Drivers of Epidemic Timing and Size in a Natural Aquatic System

by

Clara L. Shaw

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Doctoral Committee:

Professor Meghan A. Duffy, Chair
Professor Mark Hunter
Professor Edward Ionides
Associate Professor Timothy Y. James
Professor Aaron A. King

Clara L. Shaw

shawclar@umich.edu

ORCID iD: 0000-0002-0176-8519

DEDICATION

To Andrew Wood and Ruth and Frank Shaw

Thank you for your unwavering confidence in me.

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ABSTRACT

Parasite epidemics are embedded in complicated webs of interacting organisms as well as in an abiotic environment, with direct and diffuse connections among these elements impacting epidemic dynamics. In addition, these elements can be dynamic over time and space, and they may be changing due to human impacts. Yet, though myriad factors influence it, infection is fundamentally the result of an interaction between an individual host and parasite and is impacted by the particular defenses and strategies of these antagonists. Therefore, to understand drivers of epidemics, some of which can be catastrophic for host populations, we must incorporate a nuanced understanding of environmental modulators of parasite transmission as well as of the biology of host and parasite populations interacting over space and time. My dissertation elucidates some of the drivers of natural epidemics caused by two environmentally transmitted parasites, the bacterium *Pasteuria ramosa* and the fungus *Metschnikowia bicuspidata,* in Daphniid hosts. Chapters 2 and 3 focus on how abiotic factors influence disease outbreaks through effects on parasite transmission stages. In Chapter 2, I evaluated the effects of light variation on parasite infectivity. I found that both parasites were sensitive to light, though the fungus was more sensitive. I related this to natural epidemics and found that epidemics of the less sensitive bacterium started before those of the more sensitive fungus. In addition, looking across lakes that varied in clarity (and therefore light penetration into water), I found that darker lakes had larger epidemics of the bacterial parasite. In Chapter 3, I quantified parasites in the water column of lakes to link lake habitat structure with host and parasite overlap. I found that

parasites were unevenly distributed throughout the water column, and that host habitat selection behavior may influence epidemic dynamics. Chapters 4 and 5 use genetic approaches to elucidate how parasites are passed among a community of Daphniid species in lakes and how patterns of infection differ between generalist and specialist parasites. In Chapter 4, I quantified the population structure of the fungal parasite across two common host species and performed a cross infection experiment, finding that this generalist parasite in fact was composed of genotypes that specialized on the divergent hosts and that this specialization might be driven by spore size. In Chapter 5, I genotyped the bacterial parasite across populations, host species, and over time, finding that genetic variation in this parasite was structured by lake and host species, implicating low transmission across these barriers and/or local adaptation to specific host populations. In addition, parasite strain structure changed over time in outbreaks indicating rapid evolution. Overall, my research shows that epidemic dynamics are influenced by environmental conditions through their effects on environmental transmission stages, but also that epidemics depend on the success of specific interactions that occur between hosts and parasite individuals over space and time.

CHAPTER 1

Introduction

OVERVIEW

Parasites are ubiquitous, causing effects ranging from minor discomfort for individual hosts to devastating epidemics with important ecological, economic, and conservation consequences (Johnson et al. 2015). Parasite epidemics cannot be considered as simple interactions between host and parasite populations (Betts et al. 2016). The interactions between these key players are influenced by abiotic conditions (Williamson et al. 2017, Shocket et al. 2018), the physical structure of the environment (Cáceres et al. 2006), biotic community interactions (Keesing et al. 2006, Johnson et al. 2008), and the behavior (Marino and Werner 2013) and genetics (King and Lively 2012) of hosts and parasites. Furthermore, usually multiple factors from the previous list simultaneously and interactively impact the interaction between hosts and parasites (Lazzaro and Little 2009, Vale and Little 2009). These interacting factors may dampen or exacerbate epidemics. Thus, understanding the factors that impact the timing, location, and size of epidemics is a major goal of disease ecology research especially as anthropogenic impacts modify diversity of natural communities and alter environmental conditions (Harvell et al. 2009, Lafferty 2009, Altizer et al. 2013).

My research focuses on drivers of epidemic timing and size in *Daphnia*, a common and ecologically important planktonic crustacean in Midwestern lakes (Lampert 1997, Miner et al.

2012) and a model system for understanding the ecology and evolution of infectious diseases (Ebert 2011, Cáceres et al. 2014). Parasite epidemics in *Daphnia* are embedded in complicated webs of interactions that vary through time and space in lake ecosystems (Miner et al. 2012). First (chapters 2 and 3), I explore drivers that impact parasite dynamics through effects on parasite environmental stages and second (chapters four and five), I explore the challenge of parasite transmission in a multihost environment.

In order to complete their life cycle, parasites must successfully transmit from one host to another. Parasites exhibit a range of strategies for transmitting between hosts ranging from requiring direct contact between infected and susceptible hosts, to requiring vectors, to the dispersal of transmission stages that can persist long term in the environment while awaiting contact with susceptible hosts. The focal parasites of this dissertation belong to the latter category. Though they can persist in the environment, they are also at the mercy of often hazardous environmental conditions. Before encountering a host, transmission stages may be damaged by light (Overholt et al. 2012) or high temperatures (Shocket et al. 2018), they may be consumed by an animal that is not susceptible (Hall et al. 2009, Strauss et al. 2015), or they may sink out of reach of susceptible hosts (Hall et al. 2010b). Heterogeneity among lakes in conditions that affect spore mortality should impact epidemic dynamics across a landscape.

Another important determinant of parasite fitness is the encounter rate with susceptible hosts. In habitats with high diversity, hosts vary in susceptibility, posing a challenge to parasites which may perish if consumed by a host that is not susceptible (Keesing et al. 2006). Even among hosts that can become infected, different species (or genotypes) may be less competent for the parasite, decreasing parasite reproductive potential (Hall et al. 2009). Despite this, and despite theoretical predictions that generalists should be outcompeted by specialists that can be

better adapted to exploiting a single host species (Futuyma and Moreno 1988), some parasites can infect a breadth of hosts. The focal parasites in this dissertation both infect a wide range of host species in lakes, but host species likely vary in quality or defenses (Auld et al. 2017). Therefore, infecting this wide range of hosts should be a challenge, and I explore the mechanisms by which parasites navigate these multihost communities in chapters 4 and 5. By improving our understanding of these two key facets of parasite fitness (survival of transmission stages and infection success in multihost communities), I advance the understanding of drivers of disease outbreaks.

STUDY SYSTEM

We study parasite transmission in planktonic *Daphnia* hosts in Midwestern lakes. Specifically, we sample *Daphnia* populations in two regions, with 15 lakes near Ann Arbor, MI and 38 lakes near Linton, IN. Six common *Daphnia* species reside in these lakes alongside the most common host (and focal host of laboratory studies), *Daphnia dentifera*. *Daphnia* are key members of lake food webs as a crucial link between algal producers and both invertebrate and vertebrate consumers. Their life cycle is cyclically parthenogenetic, meaning that they reproduce clonally in the lab and when conditions are good in the field. (For most of our species, this is throughout the summer and into the fall). In the late fall, *Daphnia* can switch to sexual reproduction; sexual offspring are enclosed in resting eggs (known as ephippia) that overwinter in lake sediments and that can remain viable for many years (Decaestecker et al. 2007). *Daphnia* exhibit diel migration to avoid fish predation (Bohl 1979, Lampert 1989) and light (Rose et al. 2012), swimming to deep, dark, and cold waters during the day and up to warmer, resource rich

waters at night where embryo development is more rapid (Bottrell 1975) and where algal resources are more abundant (Johnsen and Jakobsen 1987).

Throughout, I focus on two common parasites of *Daphnia*, the fungus, *Metschnikowia bicuspidata*, and the bacterium, *Pasteuria ramosa*. Both cause epidemics in *Daphnia* in lakes in the late summer and fall. Despite their phylogenetic distance, these two parasites have broadly similar infection mechanisms and life cycles. Both infect their hosts when infectious stages penetrate the digestive tract after being consumed with algal food (Metschnikoff 1884, Duneau et al. 2011). Both replicate in the hemolymph of hosts, filling host bodies with spores (Ebert 2005). Eventually, both parasites kill their hosts, at which point spores can exit the decaying corpses and spread in the water column, ready to begin the infection cycle again (Metschnikoff 1884, Ebert 2005).

One major difference between these parasites is exhibited in their specificity, which impacts the population genetic structures that I document in chapters 4 and 5. *Metschnikowia* has a generalist infection mechanism. It physically pierces the gut of its host (Metschnikoff 1884, Stewart Merrill and Cáceres 2018). However, host genotypes are differentially susceptible to this parasite (Duffy and Sivars-Becker 2007). Host size is an important determinant of susceptibility because larger animals feed faster and thus contact more parasite spores (Hall et al. 2007, 2010a). Hosts can also differ in immune defenses (Stewart Merrill and Cáceres 2018). *Pasteuria*, on the other hand is a genotype-specific parasite and must be genetically compatible with host genotypes in order to attach to receptors in the host esophagus and penetrate into the host body cavity (Duneau et al. 2011). Most variation in infection is due to this specific attachment step, but additional steps in the infection process can also be important for infection success (Luijckx et al. 2014).

Differences in infection mechanisms between these parasites and in determinants of host resistance and susceptibility to them leads to different expectations for host/parasite interactions and coevolution (Auld et al. 2012). Epidemics of *Metschnikowia* have been shown to cause directional or disruptive selection on hosts for resistance and susceptibility (Duffy and Sivars-Becker 2007, Duffy et al. 2008, 2012). On the other hand, *Pasteuria* epidemics are expected to generate Red Queen dynamics where host and parasite genotypes cycle in abundance due to negative frequency dependent selection generated by the specificity of the interaction (Luijckx et al. 2013).

SUMMARY OF CHAPTERS

Chapter 2: Shedding Light on Environmentally Transmitted Parasites: Within-Lake Light Conditions Affect Epidemic Dynamics.

Energy from sunlight is both essential for life and damaging to living organisms (Paul and Gwynn-Jones 2003). In lakes, parasite spores may be particularly susceptible to damage from light (Overholt et al. 2012) because they cannot escape by swimming deeper (as their *Daphnia* hosts do (Storz and Paul 1998, Leech et al. 2005)). The depth of light penetration varies among lakes due to differing concentrations of dissolved organic matter, which turns water brown and absorbs light, acting as a sunscreen (Morris et al. 1995). We conducted a field experiment to understand how the parasites, *Metschnikowia* and *Pasteuria*, tolerate light conditions in lakes. We incubated spores of each parasite in ambient light or dark conditions in July, August, and November at two different depths in lakes across a gradient of clarity. We also analyzed data from natural epidemics to see if lake clarity influenced epidemic size and timing.

Both parasites were sensitive to ambient light, with *Metschnikowia* sensitivity persisting into the fall when light levels were waning. Consistent with this, natural epidemics of the less-sensitive *Pasteuria* started earlier in the summer when light levels were still intense. In addition, darker lakes had larger epidemics of *Pasteuria* than clearer lakes. Overall, this study shows that light may be an important factor controlling timing and size of parasite epidemics in *Daphnia*. Furthermore, these results suggest that disease outbreaks could become exacerbated by human activities which darken waters, including lake browning associated with climate change and eutrophication.

Chapter 3: How do Animals Balance Multiple Risks in Dangerous Habitats? Quantifying the Distributions of Daphniids, Their Predators, and Their Parasites in Stratified Lakes

Transmission stages of environmentally transmitted parasites are at the mercy of the abiotic environment as they await encounter with hosts (Pietrock and Marcogliese 2003). In contrast, *Daphnia* hosts can actively choose their position in the water column. However, when they select habitat, they must balance not only risks from parasites, but also from predators, and damaging abiotic conditions (Winder et al. 2004, Rose et al. 2012). We quantified parasite spores throughout water columns in three lakes and two months during epidemics using quantitative PCR on the particles in water samples preserved on filters. We also quantified the distribution of hosts and relate host habitat use during the day and night to risk from parasites to understand where hosts are at most risk of becoming infected. We found that spores are unevenly distributed in the water column, and that hosts migrate, living lower in the water column during the day, where they could have higher contact with parasite spores. Along with avoiding parasites, hosts likely also balance risks from predation with accessing beneficial habitat (with resources and

beneficial development temperatures). We therefore also quantified distributions of *Chaoborus* predators, and associated *Daphnia* and *Chaoborus* distributions with light and temperature in lakes. *Daphnia* lived higher in the water column than *Chaoborus,* indicating that *Daphnia* may select relatively predator-free space between risks from fish higher in the water column and *Chaoborus* lower in the water column. We also found that hosts selected deeper habitats in clearer lakes during the day, and *Daphnia* selected warmer habitats at night in months where lakes were stratified. Since risks and benefits are unevenly distributed across the water column, *Daphnia* habitat selection with respect to these factors may impact their exposure to parasitic spores, which could influence patterns of disease.

Chapter 4: Asymmetric Interspecific Disease Transmission Modulated by Parasite Spore Size: Parasite Traits Help Explain Host Breadth in a Virulent Fungal Pathogen.

Parasites exhibit differences in the breadth of host species they infect. Some parasites are highly specialized on a single host species, and some readily infect multiple different host species (Viana et al. 2014). *Metschnikowia* is a generalist parasite that infects most *Daphniid* species and even other planktonic species in our study lakes. To understand patterns of transmission between host species that likely differ in quality for the parasite (Auld et al. 2017), we developed microsatellite markers to detect cryptic variation between isolates infecting the host species, *Daphnia dentifera* and *Ceriodaphnia dubia*. We found that isolates infecting these host species in natural outbreaks had different genetic signatures (though strains were not completely restricted to each host type), indicating that different strains may specialize on different hosts. We then conducted a laboratory experiment that showed that parasite strains had higher fitness on the host species from which they were collected. These results were driven by

differences in spore size, which seems to be important both for infectivity and spore production within the hosts. Overall, tradeoffs seem to largely restrict these parasite genotypes to lakes where their preferred host type is dominant. Thus, by combining molecular approaches with experimental exposures, we were able to uncover a trait that is important in determining host breadth, helping explain patterns of infection in the wild.

Chapter 5: A Common Multihost Parasite Shows Genetic Structuring at the Host Species and Population Level – and Rapid Evolution During Disease Outbreaks.

Though parasite epidemics can be catastrophic for host populations (Skerratt et al. 2007, Blehert et al. 2009, Gostin et al. 2014), we know little about the origins of the parasites that cause them. In this chapter, we focus on three mechanisms that could contribute to parasite emergence: transmission over space, spillover between host species, and evolution. We use population genetic techniques to determine how variation in *Pasteuria* is structured across lakes, host species, and over time. Though *Pasteuria* is a genotype-specific parasite (Carius et al. 2001, Luijckx et al. 2013), it infects diverse hosts (Luijckx et al. 2014, Auld et al. 2017) and outbreaks occur in lakes across a landscape that vary in host community composition. We therefore used variable number tandem repeats (VNTRs) to genotype parasites in infected animals collected from outbreaks across lakes and host species in 2015 and through time within outbreaks in two lakes in 2017. We found that genetic variation in the parasite structures by lake, by host species, and by sampling date, indicating that ecological and evolutionary processes constrain movement of parasite strains among hosts and between lakes. We also found evidence for rapid evolution of the parasite within epidemics. This study improves our knowledge of the relative contribution of transmission across space, among host populations, and change over time in a natural host and

parasite metacommunity helping us understand disease risk in complex environments and over time.

REFERENCES

Altizer, S., R. S. Ostfeld, P. T. J. Johnson, S. Kutz, and C. D. Harvell. 2013. Climate change and infectious diseases: from evidence to a predictive framework. Science 341:514–9.

Auld, S. K. J. R., S. R. Hall, and M. A. Duffy. 2012. Epidemiology of a *Daphnia*-multiparasite system and its implications for the red queen. PloS one 7:e39564.

Auld, S. K. J. R., C. L. Searle, and M. A. Duffy. 2017. Parasite transmission in a natural multihost – multiparasite community. Philosophical Transactions of the Royal Society B 372:1–10.

Betts, A., C. Rafaluk, and K. C. King. 2016. Host and parasite evolution in a tangled bank. Trends in Parasitology 32:863–873.

Bohl, E. 1979. Diel pattern of pelagic distribution and feeding in planktivorous fish. Oecologia 44:368–375.

Bottrell, H. H. 1975. Generation time, length of life, instar duration and frequency of moulting, and their relationship to temperature in eight species of Cladocera from the river Thames, Reading. Oecologia 19:129–140.

Cáceres, C. E., S. R. Hall, M. A. Duffy, A. J. Tessier, C. Helmle, and S. MacIntyre. 2006. Physical structure of lakes constrains epidemics in *Daphnia* populations. Ecology 87:1438–1444.

Cáceres, C. E., A. J. Tessier, M. A. Duffy, and S. R. Hall. 2014. Disease in freshwater zooplankton: what have we learned and where are we going. Journal of Plankton Research 36:326–333.

Carius, H. J., T. J. Little, and D. Ebert. 2001. Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection. Evolution 55:1136–1145.

Decaestecker, E., S. Gaba, J. a M. Raeymaekers, R. Stoks, L. Van Kerckhoven, D. Ebert, and L. De Meester. 2007. Host-parasite "Red Queen" dynamics archived in pond sediment. Nature 450:870–873.

Duffy, M. A., C. E. Brassil, S. R. Hall, A. J. Tessier, C. E. Cáceres, and J. K. Conner. 2008. Parasite-mediated disruptive selection in a natural *Daphnia* population. BMC Evolutionary Biology 8:80.

Duffy, M. A., J. H. Ochs, R. M. Penczykowski, D. J. Civitello, C. A. Klausmeier, and S. R. Hall. 2012. Ecological context influences epidemic size and parasite-driven evolution. Science 335:1636–1638.

Duffy, M. A., and L. Sivars-Becker. 2007. Rapid evolution and ecological host-parasite dynamics. Ecology Letters 10:44–53.

Duneau, D., P. Luijckx, F. Ben-Ami, C. Laforsch, and D. Ebert. 2011. Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host-parasite interactions. BMC Biology 9:11.

Ebert, D. 2005. Ecology, epidemeology, and evolution of parasitism in Daphnia. US National Library of Medicine, Bethesda, Maryland.

Ebert, D. 2011. A genome for the environment. Science 331:539–540.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics 19:207–233.

Hall, S. R., C. R. Becker, M. A. Duffy, and C. E. Cáceres. 2010a. Variation in resource acquisition and use among host clones creates key epidemiological trade-offs. The American Naturalist 176:557–565.

Hall, S. R., C. R. Becker, J. L. Simonis, M. A. Duffy, A. J. Tessier, and C. E. Cáceres. 2009. Friendly competition: evidence for a dilution effect among competitors in a planktonic host-parasite system. Ecology 90:791–801.

Hall, S. R., L. Sivars-Becker, C. Becker, M. a. Duffy, A. J. Tessier, and C. E. Cáceres. 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. Ecology Letters 10:207–218.

Hall, S. R., R. Smyth, C. R. Becker, M. A. Duffy, C. J. Knight, S. MacIntyre, A. J. Tessier, and C. E. Cáceres. 2010b. Why Are *Daphnia* in some lakes sicker? Disease ecology, habitat structure, and the plankton. BioScience 60:363–375.

Harvell, D., S. Altizer, I. M. Cattadori, L. Harrington, and E. Weil. 2009. Climate change and wildlife diseases: when does the host matter most? Ecology 90:912–920.

Johnsen, G. H., and P. J. Jakobsen. 1987. The effect of food limitation on vertical migration in *Daphnia longispina*. Limnology & Oceanography 32:873–880.

Johnson, P. T. J., R. B. Hartson, D. J. Larson, and D. R. Sutherland. 2008. Diversity and disease: Community structure drives parasite transmission and host fitness. Ecology Letters 11:1017–1026.

Johnson, P. T. J., R. S. Ostfeld, and F. Keesing. 2015. Frontiers in research on biodiversity and

disease. Ecology Letters 18:1119–1133.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–498.

King, K. C., and C. M. Lively. 2012. Does genetic diversity limit disease spread in natural host populations? Heredity 109:199–203.

Lafferty, K. D. 2009. The ecology of climate change and infectious diseases. Ecology 90:888–900.

Lampert, W. 1989. The adaptive significance of diel vertical migration of zooplankton. British Ecological Society 3:21–27.

Lampert, W. 1997. Zooplankton research: the contribution of limnology to general ecological paradigms. Aquatic Ecology 31:19–27.

Lazzaro, B. P., and T. J. Little. 2009. Immunity in a variable world. Philosophical Transactions of the Royal Society B: Biological Sciences 364:15–26.

Leech, D. M., A. Padeletti, and C. E. Williamson. 2005. Zooplankton behavioral responses to solar UV radiation vary within and among lakes. Journal of Plankton Research 27:461–471.

Luijckx, P., D. Duneau, J. P. Andras, and D. Ebert. 2014. Cross-species infection trials reveal cryptic parasite varieties and a putative polymorphism shared among host species. Evolution 68:577–86.

Luijckx, P., H. Fienberg, D. Duneau, and D. Ebert. 2013. A matching-allele model explains host resistance to parasites. Current Biology 23:1085–1088.

Marino, J. A., and E. E. Werner. 2013. Synergistic effects of predators and trematode parasites on larval green frog (Rana clamitans) survival. Ecology 94:2697–2708.

Metschnikoff, E. 1884. A disease of *Daphnia* caused by a yeast. A contribution to the theory of phagocytes as agents for attack on disease-causing organisms. Archiv fur pathologische Anatomie und Physiologie und fur klinishe Medicin 96:177–195.

Miner, B. E., L. De Meester, M. E. Pfrender, W. Lampert, and N. G. H. Jr. 2012. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. Proceedings of the Royal Society B 279:1873–1882.

Morris, D. P., H. Zagarese, C. E. Williamson, E. G. Balseiro, B. R. Hargreaves, B. Modenutti, R. Moeller, and C. Queimalinos. 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. Limnology and Oceanography 40:1381–1391.

Overholt, E. P., S. R. Hall, C. E. Williamson, C. K. Meikle, M. A. Duffy, and C. E. Cáceres.

2012. Solar radiation decreases parasitism in *Daphnia*. Ecology Letters 15:47–54.

Paul, N. D., and D. Gwynn-Jones. 2003. Ecological roles of solar UV radiation: Towards an integrated approach. Trends in Ecology and Evolution 18:48–55.

Pietrock, M., and D. J. Marcogliese. 2003. Free-living endohelminth stages: at the mercy of environmental conditions. Trends in Parasitology 19:293–299.

Rose, K. C., C. E. Williamson, J. M. Fischer, S. J. Connelly, M. Olson, A. J. Tucker, and D. a. Noe. 2012. The role of ultraviolet radiation and fish in regulating the vertical distribution of *Daphnia*. Limnology and Oceanography 57:1867–1876.

Shocket, M. S., A. T. Strauss, J. L. Hite, M. Šljivar, D. J. Civitello, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2018. Temperature drives epidemics in a zooplankton-fungus disease system: a trait-driven approach points to transmission via host foraging. The American Naturalist 191:435–451.

Stewart Merrill, T. E., and C. E. Cáceres. 2018. Within-host complexity of a plankton-parasite interaction. Ecology 0:1–4.

Storz, U. C., and R. J. Paul. 1998. Phototaxis in water fleas (*Daphnia magna*) is differently influenced by visible and UV light. Journal of Comparative Physiology A 183:709–717.

Strauss, A. T., D. J. Civitello, C. E. Cáceres, and S. R. Hall. 2015. Success, failure and ambiguity of the dilution effect among competitors. Ecology Letters:1–11.

Vale, P. F., and T. J. Little. 2009. Measuring parasite fitness under genetic and thermal variation. Heredity 103:102–109.

Viana, M., R. Mancy, R. Biek, S. Cleaveland, P. C. Cross, J. O. Lloyd-smith, and D. T. Haydon. 2014. Assembling evidence for identifying reservoirs of infection. Trends in Ecology & Evolution 29:270–279.

Williamson, C. W., S. Madronich, A. Lal, R. G. Zepp, R. M. Lucas, E. P. Overholt, K. C. Rose, S. G. Schladow, and J. Lee-Taylor. 2017. Climate change-induced increases in precipitation are reducing the potential for solar ultraviolet radiation to inactivate pathogens in surface waters. Scientific Reports:1–38.

Winder, M., P. Spaak, and W. M. Mooij. 2004. Trade-offs in *Daphnia* habitat selection. Ecology 85:2027–2036.

CHAPTER 2

Shedding Light on Environmentally Transmitted Parasites: Within-Lake Light Conditions Affect Epidemic Dynamics

with Spencer R. Hall, Erin P. Overholt, Carla E. Cáceres, Craig E. Williamson, and

Meghan A. Duffy

ABSTRACT

Parasite fitness depends on a successful journey from one host to another. For parasites that are transmitted environmentally, abiotic conditions might modulate the success of this journey. Here we evaluate how light, a key abiotic factor, influenced spatiotemporal patterns of planktonic disease where light varies seasonally, across lakes, and with depth in a lake. In an *in situ* experiment using those three sources of variation, we tested sensitivity of spores of two parasites to ambient light (PAR and UV). Infectivity of both parasites declined in ambient light. The more sensitive parasite (the fungus, *Metschnikowia*) was damaged even under the lower ambient light during late fall (November). With this differential sensitivity established, we evaluated links between light environment and outbreaks in lakes. Consistent with the incubations, epidemics of the less sensitive parasite (the bacterium, *Pasteuria*) started earlier in the fall (under higher ambient light). In addition, more transparent lakes had smaller epidemics of *Pasteuria*. However, no such patterns arose for the more sensitive fungus: epidemic size

correlated with start date, not lake transparency. Overall, light helped to (partially) control the timing and size of disease outbreaks. Disease outbreaks could thus become exacerbated by human activities which darken waters, including lake browning associated with climate change and eutrophication.

INTRODUCTION

Free-living stages of parasites often must survive wide-ranging environmental conditions in nature while awaiting encounters with new hosts. Since outside-of-host environments often vary more extremely than within-host conditions, this free-living stage can pose challenges for parasites. Transmission stages of some parasites can be well-protected from environmental conditions (e.g., helminths: Pietrock and Marcogliese 2003; *Cryptosporidium*: King & Monis 2007). However, for many parasites with environmental stages, abiotic factors can harm their fitness, e.g., low temperatures (lungworm: Kutz et al. 2002), high humidity (influenza:Lowen et al. 2007), and low salinity (cholera: Miller et al. 1982). If changing climatic conditions alter these abiotic constraints on parasite fitness, climate change could alter the timing and magnitude of disease epidemics (Williamson et al. 2017).

Light poses a key environmental constraint on the fitness of free-living stages of parasites. Light intensity varies spatially and temporally, with dramatic consequences for populations, communities, and ecosystems. Light can damage organisms as certain wavelengths become lethal at high doses. Indeed, humans use ultraviolet (UV) radiation to kill pathogenic organisms (Yaun et al. 2004). Hence, light may mediate interactions between hosts and environmentally transmitted parasites (Häder et al. 2011). Hosts and parasites may differentially

resist or avoid light damage (e.g., through protective molecules: Karentz et al. 1991, Zellmer 1995, Jacobs et al. 2007; or migration: Bebout and Garcia-Pichel 1995, Storz and Paul 1998). They also can differ in their ability to repair damage (Roy 2000). Most pertinently here, darker conditions may weaken constraints on parasites, enabling epidemics.

Several ecosystem features affect the light environment. Exposure to potentially harmful wavelengths of sunlight in natural ecosystems is largely controlled by sun angle (latitude, time of day, time of year) and cloud cover. In aquatic environments, those features govern incident light to the water surface. Exposure to light in the water column then becomes depth-dependent, as absorption means that deeper waters experience less light. Notably, light is absorbed and scattered by dissolved and particulate compounds (including algae). Some of these compounds, especially dissolved organic matter (DOM), selectively absorb UV radiation (Kirk 1994). DOM therefore protects organisms within lakes from these potentially more harmful shorter wavelengths. The concentrations of compounds like DOM, phytoplankton, etc., vary both through time (Kalff and Knoechel 1978, Sommer 1985, Couture et al. 2012) and across a landscape (Morris et al. 1995, Dodson et al. 2000, Laurion et al. 2000, Xenopoulos et al. 2003).

Many lakes are darkening due to human activities. These activities are causing eutrophication (Taranu et al. 2015) and 'browning' (increasing concentrations of DOM; Solomon et al. 2015). Eutrophication is a consequence of intensive land use that elevates nutrient levels in lakes (Schindler et al. 2016). Additionally, several other human-caused factors have triggered browning, including climate change-driven precipitation events that wash land-derived carbon into lakes (Larsen et al. 2011, Williamson et al. 2014b, 2015, 2016). Changes to the light environment due to both eutrophication and browning may alter disease dynamics in lakes by removing light as a constraint on parasites (Williamson et al. 2017).

To evaluate this potential, we examined light effects on fitness of free-living stages of parasites and natural outbreaks along light gradients. In the focal system, *Daphnia* hosts avoid light damage behaviorally: to avoid UV, they migrate deep into the water column during the day (Williamson et al. 2001), greatly reducing their UV exposure (Storz and Paul 1998, Rhode et al. 2001, Leech et al. 2005). However, the infective propagules (hereafter: spores) of the two focal parasites, *Pasteuria ramosa* and *Metschnikowia bicuspidata* (hereafter: *Pasteuria* and *Metschnikowia*) cannot swim. Thus, they cannot behaviorally escape light exposure. While both parasites can reach epidemic prevalence in *Daphnia* populations in autumn (Cáceres et al. 2006, Auld et al. 2014), both are sensitive to UV and PAR (Overholt et al. 2012; Overholt, unpublished data). To evaluate sensitivity of these parasites to light, we experimentally incubated parasite spores in lakes in July, August, and November (i.e., decreasing incident light across the epidemic season) and then used them to infect hosts in the lab. Then, with field survey data, we examined the relationship between light and parasite dynamics in natural lakes. We expected that epidemics of the more sensitive parasite would start later in more transparent lakes (i.e., as the light constraint waned autumnally). Given that later-starting epidemics remain smaller (Overholt et al. 2012, Penczykowski et al. 2014, Shocket et al. 2018), we also predicted more transparent lakes should have smaller epidemics.

METHODS

Hosts and Parasites

The host, *Daphnia dentifera*, is common in Midwestern (USA) lakes. It is susceptible to parasites including *Pasteuria ramosa* (a bacterium) and *Metschnikowia bicuspidata* (a fungus).

Both parasites share similar infection mechanisms and life cycles. For instance, both infect their hosts by penetrating the digestive tract after being consumed (Metschnikoff 1884; Duneau *et al.* 2011). Then, both replicate in the hemolymph of hosts, filling host bodies with spores (Ebert 2005). Most important for this study, upon host death those spores are released into the water column, renewing the infection cycle (Metschnikoff 1884; Ebert 2005). During this stage of environmental dispersal, light could strongly impinge on epidemics via direct effects on spores.

Lake Transparency Measurements and Metrics

In order to quantify light exposure for parasites in the incubation experiment, we measured within-lake light attenuation and surface-level ambient light during each incubation. Within-lake light attenuation was measured directly (BIC 2104, Biospherical Instruments; Appendix A S1) and converted to a percent of incident light remaining at incubation depth (0.5 m and 2 m). Surface-level incident light was integrated over 3 minute time intervals by a radiometer (Model 2104RL, Biospherical Instruments) deployed at the Greene Sullivan State Forest ranger station located within 10 miles of all experimental lakes (Appendix A S1, Appendix Table A1). We multiplied these quantities to obtain within-lake light exposure for 320 nm UV and PAR.

For the field survey we indexed lake transparency as the depth at which 1% of incident 320 nm UV remained. To calculate this index, we measured the absorbance of 320 nm UV light of filtered lake water samples (using GF/F filtrate from lake epilimnia and a Shimadzu UV⁄ Visible UV-1650 PC spectrophotometer). With these absorbance values, we estimated light penetration in the water column (with Beer-Lambert law; Appendix A S1). More transparent

(lighter) lakes have deeper values for 1% 320 nm UV remaining. Attenuation of 320 nm UV light and of PAR light are correlated in our study lakes (r^2=0.66, p<0.001). Thus, darker lakes have both less UV and less PAR. To characterize incident light, we used UV index (UVI) data from Indianapolis (NOAA, 90 miles from the lakes; ftp://ftp.cpc.ncep.noaa.gov/long/uv/cities/). UVI data provided a visual descriptor of light throughout autumn and among years.

Spore Incubation Methods

We incubated spore slurries of each parasite in lakes to assess their infectivity after exposure to ambient sunlight *in situ* in July (20-25), August (15-20), and November (31 Oct-5 Nov 2016). We selected five lakes along a gradient of water transparency: Airline, Canvasback, Beaver Dam, Goodman, and Midland lakes (Appendix A S1, Appendix Table A1). Six quartz vials filled with *Pasteuria* or *Metschnikowia* spores in lake water were suspended at 0.5 m and 2 m depths in each lake, for a total of 24 vials per lake (6 per parasite per depth). Half of these vials were covered in dark plastic ('dark treatment'); the others were left uncovered, and hence exposed to ambient light at depth (i.e., PAR + UV; 'light treatment'; see Appendix A S2 for additional details). The incubations were suspended away from shore in order to minimize shade from shoreline trees.

After the incubation period, spores and algal food *(Ankistrodesmus falcatus*) were added to 150 mL filtered lake water. This water-algae-spore mixture was distributed among either ten (July and August incubations) or eight (November) 15 mL centrifuge tubes. Spore doses for *Pasteuria* were always 2,000 spores mL^{-1}. For *Metschnikowia* it was initially 100 spores mL^{-1} in July, but given low infection rates, we increased it to 250 spores mL^{-1} in August and November

assays. For both parasites, we placed 3-4 day old, individual *Daphnia* of a clone ('Mid37') that is susceptible to both parasites into the tubes. After 24 hours of exposure at 20° C, we moved *Daphnia* to 50 mL tubes. We maintained each individually (still at 20° C) with daily feeding and water changes every other day until visual diagnosis (Appendix A S2).

We used generalized linear mixed models (GLMMs) with binomial error structures to test the effects of light treatment (light-exposed or covered vials), depth, and month on host infection status. The first model evaluated which parasite was more sensitive to light. Hence, only parasite, light treatment, and the interaction were included as fixed effects with a lake by month interaction as a random effect. Then, we evaluated each parasite separately. The second set of models fit light treatment, depth, month, and their interactions as fixed effects for each parasite (retaining the lake by month interaction as a random effect). Finally, in a third set of models, data were analyzed for each month separately with otherwise similar fixed and random factors. All significant interaction terms were included that still allowed for model convergence. We evaluated the effects of light exposure in lakes on relative infectivity (β) of parasites (Appendix A S3). Infectivity is calculated as -log(1-proportion infected)/(spore dose*exposure time). We standardized 'relative infectivity' for vials exposed to light by dividing by the average infectivity from the corresponding dark treatment. We used linear models for each parasite to test the association between averaged (by lake and depth) relative infectivity and light exposure. All statistics were performed in R Version 3.4.2 (R Development Core Team). GLMMs were performed with the lme4 package (Bates et al. 2015).

Field Survey

We used a field survey to link light sensitivity of parasites to the timing and size of parasite outbreaks in lakes. We sampled 38 lakes in south central Indiana (Greene and Sullivan counties) approximately every two weeks, August - November, during 2014-2016 (Appendix A S4). At each sample date, we pooled three plankton tows, collected at least 25 m apart with a Wisconsin net (13 cm diameter, 153 micron). From those tows, we visually diagnosed 400+ live *D. dentifera* for late-stage infection using a dissecting microscope (40-50X). We integrated data on prevalence through time (with the trapezoid rule) to calculate 'outbreak size' in each lake. Epidemics ('large' outbreaks) 'started' on the first date at which infection prevalence reached and remained above 1% for at least one more visit (Duffy et al. 2005). 'Small' outbreaks did not maintain prevalence above 1% for more than one visit.

We compared start dates of epidemics between parasites with a linear mixed effects model. In this model, epidemic start date was the response variable, parasite identity was a fixed effect, and year and lake were random effects (nlme package in R; Pinheiro et al. 2018). A paired t-test was also used to compare start dates of parasites in lake-years where epidemics of both parasites occurred. This smaller subset of lakes controls for within-lake factors that could also influence epidemic start dates. For each parasite, we also fit linear mixed effects models to link our index of lake transparency (depth of 1% 320 nm UV remaining) to outbreak size. In these models, outbreak size was predicted by transparency, epidemic start date, and interactions as fixed effects and with year as a random effect. Since high algal production could correlate with light exposure, we included chlorophyll concentrations in additional models (Appendix A S5). Following model selection, we dropped non-significant fixed effects. To improve normality, outbreak size was log transformed. To evaluate whether high transparency inhibited epidemics ('large' outbreaks; see above), we used a generalized linear mixed effects model. Here, epidemic

presence/absence was a binomial response variable, index of lake transparency was a fixed effect, and year was a random effect (lme4 package in R; Bates et al. 2015).

RESULTS

Spore Incubations

Spores of both parasites were sensitive to ambient light, but *Metschnikowia* was more sensitive than *Pasteuria*. This differential sensitivity appeared as a significant interaction between parasite and light treatment (parasite x light: $z=2.71$, $P=0.007$). Additionally, *Metschnikowia* was still harmed by incident light in late summer (Aug) and fall (Nov; Figure 2.1D & F). In contrast, the impact of incident light on *Pasteuria* decreased as the season progressed (Figure 2.1 left panels). More specifically, compared to July incubations, light-exposed *Pasteuria* spores infected a greater proportion of hosts in August ($z=5.41$, $P<0.001$; compare Figure 2.1A & C) and November ($z=6.76$, $P<0.001$; compare Figure 2.1A & E). The diminishing seasonal impact of light on *Pasteuria* also manifested in the separate analyses of months. In July, spores exposed to ambient light were harmed (light: $z=-8.26$, $P<0.001$), especially at shallower depth where light was greater (light x depth: $z=5.60$, $P<0.001$; Figure 2.1A). When incident light declined in August (Figure 2.1G), the light x depth interaction became insignificant ($z=1.72$, $P=0.085$) but the main effect of light remained ($z=-3.41$, $P<0.001$; Figure 2.1C). In still darker November, light no longer constrained success of *Pasteuria* (light: $z=-1.00$, $P=0.318$; light x depth: $z=1.29$, $P=0.259$; Figure 2.1E). Thus, exposure to ambient light reduced infectivity of *Pasteuria* spores in summer. By late autumn, this inhibitory effect on *Pasteuria* disappeared.

The fungal parasite *Metschnikowia* was more sensitive to light. Light damaged spores throughout the epidemic season (light: $z=-4.42$, $P<0.001$) but depth provided some protection from light damage (light x depth: $z=2.50$, $P=0.013$). Both August ($z=1.96$, $P=0.050$; Figure 2.1D) and November ($z=3.27$, $P=0.001$; Figure 2.1F) showed higher overall proportion infected hosts compared to July when the spore dose was lower (Figure 2.1B; see Methods). However, light remained a strong constraint throughout autumn: even in later, darker months, spores from light-exposed treatments were less infective than spores from covered treatments (Figure 2.1B, D, & F). In each month analyzed separately, spores exposed to light infected a smaller proportion of hosts in July ($z=-1.99$, $P=0.047$; Figure 2.1B), August ($z=-3.67$, $P<0.001$; Figure 2.1D) and November ($z=-4.00$, $P<0.001$; Figure 2.1F); no depth or depth x light interactions were significant. Thus, unlike for *Pasteuria*, light continued to significantly impact the infectivity of *Metschnikowia* spores well into autumn, even in the deeper (and therefore darker) incubations.

An additional analysis also illustrated how light exposure influenced the relative infectivity of light-exposed spores. Light exposure depends upon light absorption in the water column by particulate and dissolved matter (more is darker), depth (deeper is darker), and season (later is darker). Looking across lakes and seasons, relative infectivity (β) of *Pasteuria* spores declined with higher light exposure with respect to both UV ($F_{1,26}=8.46$, $P=0.007$; Figure 2.2A) and PAR ($F_{1,26}=8.46$, $P<0.001$; Figure 2.2B). For *Metschnikowia*, relative infectivity was not associated with UV ($F_{1,22}=0.36$, $P=0.554$; Figure 2.2C) or PAR exposure ($F_{1,22}=2.2$, $P=0.153$; Figure 2.2D). *Metschnikowia* infection rates in both light-exposed and covered treatments were low, leading to high variability and relative infectivity of light-exposed treatments greater than 1 in some cases. However, relative infectivity of most light-exposed *Metschnikowia* treatments were well below 1, indicating that light had a strong impact on spore infectivity at all levels of

light exposure tested here.

Field Survey

We predicted that differential light sensitivity of parasites would impact timing of outbreaks. Specifically, because incident light levels wane in late summer and autumn, epidemics should start earlier for the less sensitive *Pasteuria* than for the more sensitive *Metschnikowia*. Indeed, the median start date for *Pasteuria* epidemics was 16 days earlier than for *Metschnikowia* in 2014 (Sept 22 compared to Sept 6), 12 days in 2015 (Sept 7 compared to Aug 26), and 24 days in 2016 (Oct 11 compared to Sept 17; Figure 2.3). Thus, epidemic start date differed significantly between parasites ($t=3.40$, $df=8$, $P=0.009$; Figure 2.3B). In lakes with epidemics of both parasites in the same year, those of *Pasteuria* started on average 24 days earlier ($t=4.0$, $df=8$, $P=0.004$; Figure 2.3C).

