

Title: Context dependent host-symbiont interactions: shifts along the parasitism–mutualism continuum

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Table 1 (after references)

Supplemental information:

Table S1. Location and maximum prevalence of each parasite in the study lakes.

Supplemental text S1. MicG infection does not affect host lifespan

Figure S2. Microscope images showing needle-shaped *Metschnikowia* spores attacking the gut epithelium of wild-caught *Daphnia*.

Supplemental text S2. Regressing X vs. Y/X

Supplemental text S3. Direct comparison of MicG-infected and uninfected *Daphnia* egg ratios

Tables S2-S9. Model results for GLMM analyses of the laboratory infection assay of MicG impacts on gut penetrability as well as field relationships among virulent parasite prevalence, resources and the relative fecundity of MicG-infected *Daphnia* in Michigan and Indiana lakes.

Supplemental text S4. Relationship between resource availability and virulent parasite prevalence.

Supplemental text S5. Variation in resource availability among the study lakes.

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Abstract

Symbiotic interactions can shift along a mutualism–parasitism continuum. While there are many studies examining dynamics typically considered to be mutualistic that sometimes shift towards parasitism, little is known about conditions underlying shifts from parasitism towards mutualism. In lake populations, we observed that infection by a microsporidian gut symbiont sometimes conferred a reproductive advantage and other times a disadvantage to its *Daphnia* host. We hypothesized that the microsporidian might benefit its host by reducing infection by more virulent parasites, which attack via the gut. In a lab study using field-collected animals, we found that spores of a virulent fungal parasite were much less capable of penetrating the guts of *Daphnia* harboring the microsporidian gut symbiont. We predicted that this altered gut penetrability could cause differential impacts on host fitness depending on ecological context. Field survey data revealed that microsporidian-infected *Daphnia* hosts experienced a reproductive advantage when virulent parasites were common while resource scarcity led to a reproductive disadvantage, but only in lakes where virulent parasites were relatively rare. Our findings highlight the importance of considering multiparasite community context and resource availability in host-parasite studies and open the door for future research into conditions driving shifts along parasitism to mutualism gradients.

Introduction

Symbiotic microbes — defined broadly to include all microbes that live in very close association with another organism — are ubiquitous and often have strong negative or positive fitness impacts on their hosts. Traditionally, ecologists have viewed these interactions as being

qualitatively fixed — a particular symbiont always increases host fitness or always decreases it. However, we now realize that these interspecific interactions can be fluid, shifting along a continuum of mutualism to commensalism to parasitism (Johnson et al. 1997; Min and Benzer 1997; Hoeksema and Bruna 2000; Denison and Kiers 2004; Stadler and Dixon 2005).

Symbioses involve both costs and benefits for each species engaged in a given interaction, and it is the net sum of these costs and benefits that determines the nature of the association (Johnson et al. 1997; Chamberlain et al. 2014; Stewart and Schnitzer 2017). Examination of the degree of flexibility seen in interspecific interactions across a range of ecosystem types and study locations has shown that both abiotic factors and the presence of a third species can influence the strength and direction of symbiotic interactions (Chamberlain et al. 2014).

However, while many studies of flexible interactions have considered shifts from mutualism to parasitism [e.g., plant-mycorrhizal (Johnson et al. 1997), plant-pollinator (Irwin et al. 2010), and ant-aphid (Stadler and Dixon 2005) interactions], we know much less about the potential for organisms that are traditionally considered parasites to shift towards mutualism (Redman et al. 2001; Canestrari et al. 2014; Lin and Koskella 2015; Savory et al. 2017; González et al. 2021).

While relatively little is known about conditions that cause parasites to become mutualists, it is well known that parasites in a particular host-parasite interaction can vary greatly in the degree of harm inflicted on their hosts, ranging from modest to severe virulence (Read 1994; Casadevall and Pirofski 1999; Hube 2004; Bouzid et al. 2013). One important factor that can influence the pathology of parasites is the multiparasite environment. Many host species

encounter multiple parasite species in their lifetime, whether sequentially or simultaneously (Cox 2001). Interspecific interactions among cooccurring parasites can affect how a given parasite influences its host (Petney and Andrews 1998; Johnson and Hoverman 2012). For example, infection by one parasite can make it either easier or more difficult for a second parasite to infect the host (Telfer et al. 2010). Once inside the host, parasite species may compete for resources and space, which can alter the outcome for the host compared with a single infection (Dobson 1985). Antagonistic within-host interactions between parasite species may benefit the host (e.g., Rafaluk-Mohr et al. 2018), while synergistic interactions may increase harm (e.g., Druilhe et al. 2005). The impacts of coinfection on host fitness can even vary based on the relative timing of the infections by the two parasites (e.g., Lohr et al. 2010).

Another finding that has emerged is that the abiotic environment can alter the strength of a particular interaction (e.g., shifting an interaction from mildly parasitic to strongly parasitic) as well as the qualitative nature of host-symbiont interactions, shifting interactions between mutualism, commensalism, and/or parasitism. Studies of plant-mycorrhizal interactions provide significant insight into the importance of environmental context in determining the nature of symbioses (Johnson et al. 1997; Johnson and Graham 2013); in particular, nutrient availability can strongly shape whether symbiotic fungi act as parasites or mutualists for their host plants (Hoeksema et al. 2010). Similarly, sunlight and nutrient availability play a key role in shaping legume-rhizobium interactions (Lau et al. 2012). Variation in the strength of host-parasite interactions can also be mediated by the availability of resources (Bedhomme et al. 2004; Johnson et al. 2010; Cressler et al. 2014; Kinnula et al. 2017). Resource availability (defined

hereafter as resource quantity and/or quality) can affect host-parasite interactions in diverse and potentially conflicting ways. For example, host fitness can be bolstered by increasing resources (Brown et al. 2000), perhaps because greater resource availability provides energy or essential nutrients needed for immune responses to infection (Lochmiller et al. 1993; Chandra 1999; Hughes and Kelly 2006). Increased resource availability can also negatively affect infected hosts, for example, by influencing host behavior or density in ways that promote pathogen transmission (Penczykowski et al. 2014) and greater host mortality (Washburn et al. 1991; Hall et al. 2009b). Pathogen load can increase [e.g., (Bedhomme et al. 2004; Trujillo-Ferrara et al. 2011)] or decrease (Furlong and Groden 2003; Gardner 2005) with greater host resource availability, or even peak at intermediate resource levels [e.g., (Lambrechts et al. 2006; Sadd 2011)]; these qualitatively different outcomes might be mediated by variation in within-host resource competition between the host immune system and the pathogen (Cressler et al. 2014). Overall, resources likely play an important role in mediating the severity of disease, but we still lack evidence of whether resource variation can tip the scales along the parasitism-mutualism continuum.

Here, we empirically examined the fluidity of host-parasite interactions over the course of individual epidemics. We took advantage of naturally occurring temporal gradients in both the prevalence of cooccurring parasite species as well as resource availability to examine the context dependency of interactions between a freshwater zooplankton host, *Daphnia dentifera*, and an undescribed microsporidian that infects its gut epithelial cells. We found that the guts of field-collected individuals infected with the microsporidian were less likely to be penetrated by

spores of a highly virulent fungal parasite that infects via the gut. In addition to altering interactions with virulent parasites, this reduced gut penetrability has the potential to alter interactions with resources. This led us to hypothesize that the fitness impact of this microsporidian might shift based on resource availability and/or prevalence of virulent parasites. Using data from naturally occurring outbreaks, we found that the fitness impact of *Daphnia*-microsporidian interactions was associated with resource availability and the prevalence of virulent parasites, with mutualism more likely when virulent parasites were common and parasitism more likely when resources were scarce and virulent parasites were relatively rare. This suggests that the ecological context in which this host-symbiont interaction occurs might explain shifts along a gradient from parasitism to mutualism.

Materials and methods

Study organisms

Our host species, *Daphnia dentifera* Forbes, is a dominant freshwater microcrustacean of lakes in the Midwestern United States (Hebert 1995). *Daphnia* are cyclical parthenogens, reproducing asexually during favorable conditions and often switching to sexual production of diapausing embryos during times of stress (Cáceres and Tessier 2004). Owing to their capacity for rapid asexual reproduction (releasing a clutch of eggs approximately every two days in summer) and their voracious consumption of phytoplankton, *Daphnia* can play an important role in controlling productivity in lake ecosystems (Miner et al. 2012). *Daphnia* encounter several environmentally transmitted symbionts in the lake water column and sediments in our study lakes (Table 1). We use the term ‘symbiont’ to refer to microorganisms that live in close

relationship with a host species, inclusive of organisms that confer a net beneficial, harmful, or neutral influence on host fitness. We refer to symbionts as ‘parasites’ if there is evidence that they harm their hosts. Our focal symbiont is a microsporidian that we commonly observe infecting the gut epithelium of *Daphnia*. We refer to this symbiont as “MicG”. Morphologically, MicG resembles the genus *Glugoides*; however, genetic sequencing revealed that this microsporidian is distinct from other sequenced microsporidian symbionts of *Daphnia*, including *Glugoides* (GenBank accession of MicG: MH635259). We have not yet succeeded in experimental transmission of MicG in the laboratory; however, other microsporidia infecting the gut epithelia of *Daphnia* have horizontal transmission, with spores shed continuously along with feces, leading to infection via feeding (Mangin et al. 1995; Ebert 2005; Fels 2005). In lab studies, *Glugoides* had a moderately negative to neutral impact on *Daphnia* host reproduction (Ebert et al. 2000; Chadwick and Little 2005). Another symbiont that commonly infects the *Daphnia* gut, *Caullerya mesnili*, is highly virulent (Bittner et al. 2002). Based on this evidence, we expected MicG to act as a parasite in our system.

Three additional parasite taxa commonly infect *D. dentifera* in our study system (Table 1); each has strong negative impacts on host fitness, but impacts on host survival and reproduction vary. These parasites include 1) *Metschnikowia bicuspidata*, a virulent fungal parasite that greatly reduces host life span and fecundity (Auld et al. 2012); 2) *Pasteuria ramosa*, a bacterium that castrates its host early in the infection process and has less impact on host lifespan compared with *Metschnikowia* (Auld et al. 2012); and 3) *Spirobacillus cienkowskii*, an extremely virulent bacterial parasite that kills its host so rapidly (within days of experimental exposure) that

infections may be difficult to detect in the field (Wale et al. 2019). All three of these virulent parasites are obligate killers, spreading to a new host only after killing their current host.

The three obligate killers are thought to share a common infection process: crossing the host gut to initiate development inside the host body cavity (Table 1). *Metschnikowia* and *Pasteuria* are both known to infect *Daphnia* when infective spores are consumed during filter feeding and penetrate the gut wall, after which they replicate in the hemocoel (Ebert et al. 1996). *Spirobacillus* is less well studied, but likely infects in a similar manner to *Pasteuria* and *Metschnikowia*. Given that MicG infects the gut epithelium, one way we expected MicG might influence other *Daphnia* parasites is by changing the physiology of the host gut in a way that affects how easily other parasites penetrate the gut.

