

BIOTIC AND ABIOTIC FACTORS INFLUENCING HOST-PATHOGEN
DYNAMICS IN A ZOOPLANKTON-FUNGUS SYSTEM

by

TAD DALLAS

(Under the Direction of John M Drake)

ABSTRACT

Host-pathogen interactions can be influenced by environmental conditions and interactions with other hosts, either directly through the modification of pathogen transmission or development inside of hosts, or indirectly by influencing host or pathogen demography, survival, or functional traits. In this dissertation, I investigate several environmental (e.g. nitrate) and ecological (e.g., competition) factors that could influence host-pathogen interactions, using a model system of *Daphnia* species infected by an environmentally-transmitted fungal pathogen. I use this system to examine 1) the effect of nitrate pollution on host demography, pathogen survival, and infection dynamics, 2) how host-pathogen interactions respond to variable environments, 3) if a critical host density is present, and predictable, 4) how competition with a non-susceptible competitor influences epidemic dynamics, and 5) how pathogen exposure and infection influences host fitness for a number of host species differing in susceptibility.

INDEX WORDS: Disease ecology, Host-parasite interactions, *Daphnia*, Fungal parasite

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B.S., Truman State University, 2009

M.S., Truman State University, 2010

A Dissertation Submitted to the Graduate Faculty
of The University of Georgia in Partial Fulfillment
of the

Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2016

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Tad Dallas

Dedication

To my parents for believing in me,
committee members for challenging me,
friends for helping me,
and Pabst Blue Ribbon for making it all more fun.

Acknowledgments

I would like to gratefully acknowledge my advisor, John Drake, for providing guidance, support, and the freedom to create and pursue my own research path. However, it takes an academic village, and I benefited greatly from the collective support, guidance, and expertise of my committee - Meghan Duffy, Vanessa Ezenwa, and Andrew Park - as a whole. Thank you.

Throughout my dissertation research, I relied largely on my fellow Drake lab graduate students, especially Reni Kaul, Sarah Bowden, and Marcus Zokan. Further, Tomlin Pulliam provided many well-reasoned answers to some really ill-formed questions, and I am incredibly thankful for his patience and friendship. I thank Drew Kramer not only for his much-needed guidance and support, but also for being a great running partner and mentor. Further, I am deeply grateful to my grandparents -June and Paul Dittmer- and my parents -David and Deb Dallas- for their love and support while I studied my “bugs.”

Lastly, and in no particular order or relative importance, I'd like to thank my cat Brody, Bill McDowell, The Hold Steady, the Odum School of Ecology, coffee, GitHub, Julie Tierney, 5 Points Bottle Shop, rOpenSci, the alligator in Bay 78, Chelle King, Pat the Bunny, the R Core Development Team, and you for reading this.

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Chapter 1

Introduction

Parasitism is one of the most common species interactions, with the potential to influence host population dynamics [3], host communities [207], and ecosystem-level processes [95]. Further, parasites may alter the rate of evolution by placing differential selection on specific genotypes [58]. Host-pathogen interactions have typically been examined by at least three distinctly different groups of people. Epidemiologists, by definition, study pathogens of humans, often with the goal of controlling infectious disease. Veterinary medicine or wildlife pathologists are another group, applying epidemiological principles to the mitigation of wildlife diseases. Lastly, disease ecologists study of interactions between host and pathogen that alter the dynamics of one or either player in the host-pathogen relationship. Further, disease ecologists tend to examine how biotic and abiotic factors mediate infectious disease risk and spread in populations of human or animal hosts. Wildlife pathologists are intermediate between epidemiologists and disease ecologists, in that the goals are typically similar to epidemiological goals (i.e., disease mitigation), while host life history and movement patterns are also considered in understanding disease risk, which aligns more with disease ecology research. However, these lines are constantly blurred, potentially as a result of the rise of ecological epidemiology [11], and the application of tools and theory from epidemiology by

ecologists. For instance, the idea of a critical population size below which a pathogen is unable to invade was originally applied to measles in England [9], but has since been applied outside of human infectious disease systems ([128] and Chapter 4 of this document). In this dissertation, I aimed to further blend these two approaches together, with the goal of examining how host and pathogen interact with the environment to affect epidemic dynamics.

Host-pathogen interactions in a variable world

Host-pathogen relationships exist in a variable world, whether by natural variation in seasonality or climate, or anthropogenic changes to the landscape (e.g. land use change). While environmental variability may alter host and population demographics in isolation [13], there is increasing evidence that environmental variability will alter the interaction between host and parasite as well [95, 121]. The degree to which environmental variability affects host and parasite individually may influence the resulting change to infection dynamics. If the parasite is more susceptible to the environmental change, infection prevalence may be reduced. Conversely, if hosts suffer higher costs of environmental change, infection prevalence may be increased. This is admittedly an oversimplification, as while an environmental change that reduces host population abundance is expected to reduce infection prevalence through decreased contact rates and transmission, environmental change may also influence host immunocompetence, thus enhancing infection prevalence [42, 149]. As parasites rely on host resources, effects of environmental change on host condition often translate into negative effects on pathogen growth and reproduction, as is the case when zooplankton hosts are fed lower quality resources [89] or are fed fewer resources [162].

Directly transmitted pathogens may not be influenced in the same manner as environmentally

transmitted pathogens, as both pathogen transmission modes are subject to the environment within a host, but the environmentally transmitted pathogen is also subject to external environment. This dissertation focuses on an environmentally transmitted pathogen, and treats the host-pathogen interaction as being influenced by the environment in three distinct ways. The environment could influence environmental pathogen populations, host populations, and the interaction between host and pathogen. While the dynamics may be similar to a macroparasite transmission model, environmentally transmitted pathogens offer several challenges when attempting to predict the response of the host-pathogen relationship to environmental change. For instance, negative effects to pathogen populations become very important, especially if successful transmission or epidemic initiation relies on a large environmental pathogen supply. Therefore, especially in the case of the environmentally transmitted pathogens, it may be beneficial to study environmental pathogen survival in the absence of host species in order to infer the potential effect of environmental variability on the host-pathogen relationship, especially when compared to inference obtained from studies of host demography in response to environmental variability. However, pathogen survival in the environment is often very different from pathogen survival inside of infected hosts [42], as infection intensity (number of pathogen cells inside of infected host) may increase as a function of environmental change while pathogen survival in the environment is reduced. Further, even when pathogen populations are disproportionately negatively affected by an environmental stressor, the reduction in transmission as a function of pathogen death may be equal to the increase in transmission rate as a function of decreased host immunocompetence, resulting in a null effect [121].

The role of model systems in the study of host-pathogen interactions

While the role of environmental variability in mediating infectious disease risk is well recognized [60, 121, 200], the study of the effects of certain environmental variables in isolation of others remains hampered by logistical and, sometimes, ethical constraints. Because of this variability, it is difficult to parse out causal relationships between environmental covariates and infection. Microcosms enable researchers to isolate environmental factors and determine how they may influence host-pathogen relationships. Further, microcosms are often small, allowing researchers to have many replicates, allowing for enhanced statistical power, and the ability to examine a finer gradient of an environmental factor of interest. Lastly, for the reasons above, microcosms may be more adept at testing ecological theory. For instance, critical host density thresholds are rarely observed in natural systems [128], where replication, spatial processes, and many other confounding effects exist. However, the theory underlying critical thresholds, and what variables may influence these thresholds are easily testable in microcosms (see Chapter 4).

Zooplankton – pathogen model system

Each experiment performed in this dissertation focused on the interactions between aquatic crustacean zooplankton hosts (*Daphnia* species) and an environmentally-transmitted fungal pathogen (*Metschnikowia bicuspidata*). The *Daphnia*-pathogen system is ideal to provide tests of ecological and epidemiological theory in controlled environments. First, the *Daphnia* hosts are easy to maintain in clonal cultures, which allows control over the amount of genetic variability. Further, populations are small and easily replicated, allowing fine environmental gradients to be examined with ample replication. *Daphnia* hosts are translucent, allowing for the easy quantification of

reproductive status (i.e., visible embryos), and infection status (i.e., opaque clusters of spores within the host; Figure 2.1). Apart from being an ideal model organism, *Daphnia* species are large-bodied grazers, and are an integral part of lake ecosystems, possessing the ability to control primary productivity (top down), and limit fish production (bottom up) [17, 135].

The environmentally-transmitted fungal pathogen is also an ideal study species, as there is little genetic variation in the pathogen, so strain-specific influences are not an issue. Further, the pathogen is a multi-host pathogen, infecting a range of at least 5 *Daphnia* host species (Chapter 6, [44]), as well as other Cladocerans [138, 192]. Fungal pathogen transmission occurs through an environmental pathogen reservoir. The pathogen is ingested during host feeding, pierces the gut wall, and proliferates inside the body cavity of the host (Figure 2.1). The pathogen does not reproduce in the environment, but a single infected host can produce over 50000 pathogen spores [87]. Infection results in shortened host lifespan, reduced fecundity, and eventual mortality. Previous studies have demonstrated that fungal epidemics can exert selective pressure on *Daphnia* hosts, potentially shaping host population genetics [58].

The influence of contaminants and resource stress on *Daphnia*-parasite interactions

The environmental context of host-pathogen interactions can determine transmission and severity of infection [42, 85, 139]. This is especially true in *Daphnia*, as hosts are sensitive to environmental changes [2, 12, 102]. Many studies examining how the environment influences *Daphnia* host-parasite interactions focus one of two types of stresses; environmental contaminants and resources. Here, contaminant is inclusive of anthropogenic additions (e.g. pesticides), commonly

occurring chemicals (e.g. nitrate), and even climatic variables (e.g. temperature). I differentiate contaminant stress from resource stress in that resource stress has to do with the presence or quality of algal resources available for *Daphnia* hosts.

The distinction between these two classes is nontrivial, as they potentially influence the host-parasite relationship differently. Specifically, I hypothesize that contaminants are more likely to influence the ability of the host to respond to the pathogen challenge, by directly influencing immunity or body condition. Meanwhile, I hypothesize that resources largely influence transmission rate by altering the host filtering rate. Many parasites of *Daphnia* are transmitted during host feeding [63], so altering host filtering or feeding rate inherently changes pathogen transmission. Resources have also been found to further alter pathogen growth within infected hosts [162]. In Table 1.1, I highlight some of the previous studies on *Daphnia* host-parasite interactions with the goal of outlining the different ways that contaminants and resources influence both infection prevalence and infection intensity. While this list may not be extensive enough to warrant a meta-analytical approach, it does provide qualitative evidence that alteration to resources is fundamentally different than altering abiotic conditions. Taken together, this suggests that environmental conditions can influence infection dynamics in *Daphnia* hosts, with reductions in resource quality tending to reduce infection prevalence, and additions of contaminants or stressors enhancing infection prevalence and intensity.

Outline of dissertation chapters

In this dissertation, I focused largely on the abiotic context of host-pathogen interactions. Using the *Daphnia* – fungal pathogen model system, I aimed to address how different biotic and

Table 1.1: The influence of environmental contaminants, and altered resources on infection prevalence (“Prev”) and intensity of several *Daphnia* parasites. Arrows indicate the direction of the effect with increasing stressor concentration.

Class	Stressor	Host	Parasite	Prev	Intensity	Citation
Contaminant	Copper	<i>D. dentifera</i>	<i>M. bicuspidata</i>	↑	↓	[30]
Contaminant	Potassium	<i>D. dentifera</i>	<i>M. bicuspidata</i>	↑	-	[31]
Contaminant	Nitrate	<i>D. dentifera</i>	<i>M. bicuspidata</i>	↑	↑	[42]
Contaminant	Pesticides	<i>D. magna</i>	<i>Pasteuria ramosa</i>	↑	↑	[39, 40, 102]
Contaminant	Temperature	<i>D. dentifera</i>	<i>M. bicuspidata</i>	↑	↑	[90]
–	–	<i>D. laevis</i>	<i>M. bicuspidata</i>	↑	↑	*
Contaminant	Predator cue	<i>D. galeata</i>	<i>M. bicuspidata</i>	↑	↑	[210]
–	–	<i>D. dentifera</i>	<i>M. bicuspidata</i>	↑	↑	[57]
Resource	Resource level	<i>D. dentifera</i>	<i>M. bicuspidata</i>	↑	↑	[34, 89]
Resource	Cyanobacteria	<i>D. dentifera</i>	<i>M. bicuspidata</i>	↓	-	[151]
–	–	<i>D. magna</i>	WBD ¹	↓	-	[38]
Resource	P limitation		<i>P. ramosa</i>	↓	↓	[75]

* This dissertation (Dallas and Drake, in review); ¹ White Bacterial Disease

abiotic factors influenced the host-pathogen relationship. Collectively, these different chapters include organization scales from single host-populations to multi-species communities, and assess multiple aspects of the host-pathogen relationship. Specifically, there are at least 3 ways an environmental variable can influence the host-pathogen interaction. The environment can influence 1) host demography or life history traits, 2) environmental pathogen survival, or 3) the transmission of pathogen or development of pathogen inside infected hosts. By example, an environmental variable could influence host abundance or pathogen survival, each of which could indirectly enhance or reduce infection dynamics. However, the environmental variable could also directly influence the host-pathogen interaction by altering host encounter rates, influencing pathogen infectiousness, or enhancing pathogen growth inside of infected hosts.

Using this conceptual framework, I examined environmental effects on hosts, pathogen, and the host-pathogen relationship in the following ways:

- **Chapter 2** examines how host demography, pathogen survival, and the host-pathogen interaction is influenced by the addition of nitrate, a common pollutant in aquatic environments.
- **Chapter 3** aims to address how temperature variability influences infection dynamics
- **Chapter 4** couples theoretical models with a microcosm study to determine the existence and predictability of the critical host density threshold, which is the number of hosts necessary for a pathogen to invade and cause an epidemic.
- **Chapter 5** examines the influence of resource competition with a non-susceptible competitor on epidemic dynamics of a susceptible, inferior competitor, using a combination of

theory and experimental epidemics.

- **Chapter 6** aims to assess the magnitude of reductions to host fitness as a result of pathogen exposure (so called “costs of resistance”) among multiple *Daphnia* host species.

Chapter 2

Nitrate enrichment alters a *Daphnia*-microparasite interaction through multiple pathways

Dallas, T. & Drake, J. M., 2014 Nitrate enrichment alters a *Daphnia*-microparasite interaction through multiple pathways. *Ecology and Evolution* **4**, 243–250.

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Abstract

Nutrient pollution has the potential to alter many ecological interactions, including host-parasite relationships. One of the largest sources of nutrient pollution comes from anthropogenic alteration of the nitrogen (N) cycle, specifically the increased rate of nitrate ($\text{NO}_3\text{-N}$) deposition to aquatic environments, potentially altering host parasite relationships. The current study aimed to assess the mechanisms through which nitrate may impact host-pathogen relationships using a fungal pathogen (*Metschnikowia bicuspidata*) parasitic to crustacean zooplankton (*Daphnia dentifera*) as a tractable model system. First, the influence of nitrate on host population dynamics was assessed along a gradient of nitrate concentrations. Nitrate decreased host population size and increased infection prevalence. Second, the influence of nitrate on host reproduction, mortality, and infection intensity was assessed at the individual host level by examining the relationship between pathogen dose and infection prevalence at ambient ($0.4 \text{ mg NO}_3\text{-N L}^{-1}$) and intermediate ($12 \text{ mg NO}_3\text{-N L}^{-1}$) levels of nitrate. Host fecundity and infection intensity both decreased with increasing pathogen dose, but elevated nitrate levels corresponded to greater infection intensities. Nitrate had no effect on host growth rate, suggesting that hosts do not alter feeding behavior in nitrate-treated media compared to ambient conditions. This study suggests that nutrient enrichment may enhance disease through increased transmission and infection intensity, but that high levels of nitrate may result in smaller epidemics through reduced transmission caused by smaller population sizes and increased pathogen mortality.

Introduction

Parasites are a structuring force to host populations [3, 198] and communities [207], with the potential to influence ecosystem level processes [95] and ecological interactions [166]. However, the influence of parasites on hosts is mediated by environmental variation, either directly through changes in host or parasite demography [107] or indirectly through modulation of host traits linked to disease or competitive interactions [205]. An understanding of how environmental variation influences disease is a central goal of disease ecology with implications for understanding epidemic dynamics. Given continued anthropogenic modifications to ecosystems, an understanding of how changes in environmental variables influence community persistence and disease dynamics is a problem of increasing importance.

Nutrient pollution is an exemplary anthropogenic modification to an environmental variable that alters ecosystem structure and function [18, 106]. Nitrate pollution, particularly, is responsible for changes in community composition [104], plant productivity [164] and pH of streams and lakes [22, 69]. While many of these effects are a result of increased phytoplankton growth, nitrate toxicity also directly influences animal populations and communities [106]. Nitrate pollution is also of human health concern, as elevated nitrate levels observed in many agricultural or industrialized areas [131] could cause methemoglobinemia, a condition affecting the ability of hemoglobin to carry oxygen [22]. Lake ecosystems may be particularly vulnerable to the influences of nitrogen pollution because of their typically longer nutrient residence time relative to terrestrial systems and rapid increase in phytoplankton biomass after nitrogen enrichment [67].

Our current understanding of the influence of nitrate on disease in aquatic ecosystems remains limited. One major unanswered question concerns the mechanisms behind an apparent increase in parasite abundance in the presence of elevated nitrate [119, 122]. Field studies on alteration of host-parasite interactions by nitrate have identified several pathways, including nitrate-induced increases in host density [107], increased infection intensity (number of parasite cells per infected host; [106]), and alterations to habitat use of both host and parasite [106]. However, few studies have been able to tease apart the potential causal mechanisms underlying the relationship between nitrate pollution and disease as a result of confounding variables in field systems. Specifically, while the influence of other contaminants such as copper [30] and potassium [33] have been examined, the direct impact of nitrate toxicity on the host-parasite relationship remains largely unexplored. Further, while other studies have tested a small subset (1 or 2) mechanistic pathways through which nutrient pollution potentially acts, the current study examines nitrate contamination at three hierarchical levels (and a total of 4 potential pathways), all of which are potentially influenced by elevated nitrate concentrations.

We used a model system comprised of *Daphnia dentifera* and a virulent yeast pathogen, *Metschnikowia bicuspidata*, to investigate the impact of nitrate on the host-pathogen relationship on three levels. First, we assessed the impact of nitrate at the host population level, by examining host population size and pathogen prevalence in *D. dentifera* microcosms exposed to the fungal pathogen along a spectrum of nitrate concentrations. Second, we assessed the impact of nitrate on pathogen populations by exposing environmental pathogen spores to a gradient of nitrate concentrations and measuring survival. Lastly, we assessed the impact of nitrate on the host-pathogen interaction at the individual host level by examining the influence of pathogen

dose and elevated nitrate concentration on infection prevalence, intensity, host growth and fecundity. These experiments address four possible causal pathways by which nitrate could enhance pathogenic infection: (i) the reduction of host population size through increased mortality or decreased fecundity, (ii) an increase in host susceptibility by stressing host populations, (iii) enhanced infection intensity as a result of nitrate promoting pathogen propagation inside of stressed hosts, or (iv) the reduction of environmental pathogen survival, as fungal spores are sensitive to nitrate when grown in culture (Pitt and Miller 1970). The host population level experiment addresses points (i) and (ii), while the pathogen survival experiment addresses hypothesis (iv). Lastly, the individual level dose-response experiment addresses hypothesis (iii) and lends support for a mechanism of hypothesis (i).

Methods

Study system Our model system consisted of a single clone of the freshwater cladoceran *D. dentifera* reared on a food resource of pulverized blue-green algae (*Spirulina sp.*). *Metschnikowia bicuspidata*, a fungal pathogen used in this study, was cultured in-host following the methods of Duffy and Sivars-Becker (2007). The needle-shaped fungus infects hosts after ingestion by piercing the host's gut wall and propagating within hosts, typically leading to host mortality between 10 and 20 days after pathogen exposure (Figure 1). Nitrate media was produced by dissolving NaNO_3 in deionized water at concentrations that varied by treatment, autoclaving the solution, and then mixing this sterile solution with filtered pondwater (30 μm filter). Control media was produced by adding sterile deionized water to filtered pondwater.

The influence of nitrate concentration on host populations *Daphnia* populations, consisting of 15 isoclonal individuals, were placed in 50ml of pondwater media supplemented with NaNO_3 and fed 70 μl of a solution of 0.2 mg *Spirulina* suspended in 100ml deionized water. A total of 90 populations (for a total of 1350 individuals) were initialized along a gradient of 6 nitrate concentrations (0.4, 2, 4, 8, 16, and 32 mg $\text{NO}_3\text{-N L}^{-1}$) to investigate the impact of elevated nitrate on population and infection dynamics. Populations were inoculated with approximately 10,000 *Metschnikowia* spores (200 spores ml^{-1} of media) at their experimental nitrate levels, allowing nitrate to influence transmission rate. Every other day, half of the experimental media from each population was replaced with fresh media to prevent the buildup of toxic metabolites and reduce the risk of algal contamination. Infection was assessed using a dissecting microscope (20 \times - 50 \times magnification) 10 days after inoculation. The influence of nitrate on host population size and susceptibility was assessed with Spearman's rank correlations, as a result of the nonlinear response.

Impact of nitrate on environmental spore survival The impact of nitrate on environmental pathogen survival was assessed at 7 nitrate concentrations (0.4, 5, 10, 15, 20, 25, and 30 mg $\text{NO}_3\text{-N L}^{-1}$) using fluorescent staining to diagnose spore viability at days 0, 1, 20, 40, and 80. Heavily infected individuals were homogenized in sterile pondwater, aliquoted into seven centrifuge tubes and adjusted to experimental nitrate concentrations. At each observation time, an 18 μl sample was mixed with 2 μl of a 0.125 mg ml^{-1} Propidium Iodide solution and allowed to incubate for 15 minutes at room temperature protected from light. Propidium Iodide infiltrates cells with compromised cell walls, which is readily observed using fluorescent microscopy. A total of 20 fields of view at 400 \times magnification were observed for each sample to quantify the proportion of

viable spores at each time step.

The impact of nitrate on environmental spore survival was assessed with a repeated measures ANOVA, with the proportion of surviving spores as the response variable, and nitrate concentration as the treatment factor.

Impact of nitrate and pathogen dose on infection dynamics and host traits The influence of nitrate on the relationship between pathogen dose and infection prevalence (i.e., the dose-response relationship) was examined at a single elevated nitrate concentration, and at an ambient control nitrate concentration. The elevated level of nitrate (12 mg NO₃-N L⁻¹) was chosen to represent a moderately polluted system, reminiscent of some agricultural systems [22]. A total of 11 pathogen doses were used, starting with 10,240 *Metschnikowia* spores per ml of media as the highest dose and diluting sequentially 10 times by a factor of two in order to obtain the remaining pathogen concentrations (10, 20, 40, 80, 160, 320, 640, 1280, 2560, 5120, and 10240). A total of 462 animals were used to assess the influence of nitrate on the dose response curve (21 replicates * 2 treatments * 11 dose levels = 462 individuals). To remove maternal effects, we first removed a generation of reproductive females from laboratory stocks. Offspring of this generation were removed and placed into individual tubes and the offspring of this generation used for experimentation. All hosts were between 24-72 hours old at the start of the experiment.

Daphnia were inoculated together in 200 ml beakers of pondwater media at their respective dose treatments, then each individual was moved to 50 ml test tubes filled with spore-free experimental media the following day to remove any influence of nitrate levels on transmission itself, but allow for nitrate to influence host behavior or immunological responses and/or pathogen fitness. During the inoculation period half of the normal concentration of food was provided

(100 μ l *Spirulina* suspension). Infection was assessed using a dissecting microscope (20 \times - 50 \times magnification) 9 days after inoculation. Intensity was assessed by homogenizing infected *Daphnia* in 200 μ l of deionized water and determining spore concentration using a hemocytometer. Offspring were counted and removed from experimental test tubes daily to obtain estimates of fecundity. To determine how nitrate influenced host growth, 40 individuals were placed individually in 50 ml culture tubes of experimental media (20 individuals in 12 mg NO₃-N L⁻¹ media and 20 individuals in pondwater media containing ambient levels of nitrate). Body length, measured from eyespot to base of tail spine, was assessed for all hosts prior to inoculation and at day 9. The difference between these measurements was used as a measure of growth over the experimental period, and was analyzed using a two sample t-test with a 0.05 significance level.

Two and three parameter Weibull and log-logistic models with lower and upper boundaries of 0 and 1 were fit to the dose-response data for nitrate and control media using the drc R package [171]. Two different formulations of the Weibull models were used, referred herein as type “a” and “b”. These formulations differ slightly in their curve fit, as examined by [187]. These models were competed against one another, with model selection based on negative log-likelihood values. The influence of pathogen dose and nitrate on infection intensity was analyzed with a simple linear regression model. Pathogen dose levels in which only one individual was infected were not considered when fitting the linear model, as these single estimates likely do not represent the true mean infection intensity for that dose treatment.

Results

The influence of nitrate concentration on host populations Nitrate influenced host populations in two notable ways. First, nitrate caused an increase in infection prevalence, altering prevalence from 53.9% at ambient conditions to 90.7% at the highest nitrate concentration examined. Second, nitrate reduced final host population size (Figure 2.2) from an average of 11 individuals at ambient conditions to approximately 5 individuals when exposed to elevated nitrate concentrations. Both the effect of nitrate on host population size and the effect of nitrate on infection prevalence were nonlinear. Elevated nitrate concentrations were correlated with reduced population sizes (Spearman's rank correlation: $R_s = -0.694$, $p < 0.0001$) and elevated infection prevalence (Spearman's rank correlation: $R_s = 0.607$, $p < 0.0001$). Infection prevalence increased to nearly 100% infection prevalence. Meanwhile, population size was reduced drastically in the treatment period between ambient levels of nitrate and 4 mg NO₃-N L⁻¹, followed by a much slower decrease in host population size (Figure 2.2).

Impact of nitrate on environmental spore survival Spore survival was reduced at each time step as a result of increased nitrate concentrations (repeated measures ANOVA; $F = 43.22$, $p < 0.0001$). Elevated nitrate severely reduced spore survival after 24 hours of exposure to nitrate enriched media, resulting in sharp declines in survival for higher nitrate concentrations followed by asymptotic approach to between 15% and 25% spore survival after 80 days (Figure 2.3).

Impact of nitrate and pathogen dose on infection dynamics and host traits Nitrate did not significantly affect the relationship between pathogen prevalence and pathogen dose (Figure 2.4). The three-parameter Weibull dose response model provided the best fits for both nitrate and

control dose response curves based on log-likelihood values (Table 2.1). Nitrate and pathogen dose both influenced the average spore load of infected individuals (i.e. infection intensity). Infection intensity decreased with increasing pathogen dose, but was enhanced by the presence of elevated nitrate levels (Figure 2.5). A generalized linear model (Gaussian family, identity link) was used to determine that infection intensity was positively related to nitrate treatment ($t = 2.39$, $p = 0.032$) and negatively related to pathogen dose ($t = -5.96$, $p < 0.0001$), providing evidence that nitrate causes increased infection intensities consistently across a gradient of pathogen doses. Nitrate treatment and pathogen dose explained 72.3% of the variation in infection intensity. A generalized linear model (Gaussian family, identity link) was used to determine if host fecundity was altered by elevated nitrate or pathogen dose ($R^2 = 0.13$). Fecundity of uninfected hosts was reduced at higher pathogen doses ($t = -7.69$, $p < 0.0001$), but this relationship was not altered by nitrate ($t = -1.89$, $p = 0.059$). Additionally, host mortality increased as a function of nitrate based on the results of a paired t-test on the number of dead individuals in each pathogen dose class before the experiment was completed ($t = -3.857$, $p = 0.0039$). In the separate group of hosts examined for changes in growth rate, we excluded those individuals that died from analysis, and found no evidence that growth rate differed as a function of nitrate treatment ($t = -0.5618$, $df = 27$, $p = 0.579$).

Discussion

Major findings The influence of nitrate pollution was examined with respect to four possible mechanistic pathways discussed in the introduction (hypotheses **i** - **iv**). Previous studies have suggested that nutrient pollution has the potential to alter host susceptibility to infection, infection

intensity, host population growth, or pathogen survival (specifically in environmentally transmitted pathogens). Through a combination of three experiments performed at three distinct scales (i.e. host population, host individual, and pathogen population), the current study provides evidence that nitrate acts via all four of these pathways, reducing population sizes of susceptible hosts, enhancing both infection prevalence and infection intensity, and reducing environmental spore survival. However, nitrate did not influence host growth or the relationship between pathogen dose and prevalence. Pathogen dose nevertheless decreased host fecundity and infection intensity, which is consistent with previous findings of pathogen-induced life history shifts in hosts [25, 142] and decreased infection intensity at higher pathogen doses [65].

Pathogen dose and infection intensity The negative relationship between pathogen dose and infection intensity has been observed previously in a *Daphnia*–microparasite system [65]. The proposed explanation being that hosts died so quickly at high pathogen doses that the parasites could not propagate within hosts. However, many hosts in our experiment were still alive at the end of the experiment, suggesting that this may not best explain the relationship between pathogen dose and infection intensity. As *Daphnia* respond strongly to cues, it is possible that hosts either recognize the pathogen threat and reduce feeding, or the pathogen is so dense that it interferes with the acquisition of algal cells, which would result in reduced feeding [113]. Either way, reduced feeding would reduce the number of infectious spores entering the host gut, and this could reduce overall infection intensity.

Synthesis At the population level, nitrate reduced host population size, and increased infection prevalence. Together, these effects may increase host extinction risk and/or alter host commu-

nity composition [71]. Further, we found that environmental pathogen concentration negatively influenced host fecundity and decreased infection intensity. The decreased infection intensity is not a result of trait-mediated effects, as *Daphnia* were raised and inoculated in ambient pond-water then placed in experimental media the following day, standardizing the exposure period of host to parasites, and removing the potential for nitrate to directly influence transmission rate (as was permitted in the population level experiment). Despite the negative relationship between pathogen dose and infection intensity, elevated nitrate resulted in increased infection intensity. Taken together, these results imply that nitrate contamination may result in an increase in environmental pathogen concentration, a corresponding decrease in host fecundity, and a dose-dependent increase in infection intensity. These factors, in turn, contribute more pathogen spores to the environmental reservoir, suggesting that increased nitrate concentrations may result in larger epidemics and reduced host population sizes.

On the other hand, we found a decrease in environmental spore survival with increasing nitrate concentrations. This indicates that large inputs to the environmental pathogen bank as a result of increased nitrate may be masked in natural populations by the influence of nitrate on pathogen survival, as the effect of reduced pathogen survival may counteract the influence of nitrate on spore propagation within hosts. This may become especially important to recurrent epidemics, as epidemics are probably primarily initiated from an environmental reservoir [56]. However, the current study examined the influence of nitrate as a stressor, deliberately controlling for the impact of nitrate on other ecosystem properties such as primary productivity. Increases in algal productivity may increase infection prevalence and fecundity [89], thereby both increasing epidemic size and reducing the likelihood of host extirpation. While the pathway by which elevated nitrate influences pathogen dynamics may be context dependent, it is nevertheless more likely that

nitrate will increase epidemic size by causing higher infection intensities and increasing infection prevalence, even if the size of the environmental pathogen bank is reduced, as environmental transmission likely occurs shortly after infected host death.

