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.

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,
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 (Pajijmans 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 (Paele & 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; Lamert, 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; Lamert, 1987). As typically dominant zooplankton and key herbivores in freshwater food webs (Lamert & Kinne, 2011), water fleas (Daphnia) are likely to be affected by these harmful blooms (Rohrback, Dittmann, Henning, Börner, & Kohli, 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; Schoeble, Telegen, Spaa, & 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 Caulerya 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 (Sanchez, 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; Sanchez et al., 2019), nutrient availability (Frost, Ebert, & Smith, 2008; Nar, Ebert, Bastille-Rousseau, & Frost, 2019) and water temperature (Cuco, Castro, Goncalves, 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 (Johann 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, 2016) 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 cocccid cyanobacterium M. aeruginosa (MaGr01, isolated from Greifensee in Switzerland; Tellenbach et al., 2016) and the filamentous cyanobacterium P. agariphii (NIVA-CYA 630, isolated from Lake Lysener 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—also hosts of 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—also hosts of 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—also hosts of 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).
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:

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

with r as the rate of population increase (/day), x the age class in days, lx the probability of surviving to age x, and mx 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 $\chi^2$ 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 $\gamma$-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 $\chi^2$ 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 ($\leq 0.05$) 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)
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, the population growth rate ($r$) was significantly affected by infection, with a higher growth rate observed in infected hosts compared to controls. This suggests that infected hosts are more likely to contribute to the parasite population growth.

### 4.2 Host lifespan

Temperature and food quality were the main drivers of host lifespan, with higher temperatures causing a decrease in lifespan. This effect was more pronounced in infected hosts, where the decrease in lifespan was more significant.

### 4.3 Host fecundity

The total number of offspring produced by infected hosts was significantly lower compared to controls, especially under higher temperatures. This indicates a negative impact of infection on host reproductive success.

### 4.4 Host growth rate ($r$)

The per capita intrinsic rate of increase ($r$) was significantly reduced in infected hosts, especially under higher temperatures, indicating a decreased ability of infected hosts to sustain the parasite population growth.
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 reduced 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 favor 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). Schistoscephalus solidus parasite prevalence and biomass intensity in threesspine 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., & Hettlinger, 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.

Codeanu, 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., & DeCastecker, 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.tb00557.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
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. L., Pielpher, 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

Paajimans, 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. (2008). Global warming and cyanobacterial harmful algal blooms. Limnology and Oceanography, 53(4), 1279–1293. https://doi.org/10.4319/lo.2008.53.4.1279

Penczynowski, 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). Plasticity, not genetic variation, drives infection success of a fungal parasite. Parasitology, 124(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/elic.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/10.1002/ja.2011.21

Sønstebo, J. H., & Roehrland, 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.

Spiedman, 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 Merril, 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., Hairson, N. G., Wolinska, J., & Spak, 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 geno-type-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 a plankton-parasite interaction. 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., & Petrushke, 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.