Hypotheses and Predictions

The fitness impact of a symbiont on its host is the result of the net balance between costs and benefits associated with the interaction. Those costs and benefits, in turn, may depend on the ecological context in which the host-symbiont interaction takes place. In our initial observations of MicG-infected *Daphnia*, we noted that infected hosts sometimes had fewer offspring than uninfected hosts (expected for parasitism) but in some cases had more (suggesting mutualism). Because MicG infects the gut epithelium – a very important site for interactions of the host with resources and highly virulent parasites – we hypothesized that variation in the fitness impact MicG has on its host might correspond with resource levels and/or with the prevalence of highly virulent parasites. First, we hypothesized that MicG infections might influence the

penetrability of the host gut. We explored this hypothesis by assessing whether the guts of MicG-infected and uninfected *Daphnia* differed in their likelihood of being penetrated by *Metschnikowia* spores. We predicted that *Metschnikowia* spores would be less able to infect the guts of MicG-infected hosts. Second, we hypothesized that, if MicG infections make the host gut less penetrable, that might benefit MicG-infected hosts by reducing the need to mount a costly immune defense in response to the three highly virulent parasites present in our system. If this is the case, we predicted that the fitness impact of MicG infections would vary with prevalence of those highly virulent parasites: at high parasite prevalence, *Daphnia* that are uninfected but exposed to one of the virulent parasites would have to invest greater resources into deploying costly immune defenses to fight off parasite infections (Little and Killick 2007; Allen and Little 2011), whereas MicG-infected hosts would not. This difference in immune cost might offset any cost associated with harboring MicG, giving MicG-infected hosts a relative fecundity advantage over uninfected hosts. Third, we hypothesized that the fitness impact of MicG infections might also vary with the resource environment. This could happen if any impacts of MicG on the gut also influence the ability to acquire resources and/or if hosts are better able to deal with MicG “stealing” resources when resources are rich. In both cases, we predict that MicG infections should have a particularly strong (and negative) impact on hosts when resources are scarce.

Laboratory infection assay

To infect its host, needle-shaped *Metschnikowia* spores must successfully penetrate the *Daphnia* gut epithelium and enter the hemocoel (body cavity) to then develop and reproduce

(Stewart Merrill and Cáceres 2018). We assessed whether MicG-infected hosts had guts that were less penetrable by *Metschnikowia* spores with a laboratory infection assay. We collected adult, asexually reproducing *Daphnia dentifera* from each of six lakes from our Indiana field survey throughout the course of the field season in 2017 (early-June to early-December). We experimentally infected 231-406 *Daphnia* from each lake (total of 1728), of which 21-62% from each lake (647 of the total) were infected with MicG while the rest were uninfected by any observable parasite (as determined by inspection at 400 × magnification; it is possible some very early MicG infections were not apparent).

Within 24 hours of collection, these wild-caught *Daphnia* individuals were inoculated with *Metschnikowia bicuspidata*. *Daphnia* were inoculated individually in 15 mL Falcon tubes filled with 10 mL of filtered lake water and were fed 1 mg C/L of high-quality algae (*Ankistrodesmus falcatus*). We exposed each *Daphnia* individual to 200 spores of *Metschnikowia bicuspidata* per mL, some of which were consumed during filter feeding. After a 24-hour inoculation period, we estimated gut penetrability by observing *Daphnia* under a compound microscope at 400 × magnification and counting the number of *Metschnikowia* spores that successfully entered the body cavity compared with the number of spores attacking the gut epithelium that failed to penetrate (Stewart Merrill *et al.* 2019; Figure S2). For those individuals that were infected with MicG, we estimated infection intensity by counting the number of MicG spore clusters occurring along the gut epithelium, scanning from the anterior diverticulum to the posterior midgut. In some individuals, intensity could not be precisely estimated (due to gut physical abnormalities or technical challenges that precluded an accurate count of spore clusters). In

those instances, intensity was not recorded.

Field study

Field sampling

We monitored prevalence of *Metschnikowia*, *Pasteuria*, *Spirobacillus*, and MicG in *D. dentifera* populations in four lakes in Livingston and Washtenaw Counties, Michigan, USA (mid-July through mid-November 2016) and six lakes in Greene and Sullivan Counties, Indiana, USA (early-June through early-December 2017). (Table S1 contains more information on the study lakes.) Every three days (Michigan) or every other week (Indiana) we estimated egg production (technically embryos, but typically called “eggs” in *Daphnia*) in each *D. dentifera* population as well as the prevalence of infection of any parasites present. *Daphnia* release a clutch of eggs when they molt, which is temperature-dependent; at 20 °C, *D. dentifera* produces a new clutch of eggs approximately every two days (Duffy et al. 2015). On each sampling visit we pooled three vertical tows of the water column collected with a Wisconsin plankton net (12 cm diameter, 153 um mesh); these whole-water-column tows were collected at least 5 meters apart in the deep basin of the lake. For each lake and sampling date, we examined a random subsample of *D. dentifera* individuals for infection and egg production (both may be viewed through the host’s transparent carapace). The Michigan samples were examined under a stereomicroscope, allowing examination of a greater number of individuals (at least 200 *Daphnia* (all ages and sexes) per sample for infection and 20 asexual adult females for egg counts) to observe the lower frequencies of infection typical of those sites. The Indiana samples were examined under a compound microscope, allowing observation of very early to late stage

infections (*sensu* Stewart Merrill and Cáceres 2018) of up to 50 adult asexual females per sample. This methodological difference between Indiana and Michigan means that some early infections (especially with *Metschnikowia*) may have been missed when diagnosing infections in Michigan populations.

Relative fecundity estimates

To quantify the fitness impact of MicG infection, we calculated ‘relative fecundity’ of MicG-infected hosts by dividing the mean number of eggs found in MicG-infected asexual adult *D. dentifera* by the mean number of eggs in uninfected (by MicG or any other parasite) asexual adult females on a given date and subtracting 1 from this value (MicG-infected egg count/ uninfected egg count) -1). A negative relative fecundity means MicG-infected animals had poorer reproductive performance than uninfected animals, and a positive relative fecundity translates into more eggs produced by MicG-infected animals relative to uninfected animals. In estimating relative fecundity, we excluded data for *D. dentifera* individuals (co-)infected with *Metschnikowia*, *Pasteuria*, or *Spirobacillus* as each of these parasites can substantially reduce host fecundity; in addition, there were too few animals in these categories to have confidence that our estimates were accurate.

Resource availability estimate

To examine whether resource-context was associated with the effects of MicG on its host, we measured resource availability for each lake and sampling date. Traditional measures of primary productivity in lakes (e.g. chlorophyll-a and total phosphorus) can be poor predictors of

the quantity and quality of food available to *Daphnia* (Tessier and Woodruff 2002). Therefore, we used an indirect but more sensitive index of resource availability (quality and quantity): the mean number of eggs present per uninfected asexual adult female (known as the “egg ratio”) (Threlkeld 1979; Kerfoot et al. 1988; Hall et al. 2009a; Civitello et al. 2015).

Statistical methods

Laboratory examination of gut penetrability

Prediction 1) *Metschnikowia* spores will be less able to penetrate the gut walls of MicG-

infected hosts. We evaluated whether MicG infection status and resource availability were associated with the penetrability of *Daphnia* gut epithelia to *Metschnikowia* spores using generalized linear mixed models (GLMMs). We included the success or failure of gut penetration by *Metschnikowia* spores attacking the gut wall for a given host individual as the binomial response variable. We evaluated whether the likelihood of *Metschnikowia* penetration was associated with resource availability (indexed by the uninfected egg ratio from the date when an experimental animal was collected), MicG infection status (infected or uninfected), or their interaction by comparing the Akaike information criterion (AIC) of the saturated model with nested simpler models containing fewer fixed effects. We used likelihood ratio tests to select which fixed effects to include in the final model, based on Zuur et al. (2009). We log transformed resource availability to improve the linearity of the relationship between the predictor and the log odds and to give less weight to a few potentially influential data points on sampling dates with unusually high resource levels.

In a separate nested set of GLMMs, we examined data from the subset of individuals that were infected with MicG, to determine whether MicG infection intensity, resource availability, and their interaction predicted the likelihood of *Metschnikowia* spores successfully penetrating the gut. All GLMM models included the random effect of sampling date nested within study lake. All statistical analyses were conducted using the statistical program R version 3.5.2 (R Core Team 2016). We used the glmmTMB package (Brooks et al. 2017) to conduct these analyses.

Field study

Data inclusion

To ensure confidence in our estimates of MicG egg counts and to minimize the influence of stochasticity when disease was rare, we only included data from dates when MicG prevalence was at least 3% for all the statistical models examining field patterns. For analyses of parasite prevalence impacts on MicG-infected *Daphnia* relative fecundity, we excluded data for lakes where the parasite of interest was never observed during the epidemic season. We also excluded data collected once sexual females appeared (near the end of our sampling period) for all of our statistical analyses, because it is difficult to distinguish between asexual adults with no eggs and sexual females about to produce their first ephippium; thus, the number of animals with zero eggs would have been inflated, changing the relationship between resource availability and egg ratio.

Relative fecundity and the multiparasite environment

Prediction 2) *The fecundity of MicG-infected Daphnia will increase relative to that of*

uninfected individuals as the prevalence of other highly virulent parasites increases. We used GLMMs to evaluate the relationship between the relative fecundity of MicG-infected and uninfected *Daphnia* hosts and the prevalence of the three virulent parasites observed in our study lakes. We included the relative fecundity of MicG-infected *D. dentifera* [(MicG-infected egg ratio/uninfected egg ratio)-1] as the response variable. We evaluated models including prevalence of either *Metschnikowia*, *Pasteuria*, or *Spirobacillus* as a fixed effect, using likelihood tests to determine the significance of these effects. Models testing predictions 2 and 3 included the random effect of study lake, and we used separate models to test relationships in the Indiana and Michigan populations.

Relative fecundity and resource availability

Prediction 3) The fecundity difference between *MicG-infected and uninfected Daphnia* will vary with the resource environment, with *MicG-infected Daphnia* having relatively lower fecundity when resources are scarce. Using GLMMs, we evaluated whether resource availability (indexed using the uninfected adult egg ratio) predicted the relative fecundity of MicG and uninfected *Daphnia* [(MicG-infected egg ratio/uninfected egg ratio)-1] using likelihood tests. However, we note that correlations of X vs. Y/X can result in a spurious negative relationship when there is no relationship between X and Y: as X increases, Y/X would tend to decrease (Brett 2004). We used permutation tests to quantify the likelihood that the coefficients describing the relationship between resource availability and MicG relative fecundity were observed by chance (Supplemental text S2). To further investigate these patterns of relative fecundity, we examined the direct relationship between uninfected egg

counts and MicG-infected egg counts (X vs Y, as opposed to X vs Y/X) in the Michigan and Indiana lakes. We evaluated to what extent this relationship fell above or below a 1:1 ratio as resource availability (i.e., uninfected adult egg ratios) increased (Supplemental text S3).

For cases where we observed significant effects of both parasite prevalence and resource availability, we examined whether resources and disease prevalence together or their interaction predicted the relative fecundity of MicG-infected and uninfected *Daphnia*. We used likelihood ratio tests to determine whether disease prevalence still explained the relative fecundity of MicG-infected animals when accounting for resource availability. We used the lme4 package to conduct all GLMM analyses of the field data (Bates et al. 2015).