Potential implications for epidemics in natural systems Many environmentally transmitted pathogens cause recurrent epidemics, initiating the epidemic from an environmental pathogen bank. This study suggested that while nitrate may cause higher spore loads in infected hosts, environmental pathogen survival may be reduced at elevated nitrate levels. Thus, while nitrate may increase disease severity post-transmission, elevated nitrate levels may also reduce the environmental pathogen reservoir, perhaps resulting in a lower likelihood of an epidemic occurring or causing a change in the timing of recurrent epidemics. If pathogen transmission occurs within 24 hours of spore liberation from infected hosts, it is likely that nitrate would stimulate epidemics in natural populations. However, if transmission occurs largely through an environmental pathogen source, composed of pathogen spores that have been exposed to elevated nitrate for longer than 24 hours, nitrate may reduce epidemic size in natural populations. Given the importance of algal resources to host population density, and the role of nitrate in promoting primary productivity, it is possible that elevated nitrate could result in higher transmission rates through density dependent transmission. However, it is important to use caution when considering applying these results to natural systems. Our experiments controlled for environmental variability, algal species composition and other factors that could influence the ecosystem level response to nitrate addition. While our experiments were not intended to mimic natural systems, we do provide an investigation of the mechanisms that underly how nitrate enrichment alters host populations, parasite populations, and host-parasite interactions.

Conclusions The current study isolates nitrate as a stressor, providing evidence that nitrate pollution can directly influence host populations and their resident pathogens. Generally, nitrate is likely to influence pathogens in different ways, and will likely not always increase disease prevalence or intensity [106]. Nitrate deposition in natural settings rarely occurs in isolation of other anthropogenic stressors, making mechanistic field studies difficult. By examining the relationship between an ecosystem level property and infection dynamics in experimental microcosms, we disentangled the mechanisms by which nitrate influences infection at the host population level and with respect to pathogen dynamics. This study represents an important test of the influence of ecosystem level properties on infection dynamics, as laboratory examinations of these interactions are rare (but see [30]). A useful next step would be to conduct field studies of how environmental factors such as nitrate act both in isolation and in concert with other environmental stressors to influence pathogen dynamics. Together, these will lead to a more complete understanding of how pathogen dynamics are altered in the face of an increasingly variable environment.

Tables

Table 2.1: Dose-response model fits compared using AIC and log-likelihood (LL). The number of parameters in the model is provided by n_p , and the two variations of the Weibull model are denoted as a or b. Based on log-likelihood values, the 3-parameter Weibull model best fit data for both control and nitrate treatments.

Model	n_p	<u>Control</u>		<u>Nitrate</u>	
		<i>LL</i>	<i>AIC</i>	<i>LL</i>	<i>AIC</i>
Weibull b	3	11.98	-15.96	13.12	-18.23
Weibull b	2	10.27	-14.54	12.79	-19.58
Log-logistic	3	10.68	-13.35	12.15	-16.29
Log-logistic	2	8.74	-11.48	11.15	-16.31
Weibull a	3	7.23	-6.45	10.86	-13.72
Weibull a	2	7.20	-8.40	9.20	-12.40

Figures

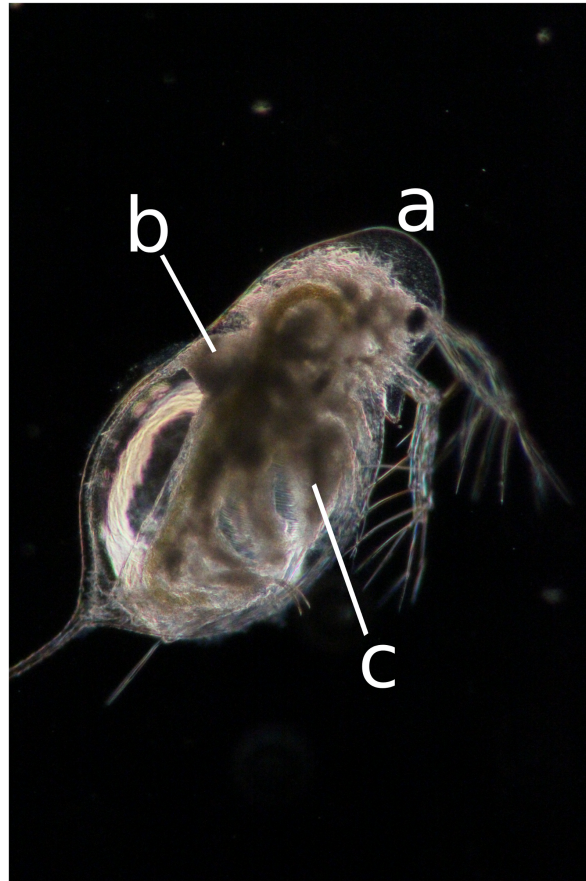


Figure 2.1: A *Daphnia dentifera* host infected with *Metschnikowia bicuspidata*, a virulent fungal pathogen. The pathogen clogs the gills (c), heart (b), and head (a) with needle-shaped spores, making the host appear opaque and white.

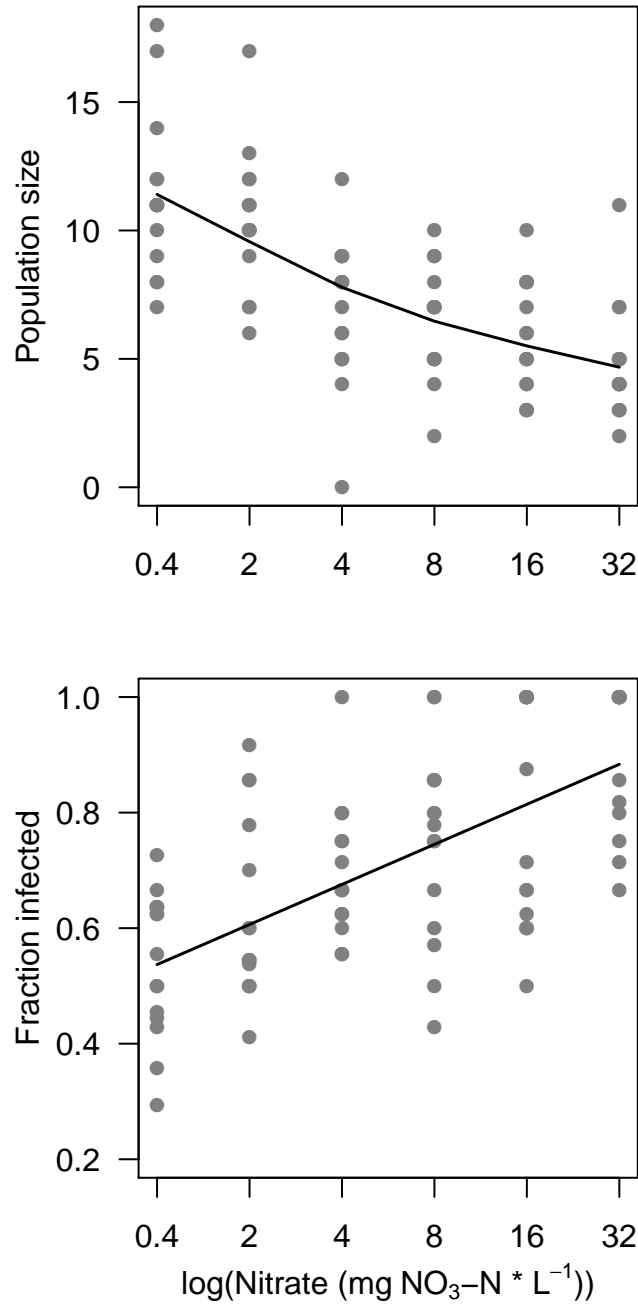


Figure 2.2: Elevated nitrate concentrations resulted in severely reduced host population size (Spearman's rank correlation: $R_s = -0.694$, $p < 0.0001$) and greater infection prevalence (Spearman's rank correlation: $R_s = 0.607$, $p < 0.0001$) after only moderate increases to nitrate concentration. Solid black lines are cubic smoothed splines fit to experimental data (grey points).

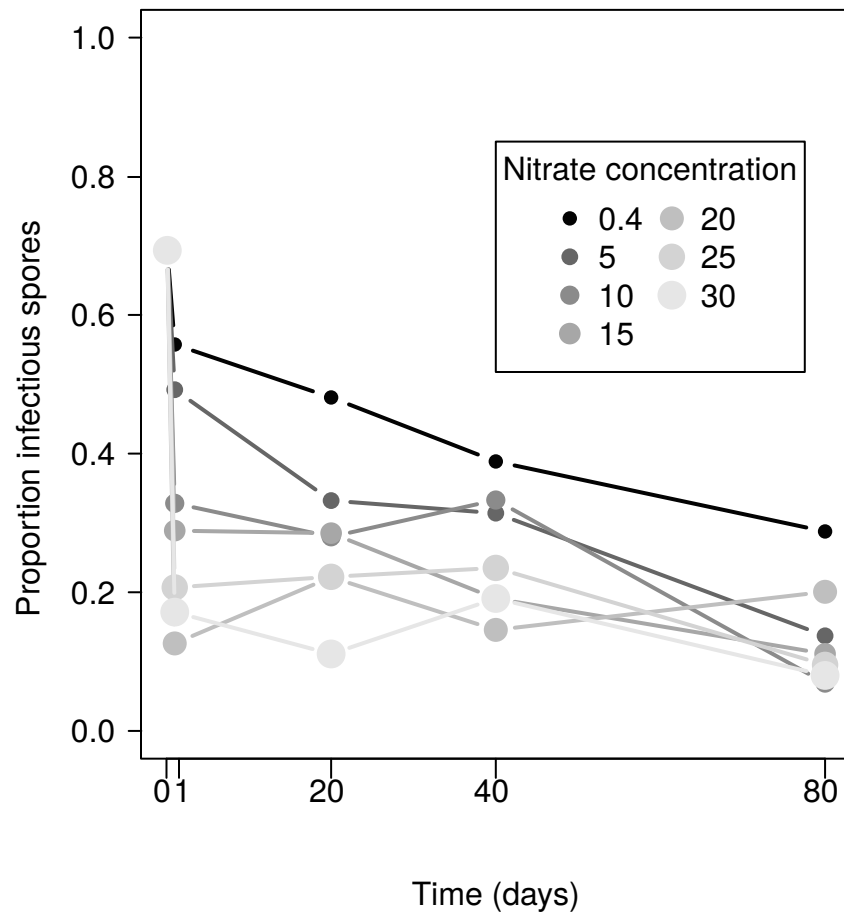


Figure 2.3: Elevated nitrate concentrations greatly reduced environmental pathogen survival after one day, suggesting that nitrate could severely reduce the size of the environmental pathogen bank, which is the the inoculum source for new infections, and may be responsible for the initiation of seasonal epidemics.

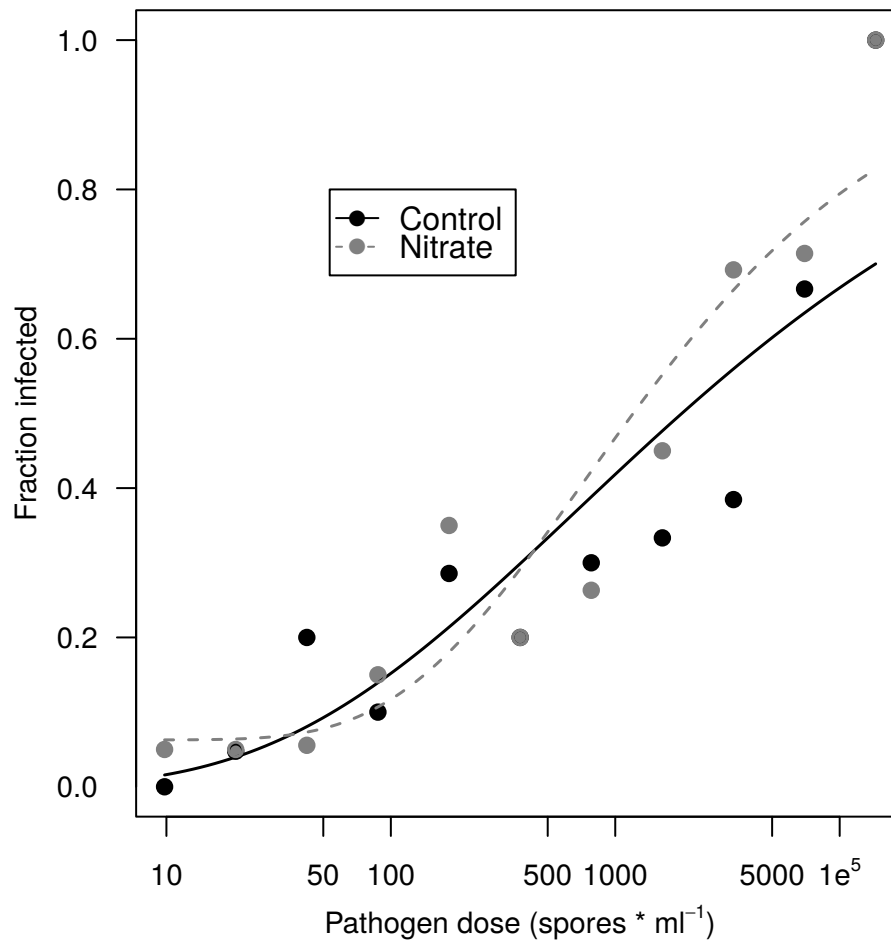


Figure 2.4: Prevalence increased with increasing pathogen dose in the typical sigmoid fashion, but there was no evidence for a difference in the shape of the dose-response relationship between individuals in control media (solid line; black points) relative to those in media containing elevated nitrate levels (dashed line; grey points). Plotted lines are model fits from a three parameter Weibull dose-response model, with the upper limit fixed at 1, determined to be the best fit model through model selection (Table 1).

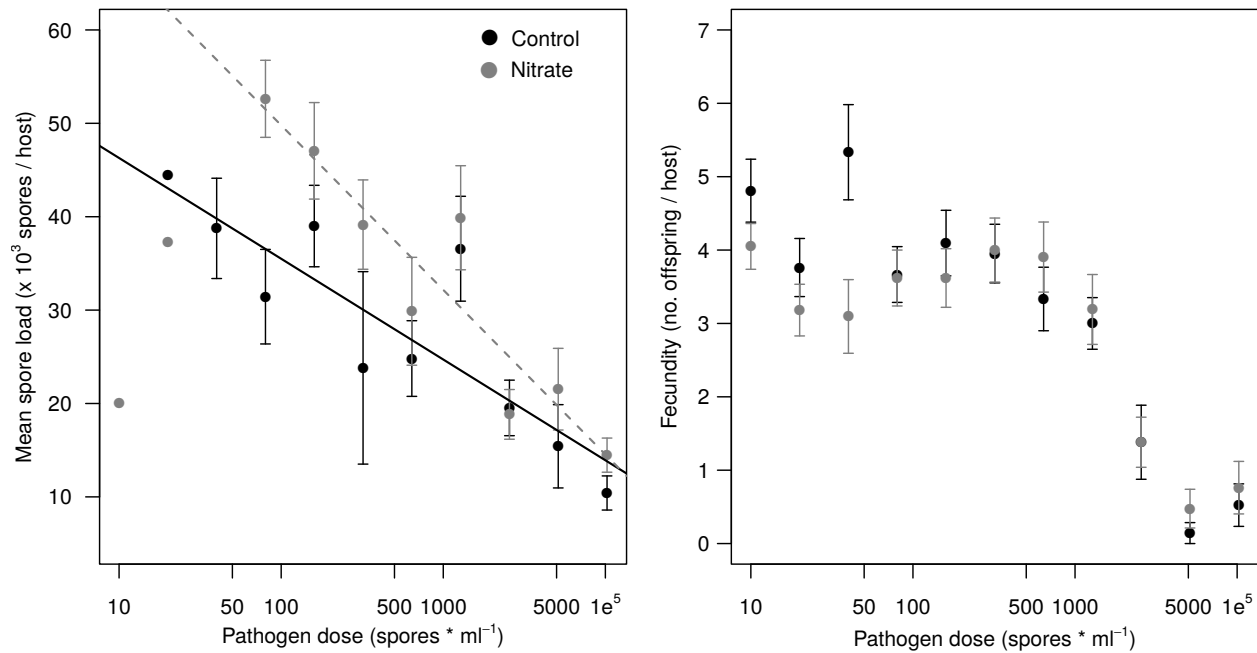


Figure 2.5: Mean spore load per infected individual declined with increasing pathogen dose (left plot), probably as a result of density-dependent pathogen competition within hosts, while individuals in nitrate media (dashed line) had higher spore loads on average than individuals in the control group (solid line). This relationship is more pronounced at lower (more realistic) pathogen doses. Plotted lines exclude dose treatments with only one infected individual. Pathogen dose decreased host fecundity, even when only considering uninfected hosts. Individuals in both nitrate (grey circles) and control (black circles) treatments did not differ in their fecundity.

Chapter 3

Temperature variability increases infection in a *Daphnia*-microparasite system

Dallas, T. & Drake, J. M., 2016 Temperature variability increases infection in a *Daphnia*-microparasite system Submitted to *Proceedings B*

Abstract

1. Environmental conditions are rarely constant, but instead vary spatially and temporally. This variation is likely to influence ecological interactions and epidemiological dynamics, yet most experimental studies examine interactions in constant conditions.
2. Here, we examined the influence of variability in temperature on the host-pathogen relationship between an aquatic zooplankton host (*Daphnia laevis*) and an environmentally-transmitted microparasite (*Metschnikowia bicuspidata*), with the goal of determining if and how temperature variability influenced infection, free-living pathogen survival, and host foraging ecology.
3. We manipulated temperature variability by exposing populations to both high and low temperatures (12 and 28 °C) for different durations of time (0, 1, 2, or 4 hours), such that the mean temperature (20 °C) remained the same among treatments. Three experiments were performed to assess the role of thermal variability on *Daphnia*-microparasite interactions. Experiments examined the impact of fluctuating temperatures on host infection prevalence and intensity, free-living pathogen survival, and host foraging ecology.
4. Temperature variability increased both infection prevalence and intensity, while reducing host population size. Further, pathogen survival did not differ as a function of temperature variability. Lastly, host filtering rate was decreased by temperature variability, counter to the expectation that filtering rate is positively related to pathogen transmission.
5. Reduced filtering rate is likely a symptom of host stress, and this effect overwhelms any effect of filtering rate on pathogen transmission. These findings suggest that *Daphnia* hosts

are more sensitive the effects of thermal variability than pathogen, and that even a modest amount of thermal perturbation will likely enhance epidemic size.

Introduction

Ecologists have long recognized the importance of temperature in influencing the strength and direction of ecological interactions [179, 202]. For instance, small changes in mean temperature over long time scales as a result of climate change affect species distributions [27], community structure [37], and ecosystem stability [10]. Ecologists have also recognized that environmental conditions fluctuate, resulting in variable environmental parameters. For instance, predation rates [20], gene expression [195], reproductive effort [181], and population stability [98] all are influenced by fluctuating environments. While the effects of mean temperature shifts on some interactions are well understood [35, 80], studies examining the influence of fluctuating temperatures on ecological interactions are rare [145, 176, 200]. While global climate models predict that mean temperatures will generally increase, they also predict an alteration to the frequency, intensity, and duration of temperature extremes, thus increasing the variability in temperature [173, 200]. This creates a clear research need, as temperature variability may be more important to shifting mean temperatures to certain ecological interactions. For instance, plant-pollinator interactions are likely to be influenced more strongly by temporal variation in temperature than in an altered mean temperature (see [167] for a review).

Host-pathogen interactions are also influenced by environmental variability [60, 121, 173]. Fluctuating environmental conditions can disrupt coevolutionary arms races (i.e. Red Queen dynamics) between host and parasite, which may have long term effects on host resistance, demography, and the rate of antagonistic coevolution between host and parasite [91]. Further, changes in abiotic variables may push a host or parasite species to a “niche edge”, where the host or parasite may have reduced survival or reproduction. Environmental stress rarely occurs

as a constant shift in mean conditions over time, but instead typically manifests as a pulse to the system, which serves to change both mean conditions and temporal variability in conditions. For instance, resource pulses have previously been linked to changes in host demography and infection dynamics in white-footed mice parasitized by intestinal helminths [150]. Variability in temperature has been linked to chytrid fungal infections of amphibians [173]. Temperature variability in particular is an important and pressing concern to animal populations and distributions [176, 200], and host-parasite interactions [1]. While many studies focus on changes in mean temperature, predicting the response of hosts and pathogens to increasingly variable temperature is an important research need [1].

The importance of changes to temperature variability relative to changes in the mean temperature has been largely overlooked (but see [176, 200]). Studies have demonstrated mixed results, as temperature variability can either reduce [60] or enhance [188] infection dynamics. This may be mediated by the effects of temperature variability on parasite emergence, development time, or transmission dynamics [97, 108, 120, 130, 148, 193], or differences in thermal tolerance ranges of host and parasite [1, 121]. If the thermal tolerance range of the host is broader than that of the pathogen, extreme hot or cold temperatures may provide a thermal refuge, where pathogen pressure is not as high [83, 132, 185]. Thermal variability may influence host behavior, feeding ecology, and survival of both host and pathogen. The sum of these effects will determine the resulting effect of temperature variability on infection dynamics. To date, few studies have attempted to determine how temperature variability influences host and pathogen populations independently, while also addressing the interaction between host and pathogen. This is especially important for environmentally transmitted pathogens, as free-living pathogen may be driving the

infection dynamics in the system, and are exposed to the same environmental conditions as the host. Lastly, previous experimental studies of temperature variability have examined a single level of variability [60], though increasing either the duration or magnitude of exposure to temperature extremes may yield interesting dynamics.

Here, we investigate the role of temperature variability using microcosm populations of an aquatic crustacean zooplankton (*Daphnia laevis*) parasitized by an environmentally transmitted fungal pathogen (*Metschnikowia bicuspidata*). We approached this interaction using three experiments to gain a mechanistic understanding of how temperature variability influences *Daphnia*-microparasite interactions. Temperature variability was examined along a gradient by altering the duration of time (either 0, 1, 2, or 4 hours) hosts or pathogen were exposed to either 12 °C or 28 °C temperature treatments. Since populations were exposed to both lower and upper temperatures, the mean temperature for all treatments is the same (20 °C). We examined three core aspects of the host-pathogen interaction. First, we examined populations of hosts exposed to pathogen and different magnitudes of temperature variability to address if temperature variability altered host demography, infection prevalence, or infection intensity. Second, we examined environmental pathogen survival as a function of temperature variability. Lastly, we examined how host foraging ecology was altered by temperature variability, as host foraging rate is directly related to pathogen transmission in the *Daphnia*-fungal pathogen system [89]. Taken together, these experiments provide evidence that temperature variability does not influence environmental pathogen appreciably, but instead acts strongly on *Daphnia* hosts, altering host mortality and filtering rate, while also influencing the interaction between *Daphnia* and microparasite, resulting in increased infection prevalence and intensity.

Methods

Host-pathogen system Our host-parasite model system consisted of *D. laevis*, a parthenogenetic crustacean grazer, parasitized by a virulent fungal pathogen, *M. bicuspidata*. Transmission of the needle-like ascospores of *M. bicuspidata* occurs when hosts ingest the spores during feeding, piercing the gut wall and proliferating in the host hemolymph, reducing host fecundity and lifespan [59, 90]. Infection by the fungus is lethal, and is horizontally transmitted from dead infected hosts. The *Daphnia* clone used in the current experiment was isolated from a small depression wetland located within the Savannah River Site (bay 40; Aiken, SC, USA). *Metschnikowia bicuspidata* was cultured *in vivo* by crushing infected *D. laevis* of this clone in deionized water. Spore concentrations were estimated using a hemocytometer and compound microscope using 200-400x magnification.

Temperature treatments A baseline temperature of 20 °C was used, as this is around the temperature range experienced by many *Daphnia* species in natural populations [90, 154]. We induced variability in temperature by exposing populations to low (12 °C) and high (28 °C) temperatures that were still well within the range of temperatures naturally experienced by *D. laevis*. Water temperatures in the Carolina bays where *D. laevis* were isolated from can experience low temperatures of approximately 5 °C and high temperatures over 30 °C [211]. Variability in temperature was altered by modifying the duration of time populations were kept at the low and high temperatures. Each day, populations were exposed to either zero, one, two, or four hours of the upper temperature, followed by the same duration exposure to the lower temperature. After high and low temperature exposure, populations were kept at 20 °C. Thus, the mean temperature across all four treatments was 20 °C. This exposure regime was repeated each day

of the experiment, using a series of incubators (Percival Scientific, Indiana, USA) to maintain temperature treatments. All three of the following experiments were subjected to this temperature treatment regime. The incubator was able to change between temperature treatments ($\Delta 8^{\circ}\text{C}$) in less than 6 minutes. Water temperature changed more gradually, taking less than 40 minutes to change between temperature treatments. This serves to reduce the duration of time hosts or parasites are exposed to the high and low temperatures, and to impose a stronger signal of temporal autocorrelation in temperature treatment (i.e. reddened environmental noise). However, both high and low experimental temperatures were reached in all treatments.

Temperature variability and host infection dynamics We first examined the relationship between temperature variability (as described above) and infection by exposing populations of susceptible hosts to free-living pathogen at each of our temperature treatments. *Daphnia* host populations ($n = 20$ per treatment) were established by placing 10 adult *D. laevis* in 50 ml of dilute (80% deionized water) EPA hardwater media [199], for a total of 80 populations and 800 individual hosts. All populations were fed 2 mg L^{-1} *Spirulina* sp. suspension each day, and kept at 12:12 L:D photoperiod. At control (20°C) conditions, all host populations were exposed to 10 *Metschnikowia* spores per ml (500 spores per population), a value determined from previous studies [32]. Experimental treatments were started two hours after host populations were initially exposed to pathogen spores. We terminated the experiment after nine days, which allows for the assessment only of primary infections, as infected hosts have not died and released pathogen spores. We quantified host population size, infection prevalence, and intensity. Infection intensity is defined here as the number of spores inside an infected host, and was quantified at the population level (i.e., the average infection intensity for an individual in each population).

All infected individuals in a population were crushed together, diluted to a known volume, and the density of spores was quantified through two replicate counts using a hemocytometer. The mean of these two independent counts was used as a measure of the mean infection intensities per infected individual in that population. We assessed the relationship between three response variables (infection prevalence, infection intensity, and population size) and temperature variability (0, 1, 2, or 4 hour exposure treatments) using linear models.

Temperature variability and environmental pathogen survival The ability of free-living pathogen to survive variable environments may enhance pathogen transmission success, especially if variable environments reduce host immunocompetence, leading to reduced infection resistance. We examined free-living pathogen survival by exposing 2 mL of pathogen spores suspended in dilute hardwater media, the same media used in host population experiment, to temperature variability treatments (0, 1, 2, or 4 hours exposure to low and high temperatures). Every other day, after pathogen populations had been exposed to their temperature variability treatments, we resuspended the spore solutions and took an 18 μ l sample. This sample was mixed with 2 μ l of 0.125 mg ml⁻¹ Propidium Iodide dye, a fluorescent dye that stains the DNA of non-living cells. Samples were allowed to incubate for 15 minutes in the absence of light before pathogen spore viability was assessed with an inverted compound microscope (200x magnification) and fluorescent filter. We counted 20 fields of view for each sample, counting the dead (fluorescent) and living (non-fluorescent) pathogen spores. Pathogen spores do not reproduce in the environment, and dead pathogen spores cannot infect. Spore survivorship was monitored for a total of 19 days. The influence of temperature variability on spore survival was tested using a repeated measures ANOVA on the proportion of pathogen spores surviving, with sampling dates serving as the

repeated measures (i.e., observations for each treatment are blocked by sampling date).

Temperature variability and host filtering rate Hosts with higher filtering rates may encounter more pathogen spores, resulting in a positive relationship between filtering rate and pathogen transmission [86]. To address how temperature variability influenced host filtering, we sequentially isolated host individuals from mothers raised singly in 50ml for at least two generations, which serves to remove maternal effects [161], to generate a cohort of individuals of a known age, between two and three days old at the start of the experiment. Experimental animals ($n = 20$ per treatment) were kept at one of the previously described temperature variability treatment levels (see *Temperature treatments* paragraph) for three days before filtering rates were assessed to allow hosts to acclimate. Animals were kept singly in 50 ml glass containers, fed 2 mg *Spirulina* dry weight L^{-1} , and kept on 12:12 light:dark cycle in incubators. For comparison, we also examined filtering rates of individuals kept at constant low (12 °C), control (20 °C), and high (28 °C) temperatures for the three day acclimation period. This enabled us to assess host filtering rates at the temperature of experimental treatment. Filtering rate trials were performed at different times for the assessment of the effect of environmental variability and a simple effect of temperature on host filtering rate. For temperature variability trials, host filtering rates were assessed at least 4 hours after daily temperature variability treatments had ended, such that all hosts were at 20 °C when filtering rates were quantified, allowing filtering rate to equilibrate to environmental conditions. However, for constant temperature treatments, we wanted to see the direct effect of temperature on host filtering rate, and assessed host filtering rates at experimental temperatures (12,20, or 28 °C).

Filtering rate was determined using the number of microspheres in both gut and gills. While

estimates of filtering rate were reduced relative to estimates from *Daphnia* fed yeast [19], they were comparable to estimates of filtering rate determined from feeding live algae [28, 48], or fluorescent microspheres [201]. In the main text, we report on filtering rate as calculated from microspheres in gut and gills, and provide further information in the Supplement. Filtering rate (ml hour^{-1}) was calculated by dividing the number of microspheres observed in a host (N) by the concentration of microspheres ($N \text{ ml}^{-1}$; B) multiplied by the duration of time hosts were exposed to the microspheres (hours; T).

$$F = \frac{N}{B * T} \quad (3.1)$$

Host filtering rate was nonlinearly related to temperature variability. To account for this, we treated temperature variability as a factor, and examined differences in filtering rates among temperature variability treatments using analysis of variance (ANOVA), followed by a Tukey HSD comparison of means. Here, we treat host filtering rate as a putative transmission mechanism, as the rate at which hosts encounter the pathogen should directly correspond to infection risk. Further, the presence of parasites is not necessary to examine host filtering rate changes as a function of temperature as a way to infer potential infection risk. This is because *Daphnia* foraging rates have been found to be insensitive to both the presence and density of parasites (see Appendix S1 of [32]).

Results

Host-pathogen interaction Higher temperature variability increased infection prevalence (adjusted $R^2 = 0.196$, $p = 0.0001$), and infection intensity (adjusted $R^2 = 0.312$, $p = 0.0003$).

Infection prevalences ranged from 0% to 30% of the population infected. Infection intensity also increased with increasing thermal variability, from around 2,000 pathogen spores per infected host to over 10,000 pathogen spores in some of the populations in the highest variability treatment. Host population size was greatly reduced in higher variability treatments (adjusted $R^2 = 0.451$, $p < 0.0001$), suggesting that temperature variability increased host mortality, reduced fecundity, or both (Figure 3.1). Population extinction was only observed in the highest variability treatment, in which 65% ($n = 13$ out of 20) of populations went extinct during the duration of the experiment. These populations were omitted from the analysis of the relationship between temperature variability and infection prevalence or intensity.

Environmental pathogen survival Environmental pathogen survival was variable over the course of the experiment (Figure 3.2). Using a repeated measures ANOVA, we found no difference in the proportion of pathogen spores surviving among temperature variability treatments ($F_{1,796} = 0.639$, $p = 0.42$), suggesting that temperature variability did not alter the environmental decay of pathogen spores. Instead, we found that environmental pathogen decayed quickly, with a half life of approximately 11 days in the absence of any predation on spores.

