



The role of varying resources on *Daphnia dentifera* immune responses

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With 3 figures

Abstract: Despite all that is known about *Daphnia* and their interactions with algal resources, questions remain as to how a changing resource environment influences a host's susceptibility to parasites. Theory and empiricism have demonstrated that increasing resource quantity can positively, negatively, and even non-linearly correlate with susceptibility. The nature of this correlation depends on the complex dynamics between the host's immune traits (which are assumed to be costly) and a parasite's ability to evade that immune system and "steal" resources from the host. We used three separate assays to examine how resources influence host immune responses and infection outcomes in eight genotypes of *Daphnia dentifera*. We challenged *Daphnia* with the fungal parasite *Metschnikowia bicuspidata* at three concentrations of the green algae *Ankistrodesmus falcatus*. In the first assay, we investigated how this resource gradient influences the number of fungal spores consumed (a measure of encounter with the parasite), host gut penetrability (a measure of resistance to the parasite), and the haemocyte response (a measure of clearance of the parasite). In the second assay, we explored how these traits combined to determine overall susceptibility to infection. Finally, our third assay investigated the potential for tolerance in this system by comparing reproduction among hosts that managed to avoid, resist, or clear infection to those that developed late-stage infections. We found that host immune responses changed non-uniformly with resources: the number of fungal spores consumed decreased with increasing resources, gut penetrability showed no relationship with resources (but was strongly driven by host genotype), and haemocyte counts peaked at intermediate resource levels. Ultimately, overall susceptibility demonstrated a strong genotype by environment interaction, with some genotypes showing the highest proportion infected in high resource environments, others in low resource environments, and one genotype had the highest proportion infected at the intermediate resource level. In all resource environments, individuals that avoided, resisted, or cleared infection had higher reproduction than those that developed late-stage infections, suggesting that *Daphnia* hosts use resistance rather than tolerance with this parasite. Our results demonstrate the importance of integrating resource supply with immunological mechanisms and examining those effects across a range of genotypes that differ in their responses to the environment.

Keywords: zooplankton; disease ecology; microbe; cladocera; resource; eco-immunology

Introduction

Our knowledge of how resources influence processes ranging from *Daphnia* fitness to ecosystem dynamics increased tremendously during Professor Dr. Winfried

Lampert's tenure as Director of the Max Planck Institute for Limnology in Plön, Germany. He and his collaborators not only uncovered mechanistic principles underlying metabolism and feeding rates and how those vary under a variety of environmental condi-

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tions (Brendelberger et al. 1986; Lampert 1987), but also used this ecophysiology approach to sharpen our understanding of how *Daphnia* physiology and behavior influence and are influenced by other community members of lakes and ponds (e.g., Lampert et al. 1986; Lampert 1987a; Lampert 1989). Not least of all, his work and that of his colleagues demonstrated the impressive amount of clonal variation in natural daphniid populations (Stibor & Lampert 2000; Mitchell et al. 2004). This research helped establish *Daphnia* as a modern model organism for which we can pair genomic information with elegant field and laboratory experiments formed by decades-long records of patterns observed in nature (Lampert 2006; Shaw et al. 2008; Altshuler et al. 2011; Colbourne et al. 2011; Lampert 2011; Miner et al. 2012).

One way in which scientists around the world have taken advantage of the power of this model system is by studying the role of resources in the ecological and evolutionary dynamics of infectious disease. *Daphnia* are ideal organisms in which to investigate these questions because, not only are they infected by a suite of bacterial, fungal, viral, and other parasites, but natural populations maintain substantial genetic variability in multiple traits underlying susceptibility to infection (Green 1974; Ebert 2005; Wolinska et al. 2009; Lampert 2011; Cáceres et al. 2014). Many parasites of *Daphnia* are acquired while feeding, thus foraging for resources and exposure to parasites are directly linked (Decaestecker et al. 2007; Hall et al. 2007; Izhar & Ben-Ami 2015). Beyond exposure, resources can influence parasite transmission through multiple mechanisms (Civitello et al. 2015). In some cases, better fed hosts are more able to resist infection (Siva-Jothy & Thompson 2002; Rolff & Siva-Jothy 2003). However, since hosts and parasites often compete for resources, increasing resources can also result in increased fitness of the parasite (Cressler et al. 2014; Pike et al. 2019). In addition, host birth rates are tied to resources, hence the size of the susceptible population should increase under increasing resources (Civitello et al. 2015), resulting in more efficient transmission (Dallas et al. 2018). In short, it is well established that resources influence both within-host growth of parasites and between-host transmission in multiple *Daphnia*-parasite systems.

