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Host-Parasite Interactions Within Food Webs

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Host-Parasite Interactions Within Food Webs

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by

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Abstract

Parasitism is one of the most common life history strategies employed in nature, yet the effects of parasites are often thought to be minimal, and the vast majority of studies fail to consider parasites and their effects on host organisms. This is likely a problem, as the magnitude of parasite-mediated effects on their hosts can be quite large. Additionally, the effects of parasites are known to extend beyond the host to affect other species interactions. I used a series of approaches to gain a more integral understanding of host-parasite interactions by studying (1) the effects of parasites on biotic interactions that hosts engage in, (2) how biotic interactions such as predation and competition can affect host immune defense, and (3) how abiotic and biotic factors within the local environment affecting the host can further mediate parasitism dynamics. Specifically, in Chapter 1 I conducted a phylogenetically informed meta-analysis of the effects of parasites on species interactions (i.e., predation, competition, mutualism, and reproduction). I found that despite a strong overall negative effect on species interactions, the effects of parasites surprisingly ranged from being strongly beneficial to strongly deleterious on host species interactions. In Chapter 2 I used larval damselflies and their dominant fish predator to test how cascading effects of predators on host competitive interactions and resource acquisition affected a critical component of damselfly immune function, the phenoloxidase (PO) cascade. I found that neither direct density-mediated effects, indirect, trait-mediated effects, nor combined effects of predators via natural selection affected total PO activity. Instead, PO levels increased with resource availability, implying resource limitation. Finally, in Chapter 3 I used two field experiments and a detailed observational study to investigate how host, abiotic, and biotic factors within the local environment affected the relationships between damselfly (*Enallagma* spp.) hosts and their water mite (*Arrenurus* spp.) ectoparasites. I found that parasitism was species-specific and did not vary with host density or host condition (i.e., immune function). Instead, parasitism was largely predicted by abiotic factors (i.e., pH). Collectively, my results indicate that parasites are key players in the complex web of species interactions that compose food webs. Furthermore, host-parasite interactions are mediated by many of the same ecological factors as

other species interactions, which has implications for parasitism dynamics within ecological communities. Future studies of food webs must incorporate parasites into their experimental and theoretical designs, and future studies of host-parasite interactions must expand beyond the focal relationship and consider the ecology of both the host and parasite.

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Hasik, A.Z.; Siepielski, A.M. Home is where the host is: the local environment enhances species-specific parasitism. Ecology. Submitted.

Introduction

The majority of organisms in nature are parasitic, infecting hosts at all trophic levels of the food web (Price 1980, Hudson et al. 2002). Although ubiquitous, effects of parasites on their hosts are often thought to be minimal, possibly because parasites tend to be aggregated in populations, with most individuals not parasitized (Crofton 1973, Shaw et al. 1998, Wilson et al. 2002). Despite progress incorporating parasitism into food web ecology (Hudson et al. 2006, Lafferty et al. 2006, 2008), the general effects of how, why, and to what extent parasitism affects the outcome of species interactions and host fitness remain poorly understood. Indeed, ecological studies do not traditionally consider parasites in their experimental designs and theoretical frameworks, despite many calls to do so (Cohen et al.1993, Marcogliese and Cone 1997, Kuris et al. 2008, Gehman et al. 2019). Yet, the commonness of parasites indirectly links them to myriad species interactions throughout the food web. Parasites may have direct effects on other species interactions such as predation and competition (Hatcher et al. 2006, 2012), and other species interactions are known to shape parasitism within host populations (Navarro et al. 2004, Raffel et al. 2010, Ostfeld et al. 2018). By not considering parasitism within food webs, we are left with a key gap in our knowledge of how a widespread species interaction modifies and is modified by other species interactions. Determining the general nature of how parasitism interacts with other species interactions within food webs is therefore a necessary step towards developing a more complete understanding of how communities are structured.

Parasites often infect organisms in a disproportionate manner due to their aggregated infection patterns, but this aggregation does not isolate parasites (or their hosts) from other species interactions (Lafferty et al. 2006). For example, predators often selectively consume parasitized prey (Duffy et al. 2005, Duffy and Hall 2008), and parasitized hosts can experience reductions in competitive ability (Grosholz 1992, Refardt and Ebert 2012). In addition to their effects on predation and competition, parasites can also affect reproductive capabilities of their hosts. Though not classically considered a species interaction, effects of parasites on host reproductive interactions have the potential to scale up and affect population dynamics through

reductions in fecundity (Chong and Oetting 2008, Botto-Mahan et al. 2017), fertility (Peng and Baer-Imhoof 2015), and effects on mate choice (Deaton 2009). The magnitude of parasite-mediated effects on host reproduction are such that they can reduce population density and drive host populations to extinction (Boots and Sasaki 2002, Hwang and Kuang 2003). Indeed, reproductive interactions among and within species affect population dynamics, scaling up to influence species coexistence, community, and ecosystem-level processes (Giery and Layman 2019). Beyond their deleterious effects on their hosts, an intriguing and understudied aspect of parasites is that they can also "benefit" the host. Host organisms still suffer the effects of a parasitic attack, but they have been known to experience parasite-mediated reductions in the negative effects of both predation (Pourian et al. 2011) and competition (Hyder et al. 2013), though little is known as to the commonality of these benefits to host species interactions.

Importantly, hosts are not passive organisms that simply endure parasitic attacks. In addition to behavioral defenses to prevent infection, one critical countermeasure against pernicious effects of parasites and pathogens are immune defenses (Sheldon and Verhulst 1996, Zuk and Stoehr 2002, Schmid-Hempel 2005, Siva-Jothy et al. 2005, Sadd and Schmid-Hempel 2009). Though they are an important mechanism for defending against parasites, host immune defenses are energetically costly and require that hosts both acquires and utilizes resources (Lochmiller and Deerenberg 2000, Zuk and Stoehr 2002, González-Santoyo and Córdoba-Aguilar 2012). Resources are often limited, however, and resource acquisition is further constrained by interactions with predators and competitors. Therefore, to understand how host immune defenses function in complex food webs it is necessary to identify ecological factors and phenotypic traits influencing resource acquisition.

Predators can mediate host competition and resource acquisition in several ways, which should in turn affect immune function. Predators impose direct effects on host populations through consumption, which lowers population densities and reduces density-dependent effects of intraspecific competition (Chase et al. 2002, Chesson and Kuang 2008). Because resource acquisition decreases with increasing intraspecific competition (McPeek and Crowley 1987,

McPeek 1998, Kobler et al. 2009), these direct effects of predators should therefore increase access to resources necessary to mount an effective immune response (Siva-Jothy and Thompson 2002, Kristan 2008, Budischak et al. 2018). In addition to their direct effects on hosts via consumption, indirect (non-consumptive) trait-mediated effects of predators are also known to affect competition (Werner and Peacor 1993, Preisser et al. 2005). Specifically, these trait-mediated effects reduce activity rates and foraging, subsequently reducing among-host competition (Werner and Peacor 1993, McPeek 2004, Strobbe and Stoks 2004, Ousterhout et al. 2018, Siepielski et al. 2020), increasing per capita resource acquisition and immune function. Predators also impose selection on host populations through their combined direct, consumptive effects and indirect, trait-mediated effects, thereby reducing resource acquisition (Strobbe et al. 2011, Ousterhout et al. 2018), and thus the strength of competition (Siepielski et al. 2020). These various predator-mediated mechanisms affecting host competitive interactions imply that direct and indirect effects of predators should strongly affect host immune function. However, unravelling these interactions is a daunting challenge, and no previous study has determined the cascading effects of predators on host immune function.

Though parasitism is a ubiquitous threat that all organisms must contend with, host organisms are embedded within complex and diverse ecological communities that contain not only parasites, but also predators, competitors, and a multitude of other species interactions. These biotic factors within the local environment are expected to mediate population-level variation in both parasite prevalence (proportion of the host population parasitized) and intensity (number of parasites per host). For example, Raffel et al. (2010) found that increasing intra and interspecific competition indirectly increased parasite prevalence, as reductions in resource acquisition suppressed host immune function. Predators can also affect parasitism dynamics by directly consuming infective stages of parasites, subsequently decreasing parasite intensity (Rohr et al. 2015). In addition to their direct effects, predators can also indirectly affect parasitism by modifying prey traits that increase contact rates with parasites (Bertram et al. 2013), thereby increasing both the prevalence and intensity of parasitism (Zukowski et al. 2020). Beyond effects

of biotic factors on parasite prevalence and intensity, spatial variation in abiotic factors such as temperature, precipitation, and water chemistry have also been linked to variation in parasitism (Preisser 2019, LoScerbo et al. 2020). Despite compelling evidence from previous observational studies linking variation in parasitism to myriad environmental factors (Gehman et al. 2017, Hanley et al. 2019, LoScerbo et al. 2020), few studies have investigated the joint influence of abiotic and biotic drivers of parasitism among host species and populations.

Although parasitism varies across environmental gradients, factors at the level of the host should also further mediate parasite prevalence and intensity. For example, host species within the same local community often share the same parasites (Lafferty et al. 2006, Ostfeld et al. 2018), yet closely-related host species are known to vary in both parasite prevalence and intensity (Mlynarek et al. 2015, Preisser 2019). Importantly, these patterns of parasitism within a given host species are not fixed across a species range (Poulin 2006), but they are repeatable to such an extent that high or low prevalence and intensity values among species are often considered an emergent property of the host species (Krasnov and Poulin 2010). This repeatability can be attributed to the host species itself, as common species with large populations tend to support larger parasite populations (Dobson 1990, Arneberg et al. 1998). Increased host density may also simply increase parasite encounter rates and subsequent infection (Detwiler and Minchella 2009). To reveal how host-parasite interactions operate within complex food webs, it is necessary to disentangle the joint influences of host and population level factors on parasitism dynamics across environmental gradients.

This dissertation focused on understanding the ecology of host-parasite interactions. To do so, I first conducted a meta-analysis to understand how parasites fit into complex food webs via their effects on host species interactions. I then utilized a combination of observational and experimental approaches with damselflies (*Enallagma* spp.) and their water mite (*Arrenurus* spp.) parasites to reveal how abiotic and biotic environmental factors within local environments shape host-parasite dynamics. I had three primary questions, each addressed in its own chapter and prepared as a publication in peer-reviewed journals:

1) What are the general effects of parasites on the species interactions of their hosts?
2) How do predators (and their cascading effects on host competition) directly and indirectly impact a critical aspect of immune function?
3) How do parasite prevalence and intensity vary among host species and populations across environmental gradients?

By addressing these questions, I can make progress on incorporating parasites into food web ecology. This work will therefore link community and disease ecology, providing a more holistic understanding of complex ecological communities.

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Chapter 1

The costs and benefits of parasites on the outcome of host species interactions

Adam Z. Hasik & Adam M. Siepielski

Abstract

Despite the ubiquitous nature of parasitism, the general effects of how parasitism alters the outcome of host species interactions such as competition, mutualism, predation, and reproduction remain unknown. Using a meta-analysis of 178 studies, we examined how the outcomes of diverse species interactions differed between parasitized and non-parasitized hosts. We also evaluated how the effects of parasitism on species interactions varied geographically with latitude. Overall, parasitism had relatively large deleterious effects on the outcome of host species interactions. However, there was considerable variation among interactions in these outcomes, with reproduction severely negatively affected, marginal effects on competition, and muted effects on predation – the latter results emanating because of the surprising tendency of parasitism to frequently reduce the effects of competition and predation. The effects of parasites did not differ between macro- and microparasites, nor did the shared evolutionary histories of hosts and parasites have an effect. Although studies capturing latitudinal variation are limited, parasites had more detrimental effects on the outcomes of species interactions near the equator and at higher latitudes, but more variable effects at temperate latitudes. These results highlight the need to better understand how parasitism can affect the multitude of complex species interactions that structure biological communities.

Introduction

Most organisms are parasitic and infect hosts at all trophic levels throughout food webs (Price 1980, Hudson et al. 2002). For example, Lafferty et al. (2006) showed that more than three-quarters of all links in an aquatic food web involved connections with parasites. Although parasites are ubiquitous, their effects are often thought to be minimal, possibly because parasites tend to be aggregated in populations, with most individuals not parasitized (Crofton 1973, Shaw et al. 1998, Wilson et al. 2002). Yet, the commonness of parasites may indirectly link them to myriad species interactions throughout the food web, or they may have direct effects on other species interactions such as predation and competition (Hatcher et al. 2006, 2012). Despite progress incorporating parasitism into food web ecology, the general effects of how, why, and to what extent parasitism affects the outcome of species interactions and host fitness remain unknown. This leaves us with a key gap in our knowledge of how a widespread species interaction modifies other species interactions. Determining the effects of parasites on species interactions is therefore a necessary step towards developing a more complete understanding of how communities are structured.

Parasites may infect organisms in a disproportionate manner due to their aggregated infection patterns, but they can also moderate the outcome of many species interactions. For example, predators often consume infected prey over uninfected individuals (Duffy et al. 2005, Duffy and Hall 2008), and parasitized organisms often experience reductions in competitive ability (Grosholz 1992, Refardt and Ebert 2012). Interestingly, parasites also have the potential to "benefit" the host in that they can also reduce predation (Pourian et al. 2011) or competition (Hyder et al. 2013), though how common these benefits are is unknown. Parasites can also reduce host fecundity (Botto-Mahan et al. 2017) and make potential mates less attractive (Gómez-Llano et al. 2020). While not classically considered a species interaction per se, parasite-mediated effects on reproductive interactions have the potential to affect population dynamics through reductions in fecundity (Chong and Oetting 2008), fertility (Peng and Baer-Imhoof 2015), or effects on mate choice (Deaton 2009), such that they can drive host populations

to extinction (Boots and Sasaki 2002, Hwang and Kuang 2003). Indeed, reproductive interactions among and within species affect population dynamics, and may scale up to influence species coexistence, community, and ecosystem-level processes (Giery and Layman 2019).

Parasite-mediated effects on species interactions and fitness components may also vary among parasite groups (i.e., macro- vs. microparasite, Anderson and May 1979). Macroparasites are disproportionately represented in food webs, with a biomass several orders of magnitude greater than microparasites (Kuris et al. 2008). In addition, trophically-transmitted parasites are often macroparasites (specifially helminths, Poulin 2010, Poulin and Randhawa 2015), dependent on consumption by one or more intermediate hosts to complete their life cycle, reducing host survival (Poulin 2013). In a recent meta-analysis on the relationship between parasite success and host genetic diversity, Ekroth et al. (2019) found a negative association between microparasite success and host diversity, but no association for macroparasites. Such differences imply that taking parasite group into consideration is important for disentangling potential interactions between parasitism and the outcome of other species interactions.

Although ubiquitous, parasites may be more common and have stronger effects in a geographically explicit manner. Indeed, parasite richness, abundance, and prevalence are all greater towards the equator (Rohde and Heap 1998, Calvete et al. 2003, Benejam et al. 2009, Kaunisto et al. 2015, but see Torchin et al. 2015), mirroring patterns of their hosts (Poulin 2014). Moreover, the negative effects of parasites often seem to increase towards the equator (Schemske et al. 2009, Robar et al. 2010), similar to the increasing intensity of other species interactions such as predation and herbivory (Roslin et al. 2017, Hargreaves et al. 2019). Combined with the increased strength of both other species interactions and parasitism towards the equator (Schemske et al. 2009), the detrimental effects of parasites on species interactions may also be accentuated closer to the equator – a hypothesis that remains untested.

The above points illustrate the complexity and context-dependent nature of parasites and their potentially pernicious effects on host organisms, which when combined with their potential for population and community level effects (Thieltges et al. 2013, Hatcher et al. 2014), highlights

the need for a comprehensive review and analysis of the effects of parasites on species interactions. Moreover, studies have not traditionally considered parasites in their experimental designs and theoretical frameworks, despite many calls to do so (Cohen et al.1993, Marcogliese and Cone 1997, Kuris et al. 2008, Gehman et al. 2019). As such, the generality of the effects of parasites on species interactions has not been comprehensively assessed. This is potentially problematic, because if the effects of a focal interaction are exaggerated when a host is parasitized, and such parasitism is not accounted for, the magnitude of the outcome of a focal interaction may be overestimated.

The goal of this study was to quantitatively assess how parasites shape the outcome of species interactions within food webs. To achieve this goal, we used a meta-analysis to quantitatively summarize how infection from a diverse range of parasites affects species interactions to address four questions (Table 1): First, we asked how parasitism affects the outcome of inter- and intraspecific interactions. Second, we investigated if different parasite groups (macro- vs. microparasites) had different effects on the outcomes of the species interactions. Third, we determined what effect parasites have on fitness components (fecundity, growth, and survival), and how these effects may differ across parasite groups. Finally, we examined if the effects of parasites on the outcome of species interactions varied latitudinally.

