Ecological and Evolutionary Dynamics of Complex Host-Parasite Communities

by

Camden D. Gowler

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Doctoral Committee:

Professor Meghan A. Duffy, Chair
Associate Professor Marisa C. Eisenberg
Professor Edward Ionides
Associate Professor Timothy Y. James
Professor Aaron A. King

Camden D. Gowler

cgowler@umich.edu

ORCID iD: 0000-0003-3596-614X

Dedication

To a few of my hometown heroes: Pres. Jimmy Carter, Rep. John Lewis, and Dr. Martin Luther King Jr. You will always give me hope.

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Abstract

Parasites are ubiquitous in nature, and embedded in complex communities of hosts and parasites. Most parasite species infect multiple host species, and most host species are infected by multiple parasite species. However, it's very challenging to study the complex web of host-parasite interactions in natural settings, and controlled lab experiments are often limited to small numbers of host or parasite species. Additionally, parasites can evolve rapidly, so host-parasite interactions change over time. In my dissertation, I used field surveys, network analyses, and lab experiments to understand how different host species influence parasite infections in another host species, how parasites differ in their ability to infect multiple host species, how hosts respond to the threat of multiple parasites, and how parasites evolve over the course of an epidemic. My general aims were to untangle the web of interactions in host-parasite communities and to understand the evolutionary consequences of those interactions. In Chapter 2, I estimated potential cross-species transmission of different parasite species and built networks of hosts and parasites connected by these interactions. In Chapter 3, I investigated the consequences of multiple parasites on host behavior, namely sexual reproduction. Lastly, in Chapter 4, I looked to see if parasites were evolving in response to ecological dynamics such as the growth phase of an epidemic. Overall, I found that particular host and parasite species may disproportionately contribute to cross-species transmission, hosts alter their reproductive behavior in response to biotic factors, and parasite virulence can evolve rapidly over the course of a natural epidemic.

Chapter 1 Introduction

Parasites are omnipresent in nature (Lafferty, Dobson, and Kuris 2006; Windsor 1998), and embedded in complex and changing communities of hosts and parasites (Rigaud, Perrot-Minnot, and Brown 2010; Pedersen and Fenton 2007). Most parasite species infect multiple host species (Petney and Andrews 1998), and most host species are infected by multiple parasite species (Pedersen and Fenton 2007). Further complicating the story, both hosts and parasites can evolve over time (Anderson and May 1982). Thus, there is a web of many interactions between hosts and parasites in communities and those interactions might change over time. My broad aims for this dissertation are (1) to untangle the web of hosts and parasites in nature by investigating which hosts are transmitting which pathogens and to whom and (2) to predict how these interactions will change over time by studying if pathogens evolve to become more or less harmful and if hosts are under pressure to alter their reproductive behavior.

Over the past six years of research, I have used *Daphnia* communities and their many parasites as a study system to address important and timeless questions about infectious disease ecology and evolution. *Daphnia* are an amazing model system for numerous and sometimes unexpected reasons. First, *Daphnia* are plankton that live in lakes with clearly defined boundaries, making it simple to designate and study communities as replicates. Second, they are cyclical parthenogens, meaning they reproduce both asexually and sexually, which is crucial because we can preserve clonal lines indefinitely in the lab. Third, an initially unexpected asset: *Daphnia* are transparent, meaning we can readily diagnose parasite infections. Finally, I

(subjectively) believe *Daphnia* and their parasites are representative of how these complex interactions actually work in nature.

Throughout my dissertation work, I've often wondered what a "typical" or "normal" community of hosts and parasites would look like. We know the interactions would be complex: even small communities of hosts can have and share many parasite species (Johnson, de Roode, and Fenton 2015). Often, the more a host species is sampled, the more parasites are found (Pappalardo et al. 2020), and likely those parasite species are infecting more than one host species. Thus, there is a diversity of parasites infecting each host species.

But what determines which parasites infect which host species? If a parasite species is adapted to a particular host species, then phylogenetic relatedness of hosts may help predict which other host species it can infect (Streicker et al. 2010). Additionally, spatial structure of different host species is important: parasites must be able to encounter multiple host species in order for cross-species transmission to occur. Life history of both the host and parasite can be especially important. For example, parasites with a complex life cycle often require a particular order of hosts. Transmission mode of the parasite is another great example (Pedersen et al. 2005). For instance, environmentally transmitted pathogens, like the bacterium that causes anthrax, can easily encounter many potential host species, whereas sexually transmitted infections depend more on the particulars of host behavior.

Reciprocally, what makes a host good at transmitting parasites? Often, "fast" life history traits—growing and reproducing quickly—are associated with harboring more parasites (Han et al. 2015). Host immunity is also important, albeit more complicated. Hosts with poor immunity may easily become infected but may die quickly as a result (e.g. insects), whereas hosts with good long-lasting immune systems may be less likely to become infected but can harbor

parasites without much of a cost (e.g. bats). Together, understanding the host and parasite interactions in a community is important on its own, but even more so because most emerging infectious diseases of humans are zoonotic in origin (Woolhouse, Haydon, and Antia 2005).

Given the complexities of host-parasite communities, we need objective and reliable means of quantifying and studying them. I argue that network-based methods, like those used in the social sciences, present a great opportunity. We can build networks by drawing connections between hosts or parasites based on samplings of their interactions in nature. Then, we can apply metrics like centrality to measure the "importance" (*e.g.*, how influential is a given node in relation to the cohesiveness of the network) of each host or parasite in the network, much like search engines rank various websites. These objective metrics allow us to create predictions about particular hosts and parasites; we can then go out and evaluate those predictions with field observations, lab experiments, and/or mathematical models.

Once we understand some of the ecological complexities, we can begin to ask more specific questions about host and parasite populations. I chose to focus on one question related to hosts and one related to parasites. From the host point of view, it's important to recognize the extensive selection pressure faced by hosts. Fortunately for hosts, there are a multitude of options for escaping or alleviating the burden of parasitism, such as evolution of resistance (Anderson and May 1982), behavioral avoidance (Buck, Weinstein, and Young 2018), and medication (Lefèvre et al. 2010). Another possible strategy hosts can employ is genetic recombination via sexual reproduction.

Ideas like the Red Queen hypothesis suggest host-parasite interactions are responsible for the maintenance of sexual reproduction (Strotz et al. 2018). The Red Queen hypothesis posits that, because parasites are continually adapting to the most common host genotypes and because

parasites exert strong selection on their hosts through virulence, hosts are under constant selection to produce more diverse offspring, which are more likely to be resistant to the dominant parasites (Ebert and Hamilton 1996). Thus, coevolutionary interactions between hosts and parasites might favor reproducing sexually as opposed to asexually. There are great examples of this in nature, such as snails from New Zealand (Lively and Dybdahl 2000) and *Caenorhabditis elegans* (Morran et al. 2011). However, few studies have incorporated the threat of multiple parasites, even though we now know that is the norm in nature. Thus, my work attempts to bridge the gap by addressing the multiple biotic factors like multiple parasites and host density, as well as known abiotic factors that are more specific to *Daphnia* biology.

On the flip side, parasites face enormous selection pressures as well. Unlike a predator, parasites are not "running for their dinner", they are running for their lives. Because parasites have short generation times and face strong selective pressure, it is not unexpected to see them evolve over short periods of time. The most important example of this is parasites evolving in response to epidemiological dynamics (the transient phase before an equilibrium state). In general, theory predicts that the two main strategies for optimal virulence are to either maximize *r* (intrinsic growth rate) or maximize R_0 (the basic reproductive number) (Bolker, Nanda, and Shah 2010). At the start of an epidemic, when susceptible hosts are plentiful, pathogens may evolve increased virulence, thus maximizing the intrinsic growth rate. However, as the epidemic wanes, lower virulence and higher R_0 might be more advantageous. Unfortunately, most studies of transient virulence evolution are either strictly theoretical or use non-native host and/or parasite species. I wanted to understand virulence evolution in more natural settings, using a combination of lab and field experiments to explore this.

More generally, to begin to understand the complexities of host-parasite communities, I focus on three main questions: (1) how are pathogens being transmitted among host species in a community context?; (2) how do hosts respond to the threat of multiple pathogens?; and (3) how do pathogens change over time in response to ecological dynamics?

SUMMARY OF CHAPTERS

Chapter 2: Using Networks to Understand Cross-Species Transmission in Daphnia Communities

Parasites are everywhere in nature. Most parasite species infect multiple host species, and reciprocally, most hosts are infected by multiple parasites. This complexity poses a challenge for observational and empirical studies, but these communities are vital to study because most emerging infectious diseases of humans are zoonotic in origin. Even the most basic of questions: like "which parasites are transmitting across different hosts" are very hard to objectively answer. Here, we attempt to address these types of questions by using *Daphnia* host-parasite communities as a model system. We used a long-term time series dataset on 8 different host species and 7 different parasite species. We leveraged the power of network-based approaches like degree and centrality to predict patterns of cross-species transmission. Our network analyses of the study communities found that parasite species varied in the degree to which they infected multiple host species and likely varied in their capacity to transmit from one host species to another. Furthermore, some host species may disproportionately transmit certain parasites, but the measure of infection (prevalence or infected host density) also mattered. In total, this information gives us insights into the dynamics of the multihost-multiparasite community as a whole, including identifying host and parasite species that might disproportionately contribute to cross-species transmission.

Chapter 3: Pluralistic Approach Reveals Biotic and Abiotic Factors Associated with Variation in Sex in Natural Populations of Daphnia dentifera

The maintenance of sexual reproduction is one of the oldest unsolved mysteries in evolutionary biology. While there has been a particular focus on the potential role of parasitism in explaining sexual reproduction, a variety of factors have the potential to influence investment in sex. Here, we take advantage of the natural history of *Daphnia* dentifera—which alternate between sex and asex—to uncover the variables associated with the wide variation in sexual reproduction in this system. We tracked host density, parasite infections, sexual reproduction, temperature, and light in 15 wild populations of *Daphnia* for three years. We found substantial variation in investment in sex, with some populations reproducing entirely asexually and others shifting almost entirely to sexual reproduction by late autumn. Moreover, we found that higher host density and parasitism were associated with greater investment in sex. Temperature and light were not as predictive of investment in sex, but may indeed play a role. While correlational, our results leverage a large time series dataset and suggest the pluralistic factors that may be responsible for sexual reproduction. Interestingly, our results suggest density (an understudied factor for the maintenance of sex, but one recognized as important in prior studies of cyclical parthenogens) was an important predictor of investment in sex in our study populations. Together, the results add to our understanding of the evolution and maintenance of sexual reproduction in nature.

Chapter 4: Virulence Evolution in Natural Epidemics of a Pathogen of Daphnia

Virulence, the degree to which a pathogen harms its host, is perhaps the most important trait of a host-pathogen interaction. However, it is not a static trait, instead depending on the ecological context and possibly evolving over short periods of time (e.g., during the course of an epidemic). In general, theory predicts that the two main strategies for optimal virulence are to either maximize *r* (intrinsic growth rate) or maximize R_0 (the basic reproductive number). At the start of an epidemic, when susceptible hosts are plentiful, pathogens may evolve increased virulence, thus maximizing the intrinsic growth rate. However, as the epidemic wanes, lower virulence and higher R_0 might be more advantageous. Although abundantly studied theoretically, there is still a lack of empirical evidence for virulence evolution in epidemics, especially in natural settings with native host and pathogen species. Here, we used a combination of field observations and lab experiments in the *Daphnia*-Pasteuria model system to look for evidence of virulence evolution in nature. Controlling for environmental conditions, we found that virulence did in fact change over the course of the epidemic, although our study is limited to a single population. Nevertheless, this provides a foundational knowledge for other studies of virulence evolution in this and other systems.

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Chapter 2 Using Networks to Understand Cross-Species Transmission in *Daphnia* Communities

with Mary A. Rogalski, Clara L. Shaw, Katherine K. Hunsberger, Marisa C. Eisenberg, and Meghan A. Duffy

ABSTRACT

Parasites are everywhere in nature. Most parasite species infect multiple host species, and reciprocally, most hosts are infected by multiple parasites. This complexity poses a challenge for observational and empirical studies, but these communities are vital to study because most emerging infectious diseases of humans are zoonotic in origin. Even the most basic of questions: like "who is infecting whom and with what?" are very hard to objectively answer. Here, we attempt to address questions about cross-species transmission by using *Daphnia* host-parasite communities as a model system. We used a multi-year time series dataset on 8 different host species and 7 different parasite species, all of which are short-lived. We leveraged the power of network-based approaches to examine patterns of cross-species transmission. Our network analyses of the study communities found that parasite species varied in the degree to which they infected multiple host species and likely varied in their capacity to transmit from one host species to another. Furthermore, some host species may disproportionately transmit certain parasites, but in some cases the results were sensitive to the measure of infection (prevalence or infected host density). In total, this information gives us insights into the dynamics of the

multihost-multiparasite community as a whole, including identifying host and parasite species that may disproportionately contribute to cross-species transmission.

INTRODUCTION

Not only are parasites ubiquitous in nature, but they are also often embedded in complex webs of interactions between different host and parasite species (Rigaud, Perrot-Minnot, and Brown 2010). In fact, most parasite species infect multiple host species in nature, and reciprocally, most host species are infected by multiple parasite species (Pedersen and Fenton 2007; Petney and Andrews 1998; Rynkiewicz, Pedersen, and Fenton 2015). This poses an enormous research challenge: how do we objectively study these complex communities and disentangle which host and parasite species are most important for issues like cross-species transmission and spillover?

It's vital to study host-parasite communities both because understanding these is vital to understanding the basic functioning of communities, but also because of the intricate links between wildlife populations and infectious diseases of humans (Rogalski et al. 2017). Cross-species transmission is common enough to have a public health impact, as most human infectious diseases are zoonotic in origin (Woolhouse, Haydon, and Antia 2005; Lloyd-Smith et al. 2009). Additionally, complex life cycle and vector-borne parasites intricately link human and wildlife populations (Dantas-Torres, Chomel, and Otranto 2012). Thus, it's crucial to identify reservoir hosts that contribute to spreading disease as well as identifying pathogens capable of transmitting across multiple host species (Plowright et al. 2008). However, with so many different host and parasite species—and the complex communities they're a part of—it is difficult to objectively quantify important ecological interactions.

Because host and parasite communities are intricate webs of interactions (Lafferty, Dobson, and Kuris 2006), it's challenging to figure out which hosts and parasites are important for spreading diseases. For instance, when controlling for all possible interactions in an experiment factorially, experiments quickly become too large, even if we ignore that, in most systems, such experiments are likely to not be feasible for logistical and/or ethical reasons. Even mathematical models become less tractable with additional host and parasite species (Dobson 2004). Observational studies offer another approach, yet hosts and parasites rarely occur in nicely replicated combinations in the wild, and species are not gained and lost from communities at random (Johnson et al. 2013). Overall, the reality of complex host-parasite communities makes them challenging to study and require creative solutions for asking important questions about multihost-multiparasite interactions, including cross-species transmission.

Network approaches (like those used commonly in social sciences) can help researchers study these complex interactions (Silk et al. 2017; Godfrey 2013; Pilosof et al. 2015; Poulin 2010). There are many benefits to using network-based analyses for host-parasite interactions: networks (1) can synthesize large amounts of data like those produced by observational studies of parasite communities (Poulin 2010), (2) generate hypotheses about which hosts and parasites might be worth studying more in experiments or models (Pilosof et al. 2014), and (3) produce useful metrics such as centrality (i.e., the relative "importance" of a species; how influential it is to the cohesiveness of the network) and degree (i.e., the number of connections of a species in a population) (Gómez, Nunn, and Verdú 2013; Dallas et al. 2017).