Yet, many questions remain unanswered, primarily about the role that resources play in various parts of the immune response of *Daphnia*. As with many arthropods, immunity in *Daphnia* consists of at least three broad categories: behavioral mechanisms that influence encounter rate, resistance to initial infection,

and clearance of established infection (Metschnikoff 1884; Hall et al. 2019; Stewart Merrill et al. 2019; Hite & Cressler 2019; Izhar et al. 2020). Swimming behavior and foraging rate are two traits that influence encounter rate. By reducing feeding rate, and selecting a vertical position in the water column away from infective stages, a host can theoretically avoid infection (Decaestecker et al. 2002; Hall et al. 2005; Izhar & Ben-Ami 2015; Johnson et al. 2018; Hite et al. 2020). However, the extent to which these two behaviors are parasite-avoidance strategies is not fully understood. Once a parasite is encountered, many need to cross the gut barrier to continue development, and the degree to which this barrier can reduce infection depends on the particular host, parasite, and the genotype of both (Izhar et al. 2020; Stewart Merrill et al. 2021a). Even after the parasite has been growing in the host's body, some host genotypes are able to clear infection before it becomes fatal (Stewart Merrill et al. 2019; Stewart Merrill et al. 2021a). However, resisting and clearing an infection is not without costs, and the costs of resistance on both mortality and fecundity likely depend on the host genotype, the environment, and in many cases the parasite genotype. How these costs compare to the costs of infection should determine whether hosts invest in preventing infection or tolerating infection (where tolerance represents a strategy to limit the fitness consequences associated with infection; Råberg et al. 2007).

We combined three assays to ask how variation in resource quantity influences the number of parasite spores consumed (encounter), gut penetrability (resistance), and the haemocyte response (clearance), and how these three immune-related traits influence susceptibility and reproduction in the *Daphnia dentifera* – *Metschnikowia bicuspidata* host-parasite system. We tested eight host genotypes that are known to differ in both susceptibility to the parasite and their response to resources (Hall et al. 2010; Stewart Merrill et al. 2021a) and raised them at three levels of the green algae *Ankistrodesmus falcatus*. By using multiple host genotypes, in three resource treatments, both in the presence and absence of infection, we sought to uncover both direct effects and potential genotype by environment interactions in encounter rate, resistance, and clearance. First, we predicted that encounter would increase with higher resources due to increased feeding rate, following theory on functional responses (Hall et al. 2007). Second, gut penetrability was predicted to increase with increasing resources, based on field observations by Rogalski et al. (2021) that *Daphnia* in environments with greater resource avail-

ability have guts that are more penetrable (less resistant) to *Metschnikowia*. Finally, given that immune defenses are thought to be energetically costly to activate, our clearance trait (haemocytes) was predicted to increase with increasing resources. Given that these three traits were predicted to shape susceptibility in different ways over the resource gradient, we did not expect a clear pattern for proportion infected among the resource treatments. Finally, we predicted that the fecundity costs of infection would be greater than those associated with immunity. Given the previously documented genetic variation in susceptibility among these host clones, we expected to uncover significant genetic variation in at least some of these traits.

Methods

Prior research with the *Daphnia dentifera*-*Metschnikowia bicuspidata* interaction has documented some roles that host genotype and resources play in host susceptibility and transmission dynamics (Hall et al. 2009a; Cáceres et al. 2014; Civitello et al. 2015; Stewart Merrill et al. 2021a). As is the case with most *Daphnia* parasites, *Daphnia dentifera* encounter infective fungal spores while foraging (Ebert 2005), at a rate based on both the host's feeding rate and spore concentration in the water column (Hall et al. 2007). A snapshot of spore consumption can be directly measured by counting the number of fungal spores in the host gut lumen following exposure to *Metschnikowia* (Stewart Merrill et al. 2019; Stewart Merrill et al. 2021b). Although the general process following encounter is similar across genotypes, the specifics of resistance are genotype-specific. Some host genotypes have a more robust gut epithelium, which can prevent attacking *Metschnikowia* spores from entering the host body cavity (Stewart Merrill et al. 2021a). This barrier response likely trades off with the host's ability to acquire nutrients (Stewart Merrill et al. 2019; Rogalski et al. 2021). For those spores that cross the gut barrier and enter the body cavity, some early infections are cleared by the host's immune response. Some genotypes are more efficient than others in clearing these infections (Stewart Merrill et al. 2021a), but the underlying immunological details, and how those are influenced by changing resources, remain a mystery.

We raised eight genotypes of *Daphnia dentifera* on three resource levels and measured susceptibility to a fungal parasite and the immune traits that underlie that susceptibility. We also measured reproduction and compared hosts that avoided, resisted, or cleared the

infection to those that developed late-stage infections (that will eventually kill the hosts). The general design of the experiment was as follows. *Daphnia dentifera* were raised for three generations under standard laboratory conditions to standardize maternal effects (Lynch & Walsh 1998). Once the third generation was achieved, < 24 hour old female neonates were collected from standardized mothers and set up individually in 50 ml falcon tubes containing 45 ml filtered lake water and incubated at 20 °C (16 light:8 dark). Experimental neonates were provided with daily food (1.0 mg C L⁻¹ of *Ankistrodesmus falcatus*) and water changes every other day. At five days of age, experimental individuals were allocated to resource treatments consisting of 0.5 (low), 1.0 (medium), or 2.0 (high) mg C L⁻¹ *A. falcatus* provided daily. These resource levels corresponded with prior experimental gradients selected to replicate natural resource variation observed in temperate lakes (Hall et al. 2007). At eight days of age, *Daphnia* were inoculated with 200 spores ml⁻¹ *Metschnikowia bicuspidata* in 15 ml tubes containing 10 ml filtered lake water for a 24-hour exposure period (following standard methods; Stewart Merrill et al. 2019; Stewart Merrill et al. 2021a). After inoculation, we transferred *Daphnia* back to 50 ml tubes containing spore-free filtered lake water. *Daphnia* remained in their designated resource treatments during exposure and for the duration of the experiment. Three assays were conducted under this general design to quantify: 1) immune defenses and traits associated with susceptibility; 2) proportion infected; and 3) reproduction.