Materials and Methods

Literature search and classifications

We performed a systematic literature search for studies investigating how parasitism affects species interactions. Figure S1 visualizes the study selection process with a PRISMA flow chart (Moher et al. 2009). We searched for relevant articles on ISI Web of Science up to 2 September 2020, using the following topic search queries in multiple, separate searches: *"effects of parasit* + fung* + bact* + hemiparasit* + holoparasit* + predat*, compet*, mutual*, or reprod*."* We also included the following terms to exclude a large number of non-relevant studies on non-

parasitic mutualistic organisms, humans, and porcine viruses: "*NOT mycorrhiz* NOT mutual* NOT endophyt* NOT Wolbachia NOT symbi* NOT vaginal NOT vaginosis NOT porcine*". These searches returned a total of 19,137 articles, of which 18,961 were excluded for not meeting inclusion criteria (see below), leaving us with 176 studies. We also included one study (Bell et al. 2004) identified from a previous meta-analysis (Flick et al. 2016) and one study (García-Corredor et al. 2016) identified by authors we had requested data from. Thus, we had a total of 178 studies.

We applied the following criteria to select relevant articles: First, the study had to measure the impact of parasitism on a species interaction: interspecific competition, intraspecific competition, mutualism, predation, or reproductive interactions within species. Studies that measured a proxy for predation, such as parasitoids consuming host biomass (Shu et al. 2015) were discarded. We also discarded studies that manipulated hosts such that they would not behave as they naturally would (e.g. decapitating females in order to limit behavioral effects on mate-choice in Wittman and Fedorka 2015). Studies included all life stages, experimental and observational studies, in addition to both field-based and laboratory studies.

Second, we defined parasitic organisms as both parasites and parasitoids because we were interested in the effect that a parasitic organism has on the species interactions that its host engaged in. We did not include studies that analyzed the effects of brood parasites or social parasites. While these organisms acquire host resources, they are not true parasites that acquire resources directly from and live on or in the host. We also excluded studies that analyzed the effects of the endosymbiotic bacteria *Wolbachia* and mycorrhizal fungi on host organisms, as both *Wolbachia* (Zug and Hammerstein 2015) and mycorrhizal fungi (Johnson et al. 1997, Hoeksema et al. 2010) are defined as mutualists that can become "parasitic" and are not true parasites, as well as excluding studies that used heat-killed parasites (as in Nystrand et al. 2017), as this limited the effects of the parasite as seen when allowed to normally infect its host.

Third, because we were interested in the effects of parasites on species interactions, not how those effects may vary with the intensity of parasitism (i.e., number of parasites per host), the

study had to measure differences in a metric of performance (e.g., competitive interactions won, percent survival) for a species interaction between non-parasitized and parasitized groups. Studies in which the non-parasitized group only had their parasite load reduced (Hoi et al 2018) or those where the authors could not detect low levels of infection (Zylberberg et al. 2015) were discarded. In the *Discussion* we return to the issue of parasite load and how it may affect species interactions.

If studies did not provide the necessary data to calculate the effect sizes needed for our meta-analysis (means, standard deviations, and sample sizes), we contacted the authors directly (n = 28 studies). If the data could not be acquired by these means, we used figures from the studies to extract the relevant information (n = 63 studies) using ImageJ ver. 1.53a (Schneider et al. 2012).

Moderators and effect size calculations

For each study, we extracted the following moderators: (1) type of species interaction, (2) fitness component, (3) parasite group, and for field-based studies (4) latitude. For the moderator species interaction, studies were grouped by the species interaction investigated: competition, mutualism, predation, and reproduction. One study (Bernot and Lamberti 2008) measured indirect effects of parasites, and as such was coded as "Other." For fitness component, studies were grouped by the fitness component (fecundity, individual growth, and survival) impacted by the parasite, allowing us to relate host performance to fitness (Arnold 1983).The fecundity fitness component included not only effect sizes extracted from reproduction studies, but also predation and competition studies. For example, Creissen et al. (2016) measured the competitive ability of parasitized and non-parasitized *Arabidopsis thaliana* by comparing seed production, while Coors & De Meester (2008) measured the effects of predators on the number of offspring produced by parasitized and non-parasitized *Daphnia magna*. Parasites were distinguished by their reproductive characteristics: microparasites reproduce and multiply within their definitive host; macroparasites reproduce but do not multiply within their definitive host. For field studies, latitude was coded as the absolute degree of latitude.

To examine the effects of parasitism, we used a single effect size measure to compare differences in mean responses between parasitized and non-parasitized groups; Hedge's *g*, or the standardized mean difference (SMD) – the difference between two groups in units of standard deviations (Hedges 1981). Hedge's *g* was calculated by subtracting the mean of the non-parasitized group from the mean of the parasitized group, then dividing the difference by the pooled standard deviation. Studies often used different scales when measuring host performance: larger or more positive values could indicate a more beneficial mean outcome (higher percent survival) or a more detrimental mean outcome (higher percent predated) for the host. As such, we converted means when necessary by multiplying them by negative one, ensuring that all were on the same scale. Additionally, some studies utilized fully-factorial designs (predator/competitor present/absent crossed with parasite present/absent) while others did not (a predator was simultaneously offered both parasitized and non-parasitized prey items, or a non-parasitized host competed directly with a parasitized host). In the case of fully-factorial designs, we calculated the mean response of the parasitized and non-parasitized groups as the difference of the difference between the predator/competitor absent and predator/competitor present treatments. We report Hedge's *g* as Θ [95% confidence interval], with negative SMD values representing a detrimental effect of parasites (i.e., higher mortality due to predation, reduced competitive ability), while positive SMD values represent an advantageous effect of parasites (i.e., reduced mortality due to predation, increased competitive ability). Hedge's *g* effect sizes were calculated using the "SMDH" measure option in the escalc function (Bonett 2009). We used the R package *metafor* (Viechtbauer 2010) to calculate effect sizes.

Statistical analysis

We constructed separate multi-level mixed-effect models (Viechtbauer 2010, Nakagawa and Santos 2012) for each of our core questions (Table 1). Categorical moderators (type of species interaction, fitness component, parasite group) were included as fixed effects. To test for an effect of latitude, we fit a meta-regression mixed-effect model (van Houwelingen et al. 2002)

using absolute degree of latitude, species interaction, and their interaction as fixed effects. We also included a quadratic term for latitude and its interaction with species interaction as fixed effects to test for non-linear relationships between the effect of parasitism and latitude.

Because most studies (n = 127) had multiple effect sizes, each effect size was not independent. Therefore, to take into account the correlated structure of this dataset, we nested each effect size within study, and included both terms as random effects (Nakagawa and Santos 2012). We used a restricted maximum-likelihood estimator to calculate the amount of residual heterogeneity (τ^2) among effect sizes (Viechtbauer 2005). All models were built with the rma.mv function in *metaphor* (Viechtbauer 2010), which weights each effect size by the inverse of its sampling variance (Gurevitch and Hedges 1999).

Incorporating phylogeny

One additional source of non-independence is the shared evolutionary histories of the hosts and parasites (Chamberlain et al. 2012). Closely related hosts could exhibit similar responses to infection for a given interaction, or two closely related parasites could have similar effects on a host's species interactions. To account for these sources of non-independence we first constructed phylogenies of the host and parasite species using the *rotl* package (Michonneau et al. 2016) to trim the Open Tree of Life (Hinchliff et al. 2015) such that it included only the host (n = 159) or parasite species (n = 96). In one case (Slattery et al. 2013) we randomly assigned the hybrid host species used to calculate an effect size to one of its sister species, as the hybrid species was not defined on the Tree of Life. Host species in Washburn et al. (1991) and Candia et al. (2014) were assigned to the genus *Ochlerotatus* and family *Verbenaceae*, respectively, as these species were flagged as uncertain on the Tree of Life. Parasite species were not as well-resolved as hosts, thus when necessary they were collapsed into higher taxonomic levels (full list in Table S1). All host and parasite taxa that were not defined at the species level were assigned to genera. Polytomies in both the host and parasite trees were randomly resolved using the *ape* package (Paradis et al. 2004). After constructing both phylogenies, we then constructed

phylogenetic correlation matrices assuming full Brownian motion evolution (Lajeunesse 2009) using default settings for the evolutionary rate parameters and starting trait values in the *ape* package (Paradis et al. 2004) and included them as random effects. Interactions between hosts and parasites are not likely to be dependent solely on either the host or the parasite, but instead relate to the shared evolutionary history of both organisms (Hadfield et al. 2014). We therefore also included a random effect of the interaction of the host and parasite phylogenies by calculating the tensor products of the correlation matrices (Lynch 1991, Hoeksema et al. 2018).

Multiple effect sizes in our dataset (n = 89 effect sizes from n = 26 studies) were extracted from studies investigating viruses, which do not have a resolved position on the Tree of Life. As such, we could not examine all of the studies in our complete dataset while controlling for phylogeny. We therefore conducted three sets of analyses on two separate datasets. The first dataset included only studies for which we could control for the phylogeny of both the host and parasite (n = 614 effect sizes from n = 172 studies), while the second dataset included viruses (n = 658 effect sizes from n = 178 studies). Differences between the two sets of analyses conducted on the first dataset would indicate that shared evolutionary histories influenced the effects of parasites on host species interactions, while differences between the analyses conducted on the first and second dataset would indicate that removing viral parasites influenced the overall estimated effect of parasites.

We did not find evidence for a publication bias towards large, negative (or positive) effects of parasites on mean responses using funnel plots (Fig. S2). Trim-and-fill analysis revealed that no effect sizes were missing, indicating there was no asymmetry in the data.

We also examined for potentially influential outliers with Cook's distance d (Cook 1977). Any effect size with values of d greater than three times the mean was considered an outlier (with phylogeny: $d > 0.0009$, n = 45 effect sizes from 18 studies; without phylogeny: $d > 0.001$, n = 50 effect sizes from 15 studies; without phylogeny and with viruses: $d > 0.0016$, n = 52 effect sizes from 26 studies), and we ran our analyses again without these outliers. After removing outliers, the sample size for the analysis with phylogeny had n = 569 effect sizes,

without phylogeny had n = 564 effect sizes, and without phylogeny and with viruses had n = 606 effect sizes. The analysis with phylogeny had a greater number of effects sizes than the analysis without phylogeny because fewer of the effect sizes from the original analysis were identified as potential outliers when controlling for phylogeny. In the majority of cases, the effects of removing outliers reduced the magnitude of effect sizes; we highlight differences in the overall trends in the *Results*.

Results

Overview of studies in the database and overall effects of parasitism

Most studies focused on the effects of parasites on intraspecific reproductive interactions (n = 116, Fig. S3) and the effects of parasites on invertebrate (n = 121) and vertebrate (n = 40) animal hosts (Fig. S3a). The majority of parasites were either fungi, platyhelminths, or arthropods (64, 27, and 26 studies respectively, Fig. S3b). Only 3 of the 178 studies measured the effect of parasites on mutualisms, and a single study (Bernot and Lamberti 2008) investigated the indirect effects of parasites, therefore we removed these four studies from further analyses beyond determining the overall effects of parasites (e.g., Fig. 1).

Overall, our analyses revealed significant and negative values for Hedge's g, indicating that on average parasites have a detrimental effect on the outcome of all species interactions when viewed collectively (Fig. 1). After removing studies with potential outliers, the overall effect of parasites on mean responses was reduced by 11.5% when controlling for phylogeny, 23.8% when not controlling for phylogeny, and 22.2% when not controlling for phylogeny and including viruses (Fig. S4). All three analyses had a high degree of heterogeneity associated with their measured effects (Table 2). In addition, we found no difference in the effect of parasites on species interactions between field-based and lab-based studies, no difference among experimental and observational studies, nor was there a difference among trophically-transmitted parasites and non-trophically-transmitted parasites (Appendix 1).

Do parasites affect the outcome of species interactions

When considering only competition, predation, and intraspecific reproductive interactions we found that parasites were largely detrimental for reproduction, yet the effects on predation and competition were much more varied, ranging from strongly deleterious to strongly beneficial (Fig. 2). Removing potentially influential outliers reduced the magnitude of the effect size for reproduction and competition, but the magnitude of the effect of parasites on predation marginally increased (Fig. S5).

Macro- and microparasites were both consistently detrimental to reproductive interactions, though their effects were more varied for interactions with competitors, with a non-significant trend for microparasites to be beneficial and macroparasites to be detrimental to interactions with predators, though there were no significant differences among the parasite groups (Fig. 3). The removal of potentially influential outliers reduced the magnitude of most relationships but did not change the overall relationships between parasite group and species interactions (Fig. S6). Thus, overall macro- and microparasites have similar effects on species interactions.

Do parasites affect the fitness components of hosts?

The effects of parasites were strongest and consistently deleterious to host fecundity and individual growth, but parasites had more variable effects on survival (Fig. 4). When considering the results without outliers, we found that the magnitude of the effect was slightly reduced for fecundity and growth, but slightly increased for survival (Fig. S7).

Between parasite groups, we found that both macro- and microparasites were detrimental to fecundity and growth when not controlling for phylogeny (Fig. S8b) and when not controlling for phylogeny and including viruses (Fig. S8c). When controlling for phylogeny, both macro- and microparasites were detrimental to fecundity, yet only microparasites were detrimental to growth (Fig. S8a). With the exception of micro- and macroparasites no longer being detrimental to growth when controlling for phylogeny and not controlling for phylogeny, respectively, these relationships were consistent after removing influential outliers (Fig. S9).

Do the effects of parasites on species interactions vary latitudinally?

Due to the limited number of field studies on the effects of parasites on competition (n = 4 competition studies, Fig. 5a) we only analyzed the effects of parasites on predation and reproduction across latitude. We found a significant effect for the interaction of species interaction and latitude (p = 0.01); thus, we analyzed the effects of parasites for each interaction separately (see Table S2). Though we included predation, we note that the majority of those studies took place at temperate latitudes, with few close to the equator. For all models, we compared linear and quadratic model fits using AIC.

We found that the effects of parasites across species interactions were most strongly negative to reproduction both at the equator and at higher latitudes, with marginal effects at temperate latitudes (ca. 40 degrees; Fig. 5). When both controlling and not controlling for phylogeny, the effects of parasites on predation were most strongly negative at temperate latitudes (ca. 35 degrees; Fig. 5b-c), becoming increasingly positive at higher latitudes until ca. 60 degrees, at which point they became more negative. When not controlling for phylogeny and including viruses we found that the effects of parasites on predation were most strongly negative at subtropical latitudes, becoming increasingly positive at higher latitudes (Fig. 5d). After removing potentially influential outliers we did not find a significant effect for the interaction of species interaction and latitude, thus we only report the overall effect of parasites on all species interactions. We found a slight, though non-significant trend for decreasing effects of parasites on species interactions with an increase in latitude (Fig. S10).

Discussion

Overall, our analysis showed that the effects of parasitism on the outcome of host interactions are relatively large, but surprisingly variable in outcome among interaction types. Although the effects of parasitism ranged from beneficial to deleterious, parasitized organisms consistently

suffered reductions in reproduction, in addition to detrimental effects on fecundity and growth. Surprisingly, parasitism had both positive and negative effects on competitive interactions and predation. There were no differences between the effects of macro- and microparasites, and both controlling for the shared evolutionary histories of hosts and parasites and including viral parasites did not qualitatively change the effect of parasites on the outcome of species interactions. We found that parasites had deleterious effects on species interactions near the equator and at higher latitudes, although these effects had much uncertainty associated with them. Below, we place our results in the broader context of understanding how parasitism influences the outcome of species interactions and provide specific examples to illustrate our main findings.

Effect of parasites on species interactions

Across studies, parasites had variable effects on how well hosts performed in species interactions. However, there were also differences in which interactions were more strongly affected. In particular, parasites had strong and consistent negative effects on reproduction: individuals that were parasitized produced fewer offspring – an expected pattern given that parasites usurp a host's resources that could otherwise go towards reproductive investment.

Interestingly, there was no consistent effect of parasites on interactions with predators. This is surprising, as parasitized hosts are often disproportionately consumed by predators due to changes in their behavior (Otti et al. 2012) or reductions in their defenses against predators (Slattery et al. 2013). Parasites also reduce predator-avoidance behaviors in their hosts, with some that cause their hosts to become attracted to predator cues, resulting in a homogenized response to predators in parasitized prey species, facilitating the transmission of the parasites to their final hosts (Berdoy et al. 2000, Benesh et al. 2008). Importantly, the apparent lack of an overall trend points to a more varied effect of parasites on predation that is not merely due to a collection of negative and null effects, but instead due to the large number of both positive and negative effects. For example, Voutilainen (2010) infected great pond snails (*Lymnaea stagnalis*)

with the trematode parasite *Diplostomum pseudospathaceum*, a trophically-transmitted parasite that uses fish as its second intermediate host. Infection with *D. pseudospathaceum* had a negative effect on host snails in the presence of fish predator cues, as they markedly increased their foraging, but infection had a positive effect in the presence of crayfish predator cues as it decreased host foraging.