Since both parasites were sensitive to light, we tested two hypotheses. First, we expected epidemics (larger outbreaks) of a given parasite to start later in 'lighter lakes' (i.e., more transparent, with deeper light penetration). In these lakes, higher light levels should more effectively kill spores during summer. Second, outbreaks should remain smaller in lighter lakes, due to timing and direct mortality effects on spores. For *Pasteuria*, the index of lake transparency was not associated with epidemic start date ($t=-0.22$, $P=0.83$; not shown) although few outbreaks qualified as epidemics (undermining the test's power). However, less transparent lakes had larger outbreaks ($t=-5.41$, $P<0.001$; Figure 2.4A). Furthermore, lakes with *Pasteuria* epidemics were less transparent than those with just minor outbreaks ($z=-2.36$, $P=0.018$; Figure 2.4A).

For *Metschnikowia*, epidemics started earlier in more transparent lakes (t=-2.45, P=0.019; Figure 2.4B), contrary to our hypothesis. Epidemic size was correlated strongly with epidemic start date: earlier starting epidemics grew larger (t=-2.53, P=0.016; Figure 2.4C). Perhaps due to these contrasting patterns, the index of lake transparency did not significantly predict epidemic size for *Metschnikowia* (t=-0.57, P=0.57; Figure 2.4D).

DISCUSSION

Light damages many environmentally transmitted parasites. Therefore, waters darkened by human activities could unleash larger disease outbreaks. To better understand this possibility, we evaluated light effects on parasite outbreaks in Midwestern lakes with an incubation experiment and a field study. Both parasites were sensitive to ambient light conditions in summer (July). For the more sensitive *Metschnikowia*, these effects persisted even into November. Consistent with this differential sensitivity, we found that *Pasteuria* epidemics began earlier in the fall (when light levels begin to decrease). Furthermore, transparent lakes had smaller outbreaks of *Pasteuria.* However, *Metschnikowia* epidemics did not behave similarly. Below, we suggest reasons that might explain this result for the more sensitive parasite.

In the incubation experiment, light damaged parasite spores (especially *Metschnikowia*), making them less infective. We did not separate UV from PAR effects here, but both likely harmed spores *in situ*. UV typically damages more, interfering with replication and transcription of DNA (Sinha and Häder 2002). However, despite only shallow penetration of UV (see Figure 2.2), spores still faced damage at 2 m depth, and *Metschnikowia* experienced damage in late autumn (when incident UV was negligible). Thus, PAR likely damaged spores, too (see also:

Hernández et al. 2006, Overholt et al. 2012), although mechanisms for this damage remain less clear (Ruiz-González et al. 2013). Differential sensitivity among parasites here may stem from differences in protective or repair mechanisms. For instance, *Pasteuria* resides in the *Bacillus* clade (Ebert et al. 1996) where species resist UV through several mechanisms (Nicholson et al. 2000, Setlow and Li 2015). Some fungi tolerate UV well (Onofri et al. 2007) due to protective pigments, etc. (Ruisi et al. 2007). However, other *Metschnikowia* species do not produce high levels of these compounds, even in high-UV Antarctic conditions (Villarreal et al. 2016).

Our field results demonstrate that lake transparency is important for the success of *Pasteuria*, since more transparent lakes had smaller outbreaks. Surprisingly, the same pattern was not observed for more sensitive *Metschnikowia*. One possible explanation involves refuge with depth. Larger *Metschnikowia* spores (Stirnadel and Ebert 1997) may be more likely to sink deeper in the water column than smaller *Pasteuria* spores. Assuming that spores spend substantial time in the epilimnion, the depth of the thermocline could modulate light received by spores. Interestingly, more transparent lakes have deeper thermoclines generally (Fee et al. 1996), and when epidemics started in our lakes (i.e., later than for the start of *Pasteuria* epidemics; Appendix A S5). Epilimnia of light lakes (with deep thermoclines) were still lighter on average than the epilimnia of darker lakes (with shallower thermoclines; Appendix A S5), but lakes with deep thermoclines might still provide a larger low light refuge.

Alternatively, other components of *Metschnikowia*'s life history may compensate for spore losses due to light damage. Parasite fitness and outbreak size can be indexed with the net reproductive ratio (R_0). In simple models, R_0 is a ratio of gains of infections (host infection risk x spores produced per host) to losses of spores (here, through light inactivation; see Bertram et al. 2013). The sensitivity of R_0 to light-induced spore losses will be minor if spore losses are high

and/or infection gains are large. Such thinking might explain why outbreaks of less sensitive *Pasteuria* responded to light. Gains of new infections of this parasite might remain small due to specificity mechanisms of infection genetics (Luijckx et al. 2013) yielding many host genotypes resistant. Hence, small infection gains with moderate light sensitivity yield greater detectability of transparency-outbreak patterns. In contrast, gains of *Metschnikowia* infections may be higher. Without similar infection specificity (Duffy & Sivars-Becker 2007) but with sufficient spore yields (Civitello et al. 2015), high gains coupled with high losses (from light) may flatten transparency-outbreak patterns. In addition to resolving the apparent paradox here, perhaps this reasoning also explains why outbreaks of *Metschnikowia* grow so much larger (here and in Auld et al. 2014b).

A third hypothesis is that *Metschnikowia* epidemic patterns are driven more strongly by other environmental factors. One important driver of epidemic size could be resource levels. In our study, *Metschnikowia* outbreak size was associated with mean chlorophyll levels (Appendix A S5). Not only could high chlorophyll protect spores from light (particularly PAR), but high levels of chlorophyll also often indicate higher food levels for *Daphnia* hosts. Since *Daphnia* produce more spores when grown on high food concentrations (Hall et al. 2009), epidemics grow larger in more productive lakes (Civitello et al. 2015). Though algal production could be increased in high DOC lakes as nutrients accompany land derived carbon, this relationship may be complicated due to a decrease in light penetrating into darker lakes (Creed et al. 2018).

Future changes to light may differentially affect parasites in this system. More frequent extreme weather and heavy precipitation associated with climate change (Easterling *et al.* 2000, Williamson et al. 2017) transport terrestrial carbon (which strongly absorbs UV) to bodies of water (Williamson *et al*. 2014). Browner lakes should have larger *Pasteuria* epidemics due to the

sunscreening effects of these dissolved compounds for parasites. Based on our field survey, it is unclear if *Metschnikowia* will be as sensitive to a browning lake environment, but its epidemics may start later. More generally, many pathogens of humans have water-borne stages that are vulnerable to light damage (e.g. *Schistosoma mansoni* (Ruelas et al. 2007), *Cryptosporidium parvum* (King et al. 2008), *Vibrio cholerae* (Berney et al. 2006)). By reducing light damage to pathogens, browning waters may unleash epidemics of aquatic parasites infecting wildlife and humans (Williamson et al. 2017). Yet, additional traits of parasites may also alter epidemics.

Environmental features that provide shade in terrestrial systems could also affect disease. For example, transmission of a virus of forest tent caterpillars was reduced in lighter environments (e.g., near edges and in patchy fragments) relative to darker interiors of forests (Roland and Kaupp 1995). Corsican Pine also showed higher rates of a fungal disease on north facing (darker) slopes, and in an artificial shading experiment (Read 1968). Furthermore, light might be shaping patterns of disease even within individuals, since most fungal diseases occur on protected parts of plants, like the undersides of leaves (Manning and Tiedemann 1995). Hence, habitat-driven light environment may shape disease in both aquatic and terrestrial systems.

In this study, light damaged spores of two parasites, and higher lake transparency was associated with reduced epidemic size for a bacterial parasite (*Pasteuria*). Global climate change is making lakes darker; thus, less transparent lakes could become sicker lakes. However, other aspects of parasite biology could compensate for spore losses. Epidemics in *Daphnia* can exert ecosystem-level effects (Duffy 2007), so larger epidemics could impact food webs and ecosystems of lakes. More broadly, human activity continues to alter light penetration into numerous systems (e.g. smog near cities; deforestation; browning of surface waters; eutrophication). These human-caused changes in light might affect disease by altering the

survival of environmentally transmitted parasites.

REFERENCES

Auld, S. K. J. R., S. R. Hall, J. Housley Ochs, M. Sebastian, and M. A. Duffy. 2014a. Predators and patterns of within-host growth can mediate both among-host competition and evolution of transmission potential of parasites. The American Naturalist 184:S77–S90.

Auld, S. K. J. R., S. R. Hall, J. Housley Ochs, M. Sebastian, and M. a Duffy. 2014b. Predators and patterns of within-host growth can mediate both among-host competition and evolution of transmission potential of parasites. The American Naturalist 184:S77-90.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models using lme4. Journal of Statistical Software 67:1–48.

Bebout, B. M., and F. Garcia-Pichel. 1995. UV B-induced vertical migrations of cyanobacteria in a microbial mat. Applied and Environmental Microbiology 61:4215–4222.

Berney, M., H. U. Weilenmann, A. Simonetti, and T. Egli. 2006. Efficacy of solar disinfection of *Escherichia coli, Shigella flexneri, Salmonella Typhimurium* and *Vibrio cholerae*. Journal of Applied Microbiology 101:828–836.

Bertram, C. R., M. Pinkowski, S. R. Hall, M. A. Duffy, and C. E. Cáceres. 2013. Trait-mediated indirect effects, predators, and disease: test of a size-based model. Oecologia 173:1023–1032.

Cáceres, C. E., S. R. Hall, M. A. Duffy, A. J. Tessier, C. Helmle, and S. MacIntyre. 2006. Physical structure of lakes constrains epidemics in *Daphnia* populations. Ecology 87:1438–1444.

Civitello, D. J., R. M. Penczykowski, A. N. Smith, M. S. Shocket, M. A. Duffy, and S. R. Hall. 2015. Resources, key traits and the size of fungal epidemics in *Daphnia* populations. Journal of Animal Ecology 84:1010–1017.

Couture, S., D. Houle, and C. Gagnon. 2012. Increases of dissolved organic carbon in temperate and boreal lakes in Quebec, Canada. Environmental Science and Pollution Research 19:361–371.

Creed, I. F., Bergstrom, A., Trick, C. G., Grimm, N. B., Hessen, D. O., Karlsson, J., Kidd, K. A., Kritzberg, E., Mcknight, D. M., Freeman, E. C., Senar, O. E., Andersson, A., Ask, J., Berggren, M., Cherif, M., Giesler, R., Hotchkiss, E. R. Kortelainen, P., Palta, M. M. Vrede, T., and G. A. Weyhenmeyer. 2018. Global change-driven effects on dissolved organic

matter composition: implications for food webs of northern lakes. Global Change Biology 24: 3692-3714.

Dodson, S. I., S. E. Arnott, and K. L. Cottingham. 2000. The relationship in lake communities between primary productivity and species richness. Ecology 81:2662–2679.

Duffy, M. A. 2007. Selective predation, parasitism, and trophic cascades in a Bluegill-*Daphnia*-parasite system. Oecologia 153:453–460.

Duffy, M. A., S. R. Hall, A. J. Tessier, and M. Huebner. 2005. Selective predators and their parasitized prey: are epidemics in zooplankton under top-down control? Limnology and Oceanography 50:412–420.

Duneau, D., P. Luijckx, F. Ben-Ami, C. Laforsch, and D. Ebert. 2011. Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host-parasite interactions. BMC Biology 9:11.

Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. Climate extremes: observations, modeling, and impacts. Science 289:2068–2074.

Ebert, D., P. Rainey, T. M. Embley, D. Scholz, P. Rainey, T. M. Embley, and D. Scholz. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. Philosophical Transactions of the Royal Society of London B 351:1689–1701.

Fee, E. J., R. E. Hecky, S. E. M. Kasian, and D. R. Cruikshank. 1996. Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. Limnology and Oceanography 41:912–920.

Häder, D. P., C. E. Williamson, S. A. Wangberg, M. Rautio, K. C. Rose, K. Gao, E. W. Halbling, R. P. Sinha, and R. Worrest. 2011. Effects of UV radiation on aquatic ecosystems and interactions with climate. Photochemical & Photobiological Sciences 10:242–260.

Hernández, K. L., R. A. Quiñones, G. Daneri, and E. W. Helbling. 2006. Effects of solar radiation on bacterioplankton production in the upwelling system off central-southern Chile. Marine Ecology Progress Series 315:19–31.

Hall, S. R., Simonis, J. L. Nisbet, R. M., Tessier, A. J., Cáceres C. E. 2009. Resource ecology of virulence in a planktonic host-parasite system: an explanation using dynamic energy budgets. The American Naturalist 174: 149-162.

Jacobs, J. F., G. J. M. Koper, and W. N. J. Ursem. 2007. UV protective coatings: a botanical approach. Progress in Organic Coatings 58:166–171.

Kalff, J., and R. Knoechel. 1978. Phytoplankton and their dynamics in oligotrophic and eutrophic lakes. Annual Review of Ecology and Systematics 9:475–495.

Karentz, D., F. S. McEuen, M. C. Land, and W. C. Dunlap. 1991. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Marine Biology 108:157–166.

King, B. J., D. Hoefel, D. P. Daminato, S. Fanok, and P. T. Monis. 2008. Solar UV reduces *Cryptosporidium parvum* oocyst infectivity in environmental waters. Journal of Applied Microbiology 104:1311–1323.

Kirk, J. T. O. 1993. Light and photosynthesis in aquatic ecosystems. Second edition. Cambridge University Press, Cambridge.

Kutz, S. J., E. P. Hoberg, J. Nishi, and L. Polley. 2002. Development of the muskox lungworm, *Umingmakstrongylus pallikuukensis* (Protostrongylidae), in gastropods in the Arctic. Canadian Journal of Zoology 80:1977–1985.

Larsen, S., T. Andersen, and D. O. Hessen. 2011. Climate change predicted to cause severe increase of organic carbon in lakes. Global Change Biology 17:1186–1192.

Laurion, I., M. Ventura, J. Catalan, R. Psenner, R. Sommaruga, N. Sep, and I. Latirion. 2000. Attenuation of ultraviolet radiation in mountain Lakes: factors controlling the among- and within-lake variability. Limnology and Oceanography 45:1274–1288.

Leech, D. M., A. Padeletti, and C. E. Williamson. 2005. Zooplankton behavioral responses to solar UV radiation vary within and among lakes. Journal of Plankton Research 27:461–471.

Lei, F., and R. Poulin. 2011. Effects of salinity on multiplication and transmission of an intertidal trematode parasite. Marine Biology 158:995–1003.

Luijckx, P., H. Fienberg, D. Duneau, and D. Ebert. 2013. A matching-allele model explains host resistance to parasites. Current Biology 23:1085–1088.

Manning, W. J., and A. V. Tiedemann. 1995. Climate change: potential effects of increased atmospheric carbon dioxide (CO2), ozone (O3), and ultraviolet-B (UV-B) radiation on plant diseases. Environmental Pollution 88:219–245.

Metschnikoff, E. 1884. A disease of *Daphnia* caused by a yeast. A contribution to the theory of phagocytes as agents for attack on disease-causing organisms. Archiv fur pathologische Anatomie und Physiologie und fur klinishe Medicin 96:177–195.

Morris, D. P., H. Zagarese, C. E. Williamson, E. G. Balseiro, B. R. Hargreaves, B. Modenutti, R. Moeller, and C. Queimalinos. 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. Limnology and Oceanography 40:1381–1391.

Moyer, B. R., D. M. Drown, D. H. Clayton, B. R. Moyer, D. M. Drown, D. H. Clayton, and D. H. Low. 2002. Low humidity reduces ectoparasite pressure: implications for host life

history evolution. Oikos 97:223–228.

Nicholson, W. L., N. Munakata, G. Horneck, H. J. Melosh, and P. Setlow. 2000. Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiology and Molecular Biology Reviews 64:1092–2172.

Onofri, S., L. Selbmann, G. S. de Hoog, M. Grube, D. Barreca, S. Ruisi, and L. Zucconi. 2007. Evolution and adaptation of fungi at the boundaries of life. Advances in Space Research 40:1657–1664.

Overholt, E. P., S. R. Hall, C. E. Williamson, C. K. Meikle, M. A. Duffy, and C. E. Cáceres. 2012. Solar radiation decreases parasitism in *Daphnia*. Ecology Letters 15:47–54.

Penczykowski, R. M., S. R. Hall, D. J. Civitello, and M. A. Duffy. 2014. Habitat structure and ecological drivers of disease. Limnology and Oceanography 59:340–348.

Pietrock, M., and D. J. Marcogliese. 2003. Free-living endohelminth stages: at the mercy of environmental conditions. Trends in Parasitology 19:293–299.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. C. Team. 2018. nlme: linear and nonlinear mixed effects models.

Read, D. J. 1968. Some aspects of the relationship between shade and fungal pathogenicity in an epidemic disease of pines. New Phytologist 67:39–48.

Rhode, S. C., M. Pawlowski, and R. Tollrian. 2001. The impact of ultraviolet radiation on the vertical distribution of zooplankton of the genus *Daphnia*. Nature 412:69–72.

Roland, J., and W. J. Kaupp. 1995. Reduced transmission of forest tent caterpillar (Lepidoptera: Lasiocampidae) nuclear polyhedrosis virus at the forest edge. Environmental Entomology 24:1175-1178

Roy, S. 2000. Strategies for the minimization of UV-induced damage. Pages 177–201 *in* S. De Mora and M. Serge Demers, editors. The Effects of UV Radiation in the Marine Environment. Cambridge University Press.

Ruelas, D. S., D. Karentz, and J. T. Sullivan. 2007. Sublethal effects of ultraviolet B radiation on miracidia and sporocysts of *Schistosoma mansoni*: intramolluscan development, infectivity, and photoreactivation. The Journal of Parasitology 93:1303–1310.

Ruisi, S., D. Barreca, L. Selbmann, L. Zucconi, and S. Onofri. 2007. Fungi in Antarctica. Reviews in Environmental Science and Biotechnology 6:127–141.

Ruiz-González, C., R. Simó, R. Sommaruga, and J. M. Gasol. 2013. Away from darkness: a review on the effects of solar radiation on heterotrophic bacterioplankton activity. Frontiers in Microbiology 4:1–24.

Schindler, D. W., S. R. Carpenter, S. C. Chapra, R. E. Hecky, and D. M. Orihel. 2016. Reducing Phosphorus to Curb Lake Eutrophication is a Success. Environmental Science and Technology 50:8923–8929.

Setlow, P., and L. Li. 2015. Photochemistry and photobiology of the spore photoproduct: a fifty year journey. Photochemical & Photobiological Sciences 91:1263–1290.

Shocket, M. S., A. T. Strauss, J. L. Hite, M. Šljivar, D. J. Civitello, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2018. Temperature drives epidemics in a zooplankton-fungus disease system: a trait-driven approach points to transmission via host foraging. The American Naturalist 191:435–451.

Sinha, R. P., and D. P. Häder. 2002. UV-induced DNA damage and repair: a review. Photochemical & Photobiological Sciences 1:225–236.

Solomon, C. T., S. E. Jones, B. C. Weidel, I. Buffam, M. L. Fork, J. Karlsson, S. Larsen, J. T. Lennon, J. S. Read, S. Sadro, and J. E. Saros. 2015. Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: current knowledge and future challenges. Ecosystems 18:376–389.

Sommer, U. 1985. Seasonal succession of phytoplankton in Lake Constance. BioScience 35:351–357.

Stirnadel, H. A., and D. Ebert. 1997. Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. Journal of Animal Ecology 66:212–222.

Storz, U. C., and R. J. Paul. 1998. Phototaxis in water fleas (*Daphnia magna*) is differently influenced by visible and UV light. Journal of Comparative Physiology A 183:709–717.

Taranu, Z. E., I. Gregory-Eaves, P. R. Leavitt, L. Bunting, T. Buchaca, J. Catalan, I. Domaizon, P. Guilizzoni, A. Lami, S. Mcgowan, H. Moorhouse, G. Morabito, F. R. Pick, M. A. Stevenson, P. L. Thompson, and R. D. Vinebrooke. 2015. Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. Ecology Letters 18:375–384.

Villarreal, P., M. Carrasco, S. Barahona, J. Alcaíno, V. Cifuentes, and M. Baeza. 2016. Tolerance to ultraviolet radiation of psychrotolerant yeasts and analysis of their carotenoid, mycosporine, and ergosterol content. Current Microbiology 72:94–101.

Williamson, C.W.; Madronich, S.; Lal, A.; Zepp, R.G.; Lucas, R.M.; Overholt, E.P.; Rose, K.C.; Schladow, G.; Lee-Taylor, J. 2017. Climate change-induced increases in precipitation are reducing the potential for solar ultraviolet radiation to inactivate pathogens in surface waters. Scientific Reports:1–38.

Williamson, C. E., J. A. Brentrup, J. Zhang, W. H. Renwick, B. R. Hargreaves, L. B. Knoll, E. P. Overholt, and K. C. Rose. 2014a. Lakes as sensors in the landscape: optical metrics as scalable sentinel responses to climate change. Limnology and Oceanography 59:840–850.

Williamson, C. E., P. J. Neale, G. Gard, H. J. De Lange, and B. R. Hargreaves. 2001. Beneficial and deterimental effects of UV on aquatic organsisms: implications of spectral variation. Ecological Applications 11:1843–1857.

Williamson, C. E., E. P. Overholt, J. A. Brentrup, R. M. Pilla, T. H. Leach, S. G. Schladow, J. D. Warren, S. S. Urmy, S. Sadro, S. Chandra, and P. J. Neale. 2016. Sentinel responses to droughts, wildfires, and floods: effects of UV radiation on lakes and their ecosystem services. Frontiers in Ecology and the Environment 14:102–109.

Williamson, C. E., E. P. Overholt, R. M. Pilla, T. H. Leach, J. A. Brentrup, L. B. Knoll, E. M. Mette, and R. E. Moeller. 2015. Ecological consequences of long-term browning in lakes. Scientific Reports:1–10.

Williamson, C. E., R. G. Zepp, R. M. Lucas, S. Madronich, A. T. Austin, C. L. Ballare, M. Norval, B. Sulzberger, A. F. Bais, R. L. McKenzie, S. A. Robinson, D.-P. Hader, N. D. Paul, and J. F. Bornman. 2014b. Solar ultraviolet radiation in a changing climate. Nature Climate Change 4:434–441.

Xenopoulos, M. A., D. M. Lodge, J. Frentress, T. A. Kreps, S. D. Bridgham, E. Grossman, C. J. Jackson, M. A. Xenopoulos, D. M. Lodge, J. Frentress, T. A. Kreps, S. D. Bridgham, E. Grossman, and C. J. Jackson. 2003. Regional comparisons of watershed determinants of dissolved organic carbon in temperate lakes from the upper Great Lakes region and selected regions globally. Limnology and Oceanography 48:2321–2334.

Yaun, B. R., S. S. Sumner, J. D. Eifert, and J. E. Marcy. 2004. Inhibition of pathogens on fresh produce by ultraviolet energy. International Journal of Food Microbiology 90:1–8.

Zellmer, I. D. 1995. UV-B-tolerance of alpine and arctic *Daphnia*. Hydrobiologia 307:153–159.

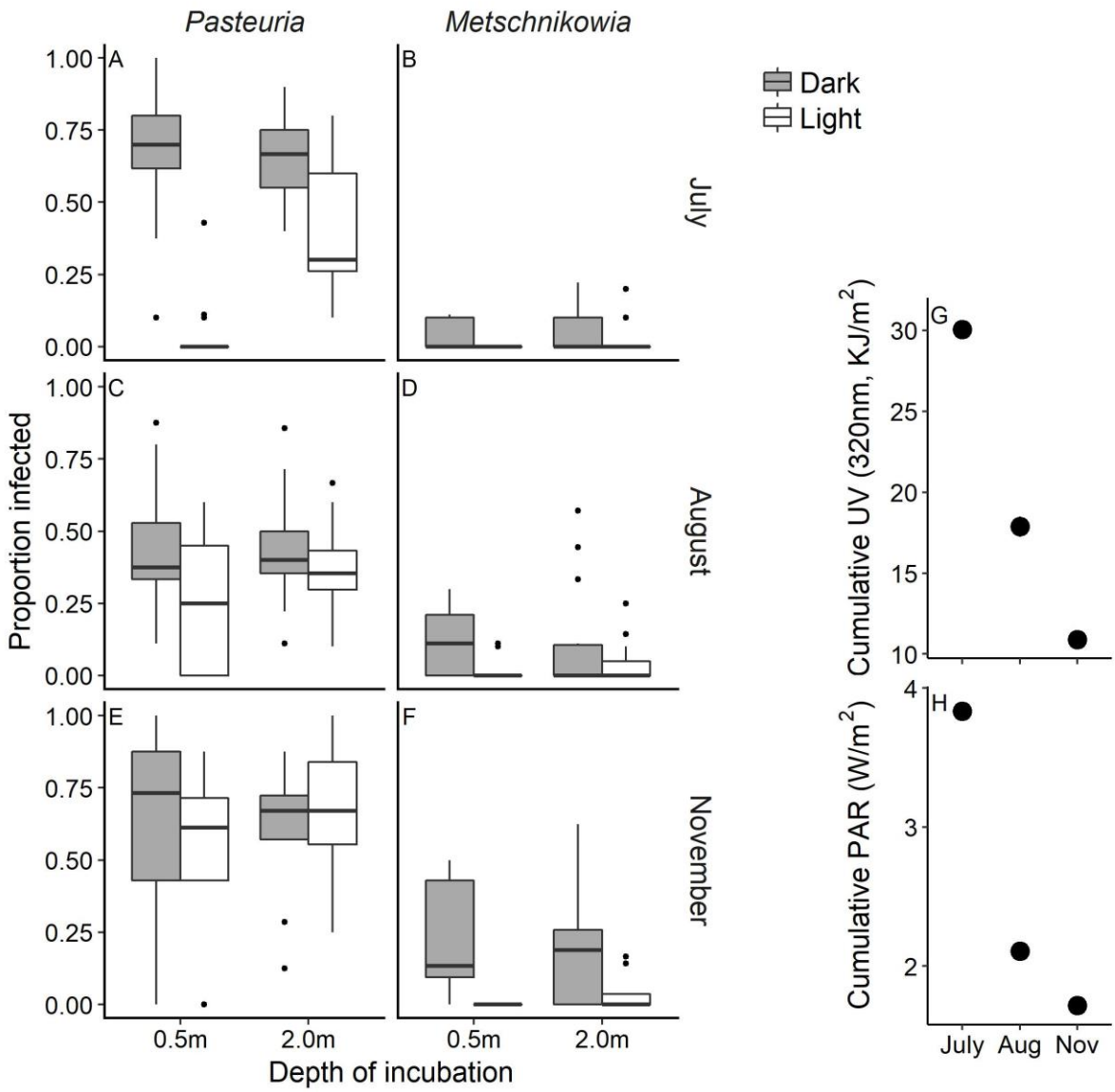

Figure 2.1: Light exposure decreased the proportion of *Daphnia* hosts infected by spores of both parasites. (A, C, & E) For the bacterium *Pasteuria*, light exposure decreased the proportion of hosts infected in (A) July and in (C) August, but not in (E) November. Additionally, for light-exposed vials (white bars), fewer animals became infected from spores incubated at lighter 0.5 than at darker 2 m depth in (A) July only. (B, D, & F) For the fungus *Metschnikowia*, light exposed spores infected fewer animals than spores in the dark treatment (grey bars) in all months (July, August, and November; no additional depth effects were found). Data from vials incubated in all lakes are pooled by light treatment in box plots. (G & H) Cumulative ambient 320 nm UV and PAR (x 10^7) in each incubation decreased as autumn progressed. Error bars (SD) correspond to cumulative differences in surface level UV and PAR along lakes.

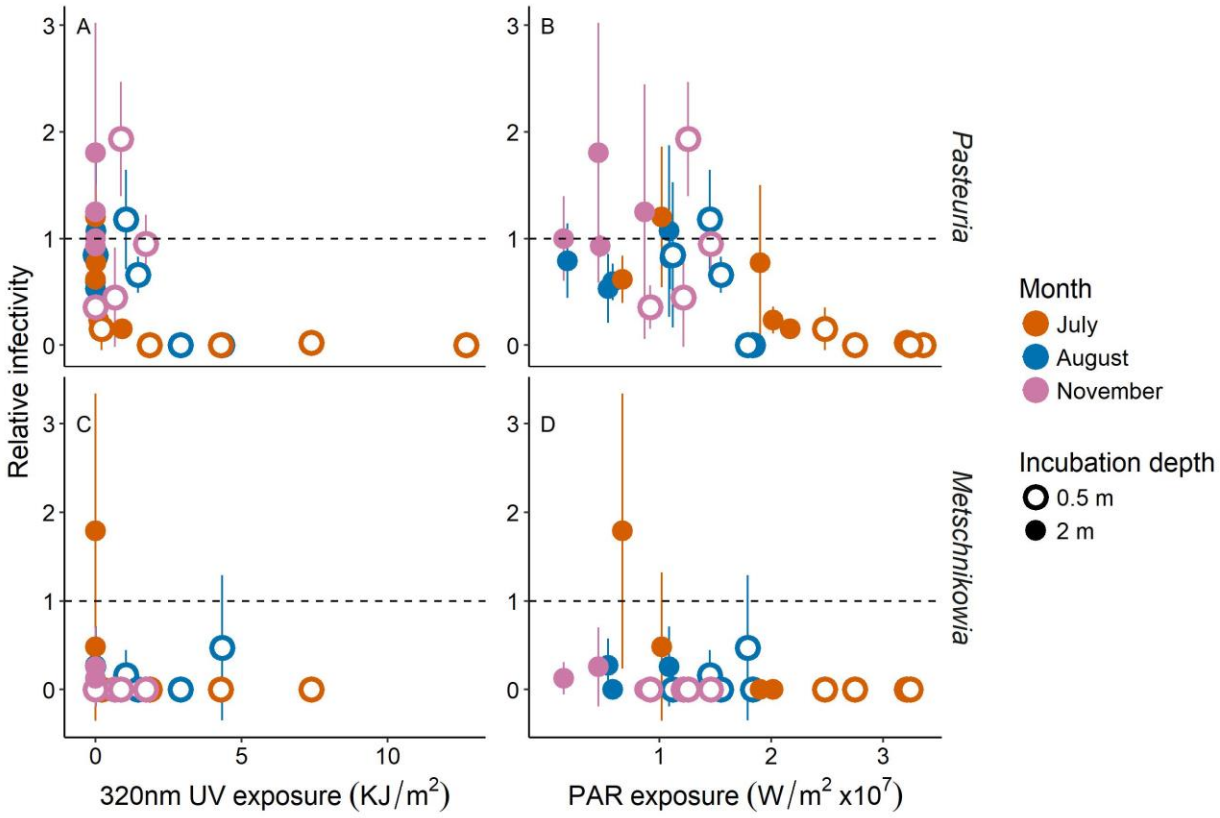

Figure 2.2: Relationship between relative infectivity, β, (± 1SD) and light exposure controlled by month (colors), depth (open [0.5 m] or filled [2.0 m] symbols), and lakes spread along a transparency gradient. (A, B) Greater light exposure (both UV and PAR) decreased relative infectivity of bacterial *Pasteuria* spores compared to dark treatments. (C, D) Variation in light exposure did not impact the relative infectivity of light-exposed fungal *Metschnikowia* spores.

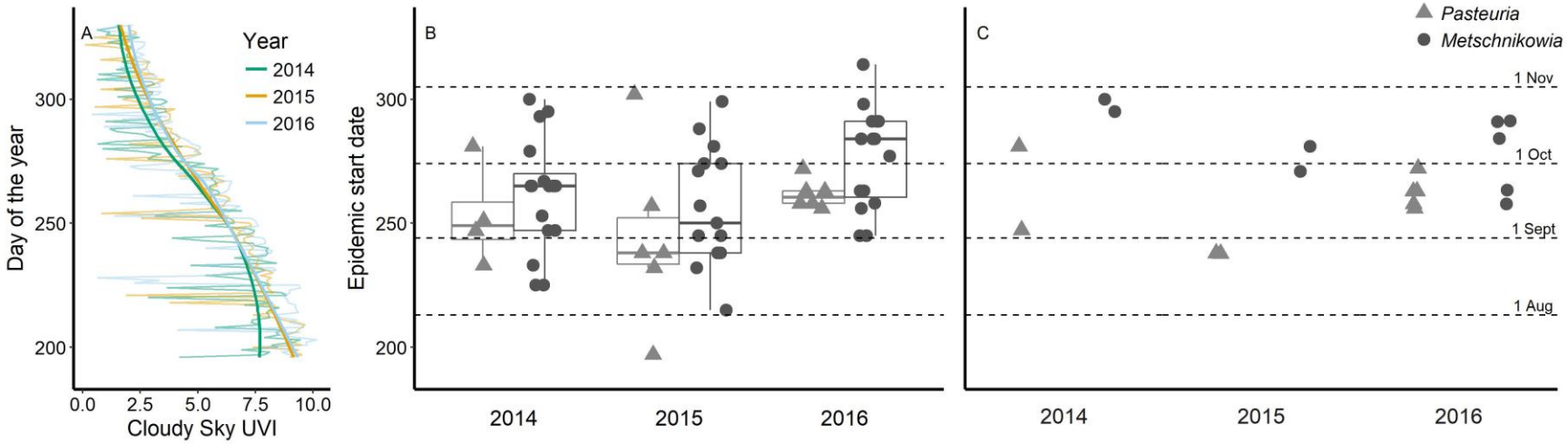

Figure 2.3: Epidemics of the less light-sensitive bacterium *Pasteuria* started earlier than those of the more sensitive fungus, *Metschnikowia.* (A) An index of incident UV intensity (daily high cloudy sky UV indexes, 'UVI') decreases autumnally near the study lakes (Indianapolis, IN). Loess trendlines accompany date from each year of the survey (2014, 2015, and 2016). Epidemics of *Pasteuria* started earlier in (B) lakes that showed epidemics of either parasite and in (C) lakes with epidemics of both in the same year. Dashed lines denote the first day of August (lowest), September, October, and November (highest day, as labeled).

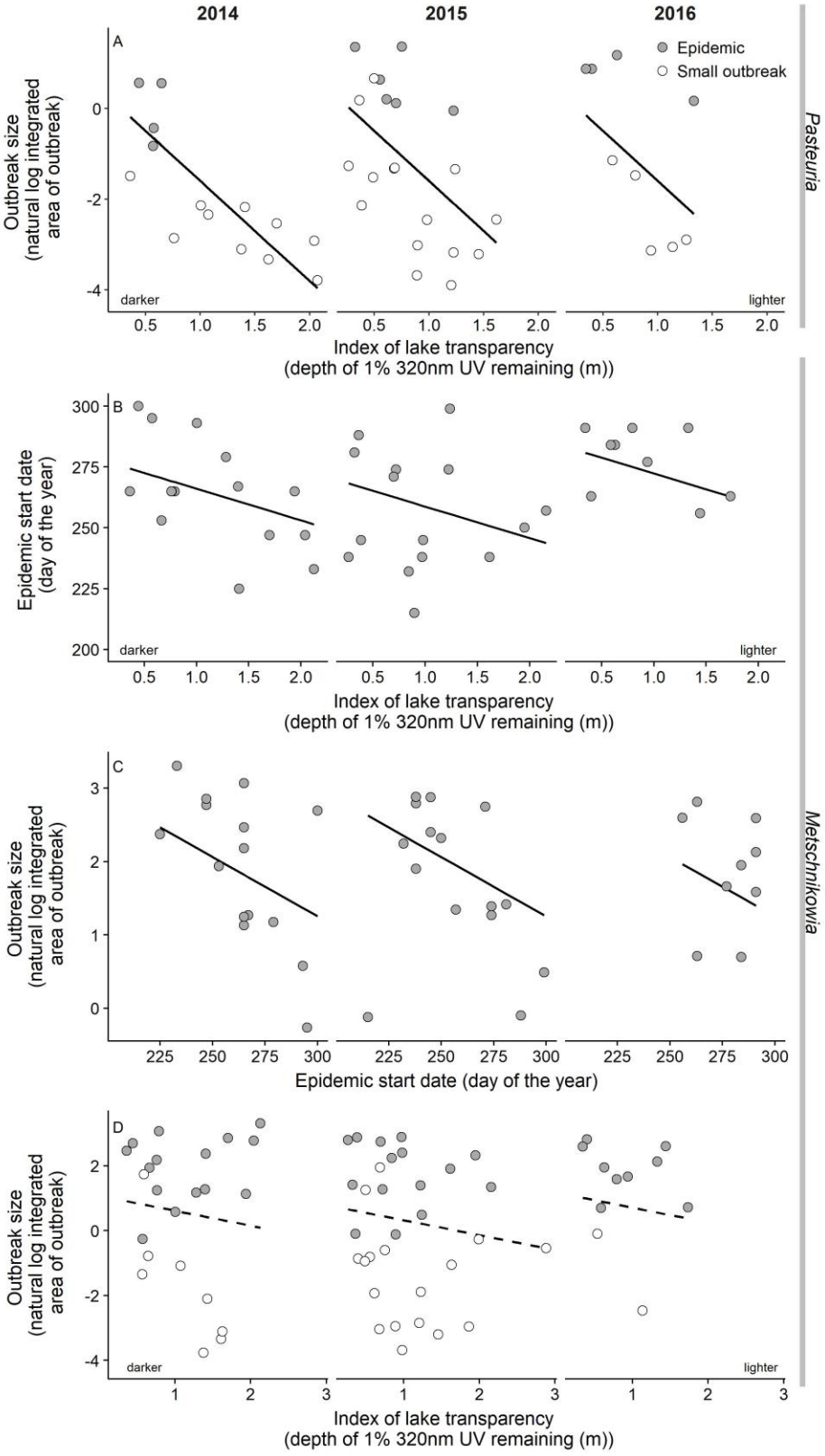

Figure 2.4. Outbreaks in lakes in 2014-2016 responded differently to the index of lake transparency (depth of 1% penetration of UV at 320 nm). (A) Outbreaks (grey and white circles) of the bacterium *Pasteuria* grew larger in darker lakes (lower index of lake transparency). (B) Contrary to predictions, epidemics (grey circles) of the fungus *Metschnikowia* started earlier in lighter lakes (i.e., those with deeper 320 nm UV penetration). (C) *Metschnikowia* epidemics became larger when they started earlier. (D) *Metschnikowia* outbreaks (including both epidemics [grey] and small [white] outbreaks) showed a non-significant (dashed) trend of becoming larger in darker lakes.

CHAPTER 3

How do Animals Balance Multiple Risks in Dangerous Habitats? Quantifying the Distributions of Daphniids, Their Predators, and Their Parasites in Stratified Lakes

with Mary A. Rogalski, Camden D. Gowler, Katherine K. Hunsberger, and Meghan A. Duffy

ABSTRACT

Landscapes vary in hazard and benefit, so animals face tradeoffs in habitat selection as they balance exposure to risks (e.g. extreme abiotic conditions, parasitism, and predation) and obtaining resources. The distribution of these detrimental and beneficial conditions may lead to avoidance of certain areas especially during times when risk levels are high. Here we studied the distribution of *Daphnia* in relation to the dangers (light, predators, and parasites) and benefits (especially warmer temperatures) in their environment. Previous work has focused on the roles of light, predation, access to resources, and temperature in driving migration behavior. As expected based on this earlier work, we found that *Daphnia* made daily migrations from daytime deep-water habitat protected from light and fish predation to surface waters at night (when these risks are lower) where resources and temperatures (and therefore embryo development time) are higher. The extent of this behavior was also associated with the presence of a second predator, *Chaoborus* (midge fly larvae) that has similar migration patterns. In contrast to earlier studies, we also quantified the distribution of another important class of natural enemy: parasites. We quantified the vertical distribution of two *Daphnia* parasites in three lakes during the day at two dates in August and October. Parasites were not evenly distributed across the water column,

which means that migration behavior likely affects disease exposure. We found that high host-parasite overlap corresponded to epidemic size, and therefore habitat selection behavior may be an important determinant of epidemic patterns.

INTRODUCTION

Animals balance risks from destructive biotic and/or abiotic conditions (e.g. exposure to predators, parasites, and extreme weather) with attaining fitness benefits such as food and mates. In so doing, they exhibit various behaviors (e.g. nocturnality, daily migrations, food selectivity, etc.) and select habitat to minimize risk and maximize benefit. Notably, animals face multiple risks simultaneously (Boeing et al. 2004, Morosinotto et al. 2010, Lone et al. 2014), adding complication to habitat use decisions. Multiple predators and parasites plague most populations, and populations may also face challenging abiotic conditions (such as extreme weather or ultraviolet light exposure); therefore, avoiding one risk may increase exposure to another (Marino and Werner 2013). How do animals balance multiple risks in dangerous habitats?

Biotic forces can strongly influence habitat choice. Predation risk can generate "landscapes of fear" where prey feed less in areas of high predator abundance (Madin et al. 2011). Analogous to this, but less well studied are "landscapes of disgust" where hosts avoid areas with aggregated or abundant parasites (Buck et al. 2018, Weinstein et al. 2018a, 2018b). For these phenomena to occur, risk must vary across space, and often variation in risk is associated with heterogeneity in habitat structure (Laundré and Hernández 2003). For example, many predators are more effective in edge or patchy environments in comparison to protected environments (e.g. dense forests, Laundré and Hernández 2003; aquatic vegetration, Gotceitas and Colgan 1987), and parasites may be associated with feces or with cadavers (Buck et al.

2018). In order to avoid these risks, focal species must sense enemies (e.g. via chemosensory cues; Ferrero et al. 2011) or have behavioral adaptations to avoid enemies when risks vary across a landscape (Laundré et al. 2010, Buck et al. 2018). Thus, adaptations to avoid commonly dangerous areas may be commonplace.