Assay 1: Immune defenses and traits associated with susceptibility

We examined *Daphnia* microscopically approximately 24 hours after inoculation with *Metschnikowia* to quantify three traits associated with susceptibility. The first trait, 'spores consumed', represents a snapshot of parasite encounter, and was measured as the number of fungal spores in the lumen of the host gut. The second trait, 'gut penetrability', indicates how resistant the gut epithelium is to attacking fungal spores. Gut penetrability was quantified as the proportion of spores attacking the gut that successfully entered the body cavity. A gut penetrability score of '1' represents high susceptibility (100 % of attacking spores entered the body cavity), while a score of '0' represents high resistance (no attacking spores entered the body cavity). The third trait, 'haemocytes per spore' was measured as the average number of host haemocytes (immune cells) found on each spore within the body

cavity. Haemocytes adhere to fungal spores early in the infection process, and greater numbers of haemocytes recruited to the site of infection decrease the probability of infection for many genotypes (Stewart Merrill et al. 2019). Each *Daphnia* (N = 195) was examined for these traits using a compound microscope at 400× magnification. *Daphnia* in the assay were not monitored following microscopic evaluation as the process was destructive.

Assay 2: Proportion infected

To investigate whether varying resource levels resulted in differences in infection outcomes, we conducted an infection assay. In this assay, *Daphnia* were maintained for ten days following inoculation with *Metschnikowia* and were provided water changes every other day to remove neonates. At 10 days post-inoculation, each *Daphnia* was examined microscopically (compound microscope; 400× magnification) for evidence of successful infection (N = 262). We denote infection as the presence of late *Metschnikowia* stages from which *Daphnia* cannot recover (the conidia and ascus stages; Stewart Merrill & Cáceres 2018).

Assay 3: Reproduction

Defense against infection – avoidance by reducing feeding, or using either the gut barrier or an activated haemocyte response – may come with fecundity costs. However, infection with a highly virulent obligate killer arguably also generates strong fitness losses. To evaluate the costs of developing late-stage infection (which is terminal) versus fighting infection immunologically, we measured reproduction in each resource treatment following inoculation with *Metschnikowia*. Each day following exposure, *Daphnia* individuals were provided with complete water changes, were recorded as “alive” or “dead”, and the number of neonates was counted. At 12 days post-inoculation, we recorded the infection status of *Daphnia*. We had strong evidence that the majority of exposed and uninfected *Daphnia* had used immune traits to defend against infection, as prior research demonstrates that the spore dose we used (200 spores ml⁻¹) results in a high percentage of individuals with spores attacking the gut and entering the body cavity (Stewart Merrill et al. 2019). Our immune trait assay confirmed these observations: 100 % of exposed *Daphnia* had consumed spores, 92 % had spores attacking their gut barriers (requiring resistance to recover and become uninfected), and 72 % had spores enter their body cavities (requiring clearance to recover and become uninfected). Our

classes for comparison were “exposed–uninfected” and “exposed–infected” and we calculated their daily reproduction as the total number of neonates produced divided by the number of days the *Daphnia* was alive following exposure (N = 173). We ended our daily reproduction evaluation at 12 days post-inoculation (before *Metschnikowia* usually kills their hosts) so that we could more directly compare the cost of infection on clutch size.

Statistical analysis

How are encounter, gut penetrability, and haemocytes affected by resources?

Using data collected from our first assay (‘Immune defenses and traits associated with susceptibility’), we used general and generalized linear models to ask whether and how each trait changed as a function of resource availability. In each model, the resource environment was coded as categorical (‘Low’ = 0.5 mg C L⁻¹, ‘Medium’ = 1.0 mg C L⁻¹, ‘High’ = 2.0 mg C L⁻¹) to allow for nonlinear responses, and to compare means among the three resource levels (in the case that there was a significant fixed effect of resources). Each model included resource level, genotype, and a resource-by-genotype interaction to capture the potential for varying reaction norms among the eight *Daphnia* genotypes. The *Daphnia* hosts, which were reared individually, served as the unit of replication in all models.

To assess how spore consumption (our proxy for encounter) changed with resources, we ran a generalized linear model with a Poisson error distribution using the function ‘glmmTMB’ in the package ‘glmmTMB’ (Brooks et al. 2017). This error distribution was selected because our response variable represented skewed count data. An observation-level random effect was also incorporated into the model because the count data were over-dispersed, and the random effect ensured a dispersion parameter of approximately one. To assess how gut penetrability changed with resources, we ran a general linear model with a Gaussian error distribution (function ‘lm’; R Core Team 2014). Because the response variable is a proportional measurement (the proportion of attacking spores that successfully crossed the gut barrier to infect the host), we arc-sin transformed gut penetrability prior to inclusion in the model. Finally, we assessed how the haemocyte response (measured as haemocytes per spore) changed as a function of resources with a general linear model with a Gaussian error distribution (function ‘lm’; R Core team).

