1. https://doi.org/10.4172/2327-4581.1000101

Rohr, J. R., & Cohen, J. M. (2020). Understanding how temperature shifts could impact infectious disease. *PLoS Biology*, *18*(11), e3000938. https://doi.org/10.1371/journal.pbio.3000938

Ruttner-Kolisko, A. (1977) The effect of the microsporid *Plistophora asperospora* on *Conochilus unicornis* in Lunzer Untersee (LUS). *Archiv für Hydrobiologie, beheifte Ergebnisse Limnologie*, *8*, 135-137. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCAL7850429317

Sadler, D. E., Brunner, F. S., & Plaistow, S. J. (2019). Temperature and clone-dependent effects of microplastics on immunity and life history in *Daphnia magna*. *Environmental Pollution*, *255*, 113178. https://doi.org/10.1016/j.envpol.2019.113178

Salvati, A., Åberg, C., dos Santos, T., Varela, J., Pinto, P., Lynch, I., & Dawson, K. A. (2011). Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(6), 818-826. https://doi.org/10.1016/j.nano.2011.03.005

Sánchez, K. F., Huntley, N., Duffy, M. A., & Hunter, M. D. (2019). Toxins or medicines? Phytoplankton diets mediate host and parasite fitness in a freshwater system. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1894), 20182231. https://doi.org/10.1098/rspb.2018.2231

Sánchez-Baracaldo, P., Bianchini, G., Wilson, J. D., & Knoll, A. H. (2021). Cyanobacteria and biogeochemical cycles through Earth history. *Trends in Microbiology*. https://doi.org/10.1016/j.tim.2021.05.008

Sanchez-Thirion, K., Danger, M., Bec, A., Billoir, E., Labaude, S., Rigaud, T., Beisel, J. N., & Felten, V. (2019). High food quality increases infection of *Gammarus pulex* (Crustacea: Amphipoda) by the acanthocephalan parasite *Pomphorhynchus laevis*. *International Journal for Parasitology*, *49*(10), 805-817. https://doi.org/10.1016/j.ijpara.2019.05.005

Sarnelle, O. (2005). *Daphnia* as keystone predators: effects on phytoplankton diversity and grazing resistance. *Journal of Plankton Research*, *27*(12), 1229-1238. https://doi.org/10.1093/plankt/fbi086

Sattar, Q., Maqbool, M. E., Ehsan, R., & Akhtar, S. (2021). Review on climate change and its effect on wildlife and ecosystem. *Open Journal of Environmental Biology*, *6*(1), 008-014. https://doi.org/10.17352/ojeb.000021

Saupe, E. E., Qiao, H., Donnadieu, Y., Farnsworth, A., Kennedy-Asser, A. T., Ladant, J. B., Lunt, D. J., Pohl, A., Valdes, P., & Finnegan, S. (2020). Extinction intensity during Ordovician and Cenozoic glaciations explained by cooling and palaeogeography. *Nature Geoscience*, *13*(1), 65-70. https://doi.org/10.1038/s41561-019-0504-6

Schampera, C., Wolinska, J., Bachelier, J. B., de Souza Machado, A. A., Rosal, R., González-Pleiter, M., & Agha, R. (2021). Exposure to nanoplastics affects the outcome of infectious disease in phytoplankton. *Environmental Pollution*, *277*, 116781. https://doi.org/10.1016/j.envpol.2021.116781

Schelske, C. L., Stoermer, E. F., Conley, D. J., Robbins, J. A., & Glover, R. M. (1983). Early eutrophication in the lower Great Lakes. *Science*, *222*(4621), 320-322. https://doi.org/10.1126/science.222.4621.320

Schindler, D. W. (1997). Widespread effects of climatic warming on freshwater ecosystems in North America. *Hydrological Processes*, *11*(8), 1043-1067. https://doi.org/10.1002/(SICI)1099-1085(19970630)11:8<1043::AID-HYP517>3.0.CO;2-5