Results

Gut Penetrability

The gut epithelia of field-collected MicG-infected *D. dentifera* individuals were less penetrable to *Metschnikowia* spores, as compared to the guts of field-collected uninfected individuals (effect of infection status: $\chi^2 = 162.98$, $p < 0.001$, Figure 1, Table S2). Moreover, resource availability altered this relationship. As resources increased, the penetrability of the gut epithelium of uninfected *D. dentifera* increased; however, for individuals infected by MicG, the gut remained largely impenetrable to *Metschnikowia* spores as resources increased (significant interaction between resource availability and infection status; $\chi^2 = 8.89$, $p = 0.003$, Figure 1A, Table S2). In addition, we observed decreasing gut penetrability with increasing MicG infection intensity ($\chi^2 = 9.45$, $p = 0.002$, Figure 1B, Table S3). For these MicG-infected *Daphnia*, gut

penetrability also increased with increasing resources ($\chi^2 = 20.55$, $p < 0.001$) but there was no significant interaction between resource availability and MicG infection intensity ($\chi^2 = 1.69$, $p = 0.193$).

Relationship between fecundity and virulent parasite prevalence

The nature of the interaction between a *Daphnia* host and its intestinal symbiont, MicG, shifted with changes in prevalence of three other much more virulent parasites. Fecundity of MicG-infected *Daphnia* relative to that of uninfected individuals increased with increasing prevalence of *Metschnikowia* in the Indiana lakes ($\chi^2 = 7.30$, $p=0.007$, Figure 2A, Table S4), *Pasteuria* in the Indiana populations ($\chi^2 = 4.05$, $p=0.044$, Figure 2C, Table S4) and Michigan populations ($\chi^2 = 5.29$, $p=0.008$, Figure 2D, Table S4), and *Spirobacillus* in the Michigan populations ($\chi^2 = 4.87$, $p=0.027$, figure 2F, Table S5). There was no relationship between the relative fecundity of MicG-infected *Daphnia* and the prevalence of *Spirobacillus* infection for the Indiana populations ($\chi^2 = 1.84$, $p=0.175$, Figure 2E, Table S4) or *Metschnikowia* infection for the Michigan populations ($\chi^2 = 0.00$, $p=0.995$, Figure 2B, Table S5); prevalence of *Spirobacillus* was low in Indiana relative to Michigan, and prevalence of *Metschnikowia* was low in Michigan relative to Indiana (Table S1), which may have limited our ability to detect an effect.

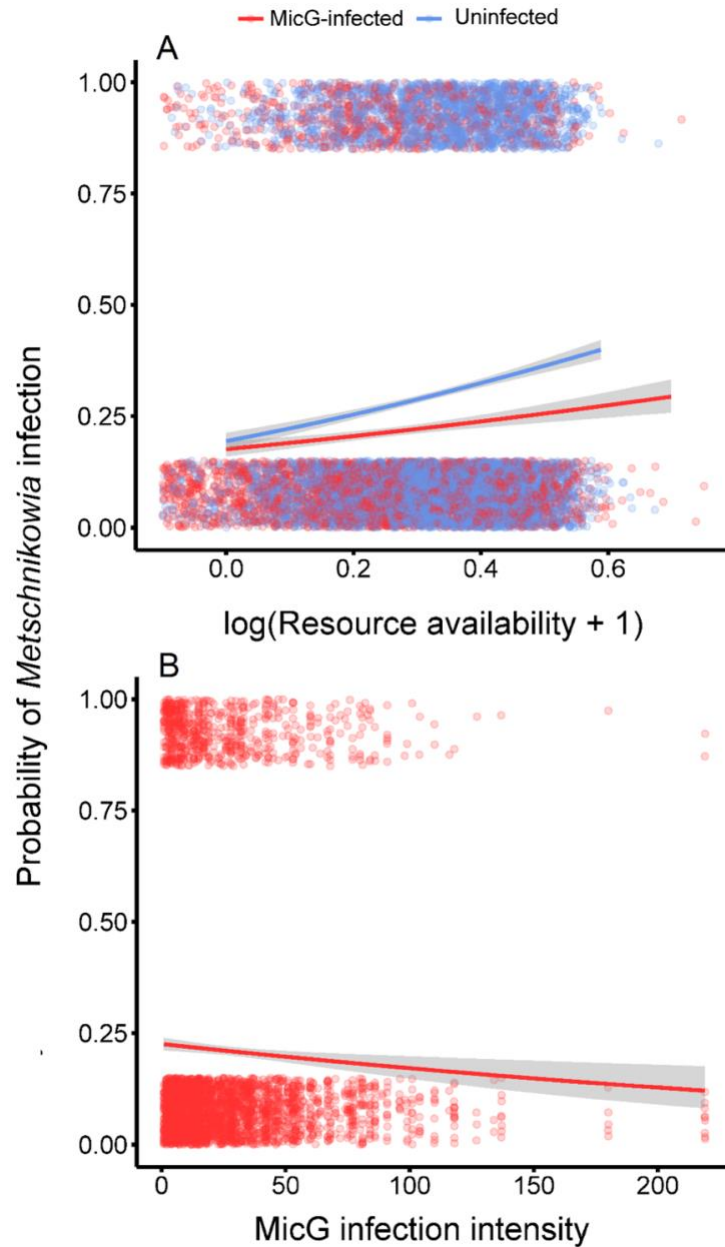


Figure 1. A) The gut epithelia of field-collected *Daphnia dentifera* infected with *MicG* were much less penetrable by *Metschnikowia* spores (red line), especially when resources were plentiful, as compared to the guts of field-collected uninfected individuals (blue line). The y-axis shows the likelihood of successful penetration of the body cavity for each individual *Metschnikowia* spore that attacked the gut epithelium of a field-collected, *MicG*-infected (red) or field-collected, uninfected (blue) *D. dentifera* individual. Logistic regression line with 95% confidence interval is shown with raw data of successes and failures in gut penetration. Resource availability is indexed as the mean egg ratio of uninfected *D. dentifera* for a given population and sampling date when a given experimental animal was collected. **B)** *D. dentifera* gut penetrability by *Metschnikowia* spores decreased with increasing *MicG* infection intensity (measured as total spore clusters occurring along the gut epithelium). Data points are slightly offset to increase

visibility.

In the Indiana lakes, where *Metschnikowia* was often highly prevalent (Table S1), the relative fecundity of MicG-infected *Daphnia* shifted from being roughly equal to that of the uninfected individuals (Figure 2A, yellow: regression line 95% confidence interval (CI) intersecting zero line) to approximately 80% higher than that of the uninfected individuals when *Metschnikowia* was most prevalent (Figure 2A, blue zone: regression line CI exceeds zero). In the Michigan lakes, MicG-infected *Daphnia* showed lower fecundity than uninfected individuals when either *Pasteuria* or *Spirobacillus* infections were rare (Figure 2B,D, red zone: regression line CI below zero) but showed no difference in fecundity compared with uninfected *Daphnia* with higher prevalence of these two parasites (Figure 2B,D, regression line CI intersects zero).

Relationship between fecundity and resources In Michigan, there was a positive relationship between resource availability and relative fecundity of infected animals ($\chi^2 = 15.16$, $p < 0.001$, Figure 3A, Table S6). The strength of this effect is likely an underestimate, given the expectation of a negative slope based on the x vs. y/x relationship (Methods and Supplemental text S2). When resources were relatively low, MicG-infected *D. dentifera* had significantly reduced fecundity compared with uninfected animals; however, as resources increased, these negative effects of MicG diminished to the point where there was no fecundity difference between infected and uninfected animals (Figure 3A). At the highest resource levels, which occurred in North Lake, MicG-infected *D. dentifera* had higher fecundity than uninfected animals (Figure 3A: blue shading).

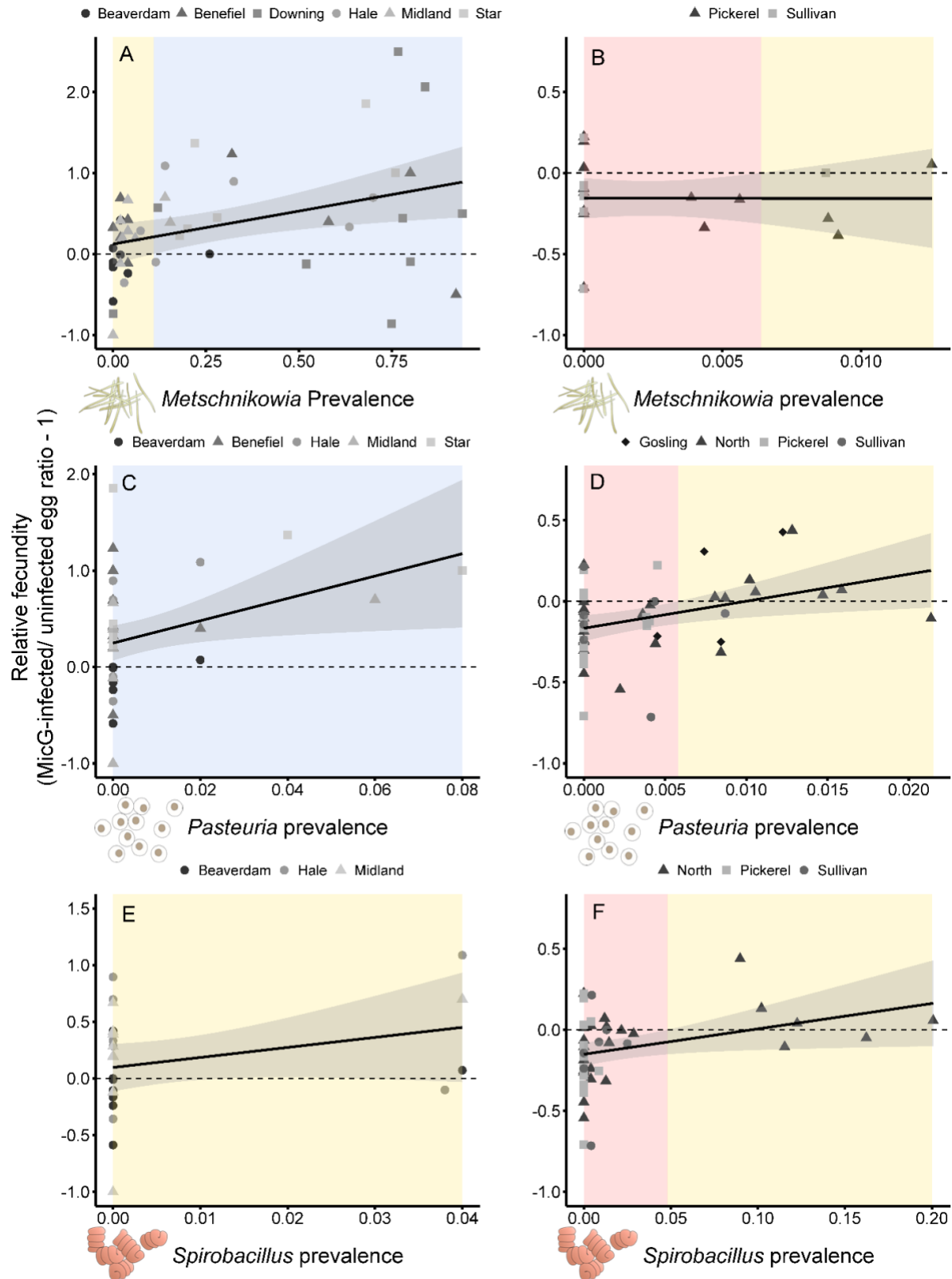


Figure 2. Relative fecundity of *Daphnia* infected with *MicG* increased with increasing prevalence of *Metschnikowia*, *Spirobacillus*, and *Pasteuria*. Positive values on the y-axis represent greater

egg production in *Daphnia* infected by MicG compared with *Daphnia* uninfected by any parasites (e.g. if $y=0.4$, MicG-infected *Daphnia* produce 40% more eggs than uninfected *Daphnia*). Each data point represents relative fecundity and parasite prevalence for a single sampling date and population, with symbol shapes and shading differing according to sampling site. Red, yellow, and blue shading delineate where the 95% CI of the regression line is below, intersects, or is above zero, respectively. Red: MicG infected *Daphnia* perform more poorly than uninfected *Daphnia* (parasitism); yellow: MicG infected *Daphnia* and uninfected *Daphnia* perform equally (commensalism); blue: MicG infected *Daphnia* have a fecundity advantage (mutualism). **A, C, E**: data from Indiana lakes; **B, D, F**: data from Michigan lakes. Note that infection prevalence on the x-axes varies among panels.