Host filtering rate Host filtering rate decreased as a result of changes to constant temperature (Figure S1) as well as increased temperature variability ($F_{6,31} = 6.70$, $p = 0.0006$), though the relationship between the degree of temperature variability and the response in filtering rate was not linear (Figure 3.3). Specifically, host filtering rate was strongly reduced even at the lowest level of temperature variability (± 1 hour treatment), and higher variability treatments did not further reduce host filtering rate greatly. Further, we failed to detect a difference in filtering rate

among temperature variability treatments, although all variable treatments (+1, 2, and 4 hour treatments) differed from constant (20 °C, also called +0 hours in the Supplement) controls (see Table S1). Lastly, host filtering rate was reduced both at low and high constant temperatures to comparable levels to temperature variability treatments

Discussion

Temperature variability influenced *Daphnia*-microparasite interactions. Specifically, we found that different levels of temperature variability altered host demography, host foraging rate, and pathogen infection success. The magnitude of temperature variability, experimentally manipulated by extending the duration of exposure to high and low temperatures, strongly influenced both host demography and host-pathogen interactions. Specifically, increasing thermal variability decreased host population sizes (Figure 3.1), and increased infection prevalence (Figure 3.3) and intensity (Figure 3.1). However, we find evidence that the pathogen may be more tolerant than the host to temperature variability (Figure 3.2), suggesting that pathogen pressure did not change as a function of temperature variability, but infection success increased. We hypothesized that the increased infection success was due to increased filtering rate resulting in enhanced transmission. However, we found the opposite relationship, as host filtering rate was reduced strongly in more thermally variable environments, as well as constant low (12 °C) and high (28 °C) temperature environments. Taken together, our findings suggest that fluctuating environments have the potential to strongly influence *Daphnia*-microparasite interactions, but that host filtering rate is at most of minor importance to the increased infection risk to *Daphnia* hosts.

Both variable and altered constant (12, and 28 °C) temperatures decreased host filtering rates. Previously, Hall et al. [86] suggested that lower filtering rate should result in reduced pathogen transmission, as reduced filtering rates may decrease the rate at which hosts encounter pathogen. However, reduced filtering rate is also a sign of host stress [30, 72]. Stressed hosts may have reduced ability to resist pathogen infection [116, 127], and, once infected, may not be able to reduce pathogen growth [121]. On the other hand, stressed hosts may limit parasite reproduction as a result of their poor condition [162, 189]. We find evidence for the former, as infection prevalence and intensity increased with higher temperature variability. This suggests that temperature variability may reduce the ability of hosts to resist infection, or the ability to limit pathogen growth after transmission. However, the effect of temperature (both constant and variable) on host filtering rate and infection dynamics are not compatible, as variable temperatures reduced filtering rate while enhancing infection prevalence and intensity, and high constant temperatures reduced host filtering rates (as reported previously by Burns [19]), where previously temperature has been related to enhanced infection in this *Daphnia*-microparasite system [86]. Taken together, this suggests that filtering rate alone cannot explain the influence of temperature variability on infection in our model system. Other environmental stressors have yielded similar relationships. For instance, pesticide exposure has been found to decrease *Daphnia* filtering rates [46, 70], but also to increase infection prevalence [39, 102] and intensity [102]. It is possible that stressors influence host filtering rate and host immunocompetence differently, such that transmission may be enhanced or reduced depending on the stressor examined. Further, host filtering rate is negatively related to gut residence time, or the duration of time that resources and pathogen spend in the host gut. Pathogen spores that spend a longer period of time in the gut may experience more opportunities to pierce the host gut wall and cause an infection. This effect would be lessened if

the stressor reduced pathogen survival. However, temperature variability did not influence environmental pathogen survival, such that an increased gut residence time could potentially increase pathogen transmission.

Previously, Hall et al. [90] found that microparasite infections of *D. dentifera* were temperature-dependent, as there was a positive relationship between temperature and infection prevalence in their experimental microcosms. Further, low temperatures (around 10 °C) reduced pathogen transmission so much that the pathogen was unable to cause a single infection [90]. Here, we added a layer of complexity, as variability in temperature was observed to influence infection dynamics, and filtering rate was reduced at high temperatures (Figure 3.3), putatively reducing transmission risk. It is likely that the relationship between temperature and infection dynamics is nonlinear, suggesting that Jensen's inequality may explain the increase in infection prevalence and intensity with increasing temperature variability [175]. Specifically, if pathogen transmission or host immunocompetence is a nonlinear increasing function of temperature, the mean effect of temperature on infection will be greater than infection at the mean temperature of the time series. This may explain why infection prevalence increased as a function of temperature variability.

Our results may not apply directly to natural systems. *Daphnia* migrate vertically as a response to light [170], or to avoid predators [129]. As a result of lake stratification, temperature may vary strongly with water depth [82]. Thus, it is possible that *Daphnia* will be exposed to as much or more thermal variability than we imposed in our experimental trials. Our aim in this study was not to apply these findings to natural systems, but to demonstrate how the magnitude of temperature variability could influence host-pathogen interactions. To do this, we used laboratory

experimental manipulations, which allowed control over experimental treatments which would otherwise be infeasible in manipulative field experiments. Further, the use of several manipulative experiments (e.g. experimental infections, spore survival study, and filtering rate trials) provided a clearer understanding of the influence of temperature variability on the *Daphnia*-microparasite interaction. However, if we had tried to predict the effect of temperature variability on infection using only information on host foraging reduction, we would've come to the opposite conclusion (i.e. that temperature variability reduces transmission). This highlights the balance between filtering rate increase as a pathogen encounter mechanism, and filtering rate decrease as a host stress mechanism. There may not be a clear link between host filtering rate and pathogen transmission when considering the influence of environment on infection. Here, we provide an instance where temperature variability influences the host far more than the free-living pathogen, resulting in enhanced infection prevalence and intensity. This contributes to a number of recent studies on the influence of temperature variability on infection dynamics in experimental [60] and natural [173] systems. More generally, we suggest that host-pathogen interactions may be influenced strongly by temperature variability, a commonly overlooked, though important aspect of the currently changing climate [200]. Our work highlights the importance of environmental variation on population and infection dynamics, and provides a case study in which the host is more sensitive to environmental change than the pathogen, resulting in smaller host populations and enhanced infection dynamics.

Figures

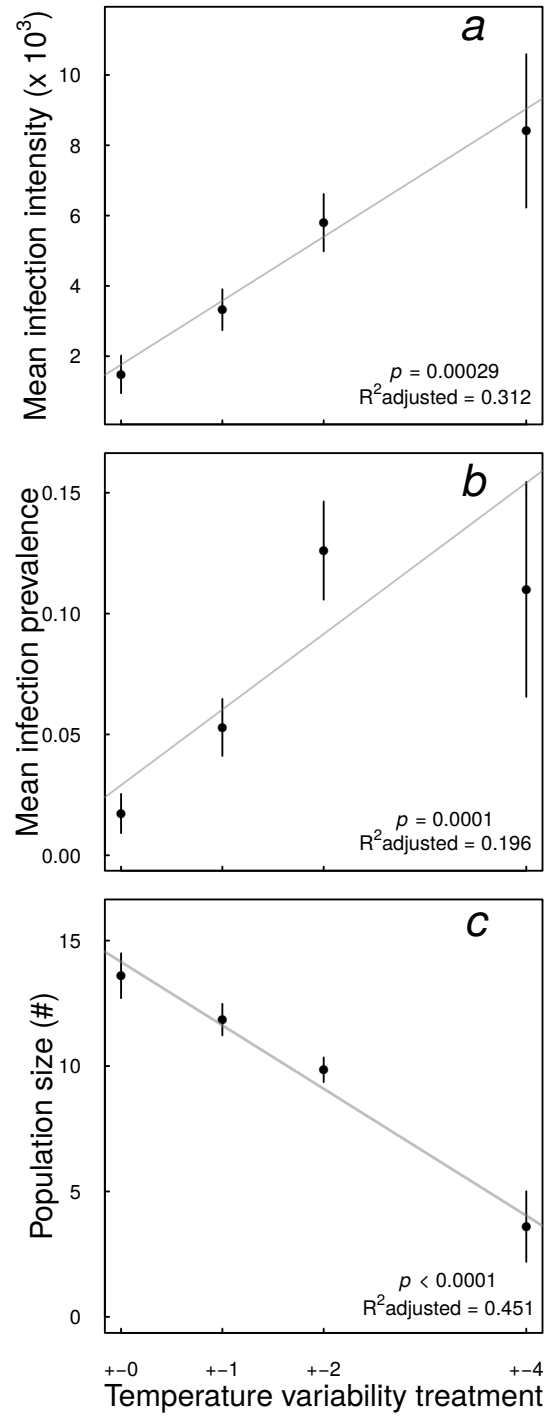


Figure 3.1: Temperature variability enhanced *Daphnia* infection intensity (number of spores inside infected host; **a**) and prevalence (fraction of hosts infected; **b**), and also reduced host population size (**c**). Plotted points are means and standard error bars. Grey lines, and within-plot text provide results for linear models fit to experimental data.

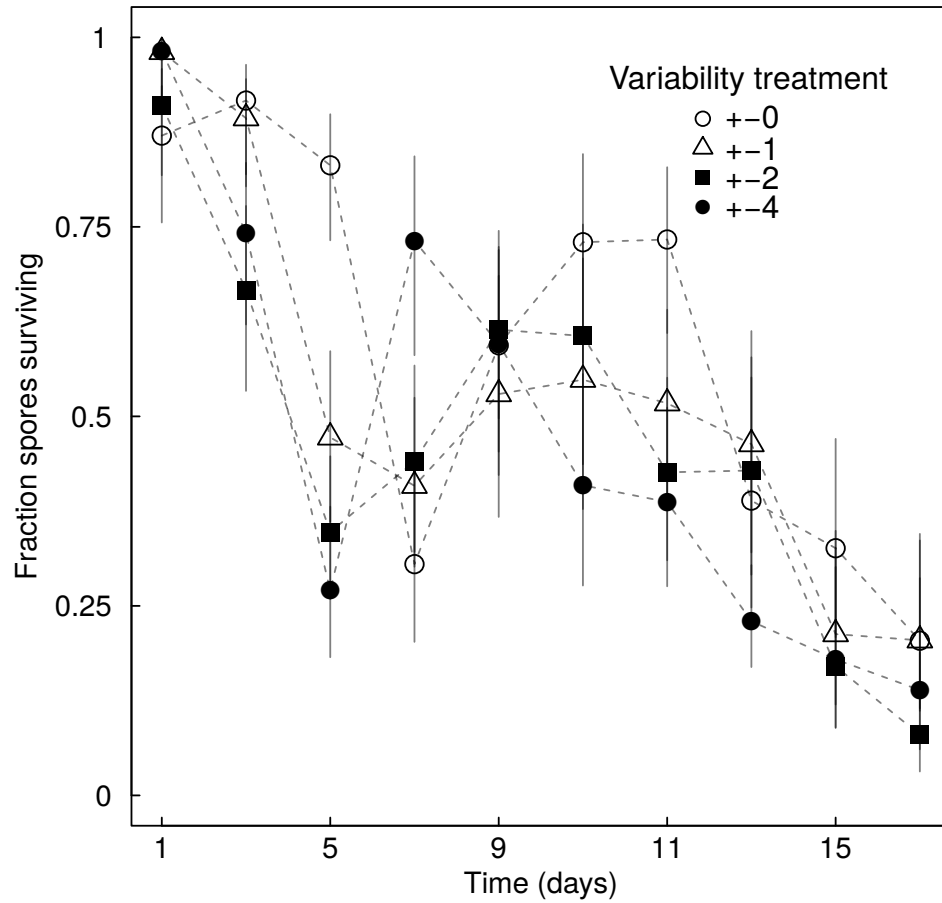


Figure 3.2: Pathogen survival was not significantly reduced as a function of temperature variability treatment, but did degrade in the environment over the course of the experiment, suggesting that a long-lived environmental pathogen bank may be unlikely in this system. Error bars represent 95% binomial confidence intervals based on replicate field of view counts of dead and alive pathogen spores.

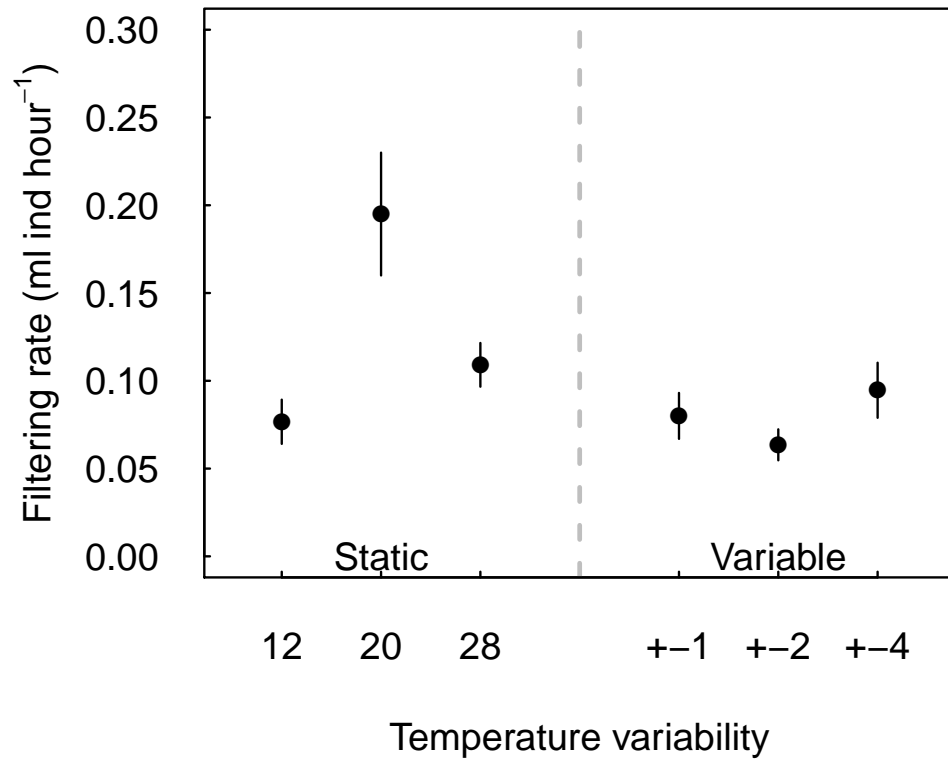


Figure 3.3: Host filtering rate was reduced as a function of temperature variability, though only the ± 0 treatment is significantly different. Host filtering rate was negatively related to infection prevalence, potentially mediated through the opposing effects of host stress on filtering rate and pathogen transmission. Plotted points are means and standard errors for both infection prevalence and filtering rates.

Chapter 4

Experimental evidence of pathogen invasion threshold in a *Daphnia*–microparasite system

Dallas, T. , Martin Krkošek, & Drake, J. M., 2015 Experimental evidence of pathogen invasion threshold in a *Daphnia*–microparasite system.

Submitted to *Proceedings B*

Abstract

Host density thresholds to pathogen invasion separate regions of parameter space corresponding to endemic or disease-free states. The host density threshold is a central concept in theoretical epidemiology and a common target of human and wildlife disease control programs, but there is mixed evidence supporting the existence of thresholds, especially in wildlife populations or for pathogens with complex transmission modes (e.g., environmental transmission). Here, we demonstrate the existence of a host density threshold for an environmentally transmitted pathogen by combining an epidemiological model with a microcosm experiment. Experimental epizootics consisted of replicate populations of naive crustacean zooplankton (*Daphnia dentifera*) hosts across a range of host densities (20 - 640 hosts L^{-1}) that were exposed to an environmentally transmitted fungal pathogen (*Metschnikowia bicuspidata*). The model, parameterized independently of the experiment, qualitatively predicted the outcome of experimental epidemics. Apart from influencing the fraction of populations invaded, host density also influenced epidemic size and peak infection prevalence in experimental epidemics. Together, we provide a demonstration of a pathogen invasion threshold in a replicated experimental system, and the ability of an epidemiological model to capture this threshold.

Introduction

Central to the study of infectious disease dynamics is the concept of a critical threshold to pathogen invasion as a function of host density (i.e., a pathogen invasion threshold), below which a pathogen is unable to invade a host population [79, 109]. This critical threshold is a commonly sought target for horticultural [79], wildlife [49], and human [11] disease control. The importance of host density to the emergence and spread of infectious disease has been demonstrated in many disease systems [45, 73, 84], but may be strongly influenced by environmental factors [123]. Apart from the influence of fluctuating environmental conditions, host density thresholds may be difficult to measure in field populations as a result of data scarcity, lack of replicated experimentation, and the effects of host ecology (e.g., behavior, social structure) [128].

Despite limited evidence for these thresholds in field populations, there is a large body of theoretical studies identifying critical thresholds for pathogen invasion using epidemiological models [103, 117, 140]. A fundamental quantity in epidemiology, the basic reproduction number (R_0), formalizes this threshold concept, providing a boundary between pathogen invasion ($R_0 > 1$) and pathogen extinction ($R_0 < 1$). The estimation of R_0 requires an epidemiological model capable of capturing host and pathogen dynamics, which is a challenging task for wildlife pathogens, or host-pathogen interactions occurring in fluctuating environments, as the environment can introduce stochasticity through changes to infectious period, host susceptibility, or host contact patterns. This challenge may be responsible for the limited support of pathogen invasion thresholds in wildlife populations [52, 128, 133, 208]. A necessary condition for a pathogen invasion threshold is the dependence of pathogen transmission on host density. This can occur simply by increased host density enhancing the probability that a host contacts an infected host or a free-

living pathogen spore. For instance, transmission of an environmentally transmitted pathogen could exhibit a threshold if hosts alter their contact patterns or feeding behavior as a function of conspecific density.

We used a *Daphnia*-microparasite system to investigate the detectability of pathogen invasion thresholds for an environmentally transmitted pathogen. This study system is ideal, as *Daphnia* are parthenogenetic (allowing for control of genetic effects), small (allowing for replicated microcosms), and well understood [61]. Previous studies of *Daphnia*-microparasite interactions have quantified many important epidemiological parameters [30, 55, 59, 65, 89], providing information on variation within key epidemiological parameters. Through a combination of replicated experimental epidemics and epidemiological modeling, we provide an experimental demonstration of a pathogen invasion threshold, and a theoretical determination of the predictability of pathogen invasion thresholds given parameter uncertainty. Specifically, we explored the sensitivity of our model results to broad ranges of several important epidemiological parameters. Taken together, these results demonstrate the existence of a critical host density threshold for an environmentally transmitted pathogen, using a combination of theoretical modeling and experimental epidemics.

Methods

Host-pathogen system We examined a model host-pathogen system consisting of a parthenogenetic freshwater cladoceran (*Daphnia dentifera*) parasitized by an environmentally-transmitted fungal pathogen (*Metschnikowia bicuspidata*). The host reproduces parthenogenetically in favorable environments, and typically produce a clutch approximately every 3-6 days after maturation, with clutch sizes varying between 5 and 20 individuals [61]. The pathogen is transmitted during

host filter-feeding, piercing the gut wall and growing inside infected hosts. During the time when the pathogen grows in an infected host, there is no pathogen shedding into the environment or host-to-host transmission. Infected hosts experience decreased fecundity and parasite-induced mortality occurs approximately 14 days after transmission [65]. Since there is no direct (host-to-host) transmission, the environment is a necessary step in the transmission process. Spores released from dead infected hosts either are ingested by susceptible hosts, or sink, and may contribute to epidemic emergence in the following year, especially if lake mixing re-suspends environmental pathogen spores.

Experimental design Populations of non-gravid adult *D. dentifera* hosts were established in 50 ml of pondwater media (25% pondwater, 75% deionized water) along an exponential gradient of host densities (20, 40, 80, 160, 320, and 640 hosts L⁻¹). Each population ($n = 15$ per treatment; $n = 90$ populations total) was fed 2 mg algal dry weight L⁻¹ of a suspension of freeze-dried pulverized *Spirulina* sp. in deionized water daily. This food concentration was constant across treatments, regardless of host density, given the logistic infeasibility and potential bias associated with feeding populations proportional to the number of individuals present. Each population was inoculated with approximately 500 pathogen spores (10 spores ml⁻¹), a value based on previous studies [30].

Populations were censused twice a week until epidemic fadeout or population extinction, using a dissecting microscope to quantify population size and infection status. Individuals were kept in media and the lowest light setting on the microscope to maximize survivorship. Dead hosts were not removed, as they may still contain small numbers of spores, and removal could impact infection dynamics. Individuals were then placed back in their original test tubes. Deionized

water was added to keep the volume in experimental populations constant. Summary statistics measuring epidemic size included the area under the infection curve, the maximum infection prevalence, and the fraction of populations infected. Area under the infection curve, hereafter referred to as epidemic size, was calculated by integrating the area under the curve of infection prevalence versus time (in days). Maximum infection prevalence was defined as the maximum fraction of hosts infected over the course of the experiment for a single population. The fraction of populations infected was considering at two time points to represent primary infection events (recorded by day 11), and secondary infections (recorded on or after day 21). Host density varied over time, which influences epidemic size and maximum prevalence, but does not influence primary pathogen invasion (infection recorded by day 11), as transmission occurred in the window where host density was experimentally determined, environmental pathogen degrades quickly [42], and there is no host-host transmission.

Epidemiological model We complemented our experimental data with a Susceptible-Infected-Pathogen (*SIP*) model to test for the existence of a host density threshold. Susceptible individuals (eq. 4.1) became infected as a function of their feeding rate (γ) and per spore infectivity (u) of the environmental pathogen (P ; eq.4.3). Infected individuals had slightly lower fecundity compared to susceptible hosts (modified by ϕ), and contributed θ pathogen spores upon death. Free-living pathogen died at rate μ , and this was the only cause of spore mortality. Parameter definitions and estimates are provided in Table 1.

The environmental pathogen population (eq. 4.3) is the sum of inputs from dead infected hosts, and losses from environmental degradation of spores at rate μ . A slightly more complicated model would incorporate loss of free-living pathogen from host foraging, as previous studies have

suggested that this could be important [31]. However, for the sake of simplicity, we present the model without spore loss through host foraging here, and provide a more detailed analysis of a model incorporating host foraging effects in the Appendix. The results are strikingly similar, and the simplified model enables us to skirt assumptions about the details of host foraging, such as the effect of gut passage on pathogen viability and the effect of fluctuations in host foraging.

$$\dot{S} = (b - d)(S + \phi I)\left(1 - \frac{S + I}{K}\right) - u\gamma SP \quad (4.1)$$

$$\dot{I} = u\gamma SP - I(d + v) \quad (4.2)$$

$$\dot{P} = I\theta(d + v) - \mu P \quad (4.3)$$

We derived R_0 from first principles, defining it as the product of the total pathogen produced by a single infected host (i.e., θ), the total number of spores consumed by hosts when $S = K = S^*$ (i.e., $\gamma u S^*$), and average free-living spore lifespan (i.e., μ^{-1}). The resulting formula (equation 4.4) is identical to the next generation R_0 (see Supplemental Material).

$$R_0 = \theta \gamma u S^* \frac{1}{\mu} = \frac{\theta \gamma u S^*}{\mu} \quad (4.4)$$

Parameter uncertainty The difficulty in measuring epidemiological parameters and the effects of environmental stochasticity can introduce uncertainty in parameter values. We used parameter estimates from our research ([42] and *unpublished data*), along with published estimates (Table 1) to determine this probabilistic range of critical threshold values. Pathogen death rate (μ) was

treated as a constant, based on data from previous studies (Table 1). We explore the influence of pathogen death rate (μ) on threshold behavior and model predictions in the Appendix (Appendix A). To explore model behavior in parameter space, we sampled values of host filtering rate (γ), per spore infectivity (u), and the number of spores produced per infected host (θ) from uniform distributions, bounded by empirical parameter estimates, except for u , for which little data was available (Table 1). These three parameters are important to pathogen transmission, as θ determines how much pathogen is present, and γ and u collectively determine pathogen transmission rate. Previous work has emphasized that θ , u , and γ can strongly influence epidemic dynamics [34, 89], and are sensitive to resource concentrations and environmental influences. Distributions of θ , u , and γ were sampled 500 times to obtain a set of possible parameters. For each parameter set, we determined whether R_0 exceeded 1 for a given host density, which generated a probability of pathogen invasion along a gradient of initial host densities. These parameter sets were also used to simulate deterministic and stochastic realizations of the model. The stochastic model was used to examine the influence of demographic stochasticity on model outcomes, while the exploration of parameter space was a way to examine the influence of environmental stochasticity.

Model simulations For each of the above parameter combinations, we simulated deterministic and stochastic realizations of our model. Pathogen death rate was treated as deterministic. The stochastic realization was computed with the next reaction method [78, 109] as implemented in the `adaptivetau` R package [105]. Deterministic and stochastic realizations of the model were run for a total of 11 time steps (i.e., days) which corresponds to the invasion window examined in our experiment. For comparison with experimental results, the criterion for pathogen invasion

in these simulations was that at least one individual had to have become infected by the end of the simulation. The probability of pathogen invasion was determined by calculating the fraction of parameter value combinations that resulted in one infected individual by day 11 divided by the total number of simulations, repeated for each initial host density. We also obtained an analytical solution (dark green region in Figure 4.2) to the probability of pathogen invasion from the stochastic model (see Appendix A).

Results

Experimental epidemics The fraction of populations infected, epidemic size (area under the infection curve), and maximum infection prevalence all increased when host density exceeded a breakpoint of 80 hosts L^{-1} , corresponding to a lower critical threshold between 40 and 100 hosts L^{-1} (Figure 4.1). When the pathogen did successfully invade, distinct waves of infection were observed (see Appendix A), a result of the latent period after pathogen transmission but before pathogen-induced host mortality and the spread of infectious spores. Demographic stochasticity could prevent secondary transmission as a result of population extinction, a process that could also influence epidemic dynamics. Population extinction was not strongly influenced by initial host population size (see Appendix Figure A3). Temporal changes in host density were related to initial host density, in which larger initial host populations maintained lower population sizes over time, potentially as a result of infection (Appendix Figure A4). Host feeding rate is positively related to transmission risk for this environmentally transmitted pathogen. At high host densities, hosts may suppress their feeding, which could reduce epidemic size and perhaps even create an upper host density threshold to pathogen invasion [31]. We found limited support for the existence of

this upper host density threshold, though the prevalence of primary infections was linearly related to host density (see Appendix A).

Epidemiological model Pathogen invasion thresholds predicted by the deterministic model, stochastic simulations, and analytical solution of the stochastic model were qualitatively similar to the pathogen invasion threshold from experimental epidemics. Specifically, the deterministic and stochastic simulations largely overlapped with the binomial confidence intervals from the experimental epidemics, in which the experimental-determined pathogen invasion probability appears to be a combination of the results from the deterministic and stochastic model predictions. The potential range of invasion thresholds (Figure 4.2) is a result of the degree of environmental stochasticity considered in terms of the broad parameter space we explored with respect to host filtering rate (γ), per spore infectivity (u), and the number of spores per infected host (θ). However, the mean model-predicted pathogen invasion threshold matches the experimentally-derived estimate. Observed differences between deterministic and stochastic realizations of the model are likely a result of demographic stochasticity, and the integer-valued nature of the stochastic process. Specifically, the model incorporating demographic stochasticity considers events instead of fractions of events (discussed in more detail in Appendix A).

Parameter uncertainty Some parameters are difficult to estimate in this experimental disease system. For instance, a range of estimates for environmental pathogen survival (μ) have been used ranging from 0.25 to 0.9 [42, 88, 90]. We explore the influence of pathogen survival on the pathogen invasion threshold in the Appendix A, finding that the position of the invasion threshold may be strongly influenced by environmental pathogen survival. Other difficult parameters

include per spore infectivity (u), and host filtering rate (γ) which combine to form the pathogen transmission term. Through our exploration of parameter space for u , γ , and θ , we provide a framework with which to investigate how infection dynamics may change as environmental forces or genetic variability in alter these important epidemiological parameters.

Discussion

We demonstrated the existence of a critical threshold to pathogen invasion for an environmentally-transmitted pathogen, using experimental microcosm populations of *Daphnia* exposed to a fungal microparasite, combined with an epidemiological model. Predictions for the critical threshold obtained from deterministic and stochastic realizations of our epidemiological model complemented experimental findings, suggesting that the critical threshold to pathogen invasion can be estimated by an epidemiological model. Pathogen invasion thresholds may depend on environmental context, which we incorporated by allowing uncertainty in estimates of host filtering rate, per spore infectivity, and number of pathogen spores produced by an infected host (and pathogen death rate in Appendix A). The range of pathogen invasion thresholds obtained by exploring the parameter space suggests that small perturbations to infection parameters can strongly influence invasion thresholds. Taken together, we provide empirical evidence for a critical threshold for pathogen invasion in an environmentally-transmitted pathogen, and present deterministic and stochastic epidemiological model realizations that capture important features of experimental epidemics.

Understanding the factors contributing to pathogen emergence remains a key goal of disease ecology and epidemiology. While previous studies have examined pathogen invasion thresholds for

directly transmitted pathogens [157], critical thresholds in environmentally transmitted pathogens are far less well understood [15, 16, 172]. The *Daphnia* host-pathogen model developed in this paper may be generalizable to other environmentally-transmitted pathogen systems. For instance, an extension of our model could consider a pathogen that reproduces in the environment, or that is able to be transmitted through direct host contact and through the environmental source [8]. Some environmentally-transmitted pathogens may have dynamics similar to directly-transmitted pathogens if pathogen is short-lived and has limited dispersal [41]. The host-pathogen system we examined is characteristic of this situation (see resulting waves of infection in Supplementary Figure A5), in which transmission occurs in a small window immediately following host death, and environmental pathogen is relatively short-lived [42]. Natural daphniid populations do not typically attain densities as high as some of the densities we examined here (but see [30]). However, the application of this framework to field populations is strongly hampered by the infeasibility or impossibility of measuring pathogen propagule pressure, and obtaining data on failed pathogen invasions. Controlled experimental studies may be able to provide predictions for natural systems (see below for further discussion). Further, despite agreement between model and experiment, the proposed model does not consider feedbacks between host density and model parameters. For instance, host density can influence host filtering rate [96]. Also, we were unable to duplicate the wave-like infection dynamics observed in natural systems, as our model does not incorporate latent periods between transmission and spore release. However, the goal was not to capture the bursty nature of fungal epidemics, but to determine the process of pathogen invasion.

Our experimental demonstration of a core concept in epidemiology provides a platform to study how shifting environments, species interactions, and pathogen pressure influence the pathogen

invasion threshold. Evidence that invasion thresholds may shift as a function of environmental context is provided by the range of threshold values observed for the wide range of parameter space sampled in our model, including many key infection parameters (u , γ , θ , and μ). This generates a number of open questions concerning how environmental covariates, species interactions, or genetic factors may influence this critical threshold. An understanding of how pathogen emergence may be influenced by environmental context is an important research area, and controlled microcosm studies offer a way to generate predictions for pathogen emergence in wildlife and human disease systems.

Tables

Table 4.1: Parameters used in our epidemiological model. Ranges are given for γ , u , and θ , three key parameters influencing R_0 .