When considering how fear and disgust impact habitat choice in complex environments, it is important to consider differences in structure in different types of habitats (e.g., lakes vs. streams vs. forests). The physical and chemical properties of water, such as its density and viscosity, affect the distribution and movement of predators (Werner and Hall 1974) and parasites (Bidegain et al. 2016). In stratified lakes, a warmer surface layer (the epilimnion) sits atop water of decreasing temperature (the metalimnion and thermocline) which is above a layer of colder water (the hypolimnion). These layers differ in light, nutrient, and oxygen concentrations in addition to temperature with cold, deep waters deficient in light and oxygen but high in nutrients (Lampert and Sommer 2007). These properties provide important structure for aquatic organisms.

In lakes and oceans, many zooplankton undergo diel vertical migration, swimming to surface waters at night to access algal food and beneficial development temperatures (Bottrell 1975, Winder et al. 2004) when risk of visual fish predation and destructive UV radiation are low (Lampert 1989, Rhode et al. 2001, Williamson et al. 2011). Migrating zooplankton obtain an important demographic advantage both from access to nutritious resources and from increased temperatures (Rinke and Petzoldt 2003). Though *Daphnia* reduce risk of predation from fish by migrating, they put themselves at risk of predation by invertebrate predators (e.g. *Chaoborus*) that follow similar diel migration patterns (Dawidowicz et al. 1990). When co-occurring with

Chaoborus, *Daphnia* may be found higher in the water column even in the presence of light (Boeing et al. 2004).

Though movement and distribution of macro organisms in lakes have been extensively studied (Lampert 1989, Winder et al. 2004, Williamson et al. 2011), we know little about the distribution of parasites within the water column of lakes and, therefore, little about how vertical habitat choice by potential hosts influences infection risk. Infectious diseases in lakes can impact species competitive interactions (Wolinska et al. 2006), trophic cascades (Duffy 2007), and potentially, nutrient cycling (Frost et al. 2008), thus, understanding the effects of habitat choice on parasitism in plankton could contribute to our understanding of impacts of parasitism on these ecological processes (Cáceres et al. 2014). Here, we focus on *Daphnia*, a common and ecologically important member of lake food webs that have been the focus of prior studies on habitat use (Leibold and Tessier 1991, Winder et al. 2004). Though most of these studies have considered the impact of predators, resources, and abiotic factors on *Daphnia* migrations, Decaestecker et al. (2002) did consider the role of parasites, finding that *Daphnia* genotypes that migrated deeper (contacting sediments) were more likely to become infected by the bacterial parasite in this study. However, Decaestecker et al. (2002) did not quantify the distribution of parasites in the water column, and more recent studies have shown that parasite transmission stages can be patchily distributed in the water column (Thomas et al. 2011). *Daphnia* can be infected by a number of environmentally transmitted parasites, and here we focus on risks imposed by two particularly common ones: the bacterium, *Pasteuria ramosa*, and the fungus, *Metschnikowia bicuspidata*. The transmission stages (spores) of these parasites are free floating and therefore, their distributions depend on water currents, sinking, and where they enter the water column when released from hosts. Both parasites infect *Daphnia* as they feed by

penetrating the digestive tract (Metschnikoff 1884, Ebert et al. 1996), and both are obligate killers (Metschnikoff 1884, Ebert et al. 1996), so spores are released only from decaying corpses or when released by predators after feeding on infected *Daphnia* (Cáceres et al. 2009, Duffy 2009). We quantified spores of these parasites throughout the water column. We also quantified habitat selection behavior of *Daphnia* and a key invertebrate predator, *Chaoborus* (midge fly larvae), in relation to temperature and light, which are potential drivers of diel vertical migration. We synthesize this information to better understand how light, temperature, and predators influence habitat use by *Daphnia* and therefore, their exposure to parasites.

METHODS

Quantifying Parasite Distributions

We quantified parasite distributions through the water columns of three lakes with sizeable epidemics of *Pasteuria*, *Metschnikowia*, or of both parasites (Mill Lake, Walsh Lake, and North Lake). We used a Van Dorn sampler (Wildco) to collect water samples at 1 m depths throughout each lake profile at the deep basin (5 m for Walsh and Mill; 16 m for North) in August and October of 2017. Water samples were pre-filtered through 63 and 35 micron mesh and then 500 mL of pre-filtered sample were vacuum filtered onto 47 mm diameter hydrophilic polyethersulfone filters with a pore size of 0.45 µm (Millipore Sigma) and frozen for later DNA extraction and quantitative PCR analysis.

We extracted DNA on filters by following the protocol from the DNeasy power water kit (Qiagen, Hilden, Germany). To compare DNA quantities on filters to known spore quantities, we counted spores of *Pasteuria* and *Metschnikowia* from infected animals cultured in the lab, put them on a filter, and extracted DNA from this filter as well. We developed primers and probes

that would detect *Metschnikowia bicuspidata* and *Pasteuria ramosa* (Table 3.1). We developed these markers with reference to closely related organisms to ensure their specificity to the parasites in this study. In 30 µl reactions 5 µl of sample, standards, or controls were amplified in 1X TaqMan Fast Virus 1-step master mix (Applied Biosystems), 900 nM forward and reverse primers, 250 nM probe, 1X TaqMan exogenous internal positive control DNA (Applied Biosystems), and 1X TaqMan exogenous internal positive control mix (Applied Biosystems). When quantifying *Metschnikowia* DNA from Walsh and Mill Lakes, we used 45 µl reactions and 10 µl of sample, standards, and controls. For samples from North Lake, we did not perform reactions to detect *Metschnikowia* in October since no animals infected with *Metschnikowia* were detected in this lake over the course of the season, and water samples from August did not have detectable levels of *Metschnikowia* by qPCR (see results). qPCR amplification conditions were: 50°C (5 min), 95°C (20 sec), followed by 45 cycles of 95°C (15 sec)/60°C (1 min) in a Quantstudio 3 system (Thermofisher). Quantstudio design and analysis software (Thermofisher) was used to calculate standard curves and to calculate the amount of parasite DNA in samples.

Quantifying Habitat Selection

To quantify *Daphnia* habitat use, we sampled seven lakes in southeastern Michigan (including the three for which we quantified spores) for zooplankton at four time points in the summer through the fall (Table 3.2). Our field sampling procedure was as follows: we located the deepest part of a lake and used a Schindler-Patalas plankton trap (30 L; Wildco) to obtain samples of *Daphnia* from every meter of the water column. Live samples of *Daphnia* and *Ceriodaphnia* were identified to species and life stage and were diagnosed for parasite infection by eye using a dissecting microscope. *Chaoborus* were also counted in samples. To understand

how *Daphnia* use habitat during the day and at night, we sampled lakes during the day (9-1 pm) and then again in the evening at least 1 hour after sunset (Table 3.2). To quantify parasite infection prevalence in *Daphnia* throughout the water column, we also took three plankton tows from three locations at least 10 m apart at the deepest part of the lake with a 12 cm Wisconsin net. Animals in subsamples of the combined tows were identified, diagnosed, and counted as above until at least 200 animals of each present species were counted or until the entire sample was processed. This sampling of the entire water column was completed every other week from mid July until mid November including on the days when Schindler trap sampling was conducted.

During the daytime sampling, we also measured temperature and dissolved oxygen levels at every meter of depth with a submersible hydrolab surveyor 4a (OTT HyrdoMet), and we used a submersible light meter (LI-250A; LI-COR, Inc) to measure the attenuation of photosynthetically active radiation (PAR) in surface waters. An epilimnetic water sample (0-2 m) was also collected with an integrated tube sampler. Upon return to lab, this water sample was filtered with ashed GF/F filters and then refrigerated until light absorbance of the filtered water could be measured. The absorbance of 320 nm UV light was quantified from the filtered water sample (Shimadzu UV⁄Visible UV-1650 PC spectrophotometer), and we used absorbance values to estimate the depth of 1% 320 nm UV remaining in the water column using the Beer-Lampert law (depth of 1% 320 nm UV = ln(0.01)/ad320).

We used the following statistical methods to understand which factors impacted *Daphnia* habitat use. Since variance in the data was overdispersed, we analyzed counts of animals at each depth with a negative binomial regression (Zuur et al. 2009) with the MASS package in R (Venables and Ripley 2002). To understand the influence of variables on *Daphnia* depth

selection, fixed effects in the model were the following set of interaction terms (each including depth): lake by depth, species by depth, sex/life stage by depth, day/night factor by depth, and month by depth as well as an offset of the natural log of the total count of a species-stage across depths in a lake. With similar models, we then analyzed data subsetted by lake (without a lake by depth term) to better understand migration in individual lakes. To understand how species and sex/life stages partitioned the water column during the day and at night, we used similar models (without a day/night by depth term) to analyze data only from day and then only from night sampling.

To understand how average depth of animals in the daytime were associated with lake clarity, we modeled average depth of *Daphnia* (sum of depths weighted by the percentage of hosts found at each depth) as a function lake clarity proxies (the depth of 1% 320 nm UV remaining in the water column and the depth 1% of PAR remaining in the water column) with linear models using gaussian error structure. We then used a linear mixed effect model to analyze the average temperature *Daphnia* experienced (sum of temperatures in the water column weighted by the percentage of a given species and sex/life stage found at that temperature) as a function of species and sex/life stage and the interaction between day/night factor and month with lake as a random effect. We then analyzed data for each month separately, modeling average temperature experienced as a function of species, sex/life stage, and day/night factor. We used the package rLakeAnalyzer (Winslow, 2018) to calculate the depth of the thermocline (defined as the deepest density gradient) from temperature profiles.

To understand relationships between *Daphnia* and *Chaoborus* distributions, we analyzed counts of *Daphnia* and *Chaoborus* at depths with a negative binomial generalized linear model with the following fixed effect interactions: type (*Daphnia* vs. *Chaoborus*) by depth, day/night

factor by depth, lake by depth, and month by depth as well as an offset of the natural log of the total count of *Daphnia* or *Chaoborus* across depths in a lake. We also compared distributions of *Daphnia* and *Chaoborus* with Kolmogorov-Smirnov tests when there were at least 10 *Chaoborus* counted in a given lake. Finally, we modeled the abundance of *Chaoborus* in lakes as a function of the depth of 1% 320 nm UV remaining in the water column using poisson error structure. Generalized linear models and linear mixed effect models were performed with the lme4 package in R (Bates et al. 2015). Negative binomial generalized linear models were performed with the MASS package in R (Venables and Ripley 2002).

RESULTS

Parasite spores were unevenly distributed across the water column and over time in the three lakes and two months for which we quantified spores. In all of these lakes, there were more *Pasteuria* spores in August than in October, corresponding with the waning of epidemics later in the fall (Figure 3.1, Figure 3.2). In August, our shallow lakes, Mill and Walsh, had peaks in *Pasteuria* spore abundance near the bottom of the lakes. Therefore, due to habitat use, *Daphnia* in Mill Lake were at greatest risk of consuming *Pasteuria* spores during the day: 84% of *D. parvula* adults and 82% of *D. parvula* juveniles overlapped with the peak *Pasteuria* spores in August, as did 59% of adult and 41% of juvenile *D. dentifera* (Figure 3.1). A smaller percentage of *D. retrocurva* overlapped with peak spore concentrations in Mill during the day (38% of adults and 25% of juveniles) since *D. retrocurva* tended to be slightly higher in the water column (Figure 3.1). In Walsh Lake, the peak *Pasteuria* spore densities coincided with lower *D. dentifera* host densities (13% of adults and 5.4% of juveniles in the day and 18% of adults and 10% of juveniles at night) since *Daphnia* in this lake were generally found higher in the water

column even during the day (Figure 3.1, Figure 3.2). Interestingly, in comparison to Mill Lake, fewer *Daphnia* in Walsh Lake became infected with *Pasteuria* (Figure 3.2); it is possible this was partially the result of lower overlap between hosts and spores. In North Lake in August, there were peaks in spore densities at two depths (4 m and 9 m); even taking this double peak into account, risk for *Daphnia* was considerably lower than in other lakes (less than 10% of each host type were exposed to *Pasteuria* spore maxima during the day), since *Daphnia* hosts were spread out more evenly through the water column in this lake. At night, the two spore peaks coincided with only 16% of *D. retrocurva* adults, 11% of *D. retrocurva* juveniles, 22% of *D. dentifera* adults, and 13% of *D. dentifera* juveniles. In October, even fewer *Daphnia* were exposed to the two spore peaks (at 3 m and 15 m) with fewer than 10% of each host type exposed to the maxima during the day and at most 11% of each host type exposed at night. *Daphnia* overlapped with *Pasteuria* spores more in Mill than in Walsh or North. This greater overlap between spores and *Daphnia* corresponds with higher infection prevalence (Figure 3.2).

Concentrations of *Metschnikowia* were low in both Walsh and Mill Lakes and *Metschnikowia* was undetectable in North lake in August. Low spore concentrations correspond with low infection prevalence at this time of year and in these lakes when infection prevalence was no more than 2% (Figure 3.2). In October, *Metschnikowia* concentrations remained low in Mill Lake, corresponding with low *Metschnikowia* infection prevalence in this lake (Figure 3.2). However, in Walsh Lake, *Metschnikowia* spores were more abundant with peak *Metschnikowia* concentrations at 2 m depth. Here, overlap with *Daphnia* was less than 10% for any group during the day and still relatively low at night (16% of adults, 13% of juveniles, and 15% of males). Higher *Daphnia* densities were found above and below this peak of *Metschnikowia* spore

concentration, though we did not have a water sample from the 1 m depth for this lake and date, so we cannot say if spore concentrations were high or low at this depth.

This study of three lakes indicated that *Daphnia* habitat selection behavior impacts their exposure to parasite spores. Therefore, we were interested in understanding how factors that are known to affect habitat selection behavior in *Daphnia* varied in these lakes and over time. We analyzed *Daphnia* habitat selection in seven lakes and across 4 months in relation to lake clarity, temperature, and *Chaoborus*. *Daphnia* in our study lakes migrated from lower in the water column during the day to higher in the water column at night, though the degree of migration varied by species, lake, and month (Figure 3.3). Counts of *Daphnia* at depths depended on lake, day/night factor, month, species, and sex/life stage (all interactive fixed effects were highly significant: depth * lake: LRT=184.6, P<0.001; day/night factor * depth: LRT=71.5, P<0.001; month * depth: LRT=53.9, P<0.001; species * depth: LRT=74.4, P<0.001; and sex/life stage * depth: LRT=26.1, P<0.001). In all but one lake (Bishop), *Daphnia* were found significantly higher in the water column at night than during the day. For Bishop lake, there was not a significant effect of day/night by depth factor, suggesting that *Daphnia* in this lake did not migrate detectably between the times that we sampled.

Daphnia species and stages selected significantly different average depths during the day (species * depth: LRT=49.53, P<0.001, stage * depth: LRT=23.10, P<0.001; Figure 3.3). Compared to *Ceriodaphnia*, *D. dubia* and *D. retrocurva* resided at the same depth (*D. dubia**depth: z=0.80, P=0.426; *D. retrocurva**depth: z=0.87, P=0.386) whereas *D. dentifera*, *D. parvula*, and *D. pulicaria* resided significantly deeper (*D. dentifera* * depth: z=2.82, P=0.005; *D. parvula* * depth: z=2.98, P=0.003; *D. pulicaria* * depth: z=3.78, P<0.001). Juveniles resided significantly higher in the water column than adult females and males (juveniles * depth: z=-5.4,

P<0.001). At night, species and stages were still found in significantly different parts of the water column (species * depth: LRT=61.0, P<0.001, stage * depth: LRT=12.9, P=0.005) with *D. retrocurva, D. dubia,* and *D. dentifera* found significantly higher than *Ceriodaphnia (D. retrocurva* * depth: z=-5.78, P<0.001*; D. dubia* * depth: z=-3.72, P<0.001; *D. dentifera* * depth: z=-3.36, P<0.001). *D. parvula* was still found significantly lower than *Ceriodaphnia (D. parvula* * depth: z=2.74, P=0.006), and *D. pulicaria* was not found at significantly different depths than *Ceriodaphnia (D. pulicaria* * depth: z=1.38, P=0.168). Juveniles were still found significantly higher in the water column (juveniles * depth: z=-3.48, P<0.001).

We expected lake clarity and temperature to influence *Daphnia* habitat choice since *Daphnia* could be protected from visual fish predation and damaging radiation in darker and/or deeper waters. On average, *Daphnia* (all species together) resided deeper during the day in clearer lakes (with respect to UV attenuation: $F_{1,26}$=56.97, P<0.001; and PAR attenuation: $F_{1,26}$=10.94, P=0.003, Figure 3.4). Two of our three clearest lakes (North and Bishop) were substantially deeper than our other study lakes, but the effects of lake clarity remained significant even when data from these lakes were excluded from models. The average temperatures of *Daphnia* distributions were also significantly different during the day and at night (LRT=12.87, P<0.001; Figure 3.5). Looking at each month separately, *Daphnia* were found in warmer waters during the night in July (LRT=15.25, P<0.001), August (LRT=5.28, P=0.022), and September (LRT=32.31, P<0.001), but not in October (LRT=0.001, P=0.97). In October, many lakes were no longer stratified, and thus temperatures were uniform throughout depths of most lakes. In each month, species and stages were found at significantly different temperatures reflecting significant differences in depth preference of different host species and stages (Figure 3.3, Figure 3.5)

Since *Chaoborus* are consumed by fish, we expected their distributions to be impacted by light levels (which are associated with fish predation risk; Wissel et al. 2003a). Light levels can be modulated by lake clarity and time of day. *Chaoborus* were most abundant in our darker lakes and at night (lake clarity: LRT=2804.3, P<0.001; day/night factor: LRT 4487.9, P<0.001; Figure 3.6). *Chaoborus* were also found significantly deeper in lakes than *Daphnia* (type * depth: LRT=57.10, P<0.001). Usually, distributions of *Chaoborus* and *Daphnia* were significantly different (Figure 3.7). When both day and night sets of distributions could be compared, distributions of *Daphnia* and *Chaoborus* were more similar at night. For example, in our darkest lake, Walsh, both day and night distributions of *Daphnia* and *Chaoborus* were significantly different. However, the distributions of *Daphnia* and *Chaoborus* were more similar at night (K-S tests: August: D=0.31, P<0.001; October: D=0.36, P<0.001) than distributions of *Daphnia* and *Chaoborus* during the day (K-S tests: August: D=0.61, P<0.001; October: D=0.78, P<0.001; Figure 3.5). In this case, *Daphnia* selected shallower habitat than *Chaoborus*, but they overlap more with *Chaoborus* at night when *Chaoborus* are likely released from fish predation risk.

DISCUSSION

Daphnia complete daily migrations from daytime habitat in colder, darker depths to shallower, warmer waters at night to minimize predation risk yet access resources and beneficial temperatures (Lampert 1989, Winder et al. 2004). However, *Daphnia* face multiple enemies in their natural habitat, and avoidance of one hazard may increase the impacts of another (Decaestecker et al. 2002, Boeing et al. 2004). We quantified distributions of two *Daphnia* parasites, finding them unevenly distributed through the water columns of three lakes. Infection risk therefore depends on habitat selection behavior of hosts. In our study, *Daphnia* tended to

live deeper in water columns during the day and also deeper in clearer lakes, which is likely a behavior that minimizes predation from fish and damage from light (Lampert 1989, Williamson et al. 2011, Rose et al. 2012). In addition, *Daphnia* were in general found higher in the water column than *Chaoborus.* The behavior of *Daphnia* in response to the challenges they face in their environment could thus impact their infection risk, and these behaviors could be manifested in epidemic patterns.

The patchy distribution of parasite spores throughout depths in lakes implicate important processes that affect disease risk in lakes. Transmission stages of parasites originate from dead, infected *Daphnia* (Metschnikoff 1884, Ebert et al. 1996). In terrestrial systems, parasites might remain near the host cadaver (Ganz et al. 2014, Turner et al. 2014), but in lakes, turbulence and mixing break apart corpses and distribute spores, expanding risk, but not homogenizing it (Bidegain et al. 2016). Spore distributions are likely dynamic as sinking, mixing, and transport on gravity currents could occur at variable rates depending on turbulence due to weather conditions (Pedrós-Alió et al. 1989, Wetzel 2001, Cáceres et al. 2006) interacting with lake depth and basin shape (Cáceres et al. 2006, Hall et al. 2010). Though the parasites studied here have similar transmission mechanisms, they differ in size and shape (*Pasteuria* cells are about 4 µm in diameter and *Metschnikowia* spores are needle-like and frequently 50 µm long), which likely are important to movement and sinking in lakes. The low density and small size of many microbes allow them to mostly remain suspended in natural lakes (Jassby 1975, Pedrós-Alió et al. 1989). We don't know the sinking speeds of the parasites in this study, but we assume that the larger *Metschnikowia* spores would sink faster. However, if parasite distribution is primarily determined by forces acting on the corpses of infected *Daphnia*, then sinking should be much more important, but no different for the two parasites.

In our study, peak concentrations of the bacterial parasite *Pasteuria* in Walsh and Mill lakes were near the bottom of these lakes, which may be the result of infected *Daphnia* corpses sinking before decomposition released spores into the water. In North Lake, there were multiple areas of high *Pasteuria* concentration in the water column. This was surprising given that we expected the epilimnion to be well mixed (as indicated by constant temperature and dissolved oxygen levels throughout in both months). Yet, in both months, spore concentrations peaked in the epilimnion (at 4 m in August and at 3 m in October) as well as lower in the metalimnion in August and near the bottom of the lake in both months. One explanation for the spike of parasite spores in the epilimnion is attachment of spores to phytoplankton; *E. coli* has been found to stick to algae (Ansa et al. 2011), and diverse bacterial assemblages have been found associated with *Microcystis* (Shi et al. 2012). Another hypothesis is that spores could be transported along subsurface gravity currents that move cooler near-shore water to offshore areas (Wetzel 2001, Hall et al. 2010). The deeper spike in spore concentration is more likely to be the result of sinking *Daphnia* corpses caught at a density barrier caused by a difference in water temperature (Wetzel 2001). Future research that quantifies the validity and relative importance of these potential mechanisms will be important for a more complete understanding of how parasite spores get distributed in lakes.

Overall, *Metschnikowia* spore concentrations were low in these three lakes except in Walsh Lake in October where there was a peak in spore concentration at 2 m depth. It's plausible that *Metschnikowia* spores were released into surface waters at night when both infected *Daphnia* and *Chaoborus* were abundant and used surface habitat. *Chaoborus* are known to release spores from infected animals as they feed (Cáceres et al. 2009). If spores sink during the day, perhaps *Daphnia* escape the highest spore concentrations through their migration behavior.

Interestingly, the peak *Metschnikowia* spore densities corresponded with a low point in *Daphnia* densities even at night. It might be possible for *Daphnia* to sense and avoid *Metschnikowia* spores, which physically pierce the gut epithelium (Stewart Merrill and Cáceres 2018), and therefore may be uncomfortable for *Daphnia* to eat. Indeed, *Daphnia* do alter migration patterns in response to the quality and edibility of algae (Reichwaldt 2007). In contrast, the high overlap between hosts and *Pasteuria* spores in Mill Lake may indicate that *Daphnia* cannot sense areas of high *Pasteuria* abundance or that risk from fish is more important than risk of parasitism.

We showed that *Daphnia* in our study lakes selected different habitats in the day and night, likely balancing their exposure to fish, *Chaoborus*, and damaging light with accessing algal food and favorable temperatures. *Daphnia* were found deeper during the day, especially in clearer lakes, suggesting that deeper habitats are protective (though not protective from parasites). Importantly, the parasites in this study are sensitive to light exposure (Overholt et al. 2012, Shaw et al. *In Review*), so clearer lakes are likely to have fewer viable parasite spores in their epilimnia than darker lakes. Thus, migration to deeper habitats may allow for irradiation of spores in surface waters during the day. Differences in average depth between *Daphnia* life stages and species may reflect differential tradeoffs faced by these animals and may also impact risk of parasitism for different groups. Small (juveniles and *Ceriodaphnia*) and helmeted (*D. dubia* and *D. retrocurva*) animals were generally found higher in the water column. The benefits of warmer waters and access to resources may outweigh visual predation risk for these animals since smaller animals are less often selected by fish (Brooks and Dodson 1965, Hansson and Hylander 2009) and helmeted animals are more defended (Brooks 1964, Swaffar and O'Brien 1996).

The other important predator in this system, *Chaoborus,* is known to migrate both in response to fish (Dawidowicz et al. 1990) and light (Persaud et al. 2003). We found that *Chaoborus* are more abundant in darker lakes, and they are also found deeper in the water column than *Daphnia*, likely because the larger size of *Chaoborus* in comparison to *Daphnia* puts them at greater risk from fish (Wissel et al. 2003b). Thus, *Daphnia* balance exposure to two predators, and live in between in relatively predator-free space. Interestingly, in Walsh lake, where *Chaoborus* were numerous, *Chaoborus*-driven habitat selection may have led to the bulk of hosts residing above the region of highest *Pasteuria* spore density.

An extensive body of work shows that resources, predation, and abiotic factors are important drivers of habitat selection in animals (Lampert 1989, Wissel et al. 2003b, Winder et al. 2004, Laundré et al. 2010, Rose et al. 2012), but recent work has brought attention to the importance of parasites for host habitat selection (Buck et al. 2018, Weinstein et al. 2018a, 2018b). Here we explored the vertical distribution of parasite spores in lake water columns as well as habitat use by their host species. We then associated host distributions to factors that likely influenced habitat use: predators, temperature, and lake clarity. At present, we cannot determine if parasites influence host habitat selection in this system. However, we find that *Daphnia* balance risk exposure with accessing beneficial conditions when selecting habitat in a complex and dangerous environment, and this could influence their exposure to parasite spores. Our study is an important step linking parasite distributions with additional hazards *Daphnia* face in their environments, and it opens doors for future investigations. Future work should address drivers of parasite distributions and explore how heterogeneity in lake structures and biological communities influence them. With these discoveries, we can gain a more

comprehensive understanding of how behavior, habitat structure, and biological community structure jointly influence parasite epidemics.

REFERENCES:

Ansa, E. D. O., H. J. Lubberding, J. A. Ampofo, and H. J. Gijzen. 2011. The role of algae in the removal of *Escherichia coli* in a tropical eutrophic lake. Ecological Engineering 37:317–324.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Bidegain, G., E. N. Powell, J. M. Klinck, T. Ben-Horin, and E. E. Hofmann. 2016. Marine infectious disease dynamics and outbreak thresholds: contact transmission, pandemic infection, and the potential role of filter feeders. Ecosphere 7:e01286.

Boeing, W. J., D. M. Leech, C. E. Williamson, S. Cooke, and L. Torres. 2004. Damaging UV radiation and invertebrate predation: conflicting selective pressures for zooplankton vertical distribution in the water column of low DOC lakes. Oecologia 138:603–612.

Bottrell, H. H. 1975. Generation time, length of life, instar duration and frequency of moulting, and their relationship to temperature in eight species of Cladocera from the river Thames, Reading. Oecologia 19:129–140.

Brooks, J. L. 1964. Predation and relative helmet size in cyclomorphic *Daphnia*. Proceedings of the National Academy of Sciences 53:119–126.

Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150:28–35.

Buck, J. C., S. B. Weinstein, and H. S. Young. 2018. Ecological and evolutionary consequences of parasite avoidance. Trends in Ecology & Evolution 33:619–632.

Cáceres, C. E., S. R. Hall, M. A. Duffy, A. J. Tessier, C. Helmle, and S. MacIntyre. 2006. Physical structure of lakes constrains epidemics in *Daphnia* populations. Ecology 87:1438–1444.

Cáceres, C. E., C. J. Knight, and S. R. Hall. 2009. Predator-spreaders: predation can enhance parasite success in a planktonic host-parasite system. Ecology 90:2850–2858.

Cáceres, C. E., A. J. Tessier, M. A. Duffy, and S. R. Hall. 2014. Disease in freshwater zooplankton: what have we learned and where are we going. Journal of Plankton Research 36:326–333.

Dawidowicz, P., J. Pijanowska, and K. Ciechomski. 1990. Vertical migration of *Chaoborus* larvae is induced by the presence of fish. Limnology and Oceanography 35:1631–1637.

Decaestecker, E., L. De Meester, and D. Ebert. 2002. In deep trouble: habitat selection constrained by multiple enemies in zooplankton. Proceedings of the National Academy of Sciences of the United States of America 99:5481–5485.

Duffy, M. A. 2007. Selective predation, parasitism, and trophic cascades in a Bluegill-*Daphnia*-parasite system. Oecologia 153:453–460.

Duffy, M. A. 2009. Staying alive: the post-consumption fate of parasite spores and its implications for disease dynamics. Limnology and Oceanography 54:770–773.

Ebert, D., P. Rainey, T. M. Embley, D. Scholz, P. Rainey, T. M. Embley, and D. Scholz. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. Philosophical Transactions of the Royal Society of London B 351:1689–1701.

Ferrero, D. M., J. K. Lemon, D. Fluegge, S. L. Pashkovski, W. J. Korzan, S. R. Datta, M. Spehr, M. Fendt, and S. D. Liberles. 2011. Detection and avoidance of a carnivore odor by prey. Proceedings of the National Academy of Sciences 108:11235–11240.

Frost, P. C., D. Ebert, and V. H. Smith. 2008. Bacterial infection changes the elemental composition of *Daphnia magna*. Journal of Animal Ecology 77:1265–1272.

Ganz, H. H., W. C. Turner, E. L. Brodie, M. Kusters, Y. Shi, H. Sibanda, T. Torok, and W. M. Getz. 2014. Interactions between *Bacillus anthracis* and plants may promote Anthrax transmission. PLoS Neglected Tropical Diseases 8.

Gotceitas, V., and P. Colgan. 1987. Selection between densities of artificial vegetation by young bluegills avoiding predation. Transactions of the American Fisheries Society 116:40–49.

Hall, S. R., R. Smyth, C. R. Becker, M. A. Duffy, C. J. Knight, S. MacIntyre, A. J. Tessier, and C. E. Cáceres. 2010. Why Are *Daphnia* in some lakes sicker? Disease ecology, habitat structure, and the plankton. BioScience 60:363–375.

Hansson, L. A., and S. Hylander. 2009. Effects of ultraviolet radiation on pigmentation, photoenzymatic repair, behavior, and community ecology of zooplankton. Photochemical & photobiological sciences 8:1266–1275.

Jassby, D. 1975. The ecological significance of sinking to planktonic bacteria. Canadian Journal of Microbiology 21:270–274.

Lampert, W. 1989. The adaptive significance of diel vertical migration of zooplankton. British Ecological Society 3:21–27.

Lampert, W., and U. Sommer. 2007. Limnoecology. Second. Oxford University Press, Oxford.

Laundré, J. W., and L. Hernández. 2003. Winter hunting habitat of pumas *Puma concolor* in northwestern Utah and southern Idaho, USA. Wildlife Biology 9:123–129.

Laundré, J. W., L. Hernández, and W. J. Ripple. 2010. The landscape of fear: ecological implications of being afraid. The Open Ecology Journal 3:1–7.

Leibold, M., and A. J. Tessier. 1991. Contrasting patterns of body size for *Daphnia* species that segregate by habitat. Oecologia 86:342–348.

Lone, K., L. E. Loe, T. Gobakken, J. D. C. Linnell, J. Odden, J. Remmen, and A. Mysterud. 2014. Living and dying in a multi-predator landscape of fear: roe deer are squeezed by contrasting pattern of predation risk imposed by lynx and humans. Oikos 123:641–651.

Madin, E. M. P., J. S. Madin, and D. J. Booth. 2011. Landscape of fear visible from space. Scientific Reports:1–14.

Marino, J. A., and E. E. Werner. 2013. Synergistic effects of predators and trematode parasites on larval green frog (*Rana clamitans*) survival. Ecology 94:2697–2708.

Metschnikoff, E. 1884. A disease of *Daphnia* caused by a yeast. A contribution to the theory of phagocytes as agents for attack on disease-causing organisms. Archiv fur pathologische Anatomie und Physiologie und fur klinishe Medicin 96:177–195.

Morosinotto, C., R. L. Thomson, and E. Korpima. 2010. Habitat selection as an antipredator behaviour in a multi-predator landscape: all enemies are not equal. Journal of Animal Ecology 79:327–333.

Overholt, E. P., S. R. Hall, C. E. Williamson, C. K. Meikle, M. A. Duffy, and C. E. Cáceres. 2012. Solar radiation decreases parasitism in *Daphnia*. Ecology Letters 15:47–54.

Pedrós-Alió, C., J. Mas, J. M. Gasol, and R. Guerrero. 1989. Sinking speeds of free-living phototrophic bacteria determined with covered and uncovered traps. Journal of Plankton Research 11:887–905.

Persaud, A. D., M. T. Arts, and N. D. Yan. 2003. Photoresponses of late instar *Chaoborus punctipennis* larvae to UVR. Aquatic Ecology 37:257–265.

Reichwaldt, E. S. 2007. Influence of food quality on depth selection of *Daphnia pulicaria*. Journal of Plankton Research 29:839–849.

Rhode, S. C., M. Pawlowski, and R. Tollrian. 2001. The impact of ultraviolet radiation on the vertical distribution of zooplankton of the genus *Daphnia*. Nature 412:69–72.

Rinke, K., and T. Petzoldt. 2003. Modelling the effects of temperature and food on individual growth and reproduction of *Daphnia* and their consequences on the population level. Limnologica 33:293–304.

Rose, K. C., C. E. Williamson, J. M. Fischer, S. J. Connelly, M. Olson, A. J. Tucker, and D. A. Noe. 2012. The role of ultraviolet radiation and fish in regulating the vertical distribution of *Daphnia*. Limnology and Oceanography 57:1867–1876.

Shi, L., Y. Cai, and F. Kong. 2012. Specific association between bacteria and buoyant *Microcystis* colonies compared with other bulk bacterial communities in the eutrophic Lake Taihu, China. Environmental Microbiology Reports 4:669–678.

Stewart Merrill, T. E., and C. E. Cáceres. 2018. Within-host complexity of a plankton-parasite interaction. Ecology 0:1–4.

Swaffar, S. M., and W. J. O'Brien. 1996. Spines of *Daphnia lumholtzi* create feeding difficulties for juvenile bluegill sunfish. Journal of Plankton Research 18:1055–1061.

Thomas, S. H., C. Bertram, K. van Rensburg, C. E. Caceres, and M. A. Duffy. 2011. Spatiotemporal dynamics of free-living stages of a bacterial parasite of zooplankton. Aquatic Microbial Ecology 63:265–272.

Turko, P., C. Tellenbach, E. Keller, N. Tardent, B. Keller, P. Spaak, and J. Wolinska. 2018. Parasites driving host diversity: incidence of disease correlated with *Daphnia* clonal turnover. Evolution 72:619–629.

Turner, W. C., K. L. Kausrud, Y. S. Krishnappa, P. G. M. Cromsigt, H. H. Ganz, I. Mapaure, C. C. Cloete, Z. Havarua, M. Ku, W. M. Getz, N. C. Stenseth, and W. C. Turner. 2014. Fatal attraction: vegetation responses to nutrient inputs attract herbivores to infectious anthrax carcass sites. Proceedings of the Royal Society B 281:1–9.

Venables, W. N., and B. D. Ripley. 2002. Modern Applied Statistics with S. Fourth. Springer, New York.

Weinstein, B. S. B., J. C. Buck, and H. S. Young. 2018a. A landscape of disgust. Science 359:1213–1215.

Weinstein, S. B., C. W. Moura, J. F. Mendez, and K. D. Lafferty. 2018b. Fear of feces? Tradeoffs between disease risk and foraging drive animal activity around raccoon latrines. Oikos 127:927–934.

Werner, E. E., and D. J. Hall. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology 55:1042–1052.

Wetzel, R. G. 2001. Limnology: Lake and River Ecosystems. Third Edit. Academic Press, San Diego, CA.

Williamson, C. E., J. M. Fischer, S. M. Bollens, E. P. Overholt, and J. K. Breckenridge. 2011. Toward a more comprehensive theory of zooplankton diel vertical migration: integrating ultraviolet radiation and water transparency into the biotic paradigm. Limnology and Oceanography 56:1603–1623.

Winder, M., P. Spaak, and W. M. Mooij. 2004. Trade-offs in *Daphnia* habitat selection. Ecology 85:2027–2036.

Wissel, B., W. J. Boeing, and C. W. Ramcharan. 2003a. Effects of water color on predation regimes and zooplankton assemblages in freshwater lakes. Limnology and Oceanography 48:1965–1976.

Wissel, B., N. D. Yan, and C. W. Ramcharan. 2003b. Predation and refugia: implications for *Chaoborus* abundance and species composition. Freshwater Biology 48:1421–1431.

Wolinska, J., K. Bittner, D. Ebert, and P. Spaak. 2006. The coexistence of hybrid and parental *Daphnia*: the role of parasites. Proceedings of the Royal Society B 273:1977–1983.

Zuur, A., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer-Verlag New York.

Table 3.1: Primers and probes used to detect parasite DNA in qPCR.

	Forward (5'-3')	Reverse (5'-3')	Probe
Pasteuria	CTT TGG TCG CAT GGC TAG AGA	GGC CGG TTA CGC ATC GTA	6FAM-CTT TGC ACT GTT TTT GGA AG-MGB NFQ
Metschnikowia	GGA TCA TTA CAG CGA AAA AGA ATA CAC	CCC GGA GGA GAA AAT GTA TGC	6FAM-ATT TGG CGT TGT GCA ACT AAC AGC TTA TTC AAG T- TAMRA

Table 3.2. Timing and conditions for lake sampling.

Month	Lake	Day	Night	Sunset
July	Cedar	7/24/2017; 10:15am; Mostly cloudy, windy	7/24/2017; 10:00pm clear, calm	8:59pm
	Crooked W	7/24/2017; 12:00 pm; cloudy, windy	7/24/2017; 10:50pm clear, calm	8:49pm
	Mill	7/25/2017 10:15 am; sunny, quite calm	7/25/2017 10:15pm; starry, clear and calm	8:58pm
	Walsh	7/25/2017 11:00 am, sunny, calm	7/25/2017 11pm, starry, clear, calm	8:58pm
	North	7/26/2017 9:45am, hazy, partly cloudy	7/26/2017 10:30pm mostly cloudy	8:57pm
	Bishop	7/27/2017 10:30am, hot, sunny, calm	7/28/2017 10:40pm; clear, starry, ¼ moon	8:56pm
	Little Appleton	7/27/2017 12:45pm; Mostly sunny, light wind	7/28/2017 11pm; clear, stars and ¼ moon.	8:56pm
August	Cedar	8/23/2017 9:45am; breezy, sunny, clear	8/23/2017 10:15pm; mostly cloudy	8:20pm
	Crooked W	8/23/2017 11:45am; partly cloudy, windy	8/23/2017 9:30pm; mostly cloudy	8:20pm
	Mill	8/24/2017 10:15am; mostly sunny, light breeze	8/24/2017 9:45pm; clear, sliver moon	8:18pm
	Walsh	8/24/2017 11:45am; cloudy, slight breeze	8/24/2017 10:30pm; clear, sliver moon	8:18pm
	North	8/25/2017 11:15am, breezy, sunny	8/25/2017 9:45 pm clear, sliver moon, calm	8:17pm
	Little Appleton	8/28/2017 11:50am; cloudy, calm, raining	8/28/2017 10:45pm; mostly cloudy, calm	8:12pm
	Bishop	8/28/2017 9:45am, cloudy, light rain, calm	8/28/2017 9:45pm; mostly cloudy calm	8:12pm
September	North	9/15/2017 10:15am; sunny breezy	9/14/2017 9:30pm; clear calm with stars	7:42pm
	Bishop	9/18/2017 10:00 am; calm, overcast, misty	9/17/2017 9pm, calm	7:37pm
	Little Appleton	9/18/2017 12:30pm; calm, overcast	9/17/2017 10:00pm, calm	7:37pm
	Crooked W	9/19/2017 11:15pm; calm, rainy	9/18/2017 9:15pm	7:36pm
	Cedar	9/19/2017 1:00pm; cloudy, calm	9/18/2017 10:15pm	7:36pm
	Walsh	9/20/2017 10:40am; partly cloudy	9/19/2017 9:15pm; raining	7:34pm
	Mill	9/20/2017 9:45am; mostly cloudy, light breeze	9/19/2017 8:30pm; calm, cloudy	7:34pm
October	Bishop	10/13/2017 11:00am; misty, calm, cloudy	10/12/2017 8:00pm; misty, calm	6:54pm
	Little Appleton	10/13/2017 9:45am; misty, calm, cloudy	10/12/2017 9:15pm; misty, calm	6:54pm
	North	10/16/2017 10:00am; sunny, clear, breezy	10/15/2017 8:30pm; windy, clear	6:49pm
	Crooked W	10/17/2017 9:15am; sunny windy	10/17/2017 8:00pm; calm	6:46pm
	Cedar	10/17/2017 10:30am; sunny, windy	10/17/2017 8:40pm; calm, starry	6:46pm
	Walsh	10/19/2017 11:00am; sunny windy	10/18/2017 8:40pm; starry, calm	6:45pm
	Mill	10/19/2017 10:00am; sunny, light breeze	10/18/2017 8:10pm; starry, light breeze	6:45pm

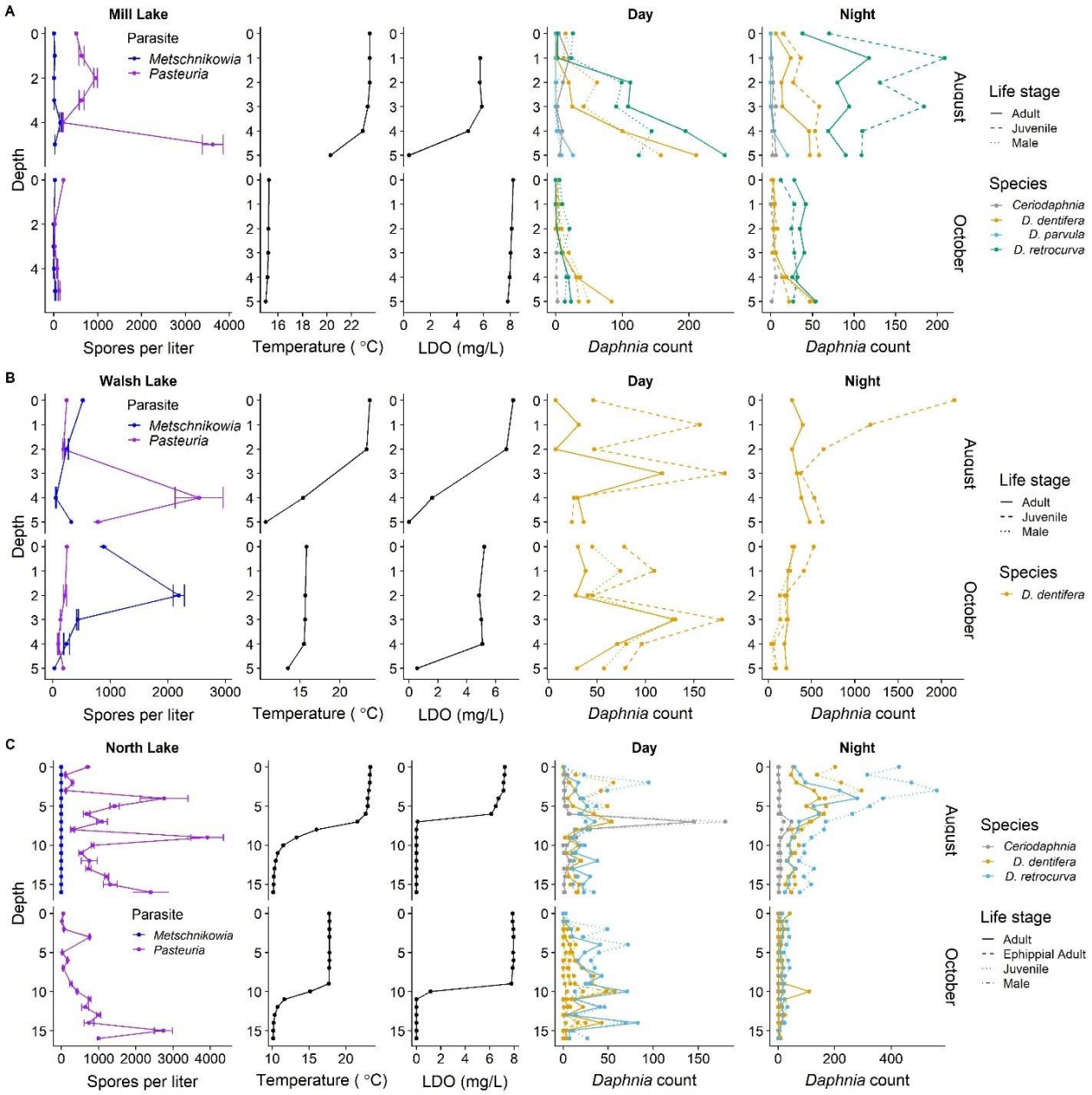

Figure 3.1. Distribution of parasite spores throughout the water column of (A) Mill, (B) Walsh, and (C) North Lakes in August and October. Accompanying from left to right are profiles of temperature, dissolved oxygen, and *Daphnia* counts during the day and night.