However, many previous studies prioritize a wide breadth of host and parasite species with limited sampling of each species and do not account for how interactions change through time (Vázquez et al. 2005; Graham et al. 2009; Dallas, Park, and Drake 2017). Here, we used a

holistic approach to study cross-species transmission in a system that is unusually well characterized and studied (though we note that some studies of mammal parasites also feature data rich, well-studied systems (Park et al. 2018; Dallas, Park, and Drake 2017)). We used a multi-year, multi-site dataset on eight different host species of *Daphnia* and seven parasite species. We applied network methods in a directed way to address specific questions about cross-species transmission in these communities. From this work, we generated hypotheses that we can then go out and test in the system with additional field studies, experiments, and mathematical models.

We set out to address three main questions: (1) what is a plausible way to estimate cross-species transmission of parasites from field data, (2) which parasite species show the most cross-species transmission and infect the widest breadth of host species, and (3) are certain host species associated with cross-species transmission of each parasite species? We took advantage of three years of multi-host, multi-parasite time series data to estimate plausible amounts of cross-species transmission. Then we used those estimates to construct networks, which we then analyzed to quantify patterns of cross-species transmission by parasite and host species, and identify host and parasite species that disproportionately contributed to cross-species transmission.

METHODS

Study system

We studied seven host species of *Daphnia* (*D. dentifera, D. retrocurva, D. dubia, D. parvula, D. pulicaria, D. ambigua,* and *D. mendotae*) and one related species of *Ceriodaphnia* (*C. dubia*) that occur at varying densities in our study lakes (hereafter: all hosts are collectively referred to as "*Daphnia*" for simplicity). *Daphnia* are small planktonic crustaceans living in

freshwater lakes, typically feeding on phytoplankton and serving as prey to small fish and invertebrate predators (Tessier and Woodruff 2002). Many parasite species infect *Daphnia*, and we focused on the seven most common species that occur in our study sites (albeit to varying degrees). One parasite is a fungus: *Metschnikowia bicuspidata* ("Metsch"), and two others are microsporidians: *Larssonia obtusa* ("Larssonia"), and *Gurleya vavrai* ("Gurleya"). Two are bacteria: *Pasteuria ramosa* ("Pasteuria") and *Spirobacillus cienkowskii* ("Spiro"). Two parasites are oomycetes: *Blastulidium paedophthorum* ("Brood"), which infects developing embryos, and an unknown oomycete ("Spider"), which grows hyphae throughout the host's body cavity. We excluded two common gut parasites of *Daphnia* (*Caullerya mesnili* and an as-yet-undescribed microsporidian species (Rogalski et al. in prep)), because they were misclassified as the same species for much of the sampling period.

We studied host and parasite communities in 15 lakes in Southeast Michigan, US over three years (2014-2016). We sampled lakes roughly every two weeks from mid-July to mid-November each year (usually 9 total sampling events), because most parasite epidemics in these lakes occur during this time period. In addition to our normal sampling efforts, we sampled four of the study sites every three days during 2016. We collected three replicate whole-water-column vertical tows from the bottom of the lake up through the surface with a 153 μm Wisconsin plankton net and sampled from three different locations in the deep basin of each lake. For one replicate sample, we visually diagnosed parasite infections in live hosts under a dissection microscope at 10x magnification (or under a compound microscope at 20 to 40x magnification for early-stage infections). As *Daphnia* are mostly transparent, many parasite infections are visibly detectable with this method. We randomly subsampled the collected hosts, surveying at least 200 individuals of each host species for possible parasite infections or surveying all

individuals of a given species when fewer than 200 of that host species were present. We preserved the other two replicate samples in 90% ethanol, and at a later date, randomly subsampled and counted one replicate to estimate the density of each host species. Density was calculated as the number of hosts throughout the water column for a given surface area of the lake (number of hosts per m2).

Quantifying potential cross-species transmission

For a plausible measure of potential cross-species transmission, we chose a method that accounts for temporal and spatial overlap of a single parasite species in multiple host species (i.e., to what degree did the same parasite species infect more than one host species on the same day in the same lake?). Our metric, which we term "epidemic overlap", measures the degree to which epidemics of a single parasite species in multiple host species overlapped within the same site over the same time period. Looking at a single parasite species in a single lake (hereafter: site) in a single year of data at a time, we compared parasite prevalence and infected host density of the parasite species in each pairwise combination of hosts (e.g., shaded region in Fig 2.1, comparing Spiro infected host density in *D. pulicaria* and *D. retrocurva* in Bruin Lake during 2014). Only including days when two hosts were infected with the same parasite, we took the average infected host density (or prevalence) of each pair of host species through time and calculated the area under the overlapping epidemic curve. In this way, epidemic overlap served as a proxy for the potential amount of cross-species transmission. In the overlapping region, infections in one host could plausibly occur due to transmission stage parasites produced by another host species. In other words, epidemic overlap quantifies the amount of synchrony of epidemics of the same parasite species in different host species.

Estimating host breadth and cross-species transmission by parasite species

We considered three network-level factors for each parasite species: (1) the number of different host species a parasite infected in a community (host breadth), (2) the number of predicted transmission pairs between different host species (epidemic overlap), and (3) a combination of the two (graph density). For each parasite species, we constructed undirected networks of host species in each site where each node represented the population of a host species in a given community. With only the host species that were present in that site as the nodes, we added edges between host species if they had overlapping epidemics of that same parasite species. To account for the breadth of the host species that a parasite infects, we added node edges to itself (loops) for each host that was infected by a given parasite (these loops are equivalent to filling a diagonal cell in the adjacency matrix). We calculated host breadth as the proportion of all hosts observed across all time points in a site that were infected with a given parasite species, and we estimated cross-species transmission using the proportion of realized connections between host species out of all possible connections in the network. Because both of these metrics are important for different reasons, we combined the two and calculated graph density as the proportion of realized edges out of all potential edges that could occur in a given network, including loops where a host connects to itself if it was infected. Additionally, since sites varied in their host community composition, we only included a host species in a given network if that species was found in that site, and sites where the parasite was not found were dropped from the analysis.

Estimating host influence on cross-species transmission

Our goal was to see if some host species were more likely to be involved in cross-species transmission of various parasite species. For each parasite species and site, we constructed undirected networks of hosts with edge connections weighted by the epidemic overlap value for each host combination, while removing host species with no connections from the network. Because some parasites produce long-lived spores capable of delaying transmission (and for others we lacked the necessary natural history information), we summed the epidemic overlap values across years for each site. Since we were purely interested in potential cross-species transmission, we did not include loops as we did for the networks described above (i.e., no connections of a host species to itself). Additionally, we dropped very small networks (2 or fewer host species), because our measures of host influence were not applicable.

As a proxy for the influence of each host species on cross-species transmission of different parasites, we calculated the eigenvector centrality for the host nodes in each network. Eigenvector centrality was useful because it considers the entire network and emphasizes second order connections; a given node is more central if it is connected to other nodes that themselves are more central (Bonacich 2007). Eigenvector centrality scores range from 0 to 1, with 1 being most central, and were calculated on weighted networks. We chose eigenvector centrality because it emphasizes connections throughout the network, which could be important in a community disease context where there might be chains of transmission across different host species (Gómez, Nunn, and Verdú 2013).

Statistical analysis

We used the Kruskal–Wallis test to see (1) if there were differences in host breadth, cross-species transmission connections, and graph density across parasite species; (2) if there

were differences in eigenvector centrality across host species; and (3) if there were differences in epidemic size of different parasites across host species. For the centrality and epidemic size data, we looked at each parasite species separately. For all comparisons, if the Kruskal–Wallis test suggested a significant main effect, we then used the Conover–Iman test with Bonferroni corrections for pairwise comparisons across parasite or host species.

We used R version 3.5 (R development core team 2018) for all of the data processing and statistical analyses. We used the bipartite package to construct host adjacency matrices and the igraph package to calculate network metrics such as graph density and centrality.

RESULTS

Host-breadth and cross-species transmission connections in different parasite species networks

All parasite-specific measures—host breadth, cross-species transmission connections, and graph density—suggest parasites in these communities vary in their ability to infect and transmit between different host species (Fig 2.2). Each of these three measures showed significant differences among parasite species (Kruskal–Wallis test, $p < 0.001$ for all), and the median values and pairwise comparisons were largely the same across metrics. Brood, Spiro, and Pasteuria were always at the top, indicating that they are roughly infecting the same number of host species. Of these three parasite species, Brood, in particular, always had higher values compared to the four lower scoring parasite species, suggesting it is more of a multi-host parasite than the others.

Comparing host breadth (Fig 2.2A) and cross-species transmission connections (Fig 2.2B) provides information that looking at the combined graph density (Fig 2.2C) values does not. For instance, Brood is the only parasite species that was found in every single host species at

one of the sites (host breadth = 1 at one site). Also, while Gurleya was found to infect multiple host species, those epidemics never overlapped in time and space (Fig 2.2B, values = 0). However, together, the same parasite species that had higher host breadth also tended to have more overlapping epidemics across host species.

Influence of each host species on cross-species transmission

Some host species were more central in the networks of overlapping epidemics, suggesting they are important for the cross-species transmission of particular parasites. The eigenvector centrality values depended on the host species, parasite species, and method of weighting network edges (i.e., epidemic overlap by prevalence or infected host density). Only three parasite species, Pasteuria, Brood, and Spiro, were sufficiently common to create epidemic overlap networks large enough to calculate centrality values. Of those parasites, cross-species transmission networks of Pasteuria showed some hosts (*D. dentifera* and *D. retrocurva*) were more central in the network (Fig 2.3A prevalence overlap: $p = 0.01$, Fig 2.3B infected host density overlap: $p = 0.005$), suggesting they are more important for driving cross-species transmission. This was true regardless of how the network edges were weighted. For Brood, host species also varied in their centrality, but only when infected host density overlap was used as the edge weight method (Fig 2.3D, Kruskal–Wallis test, $p = 0.01$). However, for Spiro, host species had similar centralities regardless of the edge weight method (Fig 2.3E prevalence overlap: $p = 0.93$, Fig 2.3F infected host density overlap: $p = 0.44$). Although each parasite (Pasteuria, Brood, and Spiro) commonly infected multiple host species, the cross-species transmission dynamics might be different because host centrality patterns differ across parasite species.

D. dentifera and *D. retrocurva* consistently had the highest centrality values, suggesting they are more important for cross-species transmission of certain parasite species. On the other hand, the host species that were least important tended to vary depending on parasite species (note letters in Fig 2.3, and see Table 1 for full list of significant pairwise comparisons). *D. parvula*, *D. pulicaria*, *Ceriodaphnia*, and *D. dubia* were all less important for transmitting at least one parasite species. Besides *D. dentifera* and *D. retrocurva*, no other host species had significantly higher centrality than another. Thus, these two host species might be of particular interest for future work on cross-species transmission.

Broadly speaking, the three common multi-host parasites—Pasteuria, Brood, and Spiro—had different patterns of cross-species transmission. For Pasteuria, a couple of host species (*D. dentifera* and *D. retrocurva*) were likely most important for cross-species transmission because they had higher centrality values on average. However, for Brood, *D. dentifera* and *D. retrocurva* were only important for cross-species transmission when total infected host density was considered. Finally, with Spiro, there were no differences across host species, suggesting that host species identity is not an important driver of patterns of cross-species transmission.

Epidemic size of different parasites in a single host species

Epidemic size—measured as the area under the prevalence curve—did not show any significant dependence on host species identity, at least for the common multi-host parasites (Pasteuria, Brood, and Spiro; Fig 2.3 A, C, and E). However, integrated area of infected host density during a single epidemic varied by host species for Pasteuria (Fig 2.3 B, $\chi^2 = 24.1$, $p < 0.001$, $df = 5$) and Brood (Fig 2.3 D, $\chi^2 = 27.1$, $p < 0.001$, $df = 5$). *Ceriodaphnia*, *D. dentifera*,

and *D. retrocurva* were all significantly different from *D. pulicaria* in terms of integrated infected host density of Brood (Conover–Iman, $p < 0.05$), and *D. dentifera* epidemics were significantly different from *D. parvula* epidemics of Brood by infected host density (Conover–Iman, $p = 0.016$). For epidemics of Pasteuria, *D. dentifera* and *D. retrocurva* were significantly different from *Ceriodaphnia* in terms of integrated infected host density (Conover–Iman, D. retrocurva-Ceriodaphnia $p = 0.004$, *D. dentifera*-*Ceriodaphnia* $p < 0.001$). Taken together with the centrality results, these results suggest density in combination with infection is predictive of which hosts are spreading parasites.

DISCUSSION

Using detailed multi-host, multi-parasite time series data across different communities of *Daphnia*, we looked at the synchrony of epidemics of the same parasite in multiple host species to estimate a plausible degree of cross-species transmission. Both parasite and host species identity were important factors for cross-species transmission networks. Our network analyses of the study communities found that parasite species varied in the degree to which they infected multiple host species and likely varied in their capacity to transmit from one host species to another. Additionally, based on network centrality, we identified that some host species may disproportionately influence cross-species transmission. However, which hosts were important depended on the parasite species and the method of quantifying infection (i.e. integrated prevalence or infected host density). In total, this information gives us insights into the dynamics of the multihost-multiparasite community as a whole, including identifying host and parasite species that might disproportionately contribute to cross-species transmission.

Multi-host, multi-parasite communities are the norm in nature (Rigaud, Perrot-Minnot, and Brown 2010; Pedersen and Fenton 2007; Poulin 2011), but they are complicated and challenging to study. Here, we used a networks approach that allowed us to synthesize this complex data to make predictions about cross-species transmission and identify host and parasite species that might be disproportionately important. The "epidemic overlap" metric we used gave us a plausible starting point for estimating cross-species transmission of parasites between pairs of host species. Eigenvector centrality then allowed us to combine all the available information and make predictions about which hosts are important. For example, our analysis suggests that *D. dentifera* is important for transmitting Pasteuria. Similarly, this approach can suggest when the identity of host species might not be important. For example, *D. parvula* might be less important for transmission of Brood to other hosts. Going forward, we can use the network results to generate hypotheses about which hosts are transmitting which parasites and design specific host and parasite combinations for lab, field, and mathematical modeling studies. For example, experimentally altering the proportions of *D. dentifera* and *Ceriodaphnia* to see how that influences epidemics of Pasteuria. It also guides us to particular comparisons that we can use to address more general questions, including: why do some parasites infect many host species, and why do some host species appear to spread more parasites?

Parasites in the study communities varied in their host breadth and the degree to which parasites plausibly transmit between different host species (Fig 2.2). Based on our field survey, the evidence strongly suggests that parasites differ in their ability to infect and transmit among different host species, yet we cannot say why some parasite species commonly infected more host species in the same site. It is possible both life history traits and taxonomic group play a role (Pedersen et al. 2005). For instance, the two bacterial parasites we surveyed, Spiro and Pasteuria,

generally had higher host breadth or cross-species transmission compared to other parasites (such as the microsporidians, Larssonia and Gurleya). Interestingly, although Brood and Spider are both oomycete parasites, Brood had significantly higher host breadth and degree of cross-species transmission than Spider. However, Brood has an uncommon transmission strategy, infecting developing embryos of hosts (Duffy, James, and Longworth 2015); moreover, it is hypothesized that infectious stages enter the brood chamber, rather than infecting via the gut as is the case for many *Daphnia* parasites. While our study cannot conclusively say whether or not these factors play a role here, taxonomic group and life history influence multi-host parasite patterns in mammals (Pedersen et al. 2005). It will only be by assembling large datasets on large multihost-multiparasite communities that we will begin to be able to identify factors associated with variation in host breadth.

Factors outside of the parasite species themselves could also drive the host breadth and cross-species transmission patterns. Parasite-driven selection for host resistance (Duffy and Sivars-Becker 2007; Duffy et al. 2012) and coevolutionary patterns (Decaestecker et al. 2007), could both alter the number of parasite propagules and susceptible hosts in a given site and change the fitness landscape of which host species most suitable. Additionally, environmental and ecological variables, such as light (Overholt et al. 2012), temperature (Mordecai et al. 2017; Shocket et al. 2018), and predation (Duffy et al. 2005) can alter parasite fitness in this system and thus possibly disrupt cross-species transmission. While the host resistance and coevolutionary patterns of the parasites in our study are unknown, other work is being done on other ecological factors that influence parasite fitness and possibly cross-species transmission. For example, one possibility is that parasites face tradeoffs in their ability to infect different

hosts: a study on Metsch found that smaller spores had higher fitness in *Ceriodaphnia* but were very unlikely to infect *D. dentifera* (Shaw 2019).