Schlotz, N., Ebert, D., & Martin-Creuzburg, D. (2013). Dietary supply with polyunsaturated fatty acids and resulting maternal effects influence host–parasite interactions. *BMC Ecology*, *13*(1), 1-11. https://doi.org/10.1186/1472-6785-13-41

Schoebel, C. N., Tellenbach, C., Spaak, P., & Wolinska, J. (2011). Temperature effects on parasite prevalence in a natural hybrid complex. *Biology Letters*, *7*(1), 108–111. https://doi.org/10.1098/rsbl.2010.0616

Scholthof, K. B. G. (2007). The disease triangle: pathogens, the environment and society. *Nature Reviews Microbiology*, *5*(2), 152-156. https://doi.org/10.1038/nrmicro1596

Scott, C., Lyons, T. W., Bekker, A., Shen, Y. A., Poulton, S. W., Chu, X. L., & Anbar, A. D. (2008). Tracing the stepwise oxygenation of the Proterozoic ocean. *Nature*, *452*(7186), 456-459. https://doi.org/10.1038/nature06811

Searle, C. L., Ochs, J. H., Cáceres, C. E., Chiang, S. L., Gerardo, N. M., Hall, S. R., & Duffy, M. A. (2015). Plasticity, not genetic variation, drives infection success of a fungal parasite. *Parasitology*, *142*(6), 839-848. https://doi.org/10.1017/S0031182015000013

Seda, J., Petrusek, A., Machacek, J., & Smilauer, P. (2007). Spatial distribution of the *Daphnia longispina* species complex and other planktonic crustaceans in the heterogeneous environment of canyon-shaped reservoirs. *Journal of Plankton Research*, *29*(7), 619-628. https://doi.org/10.1093/plankt/fbm044

Sharma, S., & Mujumdar, P. (2017). Increasing frequency and spatial extent of concurrent meteorological droughts and heatwaves in India. *Scientific Reports*, *7*(1), 1-9. https://doi.org/10.1038/s41598-017-15896-3

Shivanna, K. R. (2020). The Sixth Mass Extinction Crisis and its Impact on Biodiversity and Human Welfare. *Resonance*, *25*(1), 93-109. https://doi.org/10.1007/s12045-019-0924-z

Shocket, M. S., Strauss, A. T., Hite, J. L., Šljivar, M., Civitello, D. J., Duffy, M. A., Cáceres, C. E., & Hall, S. R. (2018a). Temperature drives epidemics in a zooplankton-fungus disease system: a trait-driven approach points to transmission via host foraging. *The American Naturalist*, *191*(4), 435-451. https://doi.org/10.1086/696096

Shocket, M. S., Vergara, D., Sickbert, A. J., Walsman, J. M., Strauss, A. T., Hite, J. L., Duffy, M. A., & Hall, S. R. (2018b). Parasite rearing and infection temperatures jointly influence disease transmission and shape seasonality of epidemics. *Ecology*, *99*(9), 1975-1987. https://doi.org/10.1002/ecy.2430

Shocket, M. S., Magnante, A., Duffy, M. A., Cáceres, C. E., & Hall, S. R. (2019). Can hot temperatures limit disease transmission? A test of mechanisms in a zooplankton–fungus system. *Functional Ecology*, *33*(10), 2017-2029. https://doi.org/10.1111/1365-2435.13403

Seda, J., & Petrusek, A. (2011). *Daphnia* as a model organism in limnology and aquatic biology: introductory remarks. *Journal of Limnology*, *70*(2), 337-344. https://doi.org/10.3274/JL11-70-2-17

Silva, A. B., Costa, M. F., & Duarte, A. C. (2018). Biotechnology advances for dealing with environmental pollution by micro (nano) plastics: Lessons on theory and practices. *Current Opinion in Environmental Science & Health*, *1*, 30-35. https://doi.org/10.1016/j.coesh.2017.10.005

Snieszko, S. F. (1974). The effects of environmental stress on outbreaks of infectious diseases of fishes. *Journal of Fish Biology*, *6*(2), 197-208. https://doi.org/10.1111/j.1095-8649.1974.tb04537.x