These results imply that the effects of parasites on predation require careful consideration of the context-dependent and system-specific nature of individual host-parasite associations. Indeed, these system-specific effects may have been why we did not find significant differences among our phylogenetically informed and naive analyses. Each host-parasite association may involve such specific nuances that closely related parasites have completely different effects on their hosts, or a given host may have a completely different relationship with a given parasite than its sister species. More generally, though, the considerable variation in the outcome of predation when simultaneously contending with parasitism demands additional study to understand the possible underlying mechanisms whereby parasites shift from being costly to beneficial. Is this merely unpredictable noise, or are there instead generalizations that can be made?

Similar to predation, another unexpected result was that parasites, though tending to reduce the competitive abilities of their hosts on average, also had more variable effects ranging from beneficial to deleterious. For example, parasitized woodlice (*Porcellio scaber)* had higher survival rates when competing with members of their own species, yet suffered reductions when competing with a congener (Grosholz 1992). Such results imply that intra and interspecific competition could be differently affected by parasitism. In the sole study investigating the effect of parasites on a fungal host's competitive interactions, Hyder et al. (2013) found that viruses could be beneficial, detrimental, or have no effect on the competitive ability of their hosts, depending on the specific host strain, virus, and competitor strain. While these studies did not explore possible explanations for these results, it could be that these apparent benefits of parasites on species interactions are due to plastic and compensatory responses to infection. For

example, Marino et al. (2016) found that infected frogs grew more than those without parasites due to increased foraging rates, and increases in mass are positively correlated with parasite tolerance (Schotthoefer et al. 2003, Holland et al. 2007) and competitive ability (Smith et al. 2004, Richter-Biox et al. 2007). The effects of parasitism on competition are not always so variable, and infected hosts can also be less competitive than uninfected counterparts. Parasitized leopard frogs (*Lithobates pipiens*) suffered reduced growth when competing for resources (Koprivnikar et al. 2008), possibly due to parasite-mediated reductions in their ability to acquire resources. Parasites were also found to impact both the fighting ability and aggression levels of horned passalus beetles (*Odontotaenius disjunctus*), due to reduced energy levels (Vasquez et al. 2015).

It is unclear why the effects of parasitism on competitive interactions are seemingly so variable and future studies should seek to better understand the causes of this variation. However, it may reflect the often context-dependent nature of competitive interactions, that competition is often a sub-lethal interaction, or the diverse ways that competition can occur (e.g., exploitative vs. interference competition). For example, loss of appetite ('illness-mediated anorexia', shown to reduce the deleterious effects of parasite infection, reviewed in Hite et al. 2020) after infection could reduce the effects of indirect resource-based exploitative competition because individuals consume fewer resources, but it could also reduce host condition and thus weaken any direct interference competition.

A key implication of our results is that the effects of species interactions such as predation and competition are often likely tempered by the additional effects of parasitism. Given how common parasitism is, this means that the effects of predators or competition in many cases may not reflect the true magnitude of the effect of predators or competitors alone. Instead, they likely reflect the combined action of how parasitism is affecting another interaction. These results are consistent with previous work that has illustrated the potential for myriad results to emerge when one interaction is considered in light of other species interactions. For example, predation can enhance, reduce, or have no effect on competition (Chase et al. 2002). Moreover, feedbacks

between interactions are common; e.g., predation not only interacts with and affects competition (Chase et al. 2002), but the strength of competition also depends on the intensity of predation (Gurevitch et al. 2000). Yet, because the cascading effects of parasitism are rarely accounted for (Cohen et al.1993, Marcogliese and Cone 1997, Kuris et al. 2008, Gehman et al. 2019) we run the risk of overestimating any direct negative effects of a focal species interaction.

Effect of parasites on fitness components

The effects of parasites on fitness components were also quite variable, as they negatively affected both fecundity and growth, yet we found substantial variation associated with the effects on survival. Much like their effects on reproduction, that parasites consistently and strongly reduced both growth and fecundity is not surprising, as parasites exploit host organisms to increase their own growth and fitness. Such exploitation reduces resources available for hosts to invest in their own growth and fecundity, thus yielding the patterns we observed.

One possible reason for the variable effects of parasites on survival is the system-specific relationships among predators, prey (hosts), and parasites. For example, when infected with an apicomplexan parasite (*Eimeria vermiformis*), mice (*Mus musculus*) preferred predator odors over neutral odors (Kavaliers and Colwell 1995), a change in behavior that reduces host survival, yet increases transmission of the parasite to its final feline host. By contrast, the survival of onion thrips (*Thrips tabaci*) parasitized by the fungus *Metarhizium anisopliae* was significantly higher than the non-parasitized thrips (Pourian et al. 2011). This apparent benefit of parasitism is because the predators (flower bugs, *Orius albidipennis*) actively avoided feeding on parasitized thrips. Though not confirmed in that study, it is possible that consuming infected prey items would infect the predator itself. Meyling & Pell (2006) found that another flower bug species (*Anthocoris nemorum*) detected and avoided the fungal parasite *Beauveria bassiana* while foraging, limiting their risk of infection. Parasitized prey items may also be a lower quality resource relative to non-parasitized prey, as Flick et al. (2016) found that the consuming infected prey reduced the fecundity, longevity, and survival of predators. These results imply that

understanding the effects of parasites on host survival also requires understanding the relationship of the parasite with potential predators.

Effect of parasite group on species interactions

We found that there was no difference in the effects of macro- and microparasites on species interactions and fitness components. This is surprising, as microparasites multiply within their host over the course of an infection and might be expected to have stronger effects, whilst macroparasite numbers are typically fixed at the time of infection (Anderson and May 1979). Although we did not analyze the effects of the duration of infection, doing so may reveal that the effects of microparasites on species interactions are indeed stronger than macroparasites as infection progresses. Conversely, macroparasites may be more detrimental to interactions with predators and have a stronger impact on host survival, as most trophically-transmitted parasites are macroparasitic helminths known for manipulating host behavior such that they increase predation (Poulin 2013). We analyzed a limited number of studies on trophically-transmitted parasites (n = 109 effect sizes from n = 36 studies), but the lack of a difference among macro- and microparasites points to a more general effect of parasites on species interactions and fitness components, regardless of parasite group.

Effect of parasites on species interactions in relation to latitude

We included studies from a wide latitudinal range (approximately 65 degrees) and found significant relationships between latitude and the effect of parasites; though we note our limited sample sizes (n = 22 effect sizes from 8 predation studies, n = 48 effect sizes from 24 reproduction studies) especially from studies closer to the equator. Previous work has shown that both the effects of parasites (Schemske et al. 2009) and the strength of other species interactions increase towards the equator (Roslin et al. 2017, Hargreaves et al. 2019). Our results suggest that these increased effects of parasites at the equator and at higher latitudes extend beyond the host to the interactions that it has with predators, in addition to reproductive interactions with

conspecifics, but that there are marginal effects at temperate latitudes. The increasing effect of parasites at higher latitudes may point to an effect of limiting resources, leaving hosts in worse condition and thus parasites are simply compounding an already stressful situation. Although we found some evidence that the effects of parasites vary with latitude, we advise caution when interpreting these results, as they are based on small sample sizes from a limited number of studies. Further studies of the effects of parasites on species interactions with broader latitudinal coverage are required to understand if these relationships are truly representative of underlying ecological patterns. The hypothesis that parasites affect species interactions in a geographically explicit manner could be further tested by examining the effects of parasites on host species' interactions with other organisms, and an especially strong test would be to examine a focal interaction, such as predation on a given species (Hargreaves et al. 2019), and see how it varies spatially with parasitism. Ideally, this test would use a broadly distributed host-parasite pair in order to limit issues from the use of different host and parasite assemblages (Schemske et al. 2009, Poulin 2014, Preisser 2019).

Limitations and future directions

In addition to the limitations noted above, here we discuss several other limitations and the opportunities these limitations present for future directions. We focused our analysis on the effects of parasite occurrence, but not on how these effects may increase with infection intensity, as this was rarely reported. Indeed, Risely et al. (2017) found that increasing infection intensity was associated with decreases in host movement, phenology, and survival. Accounting for the effects of intensity as opposed to only occurrence may help to further explain why variation in the effects of parasitism in species interactions occurred (e.g., the variable effects of parasitism on predation and competition). Future work on the effects of parasites on species interactions should therefore incorporate infection intensity. The latter is potentially important, because most parasitized individuals have few parasites (Shaw et al. 1998, Wilson et al. 2002), and as a result

the strong detrimental effects of parasitism detected may be due to over-representation by heavily-infected individuals.

Despite an exhaustive literature search reviewing >19,000 studies, we only found 20 studies of the effects of parasites on competition, and three studies on the effects of parasites on mutualisms. Although we found strong, detrimental overall effects of parasites on species interactions, we advise caution when drawing conclusions about the ubiquity of these effects on competition in particular when so few studies have been conducted. A better understanding of how parasitism affects mutualisms is also clearly warranted given that virtually all species are engaged in some form of mutualistic interaction. Hatcher et al. (2006) called for future models of competition to incorporate infection status of the competing organisms, but our results show that this needs to be extended more broadly to all species interactions.

In addition, more work is needed to understand indirect, trait-mediated effects (Werner and Peacor 1993) of parasites on species interactions. Despite collecting over 650 effect sizes on the effects of parasites on species interactions, only six of those effect sizes were extracted from some indirect effect of parasites on the mean responses of hosts to species interactions. Moreover, we found only one study that directly investigated the indirect effects of parasites on a species interaction. Bernot & Lamberti (2008) found that snail communities with increasing prevalences of a trematode parasite consumed more algae, reducing algal biomass and altering the periphyton community composition.

Future studies should also investigate a wider variety of hosts, as we found a disproportionate representation of animal hosts in studies of parasite effects on species interactions, most of which were invertebrates, with only one study using a fungal host and 16 using plants. As such, we were unable to investigate the effects of parasites among different host taxa.

Beyond true parasites, considering emerging “parasite-like” transmissible factors in tandem with species interactions offers a wealth of untapped research potential. For example, contagious prion diseases (i.e., chronic wasting disease) can result in higher predation for infected cervids (Miller et al. 2008, Krumm et al. 2010). The emergence of this disease has also coincided with

increases in both the local abundance and the geographic distribution of cougars (*Puma concolor*), a key cervid predator (Pierce and Bleich 2003). Infected predators can also drive variation in species interactions, generating diverse cascading effects. Tasmanian Devils (*Sarcophilus harrisii*) and their transmissible cancer, Devil facial tumor disease (DFTD) provide an illustrative example of the wide-ranging effects of such factors. This disease has ravaged Devil populations (McCallum et al. 2009), with population declines of up to 90%. The decline of this apex predator resulted in the loss of top-down control on their prey species (the common brushtail possum, *Trichosurus vulpecula*), which allowed the possums to shift their habitat use (Hollings et al. 2015). In addition to its effects on other species through predation, DFTD has altered the Tasmanian carrion landscape, with carcasses persisting longer in diseased regions (Cunningham et al. 2018). This disease-induced decline of Tasmanian Devils and subsequent decrease in their scavenging has released mesopredators from competition for carcasses, creating a threat to smaller wildlife in areas ravaged by DFTD (Ritchie and Johnson 2009).

Conclusions

Overall, our meta-analysis revealed detrimental effects of parasites on species interactions, with surprisingly varied effects on competition and predation, emphasizing the importance of parasites within ecological networks. These results have implications for measures of species interactions, such that any study that fails to account for the effects of parasitism on species interactions is likely to overestimate the magnitude of the effects of interactions such as competition or predation. Previous work on synthesizing multiple interaction effects has found that not only can one species interaction impact another (Chase et al. 2002), but also that the strength of species interactions are dependent on other species interactions (Gurevitch et al. 2000). Our results add to this work, as well as that incorporating the effects of parasites into ecological communities (Hatcher et al. 2006, Duffy and Hall 2008, Lafferty et al. 2008, Tompkins et al. 2011, Poulin 2013, Flick et al. 2016). Our study highlights multiple gaps in our understanding of the effects of parasites on species interactions; especially competition, indirect

effects, and mutualisms. Parasitism is defined by the negative impact that one organism has on another. However, our review has shown that this idea can be overturned when also considering how parasitism may affect the outcome of interactions that a host has with other organisms, which may ultimately determine host fitness. No species exists in an ecological vacuum. Studies investigating these relationships are needed to understand the role of parasites within species interaction networks. The interactions making up the structure of food webs are complex, and accounting for this complexity and potential for feedbacks to emerge among species interactions is necessary to develop a more complete understanding of how communities are structured.

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Figures

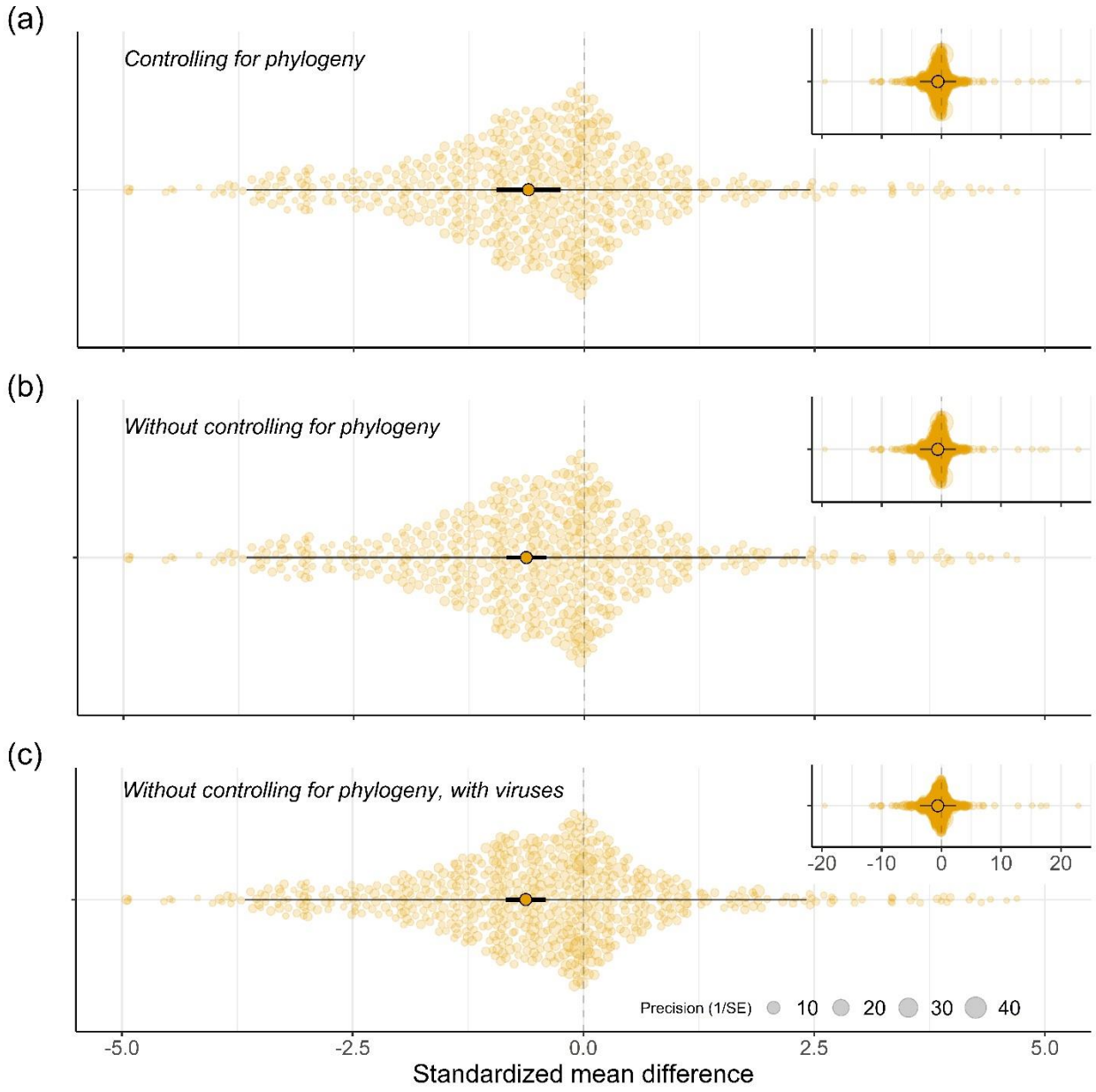

Figure 1 – Overall effect of parasitism on the outcome of species interactions across all studies and types of species interactions. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 614 effect sizes from n = 172 studies, p = 0.001), without controlling for phylogeny (b, n = 614 effect sizes from n = 172 studies, $p < 0.0001$), and without controlling for phylogeny and including viruses (c, n = 658 effect sizes from n = 178 studies, $p < 0.0001$). All main plots only show effect sizes from -5 to 5, while the inset plots show the entire range of effect sizes. The orange points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