Parameter	Units	Definition	Value	Citation
b	day^{-1}	Host birth rate	0.45	[42, 194]
d	day^{-1}	Host death rate	0.15	[42]
k	#	Host carrying capacity	$\frac{(S+I)*b}{b-d}$	–
ϕ	–	Fecundity reduction by infection	0.75	[55]
u		Per spore infectivity	0.0005 – 0.0075	[31, 86]
γ	L ind $^{-1}$ day^{-1}	Host filtering rate	0.0015 – 0.015	[19, 152]
θ	#	Mean spore load per infected host	5×10^3 – 1.5×10^4	[42, 88]
v	day^{-1}	Pathogen induced host mortality	0.05	[59]
μ	day^{-1}	Death rate of free-living pathogen	0.75	–

Figures

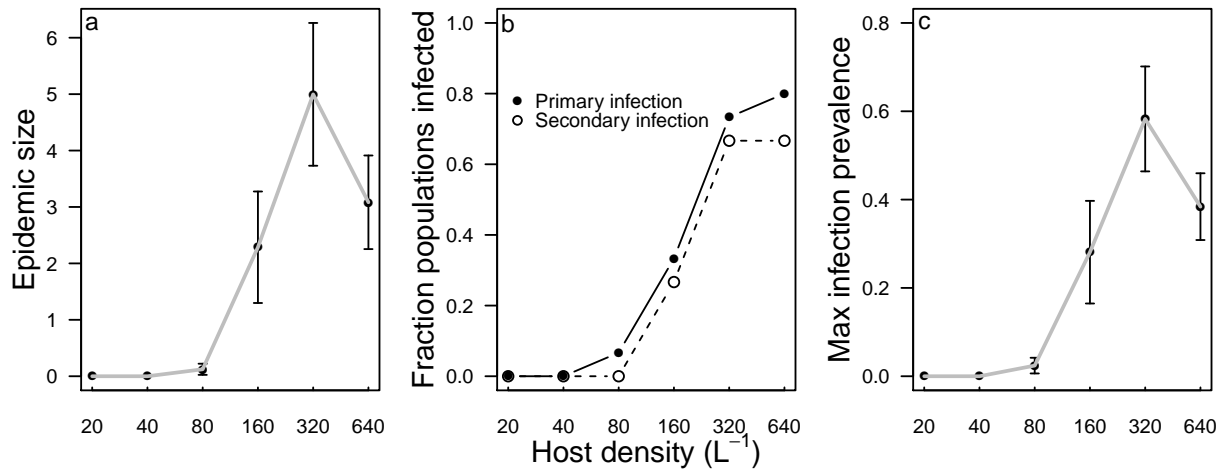


Figure 4.1: Host density strongly influenced infection dynamics and pathogen invasion in experimental epidemics, evidenced by changes in epidemic size (area under the infection curve ; panel a), fraction of populations with primary (filled circles; infection at day 11) and secondary (open circles; infection on or after day 21) infections (panel b), and maximum infection prevalence (panel c).

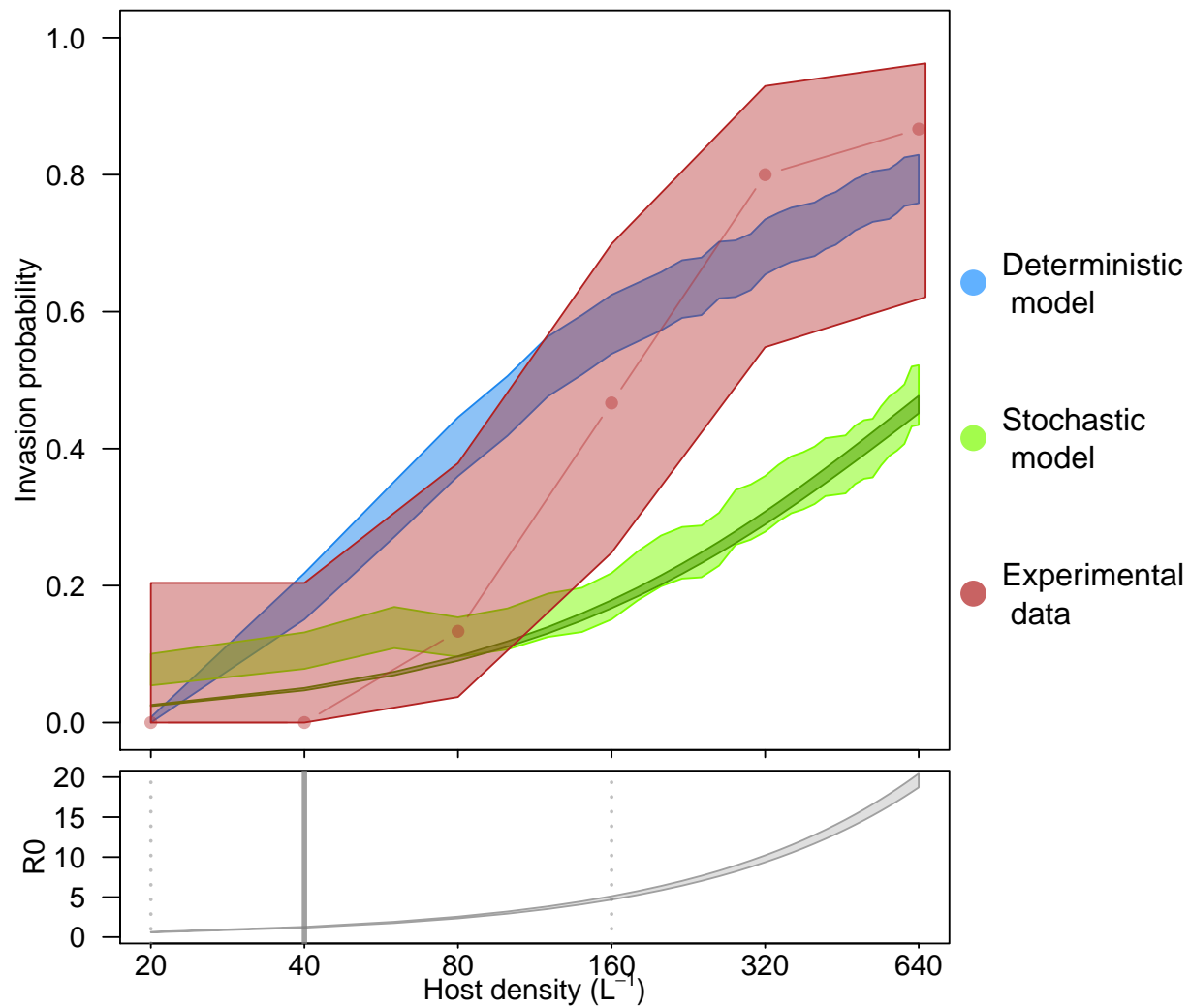


Figure 4.2: The probability of pathogen invasion (upper panel) for deterministic (blue shaded region) and stochastic (green shaded region) model realizations compared to data from experimental epidemics (red points), and associated basic reproduction number calculated from our epidemiological model (lower panel). Shaded regions represent 95% binomial confidence intervals. The darker green shaded region in the upper panel is the analytical solution of the pathogen invasion probability for the stochastic model. Grey lines in the lower panel correspond to the fraction of parameter value combinations resulting in invasion ($R_0 > 1$) for 25% (left dashed), 50% (center solid), and 75% (right dashed) of simulations.

Chapter 5

Competition-mediated feedbacks in experimental multi-species epizootics

Dallas, T., Hall, R. J., & Drake, J. M., 2016 Competition-mediated feedbacks in experimental multi-species epizootics. *Ecology* <http://dx.doi.org/10.1890/15-0305.1>

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Abstract

Competition structures ecological communities and alters host-pathogen interactions. In environmentally-transmitted pathogens, an infection-resistant competitor may influence infection dynamics in a susceptible species through the negative impacts of competition (e.g., by reducing host density or causing nutritional stress that increases susceptibility to infection) and/or the positive impacts of reducing transmission efficiency (e.g., by removing environmental pathogen stages). Thus, a non-susceptible competitor may enhance, reduce, or have no net effect on susceptible host density and infection prevalence. Here, we couple an epidemiological model with experimental epidemics to test how resource competition with a non-susceptible competitor (*Daphnia pulicaria*) influences fungal microparasite (*Metschnikowia bicuspidata*) infection dynamics in a susceptible host species (*D. dentifera*). Our model and experiments suggest that competitor density can mediate the direction and magnitude of the effect of competition on infection dynamics, with a peak in infection prevalence occurring at intermediate competitor densities. At low densities, the non-susceptible competitor *D. pulicaria* may reduce infection prevalence in the susceptible host by removing fungal spores from the environment through feeding. However, when competitor density is increased and resources become limiting, *D. pulicaria* negatively impacts the susceptible host by increasing susceptible host feeding rates, and therefore fungal spore intake, and further by reducing susceptible host population size as it is driven towards competitive exclusion. In conclusion, these results show that a tradeoff between the competitor as a consumer of pathogen, which serves to reduce epidemic size, and as a modifier of susceptible host foraging ecology, which influences infection rates, may alternately enhance or dampen the magnitude of local epidemics.

Introduction

Competition is a major structuring force of ecological communities, and the strength of interactions between competitors can determine whether coexistence or competitive exclusion occurs [4, 203]. Further, environmental conditions can influence the outcome of competition, as competitive outcomes can be shaped by predators [206], temperature [24], resource availability [168], or natural enemies [47]. Studies of competition typically focus on the long-term or equilibrium dynamics of competing species [204], but many interesting competitive interactions occur when populations are not at equilibrium, or are in the transient period before competitive coexistence or exclusion [153]. Epidemic pathogens or parasites are a prime example of this, as they disrupt host population dynamics, and can influence transient population dynamics and competitive outcomes. Pathogens may mediate the interactions between competing species by disproportionately affecting one of the competitors [159], or by altering aspects of host life history such as development time or dispersal [197]. While the influence of parasites on competitive interactions has received ample attention [95, 146, 158, 159], there have been few studies into the role of competitors on parasite populations [88].

Competitors may differ in competence [111] and susceptibility to pathogen infection [89], such that the addition of a competitor can reduce, enhance, or have no net effect on infection dynamics in another competing host. For instance, a competitor that is a more competent host species could increase parasite population size, which would potentially elevate infection rates of the other competing host. However, if the competitor is a less competent host, or if the effect of competition reduces potential pathogen transmission events to the other competitor, infection

risk may be reduced. The effect of competition therefore may offer a mechanistic explanation for some cases of the relationship between host diversity and disease risk (the “diversity-disease relationship”).

The diversity-disease relationship proposes that increases in host diversity may reduce (i.e. a dilution effect) or enhance (i.e. an amplification effect) infection risk in a focal host species [143, 144]. In theory, dilution effects may arise for many different reasons, but generally, the non-focal species are considered to be subject to “wasted” transmission events, so that pathogen fitness is reduced by infecting a less suitable host [110]. Studies of the dilution effect are typically phenomenological [177], and do not incorporate ecological interactions among species in the community. The inclusion of ecological interactions (e.g., competition) into studies of multi-host pathogen dynamics may inform a general theory for when we expect host diversity to reduce or enhance disease. Here, we use a combination of modeling and experiments to provide a link between diversity-disease relationships and parasite-mediated competition. Specifically, we investigate the impact of the addition of a non-susceptible superior competitor that consumes environmental pathogen on the infection and population dynamics of a susceptible host species.

To do this, we use a model system comprised of two sympatric zooplankton competitors, *Daphnia dentifera* and *Daphnia pulicaria*. These two species have been found to co-occur in the north temperate lakes of the United States [54]. *Daphnia dentifera* is susceptible to infection by an environmentally-transmitted yeast pathogen (*Metschnikowia bicuspidata*), and is also an inferior competitor to *D. pulicaria*, as *D. pulicaria* has larger body size, reproductive rate (un-

published data), and foraging rate [81]. Further, *D. pulicaria* has been found to outcompete *D. lumholtzi*, a formidable invasive competitor [68]. While *D. pulicaria* does not become infected, it does consume pathogen spores during foraging, potentially reducing pathogen transmission to susceptible hosts (so-called “friendly competition” ; Hall et al. [88]). Reduced resources can nutritionally stress susceptible hosts, which can result in enhanced pathogen transmission [162] as a result of increased filtering rate [89], providing a mechanistic link between host foraging ecology and pathogen transmission. Therefore, the impact of competition on infection dynamics will depend on the tradeoff between the role of the competitor as a consumer-of-pathogen and as a consumer-of-resources.

Previously, Hall et al. [88] performed an experiment in which *D. pulicaria* were allowed to graze on pathogen spores, and then this media was exposed to susceptible *D. dentifera* to see if *D. pulicaria* grazing could reduce transmission by depleting environmental pathogen spores. However, this study did not account for the role of the competitor (*D. pulicaria*) as a consumer. By reducing algal resources, the competitor may indirectly influence susceptible host foraging rate, which is intrinsically linked to pathogen transmission in this system. A theory for this complex of interactions was recently developed by Cáceres et al. [21], who examined the equilibrium outcomes of competition between a susceptible and a non-susceptible competitor, finding competitive exclusion of the susceptible host species over long time scales when the non-susceptible species is a superior competitor. While previous studies of parasite-mediated competition have focused on directly transmitted pathogens, and superior competitors that are also susceptible to parasitism [159, 160], we focus on an environmentally-transmitted pathogen, and the interaction between

a dominant competitor that does not become infected and an inferior susceptible competitor. Theory predicts competitive exclusion of the inferior competitor in the long-term, although these species coexist in natural systems, most likely through niche partitioning or complex community interactions. We focus on the transient period where both species coexist, and examine infection dynamics as a result of competition for a limiting resource altering exposure to an environmentally transmitted pathogen. These transient dynamics are important, and ecologically relevant, given that the seasonal fluctuations in both zooplankton population sizes and infection dynamics may preclude zooplankton populations from achieving equilibrium dynamics [99, 183].

Using a modified version of Cáceres et al. [21] model that more closely matches our experimental system (see Appendix B) for a comparison of our model to Cáceres et al. [21]), we extend this theory by examining the influence of competitor density on epidemic and population dynamics under non-equilibrium conditions. By examining the transient dynamics of our theoretical model, we generate several testable model predictions, and experimentally test these predictions using the same zooplankton-pathogen system examined by Hall et al. [89]. First, we predict that the extent to which the competitor enhances or reduces infection prevalence in the susceptible host will depend on the initial density of the competitor and the availability of algal resources. When resources are limiting, we predict that the competitor will enhance infection prevalence in the susceptible host species by enhancing susceptible host foraging rate, and subsequent pathogen transmission. Second, we predict that susceptible host population size will decrease as a function of competitor density, since the susceptible host will be excluded more rapidly at higher densities of the superior competitor. This reduction in susceptible host population size may reduce infection

prevalence if contact with pathogen (and therefore transmission) is reduced, or increase infection prevalence if susceptible host filtering rate is increased as a response to reduced resources. To test these hypotheses, we parameterized our epidemiological model, and compared model outputs with experimental epidemics. Experimental epidemics were initiated at three competitor densities, as increased competitor density serves to reduce resource availability through exploitative competition. We found that competition rarely benefited the susceptible host species, either enhancing infection prevalence at intermediate competitor densities, or competitively excluding the susceptible host at high competitor densities. This work highlights the importance of competitive interactions in evaluating the direction of diversity-disease relationships.

Methods

Study system Clonal lines of two sympatric freshwater cladocerans were used in this study, *D. dentifera* (provided by M. Duffy) and *D. pulicaria* (originally isolated from Oneida Lake, New York, and provided by N. Hairston Jr.). *Metschnikowia bicuspidata* is a fungal pathogen that infects *D. dentifera*, but not *D. pulicaria*. Pathogen transmission can occur when the host ingests the pathogen, allowing the pathogen to pierce the gut wall and grow within the host. Parasite-induced mortality causes the release of a multitude of infectious spores (see Table 1), which are then filtered and ingested by other hosts. Recent studies have found essentially no genetic variation in the fungal pathogen, reducing the likelihood of genotype \times genotype interactions [186]. However, genetic variation within natural *D. dentifera* populations could influence pathogen transmission dynamics through heterogeneity in resistance, or spore production per host [6, 23]. We acknowledge this as an interesting avenue for further research. However, our focus is to

elucidate patterns in infection dynamics due to competitor density. Therefore, we selected a single, well-studied *D. dentifera* clone with moderate susceptibility to infection [42] for our experiments. Model sensitivity analyses (see Appendix B) further suggest that our qualitative results are robust to variation in plausible ranges of host infection parameters.

Epidemiological model To examine the impact of a competitor on susceptible host infection dynamics, we used a two-host compartmental model, where the susceptible host species may be uninfected (S) or infected (I) by an environmentally transmitted fungal pathogen (with free-living spore population size P). This susceptible host species competes for resources (R) with a non-susceptible competitor (C). The model was formulated to correspond directly to the experimental treatments, allowing for the testing of model predictions with experimental data. The demographic and epidemic dynamics are described by the following system of differential equations:

$$\dot{S} = e_S f_S(R) R (S + I \phi) - \mu_S S - u f_S(R) S P \quad (5.1)$$

$$\dot{I} = u f_S(R) S P - \mu_I I \quad (5.2)$$

$$\dot{P} = \theta \mu_I I - \mu_P P - z_S f_S(R) (S + I) P - z_C f_C(R) C P \quad (5.3)$$

$$\dot{R} = \pi - \mu_R R - f_S(R) (S + I) R - f_C(R) C R \quad (5.4)$$

$$\dot{C} = e_C f_C(R) RC - \mu_C C \quad (5.5)$$

Susceptible (S) and competitor (C) populations grow proportionally to the rate at which individuals can acquire ($f_j(R)$, $j = S, C$) and assimilate (e_j , $j = S, C$) resources, die at rate μ_S (susceptible) or μ_C (competitor), and become infected at a rate determined by their filtering rate ($f_j(R)$, $j = S, C$) and a per spore infectivity parameter (u). Filtering rates depend on the density of algal resources (R) [21, 86] and filtering alters the rate of environmental pathogen and resource loss from the system, as well as the transmission of pathogen. Infected (I) individuals still produce susceptible offspring, but at a rate reduced by ϕ . Infected individuals die at rate $\mu_I > \mu_S$ to account for pathogen-induced mortality (i.e. virulence). Upon death, hosts release a burst of pathogen spores (θ) to the environmental pathogen bank. Environmental pathogen (P) decays at a constant rate μ_P , and is also ingested by susceptible (S), infected (I), and competitor (C) individuals at rates determined by their corresponding filtering rates ($f_j(R)$, $j = S, C$), and a parameter which determines the fraction of spores ingested that are rendered non-infectious after passage through the host gut (z_j , $j = S, C$); this matches observations of spore survival after bluegill feeding [53], and *Daphnia* hosts exposed to *Pasteuria ramosa*, a bacterial parasite [112]. Resource (R) is introduced at a constant rate (π), and decays at a per capita rate μ_R plus additional decay as a function of host foraging ($f_j(R)$, $j = S, C$).

While the exact relationship between algal resource concentration and *Daphnia* filtering rate is unclear, evidence suggests that clearance rate is negatively related to algal resource quantity,

such that it is highest when algal resources are limiting [86, 89, 156, 180]. Therefore, we use a type II functional response for filtering rates (Eq. 5.6 and 5.7). To establish the competitor as dominant, we increased the competitor's assimilation coefficient (e) and maximum filtering rate (f_{C0}) relative to the susceptible host species, reflecting the biology of the system, as the competitor is a larger-bodied grazer with an elevated filtering rate, larger clutch sizes, and faster growth. The equations for host species and competitor filtering rates are provided below, where f_{S0} and f_{C0} are the maximum filtering rates at low resource availability for susceptible and competitor species respectively, and f_{S1} and f_{C1} determine how rapidly their foraging rates decline in response to increasing resource availability.

$$f_S(R) = \frac{f_{S0}}{1 + f_{S1}R} = \frac{0.02}{1 + 4R} \quad (5.6)$$

$$f_C(R) = \frac{f_{C0}}{1 + f_{C1}R} = \frac{0.025}{1 + 4R} \quad (5.7)$$

The pathogen basic reproduction number (R_0) is a threshold quantity determining pathogen invasion. We provide it to highlight the effect of the opposing forces of spore removal through foraging (f_S and z_S), and spore creation through pathogen transmission (u) and infected host death (θ). For our system, this can be expressed as

$$R_0 = \frac{\theta u f_S(R_S) S^*}{\mu_P + z_S f_S(R_S) S^*}$$

with the derivation outlined in Appendix B.

Parameter definitions, units, and details of the parametrization are provided in Table 1; parameter values were obtained largely from the published literature. To account for uncertainty in some parameter estimates, and to investigate the generality of the simulation results, we performed a sensitivity analysis (see Appendix B). We solved this model numerically for a range of initial competitor densities (0 - 100 hosts L⁻¹). Simulations were initiated with 30 susceptible hosts, no infected hosts, and 10000 pathogen spores. Simulated epidemics were run for 70 days, corresponding to conditions in experimental epidemics. From epidemic simulations, we calculated mean infection prevalence (i.e., average infection prevalence over 70 day time series), and mean susceptible host density as our response variables to changes in competitor density.

Experimental epidemics To test our model predictions, we devised a mesocosm experiment where we manipulated competitor density as a means to modify resource availability, and therefore the effects of competition. Experimental populations were formed by dividing five gallon glass aquaria (16" x 8" x 10") in half, separating the two sides of the aquaria with partitions of 210 μ m Nitex mesh, and filling the tank with 6 L of media; a combination of 2 L filtered pondwater (30 micron filter) and 4 L deionized water. Species were separated by this mesh partition, which allowed for the flow of resources and pathogen spores, but restricted movement of individuals, thereby isolating the effects of resource competition (i.e. exploitative competition) from any

direct interaction (i.e. interference competition), and removing any confusion identifying *Daphnia* neonates to species.

Resource competition was produced by altering the density of *D. pulicaria* and restricting algal resources. Every day, each half of experimental mesocosms was fed 1 mL of a solution of 200 mg freeze-dried, pulverized *Spirulina* sp. suspended in 100 mL deionized water. We fed both partitions of the aquaria the same amount to ensure that resources were well-mixed between halves of each tank, and that the resource concentration throughout the aquaria was approximately 0.67 mg algal dry weight L^{-1} . Five mesocosms were formed for each of three initial *D. pulicaria* densities (0, 30, and 100 individuals L^{-1}) for a total of fifteen aquaria. Populations of *D. dentifera* were established in each of the fifteen experimental aquaria at a density of 30 individuals L^{-1} at the start of the experiment. Competitor densities were chosen based on our susceptible host density, where the 30 individual L^{-1} treatment corresponds to both species starting at equal densities, and the 100 competitors L^{-1} corresponding to a case where the competitor dominates the community. Both sides of the aquaria were inoculated with 10 *Metschnikowia* spores mL^{-1} one day after populations were established.

Mesocosms were sampled every 3-4 days until infection was no longer observed, which was after 70 days. We assessed infection prevalence and host density by stirring tanks and taking a 1 L water sample from each partition of each aquarium. Infection was assessed by visual inspection using a dissecting microscope (10 \times - 40 \times) under low light and keeping hosts in a minimal amount of water to reduce host mortality. Hosts are translucent, and opaque pathogen clusters are present in host heart or gills approximately one week after pathogen transmission. Hosts were returned

to their respective aquarium. Sampling with replacement is ideal in this experiment, as spores that infected hosts liberate upon death drive subsequent infections in natural systems; removal of infected individuals would artificially reduce epidemic size or duration.

We analyzed the influence of competition on epidemic dynamics and host density. To examine epidemic dynamics in *D. dentifera* in response to competition with *D. pulicaria*, we calculated two quantities meant to capture aspects of epidemic size and duration: mean infection prevalence, and epidemic duration. Mean infection prevalence was quantified as the fraction of *D. dentifera* infected averaged over the total number of sampling points in which the susceptible host population persisted. Epidemic duration was defined as the number of days epidemics had non-zero prevalence. These measures were compared among initial competitor density treatments using Kruskal-Wallis tests. These tests addressed the influence of competitor density on infection dynamics and epidemic duration. Kruskal-Wallis tests were also used to investigate the relationship between the time until *D. dentifera* population extinction and initial competitor density, which addressed the influence of competitor density on susceptible host demography and extinction dynamics. While it is possible that very small populations would not be detected in our 1 L sample, population extinction was noted only when a sample contained no hosts, and a visual inspection of the tank confirmed no living *D. dentifera* hosts.

Results

Comparison of model and experiments Equilibrium analysis of the model (see Appendix B) demonstrated that in the long term, *D. dentifera* would be excluded by *D. pulicaria*, and indeed our experimental populations went extinct within 70 days. Our epidemiological model

revealed some outcomes that were not observed in our experimental epidemics. For instance, by examining numerous algal resource input values (Figure 5.1 and 5.2), we found that the theoretical hump-shaped relationship between initial competitor density and infection prevalence in the susceptible host species was not strongly influenced by resource availability. Consideration of the pathogen basic reproductive number in the absence of the competitor suggested that the addition of a competitor could enhance or reduce epidemic risk through antagonistic effects of increasing the filtering rate (and the chance of pathogen exposure) while simultaneously reducing the number of susceptible hosts and infectious propagules. Further exploration of the conditions where competition could reduce or enhance epidemic risk is outlined in the sensitivity analysis section of Appendix B. Overall, this effort suggested that the hump-shaped relationship between competitor density and infection prevalence observed in both our experimental epidemics and epidemiological model is robust to a range of parameter values. The range of parameters in which competitor density strictly reduces infection prevalence is small, and corresponds to situations in which the competitor digests a much larger proportion of spores than the susceptible host, or when susceptible hosts produce too few infectious spores to result in sustained transmission. Infection prevalence in the susceptible host species increased when competitors were first added to the system until a threshold was reached, and then declined. When resources were less limiting, competitors were able to reduce infection prevalence in the susceptible host more strongly, and mean susceptible host population sizes were larger (Figure 5.2).

Competitor density and susceptible host epidemic dynamics Experimental epidemics were qualitatively similar to predictions derived from our epidemiological model (Figure 5.1), despite independent parameterization of the epidemiological model. The first testable prediction from

our theoretical model was that prevalence has a hump-shaped relationship with the initial density of the competitor species. In our experimental epidemics, competitor density had a strong effect on susceptible host species infection dynamics (Figure 5.3 and 5.4). Mean infection prevalence (Kruskal-Wallis test; $\chi^2 = 6.74$, $df = 2$, $p = 0.034$) and epidemic duration (Kruskal-Wallis test; $\chi^2 = 6.31$, $df = 2$, $p = 0.043$) both increased at intermediate levels of competition (30 *Daphnia* L⁻¹ for both species). Further, it is interesting to note that at the early stages of epidemics, after *Daphnia* populations were exposed to environmental pathogen spores, infection prevalence increased monotonically with competitor density (Figure 5.3), suggesting that the competitor presence increased infection prevalence over very short timescales.

Competitor density reduces susceptible host population size Our model predicts that mean susceptible host population size should decline with increasing initial competitor density (Figure 5.2). In our experiments, epidemics were smaller when *D. pulicaria* densities were at their highest (100 *D. pulicaria* L⁻¹), driven not by the removal of pathogen from the environment, but by the competitive exclusion of the inferior competitor (Figure 5.4b). The time until *D. dentifera* extinction was reduced by increasing *D. pulicaria* density, though not significantly (Kruskal-Wallis test; $\chi^2 = 4.92$, $df = 2$, $p = 0.085$). The resulting termination of epidemics with competitive exclusion is evident when examining the infection time series (Figure 5.3). However, experimental epidemics also resulted in susceptible host extinction in the absence of competition, a phenomenon not predicted in our model. This is likely an experimental artifact; a result of deteriorating water quality, limited food supply, and enhanced mortality as a result of bi-weekly sampling.

Discussion

This study investigates support for the “friendly competition” concept [88] over ecologically relevant timescales, using a theoretical model and experiments. We found that when resources are limiting, competition with a superior competitor may be entirely “unfriendly” to susceptible host populations in two different ways: by increasing infection prevalence in susceptible hosts (through increased filtering induced by nutritional stress), and by reducing resources to levels below which susceptible host populations cannot maintain themselves. Prevalence increases through increased pathogen intake occurred at intermediate competitor densities; although prevalence declined at high competitor densities (often interpreted as evidence for friendly competition), the net effect of competition was negative, reducing susceptible host populations towards competitive exclusion. Consistent with classic theory [77], our model predicts that eventual competitive exclusion of the inferior competitor is inevitable, barring niche partitioning or other coexistence mechanisms such as refugia. Overall, our findings suggest that the competitive effects of a dominant competitor are usually negative, and that any positive effect of the competitor removing pathogen from the environment may be overwhelmed by the effect of reduced resource availability on inferior competitor feeding behavior and persistence.

Our model and experimental design make several simplifying assumptions that could influence competitor effects on infection dynamics in natural systems. For instance, our model allowed filtering rate, which is crucial to pathogen transmission, to vary only with resource quantity while other factors (e.g. pesticides; Fernández-Casalderrey et al. [70]) may influence foraging ecology and therefore affect transmission independently of resource depletion by competitors. Within

hosts, gut residence time may correspond to changes in the probability that a pathogen spore will pierce the gut wall and cause infection. This might explain the findings of previous studies in which both biotic and abiotic stressors decreased filtering rate and also increased pathogen infection success [39, 40, 46, 70, 102]. Hosts experiencing stress, either through starvation [162] or from the presence of secondary compounds from competitors, may experience higher transmission success due to the inability to resist pathogen infection [122]; in this case, prevalence may continue to increase at higher competitor densities than predicted by our model. Finally, *Daphnia* feeding selectivity [48, 114], and spatial aggregation of pathogen (given that pathogen spores settle quickly after host mortality) may reduce spore encounter rates and host infection independently of resource and competitor density.

Previous studies in the *Daphnia*-microparasite system have suggested that competition with a non-susceptible host should reduce infection prevalence, resulting in so-called “friendly competition” [88]. Further, Civitello et al. [31] argued that increasing susceptible host density could inhibit disease spread as a result of pathogen consumption and host foraging interference, suggesting another instance of foraging influencing infection prevalence. Lastly, Cáceres et al. [21] examined the conditions under which “friendly competition” could result in long term persistence of the inferior competitor, using an epidemiological model nearly identical to ours. We arrived at some conclusions also supported by Cáceres et al. [21], including the fact that two hosts competing for a limiting resource are unlikely to coexist indefinitely. However, our study also considers the transient dynamics before the susceptible host was excluded. These transient dynamics are ecologically relevant [92], both to zooplankton specifically and to studies of host-parasite inter-

actions more generally [51]. Zooplankton populations are unlikely to have equilibrium population densities [134] due to seasonal and stochastic changes in resource availability and environmental conditions, which influence host demographic rates. This means that models examining equilibrium conditions may not correspond to experimental data, making comparisons of models to experiments difficult [92]. Our analysis suggests that friendly competition is unlikely to occur over shorter, biologically relevant timescales.

There are many ways that parasites can influence interactions between hosts [95]. Many studies focus on how a parasite can handicap the superior competitor, leading to parasite-mediated coexistence [74, 94, 182, 184]. However, these studies typically do not consider how pathogen uptake is influenced by changes to foraging rates due to basal resource availability. Our study suggests that competition-mediated foraging rates could increase prevalence in a pathogen-susceptible, superior resource competitor, reducing its abundance relative to the inferior competitor below that expected when pathogen transmission is assumed to be independent of resource availability. Further theoretical and empirical work in this area is warranted.

Given its importance for transmission potential of zoonoses such as Lyme Disease [144], there has been much recent interest in the role of host diversity in either diluting or amplifying pathogen transmission [34]. Many studies of diversity-disease relationships tend not to incorporate ecological interactions, most notably competition for basal resources. In simple systems where a host and non-host diluter acquire environmental pathogen stages, prevalence is predicted to decline monotonically with non-host density. Our results show that at least initially, intermediate diluter

density maximizes epidemic size by increasing host acquisition of the pathogen. The situation in which the pathogen can become a food resource introduces complexity into the study of infectious disease in ecological communities, but is not specific to our study system. Many pathogens are environmentally transmitted, and are subject to incidental predation by hosts, and non-hosts alike [147, 196]. Therefore, our results suggest that evidence for dilution or amplification may be influenced by the time scale of observation, resource availability, as well as the relative abundance or richness of lower-competency hosts.