Soares, A. M., Baird, D. J., & Calow, P. (1992). Interclonal variation in the performance of *Daphnia magna* Straus in chronic bioassays. *Environmental Toxicology and Chemistry: An International Journal*, *11*(10), 1477-1483. https://doi.org/10.1002/etc.5620111013

Søndergaard, M., & Jeppesen, E. (2007). Anthropogenic impacts on lake and stream ecosystems, and approaches to restoration. *Journal of Applied Biology*, *44*(6), 1089-1094. https://doi.org/10.1111/j.1365-2664.2007.01426.x

Sonenshine, D. E. (2018). Range expansion of tick disease vectors in North America: implications for spread of tick-borne disease. *International Journal of Environmental Research and Public Health*, *15*(3), 478. https://doi.org/10.3390/ijerph15030478

Spaak, P. (1997). Hybridization in the *Daphnia galeata* complex: are hybrids locally produced? *Hydrobiologia*, *360*(1), 127-133. https://doi.org/10.1023/A:1003157117667

Stark, J. D., & Vargas, R. I. (2005). Toxicity and hazard assessment of fipronil to *Daphnia pulex*. *Ecotoxicology and Environmental Safety*, *62*(1), 11-16. https://doi.org/10.1016/j.ecoenv.2005.02.011

Stemberger, R. S., & Lazorchak, J. M. (1994). Zooplankton assemblage responses to disturbance gradients. *Canadian Journal of Fisheries and Aquatic Sciences*, *51*(11), 2435-2447. https://doi.org/10.1139/f94-243

Sterner, R.W. (1989). The role of grazers in phytoplankton succession. In: Plankton Ecology: Succession in Plankton Communities (Ed. U. Sommer) Springer-Verlag, Berlin.

Stewart Merrill, T. E., & Cáceres, C. E. (2018). Within-host complexity of a plankton-parasite interaction. *Ecology*, *99*(12), 2864-2867. https://www.jstor.org/stable/26627163

Stewart Merrill, T. E., Hall, S. R., Merrill, L., & Cáceres, C. E. (2019). Variation in immune defense shapes disease outcomes in laboratory and wild *Daphnia*. *Integrative and Comparative Biology*, *59*(5), 1203-1219. https://doi.org/10.1093/icb/icz079

Stirnadel, H. A., & Ebert, D. (1997). Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. *Journal of Animal Ecology*, *66*, 212-222. https://doi.org/10.2307/6023

Stjernman, M., & Little, T. J. (2011). Genetic variation for maternal effects on parasite susceptibility. *Journal of Evolutionary Biology*, *24*(11), 2357–2363. https://doi.org/10.1111/j.1420-9101.2011.02363.x

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society*, *29*(1), 344-358. https://doi.org/10.1899/08-171.1

Stringer, C. (2016). The origin and evolution of Homo sapiens. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1698), 20150237. https://doi.org/10.1098/rstb.2015.0237

Studer, A., Thieltges, D. W., & Poulin, R. (2010). Parasites and global warming: net effects of temperature on an intertidal host–parasite system. *Marine Ecology Progress Series*, *415*, 11-22. https://doi.org/10.3354/meps08742

Syller, J., & Grupa, A. (2016). Antagonistic within-host interactions between plant viruses: molecular basis and impact on viral and host fitness. *Molecular Plant Pathology*, *17*(5), 769-782. https://doi.org/10.1111/mpp.12322

Tang, F. H., Lenzen, M., McBratney, A., & Maggi, F. (2021). Risk of pesticide pollution at the global scale. *Nature Geoscience*, *14*(4), 206-210. https://doi.org/10.1038/s41561-021-00712-5

Tellenbach, C., Tardent, N., Pomati, F., Keller, B., Hairston, N. G., Wolinska, J., & Spaak, P. (2016). Cyanobacteria facilitate parasite epidemics in *Daphnia*. *Ecology*, *97*(12), 3422–3432. https://doi.org/10.1002/ecy.1576