In addition to parasite species, we found that host species also show differences in their potential importance for cross-species transmission (Fig 2.3). In general, host species vary in their susceptibility, density, and infectiousness, and all of these can be influenced by ecological and evolutionary processes. For example, phylogeny can influence patterns of cross-species transmission (Streicker et al. 2010), whereby hosts might be more susceptible to parasites transmitted from more closely related species. Furthermore, host density (which is influenced by many factors, including competition, predation, and the abiotic environment) can influence cross-species transmission in multiple ways: with higher density of hosts, there are likely more total contacts with infectious stages, and higher densities of infected hosts could in turn produce more parasite propagules.

More specific to *Daphnia* hosts, both host body size (Hall et al. 2012, 2007) and habitat type (Cáceres et al. 2006; Penczykowski et al. 2014) can influence the amount of disease in a host population, and thereby possibly influence the likelihood of cross-species transmission. Variation in host body size could explain differences in centrality of hosts in Pasteuria transmission networks. For example, *Ceriodaphnia* are much smaller than *D. retrocurva* and *D. dentifera,* and *Ceriodaphnia* are significantly less important in networks derived from Pasteuria epidemic data. Additionally, habitat factors (lake basin shape, light, etc.) can influence both parasite and host fitness (Shaw 2019), so ecological variables could affect co-occurrence of particular host and parasite species, which in turn may result in certain host species being more or less important for cross-species transmission. A particularly interesting avenue for future research is studies that quantify the relative locations of host species and parasite spores in the

water column; this has the potential to strongly influence the degree of cross-species transmission.

Knowing which host species spread pathogens and which parasite species are likely to infect multiple host species is particularly valuable in the context of emerging infectious diseases. Most infectious diseases are zoonotic in origin (Woolhouse, Haydon, and Antia 2005; Lloyd-Smith et al. 2009), and being able to quickly identify potential reservoirs of disease would allow us to intervene more quickly and reduce risk to humans. As an example, if our goal was to reduce future cross-species transmission in the *Daphnia*-parasite system, we could use the results of this analysis to identify which parasites are most likely to be of concern and the identity of host species on which to focus intervention efforts. Furthermore, we have further evidence that host community composition relates to disease in this system (Hall et al. 2009; Strauss et al. 2015; Penczykowski et al. 2014). Hosts that are more central in networks, like *D. dentifera* or *D. retrocurva*, may amplify disease, whereas other species, like *Ceriodaphnia*, might dilute disease. Indeed, *Ceriodaphnia* as a diluter of a single parasite species has been demonstrated experimentally (Hall et al. 2009). Similarly, knowing when host species identity is not a factor (e.g. Spiro, Fig 2.3) is also useful in an emerging infectious disease context because targeted interventions would be a waste of resources. In all, our analyses uncovered patterns that could inform a hypothetical intervention in the system.

Our study is mainly hypothesis-generating and due to the complexity of multi-host, multi-parasite communities, it cannot definitively say whether certain host or parasite species are more involved in cross-species transmission. Genetic analysis would be ideal for inferring transmission across host species in the wild, and experimental inoculations in the lab could validate those results. However, molecular work can be prohibitively time consuming and

expensive. Additionally, as of this publication, only a subset of the host and parasite species in the study communities are culturable in the lab, making it challenging to experimentally test the full range of possible host-parasite interactions. However, analyses such as ours can help us identify which combinations of species are most likely to be of interest, thus allowing us to be more prudent in our molecular studies, and to focus culturing efforts on species that this analysis indicates should be highest priority. While our measure of "epidemic overlap" is useful, there are other means of quantifying potential cross-species transmission, such as a geometric mean instead of an arithmetic one or the correlation of the two time series. Another limitation of the present work is that, while we were able to detect differences among host and parasite species in our network analyses, it's possible do not have the power to detect more minimal differences between combinations of species. Nevertheless, the network methods proved useful and as we collect more field data, its value will only grow.

When most host species are susceptible to multiple parasite species and most parasite species are capable of infecting various host species, focusing on a single host-parasite interaction misses the big picture. However, accounting for the realistic complexity of host and parasite communities is incredibly difficult, especially with observational data alone. Network analyses can tell us where to look: which parasites consistently infect the most host species and which host species might be most important for transmitting those parasites to other host species. Since most emerging infectious diseases of humans originate from wildlife (and infect multiple host species), understanding patterns of cross-species transmission is crucial for public health and conservation. With this as our motivation, we quantified patterns of potential cross-species transmission at the species-level for natural communities, demonstrated the value of a network-based approach, and generated hypotheses for future work.

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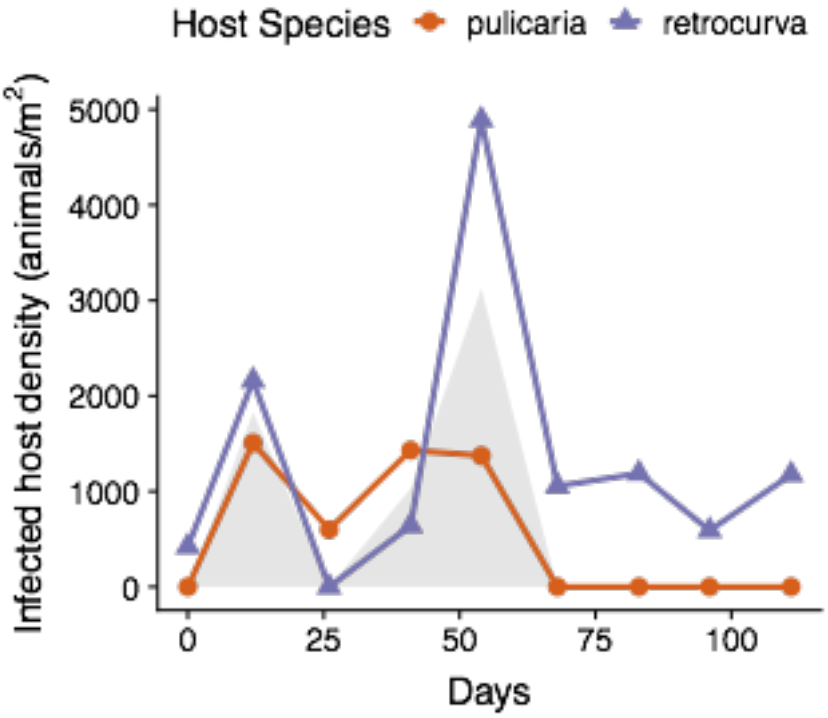

Figure 2.1: An example of the epidemic overlap calculation. Overlapping epidemics of *Spirobacillus cienkowskii* ("Spiro") in two different host species at Bruin Lake during 2014. Points denote sampling events and the estimated density of *D. pulicaria* (orange circles) and *D. retrocurva* (purple triangles) infected with Spiro through time. The shaded regions are the area calculated as epidemic overlap (*i.e.*, integrated area of the pairwise average between the two infected host densities at time points when both hosts were infected).

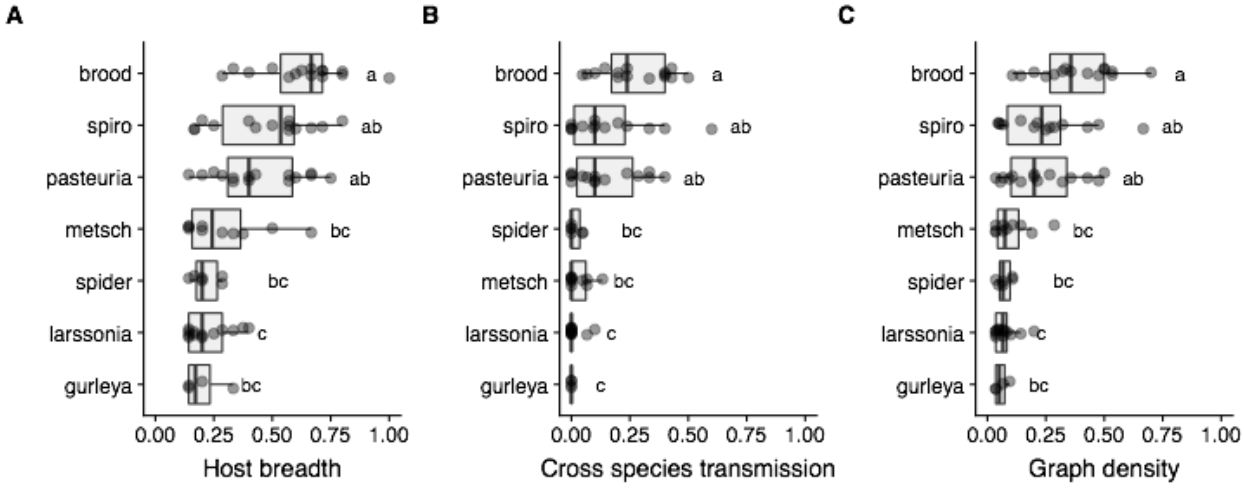

Figure 2.2: Network level estimates of host breadth (A), cross-species transmission connections (B), and graph density (C) all indicate that parasites in these communities vary in their ability to infect and transmit between different host species. Host breadth is the proportion of hosts that were infected with a given parasite, summed across years in the same site. Cross-species transmission connections show the proportion of combinations of hosts that had overlapping epidemics in the same year. Graph density is the proportion of realized connections out of all possible connections in a network. Networks were created using the host species present in each site as nodes and epidemic overlap for connections between hosts. Connections were summed across years, so each point in the figure shows data for a single site/lake. There was a significant difference in host breadth (Kruskal–Wallis test, $\chi^2 = 35.2$, $p < 0.001$, $df = 6$), proportion of cross-species transmissions (Kruskal–Wallis test, $\chi^2 = 38.3$, $p < 0.001$, $df = 6$), and graph density across parasite species (Kruskal–Wallis test, $\chi^2 = 36.2$, $p < 0.001$, $df = 6$). Letters show pairwise comparisons that were significantly different based on the Conover–Iman test with Bonferroni corrections. Note that the y-axes differ slightly between the three panels, as they are ordered based on the ranking for each particular metric.

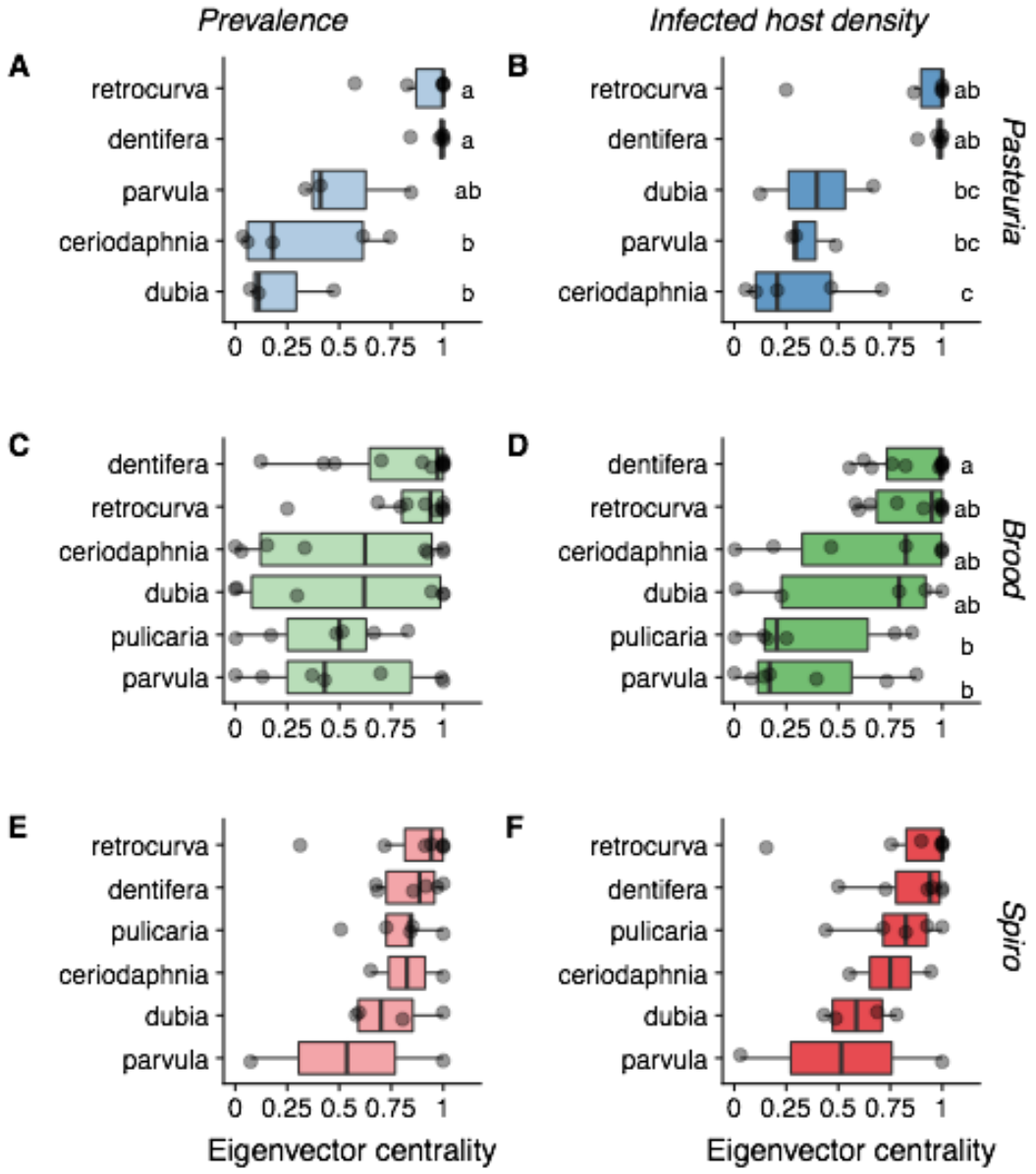

Figure 2.3: Eigenvector centrality values for host species in networks of Pasteuria (A, B), Brood (C, D), and Spiro (E, F) for overlapping epidemics by prevalence (A, C, E) or infected host density (B, D, F), showing differences by parasite species, host species, and infection metric. Each point represents the centrality values for a host in a given site for a given parasite species. Centrality values varied significantly by host species for Pasteuria prevalence (A, Kruskal–Wallis test, $p = 0.01$), Pasteuria infected host density (B, Kruskal–Wallis test, $p = 0.005$), and Brood infected host density (D, Kruskal–Wallis test, $p = 0.01$). Letters denote significant pairwise comparisons (see Table 1 for full list of significant host species comparisons). For each panel, the y-axis is arranged from host species with the highest median centrality (top) to the lowest (bottom), so the order of host species changes.