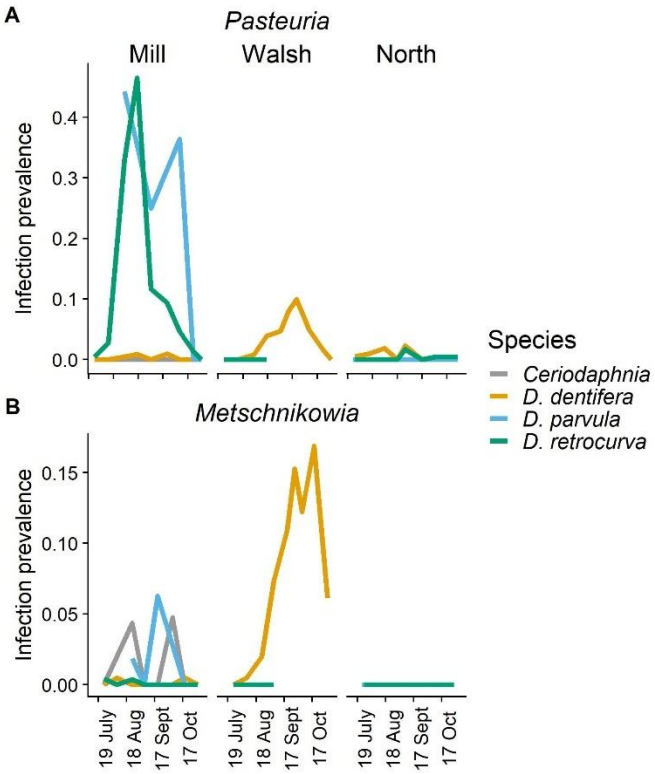

Figure 3.2. Infected (A) *Pasteuria* (B) *Metschnikowia* prevalence over time in different host species in Mill, Walsh, and North lakes.

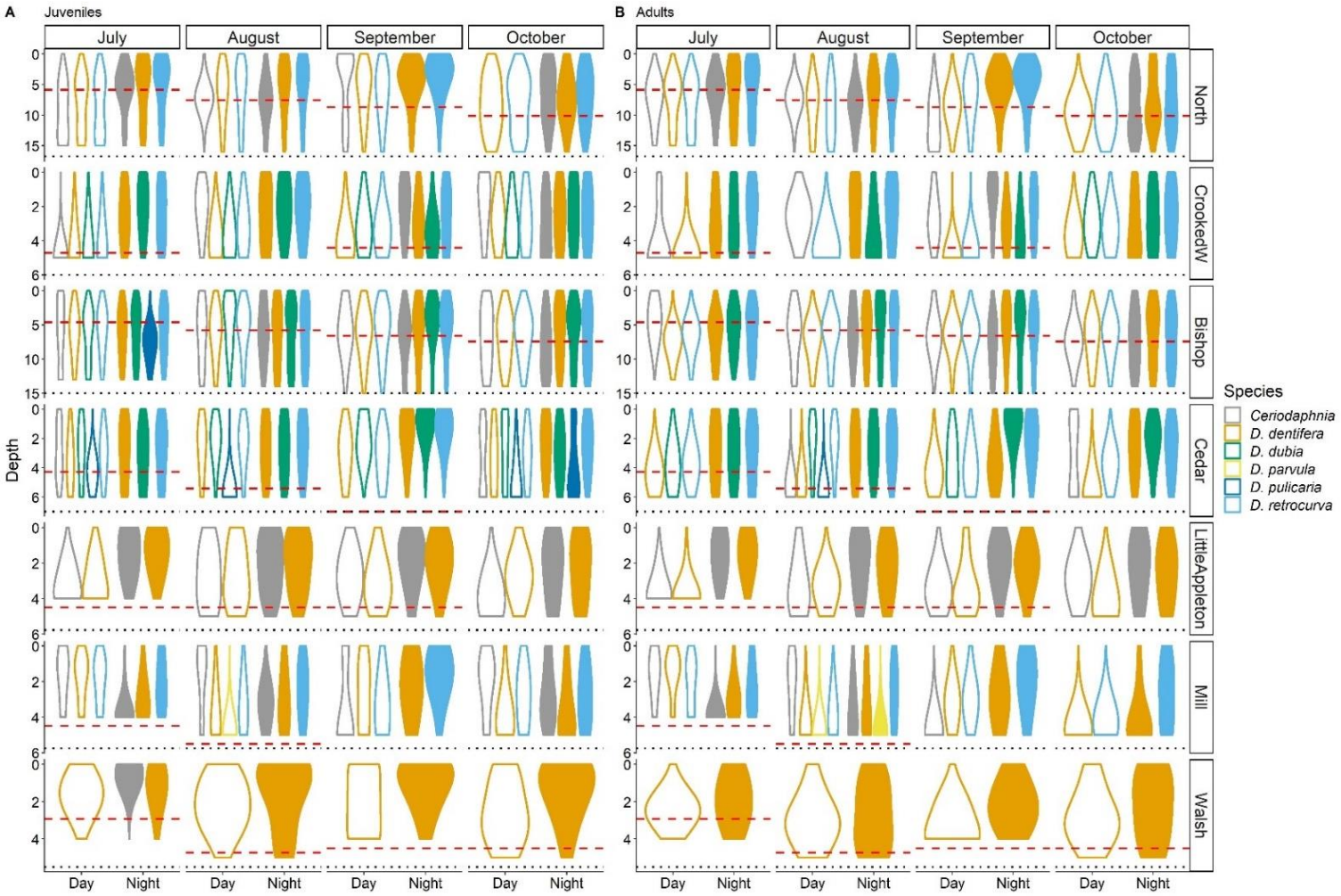

Figure 3.3. Distribution of the most common *Daphnia* species throughout water columns in the day (open violins) and night (filled violins). Width of the violins is proportional to the proportion of a given species/stage found at each depth in comparison to all animals of that type counted at the sampling time in a given lake. Dashed red lines indicate the depth of thermoclines, and dotted black lines indicate the bottom of lakes.

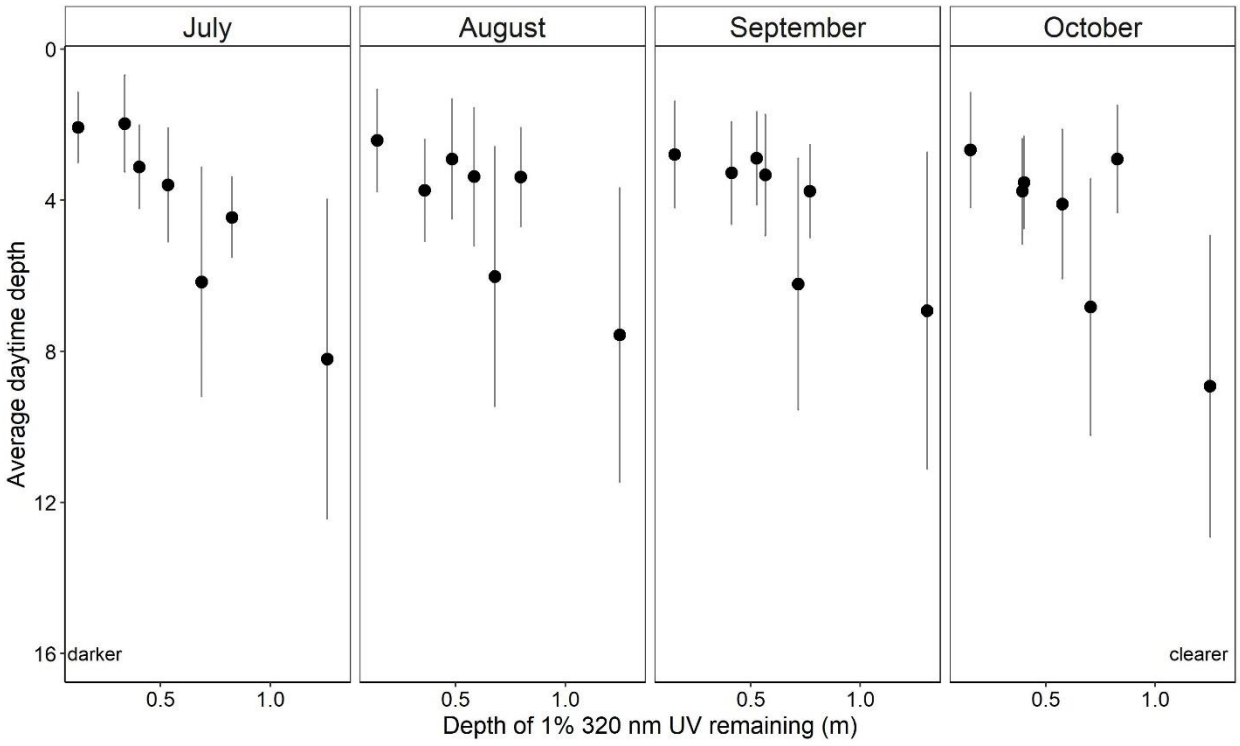

Figure 3.4. In the day, *Daphnia* lived deeper in clearer lakes (those with a deeper value for depth of 1% 320nm UV remaining). Points are weighted mean depths (averaging together all host species and sex/life stages); error bars are weighted standard deviations.

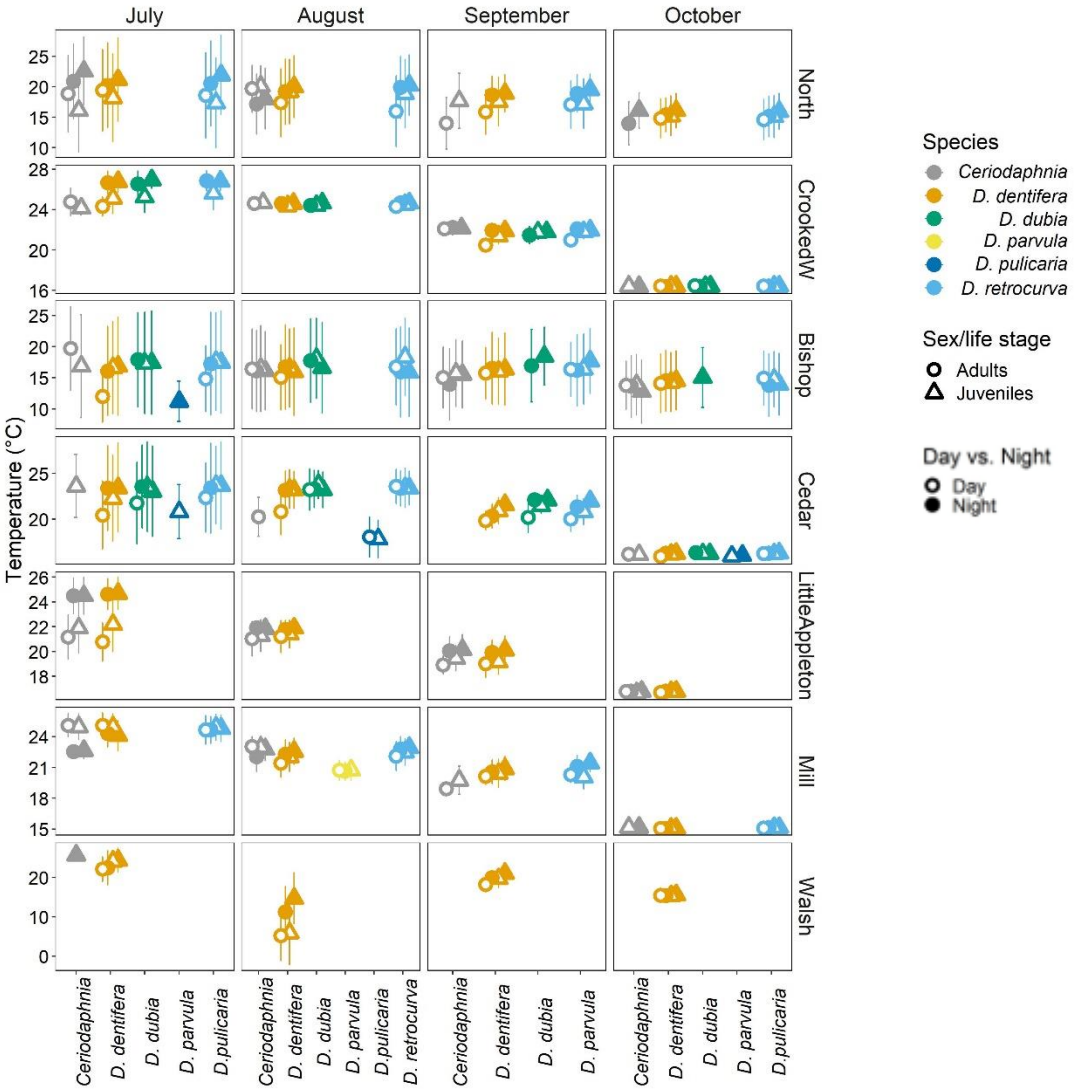

Figure 3.5. *Daphnia* resided at different temperatures during the day and at night due to their upward migration to warmer waters at night; when months were analyzed separately, this trend was significant in all months except October when many lakes were no longer stratified. Species and sex/life stages lived at significantly different temperatures in all months. Points show mean weighted temperatures and error bars show the standard deviation of weighted temperatures.

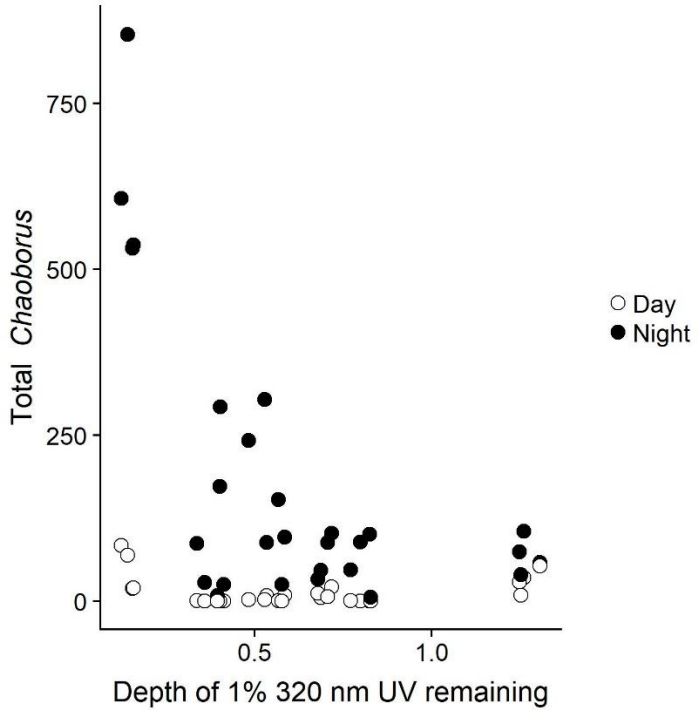

Figure 3.6. *Chaoborus* were more abundant in darker lakes and at night.

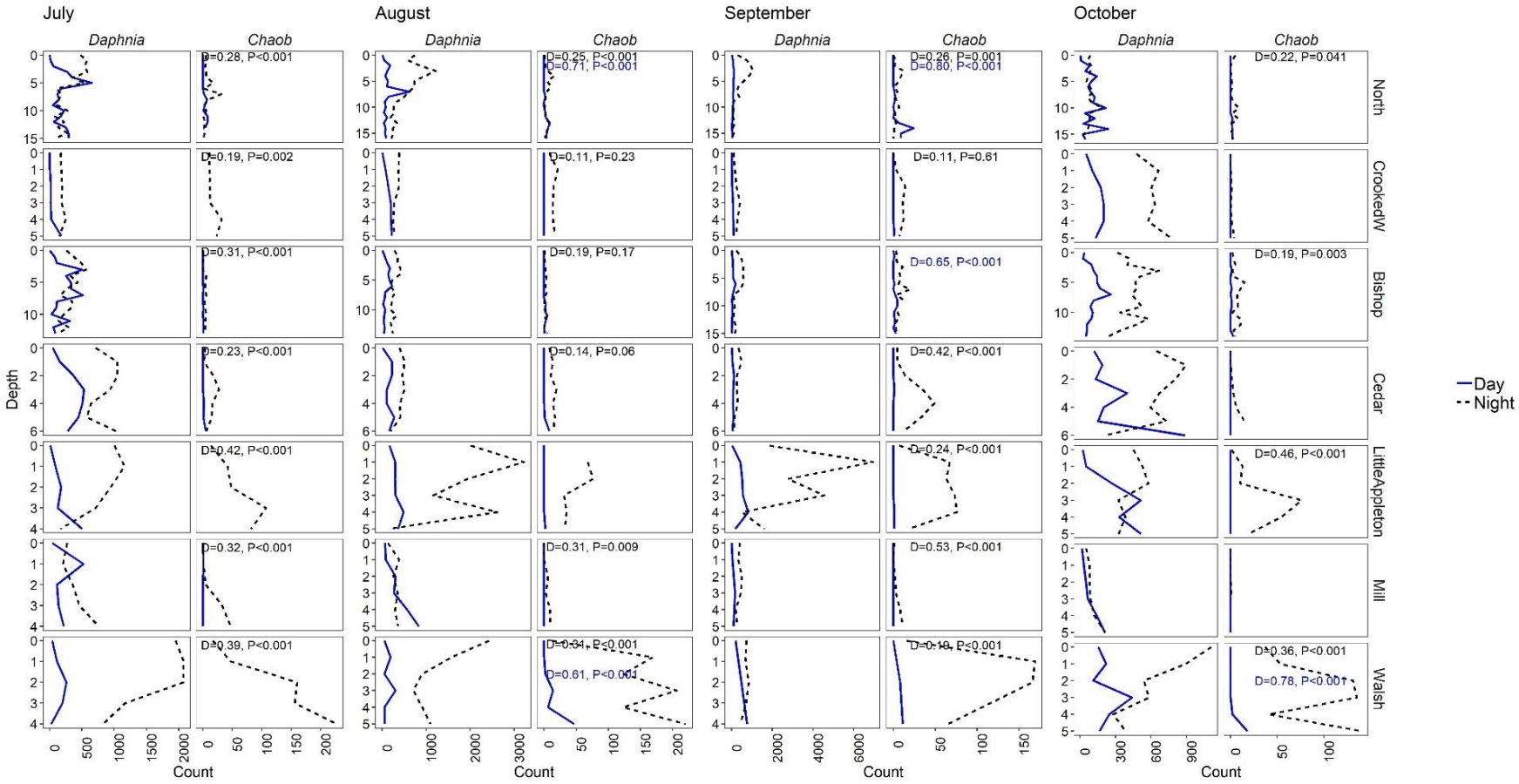

Figure 3.7. Distributions of *Daphnia* and *Chaoborus* in the water column in sampled lakes. Differences in distributions of *Chaoborus* and *Daphnia* were calculated using K-S tests when at least 10 *Chaoborus* in the lake were counted. K-S test D and P values are noted in blue for differences between *Daphnia* and *Chaoborus* distributions during the day and in black for differences between *Daphnia* and *Chaoborus* distributions at night.

CHAPTER 4

Asymmetric Interspecific Disease Transmission Modulated by Parasite Spore Size: Parasite Traits Help Explain Host Breadth in a Virulent Fungal Pathogen

with Rebecca Bilich, Bruce O'Brien, Carla E. Cáceres, Spencer R. Hall, Timothy Y. James, and Meghan A. Duffy

ABSTRACT

Parasites show a range of host breadths, with some infecting only a single host species, some primarily infecting one host species but occasionally spilling over into others, and still others routinely moving between different host species. Many ecological and evolutionary factors affect where parasites fall on this host-breadth gradient, including the diversity of hosts a parasite contacts and parasite traits that influence host exploitation. We used a combination of population genetics and a laboratory cross infection experiment to understand the factors driving host breadth. We used a common, ecologically important fungal parasite, *Metschnikowia bicuspidata*, and quantified its fitness in the two most common hosts in our study system (*Daphnia dentifera* and *Ceriodaphnia dubia*). Using microsatellite markers, we documented several parasite genotypes found in Michigan and Indiana lakes that clustered by host species and lake (which were often synonymous since the dominant host species in a given lake was most likely to become infected). There was one *Daphnia*-associated genotype that was consistent across Michigan and Indiana lakes, whereas a *Ceriodaphnia*-associated clade included one genotype that dominated in Michigan and a separate genotype that dominated in Indiana. While

both of the major clades (*Ceriodaphnia*-associated and *Daphnia*-associated) were able to infect both host species, interspecific transmission was relatively low. Our experimental results suggest that the smaller spore size of a *Ceriodaphnia*-associated genotype restricts the host breadth of this genotype in exchange for higher fitness in smaller, *Ceriodaphnia* hosts. Conversely, the larger spores of the *Daphnia*-associated genotype were able to infect *Ceriodaphnia* but had lower fitness in these smaller hosts. These tradeoffs seem to largely restrict these parasite genotypes to lakes where their preferred host type is dominant. Thus, by combining molecular approaches with experimental exposures, we were able to uncover a trait that is important in determining host breadth, helping explain patterns of infection in the wild.

INTRODUCTION

A recent uptick in emerging infectious diseases, some of which cause devasting epidemics in wildlife and human populations, motivates research into causes of disease emergence (Daszak et al. 2000, Cleaveland et al. 2001, Fisher et al. 2012, Cunningham et al. 2017). These outbreaks often occur when parasites move from one host species into another (Johnson et al. 2015, Alexander et al. 2018, Faust et al. 2018). However, understanding the mechanisms or conditions that enable parasites to spill over into new hosts remains a major challenge in the study of infectious diseases (Plowright et al. 2017). Moreover, while most parasites can infect multiple hosts, parasites vary in the degree to which they move between host species (Poulin et al. 2011, Ellis et al. 2015), and we also lack an understanding of the mechanisms that drive this variation. Fortunately, studying natural parasite population structures and parasite transmission among host types could reveal important factors affecting cross-species transmission.

Many ecological and evolutionary factors can influence where parasites fall on a specialist-to-generalist gradient. Generalist (i.e., parasites with greater host breadth) strategies may be selected in environments where parasites have frequent contact with diverse hosts (Woolhouse et al. 2001, Gandon 2004). However, specialization (narrowed host breadth) arises if trade-offs substantially constrain parasites from exploiting different hosts (i.e., when host quality differs or when hosts mount divergent defenses (Ruiz-González et al. 2012)). Moreover, a parasite's ability to navigate these trade-offs depends on its own traits or plasticity in these traits (Johnson et al. 2015, Olival et al. 2017), which may allow parasites to exploit different host resources or combat defenses of multiple host types.

Several broad patterns influence breadth of host use by parasites, but the mechanisms underlying these patterns are not well understood. Phylogenetic relationships among hosts can predict the ability of parasites to infect a set of species. Typically, parasites are more likely to infect more closely related hosts (Streicker et al. 2010, Longdon et al. 2011, Olival et al. 2017, Park et al. 2018). For instance, cross species transmission of Rabies viruses occurred more frequently among closely related bat species than among bats that were more distantly related (Streicker et al. 2010). However, habitat and environmental characteristics are important in addition to phylogenetic signals among hosts (Zukal et al. 2014), and the occasional parasite is a habitat specialist, meaning that it can infect distantly related hosts that share habitat (Clark and Clegg 2017). For example, distantly related primates shared parasites when host ranges overlapped and when host body mass and mean annual temperature were similar (Cooper et al. 2012). Second, directly transmitted parasites tend to be specialists whereas vector borne parasites and environmentally transmitted parasites are often generalists (Pedersen et al. 2005, Park et al. 2018). Third, major taxonomic groups show different average host breadths: viruses and bacteria

tend to have larger host breadth than other parasitic groups such as helminths and protozoa (Pedersen et al. 2005, Park et al. 2018). Though these broad patterns provide valuable insight into host breadth, it remains unclear mechanistically how and which parasite traits determine host breadth.

We show how variation in a key trait influences host breadth of an aquatic parasite. The parasite, the fungus *Metschnikowia bicuspidata,* infects relatively distantly related zooplankton hosts in Midwestern lakes. We focused on two common hosts, *Daphnia dentifera* and *Ceriodaphnia dubia.* We studied how *Metschnikowia* moves between these two hosts, documenting the population structure of natural parasite populations infecting *Daphnia* and *Ceriodaphnia* in lakes in Michigan and Indiana. We also quantified fitness consequences for the parasite when exploiting each host species using a cross infection experiment in the laboratory. Through this combination of approaches, we discovered a specific parasite trait, spore size, that drives host breadth variation in this multihost parasite and explains infection patterns in lake communities.

STUDY SYSTEM

The common hosts in our lakes, *Daphnia* and *Ceriodaphnia*, become infected with *Metschnikowia* via environmental transmission when hosts consume infective needle-shaped spores floating in the water. *Daphnia* and *Ceriodaphnia* overlap in lakes with high predation from fish but with a deep water predation refuge (Tessier and Woodruff 2002, Hall et al. 2010). However, they tend to thrive in different habitats with *Daphnia* preferring deeper, stratified lakes and *Ceriodaphnia* preferring shallower, warmer lakes (Desmarais and Tessier 1999). These

differences in habitat might be relevant to the frequency of cross-species transmission opportunities.

Daphnia and *Ceriodaphnia* are substantially different in body size (Figure 4.1), which could affect parasite competence (Auld et al. 2017). Within *Daphnia*, the parasite produces more spores within larger hosts (Hall et al. 2009c, Penczykowski et al. 2014, Civitello et al. 2015), perhaps due to space and/or resource constraints. *Ceriodaphnia* and *Daphnia* may differ in additional traits that could influence infection. Laboratory experiments have reported that infectivity and spore production is substantially lower in *Ceriodaphnia* than in *Daphnia* (Strauss et al. 2015, Auld et al. 2017), but these studies used parasite spores collected from infected *Daphnia*, rather than *Ceriodaphnia* hosts. We commonly observe *Metschnikowia* infecting both hosts in natural outbreaks (Appendix B Figure B1), so we hypothesized that parasites might be adapted to the host species that was most commonly infected in a given outbreak. Given the myriad challenges parasites face as they infect and propagate in varied hosts, we were interested in uncovering the mechanisms that allow *Metschnikowia* to successfully infect these two host species in the field.

METHODS

Field Survey Methods

In 2015, we surveyed 15 lakes near Ann Arbor, Michigan and 43 lakes in Greene and Sullivan Counties, Indiana. Lakes were sampled approximately every two weeks from mid July until mid November by combining 3 plankton tows from 3 locations from the deepest part of the lake. Each combined sample was subsampled until at least 200 *Daphnia* were counted and diagnosed visually (under a dissecting microscope) for infection with *Metschnikowia*. Another

sample containing 3 plankton tows from 3 locations from the deepest part of the lake was preserved in 75% ethanol, and volumetric subsamples were counted later to assess *Daphnia* and *Ceriodaphnia* densities. Though we focused on infections in dominant hosts, *Daphnia dentifera* and *Ceriodaphnia dubia*, other cladoceran and copepod species also reside in these lakes and occasionally become infected with *Metschnikowia*.

We hypothesized that larger outbreaks would be more likely to occur in the dominant host species in a given lake. In order to establish dominance quantitatively, lakes were classified as "*Daphnia* Lakes" or "*Ceriodaphnia* Lakes" according to the host species that was more common for a greater number of sampling days. To measure outbreak size, the area under the infection prevalence curve for each species was integrated using the trapezoid rule. This method was also used for the species density curves for a metric of integrated host density. A generalized linear model with binomial error structure was used to model which host species had a larger integrated infection prevalence (0 for bigger outbreak in *Ceriodaphnia*; 1 for bigger outbreak in *Daphnia*) with the lake classification as a "*Daphnia* Lake" or a "*Ceriodaphnia* Lake" as the fixed effect. Lakes were only included in this analysis if both host species were present in the lake (39 lakes met this condition). Linear models were also used to test for associations between integrated infection prevalence in each host species and integrated infection prevalence in the other host species as well as associations between integrated infection prevalence and integrated host density. Models were fit with the lme4 package in R (Bates et al. 2015).

Genotyping Methods

In order to examine the structuring of parasite populations between hosts and lakes, we genotyped *Metschnikowia* in infected hosts that were collected during the field survey (Appendix

B Table B1). The *Metschnikowia* genome was obtained using single cell genomics (Ahrendt et al. 2018). We ran the MISA script (Thiel 2003) to detect simple sequence repeats in the assembled genome, and then used Primer 3 software to develop primers (Rozen and Skaletsky 2000). We tested 24 potential primer pairs, and then selected nine that had the most consistent amplification and that showed variation between samples (Appendix B Table B2).

Infected animals from the natural outbreaks in the field were placed individually in microcentrifuge tubes in 90% ethanol. These samples were stored at -20°C until DNA extraction. DNA was extracted with the mericon DNA bacterial plus kit (QIAGEN, Hilden, Germany). Preserved infected animals were placed individually in 200 µL of the provided fast lysis buffer, and a battery powered pestle was used to homogenize each one. Each emulsified sample was transferred to a bead basher tube and vortexed at high speed for 10 minutes. Tubes were then centrifuged and the supernatant of each was saved as the DNA sample. All DNA samples were kept frozen at -20°C until PCR. PCR was performed in 96 well plates. We genotyped DNA at nine loci (Appendix B Table B2) with one reaction in each well. A M13(-21) tail was added to each forward primer, and a universal labeled 6FAM M13(-21) primer was used for detection (Schuelke 2000). PCR reactions were carried out in a final volume of 10 µL with 1X Qiagen multiplex mastermix (QIAGEN, Hilden, Germany), 15 nM forward primer with M13(-21) tails, 500 nM reverse primer, 150 nM labeled 6FAM universal M13(-21) primer, and with 1 µL of DNA. Amplification conditions were: 95°C (15 min), then 35 cycles of 94°C (30 s) / 58°C (3 min) / 72°C (1:30 min), and a final extension at 72°C for 10 min. PCR products were diluted 1:200 in water and 1 µl of diluted product was added into capillary electrophoresis loading plates containing 11 µl Hi-Di formamide and a LIZ500 size standard. Fragment analysis was performed

by the University of Michigan DNA sequencing core, and fragment lengths were read using GeneMapper (ThermoFisher Scientific).

Population genetics calculations were executed using the R package, Poppr (Kamvar et al. 2014). We calculated the index of association, I_A, among alleles in clone corrected parasite genotypes to evaluate if parasites were outcrossing or clonal (Smith et al. 1993). We then calculated Nei's gene diversity among parasites found infecting each host species (Nei 1973). This metric measures the probability that two randomly drawn alleles from a given locus in a population will be different. We calculated Prevosti genetic distance among individual genotypes, which is the fraction of allelic differences between two samples out of all loci (Wright 1978), and used this distance matrix to construct a dendrogram using the unweighted pair group method with arithmetic mean (UPGMA). To generate support for nodes, we bootstrapped, sampling 500 times (Kamvar et al. 2014). We then ran analyses of molecular variance (AMOVA). In an AMOVA, genotypes are grouped into hierarchical subdivisions, and the significance of the similarity of genotypes in each subdivision is tested (Excoffier et al. 1992). Since there was not an obvious hierarchy of groups in our study, we performed two AMOVAs. The first (AMOVA 1) designates host species as the highest level of hierarchy followed by state and lake. The second (AMOVA 2) designates state as the highest level of hierarchy followed by lake and host species.

Cross Infection Experiment Methods

We observed *Metschnikowia* infecting both hosts in natural outbreaks (Appendix B Figure B1), though previous work using *Metschnikowia* isolated from *Daphnia* had shown *Ceriodaphnia* to be a poor host (Auld et al. 2017). We therefore hypothesized that parasites

might be adapted to the host species that was most commonly infected in a given outbreak. We performed a cross infection experiment using field-collected spores from each host species and quantified performance of the parasites infecting each host species. In September 2017, we used animals collected from plankton tows to establish asexual cultures from lakes that we thought might have *Metschnikowia* outbreaks. However, only one of these (Benefiel) ended up having an outbreak in both host species. Thus, in November, we also established asexual lines from Goose Lake, where both host species were experiencing *Metschnikowia* outbreaks. We also used plankton tows collected from Benefiel Lake and Goose Lake in November 2017 to collect infected animals to be used as the source of *Metschnikowia* from *Ceriodaphnia* and *Daphnia* hosts. For the infection assay, groups of six 7-day old *Daphnia* and *Ceriodaphnia* of a given isofemale line (Table 4.1) were placed together in 80 ml of filtered lake water and exposed to spore slurries of 250 *Metschnikowia* spores/ml sourced from either the same host species or the other host species in that lake. Spore slurries were prepared by homogenizing infected *Daphnia* or infected *Ceriodaphnia* collected directly from the field. Animals were exposed to spores for 48 hours and then placed into clean filtered lake water that did not contain parasite spores. On the day of exposure, animals were fed 1,000,000 cells of algal food, *Ankistrodesmus*. On the second day of exposure, they were fed 1,500,000 cells of *Ankistrodesmus*. For the remainder of the experiment, when animals were in spore-free water, they were fed 2,000,000 cells of *Ankistrodesmus* daily. Lower food levels during parasite exposure increase parasite infection rates, but after parasite exposure, animals received saturating food. Animals were held in an incubator set to 20°C with a 16:8 hour light:dark cycle and were moved to 100 ml fresh filtered lake water twice a week. After 11 days, experimental animals were diagnosed for infection under a dissecting microscope. Infected experimental animals were placed individually in 50 µl of

nanopure water and stored at -20°C for spore counts and genotyping. For spore counts, each infected experimental animal was emulsified in 50 µl of water for 30 seconds with a battery-powered pestle. Then, three aliquots of 10 µl of the spore solution were placed on a hemocytometer and spores within the grid were counted. Average counts were used to quantify spore yield per animal. For each counted grid, one photograph was taken of spores in the view at 400x with a microscope camera (DP73, Olympus). These spores were measured with cellSens software (Olympus), and average spore length was computed across all three photographs.

We genotyped a subset of the *Metschnikowia* infections in experimental animals in order to determine which parasite genotype was responsible for infection. DNA extraction was performed on the solution remaining from spore counts again with the mericon DNA extraction kit (QIAGEN, Hilden, Germany). This time, 200 µl of fast lysis buffer was added to the remaining spore slurry and vortexed. This solution was then transferred to the bead basher tubes, vortexed, centrifuged, and the supernatant was saved as above. We genotyped experimental samples at 8 loci (Appendix B Table B2, excluding L3 because it was monomorphic in all but the Woodland (MI) samples in 2015). Due to lower DNA concentrations in experimental extractions, we altered the PCR recipe to 1X Qiagen multiplex mastermix (QIAGEN, Hilden, Germany), 10 nM forward primer with M13(-21) tails, 400 nM reverse primer, 400 nM 6FAM or HEX labeled universal M13(-21) primer, and 2 µl DNA in 10 µL reactions. Amplification conditions were: 94°C (3 min), then 10 cycles of 94°C (30 s) / 62C-53C (1°C drop each cycle; 30 s) / 72°C (45 sec), followed by 20 cycles of 94°C (30 s) / 53°C (30 s) / 72°C (45 s), followed by 8 cycles of 94°C (30 s) / 53°C (30 s) / 72°C (30 s), and a final extension at 72°C for 30 min. Amplified DNA was diluted 1:100 and loaded into prepared capillary electrophoresis plates with two (one HEX and one 6 FAM labeled) samples per well. DNA levels were much lower in these

samples, so not all loci amplified consistently in every sample, but samples were assigned to genotypes discovered in the genotyped natural samples in 2015 if at least 4 loci were amplified.

Infection results from the experiment were analyzed with generalized linear mixed effects models or linear mixed effects models using the lme4 package in R (Bates et al. 2015). Data for proportion infected were modeled with binomial error structure, while those for count and length of spores were analyzed with Gaussian error structure. The response variables (proportion infected, spores produced, and average spore length) were each modeled with an interaction between experimental host and isolation host as fixed effects (or without an interaction if the interaction was not significant) and with host clone was as a random effect. The experimental cross infections using spores from Goose Lake were run in two temporal blocks which were fit as a random effect for this lake (Table 4.1).

RESULTS

Field Survey

During naturally occurring parasite outbreaks, *Metschnikowia* preferentially infected different hosts in different lakes. Though both host species became infected with *Metschnikowia*, the host species that was more common in a given lake over the course of the sampling season tended to have larger parasite outbreaks (LRT=4.44, P=0.035, Figure 4.2A). However, within a species, outbreak size did not scale with host density (*Daphnia*: $F_{1,38}$=0.659, P=0.422; *Ceriodaphnia* $F_{1,25}$=0.238, P=0.630, Figure 4.2B & C) or with outbreak size in the other host species ($F_{1,25}$=0.518, P=0.479, Figure 4.2D).

Metschnikowia Genotypes

Genotyping parasite infections from the field revealed the presence of multiple parasite genotypes with non-random population structure. Field collected parasites grouped into six parasite genotypes within two distinct clades. On average, there were 2.78 alleles per locus, and genotypes differed on average at about 5.3 out of 9 loci. The three most common genotypes differed on average at 5.6 out of 9 loci. The clone corrected index of association was 0.995 (P=0.013) indicating that *Metschnikowia* reproduces clonally. Parasite genotypes tended to cluster by host species, though occasionally individuals of different host species in the same lake shared the same parasite genotype (Figure 4.3). Nei's gene diversity (H_S) of parasites in each host species were similarly low (*Daphnia* H_S=0.273 95% CI [0.209, 0.315]; *Ceriodaphnia* H_S=0.290, 95% CI [0.231, 0.326]), indicating low diversity of genotypes infecting each host species. When host species was the highest level of hierarchy (AMOVA 1), host species groups explained 34.56% of the variation between samples (P=0.001, Table 4.2), but when it was the lowest level (AMOVA 2) it only explained 6.35% of the variation between samples (P=0.430, Table 4.2) with lake groups accounting for 72.39% of the variation (P=0.018, Table 4.2). These results reflect that though *Daphnia* and *Ceriodaphnia* tended to get infected by different *Metschnikowia* genotypes, the infected animals were mostly from different lakes, and within lakes, there was often spillover of a given *Metschnikowia* genotype between the host species.

Of the three most abundant *Metschnikowia* genotypes, one genotype was present in both states, and found primarily infecting *Daphnia* (Figure 4.3; the single genotype in the *Daphnia*-associated clade). The other two abundant *Metschnikowia* genotypes were found primarily in *Ceriodaphnia* with one genotype common in Indiana lakes and the other genotype common in Michigan lakes (Figure 4.3; the two most common genotypes in the *Ceriodaphnia*-associated

clade). However, none of the three most prevalent *Metschnikowia* genotypes was restricted to a single host species.