Toenshoff, E. R., Fields, P. D., Bourgeois, Y. X., & Ebert, D. (2018). The end of a 60-year riddle: Identification and genomic characterization of an iridovirus, the causative agent of white fat cell disease in zooplankton. *G3: Genes, Genomes, Genetics*, *8*(4), 1259-1272. https://doi.org/10.1534/g3.117.300429

Torruella, G., De Mendoza, A., Grau-Bové, X., Antó, M., Chaplin, M. A., del Campo, J., Eme, L., Pérez-Cordón, G., Whipps, C. M., Nichols, K. M., Paley, R., Roger, A. J., Sitjà-Bobadilla, Donachie, S., & Ruiz-Trillo, I. (2015). Phylogenomics reveals convergent evolution of lifestyles in close relatives of animals and fungi. *Current Biology*, *25*(18), 2404-2410. https://doi.org/10.1016/j.cub.2015.07.053

Trevors, J. T. (2001). From chemosphere to biosphere. *World Journal of Microbiology and Biotechnology*, *17*(6), 651-655. https://doi.org/10.1023/A:1012250623041

Truscott, R., & White, K. N. (1990). The influence of metal and temperature stress on the immune system of crabs. *Functional Ecology*, 455-461. https://doi.org/10.2307/2389609

Ustabasi, G. S., & Baysal, A. (2020). Bacterial interactions of microplastics extracted from toothpaste under controlled conditions and the influence of seawater. *Science of the Total Environment*, *703*, 135024. https://doi.org/10.1016/j.scitotenv.2019.135024

Vale, P. F., Stjernman, M., & Little, T. J. (2008). Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions. *Journal of Evolutionary Biology*, *21*(5), 1418-1427. https://doi.org/10.1111/j.1420-9101.2008.01555.x

Valois, A. E., & Burns, C. W. (2016). Parasites as prey: *Daphnia* reduce transmission success of an oomycete brood parasite in the calanoid copepod *Boeckella*. *Journal of Plankton Research*, *38*(5), 1281-1288. https://doi.org/10.1093/plankt/fbw055

Van Damme, K., & Kotov, A. A. (2016). The fossil record of the Cladocera (Crustacea: Branchiopoda): Evidence and hypotheses. *Earth-Science Reviews*, *163*, 162-189. https://doi.org/10.1016/j.earscirev.2016.10.009

Van Doorslaer, W., Stoks, R., Swillen, I., Feuchtmayr, H., Atkinson, D., Moss, B., & De Meester, L. (2010). Experimental thermal microevolution in community-embedded *Daphnia* populations. *Climate Research*, *43*(1-2), 81-89. https://doi.org/10.3354/cr00894

Vandermaesen, J., Horemans, B., Bers, K., Vandermeeren, P., Herrmann, S., Sekhar, A., Seuntjens, P., & Springael, D. (2016). Application of biodegradation in mitigating and remediating pesticide contamination of freshwater resources: state of the art and challenges for optimization. *Applied Microbiology and Biotechnology*, *100*(17), 7361-7376. https://doi.org/10.1007/s00253-016-7709-z

Vidtmann, S. (1993). The peculiarities of prevalence of microsporidium *Larssonia daphniae* in the natural *Daphnia pulex* population. *Ekologija*, *1*, 61-69.

Visser, P. M., Verspagen, J. M., Sandrini, G., Stal, L. J., Matthijs, H. C., Davis, T. W., Paerl, H.W., & Huisman, J. (2016). How rising CO2 and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae*, *54*, 145-159. https://doi.org/10.1016/j.hal.2015.12.006

Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014). Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science*, *148*, 1-13. https://doi.org/10.1016/j.ecss.2014.05.027

Wang, Z., Zhang, Y., Kang, S., Yang, L., Shi, H., Tripathee, L., & Gao, T. (2021). Research progresses of microplastic pollution in freshwater systems. *Science of the Total Environment*, 148888. https://doi.org/10.1016/j.scitotenv.2021.148888