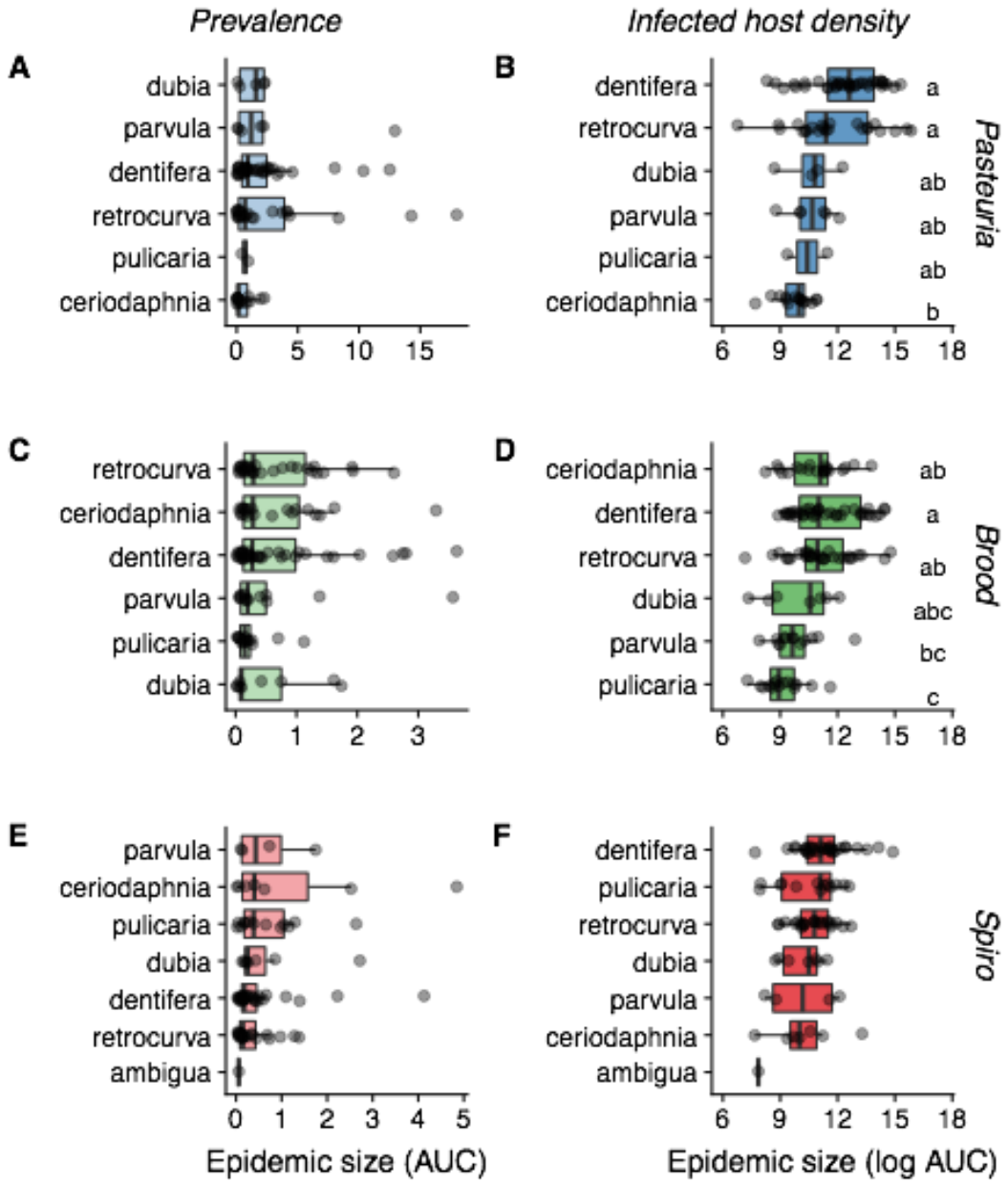

Figure 2.4: Size of a single epidemic of three common parasites in each host species varied but only for infected host density of certain parasites. Each point is the area under the curve of prevalence (A, C, E) or infected host density (log scale, B, D, F) for Pasteuria, Brood, and Spiro in a single site, in a single year. There were no differences in epidemic size by integrated area of prevalence. However, for Pasteuria (B) and Brood (D), there were significant differences in the area under the curve of infected host density across species (Kruskal–Wallis test, $p < 0.001$ for both parasite species; letters denote significantly different pair-wise comparisons). Note the differences in x-axis values for epidemic size in terms of integrated prevalence.

Chapter 3 Pluralistic Approach Reveals Biotic and Abiotic Factors Associated with Variation in Sex in Natural Populations of *Daphnia dentifera*

with Mary A. Rogalski, Clara L. Shaw, Katherine K. Hunsberger, and Meghan A. Duffy

ABSTRACT

The maintenance of sexual reproduction is one of the oldest unsolved mysteries in evolutionary biology. While there has been a particular focus on the potential role of parasitism in explaining sexual reproduction, a variety of factors have the potential to influence investment in sex. Here, we take advantage of the natural history of *Daphnia dentifera*—which alternate between sex and asex—to uncover the variables associated with the wide variation in sexual reproduction in this system. We tracked host density, parasite infections, sexual reproduction, temperature, and light in 15 wild populations of *Daphnia* for three years. We found substantial variation in investment in sex, with some populations reproducing entirely asexually and others shifting almost entirely to sexual reproduction by late autumn. Moreover, we found that higher host density and parasitism were associated with greater investment in sex. Temperature and light were not as predictive of investment in sex, but may indeed play a role. While correlational, our results leverage a large time series dataset and suggest the pluralistic factors that may be responsible for sexual reproduction. Interestingly, our results suggest density (an understudied factor for the maintenance of sex, but one recognized as important in prior studies of cyclical parthenogens) was an important predictor of investment in sex in our study populations. Together, the results add to our understanding of the evolution and maintenance of sexual reproduction in nature.

INTRODUCTION

The maintenance of sexual reproduction remains a puzzle to evolutionary biologists (Otto 2009). Sex comes with high costs: producing males which themselves don't directly reproduce, breaking up well-adapted combinations of alleles, and time, energy, and infection risk associated with mating (Jaenike 1978; Smith 1971; Otto and Nuismer 2004; Otto 2009). Given these substantial costs, sexual reproduction is surprisingly common in nature, which suggests the benefits must outweigh the significant costs (Otto 2009). Hypothesized benefits include purging deleterious alleles/mutations (Muller's Ratchet (Muller 1964), Deterministic Mutation Hypothesis (Kondrashov 1984)) or creating novel genotypes (Red Queen Hypothesis (Jaenike 1978; Van Valen 1973)). Creating novel combinations of genotypes can be beneficial in a changing environment, such as in complex biological interactions, but the factors responsible for maintaining sexual reproduction remain a major open question in evolutionary biology.

Selection by parasites is a commonly hypothesized solution to the sex riddle (Brockhurst et al. 2014; Lively and Morran 2014). In particular, parasites can exert extreme selection pressure on their hosts. If parasites are sufficiently virulent, genotypes in the host that are resistant will be favored by selection. Furthermore, if parasites adapt to infect the most common host genotypes, then rarer host genotypes might be less susceptible to the parasite. Thus, sexual reproduction might benefit the host by increasing the chances of producing a rare or resistant host genotype that will have higher fitness in the presence of parasites.

Some of the best evidence for the Red Queen Hypothesis to date comes from New Zealand snails, *Caenorhabditis elegans*, and *Daphnia* spp., all organisms that can reproduce both asexually and sexually. In the New Zealand snail system, parasites are locally adapted to snails from the same population (Lively and Dybdahl 2000) and host populations with more parasites

also reproduced more sexually (Lively 1987). However, these interactions and benefits of sexual reproduction appear to vary temporally (Gibson et al. 2018). Complementary experimental evidence in *C. elegans* shows sexual populations evolved greater resistance to a bacterial parasite than obligately asexual host populations. Meanwhile in *Daphnia* (small, freshwater crustaceans), *Daphnia* hosts and their parasites can evolve rapidly (Decaestecker et al. 2007; Duffy et al. 2012), sexually produced offspring are more fit against contemporaneous parasites (Auld, Tinkler, and Tinsley 2016), sex is more common when more parasites are around (Hite et al. 2017), more susceptible genotypes are more likely to shift to sexual reproduction (Duncan, Mitchell, and Little 2006; Mitchell, Read, and Little 2004), and common clones decrease in frequency over time in infected populations but not uninfected ones (Wolinska and Spaak 2009). This work has taken advantage of an important component of *Daphnia* biology: they reproduce by cyclical parthenogenesis, a middle ground between sex and asex where reproduction is mostly clonal but interspersed with sexual reproduction.

However, research on *Daphnia* (and other aquatic cyclical parthenogens, including rotifers) also demonstrates that other factors can be important drivers of investment in sexual reproduction. Host density is also a cue for sexual reproduction in *Daphnia* (Berg, Palsson, and Lascoux 2001; Larsson 1991; Stross and Hill 1965), along with abiotic factors like light and temperature (Stross and Hill 1965). Importantly, in these cyclical parthenogens, shifting to sexual reproduction is generally associated with a shift to producing dormant offspring. Studies on cyclical parthenogens thus allow us not only to consider the factors that influence whether organisms reproduce sexually, but also those influencing the timing of the shift to producing dormant offspring (Cáceres and Tessier 2004).

Here, we consider multiple possible drivers of sexual reproduction in order to understand the factors that influence the shift to sexual reproduction in a cyclical parthogen, *Daphnia* dentifera, with a broader goal of understanding the factors that promote the maintenance of sex. In this study, we take advantage of a large multi-year, multi-population dataset to assess parasitism and density as possible drivers of sexual reproduction in *Daphnia*. In addition to looking at multiple possible drivers, another important feature of our study is that it recognizes that populations often host multiple parasite species (Rigaud, Perrot-Minnot, and Brown 2010); most prior studies (on *Daphnia* and more generally) focus on a single parasite. Thus, our study leverages an unusually comprehensive dataset to address these knowledge gaps.

METHODS

Study system

We were interested in the drivers of variation in sexual reproduction in natural lake populations of *Daphnia dentifera*, and studied several potential factors: density, parasitism, temperature, and light. *Daphnia* are small planktonic crustaceans that live in freshwater lakes, feeding on phytoplankton and serving as prey to small fish and invertebrate predators (Tessier and Woodruff 2002). *Daphnia* are cyclically parthenogenetic, meaning they can alternate between reproducing asexually and sexually. *Daphnia* often switch to sexual reproduction at particular times of the year, when it becomes less costly (Gerber et al. 2018); the species we focused on, *D. dentifera*, shifts to sexual reproduction in autumn (Duffy et al. 2008). During sexual reproduction, female *Daphnia* create asexual males and haploid resting eggs, which the males then fertilize (Ebert 2005). The resting eggs are released by the sexually reproducing

females and remain dormant before later hatching (ideally when environmental conditions have improved (Hairston 1996)).

We studied an abundant host species of *Daphnia*, *D. dentifera*, that occurs at varying densities across our study lakes and that is infected by a suite of parasites (Duffy et al. 2010). We tracked population sizes through time, as well as infections of nine microparasites: *Metschnikowia bicuspidata* (fungus), *Pasteuria ramosa* (bacterium), *Spirobacillus cienkowskii* (bacterium), *Blastulidium paedophthorum* (oomycete), *Gurleya vavrai* (microsporidian), *Larssonia obtusa* (microsporidian), *Caullerya mesnili* (icthyosporean), an undescribed microsporidian gut parasite ("MicG"), and an unknown *Saprolegnia*-like oomycete ("spider" (Green 1974; Wolinska et al. 2008; Duffy et al. 2010; Duffy, James, and Longworth 2015; Lu et al. 2020)).

We studied host and parasite communities in 15 lakes in Southeast Michigan, US over three years (2014-2016). We sampled lakes roughly once every two weeks from mid-July to mid-November each year (usually 9 total sampling events). In addition to our normal sampling efforts, we intensively sampled four of the study sites every three days during 2016 for a study focused on population dynamics. For each lake, on each sampling date, we collected three replicate vertical tows from the bottom of the lake with a 153 μm Wisconsin plankton net and sampled from three different locations in each lake. This yielded three replicate samples per lake per sampling day, each of which contained one tow from each of the three locations within the lake. For one replicate sample, we visually diagnosed parasite infections in live hosts under a dissection microscope at 10x magnification using dark field microscopy (or under a compound microscope at 200 to 400x magnification for early-stage infections). As *Daphnia* are mostly transparent, many parasite infections are visibly detectable with this method. We also counted

males and sexually reproducing females based on morphological differences (Brooks and JL 1957). We randomly subsampled the collected hosts, surveying at least 200 individuals of each host species for possible parasite infections or surveying all individuals when fewer than 200 individuals of a host species were present. We preserved the other two replicate samples in 90% ethanol, and at a later date, randomly subsampled and counted one of the samples to estimate the density of each host species. Density was calculated as the number of hosts throughout the water column for a given surface area of the lake (number of hosts per m^2 of lake surface).

In addition to the two potential biotic cues for sexual reproduction in *Daphnia* (parasitism and total host density), we also measured two abiotic factors: temperature and light. Lake water temperature was recorded with a sonde (Hydrolab MS5 multiprobe, Hach Hydromet (now OTT Hydromet)) during nearly every sampling event. (The exceptions resulted from equipment malfunctions.) In our analyses, we used the temperature at 2 meters below the surface of the lake and averaged the temperature values within each lake for each year. For light measurements, we calculated metrics for UV (ad320) and visible light (ad440) for each lake in both 2014 and 2016, based on absorbance of filtered lake water in a spectrophotometer (Clara L. Shaw, Spencer R. Hall, Erin P. Overholt, Carla E. Cáceres, Craig E. Williamson, Meghan A. Duffy, n.d.).

In our analysis, sexual reproduction in *Daphnia dentifera* could depend on the effects of host density, parasitism, temperature, or light. For host density, we integrated the total host density for each lake in a year, integrating the area under the curve with day on the x-axis and density on the y-axis. Parasitism, calculated as the proportion of hosts infected with any parasite, was also integrated across sampling events within a lake and year. Similarly, we integrated the proportion of hosts that were sexually reproducing (ephippial) females or males. These integrated measurements are common for this type of data, and we also verified that mean and

maximum measurement choices gave similar results (see supplement). However, as light and temperature were more consistent across measurements, we simply averaged those values for each lake within a year.

In R (version 3.5), we regressed the metric of sexual reproduction on various variables that included host density, parasitism, their interaction (density*parasitism), visible light (ad440), and temperature (mean at 2m depth). We created various sub-models and then used model selection and Akaike information criteria (AIC) to compare 11 different sub-models.

RESULTS

There was substantial variation in investment in sex and parasite prevalence in the study populations of *Daphnia dentifera* (Fig. 3.1). Sexual reproduction was seasonal, with male and ephippial female production beginning in late September and generally increasing through November. In some lakes and years, we never observed any males or ephippial females, whereas in others the population shifted to nearly all (98%) being sexual. Maximum parasite prevalence was also quite variable, ranging from 0% in some lake-years to 62% in others.

Integrated host density strongly correlated with sexual reproduction of *Daphnia* in the lake (Fig. 3.2a, $F = 29.44$, $p < 0.001$, Table 3.1). As the density of hosts increased, we observed more sexual reproduction (Fig. B1a). Interestingly, it was the density of conspecific hosts—not the density of all *Daphnia* species—that correlated with sexual reproduction (sex ~ log(combined host density), $F = 0.066$, $p = 0.8$). A pluralistic, model selection approach, suggests the importance of density: all top models included log(density) as a predictor of sexual reproduction (Table 3.1).

Besides host density, parasitism appeared to influence the degree of sexual reproduction in *Daphnia* the most (sex ~ parasitism, gaussian, $F = 6.04$, $p = 0.018$). In the model comparison, the model with density and parasitism as predictors was the second best model (AIC 253.50) and performed similarly to the model with just density ($\Delta AIC = 0.37$). Moreover, the next four models ($\Delta AIC = 2.15\text{-}4.07$) all incorporated parasitism. Thus, host density and parasitism were both associated with sexual reproduction in *Daphnia dentifera*.

We also hypothesized that temperature and light might be important predictors of sexual reproduction, and they moderately improved the model fit. While the two best performing models did not incorporate abiotic factors (Table 3.1), the next four models ($\Delta AIC = 2.15\text{-}4.07$) included temperature (mean ℃ at 2m below the surface) and/or visible light (ad440). However, in linear models with a single predictor, temperature did not significantly affect sexual reproduction (sex ~ temperature, $F = 0.24$, $p = 0.63$) and neither did light (sex ~ light, $F = 2.60$, $p = 0.11$). Overall, temperature and light may help predict sexual reproduction, but the evidence is not as strong as it is for biotic factors like density and parasitism.