There were also three less common *Metschnikowia* genotypes. One was found in Sycamore Lake and Shake 1 Lake (both in Indiana). Sycamore Lake only had infections in *Ceriodaphnia,* and Shake 1 Lake had low infection levels in *Daphnia* early in the season, but not when samples were collected (Appendix B Figure B1). The other two less common *Metschnikowia* genotypes were found infecting animals in Michigan lakes, Woodland and Mill. In both of these lakes, it is possible that these infections spilled over from other host species. In Woodland Lake, two copepods from the previous year were found to be infected by the same *Metschnikowia* genotype as the infected *Daphnia dentifera* marked on the dendrogram (Figure 4.3). In Mill Lake, only two infected *Daphnia dentifera* were counted over the entire season. Though outbreaks didn't take off in any species, one infected *Ceriodaphnia*, one infected *Daphnia ambigua,* and two infected *Daphnia retrocurva* were also documented in this lake during fall 2015.

Cross Infection Experiment

We performed a cross infection experiment in order to understand if *Metschnikowia* genotypes had different fitness in each host species. This could help explain why some lakes mainly had infections in *Daphnia* and other lakes mainly had infections in *Ceriodaphnia.* The experiment was performed with hosts and *Metschnikowia* spores collected from two different Indiana lakes, Benefiel and Goose. The results differed in each lake (see below), likely because Benefiel Lake harbored multiple *Metschnikowia* genotypes, whereas Goose Lake had only one.

For the cross infections with hosts and parasites from Benefiel Lake, infection and spore production depended on the combination of exposed and source hosts. *S*pores grown within a given host species (source host) were not equally infectious to both species (Figure 4.4A; source x exposed host interaction: LRT=9.45, P=0.003). However, a post hoc test showed that the only significant difference in infection levels was between exposed *Ceriodaphnia* hosts infected with spores from *Ceriodaphnia* source hosts and exposed *Ceriodaphnia* hosts infected with spores from *Daphnia* source hosts (Tukey: z=3.18, P=0.008). *Metschnikowia* sourced from *Daphnia* produced more spores in larger *Daphnia* hosts (LRT=8.79, P=0.003; Fig. 4B). Furthermore, more spores were produced by *Ceriodaphnia* when spores were sourced from *Ceriodaphnia* (LRT=6.80, P=0.009). Moreover, *Metschnikowia* from Benefiel produced spores that were significantly different in size in exposed hosts, and this depended on both exposed and source host identity (Figure 4.4C; LRT=30.07, P<0.001). When *Metschnikowia* was sourced from *Ceriodaphnia*, spores produced in exposed *Ceriodaphnia* hosts were significantly smaller than those produced in exposed *Daphnia* hosts (from either source host; Tukey: from *Daphnia*: z=-9.78, P<0.001; from *Ceriodaphnia:* z=-8.43 P<0.001) or from those produced in *Ceriodaphnia* when sourced from *Daphnia* (Tukey: z=-9.42, P<0.001). The smaller spores belonged to the most prevalent Indiana *Ceriodaphnia*-associated genotype (in the *Ceriodaphnia*-associated clade) in the 2015 survey (Figure 4.3), and the larger spores belonged to the main *Daphnia*-associated genotype. The *Ceriodaphnia* exposed to *Metschnikowia* sourced from *Ceriodaphnia* became infected by both of these genotypes, whereas the *Daphnia* exposed to spores sourced from *Ceriodaphnia* only became infected by the main *Daphnia*-associated genotype. This pattern indicates that *Ceriodaphnia* source hosts collected directly from Benefiel Lake were infected by both parasite genotypes; then, in the experiment, exposed *Ceriodaphnia* hosts became infected

by both genotypes whereas exposed *Daphnia* only became infected by the subset of these spores that were larger (the *Daphnia*-associated genotype). Though *Ceriodaphnia* became infected by both parasite genotypes, more spores were produced in *Ceriodaphnia* experimental hosts when the spores were smaller (Figure 4.4D; spore size x host species: $F_{3,82}$=19.48, P<0.001), giving an advantage to this *Ceriodaphnia*-associated genotype in *Ceriodaphnia* hosts.

Results from the cross infection with hosts and parasites from Goose lake showed different patterns. There was no difference in infection rates of the two exposed hosts (z=1.92, P=0.14; Appendix B Figure B3A), no influence of source host on infection rate (z=-0.19, P=0.81; Appendix B Figure B3A), and no exposed by source host interaction (-0.51, P=0.71, Appendix B Figure B3A). More spores were produced in *Daphnia* hosts (LRT=3.83, P=0.05 Appendix B Figure B3B), though there was no difference in spore quantities produced by *Metschnikowia* from the two source host species (LRT=0.00, p=0.98). Spore sizes were not significantly different between the groups (Exposed species: LRT=0.168, P=0.68; Source species: LRT=1.19, P=0.28; Appendix B Figure B3C). Notably, all genotyped samples belonged to the *Daphnia*-associated genotype. If only one *Metschnikowia* genotype infected both hosts in Goose lake, it explains the lack of a source host effect on infection rate, spore yield, and spore size in this lake.

DISCUSSION

The two focal hosts for this study, *Ceriodaphnia dubia* and *Daphnia dentifera*, diverged over 200 million years ago (Colbourne and Hebert 1996), yet *Metschnikowia bicuspidata* readily infects both. Moreover, we found the same parasite also infects copepods, which diverged from the daphniid lineage in the Cambrian era (Wolfe et al. 2007, Schwentner et al. 2017), well before

the evolution of tetrapods. Thus, *Metschnikowia* appears to be a habitat specialist, infecting diverse hosts that share a habitat. In our studies of natural infections in two states, Michigan and Indiana, we found that outbreaks usually occurred in the most common host in a lake, and we identified two main parasite clades, one primarily associated with *Daphnia* and one primarily associated with *Ceriodaphnia*. Each of these genotypes 'spilled over' between hosts within a lake, indicating that parasite genotypes are not completely restricted to their favored host type. In the cross-infection experiment that included *Metschnikowia* genotypes from both clades, the *Ceriodaphnia*-associated genotype had higher fitness in *Ceriodaphnia* than in *Daphnia,* and it also had higher fitness in *Ceriodaphnia* hosts than the *Daphnia*-associated genotype. However, the *Daphnia*-associated genotype of *Metschnikowia* appears to be a more successful generalist because it was capable of infecting both host species in the experiment, whereas no *Daphnia* became infected with the *Ceriodaphnia*-associated genotype (though field collected *Daphnia* occasionally were). Our results suggest that a key parasite trait—the size of transmission stages (spores)—might drive this asymmetric transmission, providing a mechanism to explain differences in host breadth in this ecologically important parasite.

One hypothesis that could explain the asymmetric transmission of genotypes of this parasite involves the mechanics of infection. Spores usually pierce the host's gut at the anterior or posterior bends (Figure 4.1) in the beginning of the infection process (Stewart Merrill and Cáceres 2018). In other words: spores infect the host when they do not make the "turn" in the gut, but, rather, continue straight into the gut wall. Smaller spores may only be able to lodge in the gut for smaller animals; in larger animals, small spores would more easily flow around the bend in the gut without piercing the gut wall (since gut size scales with host size (Hall et al. 2007)). In contrast, the larger spores of the *Daphnia*-associated genotype of the parasite more

readily pierce guts of small and large animals. However, while the *Daphnia*-associated genotype is better able to cross the species barrier, it produces fewer spores when it does so: *Ceriodaphnia* hosts yielded more than twice as many spores from infections with the *Ceriodaphnia*-associated genotype as compared to infections with the *Daphnia*-associated genotype. Because the *Daphnia*-associated genotype spores are larger, this spore yield pattern may reflect limitations due to space and/or resources. When this spore yield result is combined with our finding that spores from *Ceriodaphnia* are much more likely to infect *Ceriodaphnia* (as compared to spores from *Daphnia*), the *Ceriodaphnia*-associated genotype should have much higher fitness than the *Daphnia*-associated genotype in an environment composed primarily of *Ceriodaphnia*.

We found the same *Daphnia*-associated genotype was present in both Michigan and Indiana lakes. This is surprising given the large population sizes within regions and the geographic distance between the two sets of lakes (about 560 km). However, it is consistent with previous work on *Metschnikowia* isolated from *Daphnia dentifera,* which did not find any heritable differences between isolates (Duffy and Sivars-Becker 2007, Searle et al. 2015). In contrast, we found several *Ceriodaphnia*-associated genotypes, at least two of which had smaller spores (Appendix B Figure B4); these were restricted either to Michigan or to Indiana. Perhaps *Metschnikowia* independently evolved smaller spores in these different locations; alternatively, diverse smaller-spored *Metschnikowia* genotypes may have moved across the region, with different small-spored *Metschnikowia* genotypes fixing in the separate metapopulations that form our two sets of study lakes. Interestingly, an earlier study also found two size morphs of *Metschnikowia* infecting *Daphnia magna* and *Daphnia pulex* in southern England (Stirnadel and Ebert 1997). More extensive sampling and genotyping of additional infected host species could help us to understand the evolutionary relationships between these genotypes as well as the

evolutionary history of *Metschnikowia* spore size. It would also help uncover the evolutionary origins of the less common *Metschnikowia* genotypes, including the one that is able to infect both *Daphnia* and copepods.

The discovery of different *Metschnikowia* genotypes raises the question of which factors cause a particular *Metschnikowia* genotype to be dominant in a given lake and under what conditions multiple *Metschnikowia* genotypes coexist. Host species composition is likely important since larger *Metschnikowia* outbreaks were more likely to occur in the more common host species in a given lake. Several additional factors are known to influence outbreak size of *Metschnikowia* including predation (Duffy et al. 2005, Cáceres et al. 2009), competition (Hall et al. 2009a, Strauss et al. 2015), resource quality (Hall et al. 2009b, Penczykowski et al. 2014), and abiotic factors (Cáceres et al. 2006, Overholt et al. 2012, Shocket et al. 2018), but it is so far unknown if these factors influence the genotypic make up of parasite populations. These factors undoubtedly also influence the structure of the host community and cause host communities to change over time (as in our system, see Appendix B Figure 2). Thus, a challenge for future work will be to determine whether it is possible to predict the success of each parasite genotype in different habitats.

It's also plausible that host body size rather than host species is the driving factor determining which *Metschnikowia* genotype is successful. *Ceriodaphnia* genotypes could be favored in lakes dominated by smaller hosts (or younger hosts), and the *Daphnia* genotype could be favored in lakes dominated by larger hosts. Host body size can also be affected by many of the factors mentioned previously: smaller hosts are expected when fish predation is high (Brooks and Dodson 1965), when resources are scarce (Gilwicz 1990) or of low quality (Sterner, 1993), and when temperature is low (Burns 1969). In fact, *Daphnia* in Gosling lake in Michigan (where

Daphnia are found infected with a *Ceriodaphnia*-associated genotype of *Metschnikowia*) are unusually small and similar in size to the *Ceriodaphnia* in that lake (personal observation, Rogalski unpublished data). In the case of the Gosling *Daphnia* infected in this study, the animal was a male, which are also usually smaller than females (Munro and White 1975).

What determines host breadth and selection on the ability to infect multiple host species? By studying parasite population structure, experimentally infecting hosts, and measuring key traits, we can uncover mechanisms driving parasite fitness across a multihost landscape. Our focal parasite, *Metschnikowia*, is environmentally transmitted in lakes with diverse and dynamic plankton assemblages, which should select for the ability to infect more than one host species (Woolhouse et al. 2001). We found that *Metschnikowia* genotypes in two clades were able to infect both focal host species. However, the smaller spores of *Ceriodaphnia*-associated genotypes may restrict the host breadth of these genotypes in exchange for higher fitness in smaller, *Ceriodaphnia* hosts. Conversely, the larger spores of the *Daphnia* genotype are able to infect *Ceriodaphnia* but have lower fitness in these smaller hosts. These tradeoffs seem to have restricted parasite genotypes to lakes where their preferred host type is dominant, but the *Daphnia*-associated genotype had a wider host breadth in our experiment likely due in part to spore size and the mechanics of infection. Our findings demonstrate how integral parasite traits are to their ability to infect different hosts, arguing that focusing on these traits can help us better understand the complexity of multihost parasite systems in nature.

REFERENCES

Ahrendt, S. R., C. A. Quandt, D. Ciobanu, A. Clum, A. Salamov, B. Andreopoulos, J. F. Cheng, T. Woyke, A. Pelin, B. Henrissat, N. K. Reynolds, G. L. Benny, M. E. Smith, T. Y. James, and I. V. Grigoriev. 2018. Leveraging single-cell genomics to expand the fungal tree of life. Nature Microbiology 3:1417–1428.

Alexander, K. A., C. J. Carlson, B. L. Lewis, W. M. Getz, V. M. Marathe, S. G. Eubank, C. E. Sanderson, and J. K. Blackburn. 2018. The ecology of pathogen spillover and disease emergence at the human - wildlife - environment interface. Page (C. J. Hurst, Ed.). Springer International Publishing.

Auld, S. K. J. R., C. L. Searle, and M. A. Duffy. 2017. Parasite transmission in a natural multihost – multiparasite community. Philosophical Transactions of the Royal Society B 372:1–10.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150:28–35.

Burns, C. W. 1969. Relation between filtering rate, temperature, and body size in four species of *Daphnia*. Limnology and Oceanography 14:693–700.

Cáceres, C. E., S. R. Hall, M. A. Duffy, A. J. Tessier, C. Helmle, and S. Macintyre. 2006. Physical structure of lakes constrains epidemics in *Daphnia* populations. Ecology 87:1438–1444.

Cáceres, C. E., C. J. Knight, and S. R. Hall. 2009. Predator-spreaders: predation can enhance parasite success in a planktonic host-parasite system. Ecology 90:2850–2858.

Civitello, D. J., R. M. Penczykowski, A. N. Smith, M. S. Shocket, M. A. Duffy, and S. R. Hall. 2015. Resources, key traits and the size of fungal epidemics in *Daphnia* populations. Journal of Animal Ecology 84:1010–1017.

Clark, N. J., and S. M. Clegg. 2017. Integrating phylogenetic and ecological distances reveals new insights into parasite host specificity. Molecular Ecology 26:3074–3086.

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London B 356:991–999.

Colbourne, J. K., and P. D. N. Hebert. 1996. The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. Philosophical Transactions of the Royal Society of London B 351:349–360.

Cooper, N., R. Griffin, M. Franz, M. Omotayo, and C. L. Nunn. 2012. Phylogenetic host specificity and understanding parasite sharing in primates. Ecology Letters 15:1370–1377.

Cunningham, A. A., P. Daszak, and J. L. N. Wood. 2017. One health, emerging infectious diseases and wildlife. Philosophical Transactions of the Royal Society of London. B. 372:4.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. Science 287:443–449.

Desmarais, K. H., and A. J. Tessier. 1999. Performance trade-off across a natural resource gradient. Oecologia 120:137–146.

Duffy, M. A., S. R. Hall, A. J. Tessier, and M. Huebner. 2005. Selective predators and their parasitized prey: are epidemics in zooplankton under top-down control? Limnology and Oceanography 50:412–420.

Duffy, M. A., and L. Sivars-Becker. 2007. Rapid evolution and ecological host-parasite dynamics. Ecology Letters 10:44–53.

Ellis, V. A., M. D. Collins, M. C. I. Medeiros, E. H. R. Sari, E. D. Coffey, R. D. Dickerson, C. Lugarini, J. A. Stratford, D. R. Henry, L. Merrill, A. E. Matthews, A. A. Hanson, J. R. Roberts, M. Joyce, M. R. Kunkel, and R. E. Ricklefs. 2015. Local host specialization, host-switching, and dispersal shape the regional distributions of avian haemosporidian parasites. Proceedings of the National Academy of Sciences:1–6.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Faust, C. L., H. I. McCallum, L. S. P. Bloomfield, N. L. Gottdenker, T. R. Gillespie, C. J. Torney, A. P. Dobson, and R. K. Plowright. 2018. Pathogen spillover during land conversion. Ecology Letters 21:471–483.

Fisher, M. C., D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw, and S. J. Gurr. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194.

Gandon, S. 2004. Evolution of multihost parasites. Evolution 58:455–469.

Gilwicz, Z. M. 1990. Food thresholds and body size in cladocerans. Letters To Nature 346:638–640.

Hall, S. R., C. R. Becker, J. L. Simonis, M. A. Duffy, A. J. Tessier, and C. E. Cáceres. 2009a. Friendly competition: evidence for a dilution effect among competitors in a planktonic host-parasite system. Ecology 90:791–801.

Hall, S. R., C. J. Knight, C. R. Becker, M. a. Duffy, A. J. Tessier, and C. E. Cáceres. 2009b. Quality matters: resource quality for hosts and the timing of epidemics. Ecology Letters 12:118–128.

Hall, S. R., J. L. Simonis, R. M. Nisbet, A. J. Tessier, and C. E. Cáceres. 2009c. Resource

ecology of virulence in a planktonic host-parasite system: an explanation using dynamic energy budgets. The American Naturalist 174:149–162.

Hall, S. R., L. Sivars-Becker, C. Becker, M. a. Duffy, A. J. Tessier, and C. E. Cáceres. 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. Ecology Letters 10:207–218.

Hall, S. R., R. Smyth, C. R. Becker, M. A. Duffy, C. J. Knight, S. MacIntyre, A. J. Tessier, and C. E. Cáceres. 2010. Why Are *Daphnia* in some lakes sicker? Disease ecology, habitat structure, and the plankton. BioScience 60:363–375.

Johnson, C. K., P. L. Hitchens, T. S. Evans, T. Goldstein, K. Thomas, A. Clements, D. O. Joly, N. D. Wolfe, P. Daszak, W. B. Karesh, and J. K. Mazet. 2015. Spillover and pandemic properties of zoonotic viruses with high host plasticity. Scientific Reports 5:1–8.

Kamvar, Z. N., J. F. Tabima, and N. J. Grunwald. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:1–14.

Longdon, B., J. D. Hadfield, C. L. Webster, D. J. Obbard, and F. M. Jiggins. 2011. Host phylogeny determines viral persistence and replication in novel hosts. PLoS Pathogens 7.

Munro, I. G., and R. W. G. White. 1975. Comparison of the influence of temperature on the egg development and growth of *Daphnia longispina* O.F. Müller (Crustacea: Cladocera) from two habitats in southern England. Oecologia 20:157–165.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70:3321–3323.

Olival, K. J., P. R. Hosseini, C. Zambrana-Torrelio, N. Ross, T. L. Bogich, and P. Daszak. 2017. Host and viral traits predict zoonotic spillover from mammals. Nature 546:646–650.

Overholt, E. P., S. R. Hall, C. E. Williamson, C. K. Meikle, M. A. Duffy, and C. E. Cáceres. 2012. Solar radiation decreases parasitism in *Daphnia*. Ecology Letters 15:47–54.

Park, A. W., M. J. Farrell, J. P. Schmidt, S. Huang, T. A. Dallas, P. Pappalardo, J. M. Drake, P. R. Stephens, R. Poulin, C. L. Nunn, and T. J. Davies. 2018. Characterizing the phylogenetic specialism-generalism spectrum of mammal parasites. Proceedings of the Royal Society B 285:20172613.

Pedersen, A. B., S. Altizer, M. Poss, A. A. Cunningham, and C. L. Nunn. 2005. Patterns of host specificity and transmission among parasites of wild primates. International Journal for Parasitology 35:647–657.

Penczykowski, R. M., B. C. P. Lemanski, R. D. Sieg, S. R. Hall, J. Housley Ochs, J. Kubanek, and M. A. Duffy. 2014. Poor resource quality lowers transmission potential by changing foraging behaviour. Functional Ecology 28:1245–1255.

Plowright, R. K., C. R. Parrish, H. Mccallum, P. J. Hudson, A. I. Ko, A. L. Graham, and J. O. Lloyd-Smith. 2017. Pathways to zoonotic spillover. Nat Rev Microbiol 15:502–510.

Poulin, R., B. R. Krasnov, and D. Mouillot. 2011. Host specificity in phylogenetic and geographic space. Trends in Parasitology 27:355–361.

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. Springer.

Ruiz-Gonzalez, M. X., J. Bryden, Y. Moret, C. Reber-Funk, P. Schmid-Hempel, and M. J. F. Brown. 2012. Dynamic transmission, host quality, and population structure in a multihost parasite of bumblebees. Evolution 66:3053–3066.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233–234.

Schwentner, M., D. J. Combosch, J. Pakes Nelson, and G. Giribet. 2017. A phylogenomic solution to the origin of insects by resolving crustacean-hexapod relationships. Current Biology 27:1818–1824.e5.

Searle, C. L., J. H. Ochs, C. E. Cáceres, S. L. Chiang, and N. M. Gerardo. 2015. Plasticity, not genetic variation, drives infection success of a fungal parasite. Parasitology 142:839–848.

Shocket, M. S., A. T. Strauss, J. L. Hite, M. Šljivar, D. J. Civitello, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2018. Temperature drives epidemics in a zooplankton-fungus disease system: a trait-driven approach points to transmission via host foraging. The American Naturalist 191:435–451.

Smith, J. M., N. H. Smith, M. O'Rourke, and B. G. Spratt. 1993. How clonal are bacteria? Proceedings of the National Academy of Sciences 90:4384–4388.

Sterner, R. W. 1993. *Daphnia* growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. Ecology 74:2351–2360.

Stewart Merrill, T. E., and C. E. Cáceres. 2018. Within-host complexity of a plankton-parasite interaction. Ecology 0:1–4.

Stirnadel, H. A., and D. Ebert. 1997. Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. Journal of Animal Ecology 66:212–222.

Strauss, A. T., D. J. Civitello, C. E. Cáceres, and S. R. Hall. 2015. Success, failure and ambiguity of the dilution effect among competitors. Ecology Letters:1–11.

Streicker, D. G., A. S. Turmelle, M. J. Vonhof, I. V Kuzmin, G. F. Mccracken, and C. E.

Rupprecht. 2010. Host phylogeny constrains cross-species emergence and establishment of rabies virus in bats. Science 329:676–679.

Tessier, A. J., and P. Woodruff. 2002. Cryptic trophic cascade along a gradient of lake size. Ecology 83:1263–1270.

Thiel, T. 2003. MISA - Microsatellie identification tool.

Wolfe, N. D., C. P. Dunavan, and J. Diamond. 2007. Origins of major human infectious diseases. Nature 447:279–83.

Woolhouse, M. E. J., L. H. Taylor, D. T. Haydon, M. E. J. Woolhouse, L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science 292:1109–1112.

Zukal, J., H. Bandouchova, T. Bartonicka, H. Berkova, V. Brack, J. Brichta, M. Dolinay, K. S. Jaron, V. Kovacova, M. Kovarik, N. Martínková, K. Ondracek, Z. Rehak, G. G. Turner, and J. Pikula. 2014. White-Nose Syndrome fungus: a generalist pathogen of hibernating bats. PLoS ONE 9:e97224.

Table 4.1. Number of replicate beakers exposed to *Metschnikowia* from each isolation host. Numbers in parentheses refer to numbers of beakers in second block.

Experimental Genotype Name	**Species**	**Number of Replicates exposed to *Metschnikowia* from *Daphnia***	**Number of Replicates exposed to *Metschnikowia* from *Ceriodaphnia***
BenefielDaphnia4	*Daphnia*	3	3
BenefielDaphnia6	*Daphnia*	3	3
BenefielDaphnia7	*Daphnia*	2	2
BenefielDaphnia14	*Daphnia*	3	3
BenefielDaphnia16	*Daphnia*	3	3
BenefielCerio13	*Ceriodaphnia*	3	4
BenefielCerio6	*Ceriodaphnia*	4	4
BenefielCerio10	*Ceriodaphnia*	2	2
BenefielCerio1	*Ceriodaphnia*	4	4
BenefielCerio15	*Ceriodaphnia*	4	4
GooseCerioB	*Ceriodaphnia*	4 (6)	4 (2)
GooseCerioA	*Ceriodaphnia*	4 (3)	3 (3)
GooseCerioC	*Ceriodaphnia*	4 (2)	3 (1)
GooseCerioI	*Ceriodaphnia*	3 (6)	3 (2)
GooseCerioJ	*Ceriodaphnia*	3 (2)	3 (1)
GooseDaphniaA	*Daphnia*	1 (1)	1 (1)
GooseDaphniaH	*Daphnia*	1 (5)	1 (4)
GooseDaphniaE	*Daphnia*	1 (0)	1 (1)
GooseDaphniaD	*Daphnia*	0 (0)	0 (1)

Table 4.2. Hierarchical analysis of variance organizing parasite samples by two hierarchical regimes. AMOVA 1 designates host type as highest level followed by state and lake. AMOVA 2 designates state as the highest level followed by lake and host type.

AMOVA 1: Hosts as highest level of hierarchy				
	Observed partition			
Variance component	Variance	% total	P[1]	ϕ-statistics
Between hosts	0.82	34.56	(greater) 0.001	$\phi_{Host-Total}$=0.35
Between states	0.28	11.88	(greater) 0.163	$\phi_{State-Host}$=0.18
Between lakes	0.94	39.54	(greater) 0.001	$\phi_{Lake-State}$=0.74
Within lakes	0.33	14.02	(less) 0.001	$\phi_{Lake-Total}$=0.86
AMOVA 2: Hosts as lowest level of hierarchy				
	Observed partition			
Variance component	Variance	% total	P[1]	ϕ-statistics
Between states	0.10	4.77	(greater) 0.309	$\phi_{State-Total}$=-0.05
Between lakes	1.46	72.39	(greater) 0.018	$\phi_{Lake-State}$=0.76
Between hosts	0.13	6.35	(greater) 0.430	$\phi_{Host-Lake}$=0.28
Within hosts	0.33	16.49	(less) 0.001	$\phi_{Host-Total}$=0.83

[1] The P values are calculated by 999 random permutations of the distance matrix (composed of Prevosti distances) between genotyped parasites. Significance is attained if the observed ϕ-statistic (and variance component) is larger or smaller than it would be by chance (Excoffier et al. 1992).

Figure 4.1. The host species in this study are substantially different in size, which might influence their competence for the parasite. The photograph shows two adult female *Ceriodaphnia dubia* (on top) and one adult female *Daphnia dentifera* (below). Arrows show curves in the gut where spores are most likely to pierce the gut wall (Stewart Merrill and Cáceres 2018; see discussion for more information). Photo Credit: Meghan A. Duffy.

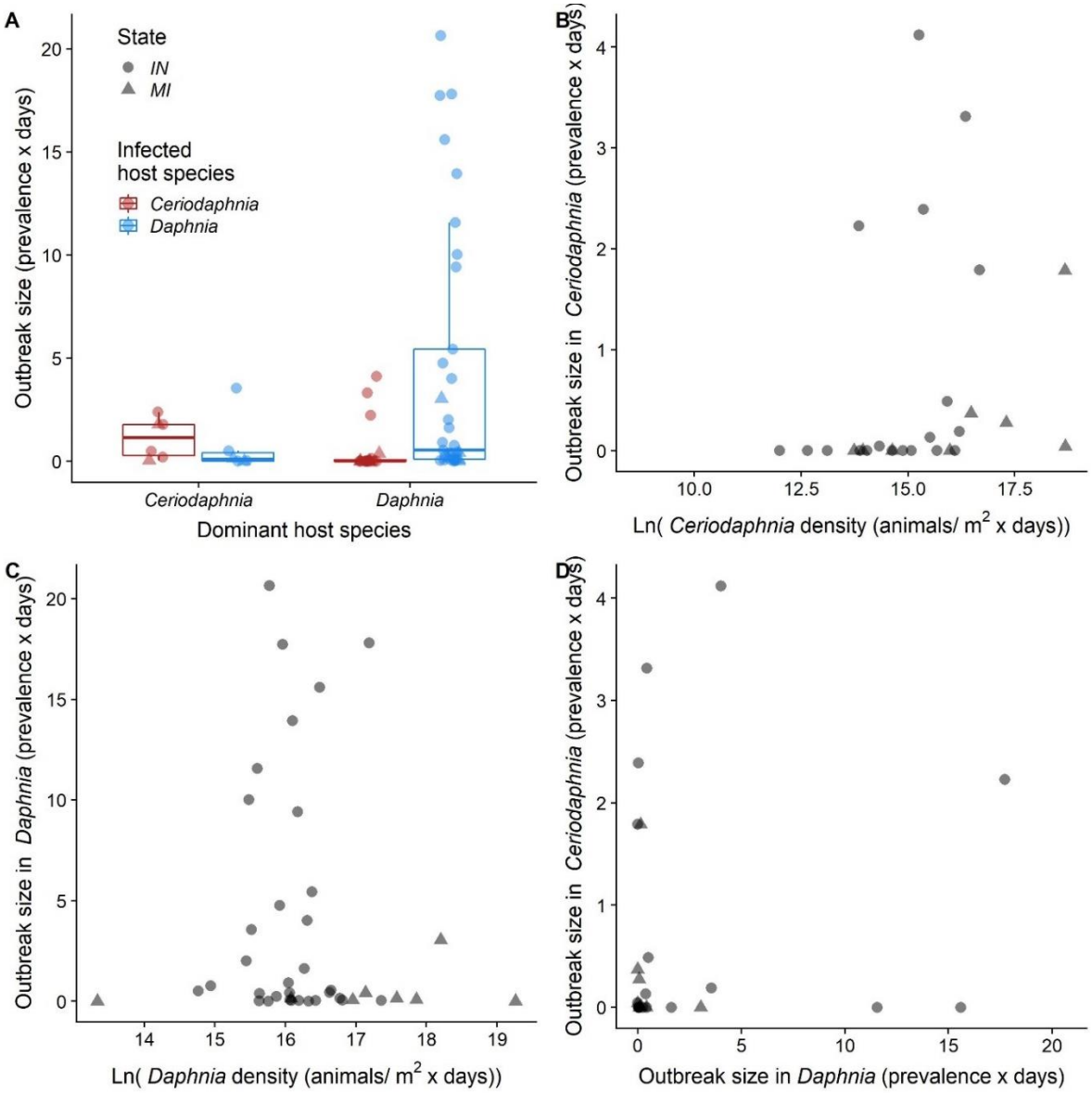

Figure 4.2. Patterns of outbreak size and host density relationships in Indiana and Michigan study lakes in 2015. A) Natural outbreaks were generally larger (in terms of integrated infection prevalence through time) in the dominant host species (i.e., the more abundant host through time). Outbreak size is the time-integrated prevalence in a given host. B) Outbreak size in *Ceriodaphnia* was not related to *Ceriodaphnia* density integrated over the sampling period, C) nor was outbreak size in *Daphnia* related to *Daphnia* density integrated over the sampling period. D) Outbreak sizes in hosts were not associated with each other.

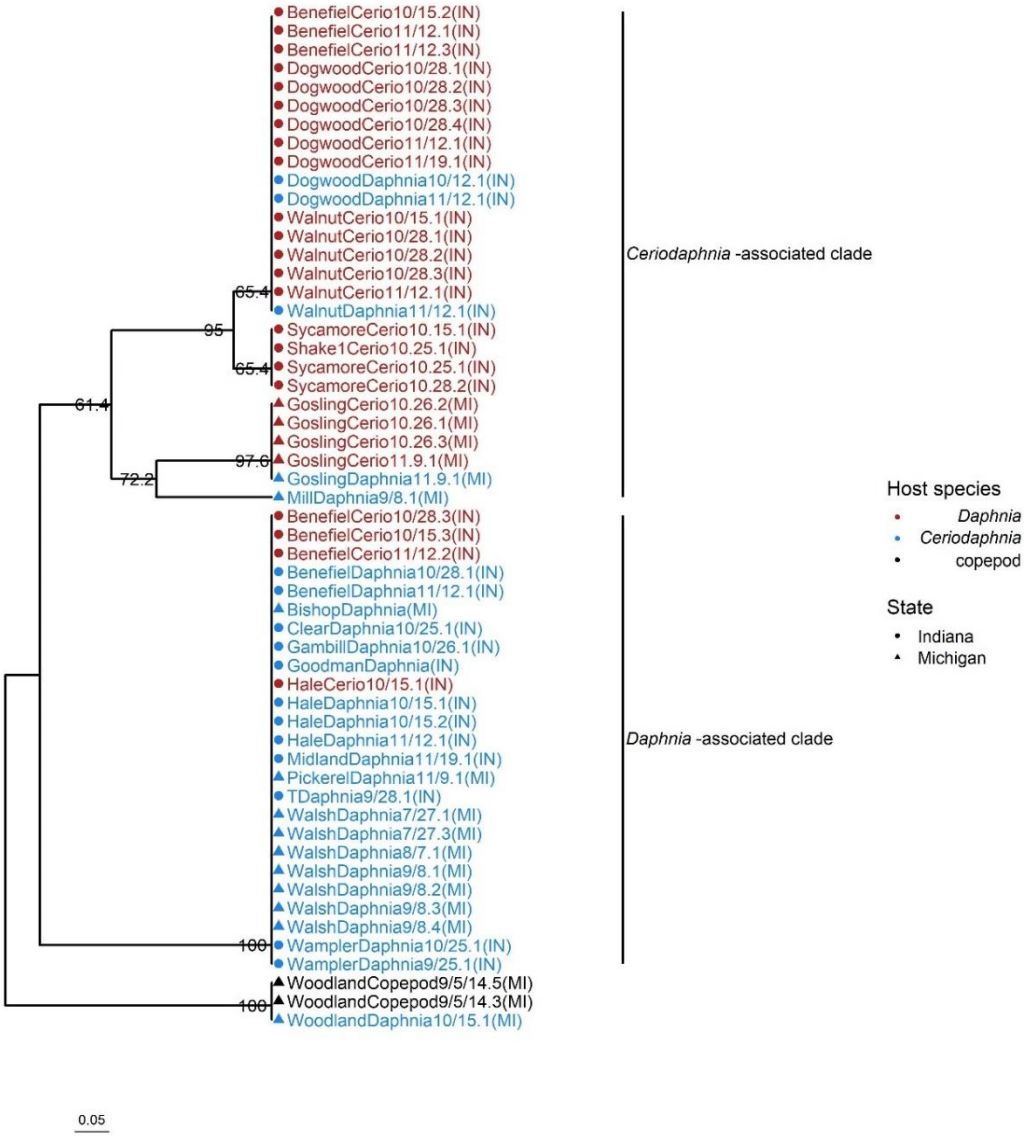

Figure 4.3. Microsatellite genotyping of *Metschnikowia* from infected *Daphnia* and *Ceriodaphnia* collected in fall 2015 in Indiana and Michigan lakes. Genotypes of two copepods collected in fall 2014 are also included. We found two main parasite clades, one associated primarily with *Daphnia* and one associated primarily with *Ceriodaphnia*. Tip labels follow the format LakeHostDate.Replicate(State). See Appendix B Table B1 for a list of samples. Prevosti distance between individuals is noted with distance bar. Bootstrapped support over 50% is documented on the nodes.

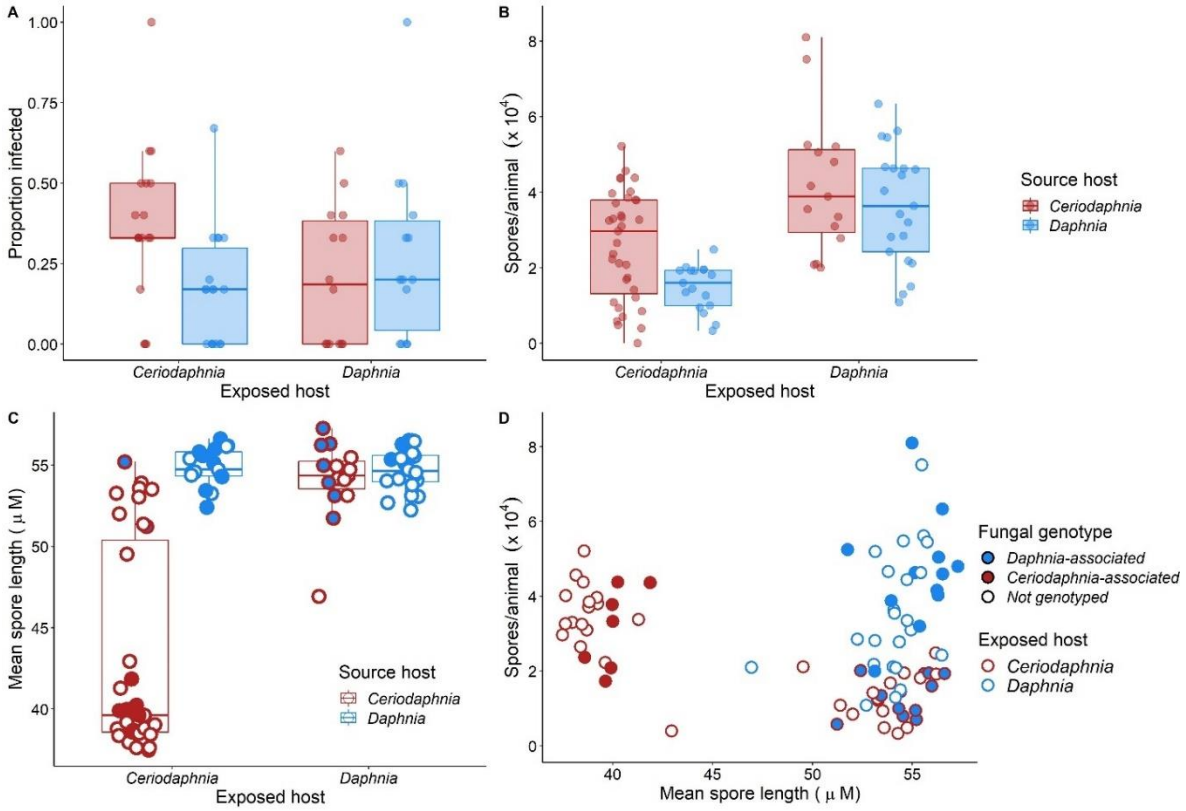

Figure 4.4. The combination of exposed and source hosts mattered for infection and spore production in the Benefiel Lake cross-infection experiment. A) The proportion of infected animals depended on an exposed x source host interaction: *Ceriodaphnia* were most infected by *Ceriodaphnia*-sourced spores. B) More spores were produced overall in *Daphnia* hosts; within *Ceriodaphnia*, more were produced in hosts that were infected with spores sourced from *Ceriodaphnia*. C) Spores in experimentally infected *Ceriodaphnia* were smaller if *Metschnikowia* was sourced from *Ceriodaphnia*. Genotyping showed that the smaller spores belonged to the *Ceriodaphnia*-associated *Metschnikowia* genotype that was found in Benefiel in 2015, and the larger spores belonged to the *Daphnia*-associated *Metschnikowia* genotype. D) *Ceriodaphnia* hosts produced more spores when the spores were smaller. *Daphnia* hosts only became infected by relatively large spores. Within these, animals that had larger spores also tended to produce more spores.

CHAPTER 5

A Common Multihost Parasite Shows Genetic Structuring at the Host Species and Population Level – and Rapid Evolution During Disease Outbreaks.

with Rebecca Bilich, Katherine K. Hunsberger, and Meghan A. Duffy

ABSTRACT

The origins of parasites invading host populations are not always clear, so studying the population structures of widespread, multihost parasites could help evaluate the relative contributions of possible sources of parasite emergence. These possible sources include transmission from other infected populations, spillover from other host species, emergence due to evolutionary changes or a combination of these. Since the genetic structure of parasite populations is determined by ecological and evolutionary dynamics, population genetic studies are useful to understand movement of parasites over space and between host species as well as evolutionary change of parasites within an outbreak. Here, we analyzed population genetic structuring of the genotype-specific parasite, *Pasteuria ramosa*, in infections in Daphniid hosts from different lakes, host species, and at different time points within outbreaks. The parasite showed structuring by host species (if nested within lake), lake (if nested within host species), and by sampling date, though we found the same strain infecting two closely related host species, and we sometimes found the same strain in nearby lakes. This structure reflects host specificity, potential adaptation to or coevolution with host populations in space, and it also indicates that

parasite strain structure is dynamic during outbreaks. To explore the latter phenomenon in greater depth, we sampled two outbreaks more thoroughly in a subsequent year and found that genetic distance between *P. ramosa* populations increased with the time between sampling, again consistent with rapid evolution during parasite outbreaks. Overall, our work supports earlier studies finding that variation in *P. ramosa* is structured at the level of the host population, indicating local adaptation. We also found that different parasite strains tended to circulate in different host species within a lake, indicating adaptation of the parasite to different host species (and barriers to transmission between them). Finally, we found evidence of rapid evolution of *P. ramosa* from multiple natural outbreaks.

INTRODUCTION

Parasite epidemics can be catastrophic for host populations (Skerratt et al. 2007, Blehert et al. 2009, Gostin et al. 2014), however the origins of the parasites that cause them are not always clear. Parasites could invade a host population by transmission from other infected populations (Jousimo et al. 2014) or spillover from other host species (Craft et al. 2009). Parasites could also emerge due to evolutionary changes in infectivity and/or virulence (Morens et al. 2004). Most likely, parasites emerge through a combination of these (and other) factors, but the relative contributions of transmission over space, spillover, and evolution to disease outbreaks are unclear. This knowledge gap should be addressed because improving our understanding of parasite origins could help to predict and prevent outbreaks. Moreover, understanding the constraints underlying each of these emergence mechanisms could help us understand disease risk in complex environments and over time (Betts et al. 2016).

One potential source of disease is the arrival of parasites to a population as they move from one host population to another across a landscape. Given heterogeneities in hosts and the environment, parasites are unevenly distributed across space (Hall et al. 2010, Laine et al. 2011, Gibson et al. 2016, Penczykowski et al. 2018). Dispersal between host populations depends on the parasite's ability to reach the new population, to colonize local host genotypes, as well as to survive in a potentially different microclimate (Ekholm et al. 2017, Penczykowski et al. 2018). Therefore, if parasite populations show strong spatial structure, this could reflect challenges for parasites to disperse over space, infect the host population they encounter, or survive in new microclimates.