Ward, J. R., Kim, K., & Harvell, C. D. (2007). Temperature affects coral disease resistance and pathogen growth. *Marine Ecology Progress Series, 329,* 115-121. https://doi.org/10.3354/meps329115

Weider, L. J., & Pijanowska, J. (1993). Plasticity of *Daphnia* life histories in response to chemical cues from predators. *Oikos, 67,* 385-392. https://doi.org/10.2307/3545351

Weigl, S., Körner, H., Petrusek, A., Seda, J., & Wolinska, J. (2012). Natural distribution and co-infection patterns of microsporidia parasites in the *Daphnia longispina* complex. *Parasitology*, 139(7), 870-880. https://doi.org/10.1017/S0031182012000303

Whisler, H. C. (1968). Developmental control of *Amoebidium parasiticum*. *Developmental Biology, 17*(5), 562-570. https://doi.org/10.1016/0012-1606(68)90005-5

Wiens, J. A., Stralberg, D., Jongsomjit, D., Howell, C. A., & Snyder, M. A. (2009). Niches, models, and climate change: assessing the assumptions and uncertainties. *Proceedings of the National Academy of Sciences*, *106*(Supplement 2), 19729-19736. https://doi.org/10.1073/pnas.0901639106

Wolinska, J., Keller, B., Bittner, K., Lass, S., & Spaak, P. (2004). Do parasites lower *Daphnia* hybrid fitness? *Limnology and Oceanography*, *49*, 1401-1407. https://doi.org/10.4319/lo.2004.49.4_part_2.1401

Wolinska, J., Keller, B., Manca, M., & Spaak, P. (2007). Parasite survey of a *Daphnia* hybrid complex: host-specificity and environment determine infection. *Journal of Animal Ecology*, 191-200. https://doi.org/10.111l/j.1365-2656.2006.0117

Wolinska, J., Giessler, S., & Koerner, H. (2009). Molecular identification and hidden diversity of novel *Daphnia* parasites from European lakes. *Applied and Environmental Microbiology*, *75*(22), 7051-7059. https://doi.org/10.1128/AEM.01306-09

Wolinska, J., Seda, J., Koerner, H., Smilauer, P., & Petrusek, A. (2011). Spatial variation of *Daphnia* parasite load within individual water bodies. *Journal of Plankton Research*, *33*(8), 1284-1294. https://doi.org/10.1093/plankt/fbr016

Wotton, B. M., Nock, C. A., & Flannigan, M. D. (2010). Forest fire occurrence and climate change in Canada. *International Journal of Wildland Fire*, *19*(3), 253-271. https://doi.org/10.1071/WF09002

Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *Wiley Interdisciplinary Reviews: Water*, *6*(5), e1373. https://doi.org/10.1002/wat2.1373

Yang, Y., Guo, Y., O'Brien, A. M., Lins, T. F., Rochman, C. M., & Sinton, D. (2020). Biological responses to climate change and nanoplastics are altered in concert: Full-factor screening reveals effects of multiple stressors on primary producers. *Environmental Science & Technology*, *54*(4), 2401-2410. https://doi.org/10.1021/acs.est.9b07040

Yevjevich, V. (1992). Water and civilization. *Water International*, *17*(4), 163-171. https://doi.org/10.1080/02508069208686135

Yin, M., Petrusek, A., Seda, J., & Wolinska, J. (2012). Fine-scale genetic analysis of *Daphnia* host populations infected by two virulent parasites–strong fluctuations in clonal structure at small temporal and spatial scales. *International Journal for Parasitology*, *42*(1), 115-121. https://doi.org/10.1016/j.ijpara.2011.11.004

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., & Oda, K. (2016). A bacterium that degrades and assimilates poly (ethylene terephthalate). *Science*, *351*(6278), 1196-1199. https://doi.org/10.1126/science.aad6359

Yuan, W., Zhou, Y., Chen, Y., Liu, X., & Wang, J. (2020). Toxicological effects of microplastics and heavy metals on the *Daphnia magna*. *Science of the Total Environment*, *746*, 141254. https://doi.org/10.1016/j.scitotenv.2020.141254

Zamparas, M., & Zacharias, I. (2014). Restoration of eutrophic freshwater by managing internal nutrient loads. A review. *Science of the Total Environment*, *496*, 551-562. https://doi.org/10.1016/j.scitotenv.2014.07.076

Zaret, T. M. (1980). Predation and freshwater communities. Yale.