For lakes sampled in Indiana, we observed a negative relationship between resource availability and the relative fecundity of MicG-infected and uninfected *D. dentifera* ($\chi^2 = 11.03$, $p < 0.001$, Table S7, Figure 3B). The negative slope we observed is what we would predict due to the x vs. y/x relationship of the data (Supplemental text S2; Table S7, Figure 3). Yet, an additional comparison of MicG-infected and uninfected *Daphnia* fecundity also supports a negative relationship between resources and MicG's impact on fecundity (supplemental text S3, figure S3). We observed greater relative fecundity of MicG-infected *Daphnia* when resources were low (95% CI of regression line is above the 1:1 line) and a shift towards lower relative fecundity for MicG-infected *Daphnia* at the highest resource levels.

For the Michigan populations, when we accounted for resources, the effects of both *Pasteuria* and *Spirobacillus* prevalence were no longer significant (Table S8, *Pasteuria*: $\chi^2 = 2.54$, $p = 0.111$; *Spirobacillus*: $\chi^2 = 1.29$, $p = 0.256$). For the Indiana populations, *Metschnikowia* and *Pasteuria* prevalence were still significant predictors of relative fecundity of MicG-infected *Daphnia* when resources were included in the models (Table S9, *Metschnikowia*: $\chi^2 = 12.67$, $p < 0.001$; *Pasteuria*: $\chi^2 = 5.43$, $p = 0.020$), but the interaction between parasite prevalence and resources was not significant (Table S9, *Metschnikowia*: $\chi^2 = 3.74$, $p = 0.053$; *Pasteuria*: $\chi^2 = 0.32$, $p = 0.575$).

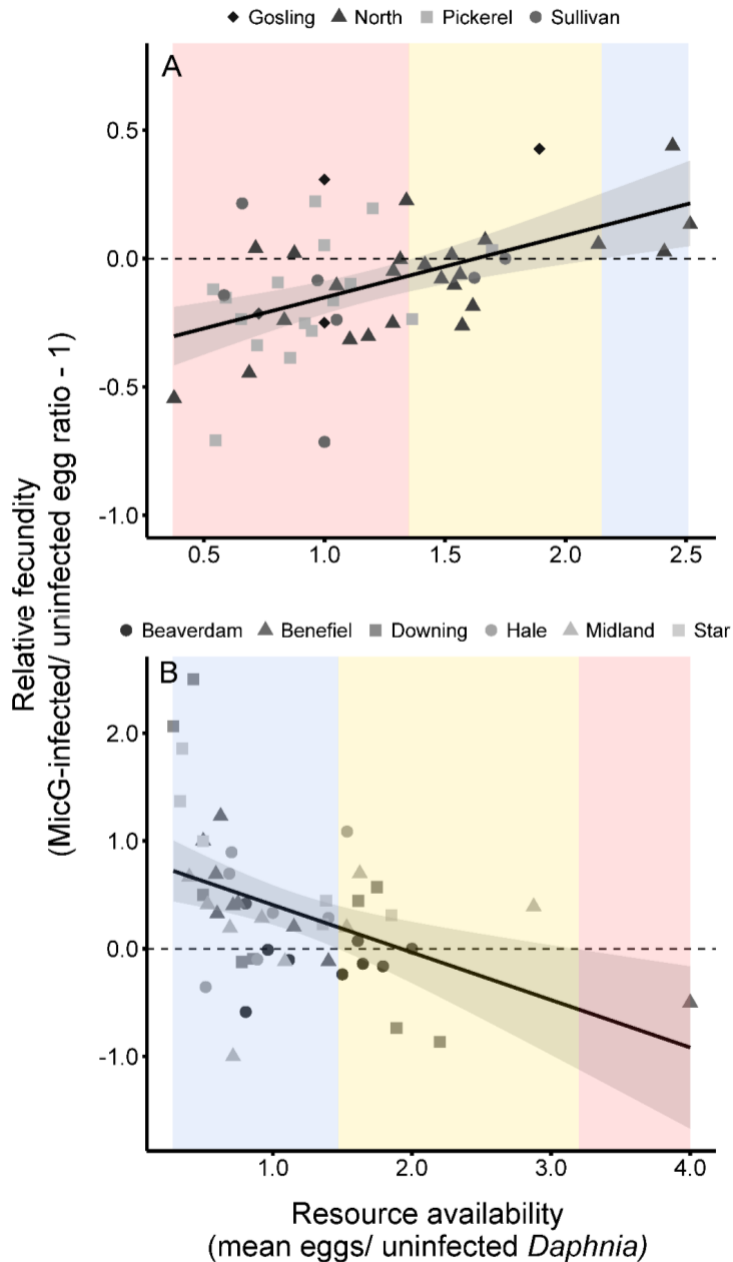


Figure 3. The relative fecundity of MicG-infected and uninfected *Daphnia* varied with resource availability. (A) MicG shifted from parasitism towards mutualism with increasing resource availability in lakes sampled in Michigan. (B) In the Indiana lakes, there was a negative relationship between resource availability and the relative fecundity of MicG-infected and uninfected *D. dentifera*. Resource availability, as indexed by egg ratios of uninfected asexual adults, increases with increasing values along the x-axis. Positive values on the y-axis represent greater egg production in infected animals compared with uninfected animals (e.g. if $y=0.4$, MicG-infected *Daphnia* produce 40% more eggs than uninfected *Daphnia*). Each data point represents average fecundity and resource availability for a single sampling date and

population. Red, yellow, and blue shading (left to right in **A**) delineate where the 95% CI of the regression line is below, intersects, or is above zero, respectively. Red: MicG infected animals perform more poorly than uninfected *Daphnia* (parasitism); yellow: MicG infected and uninfected *Daphnia* perform equally (commensalism); blue: MicG infected *Daphnia* have a fecundity advantage (mutualism).

We observed no relationship between parasite prevalence and resource availability in the Indiana populations, but we did see a weak positive correlation between resources and *Pasteuria* prevalence as well as *Spirobacillus* prevalence in the Michigan populations (Text S4, figure S4).

Discussion

The nature of the interaction between *Daphnia dentifera* and its microsporidian gut symbiont MicG varied within lakes over time and across lakes. MicG-infected *Daphnia* tended to have higher fecundity (relative to uninfected hosts) when three virulent, obligate killer parasites were more prevalent (Figure 2). In our Indiana study lakes, where the fungal pathogen *Metschnikowia* was common, MicG was particularly beneficial, shifting from a commensal to mutualist as *Metschnikowia* prevalence increased (Figure 2A). This benefit may be explained by our finding that field-collected *Daphnia* infected with MicG had guts that were much less penetrable by *Metschnikowia* spores compared with uninfected *Daphnia*, a benefit that increased with increasing MicG infection intensity (Figure 1). In addition, the relative fecundity of MicG-infected *Daphnia* varied across resource gradients, but the relationships differed between our Michigan and Indiana populations (Figure 3). In Michigan lakes, the relative fecundity of MicG-infected *Daphnia* decreased with decreasing resource availability; the interaction between *D. dentifera* and MicG was most often parasitic or commensal in nature

and only occasionally reached the mutualism threshold (when resources were highest). In contrast, in Indiana lakes, the relative fecundity of MicG-infected animals decreased with increasing resources, and MicG nearly always fell in mutualist to commensal territory. Together, our findings suggest that MicG can shift along a mutualism-parasitism continuum depending on the ecological context, with it more likely to be a mutualist in environments with high prevalence of virulent parasites.

Symbiotic interactions involve both costs and benefits for the interacting species. It is the net sum of those costs and benefits that determines whether the interaction is mutualistic, parasitic, or commensal (Johnson et al. 1997; Chamberlain et al. 2014). Due to living inside its host, MicG likely usurps resources that the host otherwise could have used for growth, maintenance, or reproduction (Hall et al. 2009b). It is plausible that as host resource availability increases, the cost of infection decreases to the point where we see no evidence of reduced fecundity (as seen in Bedhomme *et al.* 2004; Cressler *et al.* 2014). The considerable reproductive costs observed when resources are scarce in the Michigan lakes fits with this interpretation and suggests that the net negative impacts of MicG on host reproduction can be substantial. However, the negative relationship between resource availability and the relative fecundity of MicG-infected *Daphnia* in the Indiana Lakes suggests that additional factors can influence the relationship between MicG and its *Daphnia* host.

Because MicG infects gut epithelial cells, and other, more virulent parasites in our system infect via the gut (Metschnikoff 1884; Duneau et al. 2011), we hypothesized that MicG's impact

on host fitness might also depend on the prevalence of highly virulent parasites. More specifically, we hypothesized that if MicG infections reduce the likelihood of spores of other more virulent parasites crossing the gut wall, that would help protect the host from infection (including from needing to mount an energetically costly immune response). Our finding that MicG infection is associated with a greatly reduced likelihood of *Metschnikowia* spores crossing the gut wall lends support to this hypothesized benefit. This hypothesis is also supported by our observation that the reproductive benefit experienced by MicG-infected *Daphnia* increases as the prevalence of infection by *Metschnikowia* increases. While we have not examined the impact of MicG infection on penetrability by *Pasteuria* or *Spirobacillus* spores, we see the same increase in reproductive performance for MicG-infected animals when these other parasites are more common.