Does proportion infected vary among resource environments?

We used infection status data collected from the second assay ('proportion infected') to assess whether infection outcomes varied among resource levels. We ran generalized linear models (function 'glm'; R Core Team) with each individual's infection status as the response variable (binary data: '0' = uninfected, '1' = infected). We modeled the residuals with a binomial error distribution, and incorporated fixed effects of resources, genotype, and a resource-by-genotype interaction.

How do costs of defense compare to costs of infection?

We compared fecundity among individuals that fought the fungal parasite (uninfected at day 12) versus those that developed late-stage infections (infected at day 12). For this analysis, we used data collected from the third assay ('Reproduction'). We ran a general linear model with a Gaussian error distribution (function 'lm'; R Core Team) with daily reproduction as the response variable. As with prior models, we incorporated resource level (categorical: 'Low', 'Medium', 'High'), genotype, and a resource-by-genotype interaction as fixed effects. Importantly, this model also incorporated a fixed effect of infection status as well as all two-way and three-way interactions with other predictors. Individuals that died before 10 days post-inoculation were not included in this analysis because their final *Metschnikowia* statuses could not be determined (i.e., these individuals died before their infections could advance to the late conidia and ascus stages that formed our point of comparison for 'infected' versus 'uninfected').

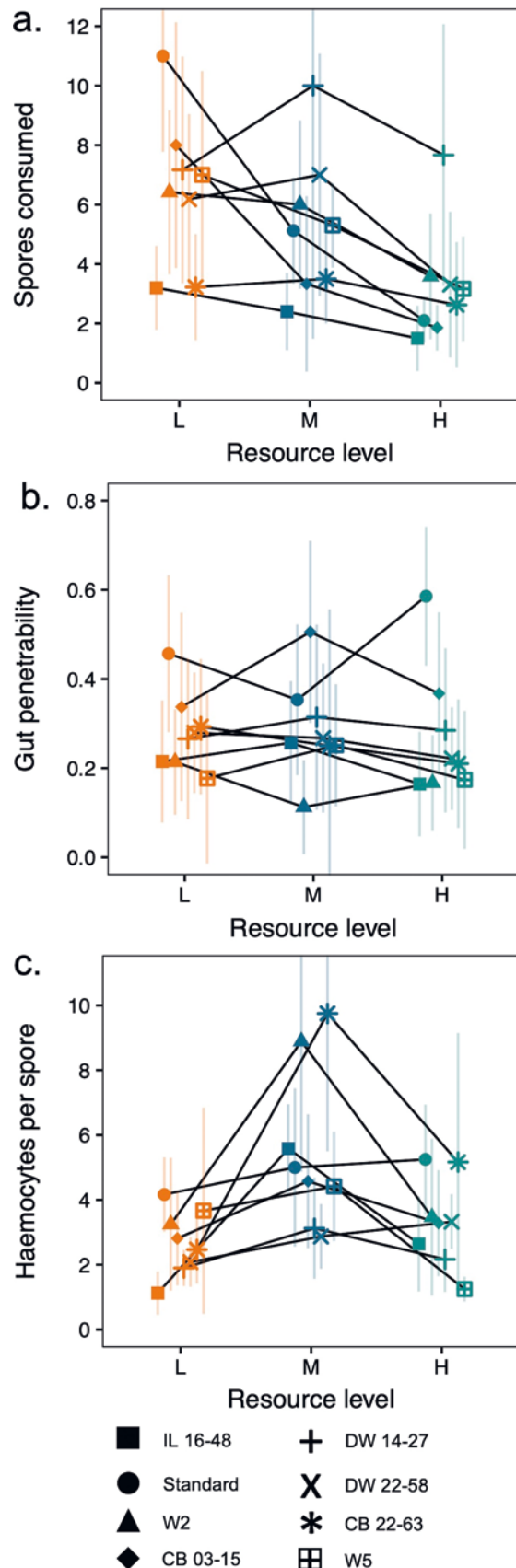
Results

We assayed 630 *Daphnia* for resource-driven variation in immune and susceptibility traits (Assay 1; $N=195$), proportion infected (Assay 2; $N=262$), and reproduction (Assay 3; $N=173$). Our three assays revealed some generic responses to resource availability, but genotypes varied in many of the infection-related processes and genotype by environment interactions were common.

Daphnia immune responses and susceptibility traits changed non-uniformly with resources. Encounter, gut penetrability, and the haemocyte response all exhibited different relationships with resource availability.