Ziajahromi, S., Kumar, A., Neale, P. A., & Leusch, F. D. (2019). Effects of polyethylene microplastics on the acute toxicity of a synthetic pyrethroid to midge larvae (*Chironomus tepperi*) in synthetic and river water. *Science of the Total Environment*, *671*, 971-975. https://doi.org/10.1016/j.scitotenv.2019.03.425

Zhu, X., Zhao, W., Chen, X., Zhao, T., Tan, L., & Wang, J. (2020). Growth inhibition of the microalgae *Skeletonema costatum* under copper nanoparticles with microplastic exposure. *Marine Environmental Research*, *158*, 105005. https://doi.org/10.1016/j.marenvres.2020.105005

Zurbenko, I., & Luo, M. (2015). Surface humidity changes in different temporal scales. *American Journal of Climate Change*, *4*(03), 226. https://doi.org/10.4236/ajcc.2015.43018

Zurbenko, I. G., & Potrzeba, A. L. (2013). Tides in the Atmosphere. *Air Quality, Atmosphere & Health*, *6*(1), 39-46. https://doi.org/10.1007/s11869-011-0143-6

Zurbenko, I. G., & Potrzeba-Macrina, A. L. (2021). Numerical predictions for rising water levels in the oceans. *World Scientific News*, *152*, 1-14. http://psjd.icm.edu.pl/psjd/element/bwmeta1.element.psjd-97fff9fd-8a46-4eac-ba64-d8dc7e051691

LIST OF PUBLICATIONS

Manzi, F., Agha, R., Lu, Y., Ben-Ami, F., & Wolinska, J. (2020). Temperature and host diet jointly influence the outcome of infection in a *Daphnia*-fungal parasite system. *Freshwater Biology*, *65*(4), 757-767. https://doi.org/10.1111/fwb.13464

Manzi, F., Halle, S., Seemann, L., Ben-Ami, F., & Wolinska, J. (2021). Sequential infection of *Daphnia magna* by a gut microsporidium followed by a haemolymph yeast decreases transmission of both parasites. *Parasitology*, *148*(13), 1566-1577. https://doi.org/10.1017/S0031182021001384

ACKNOWLEDGEMENTS

First of all, I am infinitely grateful to Justyna (and Ramsy) for conducting my initial evaluation and recruiting me into their group, back in the fall of 2017. Moving from France to Berlin was a huge step in both my personal and academic life. It was no easy decision to take, but your immediate support and warm welcome at IGB helped me realize this was indeed the right path! Thank you for always believing in me and your continuous support throughout these four years. Justyna, I'm sorry that I've never really tried to join any of your sportive/running events! I sincerely hope that my wilful contributions to the – slightly less physically demanding – board games and group activities over the years will make up for my adamant resilience to put on running shoes!

Many thanks to all past and present members of the Kinderzimmer. Alexis, Marta, Mikaël, Tarun, Jason, Félicie: you were my 'mentors', and always encouraged me to stay for one more drink (despite my less-than-stellar contributions to table soccer games)! Paul, Anna Lena, Jonas, Yile, Benji, Marcin, Joe, Samah: thank you for making this workplace so welcoming. I am glad that I was able to work throughout my entire PhD in this peaceful and cosy student's lair, which was always enlightened by your company.

Many thanks to the wider doctoral and post-doctoral community at IGB, including fellow students from MSD310 and Neuglobsow, who always took great care in organizing the multiple PhD gatherings and social events, which helped us all feel like a large and united family.