While we consistently see an increasing reproductive benefit of MicG-infection with increasing prevalence of virulent parasites, MicG-infected *Daphnia* in the Indiana lakes were much more likely to surpass the mutualism threshold. We used a lower magnification to assess infection status in Michigan lakes, and it is possible that we missed some early stage infections – for example, an animal with an early stage *Metschnikowia* infection might have been diagnosed as infected in Indiana but as uninfected in Michigan. However, this cannot account for the difference in likelihood of surpassing the mutualism threshold. Indeed, it should have biased our observations in the opposite direction, making us more likely to detect mutualism (since we would expect the “uninfected” egg ratio would be artificially low if some of the animals were actually infected). Instead, we hypothesize that the difference in the nature of the MicG-

Daphnia interaction in the Michigan and Indiana lakes relates to differences in the multiparasite environment in these two systems. While resource availability was similar among the study sites (Table S1; Figure S6), we observed striking variation in the prevalence of virulent parasites among lakes. Notably, *Metschnikowia* was commonly observed in the Indiana lakes (maximum infection rates ranging from 15-94%) but rarely observed in Michigan (maximum prevalence 0-1.3%). We posit that MicG provides a reproductive benefit by preventing virulent parasite spores from penetrating the gut wall and necessitating an immune response. At the same time, MicG exerts a reproductive cost by drawing resources from its host, and potentially by altering the ability of *Daphnia* to absorb resources. When virulent parasites are frequently encountered (e.g., *Metschnikowia* is highly prevalent), the benefits far outweigh the costs. When virulent parasites are rare, the benefit of MicG infection is smaller and rarely outweighs the costs. This may explain why we see such a strong positive effect of resources in Michigan, where virulent parasites are rare, but see little evidence of a cost of MicG-infection when resources are scarce in Indiana (but virulent parasites are common).

The patterns we have observed thus far provide compelling evidence that MicG-*Daphnia* interactions can vary from parasitic to commensal to mutualistic and that the multiparasite environment may push the interaction towards mutualism. Yet, we note that our study is limited by its observational nature. Ideally, we would experimentally test whether resource availability qualitatively changes the fitness impacts of MicG infection in *D. dentifera*, and, factorially, examine the effect of experimental MicG-infection on the ability of *Metschnikowia*, *Spirobacillus*, and *Pasteuria* to penetrate the gut wall. We have not yet been able to

experimentally infect *Daphnia* with MicG in the lab, but this is a very high priority for our future work. A study along these lines would help us understand why there was a strong positive relationship between resource levels and MicG's fitness impact in Michigan lakes but not in Indiana lakes, where resource availability appeared to have a negative effect on MicG-infected host fecundity. An alternative hypothesis explaining our observations is that *Daphnia* vary innately in gut penetrability; individuals with less penetrable guts benefit when virulent parasites are present and are also more likely to be infected by MicG. We have not been able to evaluate this hypothesis by experimentally manipulating MicG and *Metschnikowia* exposure in laboratory reared animals. However, we do know the genotype identity of the field-collected *Daphnia* from our gut penetrability experiment. We observed great plasticity in gut penetrability within genotypes: MicG-infected individuals tended to have greatly decreased gut penetrability, compared with uninfected individuals of the same genotype (unpublished data, manuscript in preparation). This supports our hypothesis that MicG influences gut penetrability rather than simply being more likely to infect *Daphnia* with a genetic predisposition to reduced gut penetrability.

A fundamental goal of ecology is to understand how organisms interact and what influences the nature of those interactions. Both resource availability and the multiparasite environment appear to be important parts of this equation. The importance of resources has been well documented in studies of shifts from mutualism to parasitism (Johnson et al. 1997). Our study supports that resource availability can also promote shifts in the opposite direction—from parasitism towards mutualism. Our work also supports existing evidence that parasite

community context may greatly impact interactions between a symbiont and its host (e.g., Petney & Andrews 1998; Johnson & Hoverman 2012). While we have relatively few studies to draw upon, evidence so far suggests that this flexibility along the parasitism to mutualism continuum shows up in a wide variety of host-symbiont taxa (*Daphnia*-microsporidian (this study), plant-fungi (Redman et al. 2001), bird nest parasitism (Canestrari et al. 2014), human-gut bacterium (Lin and Koskella 2015), plant-bacterium (Savory et al. 2017), plant-virus (González et al. 2021)), and the drivers appear to involve both abiotic context and interactions with other members of the ecological community (Redman et al. 2001; Canestrari et al. 2014; Lin and Koskella 2015; Savory et al. 2017). Further research into both the generality of these patterns and the underlying ecological and evolutionary mechanisms could provide insights into disease dynamics, with potentially important applications in disease management and human health.

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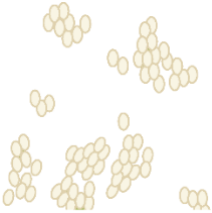
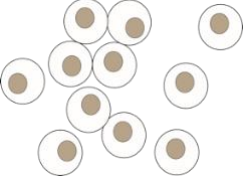
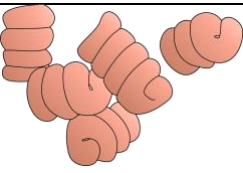
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769

770 **Table 1.** Symbionts included in this study vary greatly in their fitness impacts on hosts. This table focuses particularly on impacts of
771 these symbionts in *Daphnia dentifera*, the focal host in our study. (Illustration credit: John Megahan)

Symbiont	Symbiont cartoon used in figures	Taxon	Site of infection	Impact on host reproduction	Impact on host lifespan	Relevant references
<i>Metschnikowia bicuspidata</i>		fungus	Infects by piercing gut, develops in host hemolymph	Reduces host fecundity (by ~25-50%)	Strong	Duffy and Hall 2008; Auld et al. 2012; Stewart Merrill and Cáceres 2018
MicG (species identity not yet determined)		microsporidian	Gut epithelium	Evaluated in this study	None detected (this study; supplemental text S1, Figure S1)	Genbank accession MH635259
<i>Pasteuria ramosa</i>		bacterium	Infects via host gut (attachment to esophagus); develops in host hemolymph	Very strong (infected hosts rarely reproduce)	No significant impact (except via increased risk of fish predation)	Auld et al. 2012; Duffy et al. 2019
<i>Spirobacillus cienkowskii</i>		bacterium	Hypothesized to infect via host gut; develops in host hemolymph	Very strong (infected hosts rarely reproduce)	Very strong	Duffy & Hall 2008, Wale et al. 2019

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Table S1. Location and maximum prevalence of each parasite in the study lakes. Observation of infection by *Metschnikowia* in the Michigan lakes would only detect the more visible late stage infections, whereas data from Indiana lakes captured both early and late stage infections, owing to differences in magnification used to examine host infection status. We note the prevalence of the more visible, late stage-only *Metschnikowia* infection in parentheses for the Indiana lakes. Resource availability, as indexed by uninfected egg ratio, is shown for observations from each lake on dates included in the statistical analyses (when MicG was found at a minimum of 3% prevalence and before ephippia were observed in the late fall).

Lake	Location	MicG	<i>Metschnikowia</i>	<i>Pasteuria</i>	<i>Spirobacillus</i>	Uninfected egg ratio mean (SD/min/max)
Gosling	Livingston County, Michigan (42°26'22.2N, 84°0'11.9W)	5.0%	0.0%	1.2%	0.0%	1.15 (0.51/0.73/1.89)
North	Washtenaw County, Michigan (42°23'35.3N, 84°0'22.9W)	48.0%	0.0%	2.1%	20.1%	1.41 (0.55/0.38/2.52)
Pickerel	Washtenaw County, Michigan (42°24'37N, 83°58'58.5W)	57.0%	1.3%	0.4%	8.6%	0.93 (0.31/0.54/1.70)
Sullivan	Washtenaw County, Michigan (42°23'55.6N, 84°3'25.4W)	5.0%	0.9%	0.9%	2.5%	1.09 (0.44/0.58/1.75)
Beaverdam	Greene County, Indiana (39°5'53.5N, 87°8'45.8W)	44.0%	26.0% (0%)	2.0%	4.0%	1.36 (0.45/0.81/2.00)
Benefiel	Sullivan County, Indiana (38°58'16.9 N, 87°15'20.3 W)	82.0%	92.3% (50.0%)	2.0%	0.0%	1.15 (1.11/0.50/4.00)
Downing	Sullivan County, Indiana (39°2'24.1 N, 87°14'39.4 W)	68.0%	94.0% (42.0%)	0.0%	0.0%	1.14 (0.72/0.29/2.20)
Hale	Sullivan County, Indiana (38°58'19.8 N, 87°14'47.8 W)	46.0%	70.0% (18.0%)	2.0%	4.0%	0.96 (0.38/0.52/1.53)
Midland	Greene County, Indiana (39°7'28.4 N, 87°10'35.6 W)	56.0%	15.4% (0.0%)	6.0%	4.0%	1.15 (0.77/0.40/2.87)
Star	Greene County, Indiana (39°0'5.1 N, 87°13'6.0 W)	52.0%	76.0% (30.0%)	8.0%	0.0%	0.96 (0.65/0.33/1.85)

Supplemental text S1. MicG infection does not affect host lifespan

In the fall of 2017, we examined the effect of MicG infection on *Daphnia dentifera* host lifespan with a life-table experiment using field-collected animals. We collected 100 adult asexual *D. dentifera* individuals on October 5, 2017 from Crooked Lake (Sylvan Township, Michigan, USA) during a MicG epidemic. Fifty of these *Daphnia* were infected with MicG (and were not coinfecting with another parasite) and fifty showed no signs of infection by MicG or any other parasite. Each *D. dentifera* individual was kept at 20 °C with a 16:8 hr light:dark photoperiod in a 50 mL beaker filled with 30 mL of filtered lake water. Study animals were fed 1×10^6 cells of the green algae *Ankistrodesmus falcatus* four times per week. Individuals were monitored daily for survival and upon death were checked for infection status (to make sure they were not infected with another parasite besides MicG).

D. dentifera individuals survived between 4 and 55 days post collection. There was no difference in survival rate for uninfected *Daphnia* and those infected with MicG ($T = 0.32554$, $p = 0.7455$, figure S1).

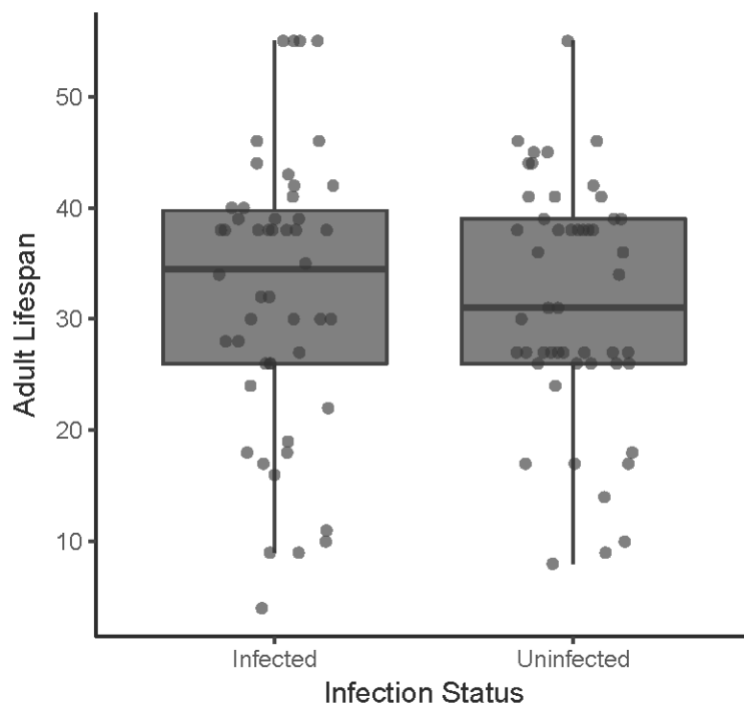


Figure S1. There was no difference in lifespan (days post field collection) of MicG-infected and uninfected adult *Daphnia dentifera*.

