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Community drivers of disease in *Daphnia*

Habitat, predators, and hosts regulate disease in *Daphnia* through direct and indirect pathways

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ABSTRACT

Community ecology can link habitat to disease via interactions among habitat, focal hosts, other hosts, their parasites, and predators. However, complicated food web interactions (i.e., trophic interactions among predators, and their impacts on host density and diversity) often obscure the important pathways regulating disease. Here, we disentangle community drivers in a case study of planktonic disease, using a two-step approach.

In step one, we tested univariate field patterns linking community interactions to two disease metrics. Density of focal hosts *(Daphnia dentifera*) was related to density but not prevalence of fungal (*Metschnikowia bicuspidata*) infections. Both disease metrics appeared to be driven by selective predators that cull infected hosts (fish, e.g. *Lepomis macrochirus*), sloppy predators that spread parasites while feeding (midges, *Chaoborus punctipennis*), and spore predators that reduce contact between focal hosts and parasites (other zooplankton, especially small-bodied *Ceriodaphnia sp.*). Host diversity also negatively correlated with disease, suggesting a dilution effect. However, several of these univariate patterns are initially misleading, due to confounding ecological links among habitat, predators, host density, and host diversity.

In step two, path models uncovered and explained these misleading patterns, and grounded them in habitat structure (refuge size). First, rather than directly reducing infection prevalence, fish predation drove disease indirectly through changes in density of midges and frequency of small spore predators (which became more frequent in lakes with small refuges). Second, small spore predators drove the two disease metrics through fundamentally different pathways: They directly reduced infection prevalence, but indirectly reduced density of infected hosts by lowering density of focal hosts (likely via competition). Third, the univariate diversity-

disease pattern (signaling a dilution effect) merely reflected the confounding direct effects of these small spore predators. Diversity *per se* had no effect on disease, after accounting for the links between small spore predators, diversity, and infection prevalence. In turn, these small spore predators were regulated by both size-selective fish predation and refuge size. Thus, path models not only explain each of these surprising results, but also trace their origins back to habitat structure.

KEY WORDS

Disease ecology, community ecology, selective predation, sloppy predation, spore predation, dilution effect, friendly competition, healthy herds, *Daphnia*, *Metschnikowia*, path analysis

INTRODUCTION

Habitat change can increase disease outbreaks (Williams et al. 2002, Patz et al. 2004). Community ecology can explain this connection by linking habitat to disease via variation in density of focal hosts and interactions among them, other hosts, their parasites, and predators (Ostfeld et al. 2008, Johnson et al. 2015). High host density can promote density-dependent disease transmission (Anderson and May 1981). Additionally, predators can drive disease by selectively culling infected hosts (Packer et al. 2003), spreading (Cáceres et al. 2009) or consuming free-living parasites (Johnson et al. 2010), or via other mechanisms less relevant here, including consumption of intermediate hosts for trophically-transmitted parasites (see Johnson et al. 2010). Furthermore, interactions among hosts can also regulate disease transmission (Holt et al. 2003). In the 'dilution effect' paradigm, higher host diversity (specifically, higher frequencies of low competency 'diluter' hosts) reduces disease, because these rarer 'diluters' interfere with disease transmission among more common, more competent focal hosts (Ostfeld and Keesing 2000b, Civitello et al. 2015a). In turn, habitat structure can regulate disease by changing each of these, i.e., through variation in host density (e.g., white nose syndrome in bats: Langwig et al. 2012), changes in predation (amphibian trematodes: Johnson and Chase 2004, schistosomiasis: Sokolow et al. 2015) or abundance of 'diluter' hosts, and hence host diversity (Lyme disease: Ostfeld and Keesing 2000b, Wood and Lafferty 2013). In these examples, links between habitat, density of focal hosts, predation, and diversity of all hosts can pinpoint *why* disease varies among habitats. Thus, these community links provide essential insights for understanding, predicting, or even managing disease across many important systems.

Unfortunately, complicated food web interactions often obscure the important pathways linking habitat to disease. For instance, habitat structure can simultaneously regulate densities of

important predators and hosts (Ostfeld et al. 1996, Orrock et al. 2011, Penczykowski et al. 2014). Thus, apparent effects of predators, focal host density, and host diversity can become correlated. Furthermore, interactions among predators and hosts can entangle direct effects on disease with indirect effects. For example, predators can consume each other (Levi et al. 2012, Rohr et al. 2015), lower focal host density (Lafferty 2004, Strauss et al. 2015), change the relative frequencies of high and low competency hosts (Borer et al. 2009), or act as more resistant hosts themselves, hence increasing diversity (Hall et al. 2010, Rohr et al. 2015). Indirect effects of predators, mediated by consumption of other key predators or hosts, can even matter more than their direct influence on disease (e.g., Borer et al. 2009). Disentangling these interactions becomes even more challenging when they depend sensitively on the metric of disease considered. For example, density of infected hosts or vectors (measurements of parasite success) may depend most sensitively on drivers that regulate overall host (or vector) density. In contrast, infection prevalence (a measurement of infection risk) may depend more on drivers that directly interfere with transmission, regardless of host density (e.g., Vanbuskirk and Ostfeld 1995, Randolph and Dobson 2012, Strauss et al. 2015). All of these complications pose major challenges for community ecologists seeking to link habitat to disease using field data.

Path models firmly grounded in natural history can provide a solution to these problems (see Grace et al. 2010). Here, we illustrate a two-step approach in a case study of planktonic disease (see Hall et al. 2010). In **step one**, we identify theoretically relevant drivers of disease and their interactions, and test all relationships with univariate field patterns. We begin by introducing our study system and the role of focal host density as a potential disease driver. Then, we review and test three general and relevant modes of predation on disease (Table 1). Next, we describe and test six types of complicating but essential links among habitat structure,

host density, predators, and host diversity. Specifically, Links 1-4) predators can be regulated by habitat structure and other predators, and Link 5) density of focal hosts and Link 6) host diversity can both be regulated by predators. In turn, host diversity also appears linked to disease. In **step two**, the univariately significant ecological links guide the creation of path models. Path models disentangle direct effects of predators from their indirect effects on disease, and distinguish spurious correlations from causal drivers. We fit separate path models to predict infection prevalence and then density of infected hosts. These separate models highlight key differences among the strengths of links (paths) from habitat to these disease metrics. With this two-step approach, we uncover the most important species interactions driving disease, and ground them in habitat structure.

STEP ONE – THEORETICALLY RELEVANT DRIVERS AND LINKS (UNIVARIATE)

Study system

Focal host and parasite

Our focal host, the cladoceran zooplankter *Daphnia dentifera*, is a dominant, non-selective grazer in many freshwater lakes in North America (Tessier and Woodruff 2002), including the southwestern Indiana lakes studied here. In many lakes, this host experiences autumnal epidemics of a virulent fungus, *Metschnikowia bicuspidata* (Overholt et al. 2012, Penczykowski et al. 2014). Hosts encounter infectious fungal spores while non-selectively filter-feeding for algal food (Hall et al. 2007). Infected hosts cannot recover and die from infection. After host death, spores are released back into the water column. Thus, *M. bicuspidata* acts as a parasitic obligate killer (Ebert and Weisser 1997). With this natural history, transmission could

increase with higher **host density** and higher density of free-living fungal spores (Anderson and May 1981).

Three Modes of Predation

Three modes of predation appear to regulate fungal epidemics in lake populations of our focal host. Each mode is grounded in general theory and arises in other host-parasite systems (Table 1). First, s**elective predators** (bluegill sunfish [*Lepomis macrochirus*]) selectively target and cull infected hosts, reducing prevalence and density of infections (Packer et al. 2003, Hall et al. 2005; the 'healthy herds' hypothesis). Fungal infection makes hosts opaque, and hence more conspicuous to fish predators (Duffy and Hall 2008). Fish then consume parasites along with infected hosts ("concomitant predation"; see Johnson et al. 2010), resulting in a net loss of fungal spores. Thus, high fish predation lowers infection prevalence of focal hosts (Hall et al. 2005, Hall et al. 2010).

Second, **"sloppy" predators** (*Chaoborus punctipennis* midge larvae) distribute infectious spores when they attack infected prey. Midge predators release spores higher in the water column, alleviating an environmental trap created when dead infected hosts sink. Focal hosts consume these dispersed spores, *increasing* infection prevalence (Cáceres et al. 2009). Midges can also induce changes in host phenotype that increase susceptibility (Duffy et al. 2011). High midge density correlates with higher infection prevalence in two sets of lakes (Hall et al. 2010, Penczykowski et al. 2014). Thus, selective and sloppy predators have opposite effects on disease spread.

Third, **spore predators** (other non-selective zooplankton [cladoceran] filter-feeders) consume free-living parasites while rarely becoming sick. Spore predation reduces contact

between focal hosts and parasites (Johnson et al. 2010). In our study system, spore predators can also compete with focal hosts, and contribute to host diversity (see more below). The most common spore predator taxa in our lakes (*Ceriodaphnia sp.*) highly resists infection, and the second most common (*D. pulicaria*) is almost completely immune. The former can reduce prevalence and density of infections in experiments, and both appear to reduce infection prevalence in lake communities (*D. pulicaria*: Hall et al. 2009, *Ceriodaphnia*: Strauss et al. 2015). Other even rarer cladoceran spore predators co-occur, but they rarely (if ever) become infected in lakes we sample (SRH, unpublished). Thus, these three modes of predation (selective, sloppy, and spore predation) each regulate disease through distinct mechanisms.

Links 1-4): Predators may be regulated by habitat structure and other predators

Refuge size, a critical habitat variable, varies among lakes and regulates selective fish predation. Visually oriented fish predators target large, conspicuous zooplankton (Brooks and Dodson 1965, Vanni 1986). However, large zooplankton can escape fish predation in the deep water refuge habitat. This refuge habitat is bounded at the top by temperature change (due to habitat choice by warm-water fishes), and at the bottom by oxygen depletion (due to physiological demands of zooplankton). Intensity of fish predation proves difficult to measure directly, but small body size of focal hosts indicates more intense predation (e.g., Mills and Schiavone 1982, Vanni 1986, Carpenter et al. 1987). Thus, smaller refuges should cause more intense fish predation (i.e., smaller focal host body size; **Link 1**).