Much like Strauss et al. [192], this study attempts to unify two concepts in disease ecology by relating dilution theory to parasite-mediated competition. We highlight the importance of ecological context (resource availability) to competitive interactions between hosts, and how this influences infection dynamics in the susceptible host through a mechanism related to host foraging ecology. Studies of diversity-disease relationships and parasite-mediated competition often do not incorporate the potentially strong effect of environmental regulation, specifically with regards to resource availability. The incorporation of resource-mediated species interactions (direct and indirect) into studies of diversity-disease relationships may yield a more mechanistic view of diversity-disease relationships and other areas of disease ecology.

Tables

Table 5.1: Parameters, definitions, and units used in our epidemiological model. We chose plausible estimates for parameters for which data were not readily available (denoted by an empty citation column). Our values of assimilation efficacy were estimated using information on *Daphnia* population growth rates [31, 190].

Variable	Units	Definition	Value	Citation
e_S	–	Assimilation efficiency (Susceptible)	26	
e_C	–	Assimilation efficiency (Competitor)	28	
μ_S	day^{-1}	Death rate (Susceptible)	0.10	1
μ_I	day^{-1}	Death rate (Infected)	0.15	2
μ_C	day^{-1}	Death rate (Competitor)	0.10	
μ_P	day^{-1}	Death rate (Pathogen)	0.25	3
μ_R	day^{-1}	Death rate (Resource)	0.25	
z_S	–	Fraction spores digested (Susceptible)	0.30	
z_C	–	Fraction spores digested (Competitor)	0.30	
ϕ	–	Fecundity reduction by infection	0.75	2
f_{S0}, f_{S1}	$ml\ day^{-1}$	Host filtering rate (Susceptible)	0.020, 4	4, 5
f_{C0}, f_{C1}	$ml\ day^{-1}$	Host filtering rate (Competitor)	0.025, 4	4, 5
u	–	Per spore infectivity	2.03×10^{-4}	4
θ	#	Mean spore load per infected host	2×10^4	3
π	$mg\ L^{-1}\ day^{-1}$	Resource supply rate	0.005 – 4	6

1: [191]; 2: [55]; 3: [42]; 4: [86]; 5: [48]; 6: [194]

Figures

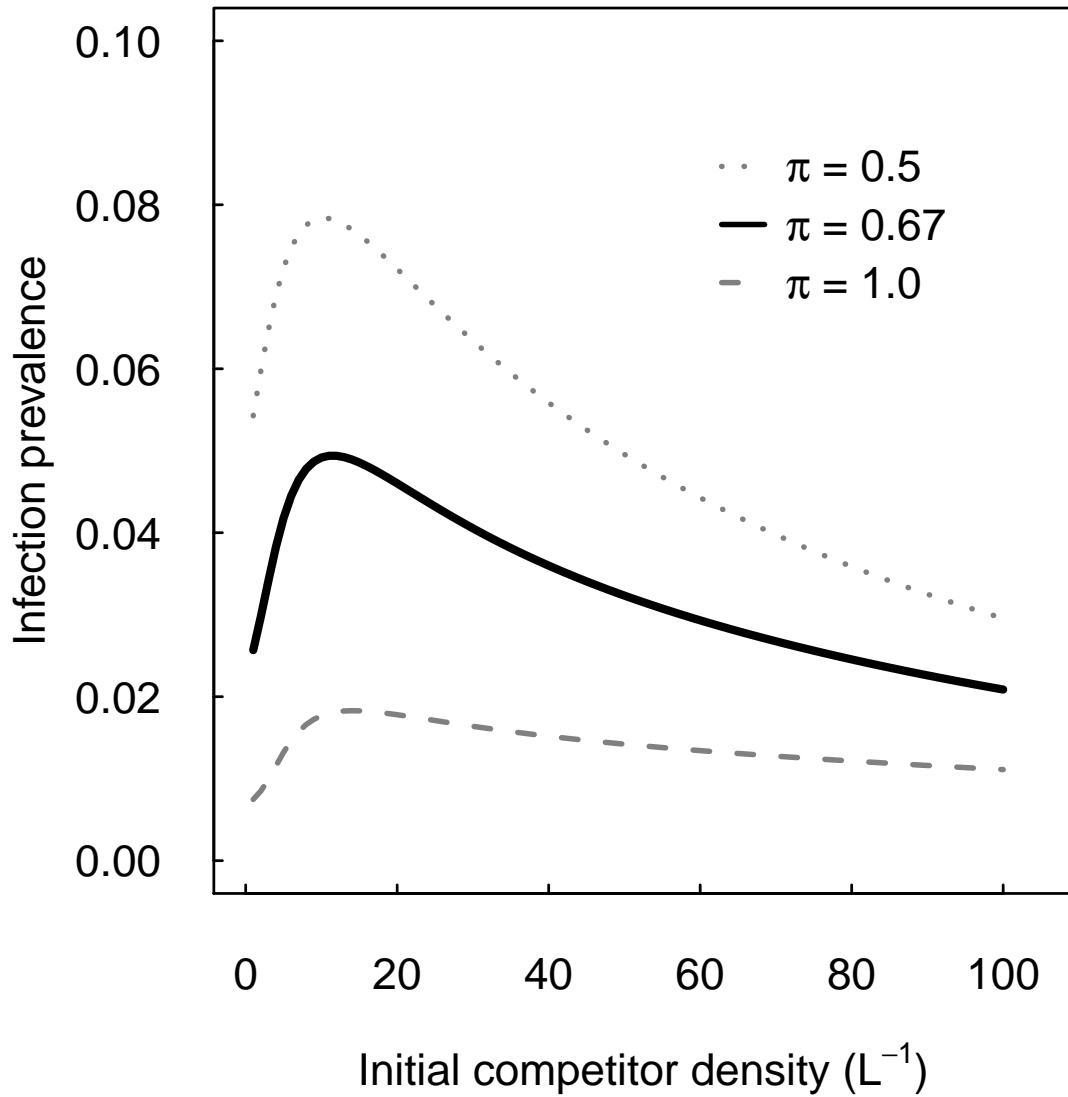


Figure 5.1: Non-monotonic relationship between mean infection prevalence and initial competitor density (x -axis) for three potential algal resource input levels (π ; y -axis). Enhanced resource input rates reduce infection prevalence by decreasing filtering rates (and hence pathogen exposure). The reduction in infection prevalence at larger initial competitor densities is a result of reduced susceptible host population sizes, and not a positive effect of the competitor removing environmental pathogen.

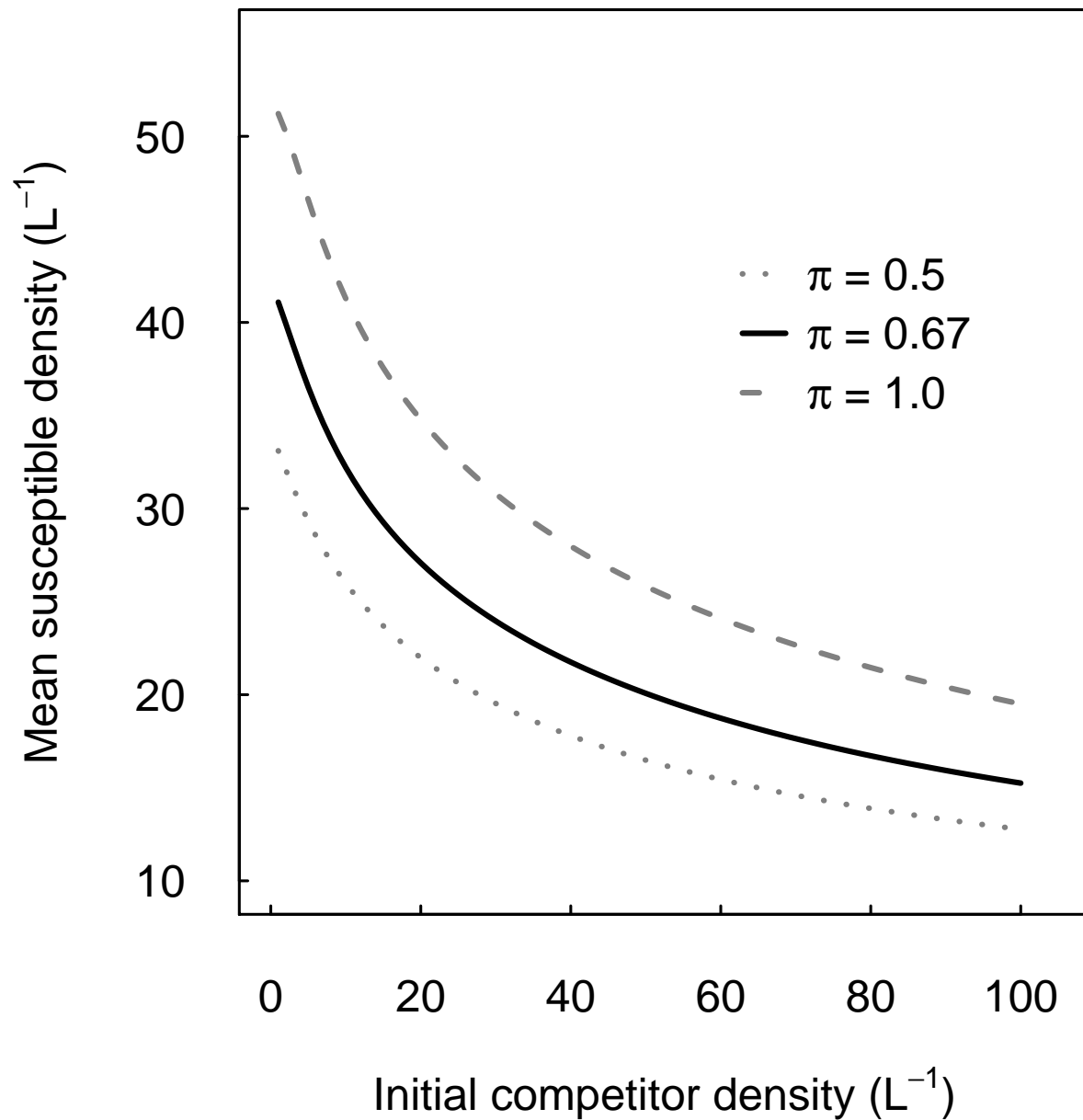


Figure 5.2: Mean population size of the susceptible host species ($S + I$) as a function of initial competitor density for three different algal resource input levels. $\pi = 0.5$ and $\pi = 1$ are the lower and upper dashed lines, respectively, and the solid black line corresponds to $\pi = 0.67$, the rate of algal resource supply in our experimental epidemics.

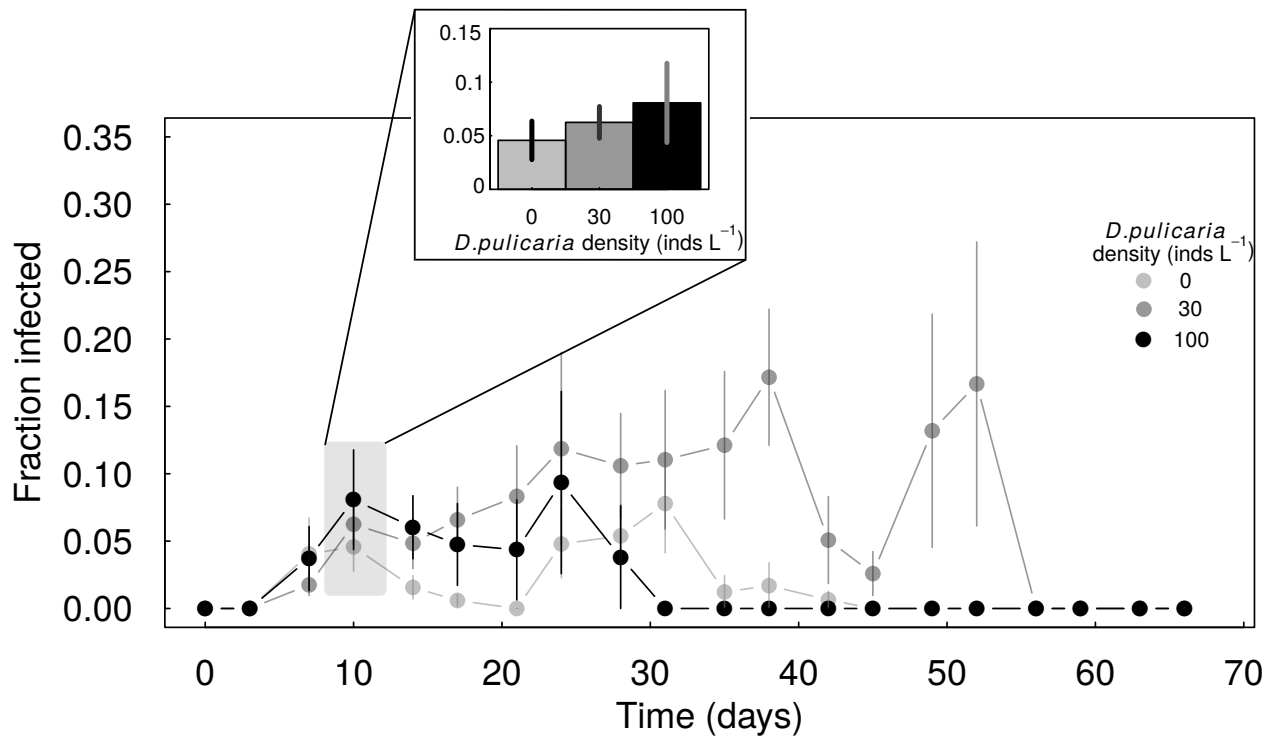


Figure 5.3: Mean infection prevalence (with standard error bars) for the epidemic time series. The inset barplot compares the infection prevalence at day 10, which corresponds to the first wave of infection, as the pathogen typically takes between 7 and 12 days to be readily identifiable, suggesting that competition initially increased infection prevalence proportional to *D. pulicaria* density.

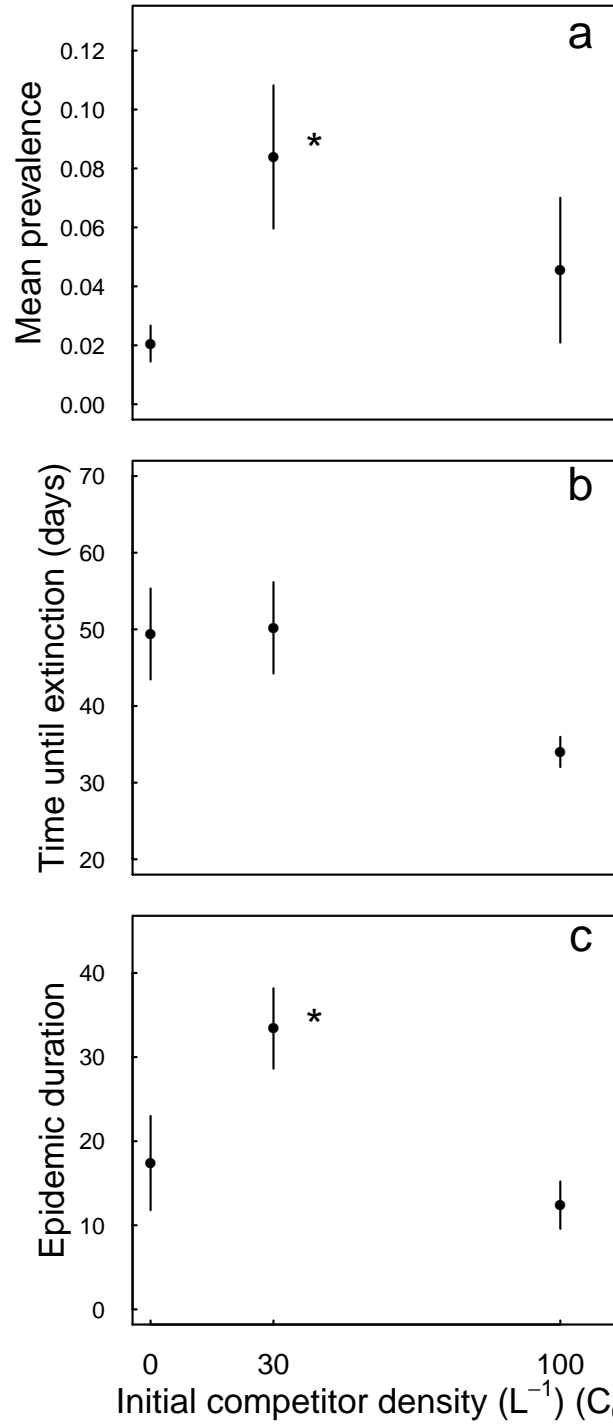


Figure 5.4: Experimental epidemics at three competitor densities revealed that intermediate levels of competition significantly increased mean infection prevalence (a) and epidemic duration (c)). There was no difference in epidemic measures between no competitor and high *D. pulicaria* competitor density treatments, driven by truncated epidemics at high competitor densities as a result of the competitive exclusion of the susceptible host (*D. dentifera*; b).

Chapter 6

Costs of resistance and infection by a generalist pathogen

Dallas, T., Holtackers, M., & Drake, J. M., 2015. Costs of resistance and infection by a generalist pathogen. *Ecology and Evolution*

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Abstract

Pathogen infection is typically costly to hosts, resulting in reduced fitness. However, pathogen exposure may also come at a cost even if the host does not become infected. These fitness reductions, referred to as “resistance costs”, are inducible physiological costs expressed as a result of a trade-off between resistance to a pathogen and aspects of host fitness (e.g., reproduction). Here, we examine resistance and infection costs of a generalist fungal pathogen (*Metschnikowia bicuspidata*) capable of infecting a number of host species. Costs were quantified as reductions in host lifespan, total reproduction, and mean clutch size as a function of pathogen exposure (resistance cost) or infection (infection cost). We provide empirical support for infection costs, and modest support for resistance costs for five *Daphnia* host species. Specifically, only one host species examined incurred a significant cost of resistance. This species was the least susceptible to infection, suggesting the possibility that host susceptibility to infection is associated with the detectability and size of resistance cost. Host age at the time of pathogen exposure did not influence the magnitude of resistance or infection cost. Lastly, resistant hosts had fitness values intermediate between unexposed control hosts and infected hosts. Though not statistically significant, this could suggest that pathogen exposure does come at some marginal cost. Taken together, our findings suggest that infection is costly, resistance costs may simply be difficult to detect, and the magnitude of resistance cost may vary among host species as a result of host life history or susceptibility.

Introduction

Pathogens are an important structuring force to host populations [3] and communities [207], with the potential to drive directional selection towards particular host genotypes [58]. Because pathogens have deleterious effects on host fitness, it is unsurprising that hosts respond to exposure through behavioral, immunological, and physiological pathways to reduce the negative effects of parasitism [14]. Typically, these host responses result in reductions to host fitness through differential resource allocation. For instance, increased immune function in response to pathogen exposure can result in lower fecundity [136]. Reductions to host fitness as a function of pathogen challenge can occur whether the host becomes infected (i.e., infection cost), or successfully evades infection (i.e., resistance cost). These costs are quantified as reductions in host fitness measures relative to unexposed, control hosts. Common host fitness measures used include host fecundity, body size, or survival [93, 212]. The magnitude of these costs may depend on host genotype [174], size of pathogen challenge, and environmental context, as seen in the dependence of the magnitude of resistance cost on the size of the pathogen challenge in a zooplankton (*Daphnia magna*) parasitized by a bacterial pathogen (*Pasteuria ramosa*; [118, 126]).

Despite the importance of these costs to host population structure and the spread of infectious disease, there is currently no consensus on the generality of resistance costs [118]. This is potentially a result of the diversity of host–pathogen interactions, or the range of deleterious effects pathogens may have on hosts [137]. The lack of consensus is perhaps most pronounced in invertebrate hosts [62, 115, 118], where linking pathogen exposure to immune function is difficult. While the existence of resistance costs in invertebrate host–pathogen interactions is unclear,

evidence of infection costs is plentiful [165]. For the purposes of this study, we define resistance costs as the negative effects resulting from pathogen challenge, but not infection, measured as differences in host fitness measures between hosts exposed to pathogen that do not become infected (hereafter referred to as “exposed-uninfected”, or “resistant”) and unexposed, susceptible hosts (hereafter referred to as “control”). This most closely corresponds to what are considered activation costs of resistance [5]. Infection costs were defined as the reductions in host fitness as a result of pathogen infection, measured by comparing control hosts to infected hosts with respect to host fitness traits. Infection likely elicits a stronger reduction in host fitness by compounding the effects of pathogen exposure and infection. Presently, few studies have examined both resistance and infection costs simultaneously (but see [29] for example). However, comparing the reductions of fitness between exposed-uninfected (resistant) hosts and infected hosts could lead to an understanding of when resistance may be advantageous. Specifically, if the costs to host fitness are equal or greater in resistant hosts relative to infected hosts, resistance is unlikely to confer an advantage. However, if resistance is not very costly, as has been previously suggested [118], then resistant individuals should have greater fitness than infected individuals.

Here, we addressed the impact of pathogen exposure and infection on host fitness using a generalist microparasite of *Daphnia* species. Many studies of resistance costs have focused on single host–pathogen pairs, which ignores the fact that pathogens tend to be able to infect multiple host species [209], and hampers our ability to identify the potential host traits associated with the presence and size of resistance costs. We examined five zooplankton host (*Daphnia*) species for the presence of resistance and infection costs to a virulent fungal pathogen (*Metschnikowia bicus-*

pidata). Resistance and infection costs were measured in terms of three host fitness measures: total reproductive output, mean clutch size, and lifespan. We found a statistically significant resistance cost (i.e., fitness difference between exposed-uninfected and control individuals) in only one host species, *D. pulicaria*, which is the least susceptible host species. Second, we found nearly universal costs of infection. However, there were no significant differences between exposed-uninfected and infected host individuals. Taken together, we found limited support for significant costs of resistance, but qualitative evidence that exposed-uninfected hosts had fitness values intermediate between infected and control hosts, suggesting that pathogen exposure can reduce host fitness, though the effects may be marginal. These nuanced costs of resistance, while not statistically significant when comparing control to exposed-uninfected hosts, add an interesting dimension, and a potential avenue for quantifying resistance costs. Specifically, the relative difference between exposed-uninfected hosts and both control and infected hosts contains information on the cost of resisting or tolerating a pathogen infection.

Methods

Origin and maintenance of hosts and pathogen Monoclonal lines of five *Daphnia* species (*D. ambigua*, *D. dentifera*, *D. laevis*, *D. mendotae*, and *D. pulicaria*) were maintained in experimental media best suited for host survival (different proportions of EPA hardwater media [199] and deionized water, *D. ambigua*, 20%; *D. laevis* and *D. mendotae*, 33%; *D. pulicaria*, 50%), except for *D. dentifera*, which were maintained in dilute pondwater (50%). Host species clones were lab-reared for many generations before this experiment, but were originally cultured from a small pond in Victoria Bryant State Park (*D. ambigua*), a Michigan Lake (provided by Meghan

Duffy of University of Michigan; *D. dentifera*), Ellenton Bay (Aiken, SC; *D. laevis*), a small pond in Northern IL (*D. mendotae*), and Oneida Lake (clone #29, provided by Hairston Lab at Cornell; *D. pulicaria*). All host cultures were fed 50 μL of a 2 g L^{-1} suspension (equivalent to 1 mg L^{-1} algal dry weight) of pulverized blue-green algae (*Spirulina* sp.), and kept on the laboratory benchtop under constant overhead lighting. Previous exposure of host clones to *M. bicuspidata* could potentially alter the expression of resistance or infection costs, but the data on previous pathogen exposure were not available for the clones studied here. However, lab clones were raised under lab conditions for more than 20 generations before their first pathogen exposure, which reduces the possibility of potential legacy effects of pathogen exposure.

The fungal pathogen used in this study (*M. bicuspidata*) was originally isolated from *D. dentifera* in Michigan lakes (provided by Meghan Duffy). The pathogen was cultured *in vivo* by exposing *D. dentifera* to infectious spores and harvesting the spores by homogenizing infected animals in deionized water. Parasite fitness may be altered by host genotype, but no heritable variation exists in the fungal pathogen studied [186]. This means that rapid pathogen evolution in response to hosts is unlikely, but also that the host genotype used to culture the pathogen could influence pathogen infectivity. To account for this, the pathogen was always cultured in a single clone of *D. dentifera*, and hosts were only exposed to the pathogen a single time (i.e., uninfected hosts from one round of pathogen exposure were not used subsequently). The host range of the fungus is unknown, but includes a variety of both terrestrial and marine organisms [36, 138]. The pathogen is environmentally transmitted during host host feeding [61, 87]. Pathogen spores pierce the gut wall, and proliferate inside the host until host death causes the release of pathogen spores into the environment. Infection is easily diagnosed, as spores form opaque clusters in the

transparent host (see [151] or journal cover image from [58]).

Experimental design To remove the confounding effects of host age and maternal environment, we sequentially isolated offspring from parthenogenetic females raised in isolation to obtain individuals of known age and maternal environment. Keeping maternal environmental conditions fairly uniform, and randomly placing individuals in experimental groups serves to reduce any effect of maternal environment. Sequential isolation was performed for three generations before hosts were used in experiments, and the resulting offspring of this process were randomly assigned to exposure treatment. Host age may influence within-host pathogen competition [101], as host immunity may change as a function of age, and fitness costs since fitness and energy allotment to growth or reproduction vary over the lifespan of the host [100]. To account for this, we sequentially isolated *Daphnia* hosts as described above for 12 days, isolating six uninfected individuals per species per day ($n = 72$ hosts of known age per species examined), creating a uniform age gradient for all host species. Animals were placed individually in 50 ml of appropriate media, and either exposed to pathogen (200 pathogen spores ml^{-1}) or a slurry of crushed *D. dentifera* as a control (sham) inoculum. This was performed because the pathogen inoculum was created by crushing infected hosts, and the presence of crushed *Daphnia* may signal an alarm response from conspecifics.

Experimental individuals were monitored daily for offspring production, mortality, and ephippia (resting egg) production. Infections are typically unobservable before seven days post infection challenge, and mortality typically occurs after 12 days or more. Infection was assessed visually daily from day seven onward, and confirmed at death by examining *Daphnia* hosts using a compound

microscope (400 \times magnification). In this approach, *Daphnia* hosts were crushed between glass microscope slide and cover slip, and examined thoroughly for the presence of pathogen spores, which normally average over 10,000 per infected host [42]. In our experiment, none of the control hosts became infected, and not all hosts exposed to pathogen spores became infected. One host species, *D. dentifera*, was excluded from the analyses as a result of excessively high host mortality. However, we reproduce manuscript plots with the inclusion of *D. dentifera* in Appendix C.

We quantified costs as relative changes in three host fitness measures; total reproduction, mean clutch size, and lifespan. Total reproductive output (total number of offspring produced per host individual) and mean clutch size (mean number of offspring per reproduction event) were both measured after the host had been exposed to the pathogen (or control inoculum). Host lifespan was measured as the total number of days from host birth to host death.

Statistical analysis To assess differences among host exposure classes (i.e. control, exposed-uninfected, and infected), we used Kruskal-Wallis rank tests. Nemenyi post hoc tests were used to examine pairwise differences between exposure classes. This analysis allowed for the separate determination of costs of resistance (control compared to exposed-uninfected host fitness), and costs of infection (control compared to infected host fitness). Further, this approach also enabled us to compare the rank distributions of exposed-uninfected hosts to infected hosts, thereby providing insight into how costly resistance is compared to infection. All analyses were performed in R [163], and Nemenyi post hoc tests were performed using the PMCMR package [155].

Results

Costs of pathogen infection Host fitness, measured as total reproduction, mean clutch size, and lifespan, was systematically reduced as a function of pathogen infection (Table 6.1), suggesting that microparasite infections were costly. Specifically, infection costs, measured as fitness differences between control (unexposed) and infected host individuals, were nearly universally significant (Table 6.2), resulting in sizable reductions to host reproductive output ($\bar{\mu}_{c-i} = 18.6$ neonates), mean clutch size ($\bar{\mu}_{c-i} = 1.1$ fewer neonates per clutch), and lifespan ($\bar{\mu}_{c-i} = 6.8$ days). The consistent finding of infection costs was not found for *D. dentifera*, which was excluded from the analyses as a result of enhanced mortality early in the experiment (see Appendix C).

Costs of resistance to pathogen Meanwhile, exposure to pathogen without infection did not cause a significant reduction in host fitness for a majority of the host species and fitness measure combinations (Table 6.2), suggesting that resistance in the *Daphnia*-microparasite system is not costly. However, significant resistance costs were observed for *D. laevis* with respect to lifespan, and in all fitness measures for *D. pulicaria* (Table 6.2). This host species does not become infected by the pathogen. For the other three species examined, exposed-uninfected individuals did not differ in fitness relative to control hosts or infected hosts, suggesting that exposed-uninfected hosts have fitness values intermediate to hosts not exposed to the pathogen, and hosts that become infected (Figure 6.1). While not statistically significant, pathogen exposure reduced mean host fitness, in terms of average host reproductive output ($\bar{\mu}_{c-r} = 13.5$ fewer total neonates), clutch size ($\bar{\mu}_{c-r} = 0.08$ fewer neonates per clutch), and lifespan ($\bar{\mu}_{c-r} = 6.9$ days).

Does host age influence costs? Host age was strongly and positively related to host fitness measures, as older hosts at the time of pathogen exposure produced more offspring, had larger mean clutch sizes, and had longer lifespans relative to hosts that were younger at the time of pathogen exposure. However, we found little evidence for variation in resistance or infection costs as a function of host age at the time of pathogen exposure, though this relationship was significantly positive in *D. laevis* hosts when costs were measured in terms of lifespan or mean clutch size (see Appendix C).

Discussion

Responding to a pathogen challenge is expected to reduce host fitness by diverting limited host resources toward pathogen resistance (i.e., an inducible cost). However, consistent evidence for resistance costs remains sparse, both in laboratory [66, 76, 118] and field [6] studies. Here, we attempted to identify resistance and infection costs for a generalist pathogen capable of infecting numerous *Daphnia* host species. We provide evidence that fungal pathogen infections come at a fitness cost to all susceptible host species, but that the fitness consequences of pathogen exposure were more nuanced. Specifically, significant resistance costs were only observed in *D. pulicaria*, a completely resistant host species. However, exposed-uninfected (resistant) hosts had fitness values intermediate between control hosts and infected hosts. This suggests that pathogen resistance still comes at a price, though this difference is insignificant based on our limited sample size. Neither resistance nor infection costs varied as a function of host age at the time of pathogen exposure, though previous studies have found an age-dependent cost in *Daphnia* parasitized by a castrating bacterial pathogen [100]. Taken together, these results support previous findings [118]

suggesting that resistance does not come at a high cost in *Daphnia*–microparasite interactions, provide one of the first examinations of costs associated with a multi-host pathogen, and suggest that host susceptibility may be related to the size of resistance costs.