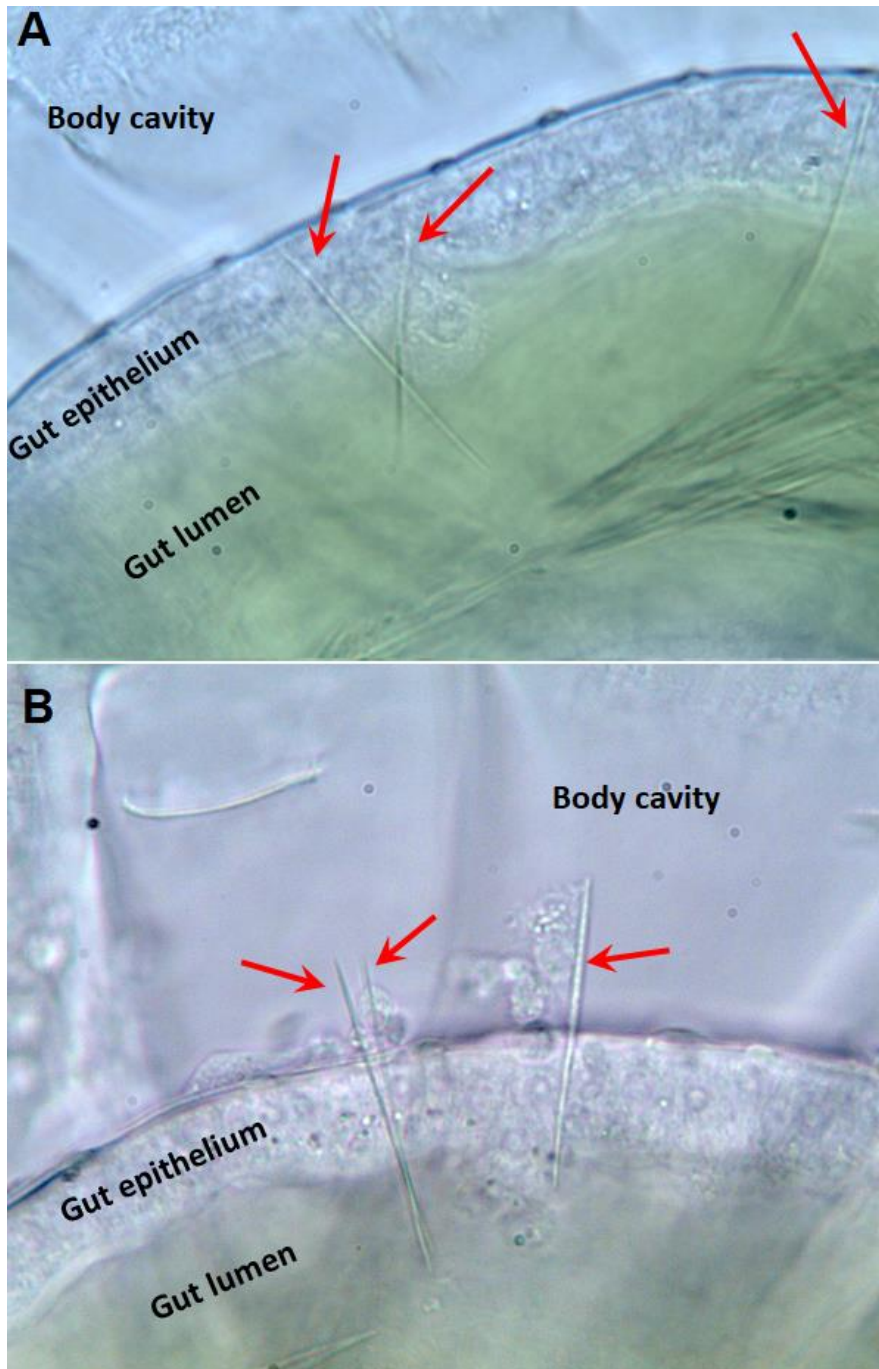


Figure S2. Microscope images showing needle-shaped *Metschnikowia* spores attacking the gut epithelium of wild-caught *Daphnia*. Spores that were consumed and moved from the gut lumen to then attack the gut epithelium are indicated with red arrows. Three spores that attacked and failed to penetrate the gut epithelium are shown in **A**; three spores that attacked the gut epithelium and successfully penetrated the body cavity are shown in **B**.

Supplemental Text S2

Regressing X vs. Y/X

In analyses where X is correlated with Y/X we might expect to see a spurious correlation, just based on the fact that as X increases, Y/X would decrease even if Y remains constant (Brett 2004). In our analysis we use the egg ratio of uninfected animals as both an index of resource availability (X) and as part of our measure of the relative fecundity of infected individuals (Y/X).

To address this issue, we conducted permutation tests to determine the likelihood that the relationship between resource availability and relative fecundity of MicG-infected *D. dentifera* is spurious. Using the statistical program R, we randomly sorted our X (uninfected egg count) and Y (MicG egg count/uninfected egg count-1) data and plotted X vs. Y to determine the correlation coefficient. This process was repeated 10,000 times with data for both the Michigan study lakes and again with the data from the Indiana study lakes. For the Michigan study lakes, the mean correlation coefficient from the permutation tests was -0.8731 (SD= 0.207, max= -0.228, min= -1.699). For the Indiana study lakes, the mean correlation coefficient was for the permutation tests -0.282 (SD=0.167, max=0.266, min= -0.886).

The slope of the relationship between resource availability and relative fecundity of MicG-infected *Daphnia* for our field data collected in Michigan was 0.241. For the permutation tests of the Michigan data, there were zero instances out of 10,000 where the simulated coefficient was positive. This suggests that there is very little chance ($p < 0.0001$) that the observed positive relationship between resource availability and relative fecundity of MicG-infected animals was due to the non-independence of our X - and Y -axis variables for the Michigan Lake data. If anything, the issues with X vs. Y/X regression likely lead to an underestimate of the slope in our linear model, providing a conservative estimate of this relationship between relative fecundity and resources.

The slope observed from our field data collected in Indiana was -0.430, which is within the 19th percentile for the coefficients observed in our permutation tests (Figure S5). Based on this, we do not feel confident in rejecting the null hypothesis that the negative relationship between resource availability and the relative fecundity of Mic-G infected *Daphnia* observed in the Indiana lakes was due to chance. However, an alternative examination of the direct relationship between egg ratios of MicG-infected and uninfected *Daphnia* suggests that indeed, there may be a negative relationship between resource availability and the relative fecundity of MicG-infected and uninfected *Daphnia* in this Indiana study system (supplemental text S3).

Reference:

Brett, M. T. 2004. When is a correlation between non-independent variables “spurious”?
Oikos 105:647–656.

Supplemental text S3. Direct comparison of uninfected and MicG-infected egg ratios.

An alternative way of viewing the relative fecundity of MicG-infected and uninfected *Daphnia* is to directly compare their egg counts and determine to what extent the ratio of MicG-infected: uninfected egg ratios falls above or below one (Figure S3). When plotted in this way, we see a similar pattern displayed in Figure 3 (main text), where we compared the uninfected egg ratio with the composite index of MicG-infected/uninfected egg ratio – 1.

In the Michigan populations, as uninfected egg ratios (and resource availability) increased, MicG-infected *Daphnia* shifted from producing relatively fewer eggs to relatively more eggs in a given lake and sampling date. In the Indiana lakes, we saw the opposite trend: MicG-infected *Daphnia* had higher fecundity on dates when uninfected egg ratios were low and a ratio equal to or below 1 at higher resource levels.

These findings support our analyses presented in the main text, suggesting that MicG shifts from providing a net cost to a net benefit in Michigan as resource availability increases and shifts from producing a net benefit to no cost or even a net cost as resources increase in Indiana.

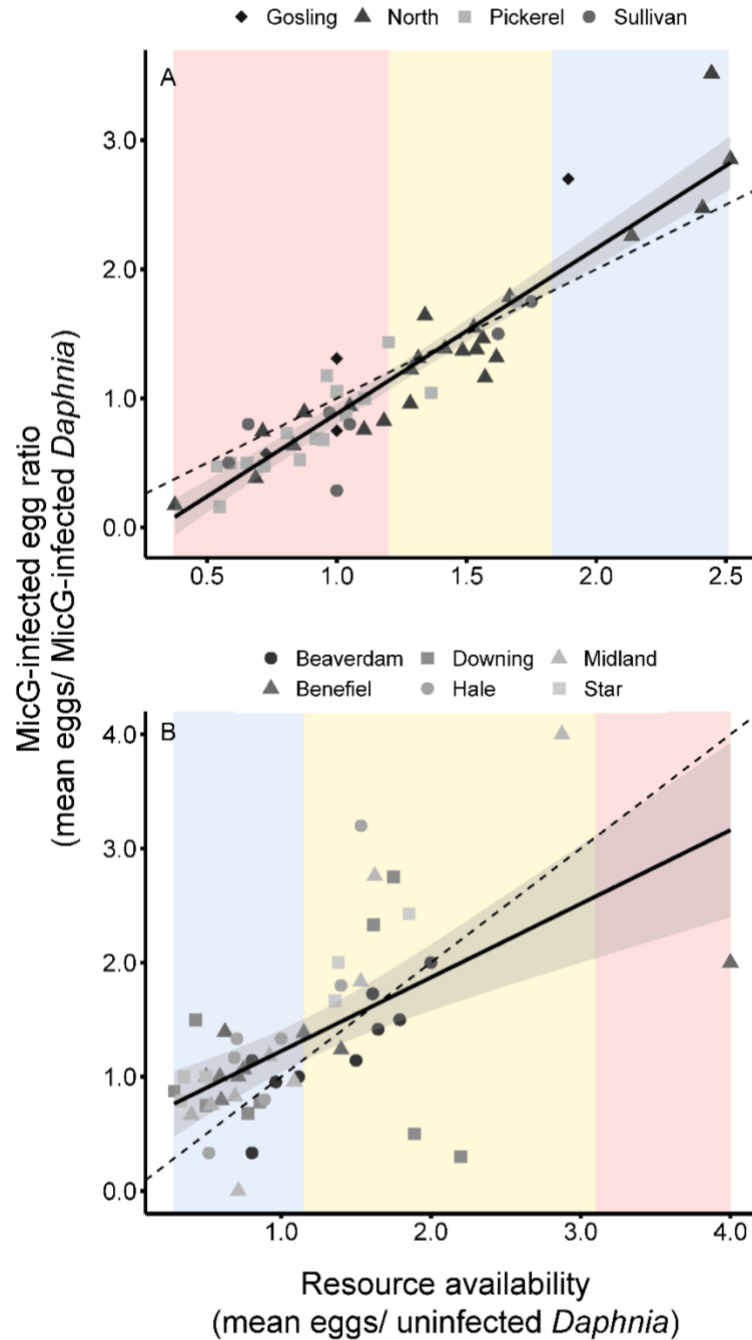


Figure S3. The relationship between MicG-infected and uninfected *D. dentifera* egg ratios diverged from a 1:1 relationship in both the Michigan (A) and Indiana populations (B). Each data point represents the ratio of the mean number of eggs produced by MicG-infected and uninfected *D. dentifera* for a given population and sampling date. Red, yellow and blue shading (left to right in panel A) delineate where the 95% CI of the regression (solid black line with gray shading) is below, intersects, or above the 1:1 line of equal fecundity (the dotted line). Red: MicG-infected *D. dentifera* produce fewer eggs than uninfected animals (parasitism); yellow: MicG-infected and uninfected *D. dentifera* produce equal numbers of eggs (commensalism); blue: MicG-infected *D. dentifera* produce relatively more eggs (mutualism).