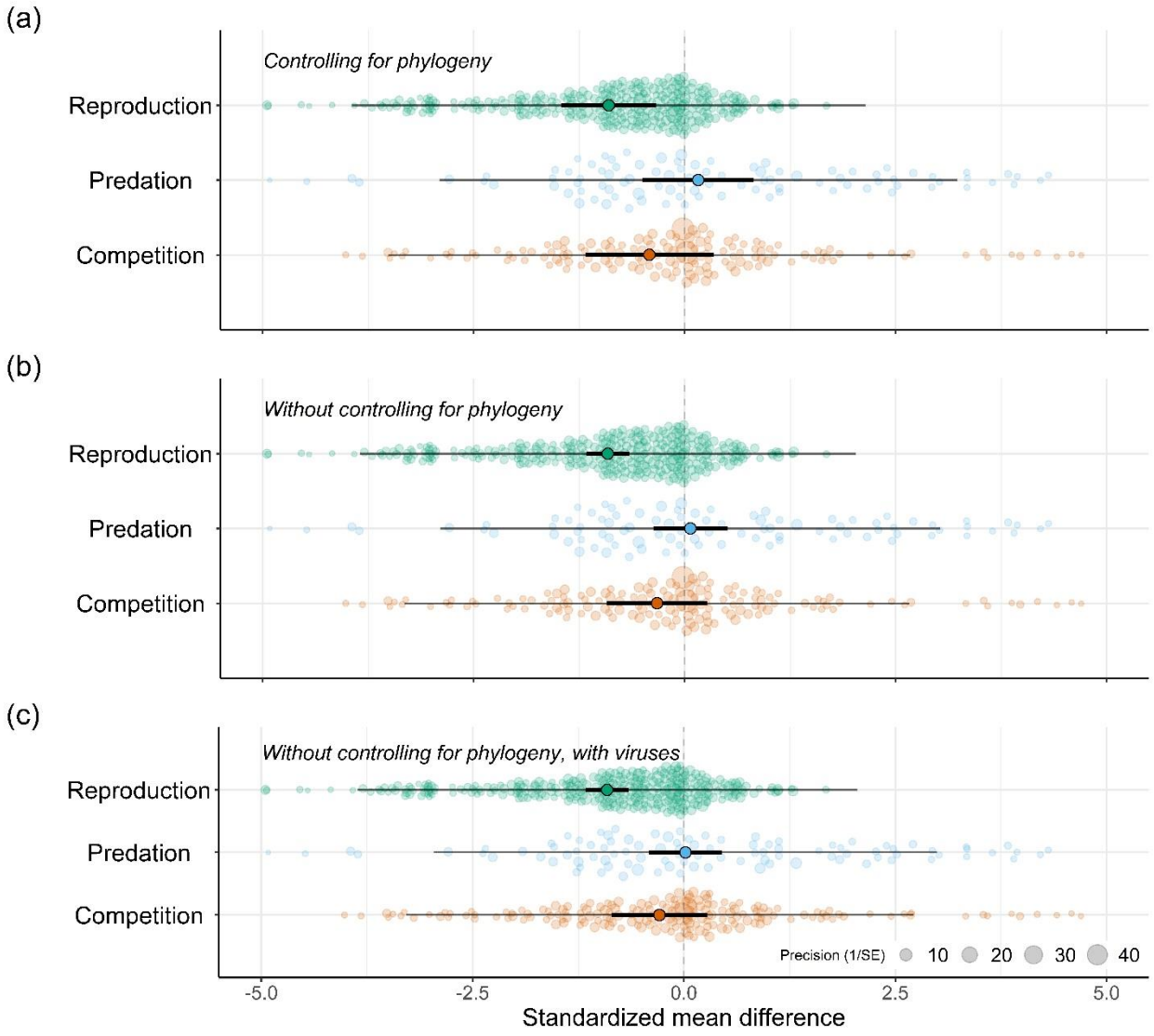

Figure 2 - Overall effect of parasitism on the outcome of species interactions among species interactions. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 603 effect sizes from n = 168 studies, $p < 0.0001$), without controlling for phylogeny (b, n = 603 effect sizes from n = 168 studies, $p < 0.0001$), and without controlling for phylogeny and including viruses (c, n = 647 effect sizes from n = 174 studies, $p < 0.0001$). All main plots only show effect sizes from -5 to 5, see Fig. 1 for the full range. The points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

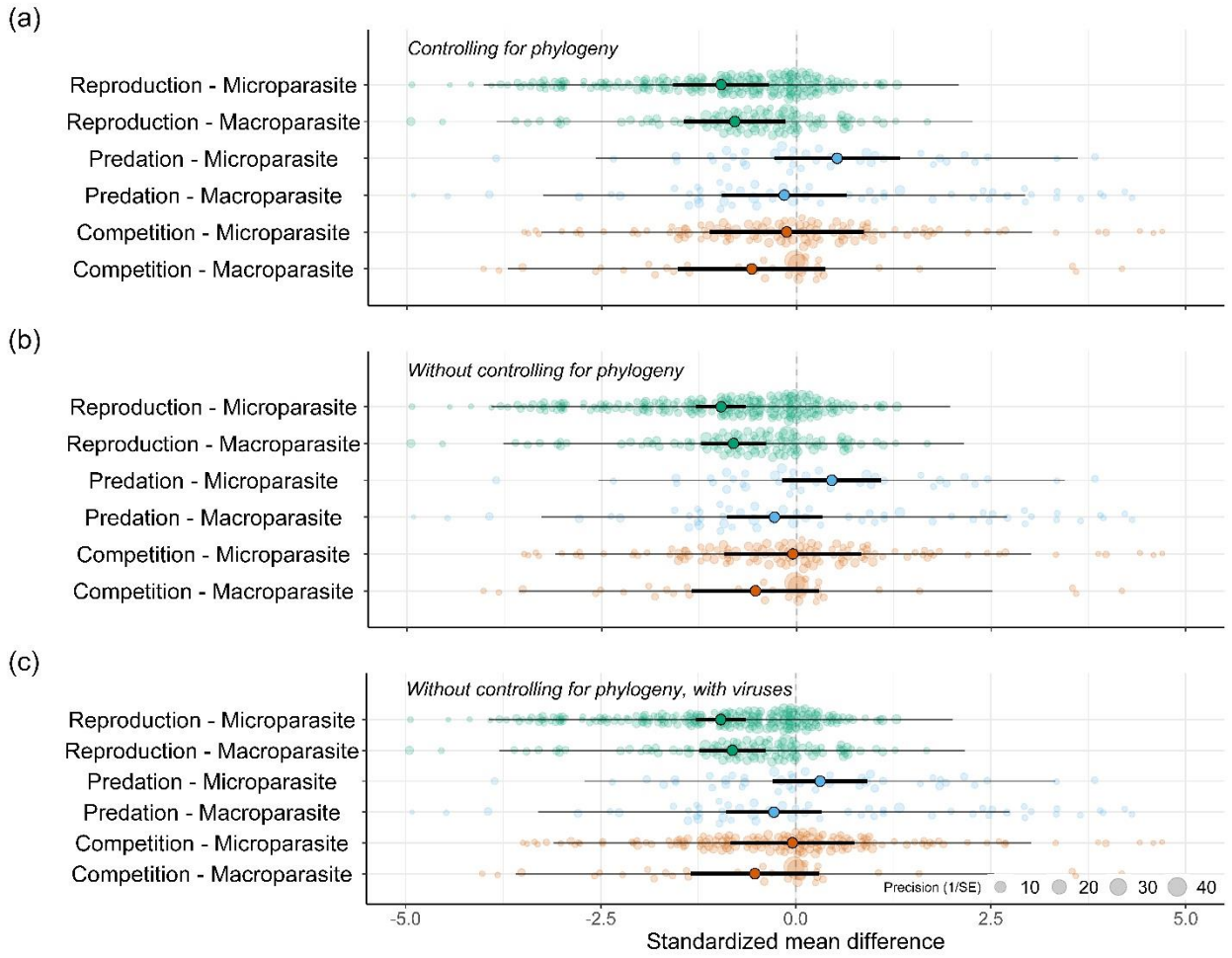

Figure 3. - Overall effect of parasite group on the outcome of species interactions among species interactions. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 603 effect sizes from n = 168 studies, p = 0.0003), without controlling for phylogeny (b, n = 603 effect sizes from n = 168 studies, p < 0.0001), and without controlling for phylogeny and including viruses (c, n = 647 effect sizes from n = 174 studies, p < 0.0001). All plots only show effect sizes from -5 to 5; see Fig. 1 for the full range. The points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

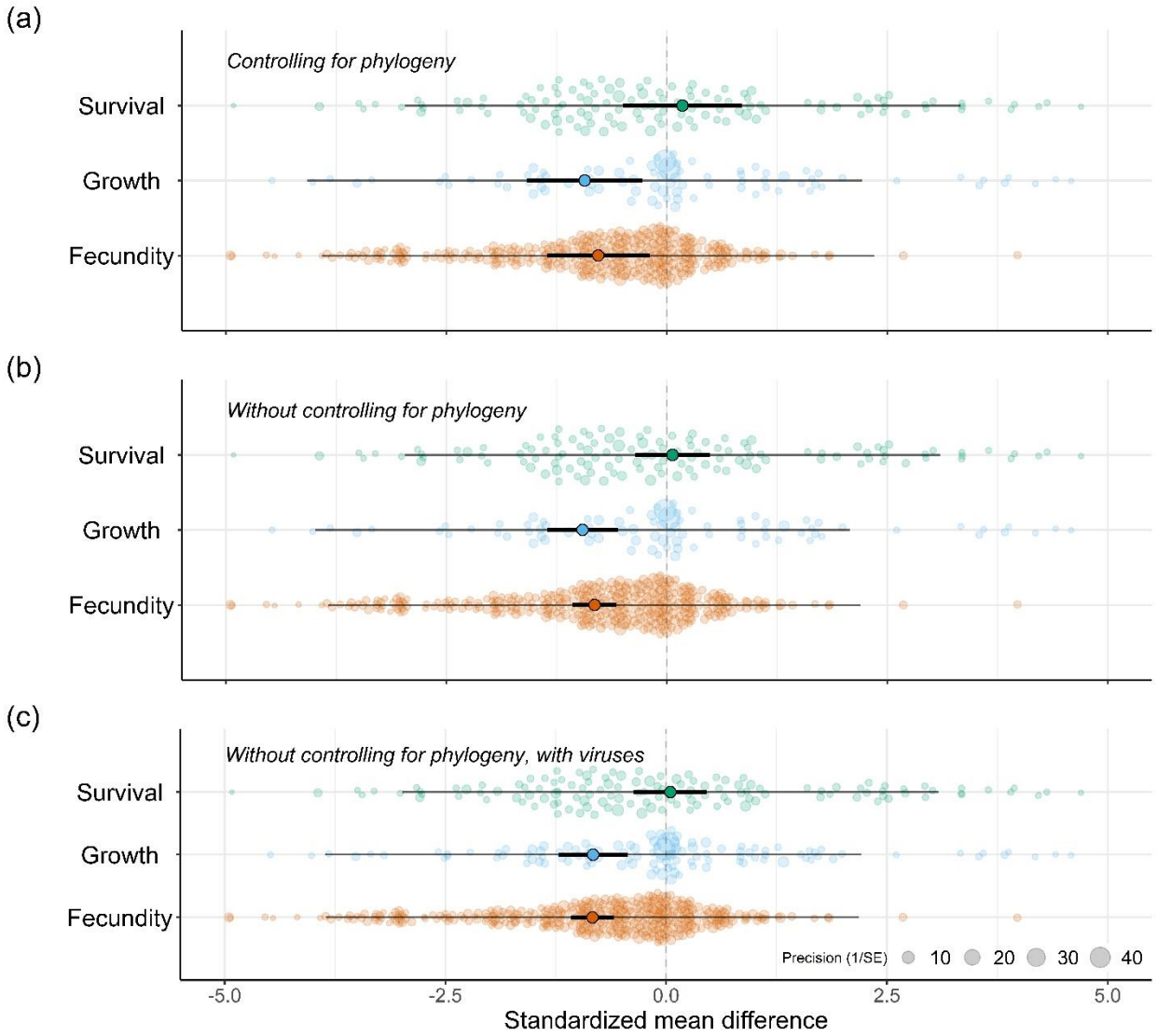

Figure 4 - Overall effect of parasitism on the outcome of species interactions among fitness components. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 603 effect sizes from n = 168 studies, $p < 0.0001$), without controlling for phylogeny (b, n = 603 effect sizes from n = 168 studies, $p < 0.0001$), and without controlling for phylogeny and including viruses (c, n = 647 effect sizes from n = 174 studies, $p < 0.0001$). All plots only show effect sizes ranging from -5 to 5, see Fig. 1 for the full range. The points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

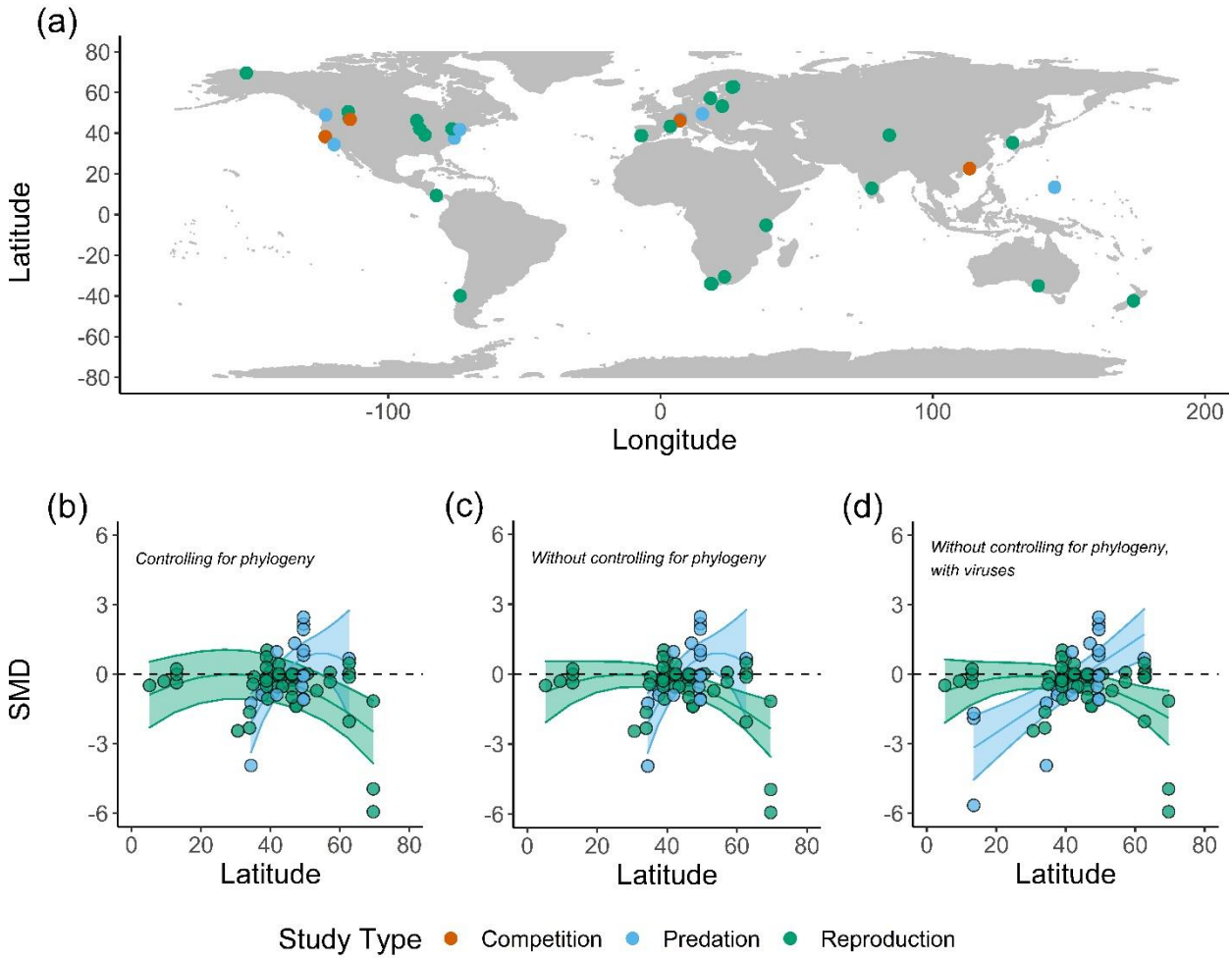

Figure 5 - Effects of parasitism on species interactions in relation to latitude. (a) shows the locations of field studies included in the meta-analysis (including the competition studies excluded from statistical analyses), (b), (c), and (d) are meta-regressions of standardized mean difference (SMD) effect sizes on absolute degrees of latitude from models with phylogeny (n = 65 effect sizes from n = 30 studies, p = 0.001 for predation, p = 0.002 for reproduction), without phylogeny (n = 65 effect sizes from n = 30 studies, p = 0.001 for predation, p = 0.009 for reproduction), and without phylogeny and with viruses (n = 70 effect sizes from n = 32 studies, p < 0.0001 for predation, p = 0.05 for reproduction). Colors indicate the species interaction, with each point representing an individual effect size. The bands denote the 95% confidence interval of the slope.