Analogous to this, parasites may disperse across time if they can remain viable without a host and later infect a non-contemporary host population (Cieslak and Eitzen 1999, Decaestecker et al. 2004). Though several theoretical studies discuss implications of long lived transmission stages for virulence evolution (Bonhoeffer et al. 1996, Gandon 1998, Kamo and Boots 2004), it is not known how spore banks affect parasite population structure. Storage effects in other systems have been shown to allow for increased diversity and coexistence of competitors (Cáceres 1997, Chesson 2000, Chesson et al. 2004, Lennon and Jones 2011), so parasite dispersal through time might increase diversity within populations and genetic structure between them if founder parasites from spore banks vary over space.

A second source of outbreaks is the transmission of parasites between host species; indeed, these events have caused some of the most devastating epidemics (Daszak et al. 2000). The circumstances leading to spillover epidemics in novel hosts are not always well understood (Lloyd-Smith et al. 2009, Plowright et al. 2017), but most parasites can infect multiple host species (Cleaveland et al. 2001, Pedersen et al. 2005, Poulin et al. 2011), and studying their

transmission across their hosts may give insight into factors that constrain or hinder cross species transmission. An obvious challenge for multihost parasites is that hosts may differ in susceptibility, competence, and defenses (Woolhouse et al. 2001, Power and Mitchell 2004, Auld et al. 2017), imposing different selective forces on parasites (Gandon 2004). However, phylogenetic relatedness of hosts and similarity of within host environments may lower barriers to transmission (Streicker et al. 2010, Longdon et al. 2011, Parker et al. 2015). The genetic structure of parasites across host populations implicates patterns of multihost parasite transmission in the wild: structure by host species indicates barriers to transmission among hosts (Wang et al. 2006) whereas lack of structure indicates frequent transmission between host species (Archie and Ezenwa 2011).

A third potential source of emerging parasites is parasite evolution. Parasite traits such as infectivity and virulence may change over time due to evolution within outbreaks (Day and Gandon 2007, Delaney et al. 2012, Osnas et al. 2015, Cressler et al. 2016). However, few studies have documented parasite evolution in natural epidemics (but see: Fenner and Fantini 1999, Koskella 2014, Park et al. 2015). Parasite evolution could be driven by selection from interacting host populations (Ebert 2008, Koskella 2014) and/or by additional environmental factors (Mitchell et al. 2005, Vale and Little 2009), or evolution could be impacted by genetic drift (Papkou et al. 2016, Kennedy and Dwyer 2018). These forces could affect parasite diversity within parasite populations (Zhu et al. 2000, Carius et al. 2001, Koskella and Lively 2008, Hall et al. 2011) and differentiation between them (Thompson and Cunningham 2002). Thus, changes in genetic structure of parasites infecting a host population indicate evolution of parasite populations.

Outbreaks might incorporate interacting aspects of transmission across space or time, and among hosts, as well as evolutionary change. For example, in many emerging outbreaks, pathogens spill over from co-occurring host species and then evolve to exploit the new host (Fenner and Fantini 1999, Delaney et al. 2012). In addition, pathogens may be introduced from other populations and then evolve to exploit hosts in a new environment (Burdon and Thrall 1999, Koskella 2014). Or, multiple mechanisms might occur at once. For example, after its introduction to the United States, West Nile Virus evolved to transmit more efficiently in new mosquito vectors, and it spread across the country (Kilpatrick and Lyon 2011). By analyzing parasite population structures, we can learn about the relative influences of these different processes, which can give us a better understanding of how parasites transmit over space and between hosts as well as how they change over time in natural epidemics. This synthesis will allow us to better understand situations that lead to emerging infectious diseases.

Here, we studied the population structure of *Pasteuria ramosa*, a wide-spread, multi-host parasite of Daphniid hosts. We investigated whether outbreaks were genetically distinct among lakes, if *P. ramosa* commonly moved between host species, and how *P. ramosa* populations changed over time. We predicted that populations of this parasite would be differentiated by lake (since we assumed transmission between lakes would be low and selection within lakes would be strong). However, we did not know if the parasite would move readily between host species. Finally, though there is some evidence that *P. ramosa* evolution may occur over the course of outbreaks (Auld et al. 2014), we did not know how parasite strain structure would change. Parasite strain diversity could decrease if parasites adapt to the host populations within a lake, or parasite diversity could be maintained if different strains are favored through time due to negative frequency dependent selection and/or by reintroduction from the long-lived spore bank.

We used variable number tandem repeats (VNTRs) to understand the population genetic structure of this parasite in natural outbreaks in multiple hosts species, multiple communities, and through time.

STUDY SYSTEM

The bacterial parasite, *Pasteuria ramosa* infects diverse Daphniid hosts in lakes across North America and Europe. Though this parasite is a genotypic specialist within host species (Carius et al. 2001), it can infect numerous Daphniid species throughout its range (Ebert 2008, Duneau et al. 2011, Luijckx et al. 2014, Auld et al. 2017). Hosts become infected after consuming environmental transmission stages (spores) floating in the water column. Susceptibility is governed by attachment of spores to the host esophagus (Duneau et al. 2011) as well as additional within-host processes that can prevent infection after the attachment step (Luijckx et al. 2014). Resistance to spore attachment is governed by one locus in the host (Routtu and Ebert 2015, Bento et al. 2017), and this locus appears to be maintained across host species (Luijckx et al. 2014). Even so, infection of two host species by the same parasite strain has been reported to be a rare event (Duneau et al. 2011, Luijckx et al. 2014) though one experiment passaged a *P. ramosa* strain through two Daphniid species (Auld et al. 2017). After infection, the parasite castrates its host and propagates itself within the host hemolymph (Ebert et al. 1996). *P. ramosa* is an obligate killer, and spores are only released from decaying host corpses (Ebert et al. 1996). These spores can remain infective for many decades in lake sediments (Decaestecker et al. 2004, 2007). Within an epidemic season, Daphniid hosts reproduce asexually yielding many asexual clutches (Smirnov 2014), only switching to sexual reproduction late in the fall towards the end of epidemics (Duffy et al. 2008, Hite et al. 2017). Sexual offspring are enclosed in

resting eggs that overwinter in sediments. Therefore, host diversity during an epidemic is governed by evolutionary forces acting on standing variation in hosts after sexual offspring hatch in the spring.

METHODS

Eight study lakes in southeastern Michigan (Table 5.1) were sampled every two weeks from mid-July until mid-November by combining 3 plankton tows (using a 12 cm Wisconsin net, 153 μm) from at least 10 m apart at the deepest part of each lake in 2015, and this process was repeated in two lakes in 2017. For infection prevalence metrics, subsamples from the combined tows were taken and all hosts (*Daphnia dentifera*, *Daphnia retrocurva*, *Daphnia parvula*, *Ceriodaphnia dubia*) were counted and diagnosed for *P. ramosa* infection using a dissecting microscope until at least 200 hosts of each species were counted or until the entire sample was processed. Another sample of three combined plankton tows was preserved in 90% ethanol and later subsampled to assess host density. Infected host density was calculated by multiplying infection prevalence by host density at each sample date.

In 2015, we collected infected hosts from the 8 lakes that we sampled. We found that *P. ramosa* strain structure changed dramatically between sample dates within a lake in 2015 outbreaks. Therefore, we collected infected hosts from two lakes (Little Appleton and Crooked P) more intensively in 2017 in order to better track parasite evolution during individual outbreaks. From these lakes, we collected up to 10 infected hosts of one species, *Daphnia dentifera,* from six sample dates, each about two weeks apart. In both years, collected infected animals were placed individually in microcentrifuge tubes in 90% ethanol for preservation. Samples were kept at -20°C until DNA extraction. For DNA extraction, preserved infected

animals were removed from ethanol and placed in sterile microcentrifuge tubes. The mericon bacteria plus DNA extraction kit (Qiagen, Hilden, Germany) was used to extract DNA. The preserved infected animals were vortexed with 200 µl fast lysis buffer with a battery-powered pestle. Once well mixed, emulsions were transferred to bead basher tubes and vortexed for 10 minutes. These tubes were then centrifuged and the supernatant was removed as the DNA sample. DNA was kept at -20°C until PCR. DNA was amplified at 11 VNTR loci (Mouton and Ebert 2008, Andras and Ebert 2013; Table 5.2). The PCR reactions took place in 10 µL volumes of 1X Qiagen multiplex mastermix (QIAGEN, Hilden, Germany), 10 nM forward primer with M13(-21) tail, 400 nM reverse primer, and 400 nM M13(-21) 6FAM labeled forward primer or M13(-21) HEX-labeled forward primer. The labeled primers allowed all loci to be visualized in fragment analysis (Schuelke 2000). Amplification conditions were: 94°C (15 min), then 42 cycles of 94°C (30 s)/ 50°C (30 s)/ 72°C (1 min), and a final extension time at 72°C for 10 min. Following PCR, 1 µl amplified product was diluted in 199 µl molecular grade water and then 1 µl diluted product was added into prepared capillary electrophoresis loading plates (UM DNA sequencing core). In cases where both HEX and 6FAM dyes were used, two distinctly labeled samples (1 µl each) were each diluted in 98 µl molecular grade water and then 1 µl of the diluted combination was added to a well in the prepared capillary electrophoresis loading plates. This allowed visualization of more samples on one plate. Prepared capillary electrophoresis loading plates contained 11 µl Hi-Di formamide and a LIZ500 (or a ROX500) size standard. Fragment analysis was performed by the University of Michigan DNA sequencing core, and fragment lengths were read by the software, GeneMapper (ThermoFisher Scientific).

To quantify parasite genetic structure, we completed diversity and population structure analyses with the Poppr package in R (Kamvar et al. 2014). We excluded one locus (Pr17) from

analyses because it was uniform across hosts. For 2015 data, we filtered our dataset to include samples that amplified at at least 8 out of 10 loci. With this filtered dataset, we condensed multilocus genotypes (MLGs) that were identical at all amplified loci, but that failed to amplify at one or more loci. First, we calculated allelic diversity (number of alleles per locus) and linkage disequilibrium (index of association, I_A) between alleles at different loci. The index of association was calculated on clone-corrected data to determine if parasites were clonal or outcrossing. This metric is defined as $I_A=V_0/V_E-1$ where V_0 is the observed variance of the number of allelic differences between individuals and V_E is the expected variance if there was no linkage disequilibrium between loci. I_A is indistinguishable from 0 if the population is large with random outcrossing (Brown et al. 1980, Smith et al. 1993). Significance of observed linkage disequilibrium was assessed with 999 permutations of the observed alleles. Then, a distance matrix between the condensed MLGs was constructed using Prevosti distance, which is the fraction of allelic differences between two samples out of all loci (Wright 1978). To show relationships among strains, we built a dendrogram from the distance matrix using the unweighted pair group method with arithmetic mean (UPGMA). The tree topology was evaluated by bootstrapping, sampling 100 times to generate support for nodes. Among the 2015 samples, 9 samples amplified two or more alleles for at least one locus. These samples were likely the result of hosts coinfected by multiple *P. ramosa* strains. We thus constructed two datasets: one counting only the alleles with the highest amplification in each sample and one that included all alleles by including two or more MLGs within coinfected animals. The same analyses were completed with both datasets and yielded results that were qualitatively similar. We report results of analyses from the former (one strain per host dataset).

To quantify the extent to which parasite strains clustered by lake, host species, and sample date, we performed a hierarchical analyses of molecular variance (AMOVA) with hosts nested within lake and sample dates nested within host species. Using the distance matrix, AMOVA partitions variation into the hierarchical groups (Excoffier et al. 1992). We then randomly permuted the distance matrix 999 times, each time calculating variance assigned to hierarchical groups to create a null distribution with which to test significance of population structure (Excoffier et al. 1992). A second hierarchical AMOVA was computed with lakes nested within host species and sample dates nested within lakes after consideration of results from the first AMOVA (see results section).

To better understand how genetic structure of *P. ramosa* changes over time, we genotyped the parasite from infected animals from 2017 focusing on outbreaks in two lakes, Little Appleton and Crooked P. Genotype data from the two lakes were analyzed separately in order to examine the change in population structure of *P. ramosa* over time in each lake. In our analysis we again excluded Pr17 and two additional loci, Pr 3 and Pr7, which did not amplify in over one quarter of the 2017 samples (in Crooked P samples, these loci did not amplify in 63.6% and 45.5% of samples respectively). We filtered the dataset to include samples that amplified at at least 6 out of 8 loci. For both lakes, MLGs were condensed if they were identical at all amplified loci. As in 2015, several coinfections were noted in 2017. We again created two datasets for each lake, one using the alleles with the highest amplification in coinfected animals, and another that included two or more MLGs within infected animals. Analysis with this coinfection dataset yielded qualitatively similar results to the dataset with a single strain per host with one exception noted in the results.

A distance matrix between individual samples was calculated with the same methods as used with the 2015 data, and separate AMOVAs were run for each lake to determine if parasite populations structured by sample date. We calculated Nei's gene diversity at each sample date for each lake (Nei 1973). This metric measures the probability that two randomly drawn alleles for a given locus in a population will be different from each other (Nei 1973). We bootstrapped values of Nei's gene diversity, resampling 1000 times and centered confidence intervals around the observed values (Marcon et al. 2012). We used linear models to assess change in gene diversity over time, and we used a t test to compare levels of gene diversity between lakes. We also calculated Prevosti distance (absolute genetic distance) between parasite populations from different sample dates for each lake (Prevosti et al. 1975). This distance metric measures the average difference in allele frequencies over all loci between two populations. Distances between the parasite populations at each date were calculated between populations at every other date. We used a linear model to test if genetic distance between parasite populations was related to the amount of time that had passed between sampling dates. All population genetics calculations were computed using the Poppr package (Kamvar et al. 2014). All statistical tests were performed in R version 3.5.3 (R core team).

RESULTS

We found *P. ramosa*-infected hosts in all eight lakes and in four host species, *Daphnia dentifera*, *Daphnia retrocurva*, *Daphnia parvula*, and *Ceriodaphnia dubia*. In the 93 *P. ramosa* samples we genotyped in 2015, we detected between 3 and 14 alleles at each of 10 loci with an average of 9 alleles per locus and a total of 32 multilocus genoytpes (MLGs), which we consider as distinct parasite strains. The clone corrected index of association was 0.44 (P=0.001),

indicating that *P. ramosa* had a primarily clonal population structure and linkage disequilibrium between alleles at different loci.

P. ramosa strains clustered by lake, host, and sample date. Genetic variation among strains between lakes accounted for 12.8% of the variation (Table 5.3, P=0.063), species within a lake accounted for 25.1% of additional variation (Table 5.3, P=0.021), and sample date within host species accounted for 15.2% of the variation (Table 5.3, P=0.002), indicating marginally significant differences between *P. ramosa* strains in different lakes, significant differences between strains infecting different host species within lakes and significant change in *P. ramosa* strain structure over time during outbreaks (Figure 5.1B). The marginal effect of lake may have been due to finding the same strains in nearby lakes (Crooked W and Cedar; Walsh and Mill; Figure 5.1B). Given this, we performed a second AMOVA with host species as the highest level of hierarchy followed by lake and sample date. Variations between host species as the highest level of hierarchy explained 8.79% of the variation (Table 5.3, P=0.057), but within species, variation between lakes explained 30.5% of the variation (Table 5.3, P=0.002) and variation between sampling dates within a lake explained an additional 14.8% of the variation (Table 5.3, P=0.002). In this alternative analysis, *P. ramosa* strains structured by lake when looking within a host species. While the same strains were most often found infecting the same host species within a lake (explaining significance of host species to structuring of parasite strains in the first AMOVA), in one lake (Mill), the same strains were found in the sister species, *D. retrocurva* and *D. parvula* (Figure 5.1A), potentially explaining the merely marginal significance of host species on parasite population structure in the second AMOVA. Given that the highest level of hierarchy in both analyses only had a marginally significant effect, but structuring within that level was significant, it's unclear which level (lake or host species) is more important for parasite

structuring. We had few lakes with large numbers of *P. ramosa* samples from multiple host species, which could be the cause of this outcome.

Because our analysis of samples collected in 2015 indicated substantial genetic structuring over time within a given lake and host species, we collected and genotyped *P. ramosa* from infected *D. dentifera* from epidemics in 2017 in two lakes, Little Appleton and Crooked P, to better understand how the strain structure of *P. ramosa* changes through an outbreak. The outbreak in Little Appleton was much larger than the outbreak in Crooked P (Figure 5.2A & B). Both allelic and MLG diversity were lower in Little Appleton than in Crooked P with an average of 3.75 alleles per locus in Little Appleton and 6.62 alleles per locus in Crooked P; there were 10 MLGs in Little Appleton (out of 38 samples) compared to 23 MLGs in Crooked P (out of 42 samples). Gene diversity remained flat over time in outbreaks (Little Appleton: $F_{1,4}$=3.34, P=0.14; Crooked P: $F_{1,4}$=0.14, P=0.71) though it was much higher in Crooked P (t=4.28, P=0.004; Figure 5.2C & D). For the outbreak in Little Appleton, parasite genotypes structured by sample date, but this was not the case for the outbreak in Crooked P (Table 5.4). However, if multiple MLGs from coinfected animals were included, genotypes did structure by sample date in this lake (Table 5.4).

For both outbreaks, genetic distance between *P. ramosa* populations at different sampling dates increased with the time between sampling, indicating that parasite populations evolved through time (Little Appleton: $F_{1,13}$=8.01, P=0.014; Crooked P: $F_{1,13}$=22.82, P<0.001; Figure 5.2E & F). For parasites in Little Appleton, this change was driven mostly by the large genetic distance between strains at the beginning of the outbreak and strain composition on the rest of the sampling dates (Figure 5.2E), whereas in Crooked P, genetic distance between populations increased more steadily as time between sampling dates increased (Figure 5.2F).

DISCUSSION

Parasites that cause disease outbreaks can originate from other populations across a landscape (Ekholm et al. 2017, Fellous et al. 2012), spill over from other host species (Daszak et al. 2000), evolve within outbreaks (Fenner and Fantini 1999), or a combination of these mechanisms can occur (Kilpatric and Lyon 2011, Delaney et al. 2012, Burdon and Thrall 1999, Koskella 2014). We studied the genetic structure of parasites in natural outbreaks between lakes, host species, and over time in order to determine the relative importance of these mechanisms to parasite outbreaks. In our study, we asked how populations of the genotype-specific parasite, *P. ramosa*, structured among lakes, host species, and over time in order to understand how this parasite navigates infection of multiple hosts in multiple populations through outbreaks. We found that all levels of hierarchy were significant or marginally significant for the structuring of parasite populations indicating that transmission between lakes and host species is low and that evolutionary processes through time shaped parasite population structure. Our genotyping from two lakes in 2017 also found that parasite populations changed significantly over time with genetic distance between *P. ramosa* populations increasing as time between sampling was greater, supporting rapid parasite evolution within these outbreaks.

We predicted that *P. ramosa* populations would show structuring between lakes for two reasons. First, *P. ramosa* strains are host genotype specific, and successful parasites must "match" local hosts in order to attach to host esophagi and infect (Luijckx et al. 2011, Routtu and Ebert 2015, Bento et al. 2017). Theory predicts that specific parasites are more likely to be locally adapted and differentiated between populations (Barrett et al. 2008). Second, *P. ramosa* spores can survive for decades in sediments (Decaestecker et al. 2007), thus the standing diversity of parasite strains in different lakes stems from outbreaks in previous years, yielding

different founder populations on which evolutionary processes work over the course of outbreaks and over greater time scales (Andras et al. 2018). Our prediction that *P. ramosa* strains would structure by lakes was generally supported, especially when looking within host species (AMOVA 2). Therefore, it was surprising to us that the same parasite strain was found in different lakes. Strains were shared between Walsh and Mill lakes (in *D. retrocurva* and *D. parvula*) and in Cedar and Crooked Lake (in *D. dentifera*). In addition, several strains were shared between Little Appleton and Crooked P in the more intensive 2017 sampling. Walsh, Mill, Cedar, and Crooked P lakes are all within 1.7 miles (2.7 km) of each other, but Little Appleton and Crooked P are about 9 miles (14.5 km) apart. After determining that these shared strains were unlikely to have been due to mistakes during lab work, we concluded that the more intensive genotyping in 2017 may have uncovered more shared strains between more distant lakes than less intensive genotyping in 2015. Much greater distances are easily traversed by waterfowl, which can move parasite spores and invertebrates (Green and Figuerola 2005). It is also possible that host communities in these lakes resemble each other due to long distance dispersal of ephippia by birds and that similar host communities could select for similar parasite assemblages.

We found that *P. ramosa* did not readily infect across certain species barriers but did across others. Previous work has shown that *P. ramosa* spores can attach to multiple host species' esophagi (Duneau et al. 2011, Luijckx et al. 2014), indicating that different host species share resistance and susceptibility alleles. However, in previous studies, the same parasite strain rarely infected two host species (despite attachment) indicating that additional steps in the hosts' resistance pathways operate differently in different host species (Luijckx et al. 2014). Here, we found that parasite strains could move between closely related host species, *D. retrocurva* and *D.*

parvula. These species are sister to each other and separated by less than a million years of evolution (Colbourne and Hebert 1996). If closely related hosts offer a more similar within-host environment and immune response (Longdon et al. 2011), this close phylogenetic relationship may explain how the parasite is able to exploit both. In the lab, we have been successful at infecting *D. parvula* with spores from *D. retrocurva,* and we have moved parasites between another closely related and hybridizing pair, *D. dentifera* and *D. mendotae* (C. D. Gowler & C. L. Shaw unpublished data). Perhaps physiological similarities between closely related species allow for infection of both host types (Parker et al. 2015).

Our study presents new evidence that *P. ramosa* evolves over the course of outbreaks though, at present, we cannot specify a mechanism. Evolution could occur due to shifts in host community structure due to parasitism (Duncan and Little 2007) or other factors (Hu and Tessier 1995, Geedey et al. 1996) or in response to selection from non-host associated factors (e.g. abiotic conditions; Mitchell et al. 2005, Vale and Little 2009, Rogalski et al. in prep). Since *P. ramosa* castrates its host, it prevents the host genotype to which it is infective from producing more progeny, thus limiting the production of more susceptible host genotypes. We might therefore expect signatures of negative frequency dependent selection (Ebert 2008). Though negative frequency dependent selection has been documented over decadal time scales in the *Daphnia-P. ramosa* system (Decaestecker et al. 2007), it's unclear how quickly these dynamics would occur in natural parasite outbreaks. However, Turko et al. (2018) found that *Daphnia* clonal turnover was associated with prevalence of a different *Daphnia* parasite and Wolinska and Spaak (2009) documented decreasing frequencies of the most common host clone between sample dates in parasitized *Daphnia* populations, a phenomenon that did not occur in unparasitized populations. This evidence of change in host populations supports negative

frequency dependent selection during disease outbreaks, suggesting that similar patterns could be found in their interacting parasite populations. However, directional selection within single epidemics could also lead to changes in strain structure. Auld et al. (2014) reported an increase in infectivity and decrease in virulence of *P. ramosa* over the course of an epidemic; this observation could have been due to underlying change in strain structure with or without negative frequency dependent selection, though, based on the design of their study, parasite plasticity (which has been found in a different *Daphnia* parasite; Searle et al. 2015) might also have contributed to the changes observed by Auld et al.

In 2017, we sampled two lakes more extensively to better understand the change in *P. ramosa* strain diversity over time. The lakes we selected had different epidemic trajectories, with Little Appleton having a much larger outbreak than Crooked P. With only two lakes we cannot explore the relationship between host diversity, epidemic size, and changes in parasite diversity, but some patterns in our data stand out as worthy of future investigation. Crooked P had lower host densities, but higher host species diversity than Little Appleton (Duffy et al. unpublished data), which may have kept the outbreak small despite high parasite diversity (Altermatt and Ebert 2008, Ganz and Ebert 2010). In contrast, Little Appleton is very densely populated with only *D. dentifera* hosts (though *Ceriodaphnia dubia* became more common over the course of the outbreak). Perhaps lower host diversity in Little Appleton allowed an explosive outbreak to occur. Evolutionary dynamics in this larger outbreak may have been responsible for lower strain diversity in comparison to the outbreak in Crooked P where strain diversity was higher. Host population diversity can impact epidemic size (Keesing et al. 2006, Lively 2010, King and Lively 2012) and therefore, selection on parasites (Gandon 2004, Day and Gandon 2007). Since selection is more efficient in large populations, low diversity host populations that become

highly infected could drive the evolution of less diverse parasite populations (Zhu et al. 2000). Further exploration of the relationships between host diversity, outbreak size, and changes in parasite diversity could help explain differential patterns of evolution between different outbreaks.

We quantified the genetic structure of the parasite, *P. ramosa*, in infected hosts during natural outbreaks across lakes, host species and over time. We found that parasites structure among lakes and host species indicating that barriers to parasite establishment are high between lakes and host species in this system. However, transmission between lakes might be more common when lakes are close together, and transmission between host species might be more common when host species are closely related phylogenetically. We also found changes in parasite strain structure over time, providing evidence of evolution within outbreaks, potentially acting on parasite diversity introduced from the spore bank. Thus, parasite population structure can implicate ecological and evolutionary forces acting on parasites and further studies of this across natural host-parasite systems could help us better understand and predict parasite outbreaks and evolution.

REFERENCES

Altermatt, F., and D. Ebert. 2008. Genetic diversity of *Daphnia magna* populations enhances resistance to parasites. Ecology Letters 11:918–928.

Andras, J. P., and D. Ebert. 2013. A novel approach to parasite population genetics: experimental infection reveals geographic differentiation, recombination and host-mediated population structure in *Pasteuria ramosa*, a bacterial parasite of *Daphnia*. Molecular Ecology 22:972–86.

Andras, J. P., P. D. Fields, and D. Ebert. 2018. Spatial population genetic structure of a bacterial parasite in close coevolution with its host. Molecular Ecology 27: 1371-1384.

Archie, E. A., and V. O. Ezenwa. 2011. Population genetic structure and history of a generalist

parasite infecting multiple sympatric host species. International Journal for Parasitology 41:89–98.

Auld, S. K. J. R., C. L. Searle, and M. A. Duffy. 2017. Parasite transmission in a natural multihost – multiparasite community. Philosophical Transactions of the Royal Society B 372:1–10.

Auld, S. K. J. R., P. J. Wilson, and T. J. Little. 2014. Rapid change in parasite infection traits over the course of an epidemic in a wild host-parasite population. Oikos 123:232–238.

Barrett, L. G., P. H. Thrall, J. J. Burdon, and C. C. Linde. 2008. Life history determines genetic structure and evolutionary potential of host-parasite interactions. Trends in Ecology and Evolution 23:678–685.

Bento, G., J. Routtu, P. D. Fields, Y. Bourgeois, L. Du, and D. Ebert. 2017. The genetic basis of resistance and matching-allele interactions of a host-parasite system: the *Daphnia magna-Pasteuria ramosa* model. Plos Genetics:1–17.

Betts, A., C. Rafaluk, and K. C. King. 2016. Host and parasite evolution in a tangled bank. Trends in Parasitology 32:863–873.

Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-zier, E. L. Buckles, J. T. H. Coleman, S. R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat white-nose syndrome: An emerging fungal pathogen? Science 323:227.

Bonhoeffer, S., R. E. Lenski, and D. Ebert. 1996. The curse of the pharaoh: The evolution of virulence in pathogens with long living propagules. Proceedings of the Royal Society B: Biological Sciences 263:715–721.

Brown, A. H., M. W. Feldman, and E. Nevo. 1980. Multilocus structure of natural populations of *Hordeum spontaneum*. Genetics 96:523–36.

Burdon, J. J., and P. H. Thrall. 1999. Spatial and temporal patterns in coevolving plant and pathogen associations. The American Naturalist 153:S15–S33.

Cáceres, C. E. 1997. Temporal variation, dormancy, and coexistence: a field test of the storage effect. Proceedings of the National Academy of Sciences 94:9171–9175.

Carius, H. J., T. J. Little, and D. Ebert. 2001. Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection. Evolution 55:1136–1145.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343–366.

Chesson, P., R. L. E. Gebauer, S. Schwinning, N. Huntly, K. Wiegand, M. S. K. Ernest, A. Sher, A. Novoplansky, and J. F. Weltzin. 2004. Resource pulses, species interactions, and

diversity maintenance in arid and semi-arid environments. Oecologia 141:236–253.

Cieslak, T. J., and E. M. Eitzen. 1999. Clinical and epidemiologic principles of Anthrax. Emerging Infectious Diseases 5:552–555.

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London B 356:991–999.

Colbourne, J. K., and P. D. N. Hebert. 1996. The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. Philosophical Transactions of the Royal Society of London B 351:349–360.

Craft, M. E., E. Volz, C. Packer, and L. A. Meyers. 2009. Distinguishing epidemic waves from disease spillover in a wildlife population. Proceedings of the Royal Society B 276:1777–1785.

Cressler, C. E., D. V. McLeod, C. Rozins, J. Van Den Hoogen, and T. Day. 2016. The adaptive evolution of virulence: A review of theoretical predictions and empirical tests. Parasitology 143:915–930.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. Science 287:443–449.

Day, T., and S. Gandon. 2007. Applying population-genetic models in theoretical evolutionary epidemiology. Ecology Letters 10:876–888.

Decaestecker, E., S. Gaba, J. A M. Raeymaekers, R. Stoks, L. Van Kerckhoven, D. Ebert, and L. De Meester. 2007. Host-parasite "Red Queen" dynamics archived in pond sediment. Nature 450:870–873.

Decaestecker, E., C. Lefever, L. De Meester, and D. Ebert. 2004. Haunted by the past: evidence for dormant stage banks of microparasites and epibionts of *Daphnia*. Limnology and Oceanography 49:1355–1364.

Delaney, N. F., S. Balenger, C. Bonneaud, C. J. Marx, G. E. Hill, P. Tsai, A. Rodrigo, and S. V Edwards. 2012. Ultrafast evolution and loss of CRISPRs following a host shift in a novel wildlife pathogen, Mycoplasma gallisepticum. Plos Genetics 8:e1002511.

Duffy, M. A., C. E. Brassil, S. R. Hall, A. J. Tessier, C. E. Cáceres, and J. K. Conner. 2008. Parasite-mediated disruptive selection in a natural *Daphnia* population. BMC Evolutionary Biology 8:80.

Duncan, A. B., and T. J. Little. 2007. Parasite-driven genetic change in a natural population of *Daphnia*. Evolution 61:796–803.

Duneau, D., P. Luijckx, F. Ben-Ami, C. Laforsch, and D. Ebert. 2011. Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host-parasite interactions. BMC Biology 9:11.

Ebert, D. 2008. Host-parasite coevolution: insights from the *Daphnia*-parasite model system. Current Opinion in Microbiology 11:290–301.

Ebert, D., P. Rainey, T. M. Embley, D. Scholz, P. Rainey, T. M. Embley, and D. Scholz. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. Philosophical Transactions of the Royal Society of London B 351:1689–1701.

Ekholm, A., T. Roslin, P. Pulkkinen, and A. J. M. Tack. 2017. Dispersal, host genotype and environment shape the spatial dynamics of a parasite in the wild. Ecology 98:2574–2584.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Fellous, S., Duncan, A. B., Quillery, E., Vale, P. F., Kaltz, O. 2012. Genetic influence on disease spread following arrival of infected carriers. Ecology Letters 15: 186-192.

Faust, C. L., H. I. McCallum, L. S. P. Bloomfield, N. L. Gottdenker, T. R. Gillespie, C. J. Torney, A. P. Dobson, and R. K. Plowright. 2018. Pathogen spillover during land conversion. Ecology Letters 21:471–483.

Fenner, F., and B. Fantini. 1999. Biological control of vertebrate pests: The history of Myxomatosis - an experiment in evolution. 2nd edition. CABI Publishing, New York.

Gandon, S. 1998. The curse of the pharaoh hypothesis. Proceedings of the Royal Society B: Biological Sciences 265:1545–1552.

Gandon, S. 2004. Evolution of multihost parasites. Evolution 58:455–469.

Ganz, H. H., and D. Ebert. 2010. Benefits of host genetic diversity for resistance to infection depend on parasite diversity. Ecology 91:1263–1268.

Geedey, C. K., A. J. Tessier, and K. Machledt. 1996. Habitat heterogeneity, environmental change, and the clonal structure of *Daphnia* populations. Functional Ecology 10:613–621.

Gibson, A. K., J. Jokela, and C. M. Lively. 2016. Fine-scale spatial covariation between infection prevalence and susceptibility in a natural population. The American Naturalist 188:1–14.

Gostin, L. O., D. Lucey, and A. Phelan. 2014. The Ebola epidemic: A global health emergency. Journal of the American Medical Association 312:1095–1096.

Green, A. J., and J. Figuerola. 2005. Recent advances in the study of long-distance dispersal of aquatic invertebrates via birds. Diversity and Distributions 11:149–156.

Hall, A. R., P. D. Scanlan, A. D. Morgan, and A. Buckling. 2011. Host – parasite coevolutionary arms races give way to fluctuating selection. Ecology Letters 14:635–642.

Hall, S. R., R. Smyth, C. R. Becker, M. A. Duffy, C. J. Knight, S. MacIntyre, A. J. Tessier, and C. E. Cáceres. 2010. Why Are *Daphnia* in some lakes sicker? Disease ecology, habitat structure, and the plankton. BioScience 60:363–375.

Hite, J. L., R. M. Penczykowski, M. S. Shocket, K. A. Griebel, A. T. Strauss, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2017. Allocation, not male resistance, increases male frequency during epidemics: a case study in facultatively sexual hosts. Ecology 98:2773–2783.

Hu, S. S., and A. J. Tessier. 1995. Seasonal succession and strength of intra- and interspecific competition in a Daphnia assemblage. Ecology 76:2278–2294.

Jousimo, J., A. J. M. Tack, O. Ovaskainen, T. Mononen, H. Susi, C. Tollenaere, and A. L. Laine. 2014. Ecological and evolutionary effects of fragmentation on infectious disease dynamics. Science 344:87–90.

Kamo, M., and M. Boots. 2004. The curse of the pharaoh in space: free-living infectious stages and the evolution of virulence in spatially explicit populations. Journal of Theoretical Biology 231:435–441.

Kamvar, Z. N., J. F. Tabima, and N. J. Grunwald. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:1–14.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–498.

Kennedy, D. A., and G. Dwyer. 2018. Effects of multiple sources of genetic drift on pathogen variation within hosts. PLoS Biology 16:1–17.

Kilpatrick, A. M., and B. Lyon. 2011. Globalization, land use, and the invasion of West Nile Virus. Science 334:323–328.

King, K. C., and C. M. Lively. 2012. Does genetic diversity limit disease spread in natural host populations? Heredity 109:199–203.

Koskella, B. 2014. Bacteria-Phage interactions across time and space: merging local adaptation and time-shift experiments to understand phage evolution. The American Naturalist 184:S9–S21.

Koskella, B., and C. M. Lively. 2008. Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. Evolution

63:2213–2221.

Laine, A. L., J. J. Burdon, P. N. Dodds, and P. H. Thrall. 2011. Spatial variation in disease resistance: From molecules to metapopulations. Journal of Ecology 99:96–112.

Lennon, J. T., and S. E. Jones. 2011. Microbial seed banks: The ecological and evolutionary implications of dormancy. Nature Reviews Microbiology 9:119–130.

Lively, C. M. 2010. The effect of host genetic diversity on disease spread. The American Naturalist 175:E149–E152.

Lloyd-Smith, J. O., D. George, K. M. Pepin, V. E. Pitzer, J. R. C. Pulliam, A. P. Dobson, P. J. Hudson, and B. T. Grenfell. 2009. Epidemic dynamics at the human-animal interface. Science 326:1362–1367.

Longdon, B., J. D. Hadfield, C. L. Webster, D. J. Obbard, and F. M. Jiggins. 2011. Host phylogeny determines viral persistence and replication in novel hosts. PLoS Pathogens 7: e1002260.

Luijckx, P., F. Ben-Ami, L. Mouton, and D. Ebert. 2011. Cloning of the unculturable parasite *Pasteuria ramosa* and its *Daphnia* host reveals extreme genotype – genotype interactions. Ecology Letters 14:125–131.

Luijckx, P., D. Duneau, J. P. Andras, and D. Ebert. 2014. Cross-species infection trials reveal cryptic parasite varieties and a putative polymorphism shared among host species. Evolution 68:577–86.

Marcon, E., B. Hérault, C. Baraloto, and G. Lang. 2012. The decomposition of Shannon's entropy and a confidence interval for beta diversity. Oikos 121:516–522.

Mitchell, S. E., E. S. Rogers, T. J. Little, and A. F. Read. 2005. Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. Evolution 59:70–80.

Morens, D. M., G. K. Folkers, and A. S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. Nature 430:242–249.

Mouton, L., and D. Ebert. 2008. Variable-number-of-tandem-repeats analysis of genetic diversity in *Pasteuria ramosa*. Current Microbiology 56:447–452.

Mouton, L., G. Nong, J. F. Preston, and D. Ebert. 2007. Variable-number tandem repeats as molecular markers for biotypes of *Pasteuria ramosa* in *Daphnia* spp. Applied and Environmental Microbiology 73:3715–3718.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70:3321–3323.

Osnas, E. E., P. J. Hurtado, and A. P. Dobson. 2015. Evolution of pathogen virulence across space during an epidemic. The American Naturalist 185:332–342.

Papkou, A., C. S. Gokhale, A. Traulsen, and H. Schulenburg. 2016. Host–parasite coevolution: why changing population size matters. Zoology 119:330–338.

Park, D. J., G. Dudas, S. Wohl, A. Goba, S. L. M. Whitmer, K. G. Andersen, R. S. Sealfon, J. T. Ladner, J. R. Kugelman, C. B. Matranga, S. M. Winnicki, J. Qu, S. K. Gire, A. Gladden-Young, S. Jalloh, D. Nosamiefan, N. L. Yozwiak, L. M. Moses, P. P. Jiang, A. E. Lin, S. F. Schaffner, B. Bird, J. Towner, M. Mamoh, M. Gbakie, L. Kanneh, D. Kargbo, J. L. B. Massally, F. K. Kamara, E. Konuwa, J. Sellu, A. A. Jalloh, I. Mustapha, M. Foday, M. Yillah, B. R. Erickson, T. Sealy, D. Blau, C. Paddock, A. Brault, B. Amman, J. Basile, S. Bearden, J. Belser, E. Bergeron, S. Campbell, A. Chakrabarti, K. Dodd, M. Flint, A. Gibbons, C. Goodman, J. Klena, L. McMullan, L. Morgan, B. Russell, J. Salzer, A. Sanchez, D. Wang, I. Jungreis, C. Tomkins-Tinch, A. Kislyuk, M. F. Lin, S. Chapman, B. Macinnis, A. Matthews, J. Bochicchio, L. E. Hensley, J. H. Kuhn, C. Nusbaum, J. S. Schieffelin, B. W. Birren, M. Forget, S. T. Nichol, G. F. Palacios, D. Ndiaye, C. Happi, S. M. Gevao, M. A. Vandi, B. Kargbo, E. C. Holmes, T. Bedford, A. Gnirke, U. Ströher, A. Rambaut, R. F. Garry, and P. C. Sabeti. 2015. Ebola Virus epidemiology, transmission, and evolution during seven months in Sierra Leone. Cell 161:1516–1526.

Parker, I. M., M. Saunders, M. Bontrager, A. P. Weitz, R. Hendricks, R. Magarey, K. Suiter, and G. S. Gilbert. 2015. Phylogenetic structure and host abundance drive disease pressure in communities. Nature 520:542–544.

Pedersen, A. B., S. Altizer, M. Poss, A. A. Cunningham, and C. L. Nunn. 2005. Patterns of host specificity and transmission among parasites of wild primates. International Journal for Parasitology 35:647–657.

Penczykowski, R. M., S. R. Parratt, B. Barres, S. K. Sallinen, and A. L. Laine. 2018. Manipulating host resistance structure reveals impact of pathogen dispersal and environmental heterogeneity on epidemics. Ecology 99:2853–2863.

Plowright, R. K., C. R. Parrish, H. Mccallum, P. J. Hudson, A. I. Ko, A. L. Graham, and J. O. Lloyd-Smith. 2017. Pathways to zoonotic spillover. Nat Rev Microbiol 15:502–510.

Poulin, R., B. R. Krasnov, and D. Mouillot. 2011. Host specificity in phylogenetic and geographic space. Trends in Parasitology 27:355–361.

Power, A. G., and C. E. Mitchell. 2004. Pathogen spillover in disease epidemics. The American Naturalist 164:S79–S89.

Prevosti, A., J. Ocaña, and G. Alonso. 1975. Distances between populations of *Drosophila subobscura*, based on chromosome arrangement frequencies. Theoretical and Applied Genetics 45:231–241.

Routtu, J., and D. Ebert. 2015. Genetic architecture of resistance in *Daphnia* hosts against two species of host-specific parasites. Heredity 114:241–248.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233–234.

Searle, C. L., J. H. Ochs, C. E. Cáceres, S. L. Chiang, and N. M. Gerardo. 2015. Plasticity, not genetic variation, drives infection success of a fungal parasite. Parasitology 142:839–848.

Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. Mcdonald, A. D. Phillott, H. B. Hines, and N. Kenyon. 2007. Spread of Chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

Smirnov, N. N. 2014. Physiology of the Cladocera. Elsevier.

Smith, J. M., N. H. Smith, M. O'Rourke, and B. G. Spratt. 1993. How clonal are bacteria? Proceedings of the National Academy of Sciences 90:4384–4388.