Table S2. Model results for GLMM analyses of the laboratory infection assay results. Models examine whether MicG-infection status, resource availability (log(uninfected adult egg ratio +1)), or the interaction of these two fixed effects influence the likelihood of *Metschnikowia* spores penetrating the gut epithelium of the field collected *Daphnia*. Resource availability was log transformed to improve the linearity of the relationship between the predictor and the log odds. The final model (as selected by likelihood ratio tests) is highlighted with bold text.

Response variable	Fixed effects	AIC	logLik	χ^2	P value
Gut penetrability	Infection status * resource availability	7804.1	-3898.0	8.89	0.003
Gut penetrability	Infection status + resource availability	7811.0	-3902.5	128.95	<0.001
Gut penetrability	Infection status	7937.9	-3967.0	162.98	<0.001
Gut penetrability	null	8098.9	-4048.5		

Table S3. Model results for GLMM analysis examining the relationship between MicG infection intensity, resource availability (log(uninfected egg ratio +1) and the likelihood of *Metschnikowia* spores penetrating the gut epithelium of the field collected *Daphnia*. The final model (as selected by likelihood ratio tests) is highlighted with bold text.

Response variable	Fixed effects	AIC	logLik	χ^2	P value
Gut penetrability	Infection intensity * resource availability	2486.3	-1239.2	1.69	0.193
Gut penetrability	Infection intensity + resource availability	2486.0	-1240.0	20.55	<0.001
Gut penetrability	Infection intensity	2504.5	-1250.3	9.45	0.002
Gut penetrability	null	2512.0	-1255.0		

Table S4 GLMM results for Indiana lakes field data examining relationships between parasite prevalence and relative fecundity of MicG-infected *Daphnia*. In each case, we compared a model that included prevalence of a single virulent parasite (*Metschnikowia*, *Pasteuria*, or *Spirobacillus*) with a null model that did not include parasite prevalence.

Response variable	Fixed effects	Coef.	AIC	logLik	χ^2	P value
MicG relative fecundity	<i>Metschnikowia</i> prevalence	0.814	102.55	-47.25	7.30	0.007
MicG relative fecundity	null		107.8	-50.90		
MicG relative fecundity	<i>Pasteuria</i> prevalence	9.497	67.34	-29.67	4.05	0.044
MicG relative fecundity	null		69.39	-31.69		
MicG relative fecundity	<i>Spirobacillus</i> prevalence	7.898	37.90	-14.95	1.84	0.175
MicG relative fecundity	null		37.74	-15.87		

Table S5. GLMM results for Michigan lakes field data examining relationships between parasite prevalence and relative fecundity of MicG-infected *Daphnia*. In each case, we compared a model that included prevalence of a single virulent parasite (*Metschnikowia*, *Pasteuria*, or *Spirobacillus*) with a null model that did not include parasite prevalence.

Response variable	Fixed effects	Coef.	AIC	logLik	χ^2	P value
MicG relative fecundity	<i>Metschnikowia</i> prevalence	-0.078	6.74	0.63	0.00	0.995
MicG relative fecundity	null		4.74	0.63		
MicG relative fecundity	<i>Pasteuria</i> prevalence	16.710	-0.57	4.28	5.29	0.008
MicG relative fecundity	null		4.43	0.79		
MicG relative fecundity	<i>Spirobacillus</i> prevalence	1.567	-3.28	5.64	4.87	0.027
MicG relative fecundity	null		-0.41	3.21		

Table S6. GLMM results for relationship between resource availability, indexed as uninfected egg ratio, and the relative fecundity of MicG-infected *Daphnia* in Michigan lakes. In this analysis, we compared a model that incorporated our index of resource availability (uninfected adult egg ratio) with a null model that did not include resource availability. See supplemental text S2 for our examination of the likelihood that this positive coefficient of 0.241 could have occurred by chance.

Response variable	Fixed effects	Coef.	AIC	logLik	χ^2	P value
MicG relative fecundity	Resource availability	0.241	-8.73	8.37	15.16	<0.001
MicG relative fecundity	null		4.43	0.79		

Table S7. GLMM results for relationship between resource availability, indexed as uninfected egg ratio, and the relative fecundity of MicG-infected *Daphnia* in Indiana lakes. In this analysis, we compared a model that incorporated our index of resource availability (uninfected adult egg ratio) with a null model that did not include resource availability. See supplemental text S2 for our examination of the likelihood that this negative coefficient of -0.430 could have occurred by chance.

Response variable	Fixed effects	Coef.	AIC	logLik	χ^2	P value
MicG relative fecundity	Resource availability	-0.430	98.78	-45.39	11.03	<0.001
MicG relative fecundity	null		107.80	-50.90		

Table S8. Model results for GLMM analyses of Michigan data examining whether *Pasteuria* or *Spirobacillus* prevalence explained variability in relative fecundity of MicG-infected *Daphnia* when accounting for any influence of resource availability (uninfected adult egg ratio). The results of the best fit models are highlighted with bold text.

Response variable	Fixed effects	AIC	logLik	χ^2	P value
MicG relative fecundity	<i>Pasteuria</i> prevalence *	-7.28	9.64	0.006	0.939
	Resource availability				
MicG relative fecundity	<i>Pasteuria</i> prevalence +	-9.27	9.64	2.54	0.111
	Resource availability				
MicG relative fecundity	Resource availability	-8.73	8.37	15.16	<0.001
MicG relative fecundity	null	4.43	0.79		
MicG relative fecundity	<i>Spirobacillus</i> prevalence	-10.08	11.04	0.43	0.510
	* Resource availability				
MicG relative fecundity	<i>Spirobacillus</i> prevalence	-11.65	10.82	1.29	0.256
	+ Resource availability				
MicG relative fecundity	Resource availability	-12.35	10.18	13.94	<0.001
MicG relative fecundity	null	-0.41	3.21		

Table S9. Model results for GLMM analyses of Indiana data examining whether resource availability (uninfected asexual adult egg ratio) explained variability in relative fecundity of MicG-infected *Daphnia* when accounting for any influence of *Metschnikowia* or *Pasteuria* prevalence. The results of the best fit models are highlighted with bold text. The qualitative results do not change if resource availability is included in the simpler model and parasite prevalence is added in the more complex model (the full model is still the best fit).

Response variable	Fixed effects	AIC	logLik	χ^2	P value
<u>MicG relative fecundity</u>	<i>Metschnikowia</i> prevalence *	90.10	-39.05	3.74	0.053
	Resource availability				
MicG relative fecundity	<i>Metschnikowia</i> prevalence	91.83	-40.92	12.67	<0.001
	+ Resource availability				
MicG relative fecundity	<i>Metschnikowia</i> prevalence	102.50	-47.25	7.30	0.007
MicG relative fecundity	null	107.80	-50.91		
<u>MicG relative fecundity</u>	<u><i>Pasteuria</i> prevalence *</u>	65.59	-26.95	0.32	0.575
	<u>Resource availability</u>				
MicG relative fecundity	<i>Pasteuria</i> prevalence +	63.91	-26.95	5.43	0.019
	Resource availability				
MicG relative fecundity	<i>Pasteuria</i> prevalence	67.34	-29.67	4.05	0.044
MicG relative fecundity	null	69.39	-31.69		

Supplemental Text S4. Relationship between resource availability and virulent parasite prevalence

We observed a weak positive relationship between *Pasteuria* prevalence and resource availability (indexed as uninfected *Daphnia* egg ratios for a given lake and date) (Figure S4d, GLMM with lake as a random effect: $\chi^2=4.535$, $p=0.033$) as well as *Spirobacillus* prevalence and resources in Michigan lakes (Figure S4f, GLMM with lake as a random effect: $\chi^2=4.294$, $p=0.038$). Otherwise we observed no significant relationship between resource availability and virulent parasite prevalence (Fig S4, *Metschnikowia* in Indiana: $\chi^2=0.0255$, $p=0.873$, *Pasteuria* in Indiana: $\chi^2=0.354$, $p=0.552$, *Spirobacillus* in Indiana: $\chi^2=0.946$, $p=0.331$, *Metschnikowia* in Michigan $\chi^2=0.244$, $p=0.621$).

This positive association between resources and *Pasteuria* and *Spirobacillus* prevalence in the Michigan lakes may help explain why *MicG* was especially beneficial when resources were plentiful in these populations. Examination of the prevalence of virulent parasites most common in the Michigan and Indiana lakes in conjunction with the relative fecundity of *MicG*-infected *Daphnia* across resource gradients (Fig S5) revealed that high prevalence of each of the parasites occurred at a range of resource availabilities. The prevalence of *Pasteuria* and especially *Spirobacillus* in the Michigan lakes appears to be relatively low when *MicG* is more virulent (i.e., the lowest relative fecundities)