To all incredibly talented BSc and MSc students we've had the chance to welcome and collaborate with in the Disease Eco Evo group: Bruno, Nesar, Charlotte, Max, Louise, Mellery, Jessica, Marc, Nele, Toni, Steve, Noemi, Paula. I hope you all find success in your future endeavours, no matter your choice of career. Thank you for always being so passionate and reliable!

To post-docs and senior researchers of our group: Ramsy, Yameng, Thijs, Beng, Maja, Jürgen, Dagmar. Your enthusiasm and exemplary work ethic were always set as driving examples for us undergrads and graduate students. Ramsy, I realized that I forgot to mention you in the acknowledgements for Chapter 4, despite providing us with an incredibly helpful method for staining our samples! Beyond our many co-authorships, I'm taking the opportunity here to thank you as well for your invaluable and frequent help with troubleshooting, relative to both microscope and statistical issues!

Infinite thanks to the ever-helpful and formidable technicians in our group: Elizabeth, Uschi, Mareike, Benjamin; magicians of the lab and masters of pipetting, I believe I speak for all of our *Daphnia* babies when thanking you for your incredible work behind the scenes.

Many thanks to our collaborators from Tel Aviv University, my second supervisor Frida and Snir, for your valuable feedback and help with the collaborative parts of our project. I received a warm welcome in Tel Aviv, and am really grateful to your working group for making this experience possible.

Many thanks as well to wider members of the IGB community, including Michael Monaghan, Klaus Knopf and regular members of our joint meetings (MTM, Parasite Discussion Club), as well as Kirsten Pohlmann, Katrin Lehmann, and Rita Adrian for your continuing support and heartful conversations.

Many thanks to regular attendees and organizers of the yearly 'PhD meetings': Adam, Miko, Dino, Sophie, Jens, Charlotte, Otti, Klaus; I have travelled to wonderful places thanks to these events, and will always remember your valuable feedback and encouraging speeches!

Beyond the scope of my formidable working environment, I cannot fail to express my most grateful thanks to my friends and family, who were always there to support me, no matter how many miles (and even sometimes continents) stood between us. À mes amis de Montpellier, les expatriés sauf un, les flamands roses authentiques, les thésaroïdes désabusés : Loïs, Bea, Elise, Maxime, Cécile, Florent, Alex. I was really lucky to have you around, either here in Berlin, or through our diverse means of communication. Thank you for our daily conversations, memes, funny pictures and videos… but most of all, thank you for your unwavering support. À mes reuleuleux : Ismaël, Nathan, Samantha. I can't express just how glad I am that we (re)connected last year. Our weekly sessions proved to be so important in keeping up high spirits and sanity, at the height of a pandemic that took us all by surprise. À mes plus vieux amis, ceux de Sarreguemines et de Sainte-Chrétienne : Caro, Philippe, Audrey, Maxime. Thank you for always putting up with my stories and grievances. To my brother (who didn't have the occasion to visit Berlin!) and my parents (who've had many occasions to visit Berlin!): thank you for always believing in my choice of career.

I would also like to thank those of my former teachers, who planted and cultivated the seed of biology, zoology and evolutionary ecology into my heart, all those years ago: Raymond Leborgne, Dominique Chardard, Emmanuel Douzery, as well as my former MSc supervisors, Bernard Godelle and Oliver Kaltz. Thank you for turning me into the scientist I hope I have become today.

Finally, I would like to give a shoutout to the musical artists and influences that followed me throughout these past few years. Between incredible new releases from my favourite bands (Gorillaz, In Flames, Opeth, Mastodon, Paradise Lost, Tool) or the unsuspected and wonderful musical discoveries I have come across throughout these last few years (Animals as Leaders, Ryo Fukui, Chet Baker, Aphex Twin, Nujabes, Tommy Guerrero, Haircuts for Men, Iron Cthulhu Apocalypse), I couldn't have hoped to work into a better soundscape than the one you have provided throughout countless hours of pipetting, counting, programming, and late-evening writing.