Trophic interactions among predators, regulated by refuge size, could confound direct (Table 1) and indirect drivers of disease. Fish predators consume sloppy midge predators, and midge predators can also seek deep water refuge from fish predation (Gonzalez and Tessier

1997). Thus, intensity of fish predation (**Link 2a**) and/or refuge size (**Link 2b**) could regulate the density of midge predators. Furthermore, midges are gape-limited, preferentially culling smaller hosts (Pastorok 1981), and can induce plastic increases in host body size (Duffy et al. 2011). Thus, midges could also potentially impact the fish predation index (body size of focal hosts). Either way, fish predation intensity and midge density should be negatively correlated.

Both fish predators and midge predators selectively consume spore predators based on body size. Visually oriented fish target larger taxa, while gape-limited midges target smaller taxa (Gonzalez and Tessier 1997, Tessier and Woodruff 2002). The most common spore predator is small, and hence less conspicuous to fish but more susceptible to midges (*Ceriodaphnia;* hereafter: small spore predators. Frequency of these small spore predators within the host community should be higher in lakes with smaller refuges (**Link 3a**), more intense fish predation (**Link 3b**), and fewer midge predators (**Link 3c**). Larger bodied *Daphnia pulicaria* (hereafter: large spore predators) are more vulnerable to fish and less to midges. Moreover, these large spore predators compete superiorly without fish predation (Leibold 1991). Thus, they should become more frequent in lakes with larger refuges (**Link 4a**), less intense fish predation (**Link 4b**), and more midge predators (**Link 4c**). Overall, variation in refuge size and predation regimes should govern the importance of these two spore predators and perhaps restrict them to different types of lakes. All of these trophic interactions create interpretation problems with univariate data, because apparent effects of predators on disease could actually arise from changes in their prey (other predators).

Link 5): Host density may be regulated by predators

When disease transmission is density dependent, species interactions that regulate host density could indirectly drive disease (Anderson and May 1981). For example, predators that consume focal hosts and reduce their density can inhibit disease spread (e.g., Lafferty 2004). Alternatively, competitors can inhibit disease spread if they reduce focal host density by depleting shared resources (e.g., Mitchell et al. 2002). Fish predators and midge predators both consume focal hosts, and spore predators compete with focal hosts for shared algal resources (Gonzalez and Tessier 1997, Tessier and Woodruff 2002, Hall et al. 2009, Strauss et al. 2015). Thus, focal host density could be lower in lakes with more intense fish predation (**Link 5a**) or more midge predators (**Link 5b**), or in lakes dominated by small spore predators/competitors (**Link 5c**) or large spore predator/competitors (**Link 5d**). These potential indirect effects mediated by host density could even exceed the direct effects of these predators on disease (Table1).

Moreover, the importance of density-mediated effects could depend on the disease metric considered. Indirect effects mediated by density of focal hosts depend on strong links between focal host density and disease. However, host density can be more closely linked to density of focal host infections than infection prevalence, for example, due to non-linear density-prevalence relationships (Civitello et al. 2013). Thus, predators that regulate focal host density may primarily drive variation in density of infected hosts. In contrast, predators that interfere with transmission through other mechanisms might more strongly drive variation in infection prevalence (see Vanbuskirk and Ostfeld 1995, Randolph and Dobson 2012, Strauss et al. 2015). Here, spore predators uniquely drive disease through two mechanisms: lowering focal host density via competition, *and* consuming of free-living parasites (Hall et al. 2009, Strauss et al.

2015). Thus, the relative importance of these two mechanisms could depend on the metric of disease considered (prevalence vs. density of infections).

Link 6): Host diversity may be regulated by spore predators (hosts themselves)

The roles of spore predators also become entangled with a potentially spurious 'dilution effect'. A dilution effect associates decreases in **host diversity** with increases in disease risk for a focal host species (Ostfeld and Keesing 2000a, Keesing et al. 2006, Civitello et al. 2015a). This pattern emerges when rarer 'diluters' interfere with transmission among more competent, more common focal hosts. Interference can occur through spore predation (Johnson et al. 2010) or competition with focal hosts (Keesing et al. 2006). Thus, spore predators may serve as potential 'diluters' in our study system. Critically however, a spurious diversity-disease correlation could merely reflect the impacts of certain spore predators reducing disease, rather than any effects of host diversity *per se* (see LoGiudice et al. 2003, Randolph and Dobson 2012). This spurious result could occur if spore predators simultaneously reduce disease and increase our index of host diversity.

Accounting for links between spore predator frequencies and host diversity may help disentangle these potential impacts of host diversity *per se* from impacts of key spore predators. Because host communities in our lakes are so uneven (see below), we represent host diversity (including both focal hosts and spore predators) with the inverse Simpson's diversity index. With focal hosts dominating most of our lake communities, host diversity should increase with higher frequencies of small spore predators (**Link 6a**), large spore predators (**Link 6b**), and other spore predators (**Link 6c**). However, as spore predators become even more frequent and begin to dominate, a higher frequency of spore predators will actually decrease the inverse Simpson's

host diversity index. By including a few of these types of lakes, we may be able to decouple host diversity (which would begin to decline) from frequencies of key spore predators (which would continue to increase). Thus, it may become possible to disentangle direct effects of host diversity from spore predation. In other words, by linking spore predators to host diversity, we can test whether host diversity *per se* drives disease, or whether a spurious dilution pattern arises merely through correlation with key, relatively rare, spore predators.

Study system summary

Three modes of predation—selective, sloppy, and spore—appear relevant to our study system (Table 1). Habitat structure could directly or indirectly regulate all of them, based on decades of natural history research. However, trophic interactions among predators and their effects on host density and diversity could confound direct effects with indirect effects of predators on disease. Altogether, six ecological links obscure the most important pathways linking habitat to disease (see Table 2). Moreover, these most important paths could depend on the disease metric examined. To continue, we must first test each of these potential disease drivers (host density, modes of predation, and host diversity) and each ecological link with univariate field patterns. Then, we can begin to synthesize disease drivers and their interactions with path analysis.

Univariate Analyses

Field Sampling Methods

We sampled lakes in Green and Sullivan counties (Southwest Indiana, USA) during epidemics of focal hosts (mid August – early December). The sampling regime differed slightly

among years: we visited 15 lakes in 2010 (visited weekly), 18 in 2009 (weekly), and 28 in 2014 (fortnightly). At each visit we collected two samples of zooplankton, each pooling three vertical tows of a Wisconsin net (13 cm diameter, 153 µm mesh). With the first sample, we measured body size (~ 40+ focal host adults) and visually screened live focal hosts (400+) for infections. Mean body size of adult hosts provides the index of intensity of fish predation. Infection prevalence was calculated as the proportion of these focal hosts that were infected.

The second sample was preserved to estimate areal densities of focal hosts and midge larvae. We also estimated frequencies of focal hosts (mean frequency: 72%; maximum: 99%) and spore predators within the host (cladoceran) community (small bodied *Ceriodaphnia sp.* [15%, 79%], large *D. pulicaria* [8%, 44%] and all others lumped together [*Bosmina sp.*:3%, 28%; *Diaphanosoma sp.*: 0.7%, 12%; , *D. parvula*: 0.4%, 10%; *Alona sp.* & *Chydorus sp.*: 0.2%,1.4%, and very rare *D. ambigua* and *Scapholebris sp.*]). We calculated inverse Simpson's diversity index of this total host community (focal hosts and all spore predators). Infection prevalence of focal hosts was multiplied by their total areal density to yield density of infected hosts. Finally, we estimated refuge size with vertical casts of a Hydrolab multiprobe, taking temperature and oxygen at every 0.5 to 1.0 m. Refuge size was calculated as the difference between the depth of the thermocline (upper bound, defined as maximum buoyancy frequency) and the oxygen threshold (lower bound, 1 mg/L) (see Penczykowski et al. 2014). For each lake x year combination, we calculated a season (Sep.-Nov.) average for each variable.

Statistical methods

All statistical models were fit using R (R Development Core Team 2010). Predation modes (Table 1) and ecological links (Table 2) were tested individually with univariate mixed

effect models in the package NLME (Pinheiro and Bates 2000). 'Lake' was included in all models as a random effect (intercept only). With only three years of data, we modeled 'year' as a fixed (rather than random) effect. With this baseline model structure, we then used likelihood ratios to test significance of each relationship. Density of sloppy midge predators was log transformed prior to analyses. However, all other data remained untransformed in order to preserve their natural variance structures. We explicitly modeled variance of all response variables with exponential or power functions to describe the heteroskedasticity in the data (see Pinheiro and Bates 2000).

Univariate disease driver results

Field patterns supported host density, all three modes of predation, and host diversity as potential disease drivers. Density of focal hosts was not correlated with infection prevalence (Fig. 1 A; $P = 0.25$). However, it was positively correlated with infected host density (Fig. 1 B; $P < 0.0001$). For all other potential drivers, impacts on infected host density (Fig. S1) qualitatively mirrored those on infection prevalence (Fig. 2). Lakes with more selective fish predation (indexed by body size of focal hosts) had lower prevalence (Fig. 2 A; $P < 0.0005$) and density of infections (Fig. S1 A; $P < 0.0004$). In contrast, lakes with higher densities of sloppy midge predators (*Chaoborus*) had higher prevalence (Fig. 2 B; $P < 0.0001$) and density of infections (Fig. S1 B; $P < 0.0001$). Furthermore, lakes with higher frequencies of small spore predators (*Ceriodaphnia*) and other spore predators had lower prevalence (Fig. 2 C & E; both $P < 0.0005$) and density of infections (Fig. S1 C & E; $P = 0.0024$, $P < 0.0001$, respectively). However, frequency of large spore predators (*D. pulicaria*) was unrelated to prevalence (Fig. 2 D; $P = 0.58$) or density of infections (Fig. S1 D; $P = 0.38$). Finally, high host diversity also