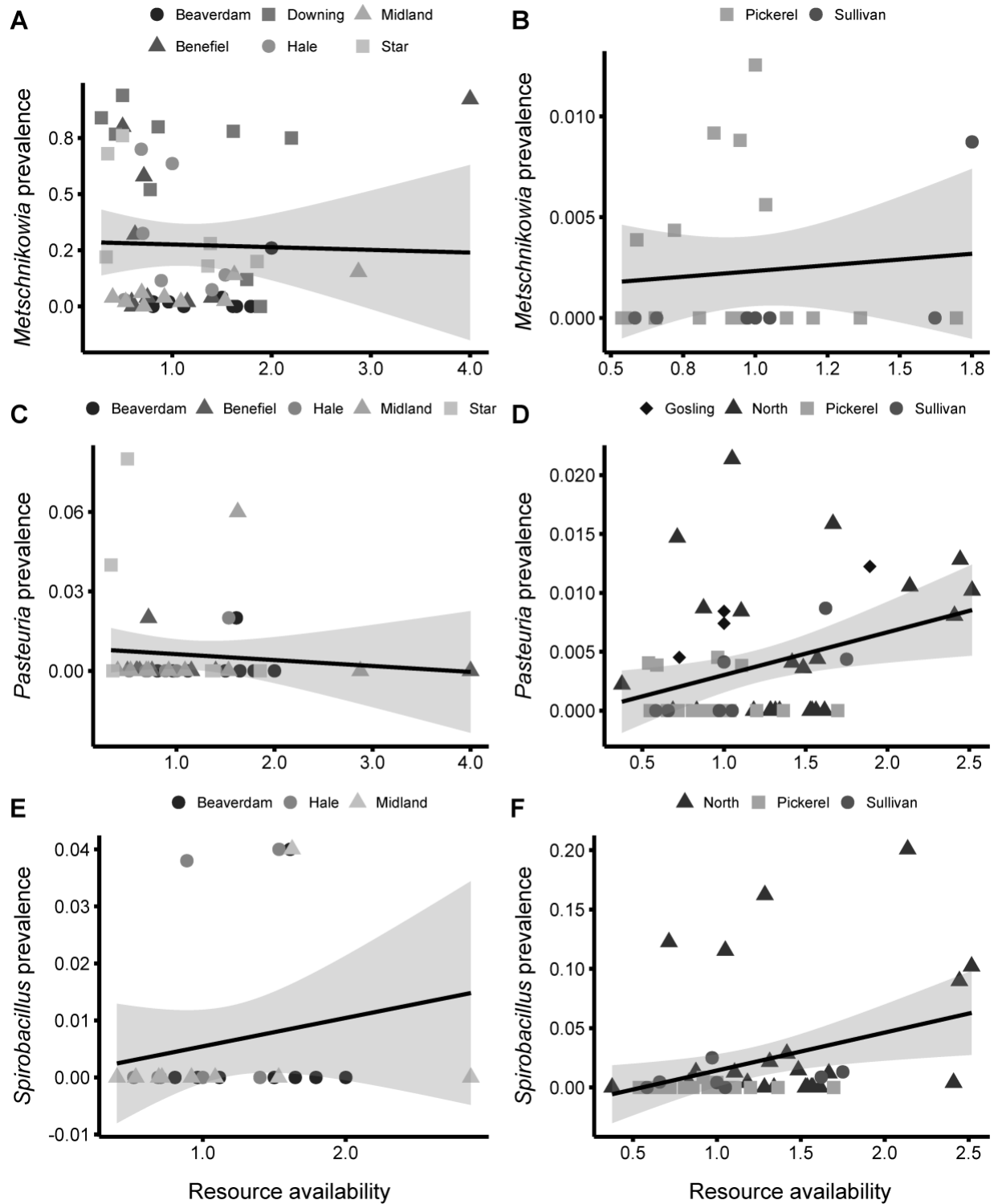


Figure S4. Relationship between resource availability, indexed by mean uninfected *D. dentifera* egg ratio and the prevalence of three common, virulent parasites in the Indiana lakes (A, C, E) and the Michigan lakes (B, D, F). Each data point represents the value for a given population and sampling date.

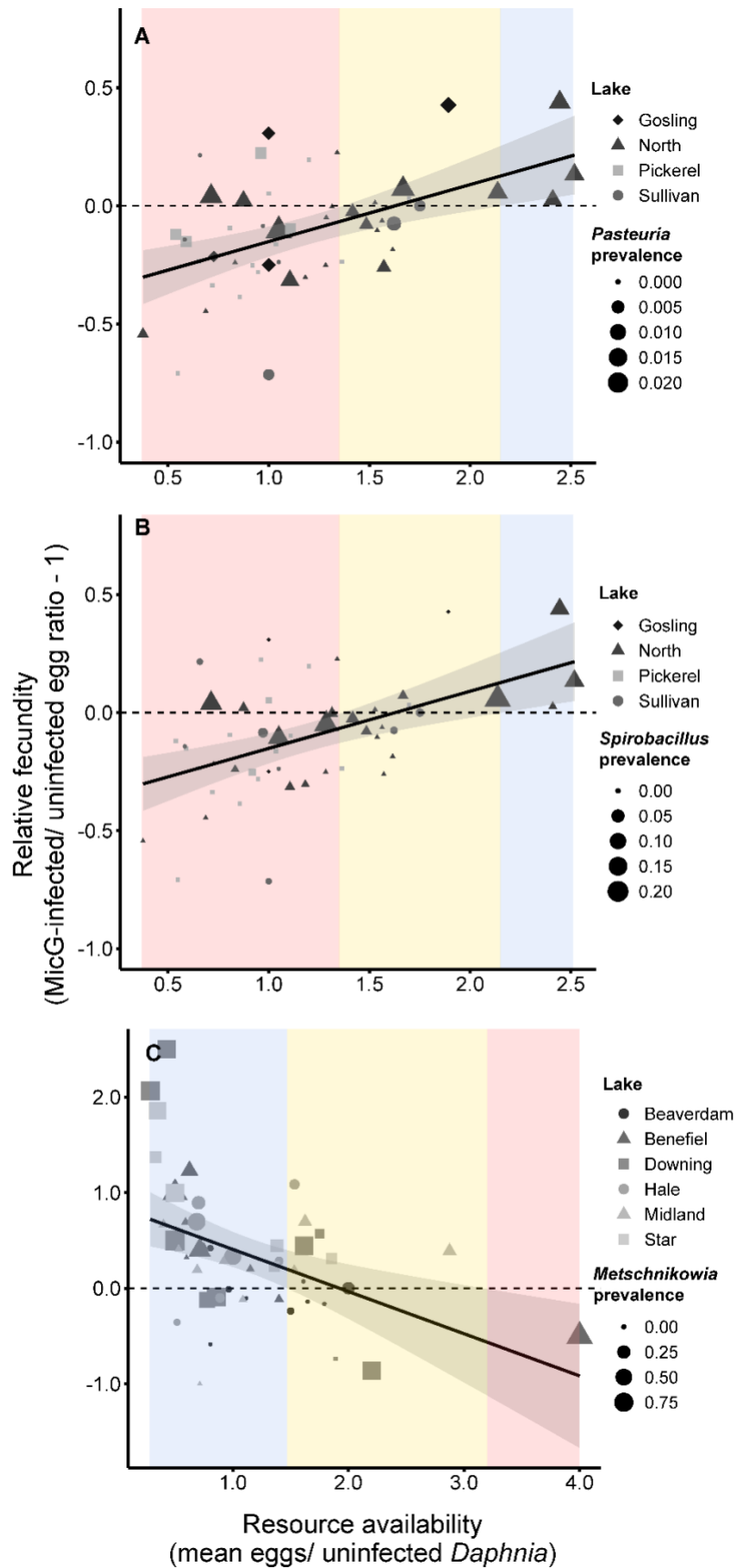


Figure S5. Relationships between resource availability and the relative fecundity of MicG-infected and uninfected *Daphnia*; the data shown here is the same as in Figure 3, but with symbol size varied to indicate prevalence of (A) *Pasteuria*, (B) *Spirobacillus*, and (C) *Metschnikowia*. (A-B) MicG shifted from parasitism towards mutualism with increasing resource availability in lakes sampled in Michigan; for *Spirobacillus* in particular, the greatest virulence of MicG infections (that is, the lowest relative fecundities). All occurred when *Spirobacillus* prevalence was low. (C) In the Indiana lakes, there was a negative relationship between resource availability and the relative fecundity of MicG-infected and uninfected *D. dentifera*; a negative slope is the null hypothesis for x vs. y/x comparisons such as this one (Supplemental text S2). Resource availability, as indexed by egg ratios of uninfected asexual adults, increases with increasing values along the x-axis. Positive values on the y-axis represent greater egg production in infected animals compared with uninfected animals (e.g. if $y=0.4$, MicG-infected *Daphnia* produce 40% more eggs than uninfected *Daphnia*). Each data point represents average fecundity and resource availability for a single sampling date and population. Red, yellow, and blue shading (left to right in panel A) delineate where the 95% CI of the regression line is below, intersects, or is above zero, respectively. Red: MicG infected animals perform more poorly than uninfected *Daphnia* (parasitism); yellow: MicG infected and uninfected *Daphnia* perform equally (commensalism); blue: MicG infected *Daphnia* have a fecundity advantage (mutualism).

Supplemental text S5. Variation in resource availability among the study lakes.

Resource availability, indexed as the mean number of eggs observed in uninfected asexual adult *Daphnia* in a given lake and sampling date, varied considerably over time (Figure S6) and among lakes (Table S1). Mean uninfected egg counts for the dates included in our analyses ranged from 0.93-1.46, and maximum counts ranged from 1.53-4.0 eggs per asexual adult female (Table S1).

While the Indiana lakes had the highest maximum and minimum egg counts, the distribution of resource availability tended to be weighted more heavily in the lower ranges (0-0.5 eggs per asexual adult female) (Figure S6).

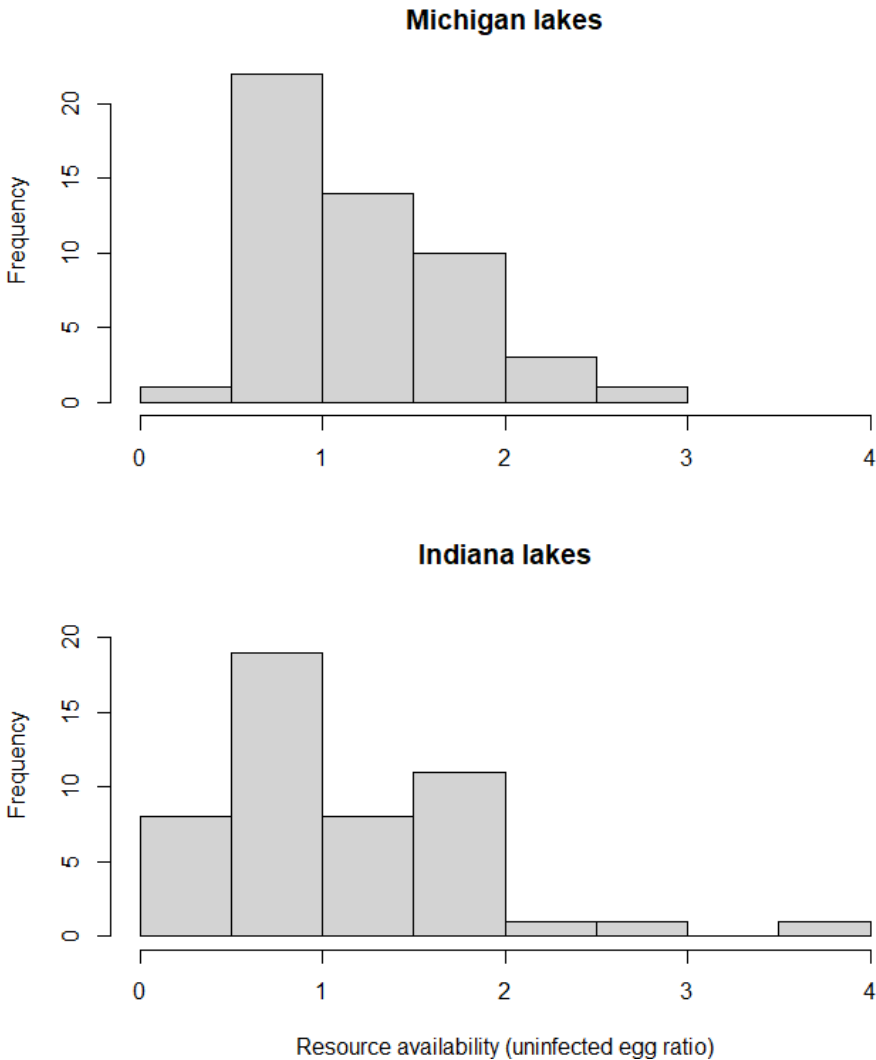


Figure S6. Variation in resources availability, indexed as mean uninfected egg counts for each sampling date and lake in the Michigan and Indiana field surveys. Data is shown for dates included in statistical analyses of field patterns, which includes MicG prevalence of at least 3% and excludes dates when ephippial production was present in late fall.