DISCUSSION

We took a pluralistic approach—measuring multiple variables that prior work suggested might be influential—to study sexual reproduction in a *Daphnia* host species in the wild. We found substantial variation in investment in sex, with some populations remaining entirely asexual and others becoming almost entirely sexual in autumn. Biotic factors like host density and parasitism were most predictive of this variation in investment in sex (Fig. 3.2, Table 3.1). However, model selection approaches revealed that abiotic factors, namely temperature and

visible light, might also play a role. Taken together, this pluralistic approach suggests multiple factors may impact investment in sexual reproduction in populations of *Daphnia dentifera*.

While many studies on the evolution of sex focus on interspecific interactions and/or spatial or temporal environmental variation, studies of cyclical parthenogens suggest the influence of density on the maintenance of sex may be underappreciated (Gerber et al. 2018). Density has long been known to be an important factor influencing this shift to sexual reproduction in cyclical parthenogens like *Daphnia* and rotifers (Berg, Palsson, and Lascoux 2001; Larsson 1991; Stross and Hill 1965; Stelzer and Snell 2003; Gilbert 2020). One possible explanation for this association is that, in many cyclical parthenogens, sexual reproduction is associated with the production of long-lasting resting stages, meaning sexual reproduction may serve as a means of temporal dispersal (Gilbert 2020; Gerber et al. 2018). However, density might be important even in organisms that are not cyclically parthenogenetic. For instance, higher densities can also be associated with more competition for resources and a greater risk of parasitism (Gerber et al. 2018). In both these cases, there may be an advantage to reproducing sexually: genetic recombination could lead to offspring with higher fitness, either because their novel genotypes are less susceptible to parasites (Auld, Tinkler, and Tinsley 2016) or because outbred individuals are more likely to be stronger competitors (Yun and Agrawal 2014). Additionally, for density-dependent transmitted parasites, perhaps a combination of parasitism and density drives sexual reproduction.

We found that parasitism positively correlated with sexual reproduction in *Daphnia*, which is consistent with the Red Queen hypothesis (Lively 1987; West, Lively, and Read 1999). As discussed in the previous paragraph, parasitism might favor sexual reproduction in *Daphnia* for both the benefits of temporal dispersal and genetic recombination. Many of the parasites we

observed are highly virulent, often dramatically reducing fecundity and/or lifespan. Virulence is an important attribute in many models of Red Queen dynamics (Lively 2016). Furthermore, some of the parasite species, such as *Pasteuria ramosa*, have well documented genetic components/interactions (Ebert et al. 2016). One would expect this matching mechanism to favor genetic recombination (and it does in Auld et al. 2016). However, it is important to note that *P. ramosa* is just one of many parasites threatening *Daphnia* (Duffy et al. 2010; Ebert 2005). Our study differs from many other studies on parasitism in that it considers the suite of parasites attacking the host populations (Fig. B4 shows results for individual parasites).

Finally, abiotic forces like temperature and light, which are known cues for *Daphnia* reproductive cycles (Stross and Hill 1965), may also influence the variation in sexual reproduction in these populations. Drawing broader conclusions from the impact of abiotic forces is more difficult because they most likely serve as cues for sex as opposed to direct selection for sexual reproduction itself. For instance, for the species we focused on in this study, *D. dentifera*, decreasing temperatures and light often precede a decrease in availability of quality food, which might favor temporal dispersal until quality food returns at a later date. However, because all our study lakes are at approximately the same latitude, the variation among our lakes in light and temperature—and, therefore, the variation associated with parasitism—is not associated with differences in climate or photoperiod. Thus, the variation in light and temperature is operating at the same scale as biotic factors like density and parasitism.

Like all studies attempting to tackle a timeless question, ours has limitations, including that no single study allows for sweeping conclusions about the origin and maintenance of sexual reproduction. Nevertheless, our study is more encompassing than many, incorporating multiple parasite species, and multiple other factors, including host density, temperature, and light.

Additionally, while this study has implications for our understanding of the factors favoring the maintenance of sexual reproduction, our study is most applicable to understanding the factors associated with a shift from asexual to sexual reproduction in cyclical parthenogens. Notably, because sex for *Daphnia* results in a dormant resting egg, it is difficult to say why sex is advantageous for *Daphnia* — both temporal dispersal (evading poor conditions) and genetic recombination occur simultaneously in the host.

The maintenance of sexual reproduction continues to puzzle researchers, but our results suggest that multiple factors, especially host density and parasitism, might explain the variation in the shift to sexual reproduction in wild *Daphnia* populations. While there has been prior work on cyclical parthenogens, including *Daphnia* and rotifers, our study is notable for the number of populations (15), the number of years of study (3), and for using a multiparasite framework. Our results suggest that future studies on the Red Queen hypothesis in particular should account for the potential confounding variable of density, especially if density dependent transmission is suspected.

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Table 3.1: Model selection results from linear models with total integrated sexual reproduction as the response variable. Models are arranged by AIC score.

	Model	*AIC*	*ΔAIC*	*Weight AIC*
1	sex ~ log(density)	253.13	0.00	0.363
2	sex ~ log(density) + parasitism	253.50	0.37	0.300
3	sex ~ log(density) + parasitism + temperature	255.27	2.15	0.124
4	sex ~ log(density) + parasitism + light	255.37	2.25	0.118
5	sex ~ log(density) + parasitism + light + temperature	257.18	4.06	0.047
6	sex ~ log(density) * parasitism + light + temperature	257.19	4.07	0.047
7	sex ~ parasitism	270.67	17.56	5.60E-05
8	sex ~ parasitism + light + temperature	273.41	20.30	1.42E-05
9	sex ~ light	273.95	20.83	1.09E-05
10	sex ~ light + temperature	275.91	22.80	4.07E-06
11	sex ~ temperature	276.34	23.22	3.29E-06

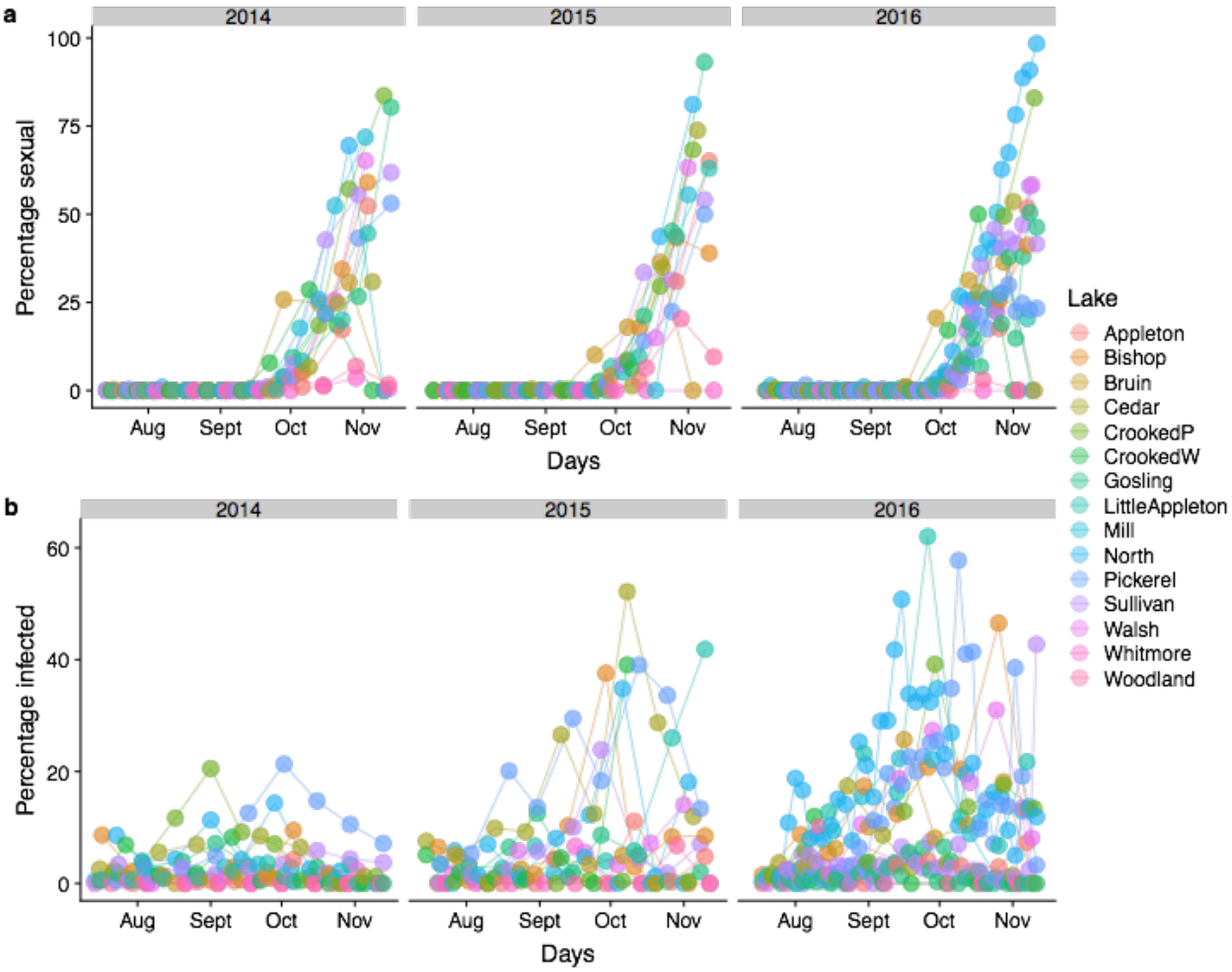

Figure 3.1: Across lakes, years, and calendar day, there was considerable variation in the percentage of *Daphnia dentifera* **(a)** reproducing sexually and **(b)** infected with at least one parasite. The percentage sexual was derived from the ratio of males and ephippial females out of the total population counted and percentage infected was calculated as the percentage of *Daphnia* with any parasitic infection, including coinfections. Each point represents a single sampling event, and colors denote different study populations.

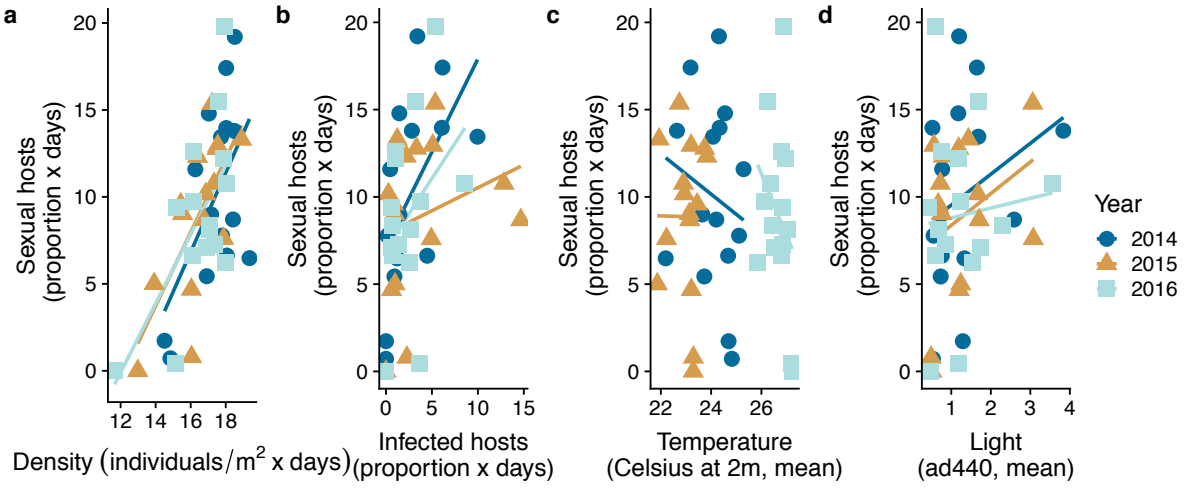

Figure 3.2: Biotic factors were most strongly associated with sexual reproduction in *Daphnia dentifera*: (a) integrated total host density on a log scale, (b) integrated area of the proportion of hosts infected with any parasite. Two abiotic factors—(c) mean temperature at 2m below the surface, and (d) light, as measured by mean ad440—were less strongly associated but modestly improved model fit. The areal density of hosts, the proportion of infected hosts, and the proportion of male and sexual female hosts values were each separately integrated across sampling events to obtain a single value (each point represents a single lake in a given year); analyses with mean and maximum host density and parasitism yielded qualitatively similar results (see Appendix B).

Chapter 4 Virulence Evolution in Natural Epidemics of a Parasite of *Daphnia*

with Haley Essington, Bruce O'Brien, Clara L. Shaw, Rebecca Bilich, and Meghan A. Duffy

ABSTRACT

Virulence, the degree to which a parasite harms its host, is perhaps the most important trait of a host-parasite interaction. However, it is not a static trait, instead depending on the ecological context and possibly evolving over short periods of time (e.g. during the course of an epidemic). In general, theory predicts that the two main strategies for optimal virulence are to either maximize r (intrinsic growth rate) or maximize R_0 (the basic reproductive number). At the start of an epidemic, when susceptible hosts are plentiful, parasites may evolve increased virulence, thus maximizing the intrinsic growth rate. However, as the epidemic wanes, lower virulence and higher R_0 might be more advantageous. Although abundantly studied theoretically, there is still a lack of empirical evidence for virulence evolution in epidemics, especially in natural settings with native host and parasite species. Here, we used a combination of field observations and lab experiments in the *Daphnia*-*Pasteuria* model system to look for evidence of virulence evolution in nature. Controlling for environmental conditions, we found that virulence did in fact change over the course of the epidemic. Thus, our study provides rare evidence for virulence evolution over the course of an epidemic, although our study is limited to a single population.

INTRODUCTION

A main reason researchers and public alike care about parasites is because, by definition, they harm their hosts. The degree to which a parasite harms it hosts, known as virulence, is a key trait: an outcome of host and parasite genotypes and the environment (Read 1994). Virulence is generally the negative effects a parasite has on host fitness, whether related to morbidity, mortality, or fecundity. Crucially, virulence can evolve over time, and the evolutionary path depends on the ecological context (Galvani 2003). Since parasites are ubiquitous in nature and interact so intimately with their hosts, it is imperative to understand and possibly even predict the evolution of virulence. Fortunately, there is extensive theory about the evolution of virulence, the majority of which centers around trade-offs (Alizon et al. 2009). On the contrary, empirical work on virulence evolution is much less common.

Most theory focuses on virulent effects on host mortality (ignoring other effects like those on fecundity), and on a virulence-transmission trade-off (Cressler et al. 2016; Alizon et al. 2009; Frank 1996). The presence of trade-offs means the environmental context is especially important. There are many reasons we might see virulence evolve in response to the environment, such as spatial structure (Boots, Hudson, and Sasaki 2004; Dennehy, Abedon, and Turner 2007), transmission mode (Bonhoeffer, Lenski, and Ebert 1996; Ebert 2013), competition (De Roode et al. 2005), and epidemiological dynamics (Bolker, Nanda, and Shah 2010). The latter of which, epidemiological dynamics, are especially important, because they can take place over short time spans and occur with all types of parasites.

In general, theory predicts that the two main strategies for optimal virulence are to either maximize r (intrinsic growth rate) or maximize R_0 (the basic reproductive number) (Bolker, Nanda, and Shah 2010). In an epidemic setting, theory predicts that at the outset, when there are many susceptible hosts around, it may be more useful for a parasite to kill its host earlier (higher

virulence) so it can transmit soon (increasing *r*) (Berngruber et al. 2013). Yet as the epidemic progresses and there are fewer susceptible hosts to infect, evolution may favor parasites that are less virulent and take time to produce more spores (maximizing R_0). Taken together, this might suggest that virulence should be highest near the peak of an epidemic. However, despite the extensive theory and commonness of parasite epidemics, there are limited examples of virulence evolution in an epidemiological context.