312 correlated with low prevalence (Fig. 2 E; $P = 0.0074$) and density of infections (Fig. S1 E; $P <$
313 0.0005), consistent with the prediction of a dilution effect.
314
315 *Univariate ecological link results*
316 Links among habitat structure, predators, host density, and host diversity complicated
317 interpretation of these potential disease drivers (see Table 2 for statistical significance of each
318 link). Smaller refuges from fish marginally (but not significantly) increased the intensity of fish
319 predation (i.e., decreased body size of focal hosts [Link 1; Fig. 3 A]). However, more intense
320 fish predation did reduce density of sloppy midge predators (Link 2a; Fig. 3 B). In turn,
321 frequency of small spore predators (*Ceriodaphnia*) increased with smaller refuges (Link 3a; Fig.
322 3 D), more intense size-selective fish predation (Link 3b; Fig. 3 E), and lower densities of gape-
323 limited midges (Link 3c; Fig. 3 F). On the opposite side of the refuge spectrum, frequency of
324 large spore predators (*D. pulicaria*) increased with larger refuges (Link 4a; Fig. 3 G), less intense
325 size-selective fish predation (Link 4b; Fig. 3 H), but lower densities of gape-limited midge
326 predators (opposite of the prediction based on natural history, but only marginally significant;
327 Link 4c; Fig. 3 I). Thus, predators were regulated by habitat structure and each other.
328 Density of focal hosts was much less responsive to these predators, however. In fact, it
329 only decreased with higher frequency of small spore predators (marginally significant Link 5c;
330 Fig. 4 C, likely due to competition). All other links with densiy of focal hosts were insignificant
331 (Links 5a,b&d corresponding to Fig. 4 A, B & D, respectively). Finally, host diversity increased
332 with higher frequencies of small (Link 6a), large (Link 6b), and other spore predators (Link 6c),
333 since all of them were relatively rare (Fig. 5 A-C, respectively). Thus, density of focal hosts and
334 diversity of host communities (two potential disease drivers) were linked via the community

composition of spore predators. This multitude of significant, univariate links (see Table 2) potentially confound disease drivers (Figs. 2 & S1). Hence, we turned to path analysis to disentangle them.

STEP TWO – SYNTHESIZING DISEASE DRIVERS

Path Analysis Methods

To work through these complicated interactions, we used path analysis. To fit path models, we used the package lavaan (Rosseel 2012), weighting observations using the package lavaan.survey (Oberski 2014) to account for non-independence of the same lakes sampled in separate years. Given the limits of our dataset, we tested three complementary models. Model 1 disentangled drivers of infection prevalence, and model 2 disentangled drivers of density of infected hosts (hence, it includes 'focal host density' [Fig. 1 B]). Unfortunately, we could not include 'host diversity' in model 2, due to collinearity among too many disease drivers. Therefore, in order to more directly compare drivers of prevalence versus density of infections, we fit a third model. Model 3 is nearly identical to model 1, but it also includes 'focal host density' and omits 'host diversity'. These modifications create a parallel structural form for comparison with model 2.

All models were constructed, fit, and assessed using a robust, pre-determined protocol. First, all significant and trending univariate patterns were included in each appropriate path model (excepting the limitations due to collinearity, described above). Two links (between the 'fish predation index' and 'midge density', and between 'small spore predator frequency' and 'focal host density') were fit as covariances, implying correlation. All other links were fit as regressions, implying causality. Additional covariances were included for correlations among

358 frequencies of spore predators (since they shared a common denominator). Second, models were
359 fit with a maximum likelihood estimator (MLM) that was robust to non-normal standard errors
360 and used a robust Satorra-Bentler chi-square test statistic (Satorra and Bentler 2001). After
361 model fitting, residual covariances were inspected in order to identify any potentially missing
362 links. Through this process, the link between refuge size and the index of fish predation (Link 1)
363 was added to all three models. Third, we assessed model fits with several robust criteria,
364 including CFI, TLI, RMSEA, and SRMR test statistics (Hu and Bentler 1999) (see Appendix S1
365 in Supporting Information for details). Finally, we extracted *P* values and standardized
366 parameter estimates (SPE's) for each relationship. These SPE's were used to compare effect
367 sizes among paths in our final models.
368
369 **Path Analysis Results**
370 Fit statistics confirmed good fits of all three path models (see Table S1). Table 2
371 delineates each ecological link, reviews theory behind the relevant natural history of the plankton
372 system, and reports its statistical significance as a univariate pattern and link in path models 1, 2,
373 and 3, where applicable (see Tables S2-S4 for parameter estimates and more details).
374
375 *Path model 1: Disease drivers & underlying ecological links*
376 Path model 1 (Fig. 6) disentangled drivers of infection prevalence (Fig. 2). Lakes with
377 small refuges had more intense fish predation (Link 1), which in turn reduced density of sloppy
378 midge predators (Link 2a). Together, small refuges (Link 3a) and more intense fish predation
379 (Link 3b) increased frequency of small spore predators. In contrast, larger refuges (Link 4a) and
380 less intense fish predation (Link 4b) increased frequency of large spore predators. Even after

accounting for these ecological links, high frequency of small spore predators (*Ceriodaphnia*) still directly reduced infection prevalence (P = 0.048; SPE = -0.231). Simultaneously, high density of sloppy midge predators (*Chaoborus*) directly increased infection prevalence (P = 0.026; SPE = 0.294). However, the index of selective fish predation no longer exerted a significant direct effect on infection prevalence (P = 0.47; SPE = 0.098), even though it appeared important univariately (Fig. 2 A). Instead, fish drove indirect effects on disease, mediated trophically through changes in small spore predators and sloppy midge predators. Furthermore, frequency of other spore predators no longer significantly reduced prevalence of infection (P = 0.103; despite the relatively strong effect, SPE = -0.332). Finally, the negative diversity-disease pattern detected univariately (a dilution effect; Fig. 2 F) now disappeared (P = 0.79; SPE = 0.063). Instead, the path model clarified that this spurious pattern merely echoed, as a correlational shadow, direct links between infection prevalence and small spore predators (see Table 2).

Path models 2 and 3: Disease drivers and underlying ecological links

Model 2 (Fig. 7 A) disentangled drivers of density of infected hosts (Figs. 1 & S1). All analogous ecological links were identical (Links 1-2) or qualitatively similar (links 3-4) to model 1 (see Table 2). Additionally, (Link 5c) frequency of small spore predators (*Ceriodaphnia*) marginally correlated with lower density of focal hosts (P = 0.070; SPE = -0.240). In contrast, disease drivers differed extensively from Model 1. High total density of focal hosts caused high densities of infected focal hosts (P < 0.001; SPE = 0.500). Neither small spore predators (P = 0.16; SPE = -0.116), sloppy midge predators (P = 0.19; SPE = 0.190), nor selective fish predation (P = 0.68; SPE = 0.054) significantly regulated density of infected hosts, even though

all appeared important univariately (Fig. S1 A-C). Instead, in this path model, the tight relationship between total and infected density of focal hosts (Fig. 1 B) washed out direct effects of those other drivers. Nevertheless, small spore predators indirectly reduced density of infections by marginally lowering density of infected hosts, most likely via competition. As in model 1, these small spore predators were regulated by habitat structure (refuge size) and fish predation (see Table 2). Thus, habitat structure still connected to disease through predator-mediated pathways. However, when predicting density of infected hosts, these connections became weaker and less direct.

Path model 3, the prevalence based analogue of model 2, largely mirrored the original model of infection prevalence (path model 1). For example, sloppy midge predators still directly influenced disease, and selective predators still exerted habitat-mediated indirect effects on infection prevalence through midges and small spore predators. However, the intentional contrasts between models 2 (Fig. 7 A) and 3 (Fig. 7 B) become uniquely informative. Both model structures linked small spore predators to focal host density and each respective disease metric. However, only the direct link to prevalence mattered in model 3 (since total density of focal hosts remained unconnected to infection prevalence). In contrast, only the indirect link mediated by density of focal hosts mattered in model 2 (since the link between densities of total and infected hosts was so strong). Thus, small spore predators reduced each disease metric through different pathways.

DISCUSSION

We disentangled drivers of zooplankton epidemics using a two-step approach, guided by theory and field data. In step one, we identified several potential disease drivers with univariate

field patterns. In this analysis, host density was correlated with density of infected hosts, but not infection prevalence (Fig. 1). Additionally, both metrics correlated with selective fish predation, sloppy midge predation, and spore predation by certain zooplankton taxa (Fig. 2 & S1 A-E). Finally, both metrics declined with higher diversity of hosts (i.e., focal hosts and all spore predators combined). This univariate diversity-disease pattern supports a dilution effect (Fig. 2 & S1 F). However, some of these strong univariate patterns proved misleading, due to complex community interactions that obscured the direct and indirect drivers of disease (Figs. 3-5). In step two, path analysis uncovered and explained these misleading patterns. Specifically, path analyses delineated three types of complicating community interactions: 1) trophic interactions among predators (see Fig. 3), 2) impacts and regulators of focal host density (see Fig. 4), and 3) a spurious diversity-disease pattern (see Fig. 5). All of these interactions were ultimately grounded in habitat structure (i.e., refuge size; see Figs. 6-7).

Path analysis improved our interpretation of univariate field patterns by breaking down each of these complicating community interactions. First, it clarified how trophic interactions among predators shaped disease. Surprisingly, in path models 1 and 3, selective fish predation did not directly reduce infection prevalence (despite Fig. 2 A). Instead, fish predation worked indirectly by decreasing density of sloppy midge predators (Link 2a; Fig. 3 B) and increasing frequency of small spore predators (Link 3b; Fig. 3 E). In turn, these indirect effects were modulated by size of the refuges from fish predators (Link 1; Fig. 2 A). Second, in path models 2 and 3, small spore predators drove the two disease metrics through fundamentally different pathways. Small spore predators directly reduced infection prevalence, but indirectly reduced density of infected hosts by lowering density of focal hosts (likely via competition, and marginally significant; Link 5c; Fig. 4 C). Finally, path model 1 undermined a causal

interpretation of the dilution effect. Instead, the spurious univariate diversity-disease pattern merely reflected the direct effects of small spore predators on infection prevalence. In turn, these small spore predators were regulated by habitat structure and fish predation. Each of these results is more thoroughly discussed in turn.