Perhaps coincidentally, species incurring the largest costs of resistance were also the least likely to become infected by the pathogen. Different clonal lines of *D. pulicaria* have also demonstrated this resistance (*unpublished data*, and [43]). Our ability to make broad generalizations about the relationship between host species susceptibility and resistance costs is limited by the examination of single representative clones of each *Daphnia* species. However, we found a consistent decline in magnitude of resistance cost with increasing host species susceptibility to infection (see Appendix C), which was significant when costs were measured in terms of change in host lifespan between control and exposed-uninfected hosts. Potentially the most obvious explanation for this relationship is that less susceptible species are less susceptible because they are able to mount an effective, though costly, behavioral or immunological response. Behaviorally, hosts could reduce feeding, which would reduce pathogen transmission, but would also reduce fitness through resource limitation. This behavioral response could also explain previous findings in natural systems, in which populations of *D. dentifera* exhibited a negative relationship between pathogen transmission rate and host birth rate [7]. This observed cost of resistance could be a result of the close relationship between *Daphnia* species feeding rate and both pathogen transmission and host birth rate. Understanding both the behavioral and immunological mechanisms contributing to resistance costs in multi-host pathogens is an important, but as yet unexplored, topic.

Host age has been hypothesized to influence the size of the host response to pathogen exposure. This has been shown previously in a castrating bacterial pathogen of *Daphnia* [100], as younger hosts had higher transmission, shorter time until castration, and higher pathogen fitness (i.e., infection intensity). There are at least two separate reasons for the difference in detectability of age-dependent costs. First, bacterial pathogens, especially castrating bacterial pathogens, could elicit a different response than fungal pathogens. This is because bacterial castrating pathogens, like the pathogen examined by [100], have strong effects on host fitness, and often exhibit co-evolutionary relationships with hosts [64]. Therefore, the existence of age-dependent costs could be a result of the type of pathogen examined, and the relative virulence of the pathogen. Second, the current study examined a narrow age range (1-12 days old) based on the survival of hosts in the lab. [100] examined a longer-lived *Daphnia* host species, and three host ages (5, 15, and 30 days old at the time of pathogen exposure). The mean lifespan of hosts, regardless of pathogen exposure, was less than 30 days, likely a result of experimental conditions (e.g., feeding live algae versus a *Spirulina* suspension). A final explanation could be the effects of pathogen dose or environmental conditions (apart from resources as described above). This explanation could explain not just the lack of detected age-dependence, but also potentially the lack of detectability of resistance costs in invertebrate systems.

There is currently no consensus about why resistance costs are detectable in some systems, and apparently absent in others, especially for invertebrate pathogens [66, 76, 118]. Environmental stress [178] and evolutionary history [124] have both been invoked as factors potentially obscuring (or promoting) the detection of costs. There are many other potential causes for the failed

detection of resistance costs in *Daphnia*, including the use of an immutable trait to quantify cost, and a limited understanding of invertebrate immunology [125]. The focus on single species host–pathogen systems also limits our understanding of resistance costs. We attempted to address this by examining multiple host species, allowing the potential for a more mechanistic examination of resistance costs. The relationship between aspects of host species (e.g. phylogenetic relatedness, susceptibility to infection, life history traits) and the magnitude of resistance costs could provide insights into why these costs are observed in some host–pathogen combinations and not in others. Lastly, because resistance costs may be mediated by changes to host phenotype, life history, behavior, or immunology [169], it is possible that costs are incurred without being detected. This may explain, in part, the mixed support for resistance costs in many animal systems, including *Daphnia* (this study; [118]), birds [141], and amphibians [26].

Investigations of resistance and infection costs incorporating the effects of environment, differential pathogen exposure (i.e., number, duration, and dose of pathogen exposure), and host life history may provide a more detailed understanding of when a host response to pathogen exposure can be costly. By examining multiple host species, we provide little evidence for resistance costs in *Daphnia*–fungal pathogen interactions, but overwhelming support for costs of infection. Resistant individuals still had reduced fitness, representing an intermediate point between unexposed control hosts and infected hosts, suggesting that resistance may still come at a cost, but that this cost may be difficult to detect. Future studies of resistance costs to multi-host pathogens in the presence of environmental stressors are necessary for the development and testing of hypotheses related to the expression and magnitude of resistance costs. Further, integrating resistance costs

into epidemiological models and experiments may be critical to developing an understanding of pathogen-mediated host competition, host community structure, and host–pathogen interactions in general.

Tables

Table 6.1: Mean and standard error for fitness measures (reproductive output, lifespan, and mean clutch size) for control, exposed-uninfected, and infected individuals. Host species are ordered from most to least susceptible to infection by *M. bicuspidata*.

Host	Infection status	n	Reproduction	Lifespan	Mean clutch size
<i>D. mendotae</i>	control	36	14.89 (2.57)	24.58 (1.53)	2.82 (0.36)
	exposed-uninfected	2	10.50 (0.50)	19.50 (0.50)	3.50 (0.17)
	infected	34	3.47 (0.77)	16.68 (0.74)	1.60 (0.24)
<i>D. ambigua</i>	control	36	31.06 (4.08)	24.67 (1.66)	3.94 (0.32)
	exposed-uninfected	10	16.80 (4.01)	18.90 (1.69)	3.39 (0.62)
	infected	26	9.77 (1.48)	17.96 (1.06)	2.65 (0.30)
<i>D. laevis</i>	control	36	36.69 (3.85)	25.53 (1.57)	4.52 (0.35)
	exposed-uninfected	12	16.58 (4.22)	18.17 (1.22)	3.49 (0.49)
	infected	24	12.33 (2.67)	19.83 (1.28)	3.05 (0.35)
<i>D. pulicaria</i>	control	36	35.92 (3.64)	32.83 (1.99)	4.29 (0.30)
	exposed-uninfected	36	14.56 (1.88)	22.11 (1.14)	3.33 (0.35)
	infected	0	–	–	–

Table 6.2: The costs of resistance and infection to a generalist microparasite. Costs are measured as reductions in lifetime reproduction, mean clutch size, and lifespan. Differences between unexposed control (*c*) hosts and both infected (*i*) and resistant (exposed-uninfected; *r*) hosts. Mean group differences are provided in columns $\mu_c - \mu_i$, where *i* corresponds to either resistant (*r*) or infected (*i*) hosts. Significance (*P*-values are in bold) was assessed at $\alpha = 0.0167$ to correct for multiple comparisons among pathogen exposure classes (i.e. control, exposed-uninfected, and infected).

Host	Covariate	$\mu_c - \mu_r$	K_{cr}	P_{cr}	$\mu_c - \mu_i$	K_{ci}	P_{ci}
<i>D. mendotae</i>	reproduction	4.39	0.77	0.848	11.42	5.03	0.001
	lifespan	5.08	0.99	0.764	7.91	5.89	< 0.0001
	mean clutch size	-0.68	1.45	0.560	1.22	3.49	0.036
<i>D. ambigua</i>	reproduction	14.26	1.77	0.423	21.29	5.06	0.001
	lifespan	5.77	2.72	0.133	6.71	4.42	0.005
	mean clutch size	0.55	1.25	0.648	1.29	4.23	0.008
<i>D. laevis</i>	reproduction	20.11	3.50	0.036	24.36	5.58	< 0.0001
	lifespan	7.36	4.14	0.010	5.69	4.33	0.006
	mean clutch size	1.02	2.65	0.146	1.46	4.50	0.004
<i>D. pulicaria</i>	reproduction	21.36	6.00	< 0.0001	–	–	–
	lifespan	10.72	6.43	< 0.0001	–	–	–
	mean clutch size	0.91	3.46	0.014	–	–	–

Figures

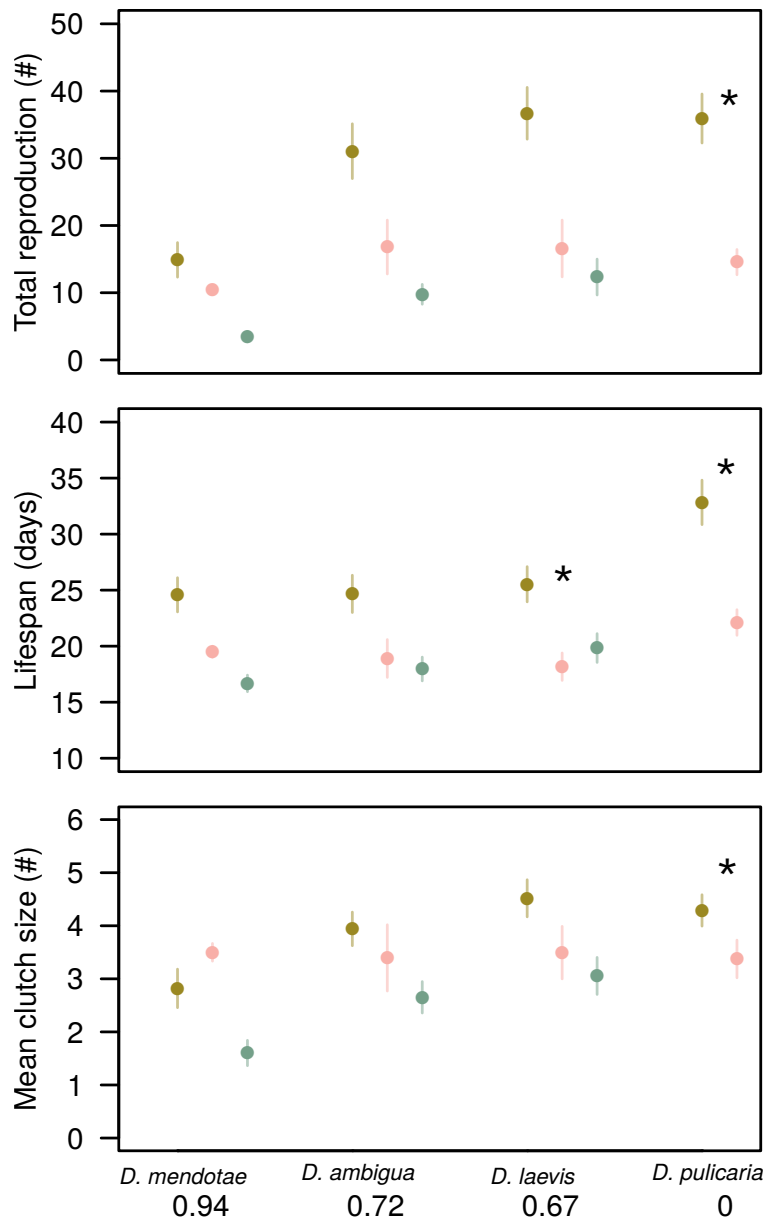


Figure 6.1: Significant costs of resistance (denoted with an asterisk; *), and infection (universal except for mean clutch size of *D. mendotae*) with respect to three host fitness measures (mean ± 1 SE). Resistance and infection costs were defined as differences between unexposed (green points) and either exposed-uninfected hosts (pink points; resistance costs) or infected hosts (blue points; infection costs). Mean clutch size and total reproduction were quantified as the number of offspring per clutch and the total number of offspring an individual produced after infection challenge. Lifespan was scored as total lifespan of the host. Host susceptibility, defined as the fraction of hosts exposed to the pathogen that became infected, is given in parentheses next to the host species name.

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Appendix A

Experimental evidence of pathogen invasion threshold in a *Daphnia*–microparasite system

Comparison of deterministic and stochastic simulations

The deterministic model and stochastic simulations predicted slightly different pathogen invasion thresholds (main text Figure 2). In both models, we incorporated environmental stochasticity by exploring the parameter space of four important model parameters (γ , u , and θ in the main text, μ below). The stochastic model incorporates demographic stochasticity, capturing the integer-based nature of the system. The waiting time until an event (e.g., birth) is a Poisson process, and the event that occurs is based on the likelihood of the different events. As population size becomes larger, the deterministic and stochastic simulations should converge to have similar dynamics, because the influence of demographic stochasticity should be reduced at larger population sizes.

Critical host density and pathogen death rate

Pathogen death rate influences the basic reproduction number, and, as such, influences the critical host density threshold. However, pathogen death rates for many environmental pathogens,

including *Metschnikowia bicuspidata*, are difficult to quantify. Environmental spore death can determine the amount of pathogen available to initiate a seasonal epidemic. Therefore, understanding how environmental pathogen can persist in the environment is potentially important to predicting timing and size of epidemics. Previous estimates of environmental pathogen loss rate have varied between 0.25 [42] to 0.9 [88]. In the main text, we used a value of 0.7, a plausible value considering the range of estimates. We incorporate variation in environmental pathogen death rate (μ) here, demonstrating the sensitivity of the basic reproduction number (R_0) and subsequently the pathogen invasion threshold ($R_0 > 1$) to changes in environmental spore survival (Figure A1).

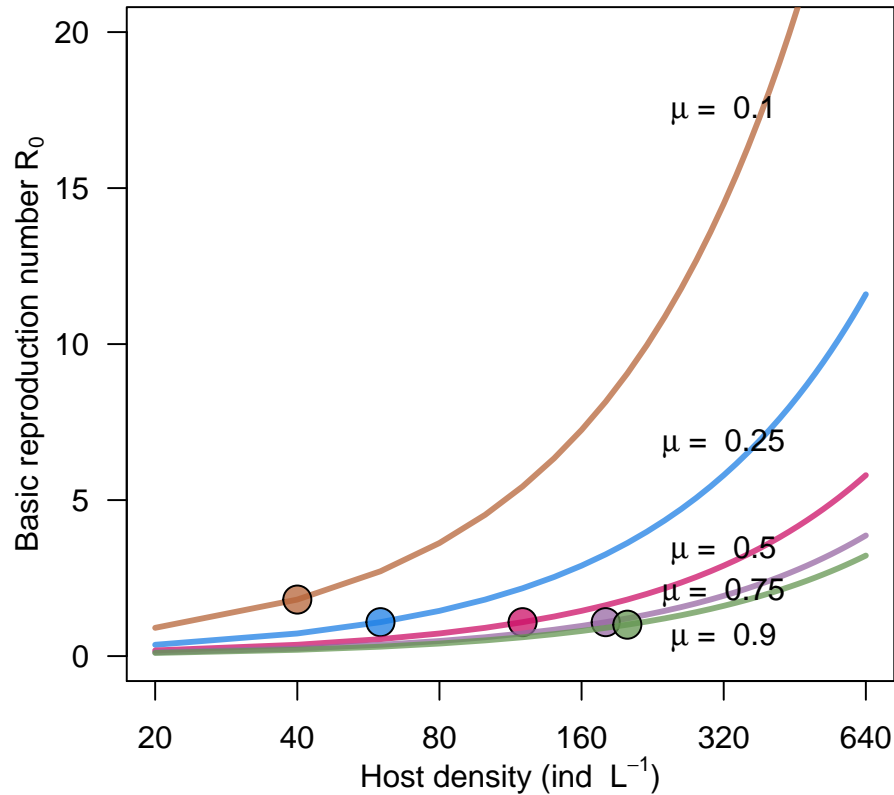


Figure A1: The rate at which pathogen is lost from the environment (μ) influences the basic reproduction number (R_0 ; solid lines), and the threshold host density necessary for pathogen invasion (indicated by shaded points). Here, we set $u = 0.002$, $\gamma = 0.00453$ and $\theta = 10000$

Analytical derivation of invasion probability from stochastic model

In the stochastic realization of our epidemiological model, the rate at which i individuals become infected by the fungal pathogen is a non-homogeneous Poisson process. This is a result of environmental pathogen death altering the potential number of infection events over time. Here, we can define the intensity function $\lambda(t)$ as

$$\lambda(t) = u\gamma P^* e^{-\mu t} \quad (\text{A.1})$$

where the per spore infectivity (u) and host filter rate (γ) influence the rate at which a host ingests infective pathogen, and the environmental pathogen (P^*) is lost from the system at rate μ . The mean intensity over the experimental time period of pathogen invasion (0 - 11 days) then becomes

$$\Lambda(t_0, t_{11}) = \int_0^{11} \lambda(t) dt \quad (\text{A.2})$$

The probability of observing n infection events in this time period is then

$$P(n) = \frac{\Lambda^n}{n!} e^{-\Lambda} \quad (\text{A.3})$$

and the probability of observing no infection events ($n = 0$), and the probability of observing one or more infection event (i.e., pathogen invasion) are

$$P(n = 0) = (e^{-\Lambda})^x$$

$$P(n \geq 1) = 1 - (e^{-\Lambda})^x$$

where x is the number of susceptible hosts in the population.

Calculation of R_0 for the SI model with environmental pathogen

Below, I outline the structural forms of two SI models with environmental pathogen (P), differing in the rate at environmental pathogen loss as a function of host foraging. Host foraging had almost no influence on critical host density for pathogen invasion (Figure A2). I provide R_0 calculations for both models, though the R_0 calculation for the SI model without spore loss through host foraging is also in the main text.

Model without spore loss through host foraging

$$\dot{S} = b - d(S + (\phi I)) \left(1 - \frac{S + I}{K}\right) - u\gamma SP \quad (\text{A.4})$$

$$\dot{I} = u\gamma SP - I(d + v) \quad (\text{A.5})$$

$$\dot{P} = I\theta(d + v) - \mu P \quad (\text{A.6})$$

Next generation matrix determination of R_0

We used the next generation matrix method [50], which is the dominant eigenvalue of the product of two matrices (F and V^{-1}). The calculation of R_0 follows the “transition” scenario of [8], in which the pathogen does not grow outside of hosts, but essentially acts as a second state of infected host. Specifically, hosts contribute pathogen to the environment, and that environmental pathogen is the only source of infection for hosts (i.e., there is no host-host transmission).

$$K = FV^{-1} = \begin{bmatrix} 0 & u\gamma K \\ 0 & 0 \end{bmatrix} \begin{bmatrix} (d+v) & 0 \\ -(d+v)\theta & \mu \end{bmatrix}^{-1} \quad (\text{A.7})$$

$$\max(\text{eig}(K)) = R_0 = \frac{\theta\gamma u S^*}{\mu} \quad (\text{A.8})$$

Model with spore loss through host foraging

$$\dot{S} = (b-d)(S + (\phi I)) \left(1 - \frac{S+I}{K}\right) - u\gamma SP \quad (\text{A.9})$$

$$\dot{I} = u\gamma SP - I(d+v) \quad (\text{A.10})$$

$$\dot{P} = I\theta(d+v) - \mu P - \gamma(S+I)P \quad (\text{A.11})$$

Heuristic formulation of R_0

In the main text, we defined R_0 from first principles. The same method can be applied to the model incorporating spore loss through host foraging. From first principles, R_0 would correspond

to the product of the total pathogen produced by a single infected host, the total number of spores consumed by hosts when $S = K = S^*$, and average environmental spore lifespan. This is the exact same formulation as in the model without host foraging on spores, but with a reduction in environmental spore lifespan proportional to the host foraging rate.

$$R_0 = (\theta)(\gamma u S^*) \left(\frac{1}{\mu + \gamma S^*} \right) = \frac{\theta \gamma u S^*}{\gamma S^* + \mu} \quad (\text{A.12})$$

Next-generation R_0 calculation

The calculation of R_0 using the next generation matrix technique is nearly equivalent for this model relative to the model without spore loss through host foraging, except that pathogen is lost from the V matrix not only through pathogen death (μ), but also through a foraging rate (γ) scaled by the equilibrium susceptible host density (S^*)

$$K = FV^{-1} = \begin{bmatrix} 0 & u\gamma S^* \\ 0 & 0 \end{bmatrix} \begin{bmatrix} (d+v) & 0 \\ -(d+v)\theta & \mu + u\gamma S^* \end{bmatrix}^{-1} \quad (\text{A.13})$$

$$\max(\text{eig}(K)) = R_0 = \frac{\theta \gamma u S^*}{(\gamma S^*) + \mu} \quad (\text{A.14})$$

Population and extinction dynamics in experimental microcosms

Host population density varied over the course of the experiment from initial experimental host densities (Figure A4). It is possible that we observed a host density threshold in our experiments as a result of small initial population sizes leading to stochastic extinctions. While it appeared

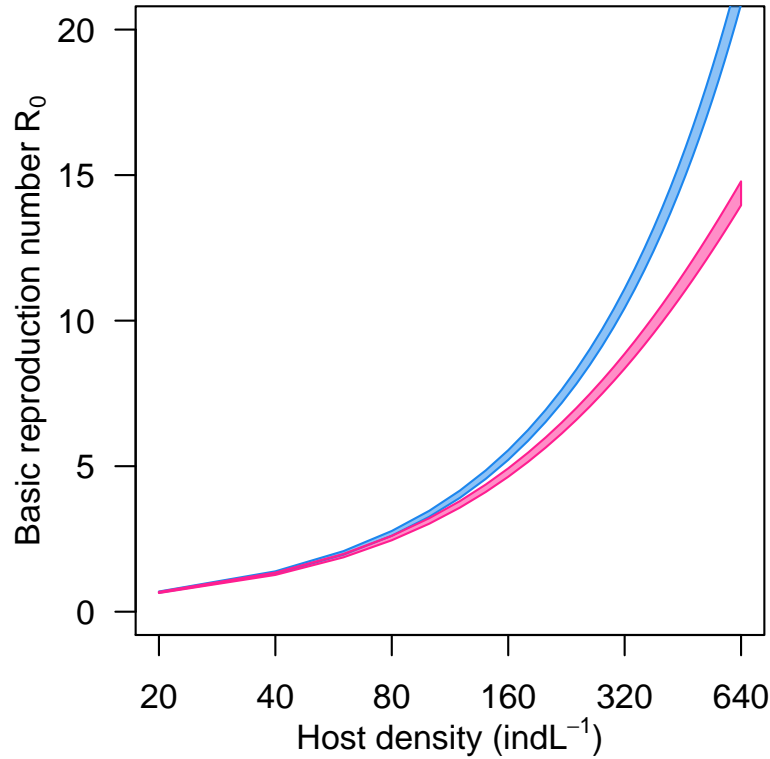


Figure A2: Model estimates of R_0 (top) and probability of pathogen invasion (bottom) were unaffected by the incorporating of spore loss through host foraging (red lines). The grey box outlines the lower ($P(\text{invasion}) = 0.25$) and upper ($P(\text{invasion}) = 0.75$) quartiles of possible invasion thresholds based on our sampling of θ and γ parameter space for the model without spore loss through host foraging.

that the lowest host density treatment (20 hosts L^{-1}) did have elevated extinction in the early days of the experiment (Figure A3), the closest host density treatment (40 hosts L^{-1}) appeared qualitatively the same as the other host density treatments in terms of extinction dynamics over time. This suggests that while extinction risk may have been increased for the lowest host density treatment, this did not drive the host density threshold we observed, as the empirical and model predicted threshold was higher than a treatment that experienced no perceivable enhanced extinction risk.

Further, the stochastic model explicitly modeled the influence of demographic stochasticity, allowing a more clear comparison of model and experiment. The differences observed between predicted invasion thresholds between deterministic and stochastic models are a result of this incorporation of demographic stochasticity, and specifically the integer-based nature of real-world population dynamics. It would appear that the probability of pathogen invasion calculated our experiment was intermediate between deterministic and stochastic predictions.

Mixed support for upper host density effects

Previous research has suggested that intraspecific interactions among hosts may result in feeding suppression, which could reduce epidemic size and perhaps even create an upper host density threshold to pathogen invasion at high host densities [31]. Further, this would result in a non-monotonic relationship between host density and the pathogen invasion threshold, as a result of suppressed host feeding at high host densities resulting in reduced pathogen transmission. We did not find support for an upper host density threshold to pathogen invasion (Figure A5 inset). On the other hand, epidemic size and max infection prevalence declined in the highest host density treatment, suggesting that epidemics weren't as severe when host density was extremely high. This suggests that dense host populations may experience smaller overall epidemics, but casts doubt on the existence of an upper host density threshold to pathogen invasion.

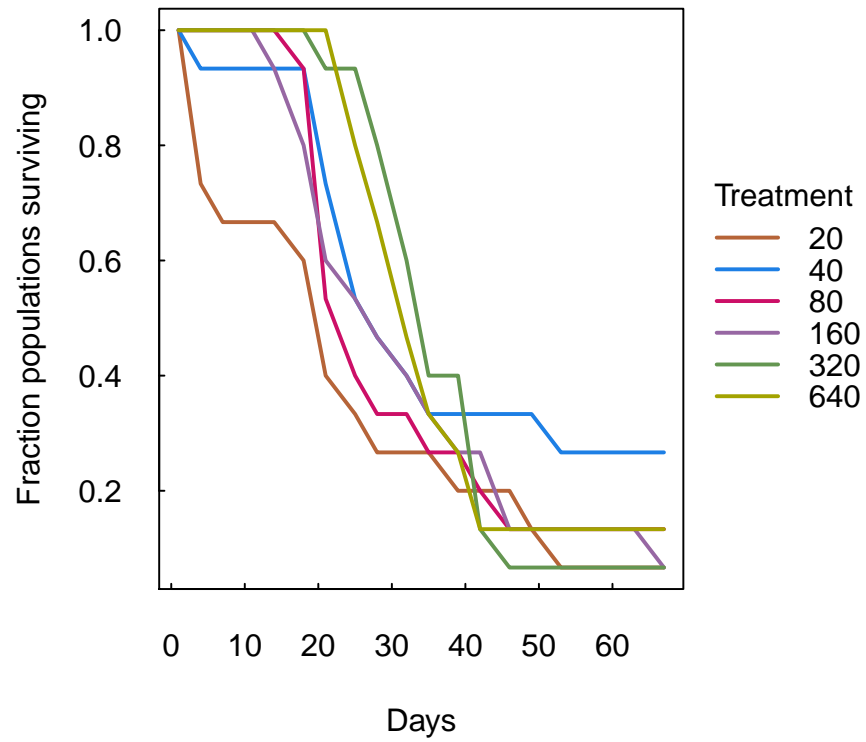


Figure A3: The fraction of host populations extinct for each treatment over the course of the 70 day experimental epizootics.

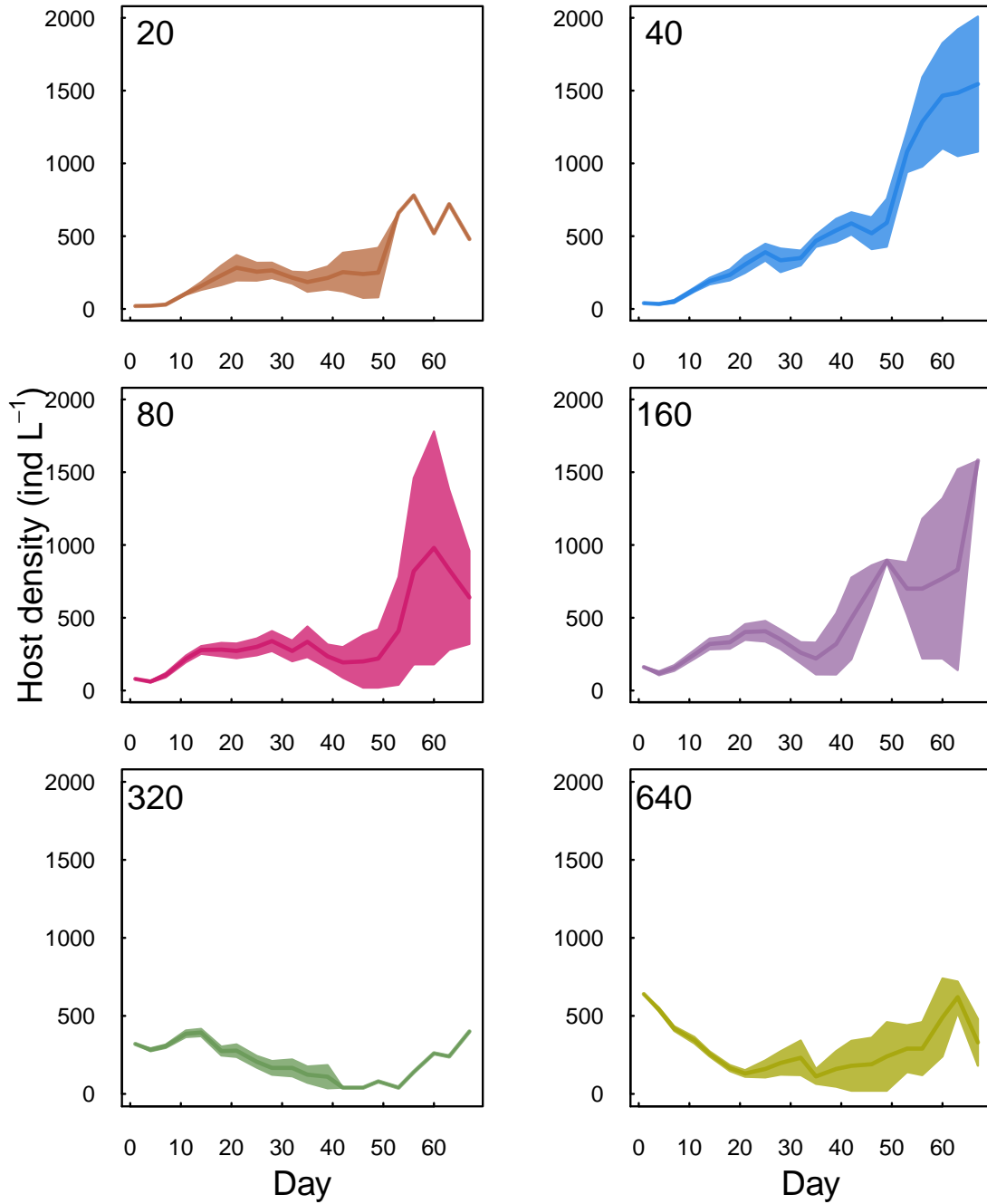


Figure A4: Population sizes for each host density treatment over the course of the experiment. Standard error bars are given by polygons, and plotted lines are mean population sizes. While the threshold behavior occurred when population size were experimentally determined, the range of host densities reached by populations is impressive, with the highest population densities achieved by the intermediate initial density groups. Populations seeded at high density had similar population sizes to the lowest initial density treatment after approximately 30 days into the experiment.

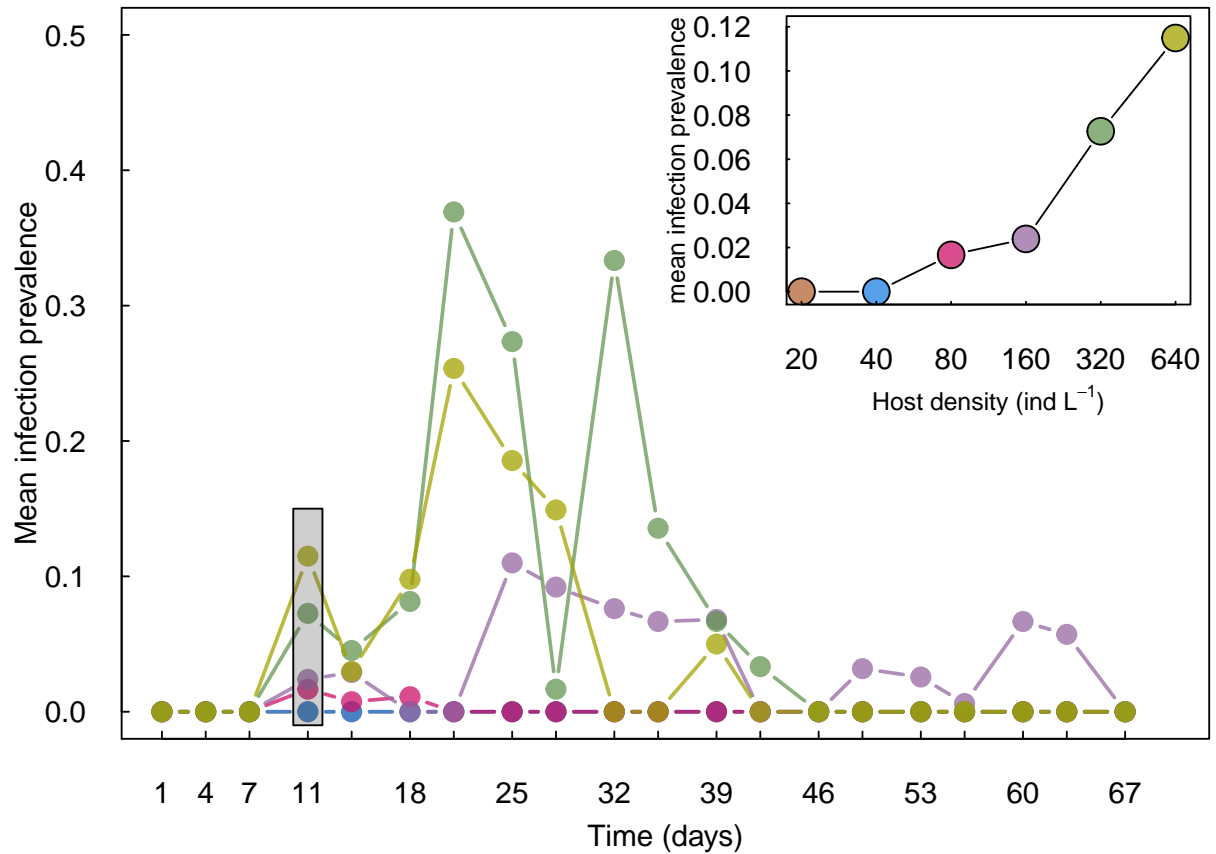


Figure A5: Time-series of experimental infections reveal wave-like infection patterns after pathogen invasion (grey box at day 11). The inset plot shows the monotonic relationship between host density and mean infection prevalence. Colors for host density treatments are conserved from Figures 3 and 4, and are also the same in the inset plot (e.g., orange corresponds to 20 host individuals L^{-1}).