Spore consumption (our proxy for encounter), which increases the probability of infection, varied with resources (Chisq = 8.87, $p=0.01$) and showed a pattern of decline with increasing resource availability (Fig. 1a). On average, *Daphnia* in high resource environments had significantly lower spore consumption than those in medium resource environments ($p=0.02$), while spore consumption did not differ among medium and low resource environments ($p=0.71$). In the encounter model, both genotype and the resource-by-genotype interaction were non-significant (genotype: Chisq = 10.79, $p=0.15$; resource*genotype: Chisq = 4.23, $p=0.99$). Gut penetrability, where more penetrable guts increase the probability of infection, did not vary among resource treatments ($F=0.17$, $p=0.84$; Fig. 1b). However, genotypes did vary in their gut penetrability ($F=3.34$, $p=0.002$), with no resource-by-genotype interaction ($F=0.39$, $p=0.98$), suggesting stronger genetic effects than environmental effects. The haemocyte response (haemocytes per spore), which decreases the probability of infection, varied among resource environments ($F=4.69$, $p=0.01$; Fig. 1c). Comparing means among treatments, haemocytes per spore were significantly higher in medium resource environments than in low ($p=0.048$) or high ($p=0.003$) resources. Haemocytes did not differ among high and low resource treatments ($p=0.298$). Additionally, haemocytes did not vary among genotypes or by a resource-by-genotype interaction (genotype: $F=1.14$, $p=0.34$; resource*genotype: $F=0.56$, $p=0.88$). Collectively, the conflicting responses of each trait to the resource gradient (Fig. 1) yielded uncertainty with regards to whether or not a host would develop late-stage infection, and how that scales with resource availability.

Proportion infected did not vary among resource environments, but genotype by environment interactions were common. Of the 262 *Daphnia* exposed to *Metschnikowia*, 142 developed late-stage *Metschnikowia* infections. The majority of infections ($N=140$) were classified by the presence of conidia or asci (patent fungal stages described in Stewart Merrill & Cáceres 2018), while two *Daphnia* possessed sporocyst infections (see Stewart Merrill & Cáceres 2018). Because *Daphnia* almost never recover from the sporocyst stage (Stewart Merrill et al. 2021a), we coded these two individuals as "infected". Results of our statistical model indicated no general differences in proportion infected among the three resource treatments (Chisq = 2.77, $p=0.25$). As expected, genotypes varied in proportion infected (Chisq = 16.03, $p=0.025$), and there was a significant resource-by-genotype interaction (Chisq = 26.44, $p=0.015$). Hence, genotypes re-



sponded to resource environments in different ways: while some genotypes demonstrated the highest proportion infected in high resource treatments (e.g., IL 16-48, Standard, and W2 in Fig. 2), high resources also led to lower levels of infection in others (e.g., CB 03-15, DW 14-27, and CB 22-63 in Fig. 2).

The costs of infection were greater than the costs of defense. In all three resource treatments, daily reproduction was higher among uninfected individuals than infected individuals ($F = 18.34$, $p < 0.001$; Fig. 3). Here, uninfected individuals represent those that were exposed to *Metschnikowia*, but did not develop late-stage infection (the majority of which had spores attacking their gut barriers, as well as spores infecting their body cavities; see ‘Assay 1: Reproduction’). Therefore, the uninfected class primarily comprised individuals that fought the fungal parasite and recovered (rather than individuals that avoided the parasite). Reproduction also varied among resource treatments ($F = 89.73$, $p < 0.001$), showing a pattern of increasing reproduction with increasing resources (Fig. 3), and genotypes themselves varied in reproduction ($F = 20.47$, $p < 0.001$). There were no significant two-way or three-way interactions in our model. In sum, fighting infection immunologically (via a resistant gut barrier or an activated haemocyte response) results in greater fitness outcomes than succumbing to this virulent obligate killer. We note that the majority of hosts in this analysis (~90 % of those assessed for infection) were alive at 12 days post-inoculation, with approxi-

Fig. 1. Assay 1: *Daphnia* immune responses and susceptibility traits change non-uniformly with resources. **a.** Encounter with the fungal parasite, *Metschnikowia bicuspidata*, here measured with ‘spores consumed’ (the number of fungal spores detected in the gut lumen of exposed *Daphnia dentifera*), decreased with increasing resource availability. Thus, the probability of infection based on encounter was lowest in high resource environments. **b.** Gut penetrability—where more penetrable guts result in a higher likelihood of infection—did not vary among resources, but did show strong variation among genotypes. *Daphnia* resistance to *Metschnikowia* may therefore be robust to acute variation in resource changes. **c.** The haemocyte response (which enables *Daphnia* to clear infections and is measured as the average number of haemocytes per fungal spore in the body cavity) peaked at medium resource levels such that the probability of infection based on this trait was highest in the low and high resource treatments. For all three panels, resource treatments correspond to low (‘L’, 0.5 mg C L⁻¹), medium (‘M’, 1.0 mg C L⁻¹), and high (‘H’, 2.0 mg C L⁻¹) levels of resource availability. Genotypes are indicated by symbols (see rightmost key). Standard error is represented by vertical lines surrounding points.