Links 1-4): Trophic interactions among predators regulate direct and indirect effects on disease

Selective fish predation, regulated by habitat (Link 1; see Fig. 3 A), structured communities of other predators in these lakes as predicted (see Table 2). In lakes with small refuges, stronger fish predation reduced midge density (Link 2a; Fig. 3 B). Small bodied spore predators (*Ceriodaphnia*) became more frequent with smaller refuges and more intense fish predation (Links 3a&b; Fig. 3 D & E), while large spore predators (*D. pulicaria*) became more common with larger refuges and less intense fish predation (Links 4a&b; Fig. 3 G & H). Despite some suggestive univariate relationships (Links 3c & 4c; Fig. 3 F & I), midges had no effect on composition of spore predators in path models. Therefore, selective fish predators had the greatest capacity to regulate disease through trophically-mediated indirect interactions (i.e., predation on midges and spore predators). In other systems, other selective predators appear to regulate schistosomiasis (Sokolow et al. 2015), salmon lice (Krkosek et al. 2011), grasshopper fungus (Laws et al. 2009), moose tapeworms (Joly and Messier 2004), and grouse nematodes (Hudson et al. 1992) (see Table 1). In most of these systems, any potential indirect effects of these predators are less clear. However, their indirect effects could even be more important than their apparent direct effects, as in our case study here.

Indeed, indirect paths linking predators to disease apply broadly. First, our larger selective predator influenced density of the smaller sloppy predator. In turn, lakes with less fish

473 predation had more disease via higher midge density (Figs. 6 & 7B). Related relationships
474 among predators regulate other diseases. For example, foxes may reduce Lyme disease by
475 lowering density of small mammal hosts that critically spread infection. However, coyotes can
476 outcompete foxes, release small mammals from predation pressure by foxes, and indirectly
477 elevate Lyme disease risk through these cascading interactions (Levi et al. 2012). Similarly,
478 lobster predators prevent epidemics in sea urchins by maintaining low densities of hosts.
479 However, overharvesting lobsters releases urchins from predation pressure, stimulates their
480 population growth, and indirectly promotes bacterial epidemics (Lafferty 2004). In all three
481 cases, top predators (fish, coyotes, humans) mediate the impacts of mesopredators (midges,
482 foxes, lobsters) on disease. Interestingly, mesopredators can then alter disease through different
483 mechanisms, either increasing it (midges: by spreading parasites during sloppy feeding) or
484 decreasing it (foxes and lobsters: by controlling density of key hosts).
485 Second, selective fish predators also regulated disease through direct shifts in the host
486 community. Specifically, higher frequencies of small spore predators (*Ceriodaphnia*) reduced
487 infection prevalence, likely via consumption of free-living parasites (Fig. 2 C). In turn, intense
488 fish predation increased frequency of these small spore predators and hence indirectly reduced
489 disease (Figs. 6 & 7B). Consumers in other systems can regulate disease via similar shifts in
490 host communities. Grazing by vertebrate herbivores can increase frequency of highly competent
491 grass hosts, and hence increase prevalence of viral disease (Borer et al. 2009). Thus, consumer
492 mediated shifts in host communities can either increase or decrease disease. Other examples
493 merit more thorough exploration. For example, variation in community structure of hosts can
494 drive hantavirus transmission (Clay et al. 2009). Predators of rodents also appear to decrease

hantavirus prevalence (Orrock et al. 2011). Could predators reduce hantavirus by regulating host community structure, by depressing density of focal hosts, or both?

Shifts in structure of host communities do not always drive disease. In our case study, large spore predators (*D. pulicaria*), had no effect on either disease metric (Figs. 2 & S1 D). This seemed surprising, since large spore predators completely resist infection and reduce transmission in experiments (Hall et al. 2009). In the field, they also reduced epidemic size in a different set of Michigan lakes (Hall et al. 2009) and delayed the start of epidemics in a subset of the present Indiana lakes (Penczykowski et al. 2014). However, using seasonal averages, they did not reduce infection prevalence among lakes in Michigan (Hall et al. 2010) or Indiana (Fig. 2 D). Perhaps seasonal declines in refuge size in these Indiana lakes squeeze out this larger spore predator just as epidemics in the focal host begin. Alternatively, *D. pulicaria* can inhabit a deeper water microhabitat (Leibold 1991), potentially below where spores are consumed by focal hosts (Cáceres et al. 2009). Either way, large spore predators somehow remained temporally or spatially irrelevant. Nonetheless, a general lesson arises here: competency assays and transmission experiments alone may not identify key species that drive disease in nature. Experiments must be paired with field data to robustly identify these taxa (e.g., Johnson et al. 2013, Venesky et al. 2014, Rohr et al. 2015). Only then can we begin to sort through the direct and indirect species interactions that regulate disease.

Overall, indirect effects overshadowed the direct effects of selective fish predation in our case study. Initially, selective fish predation seemed to strongly regulate both metrics of disease (Fig. 2A, S1A). However, these univariate patterns (especially for infection prevalence) ignored trophic interactions between fish predation, midges, and small spore predators (described above). After accounting for these indirect effects in path model 1, the direct effects of fish predation

disappeared (Figs. 6-7). Direct effects of fish predation might be more important elsewhere (e.g., in Michigan lakes: Duffy and Hall 2008, Hall et al. 2010). Alternatively, indirect effects mediated by mesoscale predators and host community structure might frequently overshadow direct effects of selective predators, even in the Michigan lakes (see Hall et al. 2010), or even more generally, in other disease systems (Table 1). Thus, our case study illustrates a common challenge for community and disease ecologists. Focusing on potential direct effects of predators is relatively simple, while unraveling complicated trophic webs requires a great amount of data and insight from natural history. Nevertheless, these indirect effects can be extremely influential (e.g., Lafferty 2004, Borer et al. 2009, Levi et al. 2012, Orlofske et al. 2012, Orlofske et al. 2014, Rohr et al. 2015).

Link 5): Impacts and regulators of focal host density

Density of focal hosts impacted the two disease metrics differentially. Univariately, density of focal hosts had no relationship with infection prevalence (Fig. 1 A). However, total and infected density of focal hosts were closely linked (Fig. 1 B). This mismatch may have arisen because high host density can depress per capita infection risk, decoupling the density-prevalence relationship (Civitello et al. 2013). These different roles of host density caused stark differences between path models disentangling infection prevalence (path model 2; Fig. 7 A) and density of infected hosts (path model 3; Fig. 7 B). Specifically, small spore predators and sloppy midge predators directly regulated infection prevalence, but no predators directly regulated density of infected hosts. Instead, these potential impacts (supported univariately) were statistically overwhelmed by the strong link between density of total and infected hosts in the path analysis. In turn, focal host density was not regulated by fishes, midges, or large spore

predators (Fig. 4 A, B & D, respectively). However, it was marginally regulated by frequency of small spore predators (Link 5c; Fig. 4 C; $P = 0.07$), who compete with focal hosts (Strauss et al. 2015) and who themselves depend on habitat structure and fish predation. Thus, these small spore predators indirectly reduced density of infected hosts, likely via competition (Fig. 7 A).

Consequently, small spore predators reduced disease in two different ways, each primarily driving a different disease metric. In general, consumption of free living fungal spores can *reduce encounters* between focal hosts and parasites, while competition can *regulate host density* (see Strauss et al. 2015). This combination of encounter reduction and host regulation defines 'friendly competition' (Hall et al. 2009, Strauss et al. 2015). Here, path analysis enabled us to partition host regulation (mediated by focal host density; Fig. 7B) versus encounter reduction (not mediated by focal host density; Fig. 7A). The partition reveals that host regulation primarily reduced density of infected hosts, while encounter reduction reduced infection prevalence. Thus, although the univariate links between *Ceriodaphnia* frequency and prevalence (Fig. 2 C) or density of infections (Fig. S1 C) looked superficially similar, they likely arose by different mechanisms. These two components of friendly competition may be quite general. Examples likely include hantavirus transmitted among rodents (Clay et al. 2009), *Schistosoma* among snails (Johnson et al. 2009), parasites in intertidal communities (Johnson and Thieltges 2010), emerging diseases in amphibians (Johnson et al. 2013, Venesky et al. 2014), and fungal pathogens and viruses in plant communities (Mitchell et al. 2002, Boudreau 2013, Lacroix et al. 2014). A similar partition between host regulation and encounter reduction could help clarify drivers of prevalence versus density of infections in all of these systems.

More generally, path analyses can attribute changes in disease to either changes in host density or changes in other drivers. This approach could be broadly useful (see Begon 2008).

For example, it could determine whether selective predators (see Table 1) reduce disease by merely reducing total host density, or also by selectively culling infected hosts (or, as in this case study, via other indirect paths). In Lyme disease, density of infected ticks depends on both total tick density and infection prevalence. In turn, both of these factors can depend on the rodent community (Vanbuskirk and Ostfeld 1995, Randolph and Dobson 2012). Path analysis could clarify whether rodents in field data drive Lyme disease more through infection prevalence or total density of ticks. Dragonfly predators regulate *Ribeiroia* infections in amphibians by both consuming free-living parasites (reducing transmission) and lowering host density via predation (elevating per-host transmission risk, because parasites seek hosts). These impacts counterbalance each other and are extremely difficult to detect in field data, but path models might tease them apart (Orlofske et al. 2014, Rohr et al. 2015). These examples exhibit a wide range of insights that can be gained with path models that distinguish between drivers of host densities and drivers of per capita transmission.

Link 6): Spurious diversity-disease pattern

The host diversity-disease pattern in our case study proved fairly misleading. In univariate regressions, higher diversity of hosts appeared to decrease prevalence (Fig. 2 F) and density (Fig. S1 F) of infections, consistent with the pattern behind the controversial dilution effect (Ostfeld and Keesing 2000a, Keesing et al. 2006, Begon 2008, Randolph and Dobson 2012). However, in path model 1 (Fig. 6), diversity had a negligible effect on disease. As such, our results support the dilution effect as spurious correlational pattern, but not a causal disease driver. Instead, path model 1 shows how small spore predators (*Ceriodaphnia*) strongly reduced infection prevalence themselves (Fig. 2 C & E). Simultaneously, frequency of all spore

predators increased host diversity (Links 6a&c; Fig. 5 A & C). Once we accounted for these links, diversity itself had a negligible effect on disease. This result makes sense since no *a priori* mechanism links diversity *per se* to disease (see LoGiudice et al. 2003, Randolph and Dobson 2012). In contrast, *Ceriodaphnia* spore predators can reduce disease mechanistically—by both consuming free-living parasite spores and competing with focal hosts (Strauss et al. 2015).