Some studies have tracked virulence evolution on epidemiological time scales, both in the lab and in nature. Perhaps the most well-known one is the introduction of myxoma virus to control invasive rabbit populations in Australia (Fenner, Ratcliffe, and Others 1965). In that system, very virulent strains of the virus predominated at the start, but parasite and host evolution led to less virulent strains taking over in the long run (Dwyer, Levin, and Buttel 1990). Another example—also of an introduced species—is the mycobacterium (*Mycoplasma gallisepticum*) that infects house finches in the United States. Both host resistance and parasite virulence changed over time (Fleming-Davies et al. 2018). From the lab, experiments with epidemics of a phage and bacterial populations demonstrated higher virulence with respect to host mortality was favored at the start of epidemic growth, but this changed as prevalence increased (Berngruber et al. 2013).

However, there are issues with drawing conclusions from these studies, primarily because the hosts and/or parasites were introduced by humans. How does virulence evolve during a natural epidemic? In addition to the phage work (Berngruber et al. 2013), laboratory experiments with *C. elegans* and bacterial parasites (White et al. 2020) or Indian meal moths and viruses (Boots and Mealor 2007) demonstrate the value in using tractable model systems to study virulence evolution. Even more so, systems like *Daphnia* and their parasites bridge the gap

between natural field studies and controlled laboratory experiments. For example, using spores produced over decades, a bacterial parasite of *Daphnia* (*Pasteuria ramosa*) was found to have evolved differences in its within-host growth rate and effects on fecundity (Decaestecker et al. 2007). On shorter time scales in a single population, *P. ramosa* evolved to grow more rapidly while the epidemic progressed, although alternative explanations (e.g., phenotypic plasticity) were not ruled out (Auld, Wilson, and Little 2014). Building on this work here, we controlled for environmental conditions and leveraged the benefits of a native species of *Daphnia* and its common bacterial parasite (*P. ramosa*) to study if and how virulence evolves over the course of an epidemic.

METHODS

Study system

We set out to see if virulence evolved over the course of a natural epidemic. We focused on a dominant host species, *Daphnia dentifera*, which occurs throughout North America (Brooks 1957). Like related species, *D. dentifera* reproduces by cyclical parthenogenesis, which makes it a tractable model system for the field and lab. These hosts live in freshwater lakes, graze primarily on phytoplankton, and become infected by a cornucopia of parasites.

One of the most common and important parasites of *D. dentifera* is the endospore-forming bacterium, *Pasteuria ramosa*. *P. ramosa* infects its host through the gut and replicates itself within the hemolymph. Additionally, this parasite exhibits strong genotype by genotype interactions with the host (Carius, Little, and Ebert 2001) and is capable of evolution over relatively short periods of time (Decaestecker et al. 2007; Auld et al. 2014). Fortunately, *Pasteuria* spores are long lasting (Decaestecker et al. 2007) and can be stored in the lab to infect

hosts at a later date (Duffy and Hunsberger 2019). Crucially, it was identified as a candidate parasite that could theoretically exhibit transient virulence evolution (Bolker, Nanda, and Shah 2010).

Field sampling

Our field observations were similar to those described in (Duffy, James, and Longworth 2015). We took three replicate samples from each lake roughly once every two weeks and visually diagnosed parasite infections under a dissection microscope or compound microscope (for early stage infections). We tracked the prevalence of multiple parasites in multiple hosts species, but *D. dentifera* was the most common host and *P. ramosa* was one of the most common parasites. Because of this and their culturability, we selected *D. dentifera* and *P. ramosa* as the focal species. We chose multiple lakes to target for extra collections of infected hosts in Fall 2017. There were initially three populations/sites, which became limited to one (Little Appleton Lake, Southeast Michigan, US). One of the other lakes was excluded because, when running that part of the study, we did not collect data that allowed us to track relative virulence. The third lake was excluded because we were unable to carry out the lab assays due to lab access limitations during the COVID-19 pandemic. After *Pasteuria* infections in the *D. dentifera* population reached a prevalence >2%, we began collecting infected hosts roughly once every three weeks until we could no longer collect them because the prevalence was too low.

We collected *D. dentifera* that were infected with *P. ramosa* so that we could experimentally quantify virulence at different time points of the epidemic. It was important to reduce the impact of environmental variation, so we could understand the evolution of virulence, separating evolutionary changes from phenotypic plasticity. At each of three time points over the

course of the epidemic, we collected up to 30 infected hosts and kept them alive in the lab. We cured them of their bacterial infections with 0.025 g/mL of tetracycline, and we maintained each one individually as clonal lines once they were uninfected. To control for epigenetic and environmental influences, we used maternal lines (Plaistow et al. 2015), taking third or later clutch hosts for at least three generations at 16:8 light:dark cycles and 1,000,000 cells *Ankistrodesmus* algae every four days a week for food in 30mL of filtered lake water in 50 mL beakers.

Additionally, we collected and froze up to 50 infected hosts per time point and preserved them in the freezer. Because temperature and time influence parasite traits (Shocket et al. 2018; Duffy and Hunsberger 2019; Searle et al. 2015), we re-cultured parasite spores by infecting *Daphnia* with spores collected from the same time point (e.g., spores from time point 2 were cultured using clones from time point 2) under standard conditions. These spores were pooled by time points and used to inoculate individual neonate (<24 hours old) *D. dentifera* with 5,000 spores per mL in 2mL well plates for 48 hours. Infected hosts were transferred to 100mL beakers of lake water in groups of 5, and collected after host death to serve as parasite stock.

Once spores had been propagated and host maternal lines reached three generations, we began exposing each treatment, save controls. For each time point (1, 2, and 3), we exposed 10 replicates of each clone to the propagated parasite stock from the same time point. The number of clones in each time point ranged from 5 to 13. However, to account for host evolution, additional hosts from time point 3 were exposed to parasites from time point 1. As uninfected controls, we also had 5 control genotypes with 5 replicates from each time point; these allowed us to calculate relative virulence (virulence of infected hosts compared to uninfected hosts).

Hosts were maintained individually in 30mL of filtered lake water, fed in the same manner as the maternal lines, and checked daily for mortality.

Statistical analysis

Our desired metric was the reduction in host lifespan due to infection with *P. ramosa*. Thus, virulence was calculated as the ratio of infected to control lifespans for each individual infected animal, subtracted from one for scaling; thus 0 indicates no reduction in lifespan when infected, whereas values above zero indicate reduced lifespan. When capable we matched control to infection treatment results by clone, otherwise taking the average of control lifespans for that time point. In our analysis, we first tested the effect of time point on virulence for contemporaneous pairs of hosts and parasites only. Additionally, for just hosts from time point 3, we tested the effect of parasite time point on virulence, because these host clones were infected with one of the two different parasite cultures. We ran these statistical tests in R (version 3.5) using a GLM with quasipoisson family structure and checked for overdispersion.

RESULTS

There was a large epidemic of *Pasteuria ramosa* in *Daphnia dentifera*. At the peak, the percentage of infected *Daphnia* reached nearly 40% of the total population (Fig. 4.1, time 2), which is a large outbreak for Midwestern lake populations. Host and parasite samples were collected from the early, middle, and later stages of the epidemic curve. Infected host density followed a similar pattern to overall prevalence (Fig C2).

Virulence (the reduction in infected host lifespan compared to that of uninfected hosts from the same time point) varied over the course of the epidemic (Fig. 4.2, $F = 3.99$, $p = 0.03$).

Generally, infected hosts had shorter lifespans than uninfected hosts (i.e. positive values of "relative virulence"). However, when assaying the virulence of parasites from a particular time point against the hosts from that same time point, it was only at time point 2 that the 95% confidence intervals of relative virulence did not overlap 0 (Fig. 4.2).

However, infected lifespan and control lifespans on their own did not vary across time points (Fig. 4.3). Generally, infection with *P. ramosa* reduced lifespan of infected hosts but to different degrees depending on the time point, hence the different virulence values in Fig. 4.2.

We also compared the virulence of parasites collected at the first and the third time points by using them to infect hosts from the last time point; in this comparison, relative virulence did not differ (compare gray box for host time point 3 with blue box; Fig. 4.3, $F = 1.47$, $p = 0.24$). The median lifespan of the first time point parasites was the same regardless of the time point of the host population it infected (compare gray bars in Fig. 4.3; $F = 1.43$, $p = 0.25$). Unfortunately, experimental constraints meant we did not have a full comparison with the second time point parasites as well.

DISCUSSION

We observed different degrees of virulence over the course of an epidemic of *P. ramosa* infecting a population of *D. dentifera*. Virulence was highest (i.e., parasites most reduced host lifespan) at the peak of the epidemic. Because virulence is influenced by both host and parasite genotype, and since we controlled for effects of the environment, these changes in virulence suggest parasite and/or host evolution.

Based on theory related to transient virulence evolution (Bolker, Nanda, and Shah 2010), we expected virulence to evolve over time. As discussed in the introduction, at the early stages of

epidemiological dynamics, theory predicts that maximizing the intrinsic growth rate (r) is the optimal strategy; in contrast, as the prevalence of susceptible hosts depletes, evolution should favor parasites that maximize R_0 by lowering their virulence. Together, this means one might expect virulence to be highest near the peak. Thus, our results appear to match up with the general theoretical predictions, though future studies with more time points would give more resolution regarding the overall pattern.

The fact that virulence changed over very short time scales and as epidemiological conditions changed means that selection may drive changes in virulence, but the timing is important. The average lifespan of an infected *D. dentifera* was about 30 days (and infection takes about 14 days to develop diagnosable spores). Thus, when we collected individuals from the wild, the spores those infected hosts had ingested could have originated much earlier, under different environmental conditions present earlier in the epidemic. For example, infected hosts at time point 2—collected at the peak of the epidemic—likely were exposed during the growth phase of the epidemic, not the peak.

There are several alternative hypotheses for why we might see the observed patterns of virulence in our study. First, there could be immigration of parasites from the sediment of the lake, in which parasite spores can lay dormant for many years (Decaestecker et al. 2004, 2007). Additionally, host evolution alone is sufficient to explain the changes in virulence through time; hosts might have evolved greater tolerance and then lost that tolerance between sampling time points. Further studies that involve more comparisons of host and parasite genotypes from within a population, and that are replicated across populations, would greatly improve our understanding of virulence evolution during epidemics in the wild.

From the public's perspective, virulence is a parasite trait of particular interest because it affects the lives of humans and other organisms. However, the evolution of virulence poses a challenge for public health. Specifically, emerging infectious diseases might be subject to selection based on epidemiological dynamics, and it would be useful to be able to predict how parasites evolve. For instance, even a small increase (or decrease) in virulence could make a substantial difference in morbidity and mortality when considering human infectious diseases. Testing the theory behind transient virulence evolution is therefore fundamental for building a better understanding of infectious disease evolution more generally, and model systems are well suited to accomplish this.

Our study lays the groundwork for future work on the evolution of virulence in natural epidemics. Limitations aside, we uncovered changes in virulence in response to epidemiological dynamics in the *D. dentifera–P. ramosa* system. Transient virulence evolution is itself understudied, and studies in natural systems even more so. Given the quantity and severity of emerging infectious diseases (like the COVID-19 pandemic that curtailed this work), understanding how virulence evolves over short time spans in an epidemic setting is vital.

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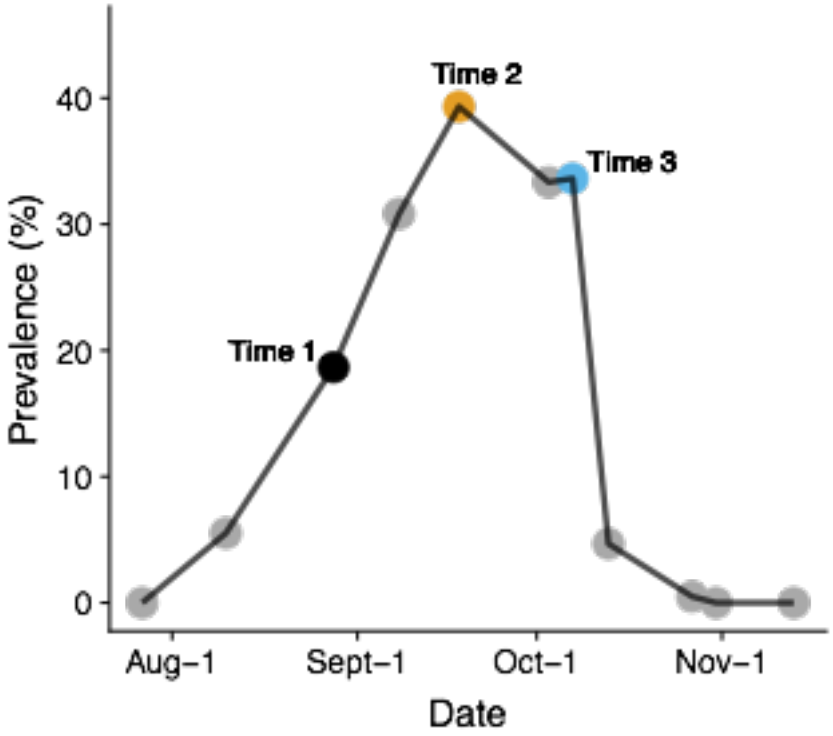


Figure 4.1: *Daphnia dentifera* in Little Appleton Lake experienced a large epidemic of *Pasteuria ramosa*; host and parasite samples were collected at three time points throughout the epidemic trajectory in the Fall of 2017. Prevalence of *P. ramosa* increased steadily from the beginning of sampling, peaked at 39% of hosts infected, and decreased more sharply during October.

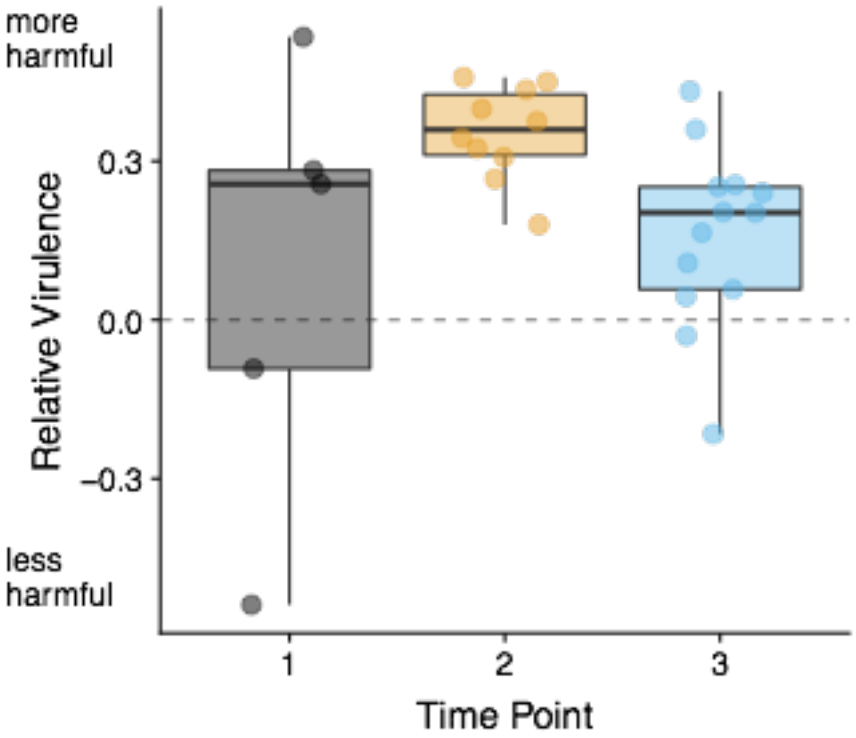


Figure 4.2: The virulence of *P. ramosa* varied across time during the epidemic in Little Appleton Lake ($F = 3.99$, $p = 0.03$), as measured by exposing hosts to parasites from the same time point. The y-axis shows virulence with respect to host mortality (1- infected host lifespan/uninfected host lifespan) of host and parasite pairs. The time points on the x-axis correspond to those marked in Fig. 1. Each point represents the mean relative virulence value of a host clone when infected with parasites from the same time point; boxplot bars show the median value.