Appendix B

Competition-mediated feedbacks in experimental multi-species epizootics

Model equations

As described in the manuscript, the full resource-host-pathogen-competitor model takes the form

$$\begin{aligned}\frac{dR}{dt} &= \pi - \mu_R R - f_S(R)R(S + I) - f_C(R)RC \\ \frac{dS}{dt} &= e_S f_S(R)R(S + \phi I) - \mu_S S - u f_S(R)PS \\ \frac{dI}{dt} &= u f_S(R)PS - \mu_I I \\ \frac{dP}{dt} &= \theta \mu_I I - \mu_P P - z_S f_S(R)P(S + I) - z_C f_C(R)PC \\ \frac{dC}{dt} &= e_C f_C(R)RC - \mu_C C\end{aligned}$$

where the filtering rate for species $j(= S, C)$ is given by

$$f_j(R) = \frac{f_{j0}}{1 + f_{j1}R}.$$

Differences from Cáceres et al. (2014)

Our epidemiological model is very similar to that of Cáceres et al. [21], despite independent derivation. However, there are a few key differences. In our model, resources (R) were not allowed to reproduce in the environment, while Cáceres et al. [21] modeled algal resources with logistic population growth. This mirrors our experimental epidemics, and presents a system in which resources are limiting, allowing the influence of competition on infection to manifest. If algal resource growth rate is large enough, resources may never become limiting. Next, Cáceres et al. [21] assumes that consumed resource and pathogen is digested and lost from the system. While we do treat algal this way, we allow a fraction $(1-z_i)$ of pathogen spores to survive host gut passage, as roughly 50% of pathogen spores survived gut passage through a bluegill predator [53]. Other small changes include the lack of predation of the susceptible and non-susceptible consumers that is included as an additional mortality source in Cáceres et al. [21] model, and the reporting of state variables in terms of number per liter, or milligrams dry weight per liter in terms of algal resources, instead of mg C L^{-1} .

Equilibria and stability determination

Here we analyze the model equilibria and their stability, when the invulnerable competitor species is the superior resource competitor. We then rescale the model and perform a sensitivity analysis

on the dimensionless parameters to explore under what conditions the transient effects of initial competitor density influence pathogen dynamics.

No consumers

In the absence of consumers, the resource density reaches an equilibrium of $R = \frac{\pi}{\mu_R}$. This is only stable if neither competing host species is able to invade (see below).

Consumer-resource, no pathogen

For a single consumer species $j = S, C$, the equilibrium resource density is

$$R_j = \frac{\mu_j}{e_j f_{j0} - \mu_j f_{j1}}$$

and the consumer density is

$$j^* = \frac{e_j}{\mu_j} (\pi - \mu_R R_j).$$

Noting that f_{j0}/f_{j1} is the filtering rate when resources are not limiting, we have two conditions for the existence of this equilibrium: (i) for R_j to be positive, we need $e_j f_{j0}/f_{j1} - \mu_j > 0$, i.e. the growth rate of j must be greater than the mortality rate when resources are not limiting; and (ii) for j^* to be positive, we need $R_j < \pi/\mu_R$, i.e. the equilibrium resource density when the consumer is present must be less than the equilibrium resource density in the absence of the

consumer. When only one consumer species is present, consideration of the Jacobian shows that the equilibrium is stable whenever it exists. This is a marked difference from the Caceres et al. (2014) model where “paradox of enrichment” consumer-resource cycles are possible, due to their assumption of logistic resource growth, where in our system we assume a constant daily resource addition to match our experimental treatments.

When both consumers but no pathogen is present, we obtain two different equilibrium values for R , and so the only possibility for coexistence is if $R_S = R_C$:

$$\frac{\mu_S}{e_S f_{S0} - \mu_S f_{S1}} = \frac{\mu_C}{e_C f_{C0} - \mu_C f_{C1}}$$

which in general will not be true. Since in our system, the non-susceptible pathogen is the superior competitor, we find the classic Tilman R^* result that C wins whenever $R_C < R_S$, i.e. when it is able to persist on a lower resource abundance than the susceptible competitor. Since the competitor experiences no negative fitness consequences due to the pathogen, there is no possibility of long-term pathogen-mediated coexistence via apparent competition. However, if these quantities are relatively similar in size, it is possible that coexistence could occur over ecologically relevant timescales (tens of generations).

Resource, susceptible consumer, and pathogen

In the absence of the competitor, the equations are

$$\begin{aligned}
\frac{dR}{dt} &= \pi - \mu_R R - f_S(R)R(S + I) \\
\frac{dS}{dt} &= e_S f_S(R)R(S + \phi I) - \mu_S S - u f_S(R)PS \\
\frac{dI}{dt} &= u f_S(R)PS - \mu_I I \\
\frac{dP}{dt} &= \theta \mu_I I - \mu_P P - z_S f_S(R)P(S + I)
\end{aligned}$$

We can use a next generation matrix to calculate the pathogen basic reproductive number, R_0 . Whenever this threshold quantity is greater than one, the pathogen is able to invade the consumer-resource equilibrium. This quantity is given by

$$R_0 = \frac{\theta u f_S(R_S) S^*}{\mu_P + z_S f_S(R_S) S^*}$$

where R_S and S^* are the disease-free resource and consumer equilibria respectively. Noting that this expression represents the product of the rate at which new spores are produced by filtering-induced infection, the susceptible host density, and the expected lifespan of environmental pathogen, $1/(\mu_P + z_S f_S(R_S) S^*)$, we can make some heuristic predictions about how the addition of a competitor will influence R_0 , and therefore infection prevalence, under non-equilibrium conditions. By reducing resource density through feeding, the competitor increases the susceptible host's filtering rate and therefore its exposure to environmental pathogen. At the same time, resource depletion by the competitor causes a drop in both the susceptible host population and the expected lifespan of environmental pathogen. Since these processes have antagonistic

effects on the effective reproductive number of the pathogen by increasing both the denominator and numerator, over ecologically relevant timescales, different initial competitor densities could either increase or decrease prevalence relative to prevalence in the absence of competitors before competitive exclusion occurs (see Fig. 1 in main text).

Sensitivity analysis and nondimensionalization

In the main text, we used plausible parameter estimates for our experimental system, and the model recreated the observed hump-shaped relationship between mean prevalence and initial competitor density. However, for some parameters, we did not have sufficient data to obtain precise estimates. In order to explore the generality of our model results, we performed extensive sensitivity analyses by rescaling the model to reduce the dimension of parameter space to be explored.

The model variables are rescaled as follows:

$$t = \frac{1}{\mu_S} \tau$$

$$R = \frac{\pi}{\mu_R} r$$

$$S = \frac{\mu_S}{f_{S0}} s$$

$$I = \frac{\mu_S}{f_{S0}} i$$

$$P = \frac{\mu_S}{u f_{S0}} p$$

$$C = \frac{\mu_C}{f_{C0}} c$$

The rescaled model then takes the form

$$\begin{aligned}
\frac{dr}{d\tau} &= \mu_{RS}(1-r) - \frac{1}{1+ar}r(s+i) - \frac{\mu_{CS}}{1+\alpha_{CS}ar}rc \\
\frac{ds}{d\tau} &= \frac{g}{1+ar}r(s+\phi i) - s - \frac{1}{1+ar}ps \\
\frac{di}{d\tau} &= \frac{1}{1+ar}ps - \mu_{IS}i \\
\frac{dp}{d\tau} &= w\mu_{IS}i - \mu_{PS}p - \frac{z}{1+ar}p(s+i) - \frac{\mu_{CS}\zeta_{CS}z}{1+\alpha_{CS}ar}pc \\
\frac{dc}{d\tau} &= \frac{\gamma_{CS}g}{1+\alpha_{CS}ar}rc - \mu_{CS}c
\end{aligned}$$

The definitions of the new dimensionless parameters are given in Table 1. We focus our sensitivity analysis on parameters relating to key infection processes in susceptible hosts, and relative differences between the two resource competitors. Specifically, key infection processes examined included pathogen virulence (μ_{IS}), relative fecundity reduction as a function of infection (ϕ), the fraction of consumed spores that are digested (z), and the mean number of infected spores produced by an infected host (w). Differences among competitors were examined by altering the relative spore digestion (ζ_{cs}), and host population growth potential (γ_{cs}). These two parameters are scaled by the susceptible host species, such that values greater than 1 indicate that the competitor has a proportionally greater value for that parameter.

Results of sensitivity analysis on key infection parameters

Pathogen virulence (μ_{IS}) was varied between 1 and 5, corresponding to a range representing equal mortality rates for susceptible and infected hosts ($\mu_{IS} = 1$) to a death rate 5 times greater for infected hosts (μ_{IS}). Since our default mortality rate for susceptible hosts was 0.1 day^{-1} , the

Table B1: The dimensionless model parameters, their definitions, default values and range explored are summarized in the table below. All parameters described as relative are measured relative to the corresponding parameter for uninfected, pathogen-susceptible hosts.

Parameter	Definition	Interpretation	Default Value	Range
μ_{RS}	μ_R/μ_S	Relative death rate (R)	2.5	
μ_{IS}	μ_I/μ_S	Relative death rate (I)	1.5	1 – 5
μ_{CS}	μ_C/μ_S	Relative death rate (C)	1	
μ_{PS}	μ_P/μ_S	Relative death rate (P)	2.5	
ϕ	ϕ	Fecundity reduction by infection	0.75	0.25 – 1
z	z_S	Fraction of spores digested (S)	0.3	0 – 0.6
ζ_{CS}	z_C/z_S	Relative spore digestion (C)	1	0.3 – 3
a	$f_{S1}\pi/\mu_R$	Maximum reduction to filtering rate (S)	10.72	
α_{CS}	f_{C1}/f_{S1}	Relative reduction to filtering rate (C)	1	
g	$e_S f_{S0}\pi/\mu_S\mu_R$	Maximum lifetime reproductive success (S)	13.4	
γ_{CS}	$e_C f_{C0}/e_S f_{S0}$	Relative fecundity (C)	1.4	0.7 – 1.4
w	$u\theta$	Mean no. of infective spores per host	4.06	2 – 6

maximum death rate we examined was 0.5 day^{-1} , corresponding to a 2 day duration of infection before infected host death. At both small and large virulence values, infection prevalence was reduced (see dotted lines on Figure B1). The peak infection prevalence over a range of competitor densities was highest at intermediate virulence values ($\mu_{IS} = 2.05$ results in the largest infection prevalence). The hump shaped relationship between initial competitor density and infection prevalence is present over the entire range of virulence values examined.

Fecundity reduction (ϕ) is the fraction of offspring that infected individuals have relative

to their susceptible counterparts. This parameter was varied between 75% fewer offspring per reproductive event for infected individuals ($\phi = 0.25$) to no reduction in fecundity as a result of infection ($\phi = 1$). Increasing ϕ increased mean infection prevalence, but the qualitative pattern of infection dynamics as a function of competitor density is unaffected (Figure B2).

The fraction of spores digested (z) influenced mean infection prevalence strongly over a range of values ($z = [0 - 0.6]$), as small values of z resulted in enhanced infection, as pathogen consumed by hosts was not digested, but simply passed through the host gut. Despite the variation in mean infection dynamics across the range of z values, there was a consistent increase in infection at intermediate competitor densities, followed by a decrease when competitor density becomes larger (Figure B3). This underscores the importance of the competitor as a remover of pathogen spores in driving infection dynamics.

The number of infectious spores per infected host w is the product of per spore infectivity (u) and the number of spores an infected host produces (θ). At low values of w , mean infection prevalence was greatly reduced (values very close to 0), while increasing w values caused an exaggeration of the peaked relationship we observed in mean infection prevalence as a function of competitor host density (Figure B4).

Results of sensitivity analysis on relative competitor differences

The last two quantities in the dimensionless model that we altered were values controlling the relative difference between competing hosts in terms of spore digestion (ζ_{cs}) and fecundity (γ_{cs}). The relationship between infection prevalence and initial competitor density remains hump-shaped even when the competitor has is able to digest double the fraction of ingested spores as the susceptible host ($\zeta_{cs} = 2$). At this parameter increases past this point ($\zeta_{cs} \geq 2$), the competing

host removes enough pathogen from the environment to reduce infection prevalence across a range of competitor densities, suggesting that it is possible that competition could be beneficial to susceptible host populations in some circumstances (Figure B5). The relative fecundity of the competitor to susceptible host species determines how rapidly the competitor population grows relative to the susceptible host. The default value from our model was 1.4, suggesting that the competitor has 40% more offspring during the generation time of the susceptible host, while a value of 1 would correspond to the competitors being equivalent, and values less than 1 mean the susceptible host is the dominant competitor. Even when the susceptible host was the superior competitor, increasing competitor density still increased mean infection prevalence (Figure B6).

Sensitivity of initial conditions

We also examined the sensitivity of our findings to initial susceptible host density (Figure B7). We found that while infection prevalence increased as a function of initial susceptible host density, the effects of competitor density remained. That is, the hump-shaped relationship between initial competitor density and mean infection prevalence remained across the gradient of initial susceptible host densities. Further, we examined the influence of the initial environmental pathogen (P) population size on infection dynamics along a gradient of competitor densities. The hump-shaped relationship between mean infection prevalence and initial competitor density was unaffected with increasing initial environmental pathogen (P) population size, though infection prevalence was enhanced with increasing initial pathogen abundance (Figure B8).

Figures

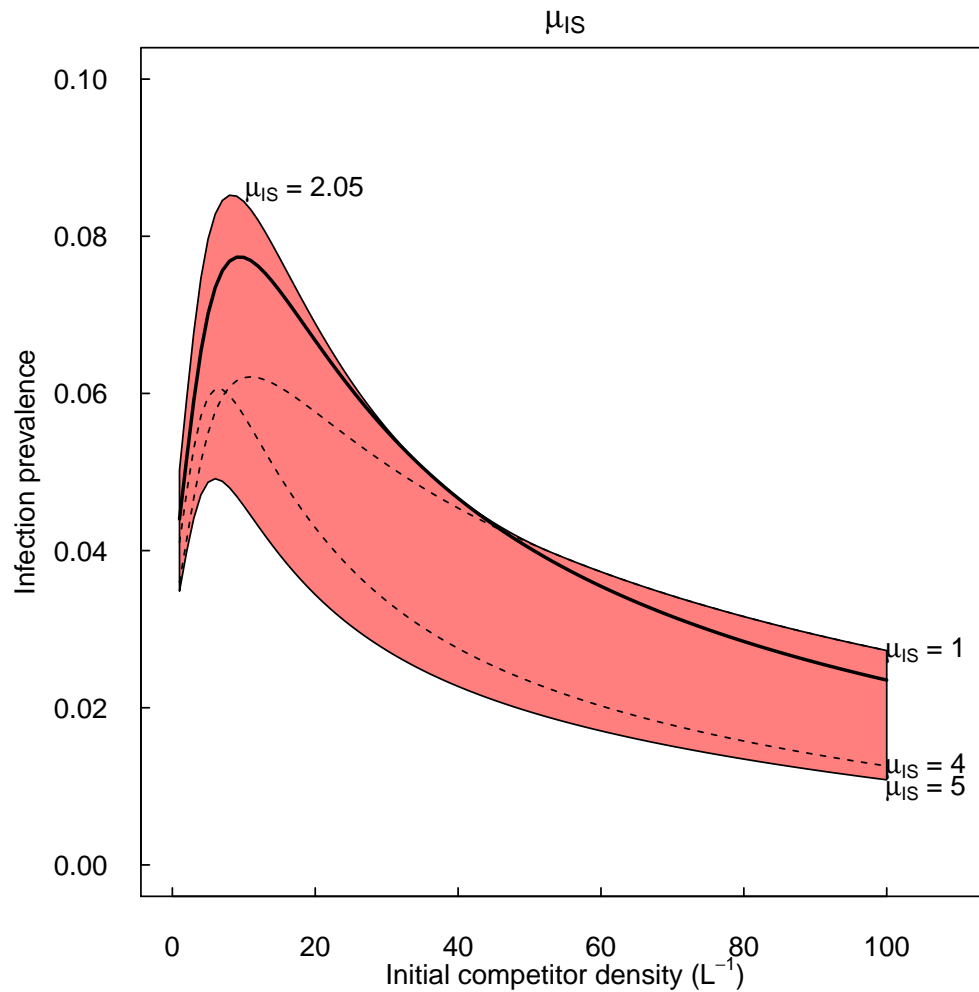


Figure B1: The hump-shaped relationship between initial competitor density and infection prevalence was conserved for a wide range of pathogen virulence (μ_{IS}) values. Shaded regions indicate ranges of infection prevalences observed across the parameter range, and solid black lines within the shaded region indicate our default model parameterization. Dotted lines show how the shape of the relationship between initial competitor density and infection prevalence changes, with the sharpest peak at an intermediate pathogen virulence ($\mu_{IS} = 2.05$).

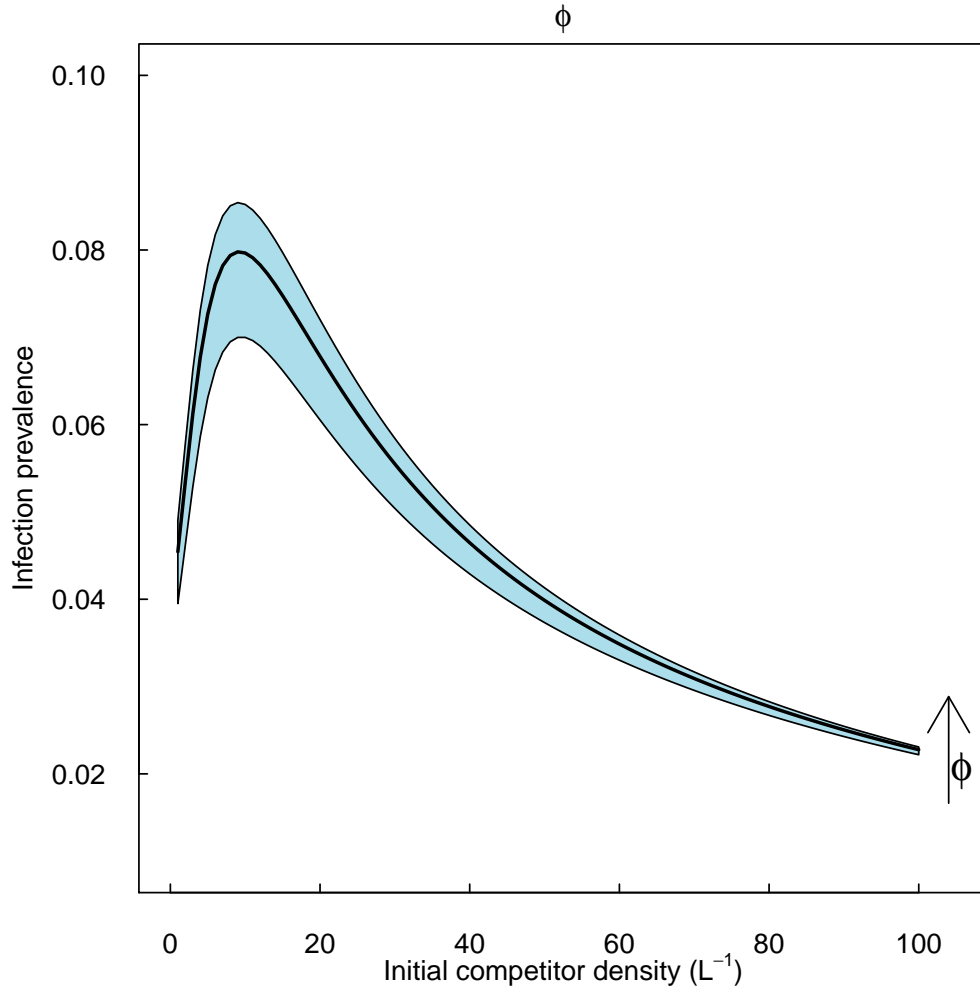


Figure B2: Fecundity reduction as a function of pathogen infection (ϕ) did not strongly influence the relationship between competitor density and mean infection prevalence, slightly increasing infection when infected individuals did not reproduce. Shaded regions indicate ranges of infection prevalences observed across the parameter range, and solid black lines within the shaded region indicate our default model parameterization.

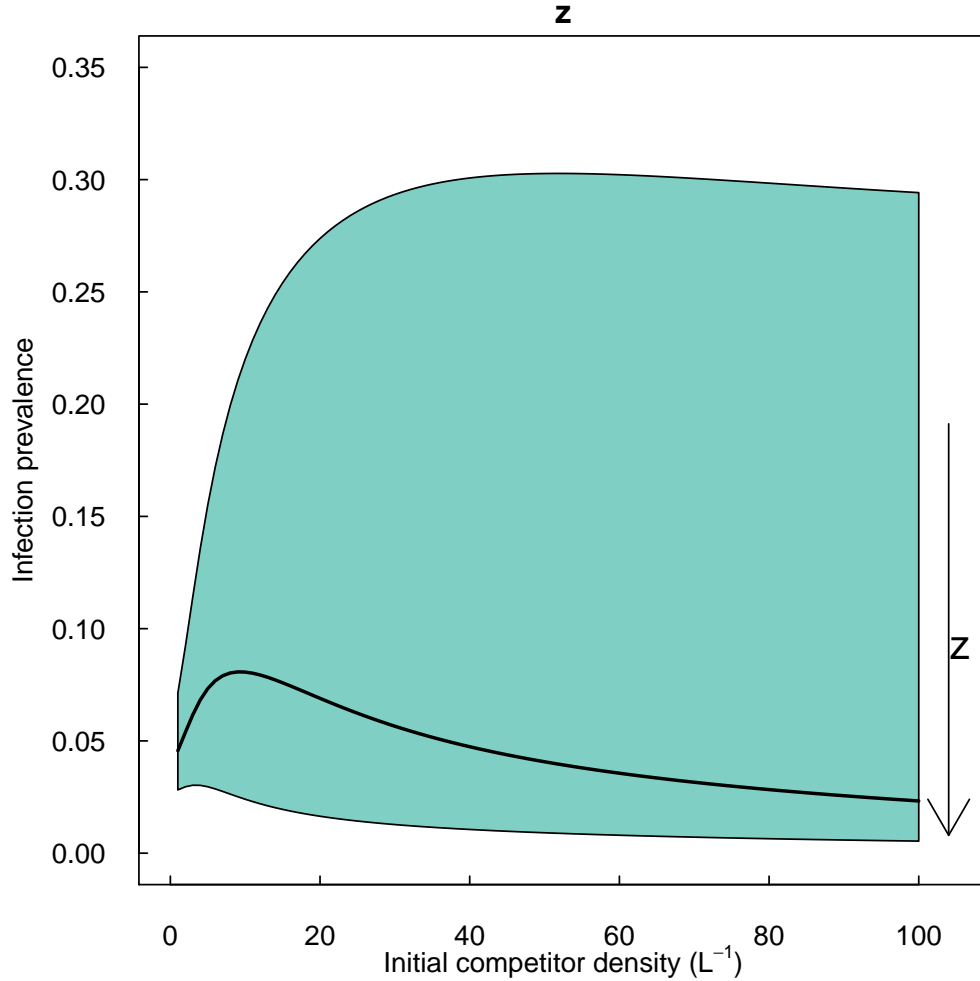


Figure B3: The fraction of spores digested influenced infection dynamics, as low values corresponded to a large pathogen population, and subsequently enhanced transmission. However, the non-monotonic relationship between initial competitor density and infection prevalence remained present across the examined parameter range. Shaded regions indicate ranges of infection prevalences observed across the parameter range, and solid black lines within the shaded region indicate our default model parameterization.

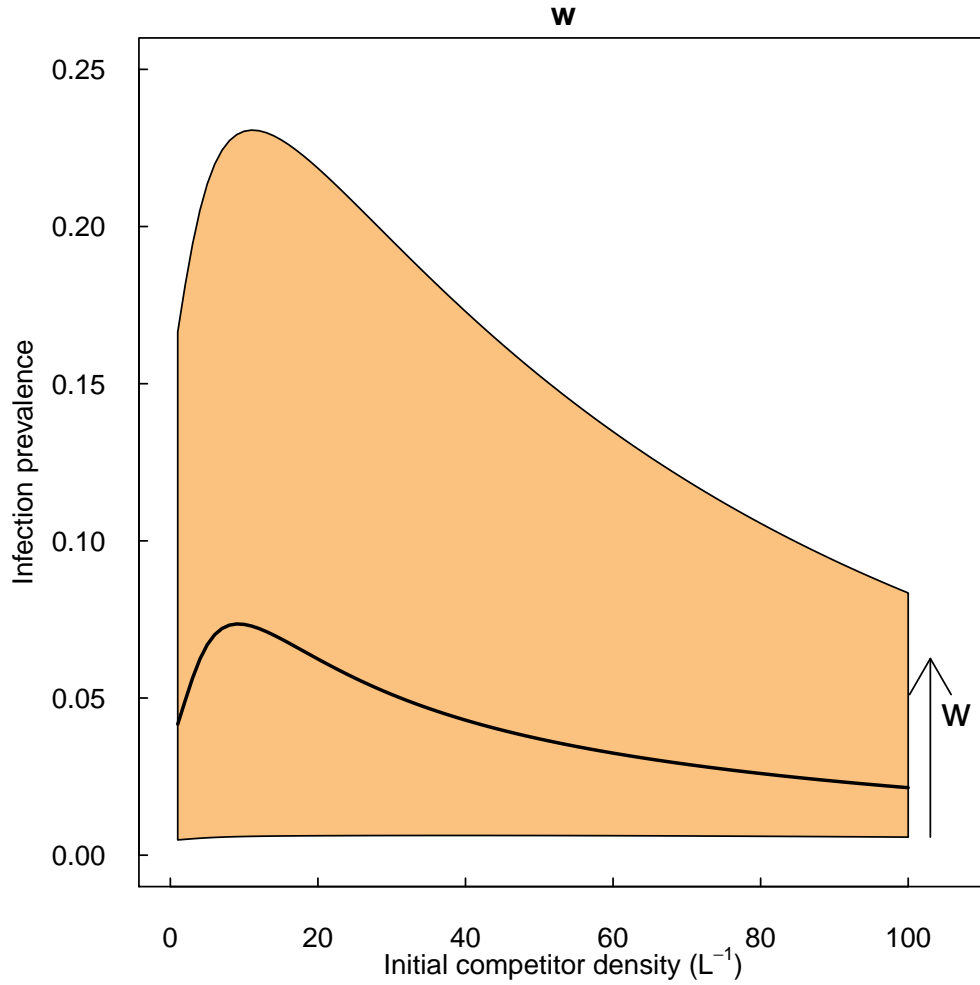


Figure B4: The mean number of infectious spores per infected host strongly influenced infection dynamics, while largely maintaining the non-monotonic relationship between infection prevalence and competitor density. Shaded regions indicate ranges of infection prevalences observed across the parameter range, and solid black lines within the shaded region indicate our default model parameterization.

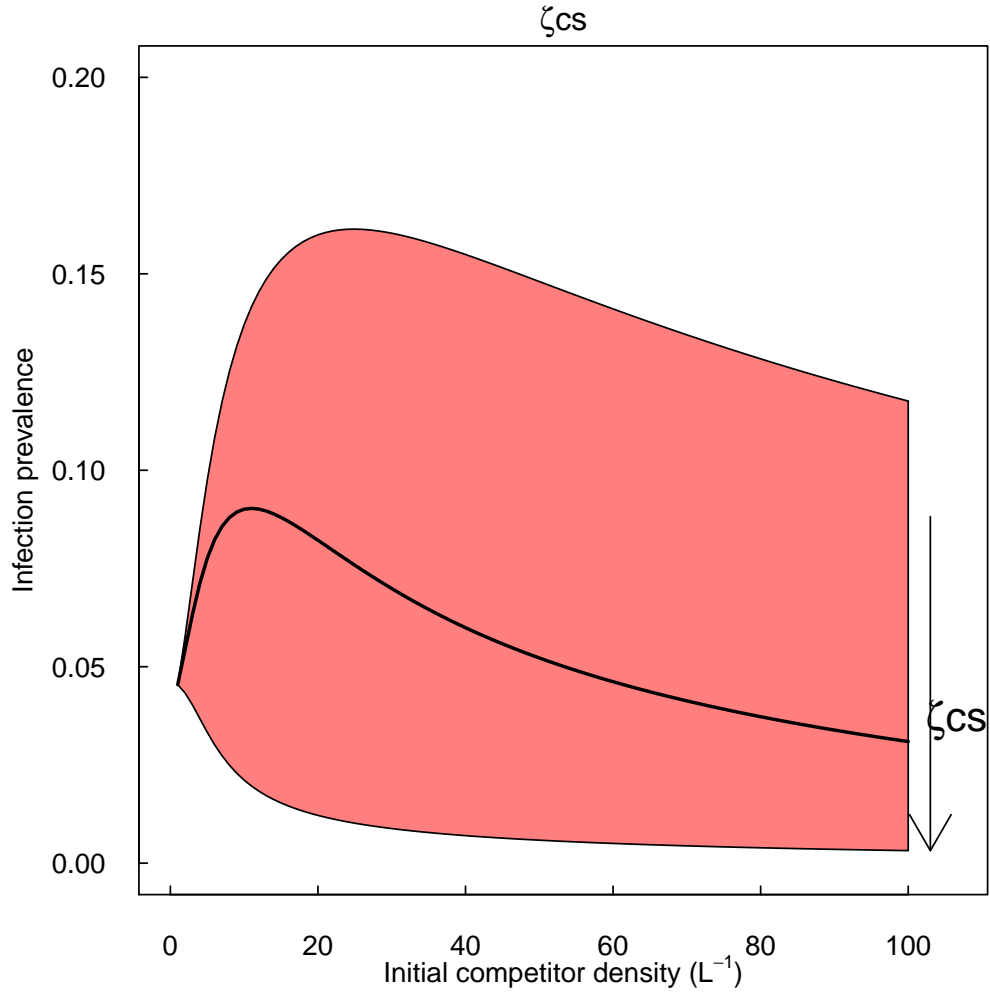


Figure B5: Competition reduced infection prevalence when the competitor digested a far greater proportion of spores relative to the susceptible host ($\zeta_{cs} \gtrsim 2.2$). However, at values smaller than this, the relationship between initial competitor density and infection dynamics was unchanged by ζ_{cs} . Shaded regions indicate ranges of infection prevalences observed across the parameter range, and solid black lines within the shaded region indicate our default model parameterization.