More generally, a similar confounding correlation between diversity and key 'diluters' can arise whenever focal hosts are common and diluters are rare (e.g., Ostfeld and Keesing 2000b, Johnson et al. 2013, Lacroix et al. 2014). Incidentally, this condition is one of the core requirements for a dilution effect (Ostfeld and Keesing 2000a, Keesing et al. 2006). Although meta-analysis demonstrates that diversity appears to broadly inhibit parasites (Civitello et al. 2015a), the mechanistic drivers of these diversity-disease patterns are rarely dissected. In the meta-analysis, 89 of 168 studies compared infection risk for host species with and without one additional species. In these cases, the design clarifies which 'diluter' species reduced disease. However, in the remaining 79 studies, it is often challenging to disentangle diversity *per se* from the identity of key diluters, especially in observational studies. Thus, compelling diversity-disease patterns of dilution effects may broadly obscure the key taxa and mechanisms driving these patterns. More experiments that independently manipulate diversity and species identity are needed to rigorously attribute 'diluting' effects to key taxa versus diversity *per se*.

Alternatively, with path analyses it even becomes possible to attribute *observational* dilution patterns to key diluter taxa. Through the same approach, we can also tease apart effects of key diluters from potential correlative changes in density of focal hosts (see Begon 2008). Finally, it becomes possible to link habitat to disease via key diluters (i.e., small predators dilute in higher predation lakes with smaller refuges). With this habitat-centered approach, we can

clarify why species diversity correlates with disease, which species drive the pattern, and how they interfere with disease transmission. This approach greatly improves upon more correlative studies between diversity and disease (e.g., Allan et al. 2009, Huang et al. 2013), although those patterns offer an important starting point.

Future directions

The habitat-centered approach here could be expanded to synthesize other community interactions. For example, other habitat variables and abiotic drivers could explain additional variation in our *Metschnikowia* disease system. Here, we grounded all drivers in size of the deep water refuge. However, midge density was not related to refuge size (Link 2b; Fig. 3 C), possibly because midge larvae can also use deep anoxic waters or sediments below the deep-water refuge (Gonzalez and Tessier 1997). Instead, lakes with more dissolved organic carbon (DOC) have more midges (Overholt et al. 2012). DOC can also structure the refuge habitat, intensity of fish predation, and frequencies of spore predators in the cladoceran community (Wissel et al. 2003, Penczykowski et al. 2014). Moreover, DOC reduces solar radiation, which can directly kill free-living fungal *Metschnikowia* spores (Overholt et al. 2012). We aim to study these interactions in future analyses armed with more data. More ambitiously, we hope to eventually synthesize our results with other, less well-documented factors among our lakes. For example, a broader synthesis could incorporate impacts of human fishing, predation by piscivorous fish, lake productivity, shifts in phytoplankton communities, or outbreaks of other parasites of zooplankton, phytoplankton, or fishes. We must first lay the groundwork to understand all of these factors' roles in the aquatic food web before we can synthesize their interactions (but see Civitello et al. 2015b)

633 Path models of other disease systems could also test other important modes of predation.
634 Most obviously, in other systems, predation of intermediate hosts could influence transmission of
635 tropically-transmitted parasites while 'micropredation' can transmit parasites when
636 micropredators act as disease vectors (see Lafferty and Kuris 2002). In our system, two
637 additional modes may occur. First, predators can change host behavior, which may in turn
638 change their exposure to parasites (Thiemann and Wassersug 2000). Fish and midge predation
639 can regulate the depths at which focal hosts and spore predators migrate and reside (Leibold
640 1991, Gonzalez and Tessier 1997), possibly influencing contact with parasites. Second,
641 predators can change host traits, rendering them either more (e.g., Katz et al. 2014) or less (e.g.,
642 Groner and Relyea 2015) susceptible to parasites. One such trait is body size: larger hosts have
643 higher exposure rates and larger spore yields, both of which can increase disease (Hall et al.
644 2007, Duffy et al. 2011, Bertram et al. 2013, Civitello et al. 2015b, Strauss et al. 2015). To
645 understand how these and other modes of predation interact, we must first clearly understand
646 their direct effects on disease (e.g., Table 1). Then, we can begin to examine their interactions.
647
648 *Summary*
649 Here, we disentangled community disease drivers of zooplankton epidemics using a two-
650 step approach. We aimed to explain the most important paths linking habitat structure to disease,
651 via changes in host density, three modes of predation, and/or host diversity. In step one, we
652 identified several potential disease drivers with univariate field patterns, motivated by natural
653 history theory. However, several of these univariate patterns proved misleading, due to complex
654 community interactions. In step two, path analysis uncovered and explained these misleading
655 patterns. For instance, we detected an apparent effect of selective predation, but then explained

it better through indirect trophically-mediated effects on sloppy and spore predators. We detected weak effects of selective, sloppy, and spore predation on density of infected hosts, but these signals were overwhelmed by the much stronger signal of total host density itself. Finally, we detected a disease-diversity pattern signaling a 'dilution effect', but then explained the pattern mechanistically by encounter reduction and host regulation from a key spore predator taxa. Ultimately, habitat structure grounded all three of these interactions in the path models. We hope that this approach to simplifying complexity will stimulate similar work in other disease systems. We must continue to disentangle these webs of interactions in order to advance our broad understanding of the community ecology of disease.

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905 DATA AVAILABILITY:

906 Data associated with this paper have been deposited in Dryad:

907 http://dx.doi.org/10.5061/dryad.4t9f2

908 **TABLES**

909 **Table 1.** Three modes of predation and their direct effects on disease: general theory, empirical examples, and natural history in the
910 study system here, with a zooplankton focal host (*Daphnia dentifera*) and a fungal parasite (*Metschnikowia bicuspidata*).

Predation Mode & General Theory	**Select Empirical Examples**	***Daphnia* / *Metschnikowia* system**
Selective Predation ***Theory:*** Selective predators target and cull infected prey, reducing prevalence, density, or intensity of infections (Hudson et al. 1992, Packer et al. 2003, Hall et al. 2005).	• Selective prawn predators target schistosome-infected snails, and appear to reduce schistosomiasis transmission (Sokolow et al. 2015). • Selective piscivorous fish target lice-infected juvenile salmon, likely lowering sea lice infection loads (Krkosek et al. 2011). • Selective spiders target fungus-infected grasshoppers, reducing parasite-driven host mortality (Laws et al. 2009). • Selective wolves appear to target moose heavily infected with tapeworms, reducing infection burdens (Joly and Messier 2004). • Selective foxes appear to target heavily infected grouse, potentially lowering nematode infection burdens (Hudson et al. 1992).	Bluegill sunfish (*Lepomis macrochirus*) predators target infected hosts because fungal infection make hosts conspicuous (Duffy and Hall 2008). Selective fish predation appears to lower infection prevalence (Hall et al. 2010).

Sloppy Predation ***Theory:*** Sloppy predators (or herbivores, or scavengers) can distribute infectious free-living parasites when they attack infected prey (Cáceres et al. 2009, Auld et al. 2014).	• Sloppy *Didinium* predators may increase infectious free living bacteria, when attacking infected *Paramecium* prey (Banerji et al. 2015). • Sloppy butterflyfish attack infected coral and enhance water-borne transmission of black-band disease (Aeby and Santavy 2006). • Sloppy beetle herbivores spread rust fungus spores (potentially long distances) after foraging on infected musk thistle (Kok and Abad 1994). • Sloppy jackal or vulture scavengers may distribute anthrax spores away from ungulate carcasses through feces (Lindeque and Turnbull 1994).	Larval *Chaoborus* midges regurgitate spores after attacking infected hosts (Cáceres et al. 2009). High midge density correlates with high infection prevalence (Hall et al. 2010).
Spore Predation *(more generally:* ***predation of free-living parasites****)*	• Zooplankton consume free-living chytrid zoospores, potentially suppressing outbreaks of algal chytrids (reviewed: Kagami et al. 2014). • Aquatic micropredators consume fungal zoospores, reducing infection rates of chytridiomycosis in amphibians (Schmeller et al. 2014).	Cladoceran spore predators inadvertently "vacuum" spores while filter-feeding. They

Theory: Predators of free-living parasites can consume parasites without becoming infected. Spore predation reduces encounters between focal hosts and parasites and can lower infection prevalence or density of infections (Johnson et al. 2010, Strauss et al. 2015).	• Damselfly nymphs consume free-living trematode larvae, reducing *Ribeiroia* infections in amphibian hosts (Orlofske et al. 2012). • Small fishes consume free-living trematode larvae, potentially reducing transmission success to final hosts (Kaplan et al. 2009). • Predatory fungi capture and consume free-living nematodes, even after passage through dog gastrointestinal tracts, offering potential biocontrol for nematodes infecting mammals (Carvalho et al. 2009). • Dung beetles feed on parasitic nematodes and protozoans, broadly reducing transmission to livestock, wildlife, and humans (reviewed: Nichols et al. 2008).	rarely (small *Ceriodaphnia sp.*) or never (large *D. pulicaria*) become infected. Both taxa appear to reduce prevalence and/or density of infections (Hall et al. 2009, Hall et al. 2010, Penczykowski et al. 2014, Strauss et al. 2015).