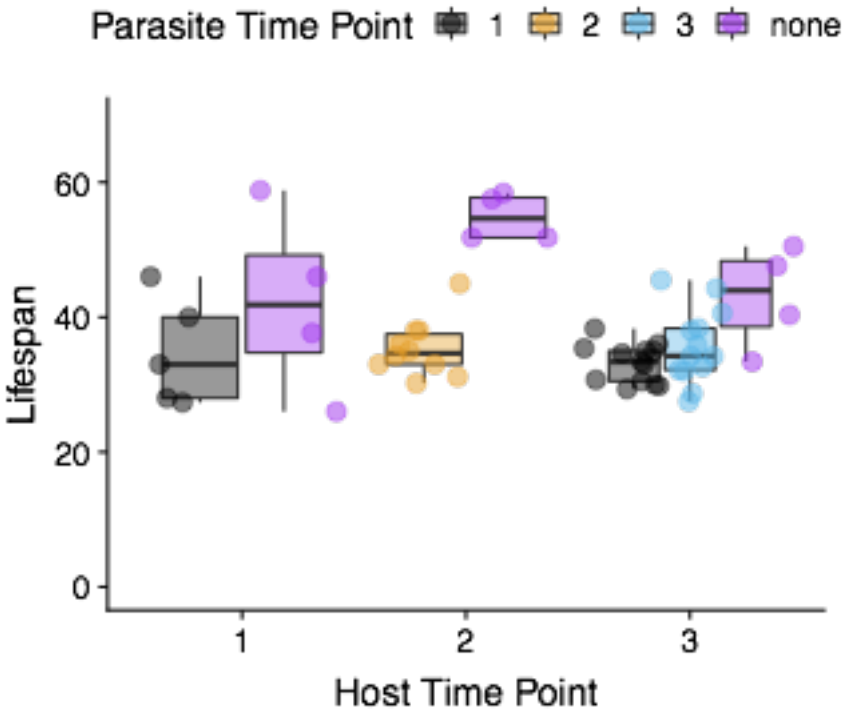


Figure 4.3: Infection with *P. ramosa* reduced host lifespans compared to uninfected controls (compare other colors to purple bars, labeled "none"). There was no discernible difference between infected host lifespan across contemporaneous time points ($F = 0.40$, $p = 0.96$), and no difference across control treatments (compare purple bars, $F = 2.08$, $p = 0.18$).

Chapter 5 Conclusion

SUMMARY

I set out to study the ecological dynamics of complex multi-host, multi-parasite communities and draw conclusions about the evolutionary consequences of those interactions. As one might expect, my work generated just as many questions as answers. Nevertheless, I created cross-species transmission networks of hosts and parasites, investigated the biotic and abiotic drivers of sexual reproduction in certain hosts, and quantified changes in virulence of a common bacterial parasite. Not included here, but additional work I did during my time in graduate school used simulations and sensitivity analysis to test basic model assumptions of healthcare-associated infections in a compartmental model framework.

As my study system, I used communities of *Daphnia* and the suite of parasites found to infect them in nature. In addition to all the benefits of being a model system, *Daphnia* are also key zooplankton species in lake ecosystems, so understanding their ecology and evolution is important on its own. I used a combination of field observations, laboratory experiments, and modeling approaches to study the infectious disease ecology and evolution in *Daphnia*-parasite communities.

First, I worked to unpack the complexities of cross-species transmission of multiple parasite species in communities of *Daphnia*. Using a high-quality time series dataset, I quantified the synchrony of epidemics as a plausible metric of cross-species transmission. Then, I applied network methods to objectively predict traits like host breadth or parasites and the "importance" of certain hosts at spreading those parasites.

Second, I addressed a timeless question about the evolution of sex, but in a way that has not really been done before by focusing on the influence of multiple parasite species. I also compared and contrasted other biotic factors like host density with abiotic factors like light and temperature that are known cues for *Daphnia* reproduction.

Third, I studied what I believe to be the most important question in infectious disease evolution: transient virulence evolution. There are many reasons one might expect virulence to evolve, and of particular interest is evolution during the growth phase of an epidemic because this mimics patterns observed in many human infectious diseases. There are few examples of studies quantifying virulence evolution in the wild, and even fewer in natural (i.e. non-introduced) host and parasite species.

Here, I summarize my findings from each chapter:

Chapter 2: Using networks to understand cross-species transmission in Daphnia communities

Using 8 different host species of *Daphnia* and 7 different parasites, I estimated cross-species transmission by quantifying the degree to which epidemics of the same parasite species overlapped in different host species. I used these estimates to build networks of hosts and parasites, constructed by the degree of plausible cross-species transmission. I quantified traits of parasites such as host breadth (how many different host species does a parasite infect in nature), finding that some parasites consistently infected many hosts and may transmit among different host species more readily than other parasite species. Additionally, I used centrality to measure the relative "importance" of each host species at spreading each parasite. The results varied by

parasite species and the way we quantified infection (prevalence vs infected host density), but often the most common host species were deemed the most important spreaders.

These results generated many new hypotheses, which other researchers can go out and test. For instance, genetic approaches and lab experiments could test if *Daphnia dentifera* is spreading *Pasteuria ramosa* to most other host species. More generally, testing network methods in a predictive disease framework is useful from a public health context.

Chapter 3: Pluralistic approach reveals biotic and abiotic factors associated with variation in sex in natural populations of Daphnia dentifera

Motivated by the Red Queen hypothesis, I sought to test if multiple parasite species influences sexual reproduction in a species of *Daphnia*. *Daphnia* are cyclical parthenogens, alternating between asex and sex over short time scales. Combined with the fact that sexual reproduction in *Daphnia* is well-studied, they are an ideal study species in which to examine the evolution of sex. Using three years of long-term field sampling data and model selection techniques, I assessed the relationship between biotic and abiotic variables — multiple parasites, host density, light, and temperature — and sexual reproduction in *Daphnia dentifera*. While there was most support for biotic factors like density and parasitism as being important drivers of variation in sexual reproduction, there was some evidence that abiotic factors of light and temperature might be important as well.

These results used a pluralistic approach to study the evolution and maintenance of sex, in which all the relevant factors were tested simultaneously. We demonstrate that many factors, possibly acting together, might influence sexual reproduction in *Daphnia*. While consistent with the Red Queen hypothesis, these results show there is much more work to be done. For instance,

an experiment that manipulates multiple factors at once would complement these correlative results.

Chapter 4: Virulence evolution in natural epidemics of a pathogen of Daphnia

Virulence (i.e. the harm a parasite does to its host) is known to evolve over time, but most studies have focused on virulence evolution under stable disease levels, rather than during the growth phase of an epidemic. Moreover, several of our most important studies of virulence evolution in nature come from introduced parasites. Using the biology of *Daphnia* hosts and its parasite (*Pasteuria ramosa*) to our advantage, I was able to collect and maintain both host and parasite genotypes from different parts of a natural epidemic in the lab. Then, upon controlling for environmental conditions, I tested the virulence of host-parasite pairs through time. I found that virulence with respect to effects on host lifespan changed over the course of the epidemic and was highest (i.e., most harmful) at the peak of the epidemic.

Demonstrations of virulence evolution in nature are quite rare and while limited to a single population, our results are an important addition to the scientific community. There are many clear avenues for future research in this system such as testing different populations, host species, and parasite species.

Extensions of this work

Like all research, my work builds upon other people's discoveries, and hopefully my work will be of use to others. There are many natural extensions to this dissertation: some more local in the realm of *Daphnia* and their parasites, and others more broad for infectious disease ecologists and the general public.

On its own, the data I have collected and helped collect is useful to many disease ecologists. In Dr. Meghan Duffy's lab, I helped to generate one of the best multiple host, multiple parasite time series datasets in existence (prevalence data for 8 different host species and up to 9 different parasite species from 15 sites through time, since 2014). Furthermore, a lot of work was not included in this dissertation because of time constraints, including intensive field sampling where we collected data for birth and death rates of hosts, further identification of a previously unknown *Daphnia* parasite, and many more years of the typical field sampling observations. Knowing others in the lab and elsewhere can use and build upon this knowledge base is very satisfying.

For researchers asking similar questions in similar systems, my work using cross-species transmission networks of different parasites is especially relevant. I've helped identify parasites that infect a broad range of host species and hosts that appear to disproportionately impact transmission of those same parasites. This allows future researchers to focus on particular hosts and parasites for experiments, observations, and mathematical models. Similarly, the work on sexual reproduction in *Daphnia dentifera* is easily extendable to other *Daphnia* species. Furthermore, my research on the virulence evolution of *Pasteuria ramosa* can be tested with other host and parasite species in the same or related systems quite easily.

In the field of ecology and evolutionary biology, my work informs future efforts to study cross-species transmission, the evolution of sex, and virulence evolution. These are all challenging questions for any one study or dissertation to tackle, but adding to the body of literature is useful and the results may be well-suited for meta-analyses.

Of concern to the public, my work has implications for zoonotic and emerging infectious diseases. Knowing which parasites are likely to transmit across host species and which hosts are spreading those infections is crucial for predicting new emerging diseases. My work explores some of the basic tools we can use to study natural host and parasite communities. Additionally, virulence evolution is of particular interest because the harm a parasite does to its hosts is of natural public health concern. Relatedly, during my time in graduate school I also did some more applied work as a guest researcher at the Centers for Disease Control and Prevention, where I used simulations to test different assumptions about asymptomatic colonization about healthcare-associated infections like methicillin-resistant *Staphylococcus aureus*.

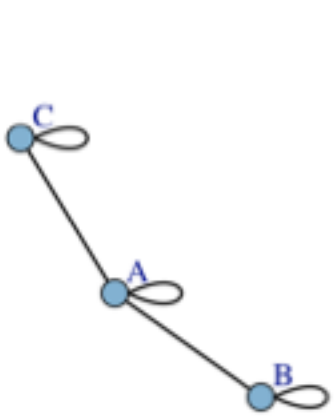
In conclusion, my dissertation research helps uncover some specifics of cross-species transmission, sexual reproduction, and virulence evolution in *Daphnia* and their parasites, touching on big picture questions that have consequences for the rest of the scientific community and the public.

Appendix A

Chapter 2 Supplemental Information

Table A1: Significant pairwise comparisons for host centrality values. Conover–Iman p values are adjusted for multiple comparisons with Bonferroni corrections.

Parasite Species	Epidemic overlap method	Overall Kruskal–Wallis p value	Overall Kruskal–Wallis chi square	Pair-wise comparison	Conover–Iman p value
brood	Infected host density	0.018	13.65	dentifera - pulicaria	0.029
brood	Infected host density	0.018	13.65	pulicaria - retrocurva	0.043
pasteuria	prevalence	0.013	12.61	ceriodaphnia - dentifera	0.020
pasteuria	prevalence	0.013	12.61	dentifera - dubia	0.018
pasteuria	prevalence	0.013	12.61	dubia - retrocurva	0.043
pasteuria	Infected host density	0.007	13.95	ceriodaphnia - dentifera	0.003
pasteuria	Infected host density	0.007	13.95	dentifera - dubia	0.029
pasteuria	Infected host density	0.007	13.95	dentifera - parvula	0.017
pasteuria	Infected host density	0.007	13.95	ceriodaphnia - retrocurva	0.022



$$\begin{pmatrix} 1 & 1 & 1 & 0 \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

Fig A1: Example network of hypothetical hosts (A through D) infected with a hypothetical parasite. On the left, the network visualization showing how we measure “self loops” in addition to epidemic overlap. On the right, the host adjacency matrix for the same pattern.

Appendix B

Chapter 3 Supplemental Information

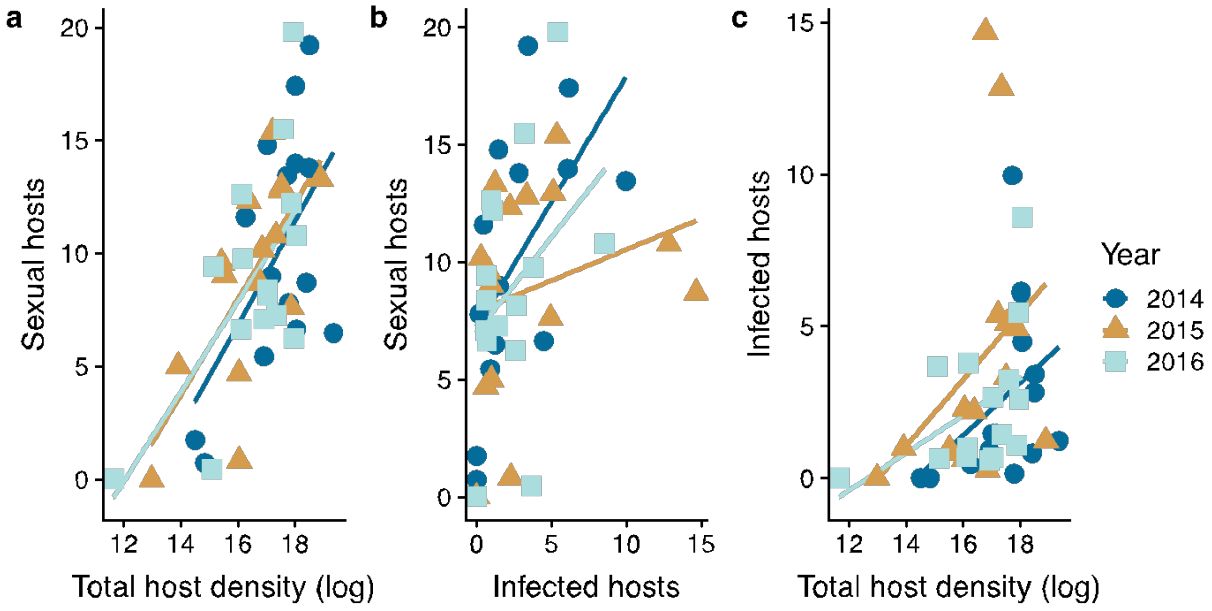


Figure B1: Variation in investment in sexual reproduction is best predicted by host density, not parasitism. **(a)** Populations of *Daphnia dentifera* with higher host densities had higher sexual reproduction (sex ~ log(density), *gaussian*, $F = 29.44$, $p < 0.001$). **(b)** Populations with more total parasitism tended to have more sexual reproduction (sex ~ parasitism, *gaussian*, $F = 6.04$, $p = 0.018$). However, **(c)** lakes where *Daphnia dentifera* was at high densities typically had more total parasite infections (parasitism~ log(density), *negative binomial*, $z = 3.57$, $p = 0.02$). In a model selection approach, host density consistently came out as a main predictor, followed by parasitism (see Table 3.1). The areal density of hosts, the proportion of infected hosts, and the proportion of male and sexual female hosts values were each separately integrated across sampling events to obtain a single value (each point represents a single lake in a given year).