Tables

Table 1. Models used to address each question from the meta-analysis. For each model, we used standardized mean difference as the effect size to analyze the effects of parasites on host mean response. The random effects among models controlling for phylogeny were the same and included effect size ID nested within study ID, host phylogeny, parasite phylogeny, and the interaction of host phylogeny and parasite phylogeny. Models not controlling for phylogeny only included effect size ID nested within study ID.

Question	Model
Do parasites affect the outcome of species interactions?	Effect Size ~ Species Interaction
Is the effect of parasites on species interactions dependent on parasite group (macro- vs. microparasite)?	Effect Size ~ Species Interaction + Parasite Group + Species Interaction × Parasite Group
Do parasites affect the fitness components of hosts?	Effect Size ~ Fitness Component
Is the effect of parasites on fitness components dependent on parasite group?	Effect Size ~ Fitness Component + Parasite Group + Fitness Component × Parasite Group
Do the effects of parasites on species interactions vary latitudinally?	Effect Size ~ Latitude + Species Interaction + Latitude^2 + Latitude × Species Interaction + Latitude^2 × Species Interaction

Table 2. Summary statistics for the heterogeneity index (I^2), which is a measure of the total variability in the effect size estimates which can be attributed to heterogeneity among true effects. Each analysis is a measure of the overall effects of parasites on species interactions, I^2_{total} represents the heterogeneity of the full model, while the other I^2 values represent the amount of heterogeneity explained by the random effects in each model.

Analysis	Heterogeneity Index (I^2)
Controlling for Phylogeny, No Viruses	I^2_{total} = 97.39%
	$I^2_{among\ studies}$ = 62.60%
	$I^2_{within\ studies}$ = 32.31%
	$I^2_{host\ phylogeny}$ = 2.49%
	$I^2_{parasite\ phylogeny}$ = ~ 0%
	$I^2_{host\ phylogeny\ x\ parasite\ phylogeny}$ = ~ 0%
Without Controlling for Phylogeny, No Viruses	I^2_{total} = 97.37%
	$I^2_{among\ studies}$ = 64.71%
	$I^2_{within\ studies}$ = 32.65%
Without Controlling for Phylogeny, With Viruses	I^2_{total} = 97.31%
	$I^2_{among\ studies}$ = 63.77%
	$I^2_{within\ studies}$ = 33.54%

Appendices

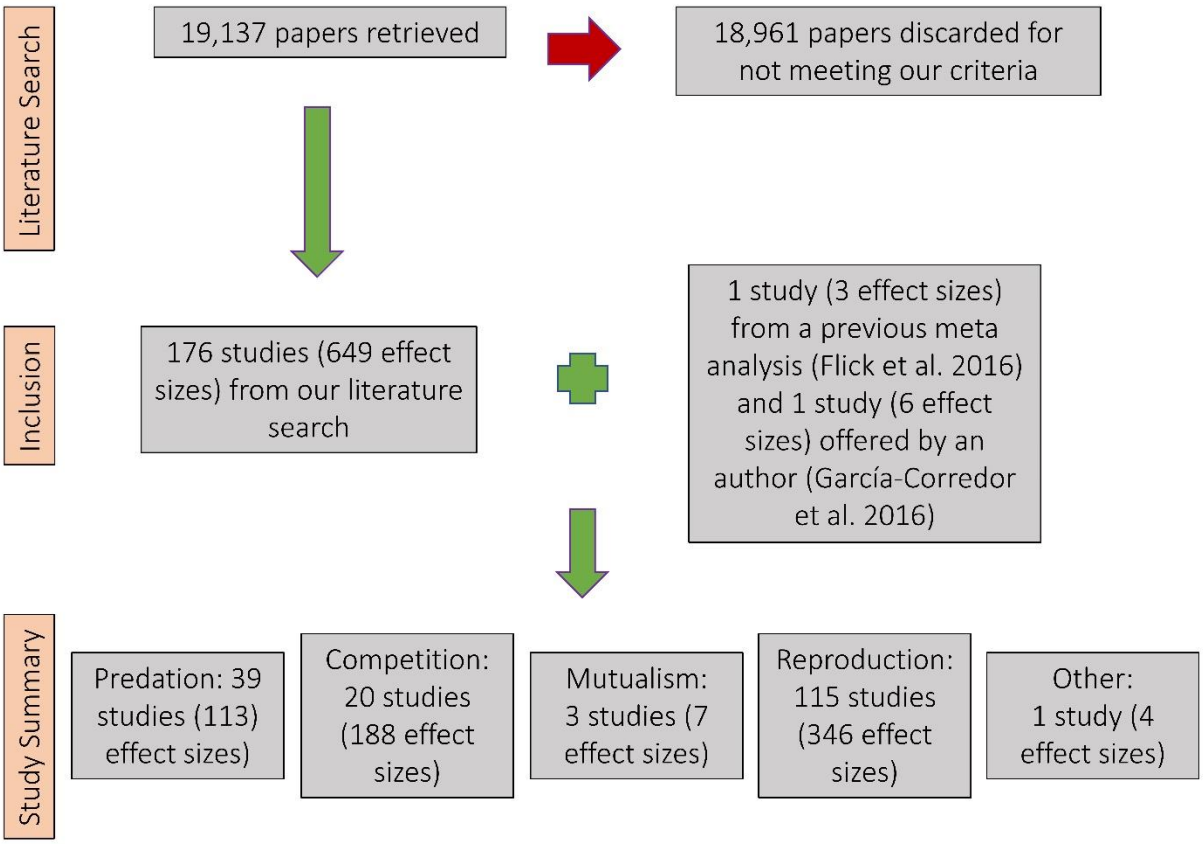

Figure S1. – PRISMA flow chart of the study inclusion process.

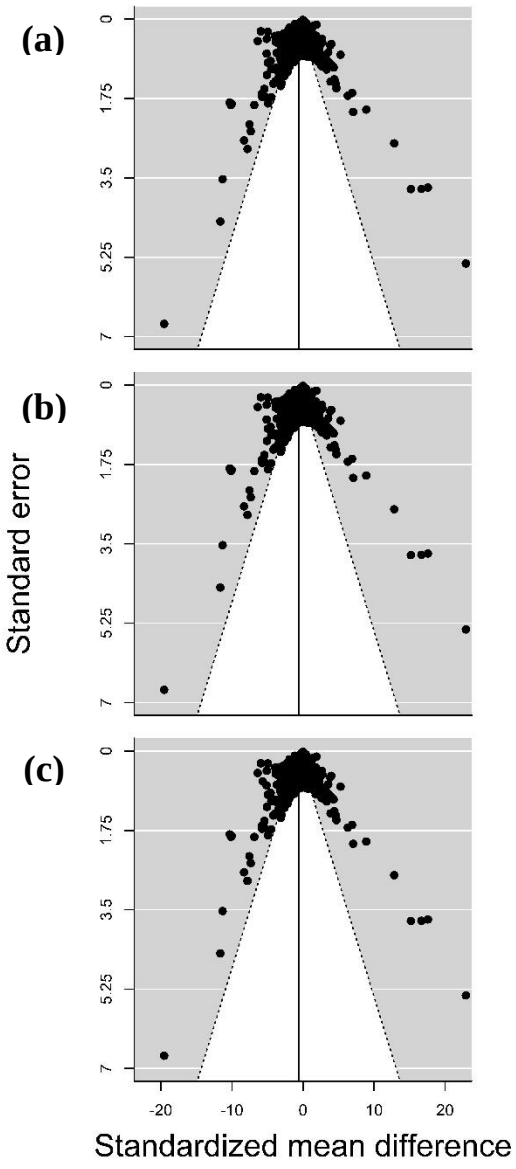

Figure S2. – Funnel plots for models that controlled for phylogeny (a), did not control for phylogeny (b), or did not control for phylogeny and contained viruses (c). The tails on the left and right sides of the plot indicate no publication bias for detrimental effects of parasites on species interactions in the models.

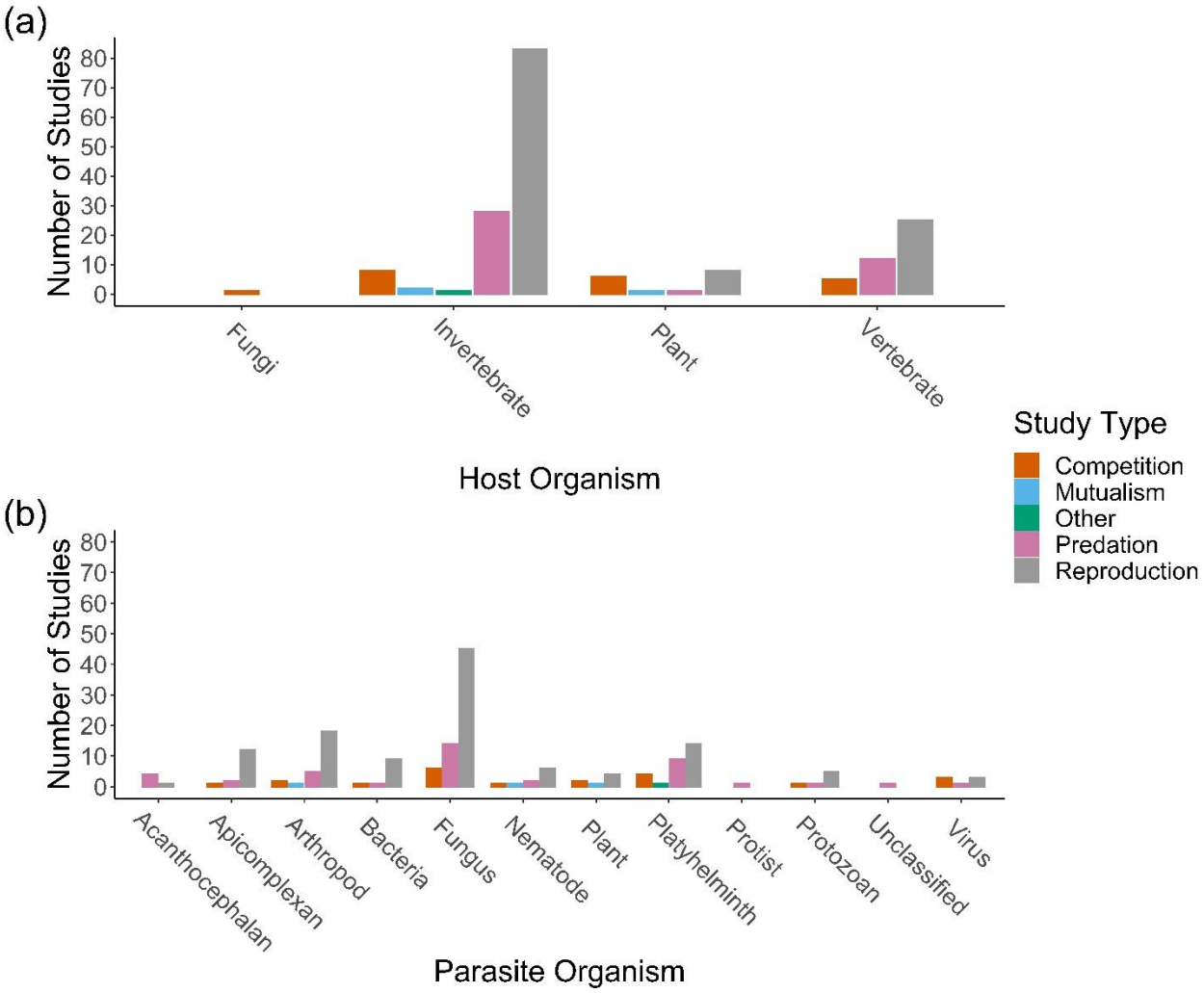

Figure S3. – Counts of the number of studies analyzing each species interaction included in our database separated by host (a) or parasite organism (b).

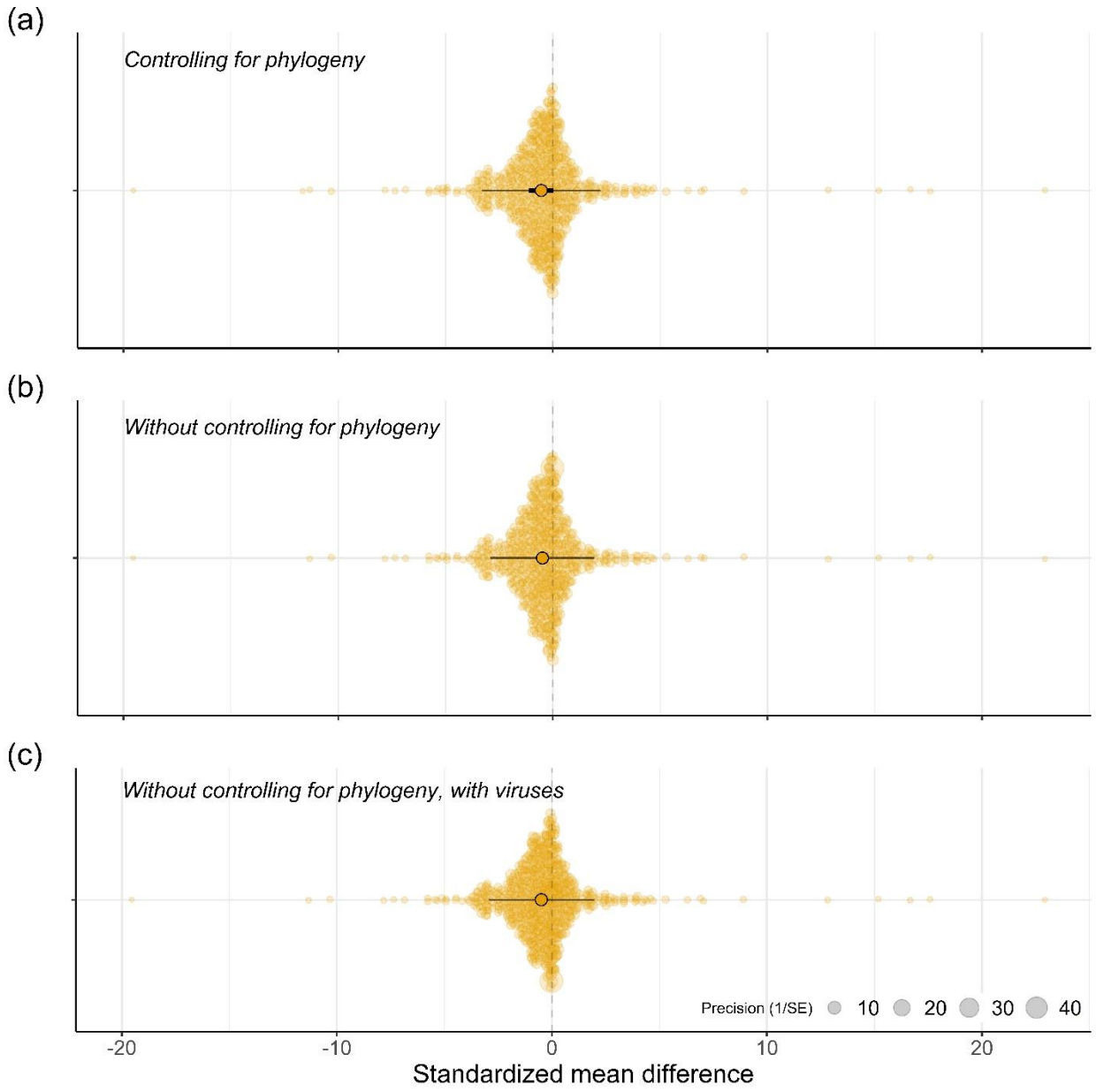

Figure S4. – Overall effect of parasitism on the outcome of species interactions across all studies and types of species interactions without influential outliers. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (*a*, n = 569 effect sizes from n = 154 studies, p = 0.07), without controlling for phylogeny (*b*, n = 564 effect sizes from n = 147 studies, $p < 0.0001$), and without controlling for phylogeny and including viruses (*c*, n = 606 effect sizes from n = 152 studies, $p < 0.0001$). The analysis controlling for phylogeny had a greater number of effects sizes than the analysis not controlling for phylogeny because fewer of the effect sizes from the original analysis were flagged as potential outliers. The orange points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