915 **Table 2.** Six ecological links among habitat, predators, density of focal hosts, and diversity of the host community complicate
916 disease drivers in the study system with zooplankton focal hosts (*Daphnia dentifera*) and fungal parasites (*Metschnikowia*
917 *bicuspidata*). Column 1 delineates each link, column 2 reviews relevant natural history theory, and column 3 reports statistical
918 significance as a univariate pattern. Columns 4 and 5 report *P* values and standardized parameter estimates with links as paths in path
919 model 1(disentangling drivers of infection prevalence), and path model 2 (disentangling drivers of density of infected hosts).
920 Ecological links in path models 2 and 3 are quantitatively identical (column 5). Significant and trending *P* values ($P < 0.1$) are bold.
921

Ecological Link	Natural History Theory	Univariate Result	Path Model 1 (Fig. 6)	Path Models 2 & 3 (Fig. 7 A & B)
Link 1: Regulators of Intensity of Selective Predation (Fish, e.g., *Lepomis macrochirus*):	**1)** Prey escape fish predation in the refuge. Small refuges should increase[1]	*P* = 0.11 Fig. 3 A	***P* = 0.004** SPE = 0.297	
Link 2: Regulators of Density of Sloppy Predators (Midge, *Chaoborus punctipennis):*	**2a)** More intense fish predation should decrease (via predation)[2]	***P* = 0.017** Fig. 3 B	***P* = 0.052** SPE = 0.281	
	2b) Larger refuges from fish predation should increase[2]	*P* = 0.98 Fig. 3 C	*Univariate relationship not significant or trending*	

Link 3: Regulators of Frequency of Small Spore Predators (Zooplankton, *Ceriodaphnia sp.*):	**3a)** Smaller refuges from fish should increase (small = inconspicuous)[2]	***P* < 0.0001** Fig. 3 D	***P* = 0.009** SPE = -0.251	***P* = 0.037** SPE = -0.211
	3b) More intense fish pred. should increase (small = inconspicuous)[1]	***P* = 0.0064** Fig. 3 E	***P* = 0.002** SPE = -0.351	***P* = 0.09** SPE = -0.358
	3c) Lower gape-limited midge density should increase (small = susceptible)[3]	***P* = 0.0072** Fig. 3 F	*P* = 0.75 SPE = -0.039	*P* = 0.89 SPE = -0.016
Link 4: Regulators of Frequency of Large Spore Predators (Zooplankton, *Daphnia pulicaria*):	**4a)** Larger refuges from fish should increase (large = conspicuous)[4]	***P* < 0.0001** Fig. 3 G	***P* < 0.001** SPE = 0.600	***P* < 0.001** SPE = 0.608
	4b) Less intense fish predation should increase (large = conspicuous)[1]	***P* < 0.0005** Fig. 3 H	***P* = 0.002** SPE = 0.254	***P* = 0.003** SPE = 0.236
	4c) Higher gape-limited midge density should increase (large = resistant)[2]	****P* = 0.062** Fig. 3 I	*P* = 0.30 SPE = -0.075	*P* = 0.35 SPE = -0.070
Link 5: Regulators of Density of Focal Hosts (Zooplankton, *Daphnia dentifera*):	**5a)** More intense fish predation should decrease (via predation)[2]	*P* = 0.73 Fig. 4 A	*Univariate relationship not significant or trending*	
	5b) Higher midge density should decrease (via predation)[2]	*P* = 0.46 Fig. 4 B	*Univariate relationship not significant or trending*	

	5c) Higher freq. small spore pred.	***P* = 0.070**	*Host density not*	***P* = 0.070**
	should decrease (via competition)[4]	Fig. 4 C	*important (Fig. 1 A)*	SPE = -0.240
	5d) Higher freq. large spore pred.	*P* = 0.18	*Univariate relationship not*	
	should decrease (via competition)[5]	Fig. 4 D	*significant or trending*	
Link 6: Regulators of Host	**6a)** Higher freq. small spore pred.	***P* < 0.0005**	***P* < 0.001**	
Diversity (Zooplankton: Focal	should increase (because rare)	Fig. 5 A	SPE = 0.365	
Hosts and Spore Predators):	**6b)** Higher freq. large spore pred.	***P* = 0.037**	***P* < 0.001**	† *collinearity*
	should increase (because rare)	Fig. 5 B	SPE = 0.479	*among disease*
	6c) Higher freq. rare spore pred.	***P* < 0.0001**	***P* < 0.001**	*predictors*
	should increase (because rare)	Fig. 5 C	SPE = 0.664	

922 * = univariate trend detected in the opposite direction than predicted from theory (Link 4c)

923 † = links not included, because inclusion of the 'dilution effect' link between diversity and disease created collinearity among disease

924 predictors (path models 2 and 3)

925 References: [1] (Tessier and Woodruff 2002). [2](Gonzalez and Tessier 1997). [3] (Wissel et al. 2003). [4](Tessier and Welser 1991). [4](Strauss

926 et al. 2015).[5] (Hall et al. 2009).

FIGURE LEGENDS

Figure 1. Overall density of focal hosts (*Daphnia dentifera*) **A)** does not drive infection prevalence, but **B)** does drive density of infected focal hosts. Each point is a lake population in a given year (2009, 2010, and 2014). Infection prevalence is mean proportion of focal hosts infected during an epidemic season. Infected host density is mean density of infected focal hosts over the same time period. Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

Figure 2. Three modes of predation (Table 1) correlate with infection prevalence of the focal host zooplankton (*Daphnia dentifera*). Infection prevalence is mean proportion of focal hosts infected during an epidemic season. Each point is a lake population in a given year. **A)** *Selective Predation:* Fish predation is indexed by body size of adult focal hosts (mm). Smaller size = more fish predation (↑); larger size = less (↓). More selective fish predation (left on x-axis) correlated with lower infection prevalence. **B)** *Sloppy Predation:* More sloppy midge predators (*Chaoborus*) correlated with higher infection prevalence. **C-E)** *Spore Predation:* **C)** High frequencies within the host community of small spore predators (*Ceriodaphnia*) correlated with lower infection prevalence. **D)** Frequency of large spore predators (*D. pulicaria*) did not, but **E)** frequency of other spore predators also did. *Host Diversity:* Finally, **F)** higher host diversity (focal hosts and spore predators) also correlated with lower infection prevalence, consistent with a dilution effect. Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

Figure 3. Predators were regulated by habitat structure and trophic interactions with other predators (Links 1-4; see Table 2). Each point is a lake population in a given year. **A)** Small refuge habitats had only marginally more fish predation. **B)** More intense fish predation (smaller adult focal host size; left on x-axis) correlated with fewer sloppy midge predators (*Chaoborus*). However, **C)** refuge size did not predict midge density. Small spore predators were more frequent when **D)** refuge size was smaller, **E)** fish predation intensity was higher, and **F)** midge density was lower. In contrast, large spore predators were more frequent when **G)** refuge size was larger, **H)**, fish predation intensity was lower, and **I)** midge density was lower (marginally). Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

Figure 4. Focal host density (*Daphnia dentifera*) was only marginally regulated by small spore predators (Link 5, see Table 2). Each point is a lake population in a given year. Focal host density was not reduced by **A)** fish predation intensity or **B)** midge predator density (both are predators of focal hosts). **C)** Focal host density was marginally lower in lakes with higher frequencies of small spore predators (*Ceriodaphnia*), but **D)** not in lakes with higher frequencies of large spore predators (*D. pulicaria*) (both spore predators compete with focal hosts). Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

Figure 5. Diversity of the host community (i.e., focal hosts [*Daphnia dentifera*] and spore predators) was strongly regulated by frequency of each group of spore predators. Spore predators are themselves hosts, but are all rarer than focal hosts. Each point is a lake population

in a given year. Higher frequencies of **A)** small spore predators (*Ceriodaphnia*), **B)** large spore predators (*D. pulicaria*), and **C)** other spore predators all increased host diversity. Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

Figure 6. Path model 1 disentangles drivers of infection prevalence in a focal host (*Daphnia dentifera*). Ecological links among habitat, predators, and host diversity (Links 1-4 & 6, Table 2; Figs. 3 & 5) synthesize three modes of predation (Table 1; Fig. 2). From the bottom, moving up: **1)** Small refuges led to intense selective fish predation. **2a)** Intense fish predation correlated with low density of sloppy midge predators *(Chaoborus*). **3a)** Small refuges & **3b)** intense fish predation increased frequency of small spore predators (*Ceriodaphnia*) in the host community. **4a)** Large refuges & **4b)** less intense fish predation increased frequency of large spore predators (*D. pulicaria*). **6a-c)** Frequencies of all spore predators increased host diversity. **Disease Drivers:** Sloppy midge predators and small spore predators (*Ceriodaphnia*) had large, significant, and direct effects on infection prevalence. Selective fish predation did not directly drive infection prevalence, but indirectly mediated density of sloppy midge predators and frequency of small spore predators. Other spore predators reduced disease, but not significantly. The dilution effect pattern was not significant, once accounting for the direct effects of small spore predators and other spore predators. Model fit statistics: Satorra-Bentler chi square P = 0.903; CFI = 1.000; TLI = 1.152; RMSEA = 0.000; SRMR = 0.044.

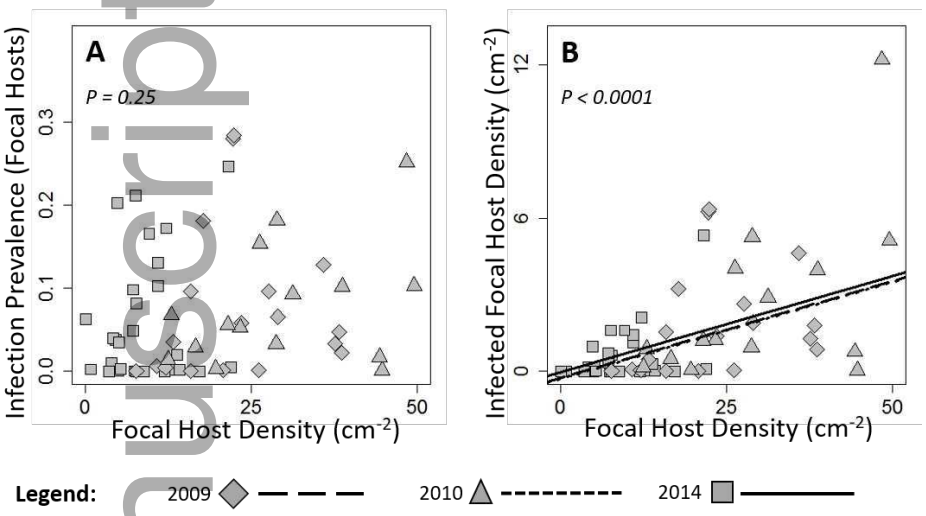
Figure 7. A) Path model 2 disentangles drivers of infected focal host density (*Daphnia dentifera*). **B)** Path model 3 mirrors the structure of model 1 (Fig. 6), but without 'host

diversity', in order to facilitate direct comparisons with path model 2. **Both models:** Ecological links among habitat, host density, and predators (Links 1-5, Table 2; Figs. 1, 3 & 4) synthesize three modes of predation (Table 1; Fig. S1). Links 1-4 are qualitatively identical to Fig. 6. Additionally, **5c)** high frequencies small spore predators (*Ceriodaphnia* competitors) marginally correlated with low focal host densities. **Model 2):** Neither spore predators, sloppy predators, nor selective predators regulated density of infected hosts. Instead, it depended only on total density of focal hosts. **Model 3):** Drivers are qualitatively identical to model 1 (Fig. 6). Model 2 fit statistics: Satorra-Bentler chi square $P = 0.317$; CFI = 0.985; TLI = 0.948; RMSEA = 0.053; SRMR = 0.070. Model 3 fit statistics: Satorra-Bentler chi square $P = 0.404$; CFI = 0.997; TLI = 0.990; RMSEA = 0.022; SRMR = 0.066.