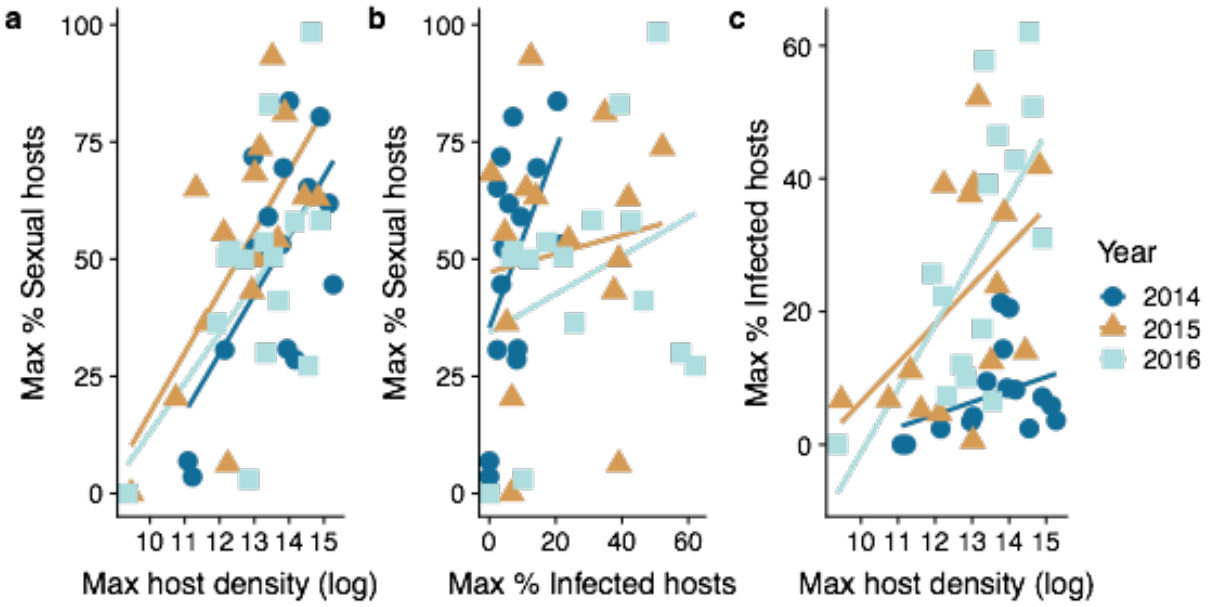


Figure B2: Analyses with maximum host density and parasitism yielded qualitatively similar results.

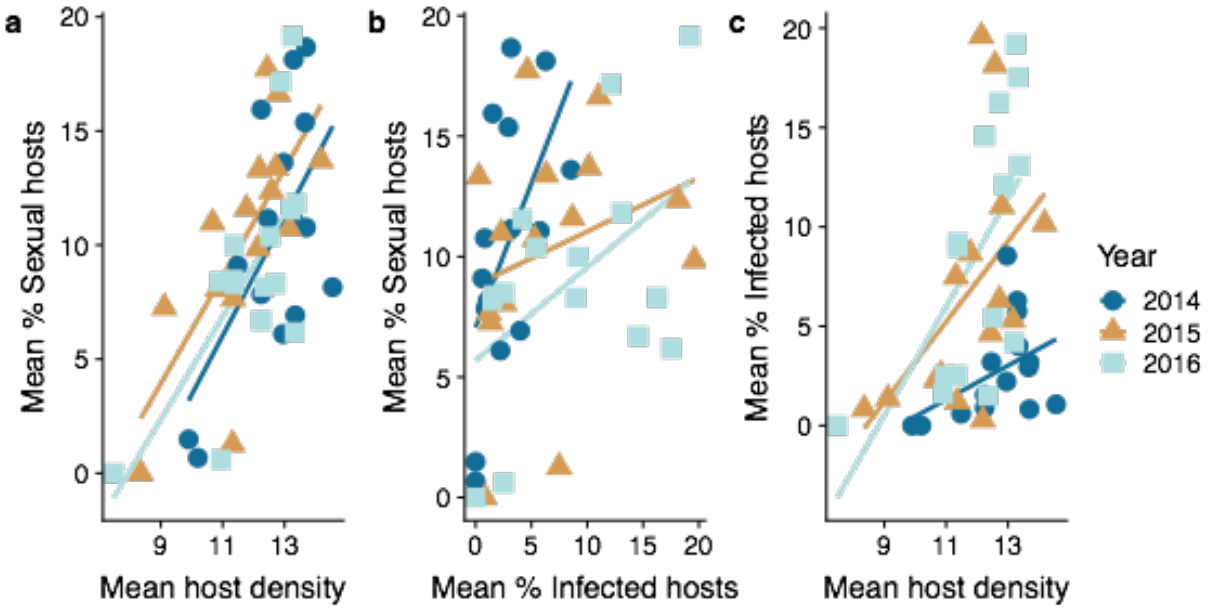


Figure B3: Analyses with mean host density and parasitism yielded qualitatively similar results.

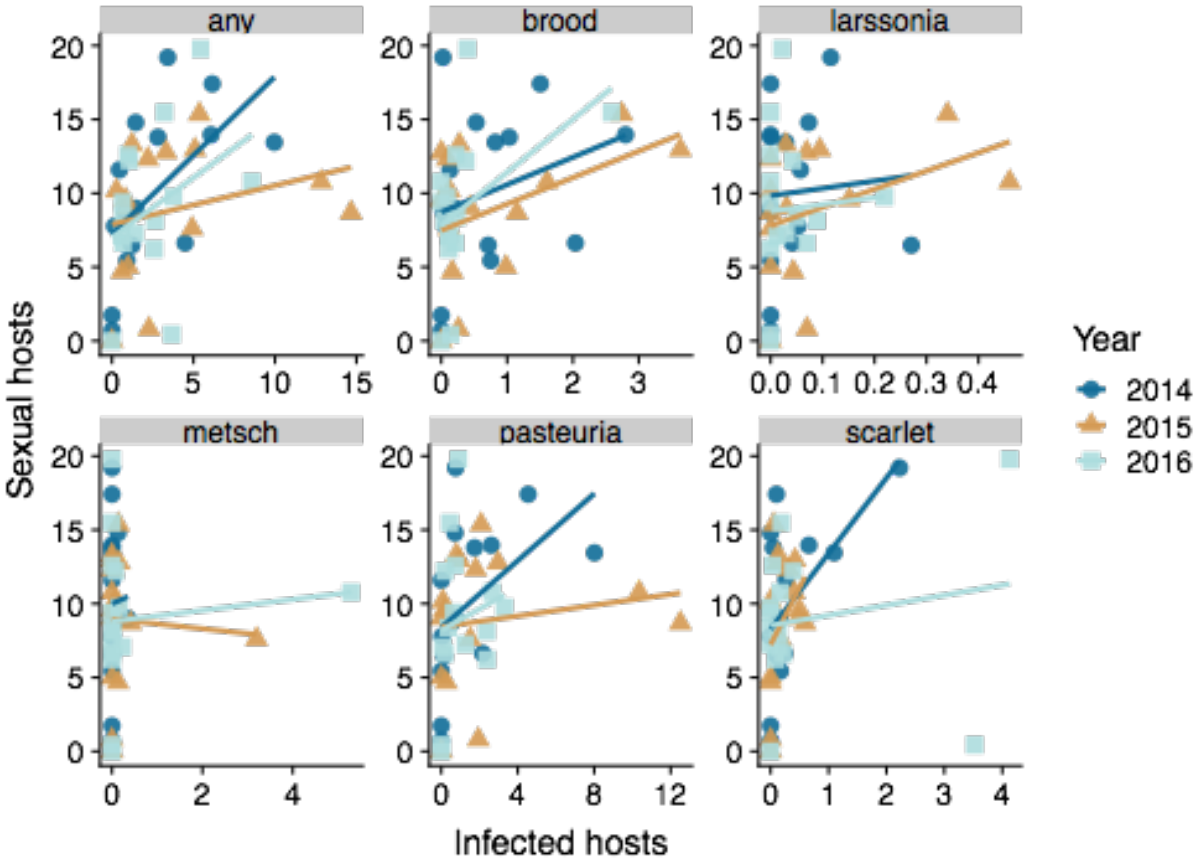


Figure B4: Total integrated sexual hosts compared to integrated infected hosts with different parasite species.

Appendix C

Chapter 4 Supplemental Information

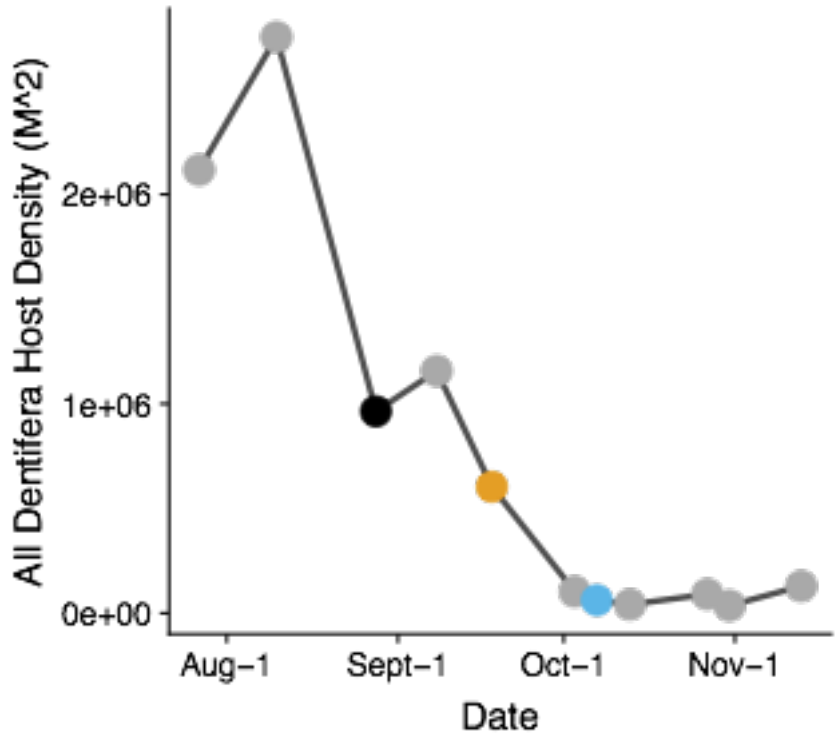


Figure C1: Densities of all *D. dentifera* decreased through time. Black, yellow, and blue points represent dates in which host and parasite samples were collected for the experiment.

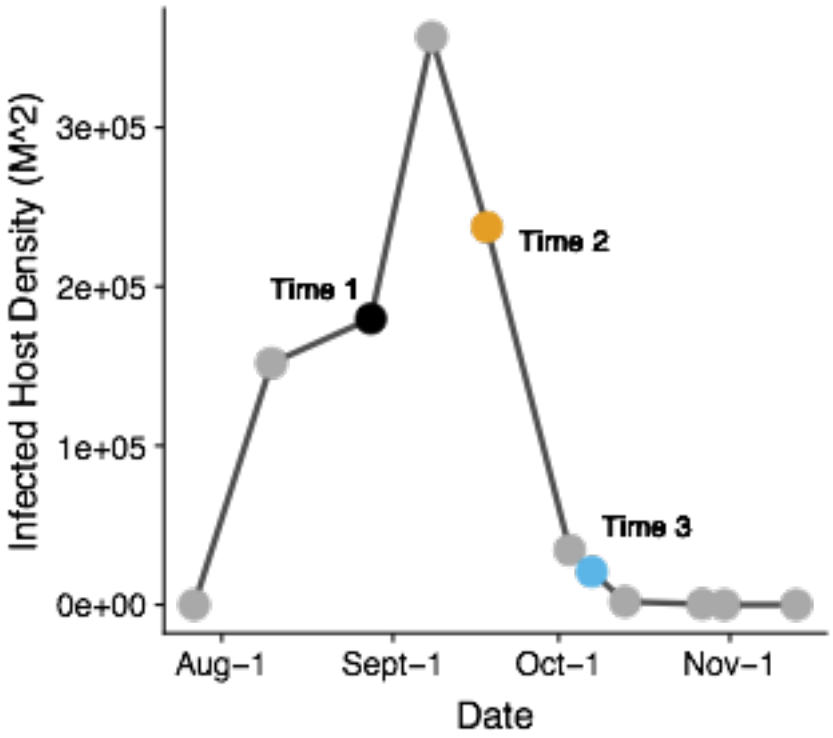


Figure C2: *Daphnia dentifera* in Little Appleton Lake experienced a large epidemic of *Pasteuria ramosa*; host and parasite samples were collected at three time points throughout the epidemic trajectory in the Fall of 2017. The y-axis represents the infected host density (the product of aerial density/m^2 and infection prevalence).

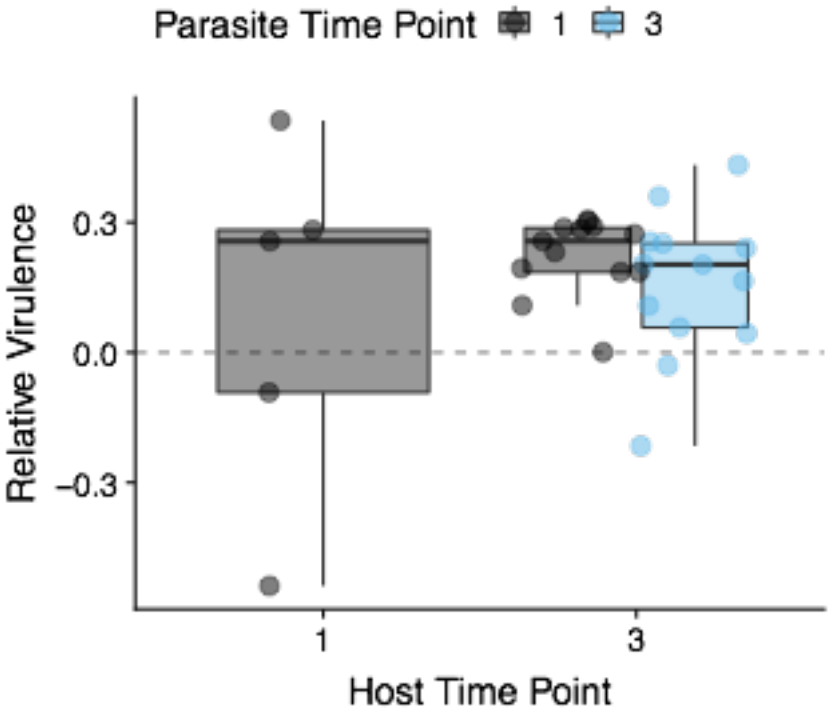


Figure C3: When hosts from time point three were tested with parasites from different time points (time point 1 or time point 3), there were no differences in the relative virulence of the parasites ($F = 1.47$, $p = 0.24$). In comparison, parasite spores from the first time point were similarly virulent when tested against first time point and third time point hosts.

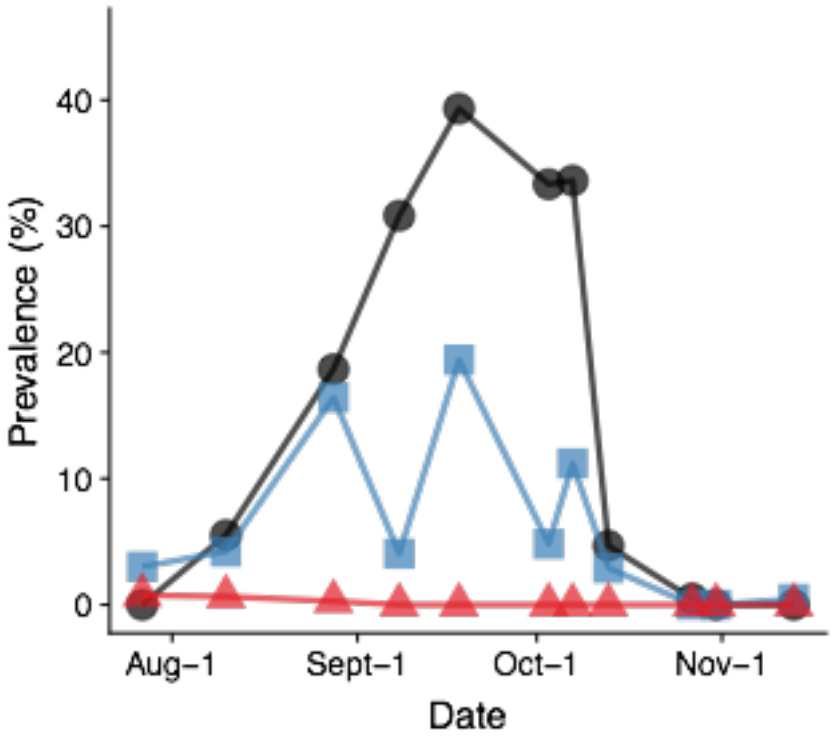


Figure C4: Prevalence of all parasites of *D. dentifera* in Little Appleton Lake, 2017. Black circles = *Pasteuria ramosa*, blue squares = MicG, and red triangles = *Spirobacillus cienkowskii*.