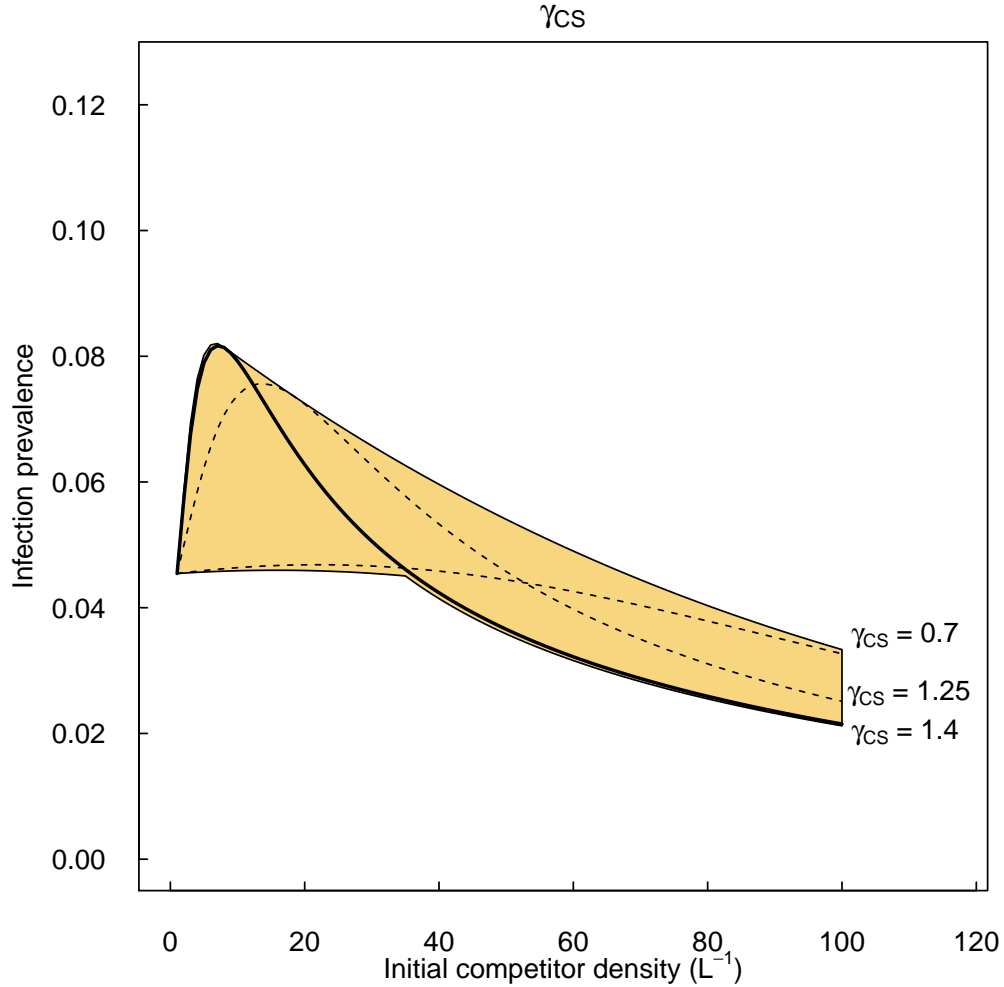


Figure B6: The proportional fecundity of the competitor served to move the competitor density at which infection prevalence was maximized, but did not alter the overall non-monotonic relationship between initial competitor density and infection prevalence for the range of γ_{cs} parameter values examined. Shaded regions indicate ranges of infection prevalences observed across the parameter range, and solid black lines within the shaded region indicate our default model parameterization. Dotted lines show how the shape of the relationship between initial competitor density and infection prevalence changes along the proportional fecundity gradient, as smaller values tend to flatten the curve (dotted line associated with $\gamma_{cs} = 0.7$).

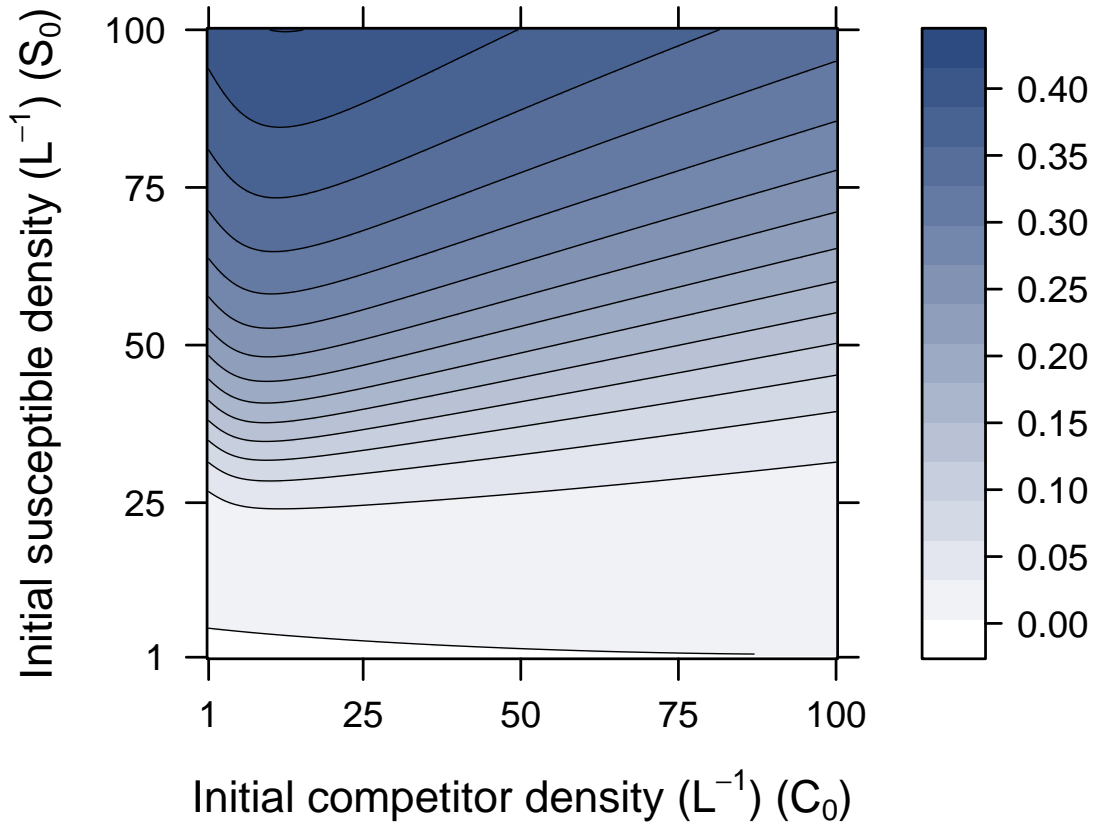


Figure B7: Mean infection prevalence as a function of initial competitor density (x -axis), and susceptible density (y -axis). Mean infection prevalence increases with increasing initial susceptible host density. The hump-shaped relationship between infection prevalence and competitor density is maintained across the gradient of initial susceptible host densities.

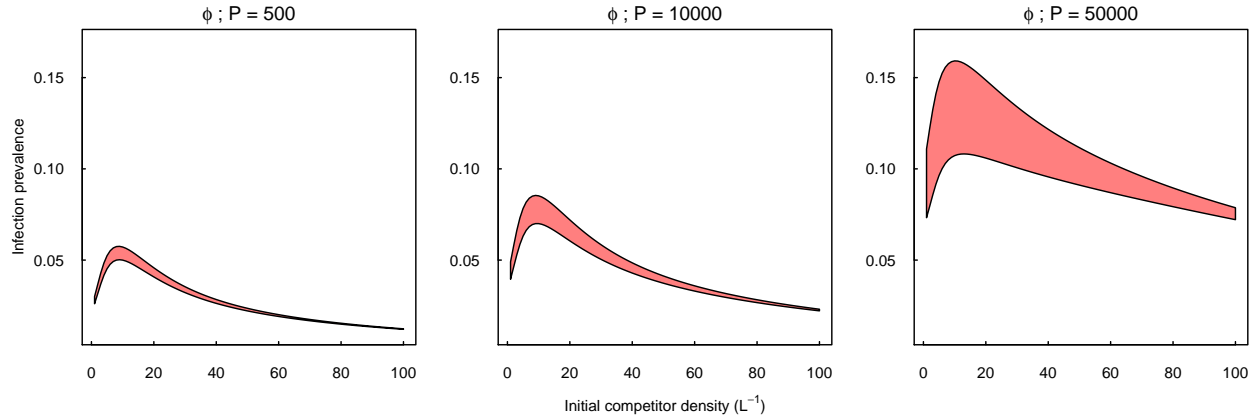


Figure B8: Mean infection prevalence as a function of initial competitor density (x -axis), for three different starting conditions of the environmental pathogen population (smallest on the left, to largest on the right). The hump-shaped relationship between infection prevalence and competitor density is maintained regardless of initial environmental pathogen population size. Shaded regions indicate ranges of infection prevalence for a range of pathogen death rates ($\mu_P = [0.25 - 0.9]$), demonstrating that the hump-shaped relationship is conserved for a wide range of pathogen mortality values.

Appendix C

Costs of resistance and infection by a generalist pathogen

The aberrant fifth host species

In the main text, we report on the results from four *Daphnia* host species, but the experiment actually included a fifth species (*D. dentifera*). However, this host species suffered high mortality, and had an average lifespan of around 15 days, and twenty individuals surviving for fewer than ten days (Figure C1). Apart from mortality, 44% of hosts did not reproduce, and clutch sizes of those that did successfully reproduce tended to be small. As a result of high mortality, replication was insufficient to test for resistance and infection costs. Because of all the reasons above, we excluded *D. dentifera* from analysis. However, for completeness, we recreate Table 1 (see Table C1) and Figure 1 (see Figure C2) from the main text here with *D. dentifera* included.

The effect of host age at exposure on the magnitude of resistance and infection costs

Hypothetically, older hosts should respond to pathogen challenge differently than younger hosts, as resistance should tradeoff with host fitness, which is intrinsically related to host age for most organisms. Therefore, older hosts would be expected to not mount a large resistance response. To examine the effect of host age on resistance and infection costs, we fit linear models to the relationship between host age at pathogen exposure and the relative difference in total reproduction, mean clutch size, and lifespan between exposed-uninfected and infected individuals of the same age (infection costs), and exposed-uninfected and resistant hosts of the same age (resistance costs). We found no evidence that host age at pathogen exposure influenced resistance (Figure C3) or infection (Figure C4) costs, except for a positive relationship between host age and the magnitude of resistance cost in terms of total reproduction for *D. pulicaria*. This means that there was a greater difference in total reproduction between resistant and control hosts when hosts were older. The fact that *Daphnia pulicaria*, a host that has never, to our knowledge, been observed to be infected, incurred such a great cost of resistance, is curious and seemingly maladaptive, when the probability of becoming infected is low (or null).

The potential relationship between host susceptibility and resistance costs

The relative per-species difference between exposed-uninfected host individuals and control individuals (i.e. resistance cost size) was dependent on host species susceptibility (Figure C5) after excluding data from *D. dentifera* as a result of the high mortality observed for this species. This

difference was calculated by sampling control and resistant hosts of a single species, truncating the control host samples to be the same length as the resistant host samples, and taking the difference between the means. This was performed 1000 times for each host species and fitness metric combination, which allowed the plotting of both mean and standard deviation of the mean difference between control and resistant hosts.

The use of a single clone of each host species examined makes interspecific comparisons difficult, as there could be large intraspecific variation in physiological responses to pathogen exposure. We therefore do not make any claims regarding the generality of the relationship between resistance cost and host susceptibility. However, this is an interesting open question, as the answer could potentially provide a more mechanistic or evolutionary perspective on interspecific differences in resistance costs. Specifically, perhaps host species are less susceptible because they mount such a large resistance effort. Understanding the mechanistic basis of interspecific variation in resistance costs is an interesting, and currently largely unexplored research area.

Figures

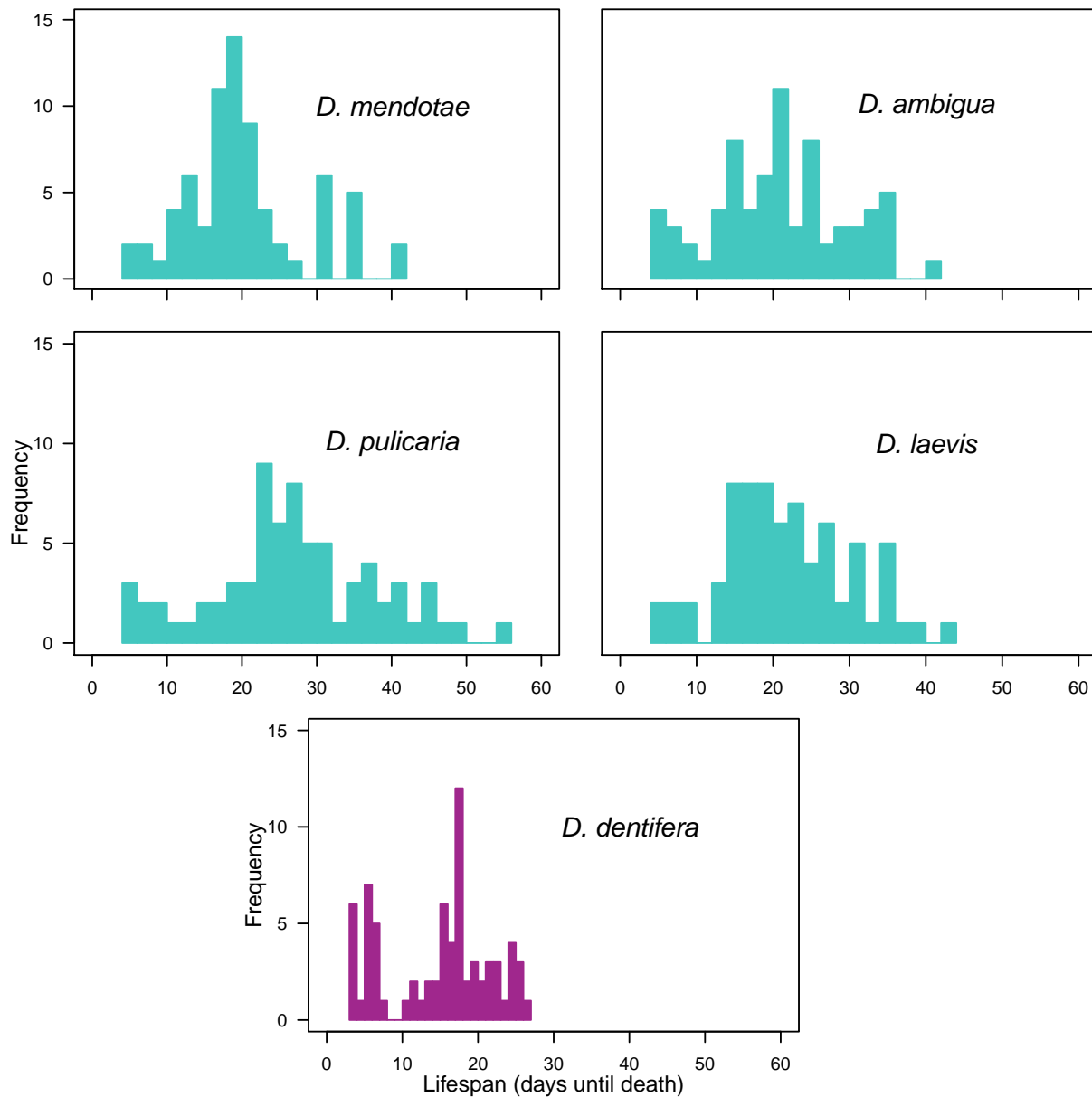


Figure C1: The distribution of lifespans was variable for some host species. High mortality in *D. dentifera* resulted in the exclusion of this species from the analysis of the main text. Note the clump of *D. dentifera* hosts (bars are colored fuchsia to highlight panel) with lifespans less than 10 days. Further, no *D. dentifera* host lived longer than 30 days.

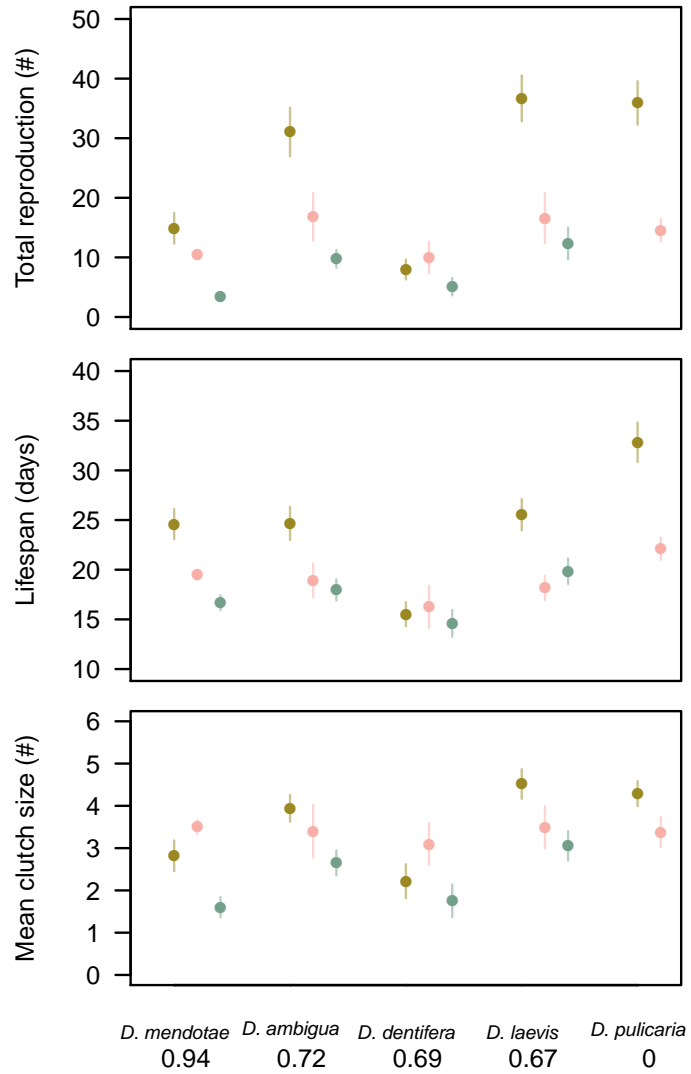


Figure C2: Observed costs of resistance and infection to a generalist fungal pathogen. This is the same as the main text Figure 1, but includes *D. dentifera*, who was excluded because of high mortality observed.

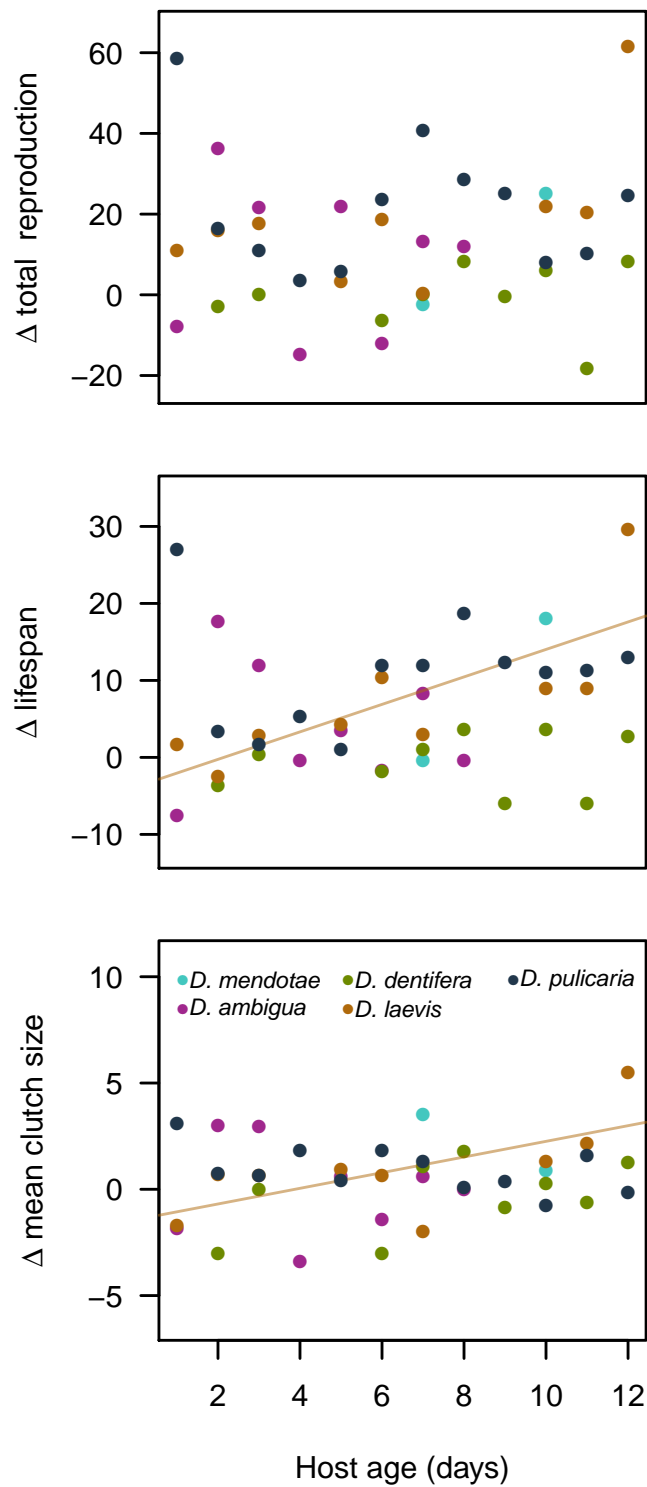


Figure C3: Resistance costs along a gradient of host age at pathogen exposure. Host age at pathogen exposure significantly influenced resistance costs in one host (*D. laevis*) for two (lifespan and mean clutch size) of the three fitness measures examined.

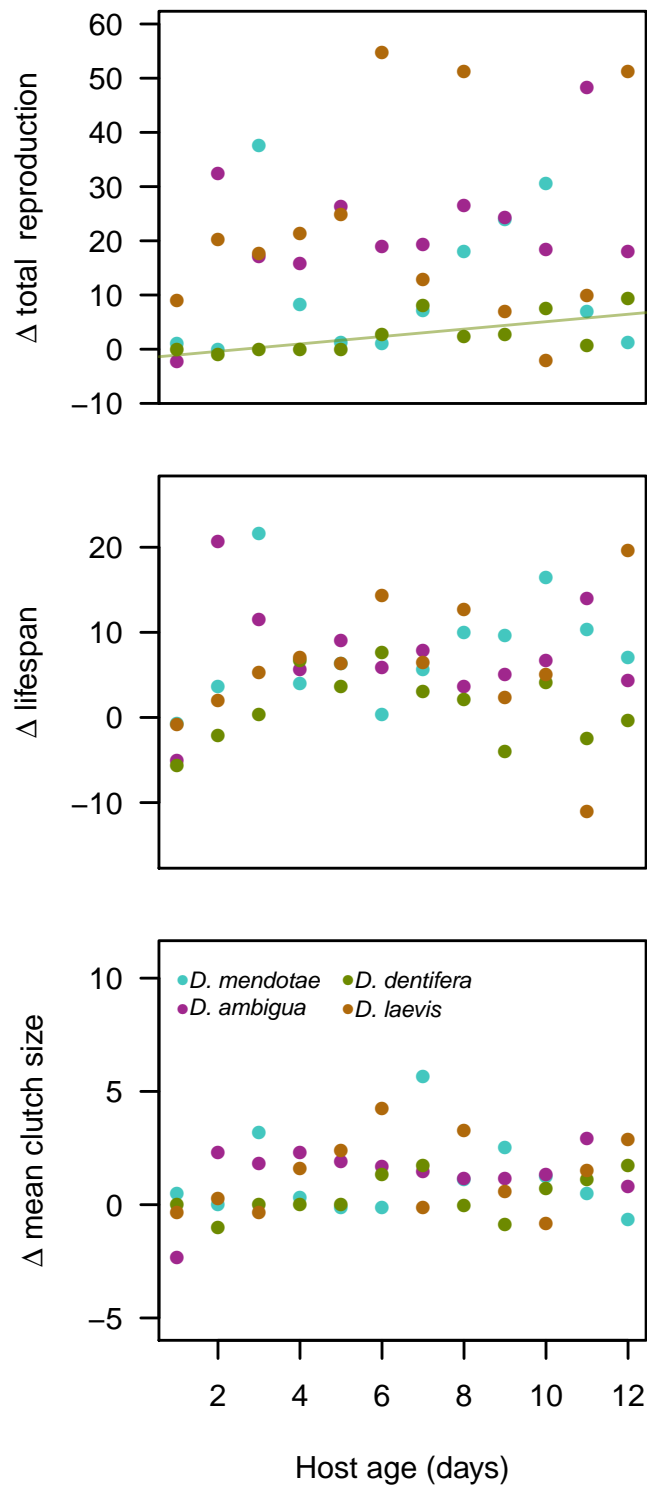


Figure C4: Infection costs along a gradient of host age at pathogen exposure. Infection costs were unrelated to host age at pathogen exposure, except for reproduction of *D. dentifera*, though this host was excluded from analyses, and the age effect on change in total reproduction is small.

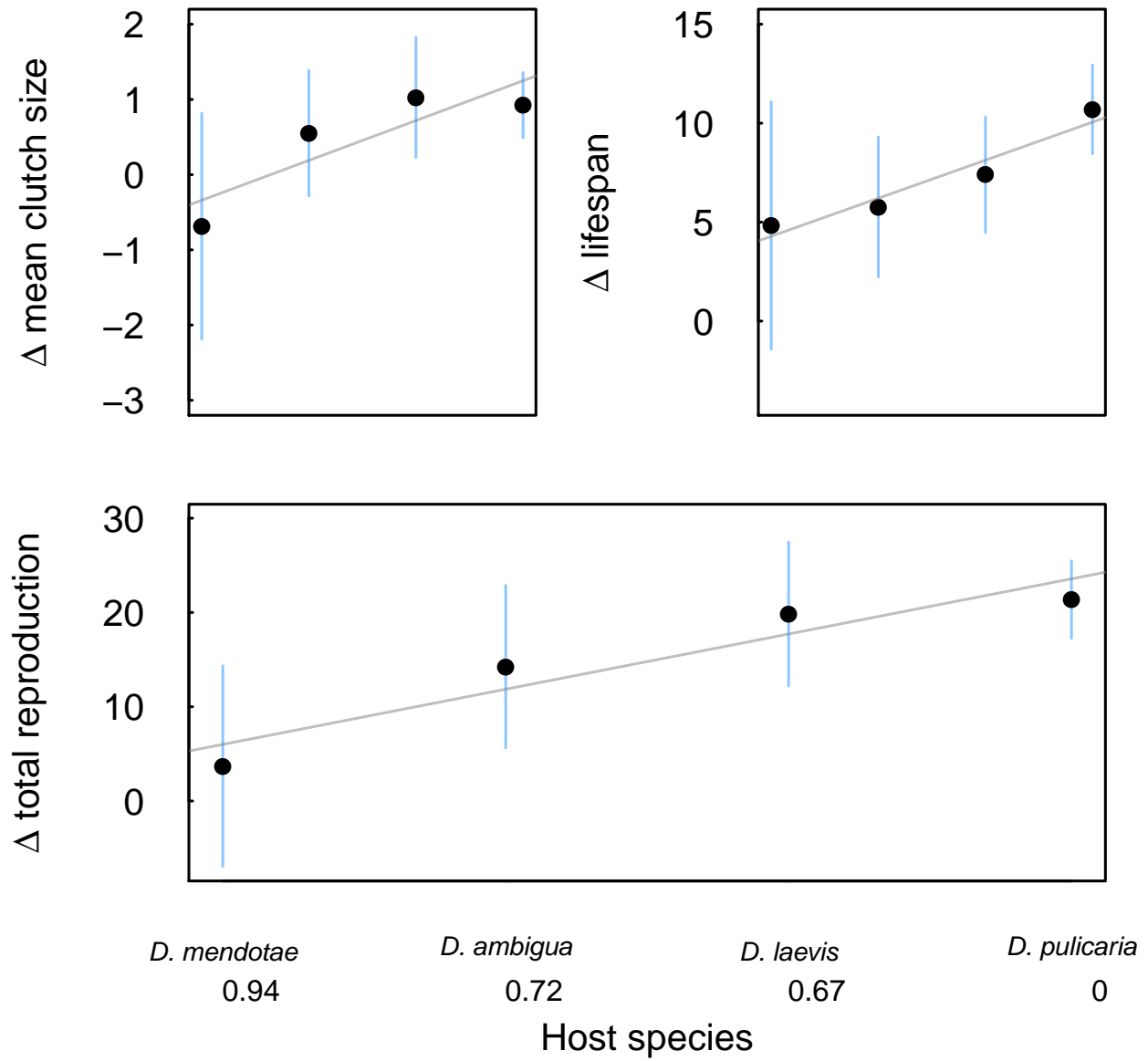


Figure C5: Resistance costs scale with host susceptibility. The difference between means (calculation described above) is plotted for 1000 bootstrapped samples. Plotted points are mean differences ± 1 standard deviation. Grey lines are linear models for illustrative purposes, though the relationship is significant for lifespan (adj. $R^2 = 0.911$, $p = 0.03$).

Tables

Table C1: Mean and standard error for fitness measures (reproductive output, lifespan, and mean clutch size) for control, exposed-uninfected, and infected individuals. Host species are ordered from most to least susceptible to infection by *M. bicuspidata*.

Host	Infection status	n	Reproduction	Lifespan	Mean clutch size
<i>D. mendotae</i>	control	36	14.89 (2.57)	24.58 (1.53)	2.81 (0.36)
	exposed-uninfected	2	10.5 (0.50)	19.50 (0.50)	3.50 (0.17)
	infected	34	3.47 (0.77)	16.68 (0.74)	1.61 (0.23)
<i>D. ambigua</i>	control	36	31.67 (4.08)	24.67 (1.66)	3.88 (0.31)
	exposed-uninfected	10	16.80 (4.01)	18.90 (1.69)	3.39 (0.62)
	infected	26	10.85 (1.55)	17.96 (1.06)	2.67 (0.26)
<i>D. dentifera</i>	control	36	7.97 (1.67)	15.53 (1.21)	2.22 (0.40)
	exposed-uninfected	11	10.00 (2.64)	16.27 (2.12)	3.10 (0.49)
	infected	25	5.08 (1.45)	14.60 (1.35)	1.75 (0.39)
<i>D. laevis</i>	control	36	36.97 (3.90)	25.53 (1.57)	4.48 (0.35)
	exposed-uninfected	12	23.83 (6.89)	19.50 (0.90)	4.87 (1.18)
	infected	24	12.83 (2.64)	19.83 (1.28)	3.02 (0.34)
<i>D. pulicaria</i>	control	36	35.92 (3.64)	32.83 (1.99)	4.29 (0.30)
	exposed-uninfected	36	14.56 (1.88)	22.11 (1.14)	3.33 (0.35)
	infected	0	–	–	–