1007 **FIGURES**

1008

1009 **Figure 1.**



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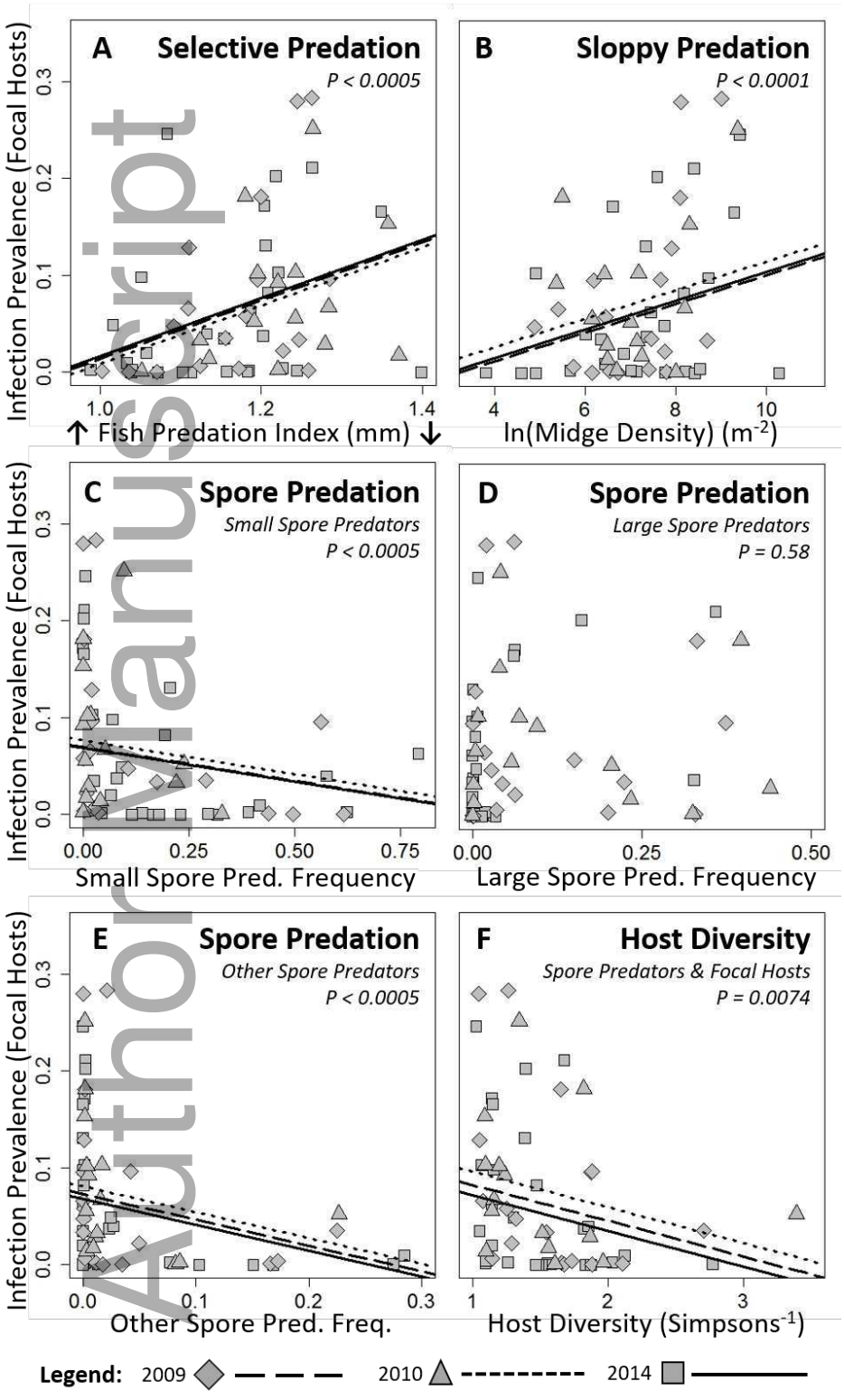

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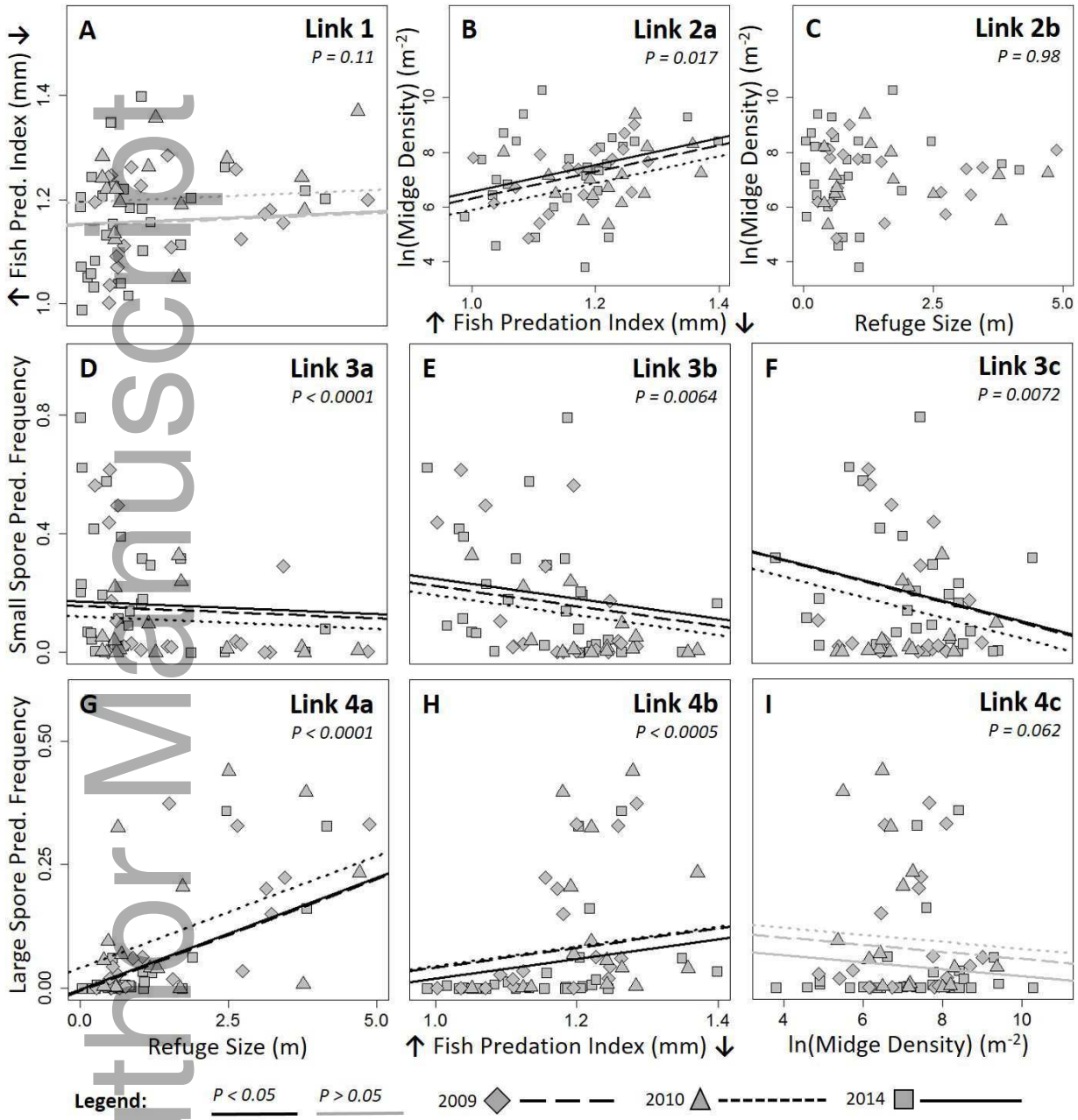

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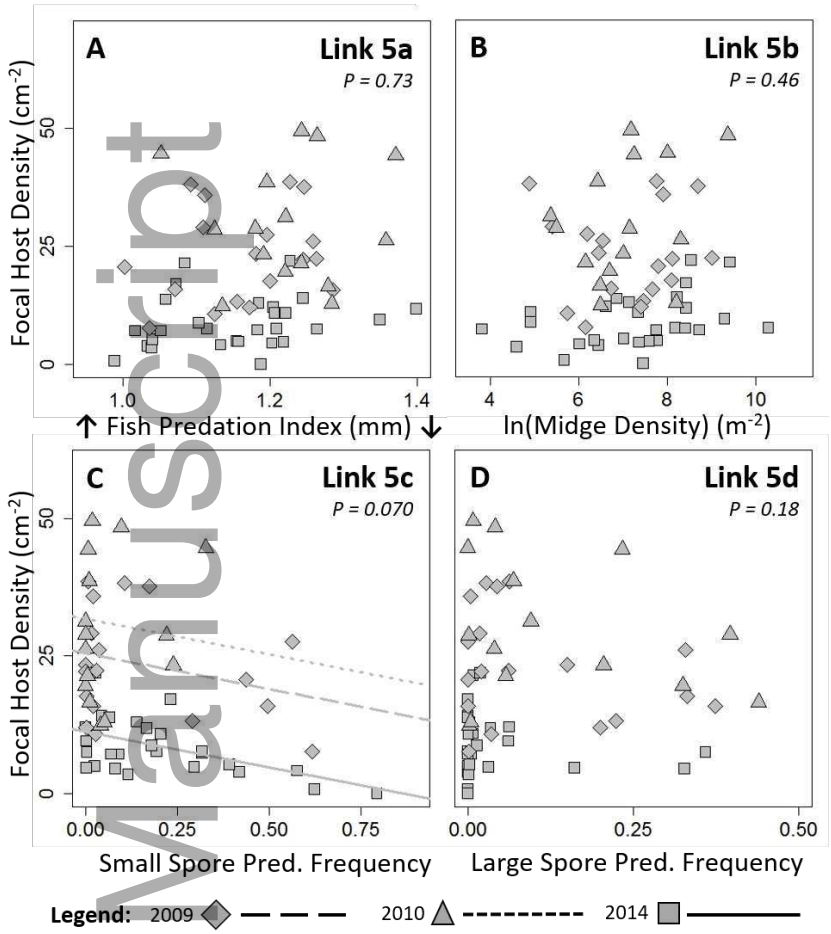