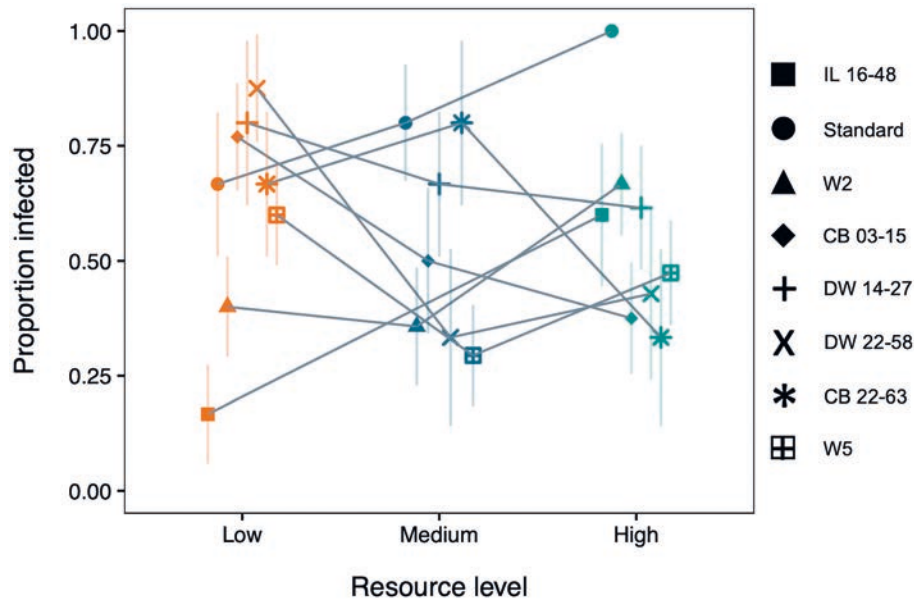


Fig. 2. Assay 2: Proportion infected does not vary among resource environments, but genotype by environment interactions are common. At ten days post-inoculation with *Metschnikowia bicuspidata*, we observed no difference in the proportion of *Daphnia dentifera* that were infected among the three resource treatments (Low: 0.5 mg C L⁻¹; Medium: 1.0 mg C L⁻¹; High 2.0 mg C L⁻¹). Rather, there was a strong statistical interaction between resource level and genotype. Some genotypes showed highest proportion infected under high resource conditions (e.g., IL 16-48, Standard and W2), while others showed highest proportion infected under low resource conditions (e.g., CB 03-15, DW 14-27, and CB 22-63). Genotypes are indicated by symbols (see rightmost key). Note that IL 16-48 (represented with filled squares) was not assayed under medium resource conditions due to limited experimental individuals available for this genotype. Standard error is represented by vertical lines surrounding points.

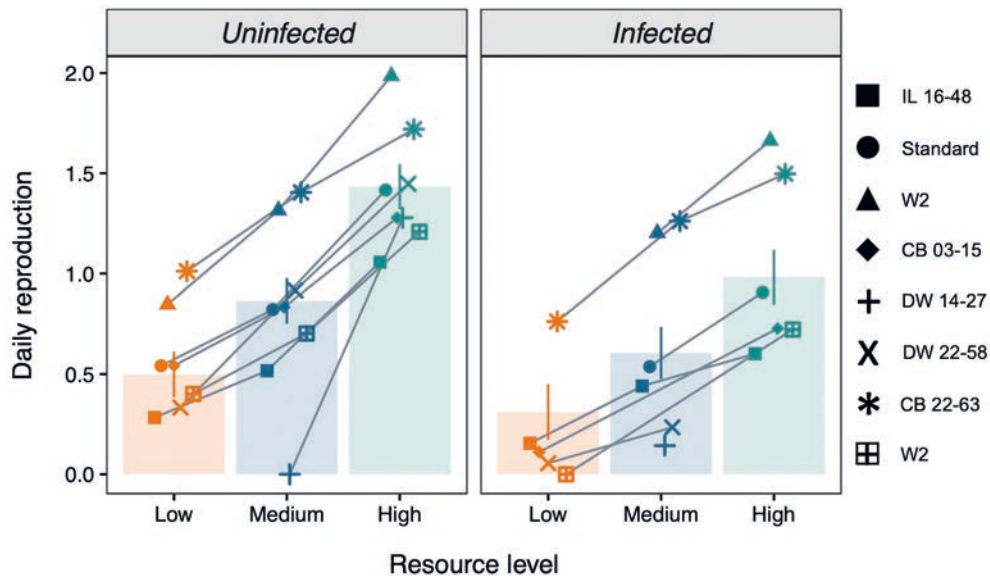


Fig. 3. Assay 3: The costs of infection are greater than the costs of defense. Daily reproduction (neonates per day) among *Daphnia dentifera* that developed late-stage infections with *Metschnikowia bicuspidata* ('Infected') was lower than daily reproduction of *Metschnikowia*-exposed *Daphnia* that fought infection and were ultimately uninfected ('Uninfected'). As expected, daily reproduction increased with resource availability and genotypes themselves varied in their daily reproduction. Resource treatments represent 'Low' (0.5 mg C L⁻¹), 'Medium' (1.0 mg C L⁻¹), and 'High' (2.0 mg C L⁻¹) levels of resource availability. Genotypes are indicated by symbols (see rightmost key). Note that some genotypes could be absent from particular resource-infection combinations if they did not survive to the point where infections could be diagnosed. Additionally, if all individuals of a genotype developed late-stage infections, then we could not evaluate reproduction in individuals that were ultimately uninfected. Similarly, when all individuals of a genotype resisted or cleared an infection, we could not evaluate reproduction in those with late-stage infections. Standard error of the mean within a resource treatment is represented by vertical lines on shaded bars.

mately 10 % dying prior to day 12 (14 died at 11 days post-inoculation; 3 died at 12 days post-inoculation). Because mortality in these individuals was low and limited to the final two days of the assay, our daily reproduction analysis and results were not impacted by mortality.