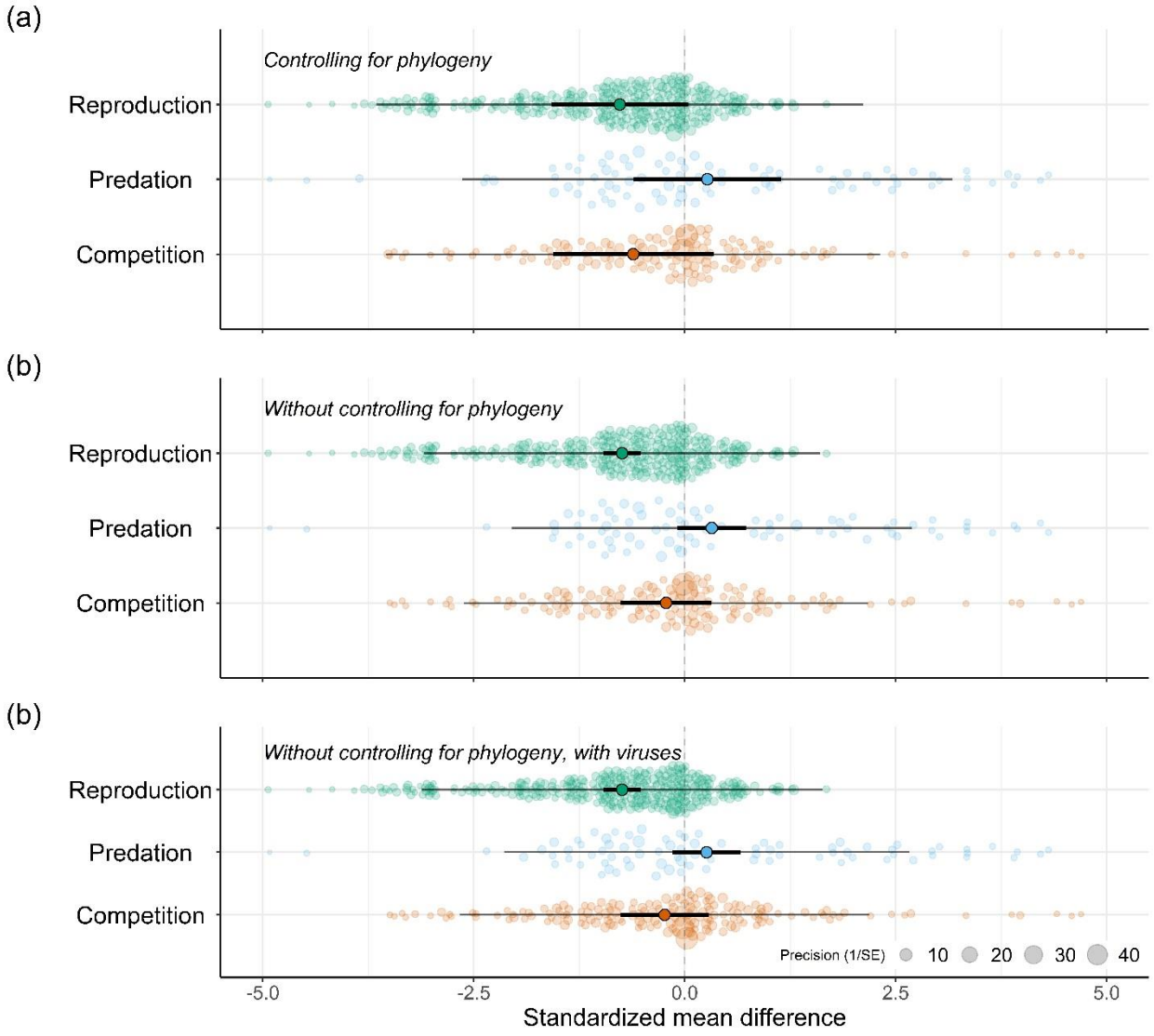

Figure S5. - Overall effect of parasitism on the outcome of species interactions among species interactions without influential outliers. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 559 effect sizes from n = 151 studies, p = 0.0001), without controlling for phylogeny (b, n = 554 effect sizes from n = 143 studies, p < 0.0001), and without controlling for phylogeny and including viruses (c, n = 596 effect sizes from n = 148 studies, p < 0.0001). All plots only show effect sizes from -5 to 5; see Fig. S4 for the full range. The points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

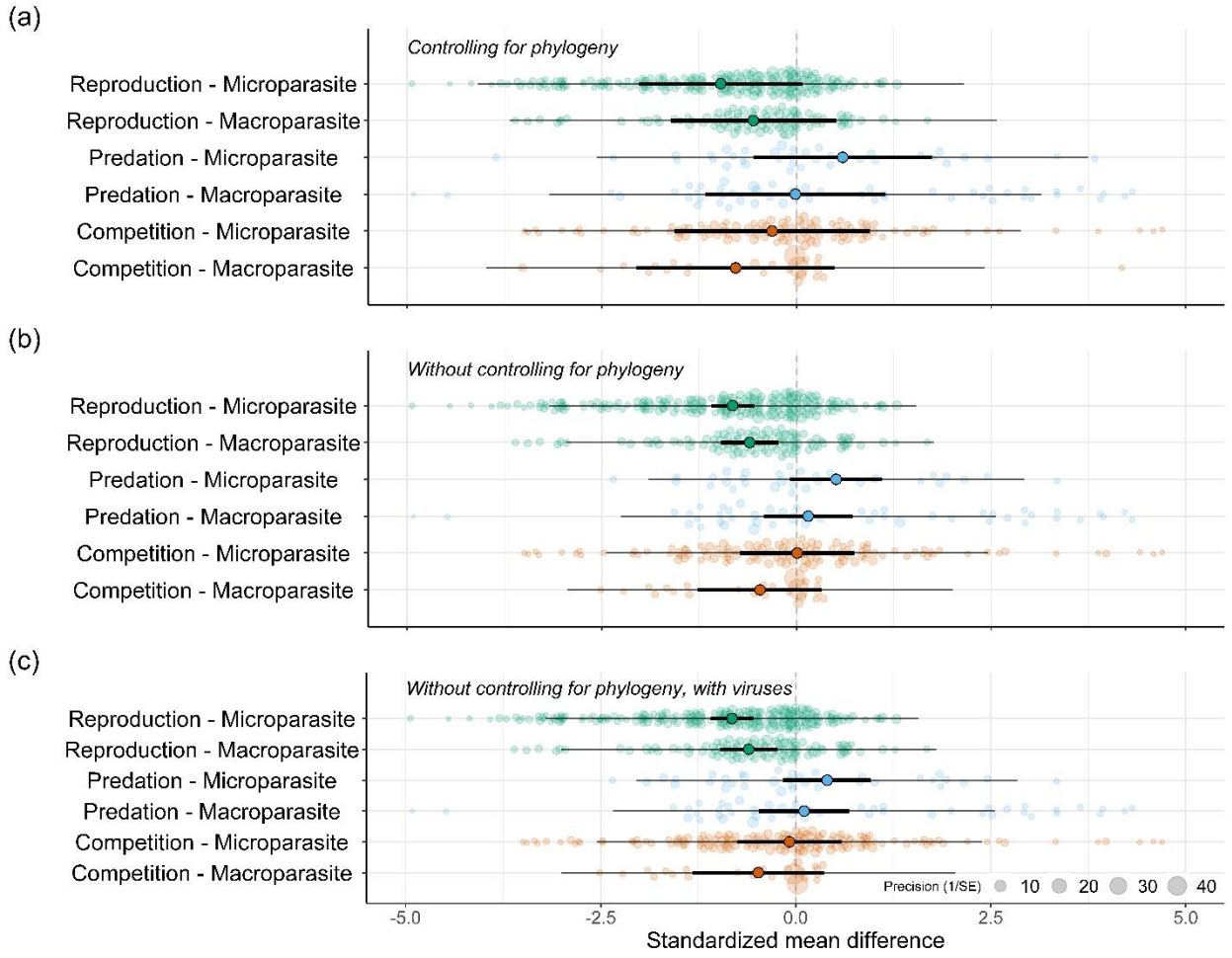

Figure S6. - Overall effect of parasite group on the outcome of species interactions among species interactions without influential outliers. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 559 effect sizes from n = 151 studies, p = 0.0002), without controlling for phylogeny (b, n = 554 effect sizes from n = 143 studies, $p < 0.0001$), and without controlling for phylogeny and including viruses (c, n = 596 effect sizes from n = 148 studies, $p < 0.0001$). All plots only show effect sizes from -5 to 5; see Fig. S4 for the full range. The points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

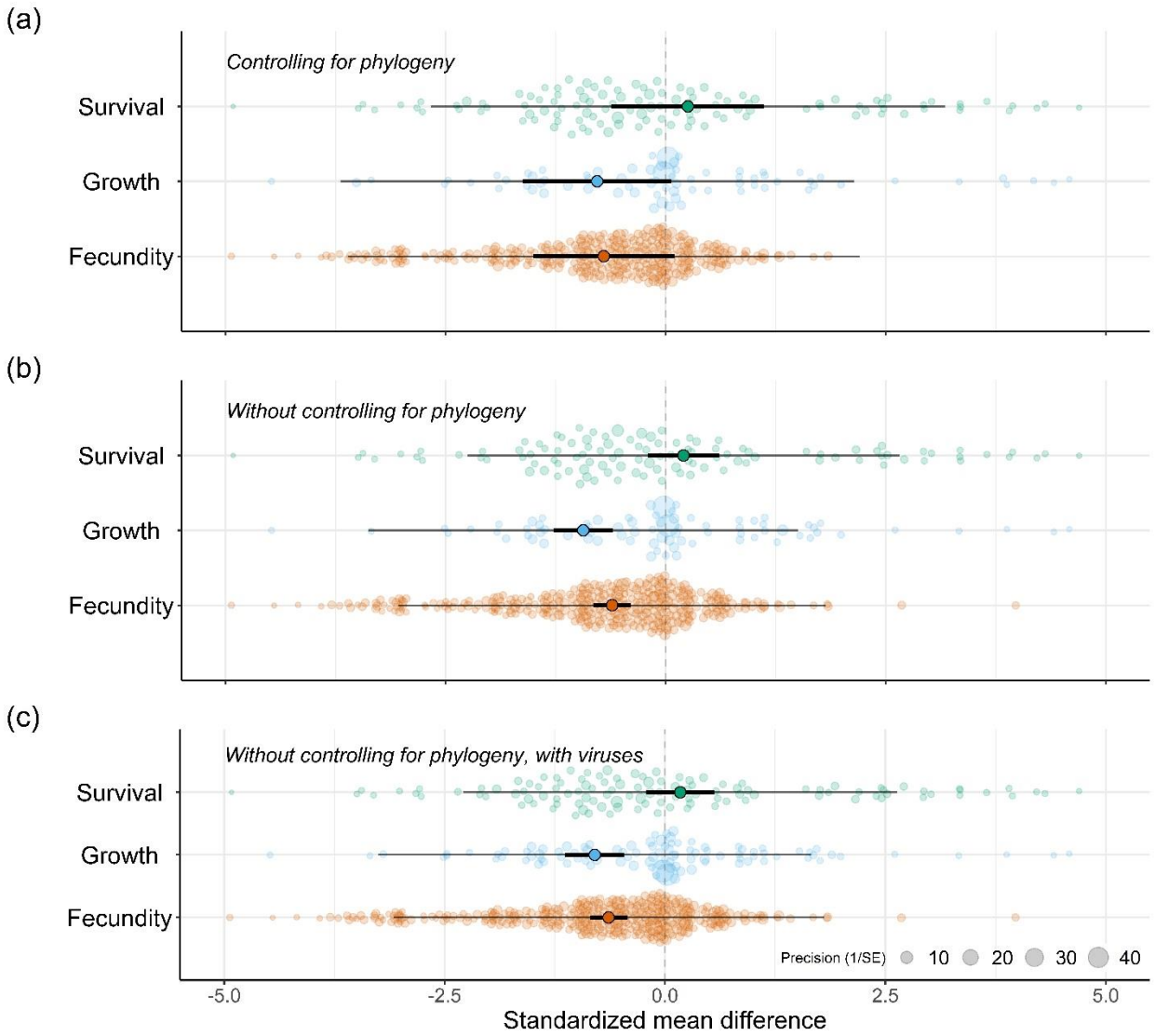

Figure S7. - Overall effect of parasitism on the outcome of species interactions among fitness components without influential outliers. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 559 effect sizes from n = 151 studies, p = 0.0001), without controlling for phylogeny (b, n = 554 effect sizes from n = 143 studies, p < 0.0001), and without controlling for phylogeny and including viruses (c, n = 596 effect sizes from n = 148 studies, p < 0.0001). All plots only show effect sizes from -5 to 5; see Fig. S4 for the full range. The points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

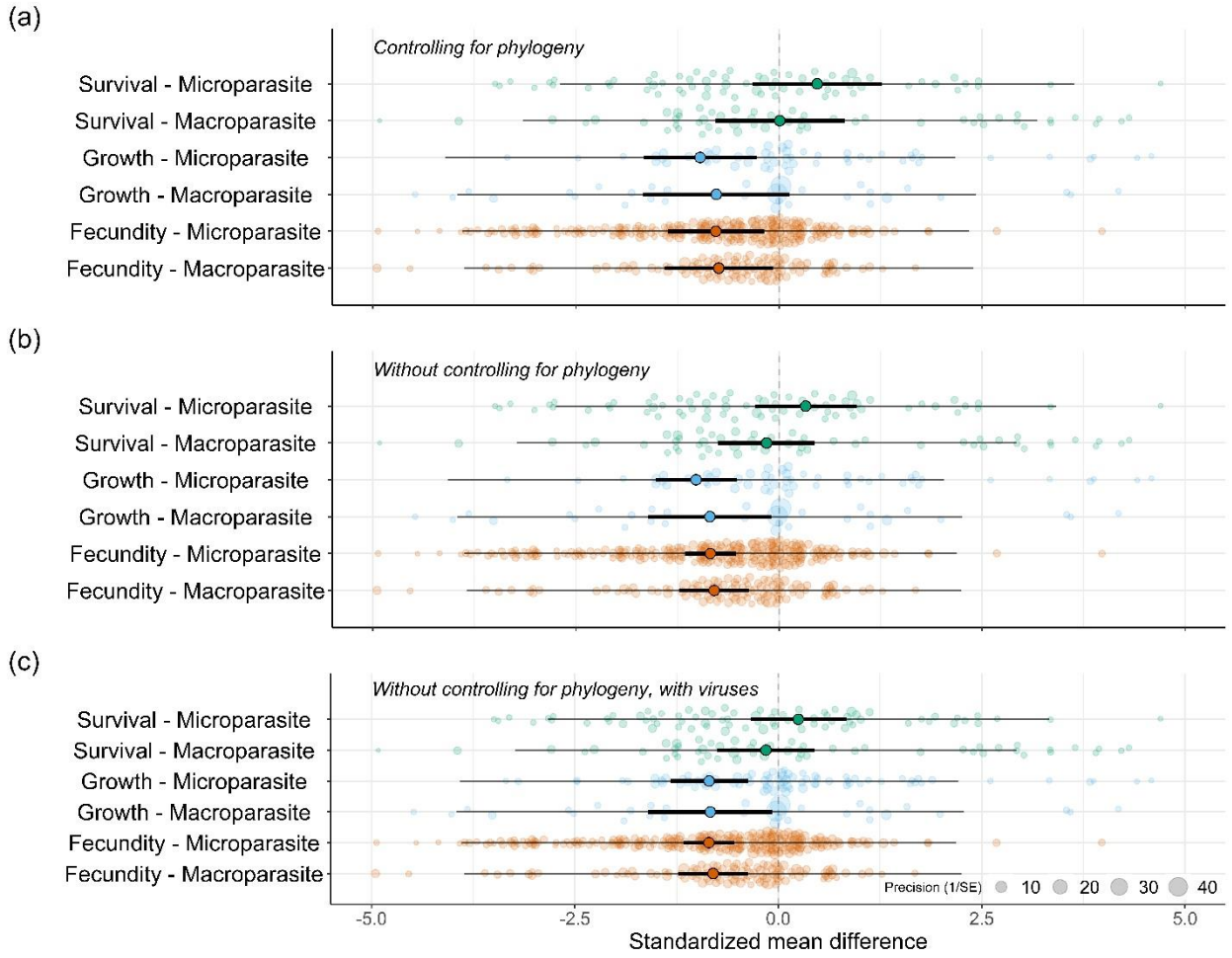

Figure S8. - Overall effect of parasite group on the outcome of species interactions among fitness components. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 603 effect sizes from n = 168 studies, p = 0.0004), without controlling for phylogeny (b, n = 603 effect sizes from n = 168 studies, p < 0.0001), and without controlling for phylogeny and including viruses (c, n = 647 effect sizes from n = 174 studies, p < 0.0001). All plots only show effect sizes from -5 to 5; see Fig. 1 for the full range. The points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