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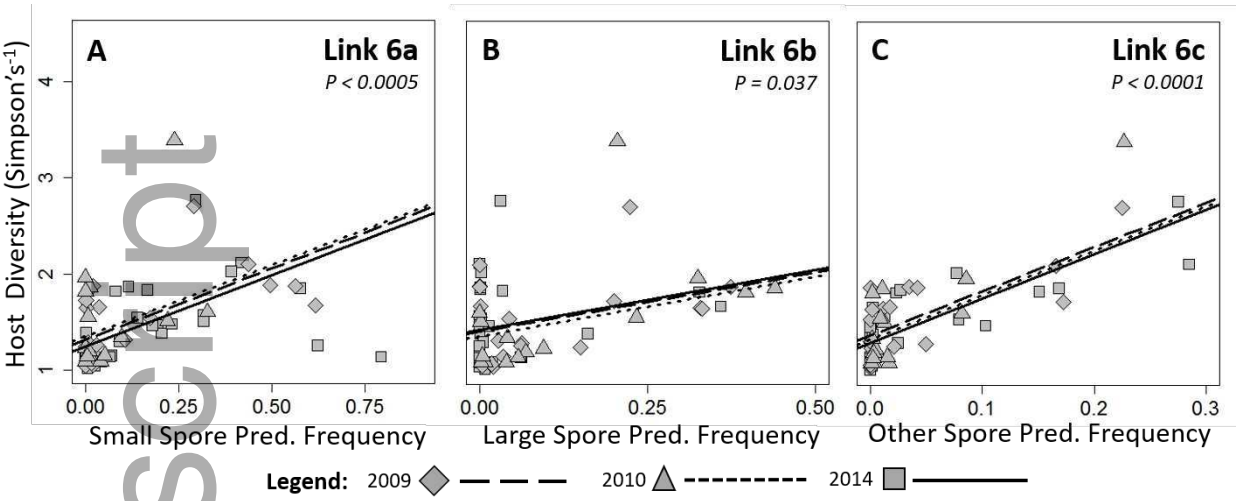

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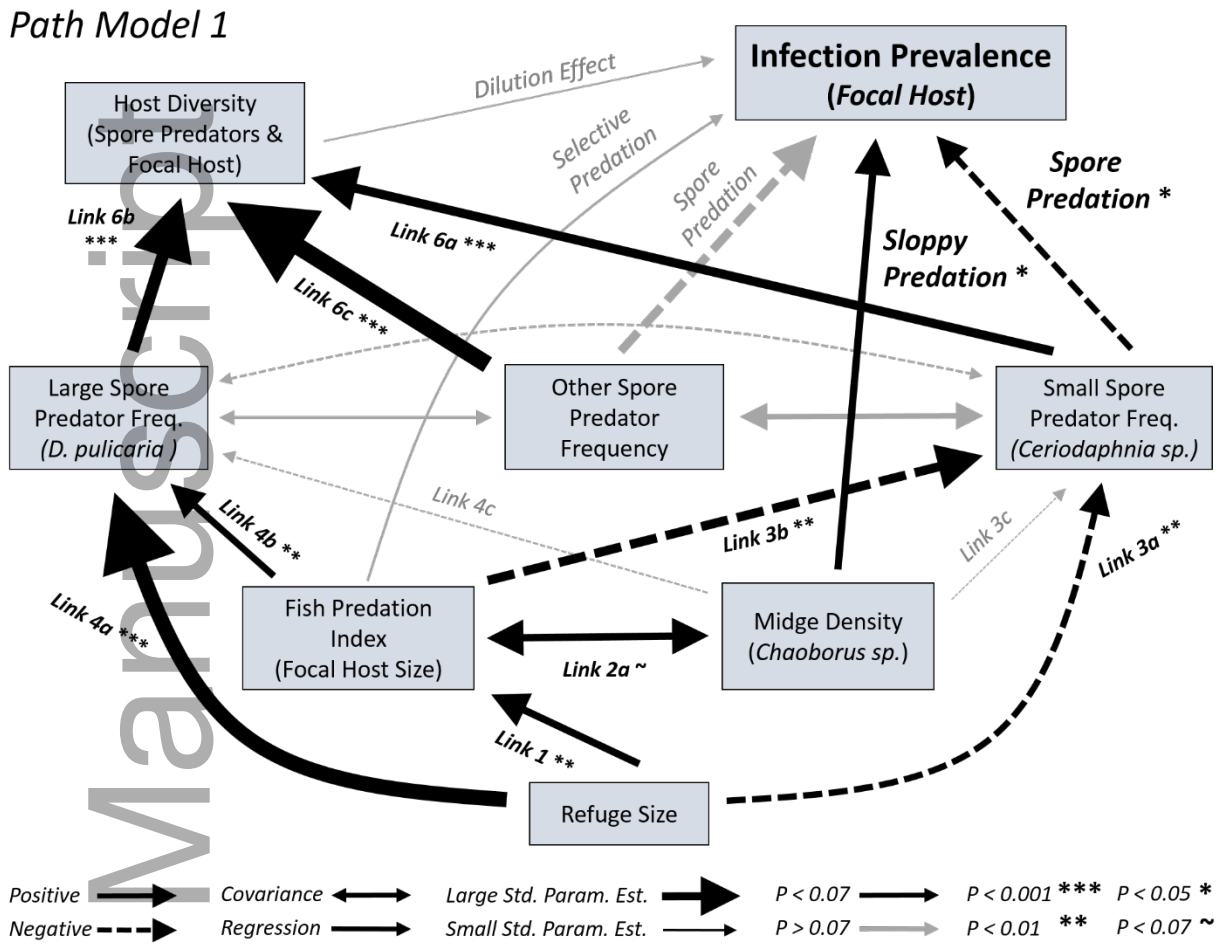

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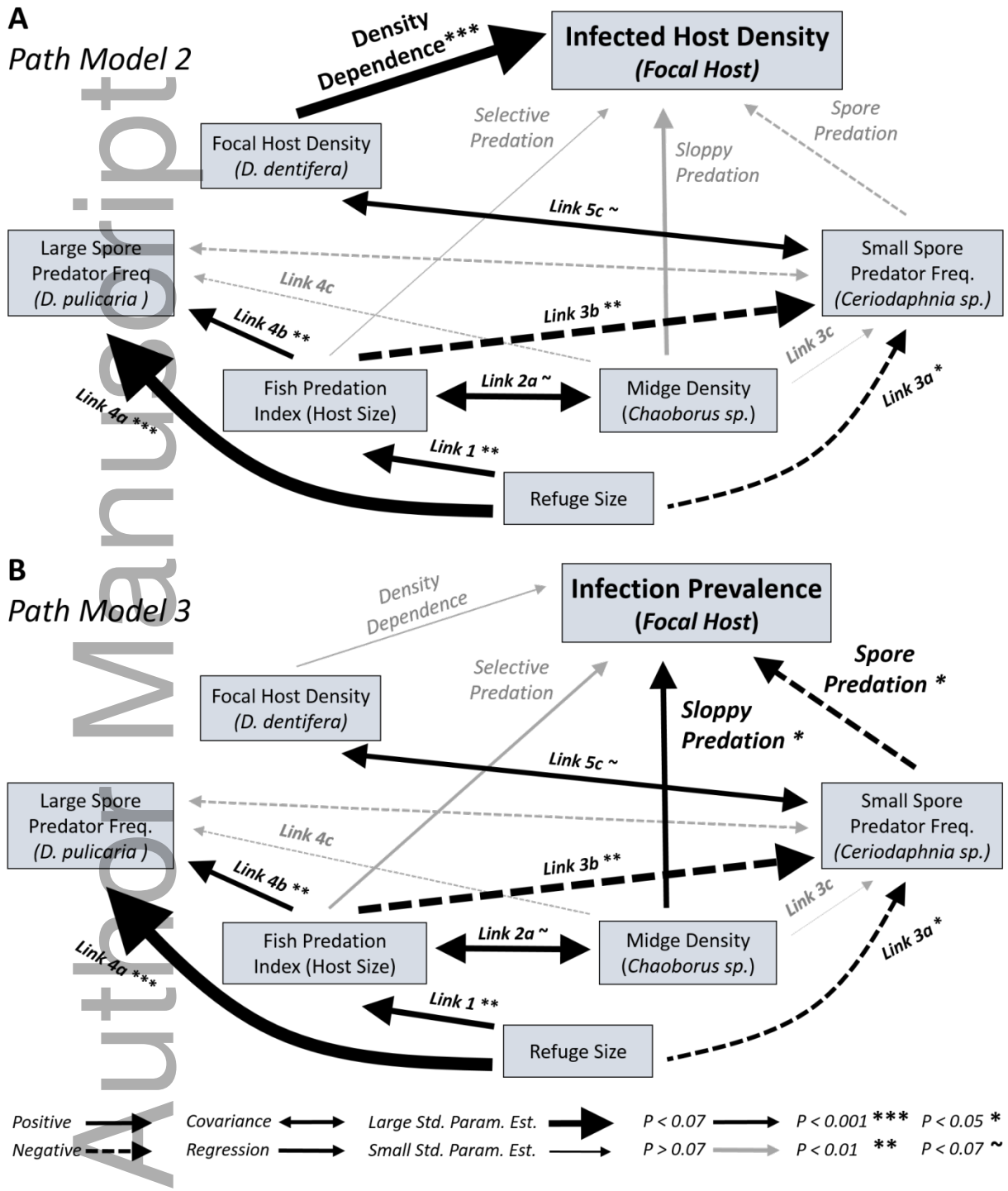

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