Streicker, D. G., A. S. Turmelle, M. J. Vonhof, I. V Kuzmin, G. F. Mccracken, and C. E. Rupprecht. 2010. Host phylogeny constrains cross-species emergence and establishment of rabies virus in bats. Science 329:676–679.

Thompson, J. N., and B. M. Cunningham. 2002. Geographic structure and dynamics of coevolutionary selection. Nature 417:735–738.

Turko, P., C. Tellenbach, E. Keller, N. Tardent, B. Keller, P. Spaak, and J. Wolinska. 2018. Parasites driving host diversity: incidence of disease correlated with *Daphnia* clonal turnover. Evolution 72:619–629.

Vale, P. F., and T. J. Little. 2009. Measuring parasite fitness under genetic and thermal variation. Heredity 103:102–109.

Wang, T. P., J. Shrivastava, M. V. Johansen, S. Q. Zhang, F. F. Wang, and J. P. Webster. 2006. Does multiple hosts mean multiple parasites? Population genetic structure of *Schistosoma japonicum* between definitive host species. International Journal for Parasitology 36:1317–1325.

Wolinska, J., and P. Spaak. 2009. The cost of being common: evidence from natural *Daphnia* populations. Evolution 63:1893–1901.

Woolhouse, M. E. J., L. H. Taylor, D. T. Haydon, M. E. J. Woolhouse, L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science 292:1109–1112.

Wright, S. 1978. Evolution and the Genetics of Populations. Volume 4. University of Chicago Press, Chicago, IL.

Zhu, Y., H. Chen, J. Fan, Y. Wang, Y. Li, J. Chen, J. Fan, S. Yang, L. Hu, H. Leung, T. W. Mew, P. S. Teng, Z. Want, and C. C. Mundt. 2000. Genetic diversity and disease control in rice. Nature 406:718–722.

Table 5.1. Lake names and locations.

Lake	Michigan Township	Lat, Long
Bishop	Hamburg Township	42.501259, -83.839804
Cedar	Sylvan Township	42.314426, -84.077480
Crooked P[1]	Dexter Township	42.420654, -83.982422
Crooked W	Sylvan Township	42.326613, -84.111816
Gosling	Putnam Township	42.439565, -84.003322
Little Appleton	Hamburg Township	42.506705, -83.838634
Mill	Sylvan Township	42.329787, -84.090868
North	Dexter Township	42.393928, -84.006628
Walsh	Sylvan Township	42.337922, -84.080098

[1]Samples from Crooked P were only collected in 2017 whereas samples from all other lakes were collected in 2015.

Table 5.2. Forward and reverse primers used to genotype each locus. Forward primers all begin with the M13(-21) sequence (first 18 base pairs), allowing binding with a fluorescently labeled (6FAM or HEX) M13 primer (Schuelke 2000).

Locus	Forward (5'-3')	Reverse (5'-3')
Pr 1[1]	TGTAAAACGACGGCCAGTACCTAAAGAACAG GAATATCTGGA	GCATGGAATGATTTTTGCTG
Pr 2[1]	TGTAAAACGACGGCCAGTCTGCTGGATGGAT GGACTACGTGA	ACCGGTCCCGTAGGTATAGG
Pr 3[1]	TGTAAAACGACGGCCAGTGGACCAATCGAAC CAGGTAT	AACGGTTTCTTCGCTTGTTG
Pr 4[1]	TGTAAAACGACGGCCAGTGGTAACCCTGGAT GTCCTGA	ATCCCGTTACAAATGGGACA
Pr 7[1]	TGTAAAACGACGGCCAGTAACGTACTGACAA ACCAAACCA	AATTTTTCTTAGATTGCTAGGTT G
Pr 11[1]	TGTAAAACGACGGCCAGTCAAGCCAAATAAA CGCATCC	TAGCGAAGAACACCAACGTG
Pr 12[1]	TGTAAAACGACGGCCAGTTCTTTAGTAGTTGC TTTGCTTGAA	AACATCTTGGCACCCCTTTA
Pr 16[1]	TGTAAAACGACGGCCAGTGGCAGGAACAAAA ATTAAGCA	CGTTCCAAAGCGTTTTATGG
Pr 17[2]	TGTAAAACGACGGCCAGTCACACACTTGCTCC ATGGTC	AAACTAGATAGCGAAAAA
Pr 18[2]	TGTAAAACGACGGCCAGTAAAGAAAGCTTCG TTTTAACGTG	CATTATCCACCCCCAAATCA
Pr 19[2]	TGTAAAACGACGGCCAGTACGACCCAATCCG TTGATAG	CCAAGGCACGTTAGAAGAAA

[1] Reported in Mouton et al. 2007; [2] Reported in Andras and Ebert 2013.

Table 5.3. Hierarchical analysis of variance organizing parasite samples by two hierarchical regimes. AMOVA 1 designates lake as the highest level followed host species and sample date. AMOVA 2 designates host species as the highest level followed by lake and sample date.

AMOVA 1: Lakes species as highest level of hierarchy				
Variance component	Variance	% total	P[1]	ϕ-statistics
Between lakes	0.93	12.77	(greater) 0.063	$\phi_{Lake-Total}$=0.13
Between host species	1.82	25.08	(greater) 0.021	$\phi_{Host-Lake}$=0.29
Between dates	1.10	15.16	(greater) 0.002	$\phi_{Date-Host}$=0.24
Within dates	3.42	46.99	(less) 0.001	$\phi_{Date-Total}$=0.53
AMOVA 2: Host species as highest level of hierarchy				
Variance component	Variance	% total	P[1]	ϕ-statistics
Between host species	0.65	8.79	(greater) 0.057	$\phi_{Host-Total}$=0.09
Between lakes	2.27	30.51	(greater) 0.002	$\phi_{Lake-Host}$=0.33
Between dates	1.10	14.81	(greater) 0.002	$\phi_{Date-Lake}$=0.24
Within dates	3.42	45.89	(less) 0.001	$\phi_{Date-Total}$=0.54

[1] The P values are calculated by 999 random permutations of the distance matrix (composed of Prevosti distances) between genotyped parasites. Significance is attained if the observed ϕ-statistic (and variance component) is greater or smaller than it would be by chance (Excoffier et al. 1992).

Table 5.4. Results of AMOVA on structure over time in lakes in 2017.

	Variance	Percent of total variance	P value[1]	ϕ
Little Appleton	Between dates: 0.61 Within dates: 2.23	Between dates: 21.47% Within dates: 78.53%	P=0.001 (greater)	$\phi_{dates\text{-}total}$=0.21
Crooked P	Between dates: 0.29 Within dates: 6.16	Between dates: 4.36% Within dates: 95.64%	P=0.108 (greater)	$\phi_{dates\text{-}total}$=0.044
Crooked P including coinfections	Between dates: 0.44 Within dates: 6.13	Between dates: 6.65% Within dates: 93.35%	P=0.03 (greater)	$\phi_{dates\text{-}total}$=0.066

[1]P value based on comparison of variance components with null distribution created with 999 random permutations of the distance matrix.

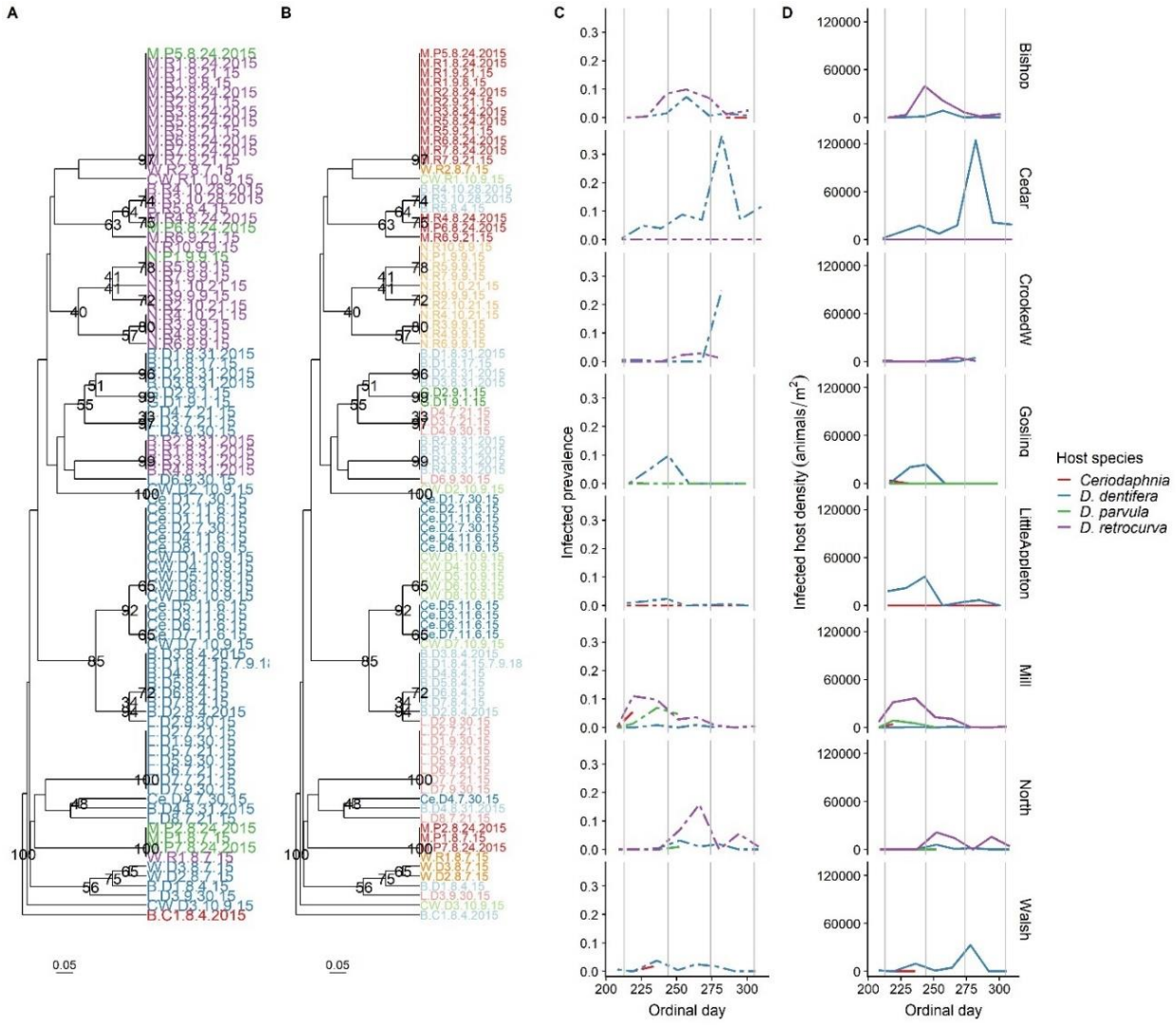

Figure 5.1: In natural outbreaks, *P. ramosa* strains clustered by lake, host species and by sampling date. (A) Dendrogram of *P. ramosa* isolates colored by host species (purple: *D. retrocurva**, blue: *D. dentifera*, green: *D. parvula**, red: *Ceriodaphnia*). Samples are named with the scheme: LakeCode.SpeciesCodeSampleNumber.SampleDate. Lake Codes are M=Mill Lake, CW= Crooked Lake (Waterloo), B=Bishop Lake, L=Little Appleton Lake, G=Gosling Lake, Ce=Cedar Lake, N=North Lake, W=Walsh Lake. Species codes are R=*D. retrocurva*, D=*D. dentifera*, P=*D. parvula*. **D. retrocurva* and D. *parvula* are sister species (Colbourne and Hebert 1996). Bootstrap support above 30% is shown on nodes. (B) Dendrogram of *P. ramosa* isolates colored by lake. (C) *P. ramosa* prevalence dynamics in above lakes in 2015. From left to right, grey lines indicate Aug 1, Sept 1, Oct 1, and Nov 1. (D) Density of hosts infected by *P. ramosa* in each lake in 2015.

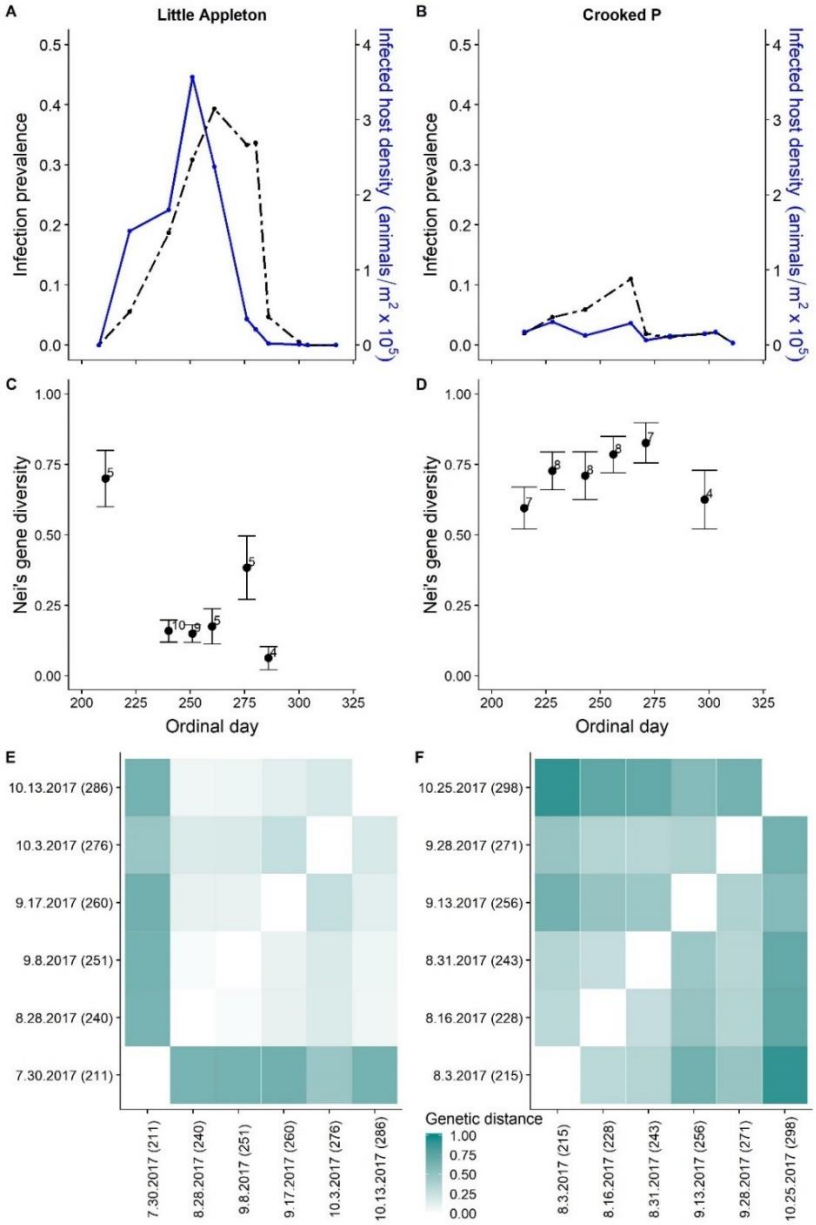

Figure 5.2. Epidemic size differences in Little Appleton and Crooked P may be associated with patterns of gene diversity and genetic distance between parasite populations over time within outbreaks. Outbreak size was much larger in Little Appleton than in Crooked P both in terms of (A, B) infection prevalence (left axis, dashed black line) and infected host density (right axis, solid blue line). There was no clear pattern of changing gene diversity over time in C) Little Appleton or D) Crooked P. Though gene diversity was comparable between the lakes early in each outbreak, it decreased in Little Appleton and remained high in Crooked P; this yielded higher gene diversity in Crooked P when all sample dates were considered. Sample sizes are noted next to points; error bars show centered bootstrapped 95% confidence intervals. Prevosti distance between *P. ramosa* strain populations increased with the amount of time between sampling dates for both lakes. E) For Little Appleton, this pattern is driven mostly by the difference between the strains at the beginning of the season and those from later in the season, F) whereas for Crooked P, genetic distance between populations increased steadily with time between sampling dates. Ordinal date is noted in parentheses. Note that Nei's gene diversity (C,D) is calculated within populations and measures the probability that two randomly drawn alleles from within a population will be different from each other (Nei 1973), while genetic distance (E,F) is calculated between populations and is the average difference in allele frequencies between the populations summed over all loci (Prevosti et al. 1975).

CHAPTER 6

Conclusions

SUMMARY

In this dissertation, I studied drivers of parasite epidemics in *Daphnia* hosts. Since host-parasite interactions are embedded in dynamic environments and complex webs of interacting species (Miner et al. 2012, Cáceres et al. 2014), I aimed to better understand factors that modulate epidemic timing, size, and patterns across a landscape. *Daphnia* are important zooplanktonic grazers in lake food webs (Lampert 1997), therefore disease in these hosts could have cascading effects through ecosystems, impacting host diversity (Duffy et al. 2008, Wolinska and Spaak 2009), densities of interacting species (Duffy 2007), and potentially ecosystem processes and nutrient cycling in lakes (Cáceres et al. 2014).

I studied the survival and success of the transmission stages of two environmentally transmitted parasites, *Pasteuria ramosa* and *Metschnikowia bicuspidata*. Transmission stages of these parasites are vulnerable to various damaging abiotic conditions (Overholt et al. 2012, Shocket et al. 2018), at the mercy of physical forces in lakes (Hall et al. 2010), and threatened by contacts with non-susceptible hosts (Hall et al. 2009, Strauss et al. 2015). Studying when and how parasites overcome these challenges allows us to understand factors that constrain or unleash epidemics.

First, I considered how parasite transmission stages are impacted by one abiotic hazard: light exposure in lakes. Second, I evaluated a jointly physical and biological challenge for these

parasites: contacting susceptible hosts in lakes where hosts can select habitat and parasites cannot. Third, I focused on the challenge of infecting hosts in a multihost environment. Finally, I studied which transmission barriers are important for parasite establishment across a landscape and among host species as well as how parasite populations change over time within epidemics. Together, my work centers on understanding challenges parasite transmission stages encounter and conditions that allow for parasites to complete their lifecycle and create epidemics in host populations.

I used a combination of approaches to answer the questions outlined above. Each study was based on observations of natural epidemics which take place in late summer and fall in Midwestern lakes. I took advantage of differences between lakes (e.g. differences in lake clarity and differences in host species composition) to test statistically how these conditions impact natural epidemic dynamics. I also used field incubations and laboratory infection experiments to test my hypotheses, and I used molecular methods (microsatellite and VNTR genotyping and quantitative PCR) to document the population structure of parasites and to quantify parasite DNA in water samples. This combination of approaches allowed me to study both parasite traits that modulate infection as well as patterns of infection across a landscape.

Taken together, my work indicates that parasite transmission stages navigate challenges within lakes and when moving between lakes and host species. These challenges affect epidemic dynamics and shape the distribution of parasites across a landscape. Below I summarize and connect my studies and point to future directions

Chapter 2. Shedding Light on Environmentally Transmitted Parasites: Within-Lake Light Conditions Affect Epidemic Dynamics.

Light is essential in aquatic communities as it is the source of the energy for aquatic food webs. However, light (particularly UV radiation) can be damaging to organisms (Häder et al. 2015). In this chapter, I explored the effects of ambient within-lake light (driven by lake clarity, which is influenced by concentrations of dissolved organic carbon (DOC), depth, and season) on parasite infectivity. I related this to epidemic size and timing of two parasites, *Pasteuria* and *Metschnikowia*. I found that the parasites are both sensitive to light when exposed within lakes. This sensitivity waned throughout the fall for *Pasteuria* as ambient light decreased, but sensitivity in *Metschnikowia* persisted into late autumn. Patterns of natural epidemic start dates were consistent with this differential sensitivity: *Pasteuria* epidemics began earlier in the autumn than epidemics of *Metschnikowia*. In addition, lake clarity was an important modulator of epidemic size for *Pasteuria* as epidemics grew larger in darker lakes. However, I did not find the same pattern for more sensitive *Metschnikowia*. In fact, contrary to expectations, I found that epidemics of *Metschnikowia* started earlier in clearer lakes. The hypotheses I outline in Chapter 2 to explain this pattern are interesting avenues for future research and might explain differences in epidemic patterns between *Pasteuria* and *Metschnikowia*. The first is that clearer lakes have deeper thermoclines (Fee et al. 1996), so though light penetrates deeper, epilimnetic spores could still gain refuge with depth in clearer lakes. The other hypothesis is that *Metschnikowia* might compensate for high spore mortality with gains in infections powered by a lack of host specificity (in comparison to *Pasteuria*) and reasonable spore yields from infected hosts. Future work could address these hypotheses to better predict when lake clarity should be important and cases when it is not.

In general, a more complete understanding of the effects of DOC on lake chemistry and species interactions is called for. DOC attenuates light (Morris et al. 1995), but it also impacts lake physical structure (Fee et al. 1996, Strock et al. 2017), chemistry (Robidoux et al. 2015), and trophic status (Creed et al. 2018, Fitch et al. 2018, Mariash et al. 2018). Thus, through these routes, DOC has additional potential to impact *Daphnia*, parasites, and their interactions. These impacts will be important to understand as climate driven lake browning intensifies across midwestern lakes (Williamson et al. 2015, Solomon 2017).

Chapter 3: How do Animals Balance Multiple Risks in Dangerous Habitats? Quantifying the Distributions of Daphniids, Their Predators, and Their Parasites in Stratified Lakes

Animals modify their habitat use balancing risks (such as predation and damaging abiotic conditions) with beneficial conditions (such as access to resources and mates). Though extensive previous research has documented the importance of these forces for habitat selection (e.g., Gliwicz and Pijanowska 1988, Leibold and Tessier 1991, Winder et al. 2004, Laundré et al. 2010), more recent research has drawn attention to the importance of parasites for host habitat selection behaviors (Weinstein et al. 2018a). In this chapter, I quantified the vertical distribution of parasite spores in the water columns of three lakes and at two points during epidemics. I found that the parasites were unevenly distributed throughout the water columns of these lakes and that host migration behavior (which was influenced by light, *Chaoborus* predators, and water temperature) should impact exposure to parasite spores. Thus, it is possible that habitat selection behavior and the amount of spatial overlap between hosts and parasites could affect the size of parasite epidemics and which host species succumb.

Future work could address how parasites get distributed in water columns by measuring physical and biological processes as well as factors such as lake basin shape and depth that might influence parasite distributions. Connecting these factors with more frequent assessments of parasite distributions could help elucidate the forces behind the uneven parasite distributions that I observed. A link between chapters 2 and 3 could focus on circumstances when spores are most at risk of light damage. Better understanding how weather conditions could hinder or unleash epidemics might allow us to explain variability in epidemic patterns from one year to the next.

Chapter 4: Asymmetric Interspecific Disease Transmission Modulated by Parasite Spore Size: Parasite Traits Help Explain Host Breadth in a Virulent Fungal Pathogen.

Many parasites infect multiple host types (Cleaveland et al. 2001, Lloyd-Smith et al. 2009, Viana et al. 2014), but theory suggests that this should be difficult as hosts differ in parasite competence and mount divergent defenses (Futuyma and Moreno 1988). In Chapter 4, I documented the population structure of the generalist parasite, *Metschnikowia*, across two host species and between Michigan and Indiana metapopulations. I found that *Metschnikowia* infects host species that are distantly related phylogenetically, but that parasite genotypes tended to specialize on the two host types in the study, *Daphnia* and *Ceriodaphnia*. In a cross-infection experiment using parasites gathered from a lake with both genotypes, I found that spores sourced from smaller *Ceriodaphnia* hosts were both more infective to and produced more spores within exposed *Ceriodaphnia* than spores from *Daphnia* hosts. I also found that parasite spores of the genotype associated with *Ceriodaphnia* were smaller. We hypothesize that spore size modulates infectivity and spore production across host species.

Explaining the distribution of large and small-spored *Metschnikowia* genotypes across lakes would be an exciting aim of future research. I predicted that host community structures would influence the success of each genotype or facilitate genotype coexistence, but further study of genotype distributions along with community characteristics could either support this hypothesis or point to other factors that could influence parasite distributions.

Chapter 5: A Common Multihost Parasite Shows Genetic Structuring at the Host Species and Population Level – and Rapid Evolution During Disease Outbreaks.

The importance of understanding origins of parasites that begin outbreaks has been highlighted with the recent increases in emerging infectious diseases (Morens et al. 2004, Cunningham et al. 2017). Since so many challenges shape parasite distributions, understanding how parasites move across a landscape and across hosts could point to parasite mechanisms for eluding these challenges. Studying parasite population structure can help us understand the relative contributions of movement across space, across host species, and of evolution within outbreaks to disease occurrence. I found that the population structure of the genotype-specific parasite, *Pasteuria*, is structured by lake, by host, and over time, indicating that there are constraints for moving between lakes and between host species within a lake and that parasite evolution occurs during outbreaks. This genetic change is likely driven by selection for parasites that generate successful infections in the local host community.

Future work could study drivers of differences in evolutionary dynamics between lakes and the importance and consequences of spore banks. Host diversity and epidemic size may impact parasite evolution in important ways, potentially with larger epidemics more efficiently

selecting on parasites. Furthermore, this could have between-epidemic consequences as the outcomes of evolution within epidemics (locally adapted parasites) are preserved over time in sediments. Better understanding how parasite diversity changes within and between epidemics is an exciting avenue for future research.

Extensions of this work

The *Daphnia*-parasite system is a powerful one for studying important questions in disease ecology. *Daphnia* have been studied for over a hundred years, so there is a wealth of knowledge on their ecology, behavior, genetics, and interactions with community members on which to build (Lampert and Sommer 2007, Ebert 2011, Cáceres et al. 2014). Furthermore, natural populations are studied relatively easily, and animals are amenable to study in laboratory settings.

Though my discoveries regarding drivers of disease were made with *Daphnia* and their parasites, my findings are readily applicable to other systems and to disease dynamics more broadly. My finding that light imposes important constraints on parasite epidemics in the *Daphnia*-parasite system informs our understanding of how light could impact disease dynamics in other systems. Light impacts a number of water-transmitted human pathogens, so lake browning will likely lead to increased disease as disinfection of water bodies by light is curtailed (Williamson et al. 2017). Light also constrains disease outbreaks in terrestrial systems (Read 1968, Roland and Kaupp 1995). Second, I found that host habitat selection behavior might drive patterns of infection, and this is likely the case in other systems especially if habitat selection drives the aggregation of hosts, which could facilitate the spread of parasites (Park et al. 2002).

Studies of how parasites influence habitat choice are relatively new (Buck et al. 2018, Weinstein et al. 2018a, 2018b), and my research shows that it will be important to consider the physical structuring of aquatic habitats in future studies of the "landscape of disgust" in aquatic habitats. Third, parasite spillover can cause catastrophic epidemics, and cross-species transmission events are increasing in frequency as human encroachment on natural systems increases contact between humans and wild populations (Faust et al. 2018) and as we transport hosts and their parasites around the world (Rogalski et al. 2016). It is therefore important to understand how parasite traits facilitate cross species transmission. I found that spore size might be important to the amount of cross species transmission for *Metschnikowia*, with larger spores more infective to both large and small hosts. In viruses, plasticity and mutation rates are often implicated in spillover propensity (Johnson et al. 2015), but more traits of multihost pathogens should be determined in order to identify candidate parasite traits to target with preventative measures. Finally, with emerging epidemics becoming increasingly common (Cunningham et al. 2017), it is necessary to identify the sources of the parasites that start them in order to combat them effectively. I found that pathogen transmission occurs when hosts overlap in space and when hosts are closely related, results that bolster similar findings of other investigators (Streicker et al. 2010, Cooper et al. 2012, Zukal et al. 2014). In conclusion, my dissertation research on drivers of epidemics in *Daphnia* not only improves our understanding of that model system but enriches our understanding of fundamental disease dynamics that are generalizable to a wide variety of organisms and situations.

REFERENCES

Buck, J. C., S. B. Weinstein, and H. S. Young. 2018. Ecological and evolutionary consequences of parasite avoidance. Trends in Ecology & Evolution 33:619–632.

Cáceres, C. E., A. J. Tessier, M. A. Duffy, and S. R. Hall. 2014. Disease in freshwater zooplankton: what have we learned and where are we going. Journal of Plankton Research 36:326–333.

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London B 356:991–999.

Cooper, N., R. Griffin, M. Franz, M. Omotayo, and C. L. Nunn. 2012. Phylogenetic host specificity and understanding parasite sharing in primates. Ecology Letters 15:1370–1377.

Creed, I. F., A. K. Bergstrom, C. G. Trick, N. B. Grimm, D. O. Hessen, J. Karlsson, K. A. Kidd, E. Kritzberg, D. M. Mcknight, E. C. Freeman, O. E. Senar, A. Andersson, J. Ask, M. Berggren, M. Cherif, R. Giesler, E. R. Hotchkiss, P. Kortelainen, M. M. Palta, T. Vrede, and G. A. Weyhenmeyer. 2018. Global change-driven effects on dissolved organic matter composition: implications for food webs of northern lakes. Global Change Biology 24:3692–3714.

Cunningham, A. A., P. Daszak, and J. L. N. Wood. 2017. One health, emerging infectious diseases and wildlife. Philosophical Transactions of the Royal Society of London. B. 372:4.

Duffy, M. A. 2007. Selective predation, parasitism, and trophic cascades in a Bluegill-*Daphnia*-parasite system. Oecologia 153:453–460.

Duffy, M. A., C. E. Brassil, S. R. Hall, A. J. Tessier, C. E. Cáceres, and J. K. Conner. 2008. Parasite-mediated disruptive selection in a natural *Daphnia* population. BMC Evolutionary Biology 8:80.

Ebert, D. 2011. A genome for the environment. Science 331:539–540.

Faust, C. L., H. I. McCallum, L. S. P. Bloomfield, N. L. Gottdenker, T. R. Gillespie, C. J. Torney, A. P. Dobson, and R. K. Plowright. 2018. Pathogen spillover during land conversion. Ecology Letters 21:471–483.

Fee, E. J., R. E. Hecky, S. E. M. Kasian, and D. R. Cruikshank. 1996. Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. Limnology and Oceanography 41:912–920.

Fitch, A., C. Orland, D. Willer, E. J. S. Emilson, and A. J. Tanentzap. 2018. Feasting on terrestrial organic matter: Dining in a dark lake changes microbial decomposition. Global Change Biology:5110–5122.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics 19:207–233.

Gliwicz, M. Z., and J. Pijanowska. 1988. Effect of predation and resource depth distribution on vertical migration of zooplankton. Bulletin of Marine Science 43:695–709.

Häder, D.-P., C. E. Williamson, S. Å. Wängberg, M. Rautio, K. C. Rose, K. Gao, E. W. Helbling, R. P. Sinha, and R. Worrest. 2015. Effects of UV radiation on aquatic ecosystems and interactions with other environmental factors. Photochem. Photobiol. Sci. 14:108–126.

Hall, S. R., C. R. Becker, J. L. Simonis, M. A. Duffy, A. J. Tessier, and C. E. Cáceres. 2009. Friendly competition: Evidence for a dilution effect among competitors in a planktonic host-parasite system. Ecology 90:791–801.

Hall, S. R., R. Smyth, C. R. Becker, M. A. Duffy, C. J. Knight, S. MacIntyre, A. J. Tessier, and C. E. Cáceres. 2010. Why Are *Daphnia* in some lakes sicker? Disease ecology, habitat structure, and the plankton. BioScience 60:363–375.

Johnson, C. K., P. L. Hitchens, T. S. Evans, T. Goldstein, K. Thomas, A. Clements, D. O. Joly, N. D. Wolfe, P. Daszak, W. B. Karesh, and J. K. Mazet. 2015. Spillover and pandemic properties of zoonotic viruses with high host plasticity. Scientific Reports 5:1–8.

Lampert, W. 1997. Zooplankton research: the contribution of limnology to general ecological paradigms. Aquatic Ecology 31:19–27.

Lampert, W., and U. Sommer. 2007. Limnoecology. Second. Oxford University Press, Oxford.

Laundré, J. W., L. Hernández, and W. J. Ripple. 2010. The landscape of fear: ecological implications of being afraid. The Open Ecology Journal 3:1–7.

Leibold, M., and A. J. Tessier. 1991. Contrasting patterns of body size for *Daphnia* species that segregate by habitat. Oecologia 86:342–348.

Lloyd-Smith, J. O., D. George, K. M. Pepin, V. E. Pitzer, J. R. C. Pulliam, A. P. Dobson, P. J. Hudson, and B. T. Grenfell. 2009. Epidemic dynamics at the human-animal interface. Science 326:1362–1367.

Mariash, H. L., M. Cazzanelli, M. Rautio, L. Hamerlik, M. J. Wooller, and K. S. Christoffersen. 2018. Changes in food web dynamics of low Arctic ponds with varying content of dissolved organic carbon. Arctic, Antarctic, and Alpine Research 50:e1414472.

Miner, B. E., L. De Meester, M. E. Pfrender, W. Lampert, and N. G. H. Jr. 2012. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. Proceedings of the Royal Society B 279:1873–1882.

Morens, D. M., G. K. Folkers, and A. S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. Nature 430:242–249.

Morris, D. P., H. Zagarese, C. E. Williamson, E. G. Balseiro, B. R. Hargreaves, B. Modenutti, R.

Moeller, and C. Queimalinos. 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. Limnology and Oceanography 40:1381–1391.

Overholt, E. P., S. R. Hall, C. E. Williamson, C. K. Meikle, M. A. Duffy, and C. E. Cáceres. 2012. Solar radiation decreases parasitism in *Daphnia*. Ecology Letters 15:47–54.

Park, A. W., S. Gubbins, and C. A. Gilligan. 2002. Extinction times for closed epidemics: The effects of host spatial structure. Ecology Letters 5:747–755.

Read, D. J. 1968. Some aspects of the relationship between shade and fungal pathogenicity in an epidemic disease of pines. New Phytologist 67:39–48.

Robidoux, M., P. del Giorgio, and A. Derry. 2015. Effects of humic stress on the zooplankton from clear and DOC-rich lakes. Freshwater Biology 60:1263-1278

Rogalski, M. A., C. D. Gowler, C. L. Shaw, R. A. Hufbauer, and M. A. Duffy. 2016. Human drivers of ecological and evolutionary dynamics in emerging and disappearing infectious disease systems. Philosophical transactions of the Royal Society B 372:1–9.

Roland, J., and W. J. Kaupp. 1995. Reduced transmission of forest tent caterpillar (Lepidoptera: Lasiocampidae) nuclear polyhedrosis virus at the forest edge. Page Environmental Entomology.

Shocket, M. S., A. T. Strauss, J. L. Hite, M. Šljivar, D. J. Civitello, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2018. Temperature drives epidemics in a zooplankton-fungus disease system: a trait-driven approach points to transmission via host foraging. The American Naturalist 191:435–451.

Solomon, C. T. 2017. Dissolved organic matter causes genetic damage in lake zooplankton via oxidative stress. Functional Ecology 31:806–807.

Strauss, A. T., D. J. Civitello, C. E. Cáceres, and S. R. Hall. 2015. Success, failure and ambiguity of the dilution effect among competitors. Ecology Letters:1–11.

Streicker, D. G., A. S. Turmelle, M. J. Vonhof, I. V Kuzmin, G. F. Mccracken, and C. E. Rupprecht. 2010. Host phylogeny constrains cross-species emergence and establishment of rabies virus in bats. Science 329:676–679.

Strock, K. E., N. Theodore, W. G. Gawley, A. C. Ellsworth, and J. E. Saros. 2017. Increasing dissolved organic carbon concentrations in northern boreal lakes: implications for lake water transparency and thermal structure. J. Geophys. Res. Biogeosci. 12:1022–1035.

Viana, M., R. Mancy, R. Biek, S. Cleaveland, P. C. Cross, J. O. Lloyd-smith, and D. T. Haydon. 2014. Assembling evidence for identifying reservoirs of infection. Trends in Ecology & Evolution 29:270–279.

Weinstein, B. S. B., J. C. Buck, and H. S. Young. 2018a. A landscape of disgust. Science 359:1213–1215.

Weinstein, S. B., C. W. Moura, J. F. Mendez, and K. D. Lafferty. 2018b. Fear of feces? Tradeoffs between disease risk and foraging drive animal activity around raccoon latrines. Oikos 127:927–934.

Williamson, C. E., E. P. Overholt, R. M. Pilla, T. H. Leach, J. A. Brentrup, L. B. Knoll, E. M. Mette, and R. E. Moeller. 2015. Ecological consequences of long-term browning in lakes. Scientific Reports:1–10.

Williamson, C. W., S. Madronich, A. Lal, R. G. Zepp, R. M. Lucas, E. P. Overholt, K. C. Rose, S. G. Schladow, and J. Lee-Taylor. 2017. Climate change-induced increases in precipitation are reducing the potential for solar ultraviolet radiation to inactivate pathogens in surface waters. Scientific Reports:1–38.

Winder, M., P. Spaak, and W. M. Mooij. 2004. Trade-offs in *Daphnia* habitat selection. Ecology 85:2027–2036.

Wolinska, J., and P. Spaak. 2009. The cost of being common: evidence from natural *Daphnia* populations. Evolution 63:1893–1901.

Zukal, J., H. Bandouchova, T. Bartonicka, H. Berkova, V. Brack, J. Brichta, M. Dolinay, K. S. Jaron, V. Kovacova, M. Kovarik, N. Martínková, K. Ondracek, Z. Rehak, G. G. Turner, and J. Pikula. 2014. White-Nose Syndrome fungus: a generalist pathogen of hibernating bats. PLoS ONE 9:e97224.

APPENDIX A

Chapter 2 Supplemental Information

Section 1. Additional lake transparency methods

In this section, we explain how we calculated metrics of light exposure and the index of lake transparency in the main text.

Incubation experiment: Within-lake light attenuation
Diffuse attenuation coefficients (k_{d320} and k_{dPAR}) measure light attenuation in water, accounting for the contribution of both dissolved and particulate substances. Diffuse attenuation coefficients were calculated from profiles generated by a submersible radiometer (BIC 2104, Biospherical Instruments, Inc., San Diego, CA). We use these values to estimate the percentage of ambient light (320 nm UV and PAR) remaining at the incubation depths of 0.5 and 2 m in each lake (see equation A1; Rose et al. 2009).

$$\% \text{ Light remaining} = e^{-\text{depth} * kdPAR} * 100\% \qquad (A1)$$

We measured diffuse light attenuation coefficients in lakes with the experimental incubations in July and August, but not in November. In the analysis, we used diffuse light attenuation coefficients from the relevant month if possible. Otherwise, we used the diffuse light attenuation coefficient from the proximate month measured. To justify, light attenuation coefficients did not change significantly through autumn in a linear mixed effects model with month as a fixed effect, and lake and year as random effects (k_{d320}: Likelihood Ratio Test (LRT)=1.24, P=0.27; k_{dPAR}: LRT=0.25, P=0.62).

Incubation experiment: Ambient Light
During each incubation, a radiometer (Model 2104RL; Biospherical Instruments) measured ambient incident radiation integrated over 3-minute time intervals. The instrument was deployed at the Greene Sullivan State Forest ranger station located 10 miles from all experimental lakes. We report both the maximum 320 nm and PAR irradiances within a 3-minute time interval and the cumulative irradiances (summed over the deployment) while the incubations were deployed in each lake. Due to time needed for deployment and recovery of the incubations, light measurements are slightly different for each lake (Appendix Table A1, Appendix Table A2).

Table A1. Light and temperature conditions at incubation depths and incident light conditions above the water surface during experimental incubations. Canvasback Lake was not included in the November incubation.

	Average % PAR remaining at incubation depth (standard deviation)		Water temperature (°C) at 0.5 m (shallower, lighter), 2.0 m (deeper, darker)			Cumulative incoming light, UV (at 320 nm, KJ/m^2) and PAR ($W/m^2 \times 10^7$)		
Lake	0.5m	2m	Jul	Aug	Nov	Jul	Aug	Nov
Airline[1]	84.7% (0.5%)	51.4% (1.3%)	28.7, 28.6	28.4, 28.4	18.1, 18.1	29.23 3.749	18.61 2.181	11.01 1.734
Beaver Dam[2]	77.3% (5.3%)	36.7% (10.3%)	28.6, 27.9	27.7, 27.3	17.1, 17.1	29.93 3.820	17.00 2.018	10.72 1.688
Canvasback[3]	83.4% (3.2%)	48.9% (7.1%)	29.4, 28.9	28.4, 28.4		30.46 3.887	17.88 2.107	
Goodman[1]	75.5% (3.5%)	32.9% (5.8%)	28.6, 28.1	27.7, 27.7	17.3, 17.3	30.41 3.881	18.31 2.151	11.06 1.742
Midland[2]	64.5% (6.9%)	18.5% (8.4%)	28.7, 28.6	28.1, 28.1	16.8, 16.8	30.18 3.842	17.56 2.075	10.82 1.703

[1] Greene-Sullivan State Forest, Greene County, IN; [2] Hillenbrand Fish and Wildlife Area, Greene County, IN; [3] Sullivan County, IN.

Table A2. Time of deployment and recovery of incubation set up.