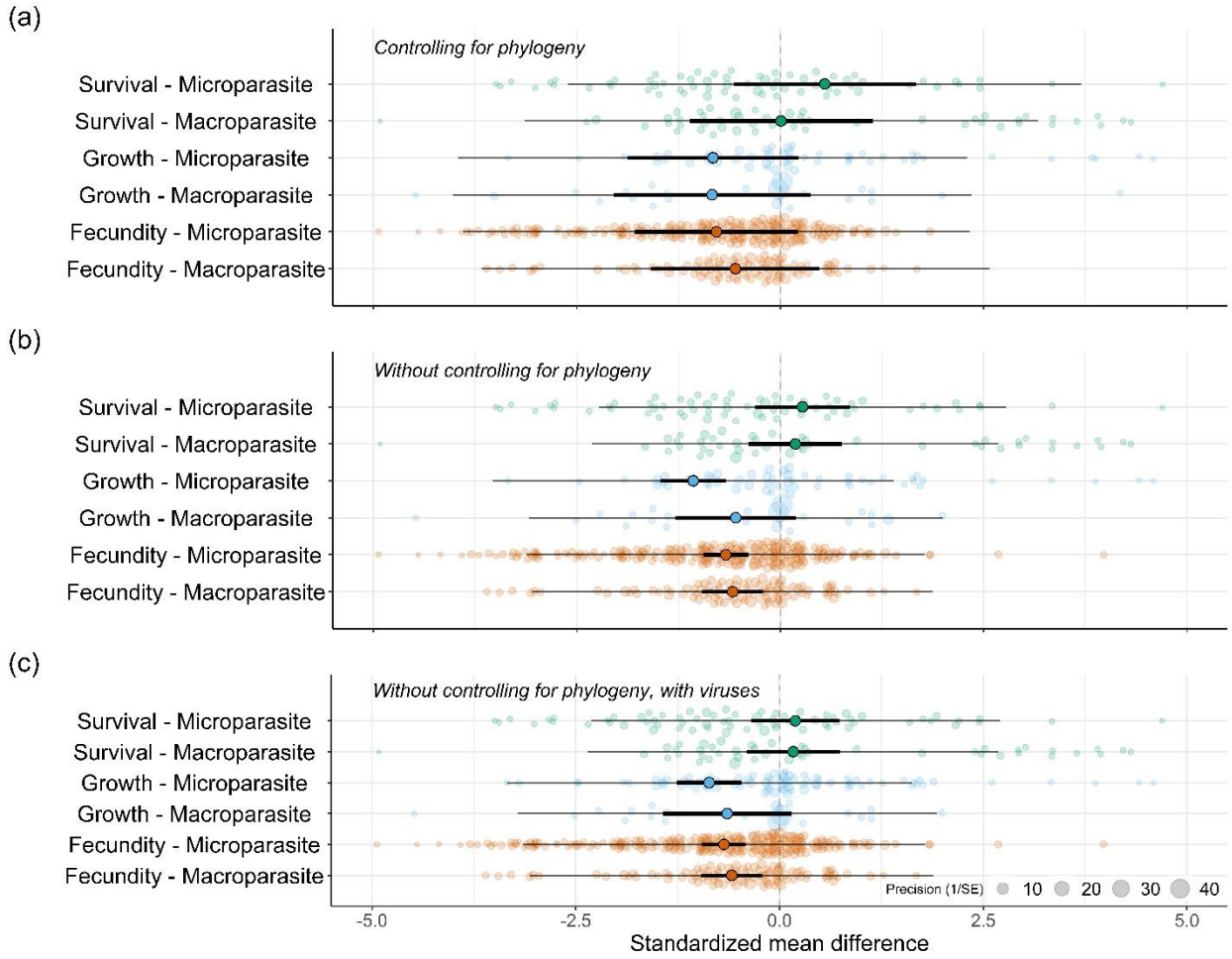

Figure S9. - Overall effect of parasite group on the outcome of species interactions among fitness components without influential outliers. Shown are orchard plots of standardized mean difference effect sizes when controlling for phylogeny (a, n = 559 effect sizes from n = 151 studies, p = 0.0006), without controlling for phylogeny (b, n = 554 effect sizes from n = 143 studies, p < 0.0001), and without controlling for phylogeny and including viruses (c, n = 596 effect sizes from n = 148 studies, p < 0.0001). All plots only show effect sizes from -5 to 5; see Fig. S4 for the full range. The points represent the individual effect sizes from each model, thin bars represent 95% prediction intervals, and thick bars represent 95% CI's.

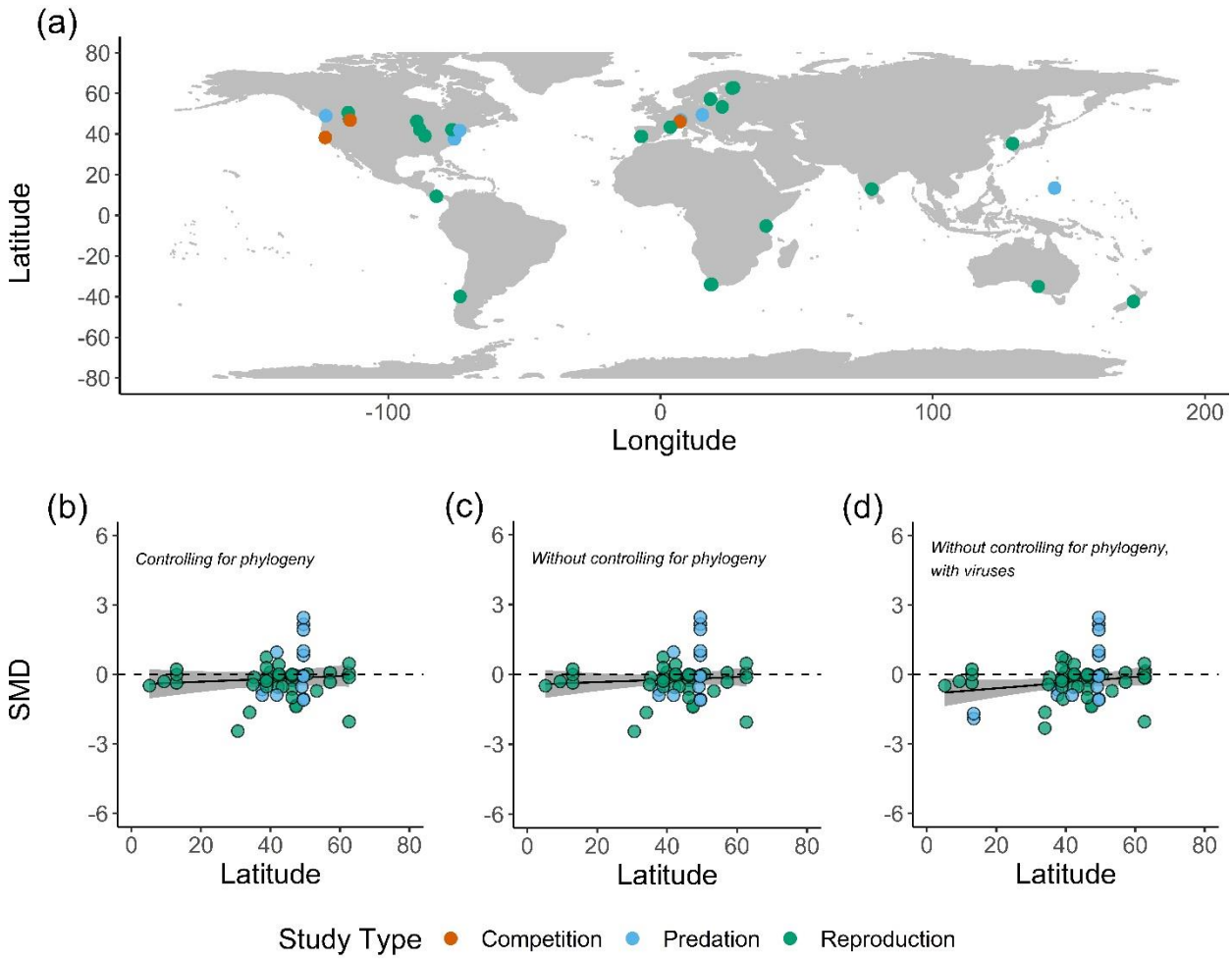

Figure S10. - Effects of parasitism on species interactions in relation to latitude without influential outliers. (a) shows the locations of field studies included in the meta-analysis (including the competition studies excluded from statistical analyses), (b), (c), and (d) represent the meta-regression of standardized mean difference (SMD) effect sizes on absolute degrees of latitude from models with phylogeny (n = 54 effect sizes from n = 25 studies, p = 0.45), without phylogeny (n = 54 effect sizes from n = 25 studies, p = 0.51), and without phylogeny and with viruses (n = 59 effect sizes from n = 27 studies, p = 0.12), respectively. Colors indicate the species interaction, with each point representing an individual effect size. The bands denote the 95% confidence interval of the slope.

Table S1. – Information on taxonomic assignments for the subset of parasite species assigned to higher taxonomic levels for inclusion in the phylogenetic analysis. Shown are the study, parasite species given in the study, and taxonomic assignment in the parasite phylogeny.

Study	Parasite	Assigned Taxa for Phylogeny
Allander & Bennett 1995	Protozoan blood parasite	Apicomplexa
Altizer & Oberhauser 1995	*Ophryocystis elektroscirrha*	Apicomplexa
Barthelemy et al. 2004	*Plasmodium chabaudi chabaudi*	Apicomplexa
Benesh et al. 2008	*Echinorhynchus borealis*	*Echinorhynchus cinctulus*
Botto-Mahan et al. 2017	*Trypanosoma cruzi*	*Trypanosoma*
Branson 2003	*Eutrombidium locustrum*	*Eutrombidium*
Chong & Oetting 2008	*Anagyrus* spp.	*Anagyrus*
Cloutier et al. 2011	*Plasmodium* spp.	Apicomplexa
Crabb & Pellmyr 2006	*Digonogastra* spp.	*Digonogastra*
Dyrcz et al. 2005	*Trypanosoma* spp.	*Trypansoma*
Fellet et al. 2014	*Trypanosoma cruzi*	*Trypanosoma*
Ferguson & Smith 2014	*Hepatozoon sipedon*	Apicomplexa
Garcia et al. 2018	*Oomycete* spp.	Peronosporomycetes
Gismondi et al. 2017	*Dictyocoela duebenum*	*Pleistophora*
Han et al. 2011	*Batrachochytrium dendrobatidis*	Peronosporomycetes
Hernaez et al. 2010	Bopyrid parasite	Bopyridae
Herrick et al. 2008	*Cotesia plutellae*	*Cotesia vestalis*
Hogg & Hurd 1997	*Plasmodium falciparum*	Apicomplexa
Islam et al. 1997	*Anagyrus pseudococci*	*Anagyrus*

Table S1. (Cont.)

Study	Parasite	Assigned Taxa for Phylogeny
Jahan & Hurd 1997	*Plasmodium yoelli nigeriensis*	Apicomplexa
Kavaliers & Colwell 1995	*Eimeria vermiformis*	Apicomplexa
Koprivnikar et al. 2008	*Echinostoma trivolis*	*Echinostoma*
Laws 2009	Mermithid spp.	Mermithidae
Lee et al. 2016	*Argeia pugettensis*	Bopyridae
MacNeil et al. 2003	Microsporidian spp.	*Pleistophora*
Marino et al. 2016	*Echinostoma* spp.	*Echinostoma*
Marzal et al. 2008	*Haemproteus* spp.	Apicomplexa
Mathis & Tsutisui 2016	*Pseudacteon lasciniosus*, *Pseudacteon planidorsalis*	*Pseudacteon*
Mottern et al. 2004	*Pseudacteon tricuspis*	*Pseudacteon*
Pasternak et al. 1999	*Triaenophorus* spp.	*Triaenophorus*
Pelabon et al. 2005	Microsporidian spp.	*Pleistophora*
Raveh et al. 2015	*Oropsylla* fleas	*Oropsylla*
Refardt & Ebert 2012	Microsporidian spp.	*Pleistophora*
Reyserhove et al. 2017	*Pasteuria ramosa*	Bacteria
Rosenkranz et al. 2018	*Apatemon* spp., *Plagiorchioid* spp.	*Apatemon*, Plagiorchioidea
Simmons 1994	Protozoan gut parasite	Apicomplexa
Soh et al. 2013	*Toxoplasma gondii*	Apicomplexa
Tseng 2004	*Ascogregarina taiwanensis*	Apicomplexa

Table S1. (Cont.)

Study	Parasite	Assigned Taxa for Phylogeny
Van Goor et al. 2018	*Parasitodiplogaster* spp.	*Parasitodiplogaster*
Vasquez et al. 2015	*Chondronema passali*	Capillariidae
Voutilainen 2010	*Plagiorchis elegans*	Plagiorchioidea
Yaro et al. 2012	*Plasmodium falciparum*	Apicomplexa
Zakikhani et al. 1998	*Plagiorchis elegans*	Plagiorchioidea
Dostal 2010	Seed fungal pathogens	*Sclerotinia sclerotiorum*
Creissen et al. 2016	*Hyaloperonospora arabidopsidis*	Peronosporomycetes
Pelizza et al. 2013	*Leptolegnia chapmannii*	Peronosporomycetes
Albuquerque Tomilhero Frias et al. 2020	*Candidatus Liberibacter solanacearum*	*Rhizobium sp. XJ-L72* + *Gellertiella*

Table S2. – Parameter estimates from meta-regression models for the analyses of the effects of parasites in relation to latitude (see Fig. 5 of the main text). Shown are results from models when controlling for phylogeny, without controlling for phylogeny, and without controlling for phylogeny and including viruses.

Model	Coefficient	Estimate	SE	*p*	CI Lower	CI Upper
With phylogeny:						
Predation	Intercept	-21.03	8.56	0.01	-37.80	-4.25
	Latitude	0.80	0.37	0.03	0.07	1.53
	Latitude^2	-0.01	0.004	0.07	-0.02	0.001
Reproduction	Intercept	-1.31	0.84	0.12	-2.95	0.34
	Latitude	0.09	0.04	0.01	0.02	0.16
	Latitude^2	-0.002	0.001	0.002	-0.003	-0.001
Without phylogeny:						
Predation	Intercept	-21.03	8.56	0.01	-37.80	-4.25
	Latitude	0.80	0.37	0.03	0.07	1.53
	Latitude^2	-0.01	0.004	0.07	-0.02	0.001
Reproduction	Intercept	-1.13	0.86	0.19	-2.81	0.56
	Latitude	0.08	0.05	0.08	-0.01	0.17
	Latitude^2	-0.001	0.001	0.02	-0.003	-0.0003
Without phylogeny, with viruses:						
Predation	Intercept	-4.52	0.99	< 0.0001	-6.46	-2.58

Table S2. (Cont.)

Model	Coefficient	Estimate	SE	*p*	CI Lower	CI Upper
	Latitude	0.10	0.02	<0.0001	0.06	0.14
Reproduction	Intercept	-1.04	0.89	0.24	-2.79	0.71
	Latitude	0.07	0.05	0.15	-0.02	0.16
	Latitude^2	-0.001	0.00	0.05	-0.002	0.00

Chapter 2

A common measure of prey immune function is not constrained by the cascading effects of predators

Adam Z. Hasik, Simon P. Tye, Taylor Ping, & Adam M. Siepielski

Abstract

Simultaneously defending against predators, stymieing competitors, and generating immune responses can impose conflicting demands for host species caught in the entanglement of a food web. Predators, in particular, can affect resource acquisition necessary for hosts to mount energetically costly immune responses. Identifying the links between predators and host immune responses determined by resource acquisition is a complex affair, because predators can (i) reduce host density and thus competition among hosts, (ii) exert non-consumptive trait-mediated effects on host resource acquisition behavior, and (iii) generate natural selection on host resource acquisition behavior. To examine the relative contributions of these potential predator driven density- and trait-mediated effects on a key aspect of immune function (total phenoloxidase activity, total PO), we conducted mesocosm and field experiments with larval damselflies (*Enallagma signatum*) and their dominant fish predator (*Lepomis macrochirus*). Contrary to our expectations, we found no support for the prediction that total PO activity would vary as a result of either non-consumptive trait-mediated effects or selection on damselfly foraging activity underlying resource acquisition. Similarly, although we expected to observe declines in total PO activity with increases in damselfly density, we found no relationship between density and total PO activity. Despite the lack of trait- or density-mediated effects, we did find that total PO activity increased with damselfly prey density among lakes, implying resource limitation for this aspect of immune function. These unexpected results point to the need to better understand the ecological conditions whereby predators and competitors constrain immune functions necessary for species to defend themselves in complex food webs.