Discussion

We found counteractive effects of resources on encounter and susceptibility in eight genotypes of *Daphnia dentifera* when challenged with *Metschnikowia bicuspidata*. Despite our initial prediction, the number of ingested spores per host decreased with resources, leading to lower infection likelihood. Also contrary to our initial prediction, we found that *Daphnia* gut penetrability did not vary along the resource gradient. As with many traits, however, we did find that it had a strong genotype signature, with a nearly three-fold spread in average penetrability. Although this primary resistance trait did not vary with resources, we found a relationship between resources and our clearance trait; average haemocyte responses peaked at intermediate resource levels. As a result of differing encounter, resistance, and clearance responses, proportion infected did not vary by resource treatment. However, there was a strong genotype by resource interaction, with some genotypes exhibiting higher levels of infection in high resources and others exhibiting the lowest proportion infected in high resources. Individuals that avoided, resisted, or cleared infection had higher reproduction than those that developed late-stage infections. Not surprisingly, higher resources increased reproduction. Our results demonstrate the importance of integrating resource supply with immunological mechanisms and examining those effects across a range of genotypes that differ in both immunological and foraging traits.

The role of resources in host-parasite dynamics in the plankton remains murky, with increasing resources both fueling and reducing epidemics depending on the system studied and the empirical approach employed (Pulkkinen & Ebert 2004; Hall et al. 2009a; Civitello et al. 2015). The particular experimental design employed certainly explains some of this variation. For example, we introduced resource treatments three days *prior* to exposure which allowed resources to shape immune responses before parasite challenge. In contrast, other studies introduced *Daphnia* to resource treatments at the point of or 24 hours after exposure to parasites such as *Pasteuria*, *Metschnikowia*, and *Glugoides* (Pulkkinen & Ebert 2004; Hall et al.

2009a; Hall et al. 2009b; Vale et al. 2013; Nørgaard et al. 2021). When resource treatments are administered after parasite challenge, parasite production often increases as a function of resources, suggesting that host energy acquired at this late time does not combat the parasite, but is seized by the parasite for its growth (Hall et al. 2009a; Hall et al. 2009b; Valet et al. 2013; Nørgaard et al. 2021; but see also Pulkkinen & Ebert 2004 where per-individual parasite production does not vary with resources). Furthermore, several previous studies on within-host interactions between *Daphnia* and their parasites use a single host genotype that is often highly resistant or highly susceptible (Ebert 2000; Pulkkinen & Ebert 2004; Hall et al. 2009a; Hall et al. 2009b; Schoebel et al. 2014; Nørgaard et al. 2021; two notable exceptions are Vale et al. 2013 and Hall et al. 2012). This single genotype approach allows for increased sample size and number of treatments, yet prevents documenting the substantial genotype by environment interactions that are often observed and likely influence between-host transmission. For example, given the potential role of superspreaders and other non-linearities in determining disease dynamics (Vale et al. 2013; Hall 2019; Elderd et al. 2022), including genetic variation is essential to understanding the dynamics of disease systems.

Several previous studies have also found reduced encounter with increasing resources (Hall et al. 2007; Hall et al. 2009a). The explanation is that, while feeding rate increases with resource density via the Holling functional response, clearance rate of the water decreases, which should limit the number of spores an individual can consume per unit of time. Alternatively, hosts may alter their feeding behavior in the presence of parasites, either as an exposure-induced strategy to reduce further encounter or perhaps as a way to starve the parasite (so called “adaptive anorexia” e.g., Hite & Cressler 2019; Hite et al. 2020). Strauss et al. (2019) provided evidence that some *Daphnia dentifera* dramatically reduce feeding following exposure, and hypothesize that this reduction in feeding may be an avoidance strategy. It could be that *Daphnia* in the high resource treatments, because they have sufficient energy stores, are more able to adopt this strategy than small, resource-deprived *Daphnia*. The model by Greenspoon et al. (2018), where parasite load is determined by both top-down (immune function) and bottom-up factors (resources), provides theoretical support for adaptive anorexia across a range of intake rates.

We predicted that gut penetrability would increase with increasing resources based on positive

relationships observed between gut penetrability and resources in field populations of *Daphnia* (Rogalski et al. 2021). Although we found significant genetic variation in average gut penetrability, the guts of hosts in this laboratory study showed no plasticity in gut penetrability across the controlled resource gradient. There are several possible explanations for these differing results. It is likely that duration of exposure to the parasite and the resource treatment matter. In our experiment, hosts were only exposed to the resource gradient for three days prior to being challenged with the parasite, whereas in the field, grandmaternal, maternal, and early neonatal conditions are well known to influence traits in both *Daphnia* hosts and their parasites (Little et al. 2007; Prior et al. 2011; Grabutt & Little 2014; Coakley et al. 2017; Shocket et al. 2018). An experiment that untangles acute and chronic exposure to both the parasite and resource quantity may provide different results, especially since host age is known to influence susceptibility in *Daphnia magna* and other invertebrates (Izhar & Ben-Ami 2015; Ben-Ami 2019; Izhar et al. 2020). Moreover, laboratory diets do not mimic natural food conditions, and based on field egg ratios data, the field populations in Rogalski et al. (2021) experienced lower resource conditions, on average, and a much greater range of variation than our laboratory experiment.