Lake	July	August	November
Airline	7/20/16 12:30pm – 7/25/16 8:20am	8/15/16 11:15am – 8/20/16 8:10am	10/31/16 10:00am – 11/5/16 8:46am
Beaver Dam	7/20/16 11:00am – 7/25/16 7:45am	8/15/16 2:30pm – 8/20/16 7:00am	10/31/16 11:30am – 11/5/16 8:08am
Canvasback	7/20/16 3:30pm – 7/25/16 9:00am	8/15/16 1:30pm – 8/20/16 7:30am	
Goodman	7/20/16 1:45pm – 7/25/16 8:40am	8/15/16 12:30pm – 8/20/16 8:30am	10/31/16 9:30am – 11/5/16 9:01am
Midland	7/20/16 9:20am – 7/25/16 7:20am	8/15/16 2:30pm – 8/20/16 6:30pm	10/31/16 11:00am – 11/5/16 7:45am

Analysis of field survey: Index of lake transparency

Like diffuse attenuation coefficients above, dissolved absorption coefficients quantify the attenuating properties of dissolved substances in water, but they do not incorporate the attenuating properties of particulate substances in water samples. Hence, these values can be lower than the dissolved absorption coefficients. In this study we measured the dissolved absorption coefficient for 320 nm UV (a_{d320}). Dissolved absorption coefficients are calculated as equation A2.

$$a_{d\lambda} = 2.303D/r \qquad (A2)$$

where D is the absorbance of the water sample at wavelength (λ) defined as $D=\log_{10}(I_0/I)$ where I_0 is the incident light intensity, and I is the light remaining after passing through the sample, r is the pathlength in the spectrophotometer (in cm), and 2.303 converts base e to base 10 logarithms (Kirk 1993). To measure a_{d320}, we collected epilimnetic water samples and filtered them with pre-combusted Whatman GF/F filters. The filtrates were kept refrigerated until analysis by spectrophotometry (Shimadzu UV⁄Visible UV-1650 PC Spectrophotometer). For the field survey, in 2014, we collected water samples from each lake throughout the season; in 2015 water samples were collected in July and in October, whereas in 2016 water samples were collected in August only. For years with more than one water sample, a_{d320} was averaged.

Diffuse attenuation coefficients for UV (k_{d320}) were not measured in all of our study lakes in every year. Hence, we estimated k_{d320} when not measured using its relationship with the dissolved absorption coefficient (a_{d320}) for UV (Figure A1, Equation A3).

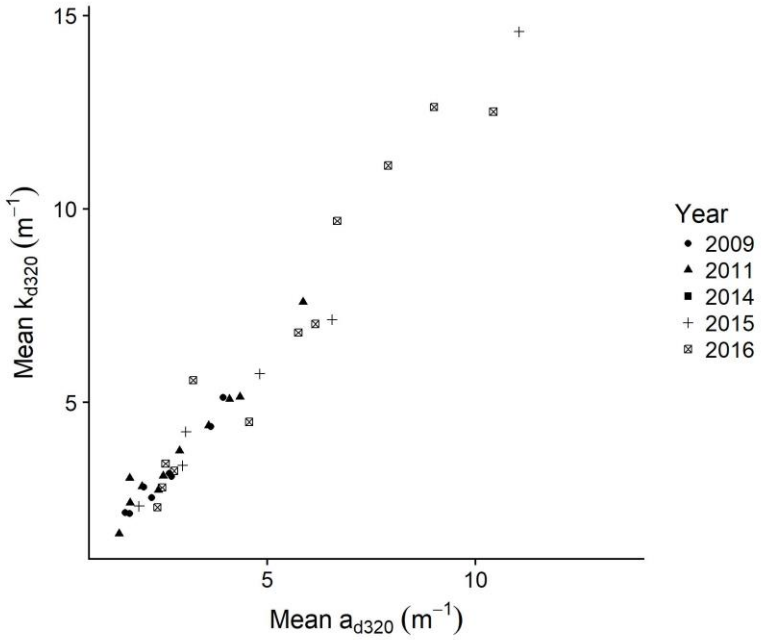

Figure A1. Mean values of a_{d320} and k_{d320} in lakes where both were measured in a given year were correlated (r^2=0.963, P<0.001).

The linear relationship (equation A3, Figure A1) used to estimate k_{d320} values was:

$$\text{Estimated } k_{d320} = -0.11896 + 1.29414 * a_{d320} \qquad (A3)$$

With these k_{d320} estimates, we then calculated the depth of 1% of remaining 320nm UV light (by solving for depth in parameterized equation A1). We used this depth as our index of lake transparency.

Section 2. Additional experimental notes and procedures

Incubation Preparation

Parasite spore slurries were composed of homogenized infected animals that were infected with laboratory strains of each parasite and maintained in the lab. Quartz vials held a volume of about 0.8 mL. For *Pasteuria*, 300,000 spores were placed in each vial in each month. Volumes of spore slurry placed in each vial in July, August, and November were: 0.448 mL, 0.570 mL, 0.318 mL respectively. For *Metschnikowia*, 15,000 spores were placed in each vial in July, and 37,500 spores were placed in each vial in August and November. Volumes of spore slurry placed in each vial in July, August, and November were 0.240 mL, 0.600 mL or 0.800 mL, and 0.341 mL respectively. After the incubations, these spore slurries were transferred to 150 mL of filtered lake water, yielding spore concentrations of 2,000 spores/mL of *Pasteuria* and 100 spores/mL of *Metschnikowia* in July and 250 spores/mL of *Metschnikowia* in August and November.

Lost Samples

We lost uncovered vials of *Metschnikowia* spores at 2 m in Goodman Lake in the November incubation. We also do not have data for a vial of *Pasteuria* from Canvasback Lake in the covered treatment at 0.5 m in July, a vial of *Pasteuria* from Canvasback Lake that was exposed to light at 2 m in August, and a vial of *Metschnikowia* from Midland Lake that was exposed at 2 m in November. In addition, we lost physical access to Canvasback Lake during the fall, so this lake was also left out of the November incubation.

Notes on Daphnia *maintenance*

In July, animals were maintained in the Hall Lab at Indiana University, and in August and November, animals were maintained in the Duffy Lab at the University of Michigan. Due to differences in lab methods for *Daphnia* care, in the Hall lab, *Daphnia* were fed 1 mg *Ankistrodesmus falcatus* per liter on the day of exposure and 2 mg *Ankistrodesmus* per liter every day after exposure, and in the Duffy lab, *Daphnia* were fed 1x10^6 cells (1.63 mg L^{-1}) of *Ankistrodesmus* every day. All levels of food are considered 'high' for *Daphnia*.

Notes on experimental take down

We maintained *Daphnia* only until infection could be ascertained, as described below.

> July incubation: No new *Metschnikowia* infections were observed after the 14th day post infection, so all animals were diagnosed by the 16th day post infection. For *Pasteuria*, some animals had discernable infections as early as 14 days post infection, and they were

taken down. Remaining animals were checked every other day until 20 days post infection, at which point all were diagnosable as infected or uninfected.

August incubation: Late – stage *Metschnikowia* infections could be discerned on the 10th day post infection, and remaining animals were taken down 13 days post infection at which point 4 additional animals were found to have *Metschnikowia*, but the others were clearly uninfected. Two questionable animals were maintained and found later to have been infected with *Pasteuria*. These animals and two more which had been noted to be questionable at take down were classified as uninfected for the analysis. However, if these animals had been counted as infected, there would be no qualitative change in the results presented here. Contamination was likely due to movement of spores during an early water change. *Pasteuria* infections were discernable 14 days post infection. Like in the July incubation, animals in the *Pasteuria* treatment that were clearly infected were taken down at or before 20 days post infection. However, even with examination under the microscope, 53 (of 590 total *Pasteuria*-exposed animals) could not be confidently diagnosed at this date; these were kept until 29 days post-infection, at which point 16 were clearly infected. If the animals which were classified as infected after the 20 day mark had been classified instead as uninfected, there would be no change in the qualitative results reported here for the comparison between parasites or for the *Pasteuria* results.

November: For the November incubation, *Metschnikowia* infections were discernable by 11 days post infection. All animals in this treatment were taken down 17 days post infection, and no additional infected animals were found at take down. For *Pasteuria*, infected animals were discernable 15 days post exposure to the parasite. Remaining animals in the *Pasteuria* treatment were maintained until clearly infected or until 28 days post infection at which point infections were clearly detectable; only three additional *Pasteuria* infections were found at this date.

Section 3. Relative infectivity

We calculated infectivity (β) for each incubated vial (see equations A4-6 below). For relative infectivity, we divided infectivity for each light-exposed vial by the average infectivity for the corresponding dark treatment.

We assume that susceptible hosts, S, get infected as they contact spores, Z, at an infectivity, β.

$$dS/dt = -\beta S Z \qquad (A4)$$

The solution to this equation is

$$S_t = S_0 \exp(-\beta Z_0 t_E) \qquad (A5)$$

where S_t is the number of hosts remaining after exposure time t_E and exp() is the exponential function. We assume that spore concentration does not change over the exposure period, so here, Z_0 is initial dose (spores/L).

Solving for β, we obtain

$$\beta = - \ln(1 - p) / (Z_0 \ t_E) \qquad (A6)$$

where p is the proportion of exposed hosts that became infected and ln is a natural log transform.

Since ln(0) is undefined, vials that had 100% infection were converted to the proportion, 0.99, for this analysis. In addition, if average infectivity in the dark (no light control) treatment was 0, the relative infectivity of the light treatment was infinite or undefined and dropped from the analysis. This was the case for 4/28 *Metschnikowia* treatments. There was one *Pasteuria* dark treatment vial (out of 83) where no exposed hosts got infected, and this vial (*Pasteuria*, dark treatment, Goodman lake, 0.5m) was dropped from this part of the analysis. Relative infectivity for the corresponding light-exposed vials was calculated with respect to the two covered vials that showed infection.

Section 4. Additional Field Methods

Lake Sampling

Lakes and the timing of sampling varied among years. Figure A2 shows when each lake was sampled in 2014, 2015, 2016.

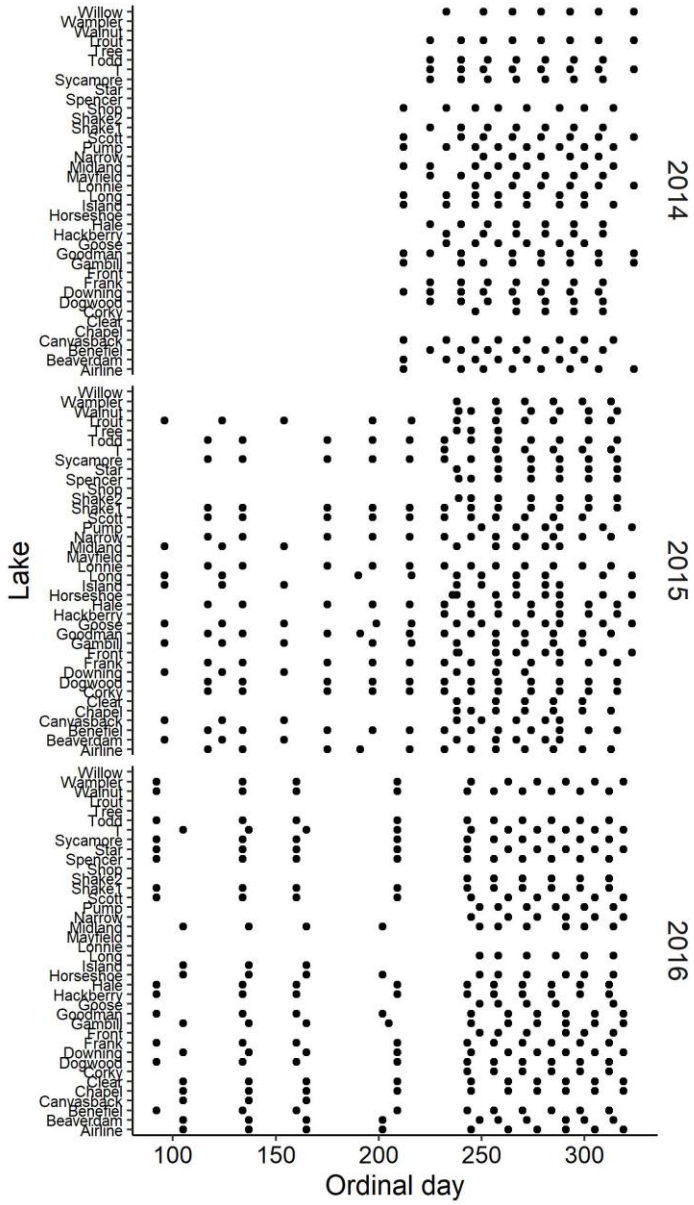

Figure A2. Sampling dates of Indiana lakes used in analysis in 2014, 2015, and 2016.

Sampling timing could affect epidemic size and start calculations: when comparing lakes with comparable maximum infection levels, lakes sampled earlier could have larger integrated areas than lakes sampled later, and lakes sampled earlier could have earlier start dates than those sampled later. To account for potential spurious results associated with sampling timing, we checked whether variation in sampling timing was correlated with lake transparency (Figure A3). Since we did not expect to see infections before day 200 (July 19 in 2014 and 2015, and July 18 in 2016), we ran a linear regression for each year with first sample date after ordinal day 200 as the response variable and the depth of 1% 320 nm radiation remaining as a fixed effect. The first sample date after day 200 was not associated with lake transparency in 2014 ($F_{1,25}$=1.69, p=0.205), 2015 ($F_{1,33}$=0.03, p=0.874), or in 2016 ($F_{1,12}$=0.25, P=0.624).

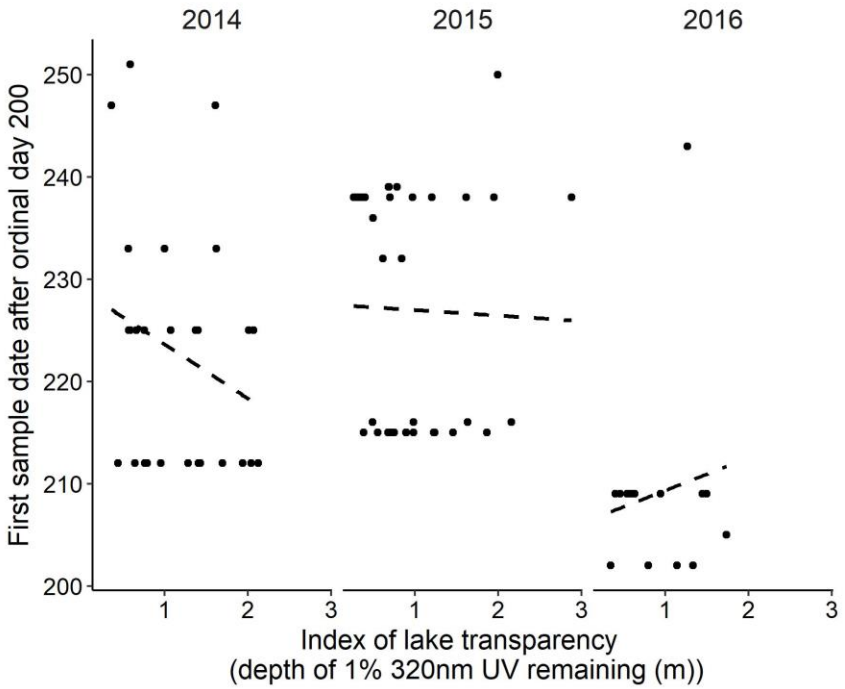

Figure A3. Association between the first sampling dates after earliest anticipated start (day 200) and lake transparency (depth of 1% 320 nm UV remaining). No significant associations were detected in any year of sampling (i.e., dashed trend lines).

Section 5. Additional Field Models

Considering chlorophyll

In our study lakes, our index of lake transparency (depth of 1% 320 nm UV remaining) is correlated with mean total chlorophyll concentrations during the sampling period ($t=-8.32$, $P<0.001$; Figure A4). To confirm that chlorophyll concentrations were not driving our results, we ran addition models. Mean chlorophyll concentration was not a significant predictor of *Pasteuria* outbreak size when lake transparency was also included in the model ($t=-1.17$, $P=0.25$), though it was on its own ($t=-5.18$, $P<0.001$). Following Legendre and Legendre (1998), we partitioned the variation in outbreak size (marginal r^2 attributed to fixed effects) between lake transparency and mean chlorophyll. Lake transparency accounted for 31% of the variance in *Pasteuria* outbreak size and mean chlorophyll accounted for 1.2%. 10.7% of variance could not be split between these variables and reflects that lake transparency and chlorophyll are biologically connected. Mean total chlorophyll was also associated with *Metschnikowia* outbreak size on its own ($t=2.13$, $P=0.037$), but including mean total chlorophyll did not allow lake transparency to become a significant predictor. When only large outbreaks (epidemics) were considered, chlorophyll was no longer a significant predictor of epidemic size ($t=0.80$, $P=0.429$) indicating that shading effects from algae (or lack thereof) may be most important for smaller outbreaks. Therefore, the results presented in the main text with regards to lake transparency

remain qualitatively the same when mean total chlorophyll concentrations are added to the models.

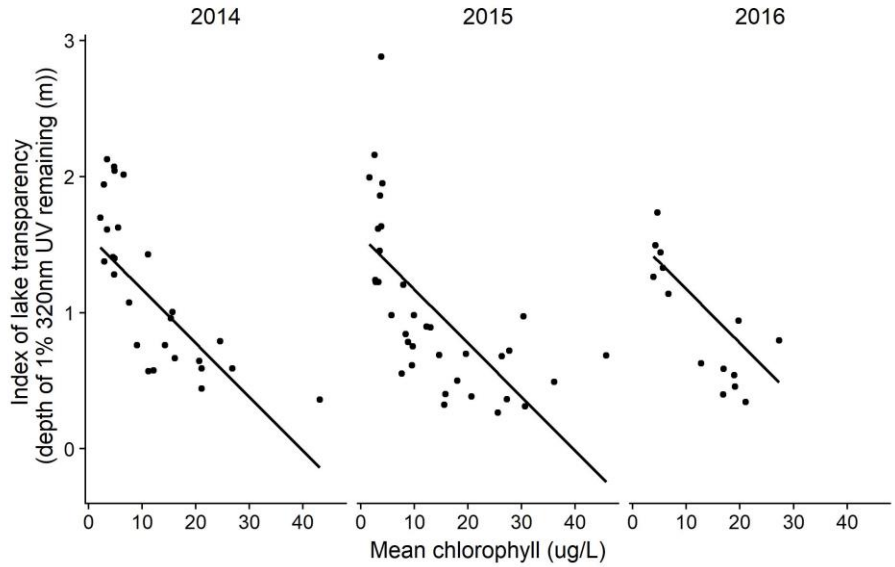

Figure A4. Mean chlorophyll concentrations during the sampling period (µg/L) are correlated with the index of lake transparency (depth of 1% 320 nm UV remaining).

As noted in the discussion, thermocline depth is negatively correlated with our index of lake transparency (lighter lakes have deeper thermoclines: $t=6.12$, $P<0.001$; Figure A5). One might assume that the mean light in the epilimnion could be lower for lighter lakes with deep thermoclines than for darker lakes with shallow thermoclines. However, the mean proportion of incident light in the epilimnion is still larger in lakes with a larger index of transparency (in general, those with deeper thermoclines) than in darker lakes with shallower thermoclines ($t=12.3$, $P<0.001$). Therefore, results presented in the main text remain qualitatively the same when using mean proportion of incident light in the epilimnion instead of depth of 1% UV penetration as the index of lake transparency.

Figure A5. Thermocline depth is negatively correlated with our index of lake transparency (depth of 1% 320 nm UV). We estimate the thermocline depth for each lake on the date of the mean *Metschnikowia* epidemic start date for that year (among all lakes that had epidemics). A spline was used to estimate the thermocline for mean start dates in between sampling dates. Trend lines indicate the predicted values from the linear mixed effects model.

References

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models using lme4. Journal of Statistical Software 67:1–48.

Kirk, J. T. O. 1993. Light and photosynthesis in aquatic ecosystems. Second edition. Cambridge University Press, Cambridge.

Legendre, P., and L. Legendre. 1998. Numerical ecology. Elsevier S. Amsterdam, The Netherlands.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. Core Team. 2018. nlme: linear and nonlinear mixed effects models.

Rose, K. C., C. E. Williamson, J. E. Saros, R. Sommaruga, and J. M. Fischer. 2009. Differences in UV transparency and thermal structure between alpine and subalpine lakes: implications for organisms. Photochemical & Photobiological Sciences 8:1244.

APPENDIX B

Chapter 4 Supplemental Information

Table B1. List of genotyped parasites for Figure 4.3 in the main text.

Sample	Host	Lake	Date
BenefielCerio10/15.2(IN)	*Ceriodaphnia*	Benefiel (IN)	10/15/15
BenefielCerio10/15.3(IN)	*Ceriodaphnia*	Benefiel (IN)	10/15/15
BenefielCerio10/28.1(IN)	*Ceriodaphnia*	Benefiel (IN)	10/28/15
BenefielCerio10/28.3(IN)	*Ceriodaphnia*	Benefiel (IN)	10/28/15
BenefielCerio11/12.1(IN)	*Ceriodaphnia*	Benefiel (IN)	11/12/15
BenefielCerio11/12.2(IN)	*Ceriodaphnia*	Benefiel (IN)	11/12/15
BenefielCerio11/12.3(IN)	*Ceriodaphnia*	Benefiel (IN)	11/12/15
BenefielDaphnia10/28.1(IN)	*Daphnia*	Benefiel (IN)	10/28/15
BenefielDaphnia11/12.1(IN)	*Daphnia*	Benefiel (IN)	11/12/15
BishopDaphnia(MI)	*Daphnia*	Bishop (MI)	
ClearDaphnia10/15.1(IN)	*Daphnia*	Clear (IN)	10/15/15
DogwoodCerio10/28.1(IN)	*Ceriodaphnia*	Dogwood (IN)	10/28/15
DogwoodCerio10/28.2(IN)	*Ceriodaphnia*	Dogwood (IN)	10/28/15
DogwoodCerio10/28.3(IN)	*Ceriodaphnia*	Dogwood (IN)	10/28/15
DogwoodCerio10/28.4(IN)	*Ceriodaphnia*	Dogwood (IN)	10/28/15
DogwoodCerio11/12.1(IN)	*Ceriodaphnia*	Dogwood (IN)	11/12/15
DogwoodCerio11/19.1(IN)	*Ceriodaphnia*	Dogwood (IN)	11/19/15
DogwoodDaphnia10/12.1(IN)	*Daphnia*	Dogwood (IN)	10/12/15
DogwoodDaphnia11/12.1(IN)	*Daphnia*	Dogwood (IN)	11/12/15
GambillDaphnia10/26.1(IN)	*Daphnia*	Gambill (IN)	10/26/15
GoodmanDaphnia(IN)	*Daphnia*	Goodman (IN)	
GoslingCerio10.26.1(MI)	*Ceriodaphnia*	Gosling (MI)	10/26/15
GoslingCerio10.26.2(MI)	*Ceriodaphnia*	Gosling (MI)	10/26/15
GoslingCerio10.26.3(MI)	*Ceriodaphnia*	Gosling (MI)	10/26/15
GoslingCerio10.26.4(MI)	*Ceriodaphnia*	Gosling (MI)	10/26/15
GoslingDaphniaMale11.9.1(MI)	*Daphnia*	Gosling (MI)	11/9/15
HaleDaphnia10/15.1(IN)	*Daphnia*	Hale (IN)	10/15/15
HaleDaphnia10/15.2(IN)	*Daphnia*	Hale (IN)	10/15/15
HaleCerio10/15.1(IN)	*Ceriodaphnia*	Hale (IN)	10/15/15
HaleDaphnia11/12.1(IN)	*Daphnia*	Hale (IN)	11/12/15
MidlandDaphnia11/19.1(IN)	*Daphnia*	Midland (IN)	11/19/15
MillDaphnia9/8.1(MI)	*Daphnia*	Mill (MI)	9/8/15
PickerelDaphnia10/4.1(MI)	*Daphnia*	Pickerel (MI)	10/4/15
Shake1Cerio10.25.1(IN)	*Ceriodaphnia*	Shake 1 (IN)	10/25/15
SycamoreCerio10.15.1(IN)	*Ceriodaphnia*	Sycamore (IN)	10/15/15
SycamoreCerio10.25.1(IN)	*Ceriodaphnia*	Sycamore (IN)	10/25/15
TDaphnia9/28.1(IN)	*Daphnia*	T (IN)	9/28/15
WalnutCerio10/15.1(IN)	*Ceriodaphnia*	Walnut (IN)	10/15/15
WalnutCerio10/28.1(IN)	*Ceriodaphnia*	Walnut (IN)	10/28/15
WalnutCerio10/28.2(IN)	*Ceriodaphnia*	Walnut (IN)	10/28/15
WalnutCerio10/28.3(IN)	*Ceriodaphnia*	Walnut (IN)	10/28/15
WalnutCerio11/12.1(IN)	*Ceriodaphnia*	Walnut (IN)	11/12/15
WalnutDaphnia11/12.1(IN)	*Daphnia*	Walnut (IN)	11/12/15
WalshDaphnia9/8.1(MI)	*Daphnia*	Walsh (MI)	9/8/15
WalshDaphnia9/8.2(MI)	*Daphnia*	Walsh (MI)	9/8/15
WalshDaphnia9/8.3(MI)	*Daphnia*	Walsh (MI)	9/8/15
WalshDaphnia9/8.4(MI)	*Daphnia*	Walsh (MI)	9/8/15
WalshDaphnia7/27.1(MI)	*Daphnia*	Walsh (MI)	7/27/15
WalshDaphnia7/27.3(MI)	*Daphnia*	Walsh (MI)	7/27/15
WalshDaphnia8/7.1(MI)	*Daphnia*	Walsh (MI)	8/7/15
WamplerDaphnia10/25.1(IN)	*Daphnia*	Wampler (IN)	10/25/15
WamplerDaphnia9/25.1(IN)	*Daphnia*	Wampler (IN)	9/25/15
WoodlandDaphnia10/15.1(MI)	*Daphnia*	Woodland(MI)	10/15/15
WoodlandCopepod9/5/14.5(MI)	*Copepod*	Woodland(MI)	9/5/14
WoodlandCopepod9/5/14.3(MI)	*Copepod*	Woodland(MI)	9/5/14

Table B2. Primer sequences used for amplifying the 9 *Metschnikowia* loci used in this study. Note that all forward primers contain the M13(-21) tail (Schuelke 2000).

Name	Forward (5'-3')	Reverse (5'-3')
L3	TGTAAAACGACGGCCAGTCAAGAGAGACAAGCGGAAGG	GAACAACACGCTCGCTACAA
L7	TGTAAAACGACGGCCAGTAGGATGCAGGTTTTCTGACG	TCAGGTGGACTACATTGGCA
L8	TGTAAAACGACGGCCAGTTCGACACACTTCAACGAAGC	ACAGGTCCTTCAACCTGGTG
L9	TGTAAAACGACGGCCAGTCGTTTCACTAAAAACCCCCA	TCAGTTGTTTGCCACTGGAC
L10	TGTAAAACGACGGCCAGTGGGTCGTTGATAAGCGAAGA	TTTAGGGTATTCACGCCGTC
L11	TGTAAAACGACGGCCAGTTGGGTAAATTGTGTGGCAGA	TTCGACAAAAACGGATCCTC
L12	TGTAAAACGACGGCCAGTCTGCCAGTACTCCTGCATCA	TTCATCACGTTCGACACCAT
L17	TGTAAAACGACGGCCAGTTACTCGCTCAATGCATCAGG	CTCCGCAAGGACTTTGCTAC
L19	TGTAAAACGACGGCCAGTATTTGCTCGAGACGCTGTTT	AATGAAATTGCGGACACACC

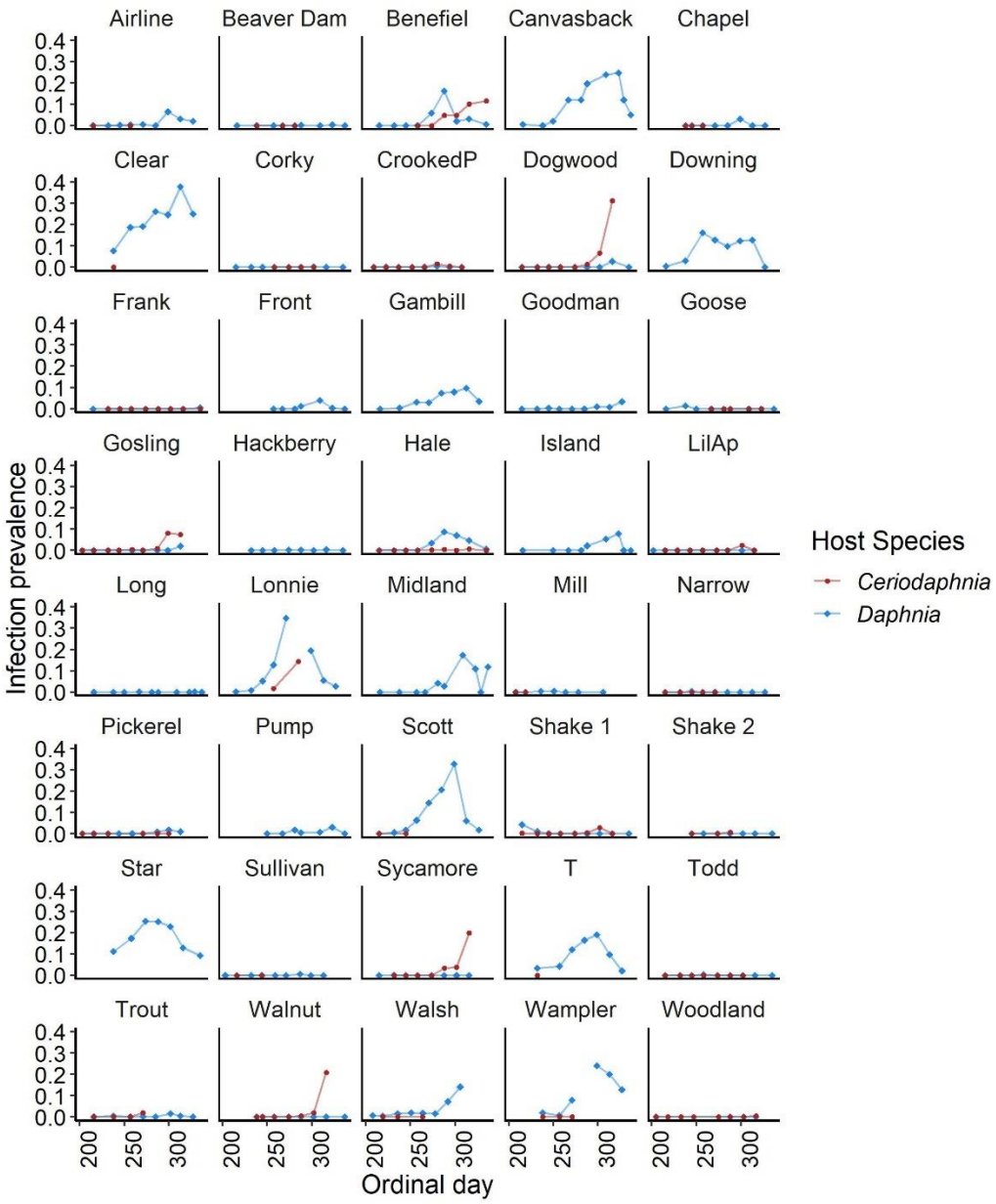

Figure B1. Epidemics of *Metschnikowia* occurred in either or both host species, *Daphnia* and *Ceriodaphnia*, during the summer through fall of 2015. Infection prevalence was calculated if at least 20 of the host type were counted on a given sampling day. Airline, Benefiel, Chapel, Clear, Corky, Dogwood, Downing, Frank, Front, Gambill, Goodman, Goose, Hackberry, Hale, Long, Lonnie, Narrow, Pump, Scott, Shake 1, Shake 2, Star, Sycamore, T, Trout, Walnut, and Wampler Lakes are in the Greene-Sullivan State Forest, Greene Country, IN. Beaver Dam and Midland Lakes are in the Hillenbrand Fish and Wildlife Area, Greene County, IN. Canvasback Lake and Island Lake are in Sullivan County, IN. Crooked, Gosling, Pickerel Lakes, and Sullivan are in the Pinckney Recreation Area, Livingston County, MI. Crooked Lake is labeled "CrookedP" on the figure since we routinely sample two Crooked Lakes; the "P" indicates this is the one in the Pinckney Recreation Area (the other lake is not included in this study). Little Appleton ("LilAp") is in Brighton Recreation Area, Livingston County, MI. Walsh and Mill are in Waterloo State Recreation Area, Washtenaw County, MI. Woodland Lake is in Brighton Township, Livingston County, MI.

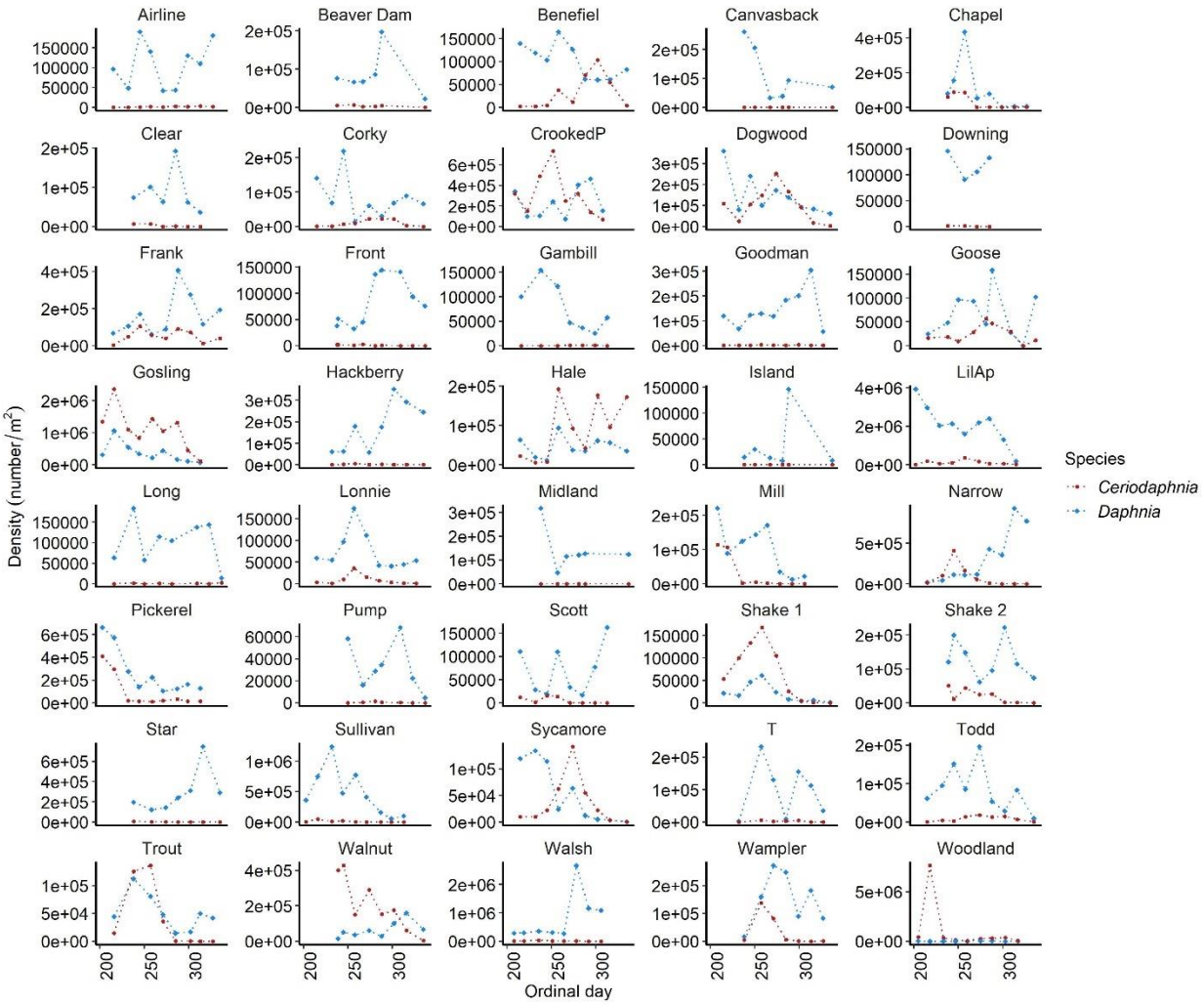

Figure B2. Densities of *Daphnia* and *Ceriodaphnia* fluctuated throughout summer and fall of 2015. See Figure B1 caption for location of lakes.

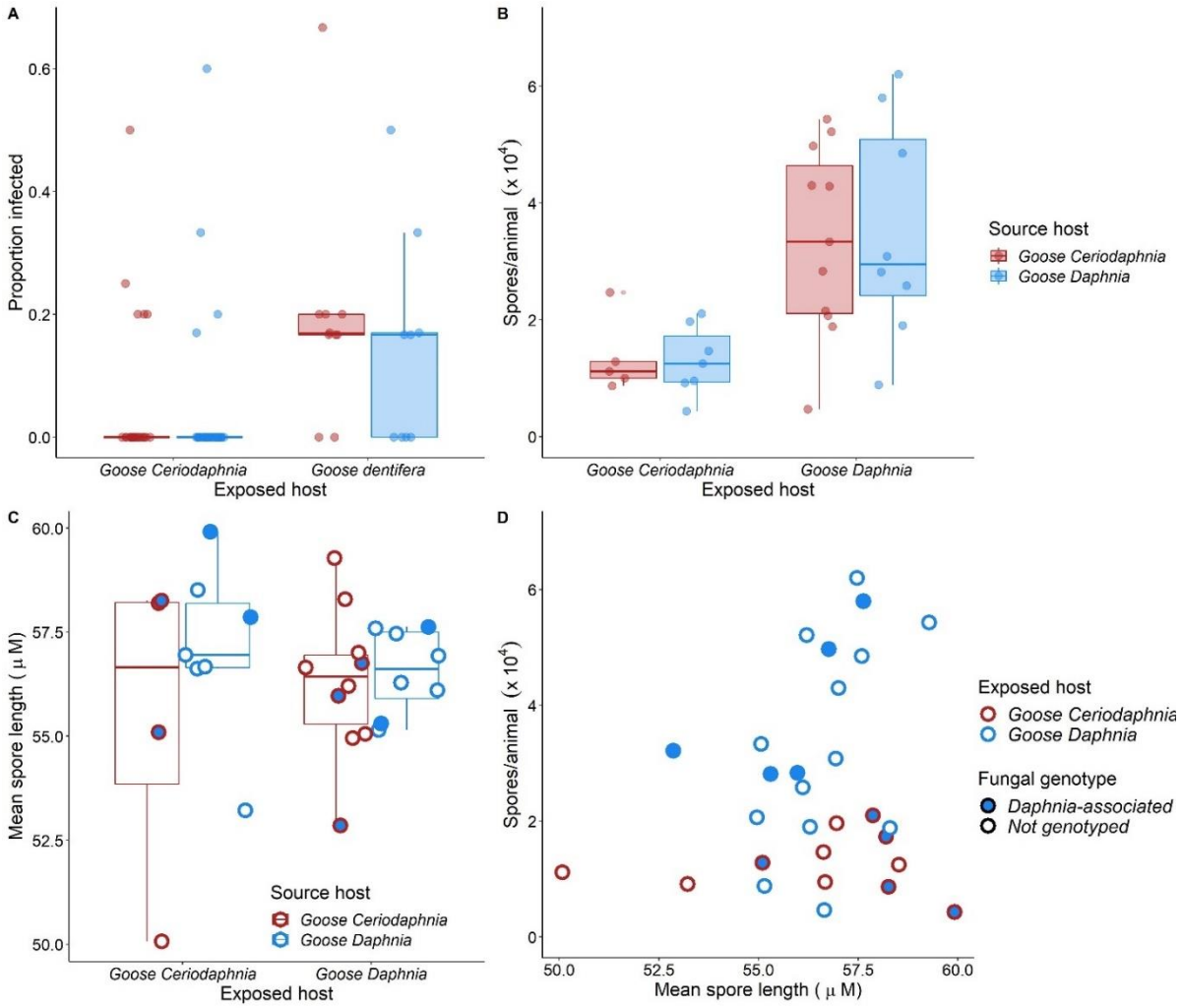

Figure B3. The combination of exposed and source hosts yielded different results shown here from Goose lake than from Benefiel Lake (presented in the main text; Figure 4.4), likely because this lake had only the *Daphnia*-associated genotype. A) There was not a significant difference in infection rates between the two exposed host species or due to identity of the source host species. B) More spores were produced in *Daphnia* hosts, but the origin of spores did not affect spore production. C) There was not a significant difference in spore size between the exposed groups. Furthermore, all genotyped infections belonged to the *Daphnia*-associated genotype. D) Spore length did not significantly influence spore production in either *Daphnia* or *Ceriodaphnia*, likely because all spores were large relative to spores of the *Ceriodaphnia*-associated genotypes.

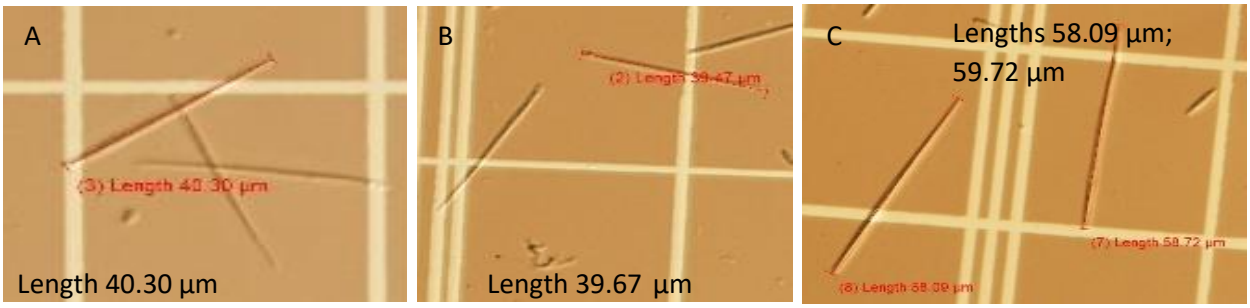

Figure B4. *Metschnikowia* spores show variation in length. A) Spores from Benefiel (IN) *Ceriodaphnia* B) Spores from Gosling (MI) *Ceriodaphnia*. C) Spores from Benefiel (IN) *Daphnia*.