Prior studies have provided conflicting information regarding how resources influence the invertebrate immune response. Some studies document increased haemocyte responses in non-*Daphnia* invertebrates exposed to higher resources (Triggs & Knell 2012; McKay et al. 2016). Others have found that resources do not influence haemocyte number (Schöbel et al. 2014). Finally, we found that haemocytes peaked at intermediate resource levels. In resolving these differences, the particular host-parasite system under investigation certainly matters, and a greater understanding of the metabolic needs of both host and parasite will likely uncover the mechanisms associated with resource dependence. Establishing this resource dependence is key for understanding the host-parasite relationship. The extent to which hosts can mount a cellular response can influence not only the virulence costs on host fitness, but also between-host transmission. For example, Vale et al. (2013) found that poorly fed hosts across a range of genotypes uniformly produced few spores. However, under higher resource conditions, there was significant variance in the number of infective spores per host, which has the potential to influence epidemic trajectories by generating super-spreaders (Lloyd-Smith et al. 2005). In con-

trast, Rivera-Quinones et al. (in review) documented that well-fed hosts from a diverse range of genotypes tended to produce fewer spores per individual (potentially as a result of cellular responses or a different resource-related defense), and in their transmission model, increasing resource availability then led to smaller epidemics. Identifying how *Daphnia* cellular immune responses determine infection outcomes and parasite growth will allow us to hone in on physiological mechanisms of infection in individuals, and establish connections between resources and disease in populations.

Immune traits are assumed to be costly (Schmid-Hempel 2003; Rolff & Siva-Jothy 2003; Siva-Jothy et al. 2005), but we predicted that *Daphnia* that defended against infection would still have higher reproduction than those that developed late-stage *Metschnikowia* infections. In support of our prediction, we found that *Daphnia* that avoided, resisted, or cleared infection had higher daily reproduction than those that advanced to late stages of infection, and this difference was consistent across resource levels. Reproductive differences over the course of infection are often used to determine whether a species or genotype exhibits tolerance as a strategy, where tolerance limits the fitness costs associated with infection (Råberg et al. 2007). Studies documenting tolerance often show increased fitness among infected individuals, and our results conflict with this, suggesting that tolerance is not operating in the *Daphnia-Metschnikowia* interaction. Defending against infection immunologically may be more adaptive in this system given the guarantee of host mortality if *Metschnikowia* develops to its final stages (Ebert 2005). An important comparison worth making (although we did not attempt it in the current study) is how fitness varies among *Daphnia* that prevented infection and those that were never exposed to the parasite. There is evidence that, in this case, *Daphnia* hosts that manage to resist infection have lower offspring production (Sánchez et al. 2019). Such costs could generate effects at the population level if parasite exposure is high and immune defenses are continuously deployed. Future experimental work should quantify the fitness of uninfected *Daphnia*, *Daphnia* that resist with barriers, and *Daphnia* that clear infections to evaluate the costs associated with each form of defense.

Our results point to several exciting questions for future research into how resources influence encounter, resistance, and clearance and how those traits influence prevalence and between-host transmission. First, we recognize that *Daphnia* in the field are almost

never limited by the quantity of available resources, but food quality can have major impacts on fitness and population dynamics (Sterner & Schulz 1998), and influence host-parasite dynamics (Frost et al. 2008; Schlotz et al. 2013; Dallas & Drake 2014; Lange et al. 2014). Poor quality resources, in addition to providing fewer nutrients, may interact with the parasite and the host's immune response in unique ways. For example, diets containing cyanobacteria have been found to both increase (Tellenbach et al. 2016) and decrease (Sánchez et al. 2019) susceptibility of *Daphnia* to parasites, indicating that much more needs to be done to understand the role of different algal species in disease of zooplankton hosts. Second, changing resources can drive behavioral changes in the host, which can alter disease processes (Altizer et al. 2018). In addition to the direct effects that altering foraging behavior may have on disease, swimming behavior (in particular, diel vertical migration) also varies among hosts and likely influences both within-host and between-host dynamics. Johnson et al. 2018 found that *Daphnia* infected with sporangia cluster disease had altered swimming behavior and remained lower in the water column. We have observed similar results for *Daphnia* infected with *Metschnikowia* (unpublished data). Third, genomic techniques to uncover the molecular basis of immune defenses and their resource-dependence exist (e.g., Labbé et al. 2009; McTaggart et al. 2009), but have yet to be fully explored. Finally, the influence of host immunity is often missing from theory designed to predict disease outbreaks in plankton, despite the fact that host immune defenses are often resource-dependent. At their core, these questions build on and are inspired by Dr. Lampert's timeless legacy.

Authors' contributions

Both authors planned the study. TESM conducted the experiments and analyzed the data. The authors collaborated in writing the paper. The research was funded by grants to both authors.

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