Introduction

Immune defenses are critical components of host defense against parasites and pathogens (Sheldon and Verhulst 1996, Zuk and Stoehr 2002, Schmid-Hempel 2005, Siva-Jothy et al. 2005, Sadd and Schmid-Hempel 2009). However, immune defenses are energetically costly and depend on the ability of hosts to acquire and utilize resources (Lochmiller and Deerenberg 2000, Zuk and Stoehr 2002; González-Santoyo and Córdoba-Aguilar 2012). Yet resources are often limited, and resource acquisition is further constrained by the ecological milieu (e.g., interactions with predators, competitors, mutualists) species exist in. Consequently, identifying the ecological factors and phenotypic traits influencing resource acquisition can provide insight into understanding how host immune defenses can function in complex food webs. Here we ask how the cascading effects of predators on host competitive interactions shapes a common component of immune function.

We focus on predators because they can have a role in shaping the interplay between host competition and resource acquisition, and therefore immune function via several paths (Fig. 1). First, direct consumptive effects of predators lower host population densities, which can reduce density-dependent effects of intraspecific competition (Chase et al. 2002, Chesson and Kuang 2008). Because increasing competition reduces resource acquisition (McPeek and Crowley 1987, McPeek 1998, Kobler et al. 2009), predator-mediated reductions in competition should increase resource access, enhancing host immune function (Siva-Jothy and Thompson 2002, Kristan 2008, Budischak et al. 2018). Second, predators can generate indirect (non-consumptive) trait-mediated effects that influence competition (Werner and Peacor 1993, Preisser et al. 2005). For example, many prey respond to the presence of predators by reducing activity rates (e.g., foraging) and such reduced activity can decrease competition (Werner and Peacor 1993, McPeek 2004, Strobbe and Stoks 2004, Ousterhout et al. 2018, Siepielski et al. 2020). Finally, natural selection exerted by predators, which couples the consumptive and trait-based effects of reduced activity, can also reduce prey resource consumption (Strobbe et al. 2011, Ousterhout et al. 2018), and thus the strength of competition (Siepielski et al. 2020). While these disparate paths between

predators and host competitive interactions imply that the direct and indirect effects of predators should strongly affect host immune function, revealing these paths is a complex affair.

A wealth of studies in *Enallagma* damselflies have uncovered many of the phenotypes and mechanisms linking predation and competition to understand how *Enallagma* persist in food webs, acquire resources, and complete their life cycle (McPeek 1990, 1998, Stoks and McPeek 2006, Siepielski et al. 2010, 2011, Siepielski and McPeek 2013, McPeek 2017, Siepielski et al. 2020). *Enallagma* are aquatic insects that inhabit the littoral zone of waterbodies for most of their life cycle. Species found in lakes with fish as the top predator are adapted to coexisting with these predators by being relative inactive, as reduced activity helps them avoid detection (Strobbe et al. 2011, Swaegers et al. 2017, Ousterhout et al. 2018). Consequently, selection by fish favors less active individuals, which consume fewer prey items and may therefore experience greater resource limitation (Strobbe et al. 2011, Ousterhout et al. 2018, Siepielski et al. 2020). Indeed, damselflies in fish lakes are food limited and show consistent declines in growth rates with increasing densities (Anholt 1990, McPeek 1990, 1998, Siepielski et al. 2010, Ousterhout et al. 2019, Siepielski et al. 2020). This negative density-dependent response results from both direct interference competition (e.g., stress responses to conspecifics, McPeek et al. 2001) and indirect resource-based competition (McPeek 1990, 1998, Siepielski et al. 2020).

Numerous studies have also examined how some of these same ecological mechanisms affect damselfly immune function (Contreras-Garduño et al. 2006, Mikolajewski et al. 2008, Jiménez-Cortés et al. 2012). In particular, several studies have investigated the ecological basis for population-level variation in a vital, resource-limited component of their immune system - the phenoloxidase (PO) cascade (Marmaras et al. 1996, González-Santoyo and Córdoba-Aguilar 2012). This enzymatic cascade begins with the activation of PO and produces melanin as its end product, which encases and kills foreign bodies such as parasites and parasitoids (reviewed in González-Santoyo and Córdoba-Aguilar 2012). Total PO (PO measured in the absence of an immune challenge) has been well-investigated in the context of understanding interactions between damselfly hosts and ectoparasitic mites. These studies have found that competitors and

predators can limit the strength of this aspect of their immune function, either by diverting resources for use in competition at the adult life stage (Contreras-Garduño et al. 2006), by prioritizing growth under predation risk (Stoks et al. 2006), or by reducing access to resources (Jiménez-Cortés et al. 2012). Indeed, resource-limitation directly reduces total PO in damselflies (Campero et al. 2008, De Block and Stoks 2008). As of yet, though, how the direct, indirect, and combined effects of predators manifested through natural selection influence host competition and immune function has not been explored.

To begin unraveling these complexities, we previously used a series of mesocosm and field experiments to investigate how a fish predator (*Lepomis macrochirus*) shaped the strength of intraspecific competition in a larval damselfly species (*E. signatum*) via direct, indirect, and combined effects of natural selection (Siepielski et al. 2020). In that study we did not examine how these effects shaped damselfly immune function. However, those experiments provided an ideal opportunity to investigate this. Below, we present the major results of those experiments and use them to frame and generate two sets of predictions for how a key component of damselfly immune function (total PO) should vary in response to the effects of predation and competition.

First, we used a mesocosm experiment to parse out: (i) the direct consumptive effects of fish predators reducing damselfly density, (ii) the indirect non-consumptive trait mediated effects reducing activity rates, and (iii) the combined consumptive and trait-based effects generated by fish exerting selection favoring less-active individuals. We found that reducing damselfly densities had the greatest effect on damselfly growth rates and thus the strength of intraspecific competition. However, this effect depended on the strength of selection on damselfly activity rates, since density-dependent growth in damselflies weakened as selection for less active individuals increased; less active individuals had lower attack rates and higher handling times associated with prey capture. Reductions in activity alone (a pure trait-mediated effect) had minimal effects on growth rates. From these combined results, we predict that i) immune

function (total PO activity) should increase as damselfly densities decrease, and ii) this effect should be greatest as the strength of selection favoring less-active damselflies increases.

Next, we conducted field experiments to examine how the strength of competition damselflies experienced varied among lakes differing in fish and damselfly prey densities. We found that damselfly activity rates declined as fish densities increased among lakes (Siepielski et al. 2020), presumably as an adaptive response to more intense selection (Ousterhout et al. 2018, see also Benkman 2013). Thus, we expected that the strength of negative density dependence in damselfly growth rates should also decline as fish densities increased – a pattern we found support for. We also found that damselfly growth rates increased with natural prey density in these lakes, but the strength of density dependence was not associated with prey densities. That is, damselflies grew faster in lakes with more prey, implying resource limitation (e.g., McPeek 1998), but this effect did not depend on damselfly density. From these results we predict that as fish densities increase, and the strength of competition declines because of reduced activity rates, that total PO should increase. Similarly, we predict that as prey densities increase among lakes, total PO should also increase. Evaluating these predictions provides a key test of how predators can shape host competitive dynamics and in turn influence how a key aspect of immune function in complex food webs where species face conflicting ecological demands (Rigby and Jokela 2000).

Materials and Methods

To test these predictions, we saved and used the same larval damselflies from the above experiments. Because the methods for these experiments (save the PO assays) have been previously published (Siepielski et al. 2020), we only briefly summarize them here.

Prediction 1: Effects of predator consumption, trait, and selection on immune function.
This first experiment was designed to isolate the effects of predator consumption, non-consumptive trait-mediated effects on damselfly activity rates, and selection on damselfly

activity rates on damselfly total PO activity levels. To accomplish this, we used a fully factorial design crossing damselfly density with differences in average damselfly activity rates. A full description of these methods and the experimental design can be found in Siepielski et al. (2020). Below, we include the most salient details. Differences in total PO activity because of damselfly density would reflect changes in the competitive environment driven by predator consumption (e.g., reducing damselfly density). Differences in total PO because of damselfly activity alone, controlling for density, mimic non-consumptive effects of predators. By crossing these two factors, the interaction between them captures how selection imposed by predators can alter total PO activity levels (e.g., the effect of depressing density via consumption when combined with changes in activity is equivalent to a covariance between fitness (survivorship) and traits).

To quantify activity rates, we gathered 400 late instar *E. signatum* larvae from two lakes with low fish densities (Charleston and Greenwood) in west-central Arkansas, USA (Ousterhout et al. 2019). Damselflies in these lakes were under weak selection by fish (Ousterhout et al. 2018) and thus represented a broad distribution of activity rate phenotypes. Activity rates were quantified using open field tests (Johansson and Rowe 1999, Brodin and Johansson 2004, Start and Gilbert 2017). All assays were conducted in a greenhouse under natural lighting and temperature conditions with a fan constantly circulating air. We assayed the activity rates of individual larvae by placing a single larva in a petri dish (10-cm diameter) filled with filtered pond water. Larvae acclimated for 12-15 hours, after which we recorded their position every 20 minutes for 3 hours. Activity rate was quantified as the sum of minimum distances between successive locations, expressed as mm moved/three hours. Importantly, activity rates of individual larvae saved from the previous experiment were a repeatable phenotype (repeatability = 0.567, 95% CI: 0.270, 0.767, Siepielski et al. 2020, see also Start 2018).

Directional selection by predators on activity levels works by generating differences in mean activity rates before relative to after consumption by a predator. Given our extensive knowledge of how selection by fish acts on damselfly activity (Strobbe et al. 2011, Swaegers et al. 2017, Ousterhout et al. 2018, Siepielski et al. 2020), we simulated such selection by ranking the

activity rates of the 400 assayed damselflies and then dividing them into four groups (hereafter 'activity levels', see Fig. 2) corresponding to significant differences in mean activity rates (Fig. 1, Fig. S2 in Siepielski et al. 2020). The difference between the mean activity level across all groups (e.g., before 'selection' occurred) and the mean in each group (e.g., equivalent to activity levels of the 'survivors' after selection occurred) represents the effect of selection, with each group reflecting a different intensity of selection. These groups represent population-level variation in activity levels commonly observed in odonates (Start 2018) and should represent differential survival across natural fish densities (Ousterhout et al. 2018).

To determine the effects of variation in damselfly density, we used 5.5L plastic tray mesocosms (0.25m diameter, bottom area = $0.05m^2$) filled with filtered lake water, macrophytes (*Ceratophyllum* spp.), and *Daphnia* prey. Mesocosms were housed in a greenhouse under natural lighting and temperature conditions, with a fan circulating air. After completing the activity rate assays, damselflies were established at densities of 1, 2, 4, or 10 per mesocosm, which are equivalent densities of 20-198 damselflies/m^2, and similar to natural larval densities in this area (Ousterhout et al. 2019). Each of the four activity levels was then crossed with density in a factorial design with five replicates each (n = 80 total mesocosms). Larvae were collected after 21 days, which is sufficient time to allow for competitive effects to affect damselfly growth rates (Siepielski et al. 2020) and for differences in total PO values to manifest (De Block and Stoks 2008), then were stored at -80°C to later conduct PO assays.

Prediction 2: Density dependent immune function along predator and prey density gradients. This second experiment was designed to examine how density-dependent responses of damselflies to intraspecific competition varied along gradients of fish predators and damselfly prey resources. Damselfly activity rates decrease with increasing fish predator density (McPeek 2004, Strobbe et al. 2011, Ousterhout et al. 2018, Siepielski et al. 2020), and the strength of density-dependent competition decreases with increasing fish density (Siepielski et al. 2020). These patterns, combined with variation in natural prey densities among lakes (Ousterhout et al.

2019) imply that damselflies not only experience spatial variation in resource availability, but also spatial variation in the strength of competition for these resources. Because immune function is tied to host resource acquisition (Siva-Jothy and Thompson 2002, Budischak and Cressler 2018, Budischak et al. 2018), spatial variation in damselfly prey resource availability (as dictated by natural prey densities) and acquisition (as dictated by damselfly activity levels) should explain variation in total PO activity among damselfly populations.

Thus, to examine how the effects of competition (as generated through negative density dependence in damselfly growth rates, McPeek 1990, 1998, Siepielski et al. 2010, 2011, 2020) affected damselfly immune function (total PO activity) among lakes varying in fish and prey densities, we established 20 submerged cages in the littoral zone of six lakes (n = 120 total cages). A full description of these methods and the experimental design can be found in Siepielski et al. (2020). Below, we include the most salient details. These lakes varied in both fish (range: 1.08-16.49 fish/m^2, based on the mean of three replicate seine hauls per lake) and prey densities (range: 11.85-121.68 prey/L, based on the mean of six replicate samples with a 6 L box sampler [100-µm mesh] placed over macrophytes where damselflies forage). Complete details for methods used to generate these estimates can be found in Ousterhout et al. (2019). Cages were constructed with PVC pipe (2.1cm diameter) and enclosed in mesh netting (0.6 x 1.2 mm mesh), allowing prey to colonize the cages but keeping non-experimental larvae out. Each cage was stocked with macrophytes (*Justica americana*) to provide a foraging substrate for damselflies. Density treatments were 1, 2, 4, or 10 larvae per cage. Larvae were removed after 21 days and stored at -80°C to later conduct PO assays.

Quantification of total PO activity

To measure innate immune function we measured total PO activity using a modified protocol (Iserbyt et al. 2012, Mlynarek et al. 2015). Larvae were placed into microcentrifuge tubes with 300µl of cacodylate buffer (0.01M $C_2H_6AsNaO_2$–0.005M $CaCl_2$), crushed in the cooled buffer, after which they were centrifuged at 15000rpm for 10 minutes at 4°C. After centrifugation, 100µl

of the supernatant was placed into a well of a 96-well plate containing 35µl of 50mM PBS buffer, after which 5µl of α-chymotrypsin (Sigma Aldritch #C4129) was added. After reacting for 5 minutes at room temperature, 60µl of L-DOPA (10mM/L of dihydroxyphenyl-L-alanine (Sigma Aldritch #D9628) in cacodylate buffer) was added as substrate for the reaction. Total PO values were measured in duplicate, and the mean of the readings was used for analyses.

The PO reaction was measured in a spectrophotometer (SprectraMax 190 Microplate Reader, Molecular Devices) for 30 minutes at 30° C and read at 485nm. A reading was taken every 20 seconds, and the plate shaken between each reading. Total PO activity values were measured as the slope of the reaction curve. As in previous studies (e.g., Iserbyt et al. 2012, Mlynarek et al. 2015), to control for variation in body size, we measured the protein content of each damselfly using a modified Bradford protocol (Bradford 1976). Using the supernatant from the PO assay, we prepared the protein assay on a 96-well plate as follows: 40µl of dye (Bio-Rad #5000006), 155µl of Milli-Q water, 5µl of supernatant, and 40µl of Bradford solution. The plate was read at 595nm at 30° C after 6 minutes of continuous shaking. Protein content was measured once at the endpoint and compared with a standard curve with Bovine serum albumin (Bio-Rad #500-0005). Protein was measured in duplicate, and the mean of the readings used for statistical analyses.

Statistical analysis

Ideally, we would have tracked individuals separately so that we could determine the relationship between individual activity rates and total PO values. However, except for the single individual density treatments it is impossible to mark individual larvae over the duration of the experiments (damselflies grow by molting, so any marks would be lost). Thus, we pooled all larvae from each replicate and used mesocosm mean (experiment 1) or cage mean (experiment 2) total PO values in all statistical analyses, which is the appropriate experimental unit. To examine if mean values were generally representative of individual-level patterns we also analyzed the correlation between activity levels and total PO values from the single density treatments in the mesocosm experiment.

We had mortality from unknown causes (cannibalism was possible, but mortality occurred across all densities). Any change in density may have affected resource acquisition, but because it was impossible to determine when individuals died, we used initial densities as in Siepielski et al. (2020). Importantly, despite these occasional losses, the density treatments still resulted in declines in damselfly growth rates (e.g., Siepielski et al. 2020). During the field experiment our lakes also experienced intense storms and all cages from the 1 and 4 larvae density treatments were lost from Lake Fayetteville. In addition, we were unable to use data from several protein assays due to unsatisfactory standard curves ($r < 0.90$). In total, we used data from 58 mesocosms for experiment 1 and 78 cages from experiment 2.

Prediction 1: Effects of predator consumption, trait, and selection on immune function.
Here, we wanted to evaluate the direct effects of predator consumption (depressing damselfly densities), non-consumptive (indirect) trait-mediated effects on damselfly activity rates, and selection on damselfly total PO activity. To do so, we constructed a general linear model (GLM) of the form: mesocosm mean total PO activity as the response variable with activity level, damselfly density, and their interaction as predictors. Mesocosm mean damselfly protein content was included as a covariate to control for potential differences in size (Mikolajewski et al. 2008). Although activity rate was treated as a categorical term, it does represent differences in average damselfly activity rates (see Siepielski et al. 2020). For this model, a significant interaction term would indicate that the effect of damselfly density on total PO level depended on the activity level, thus revealing an effect of predator-driven selection on immune function.

We also evaluated if total PO levels were condition-dependent (e.g., as reflected by differences in growth rates). To do so, we tested for a correlation between mesocosm mean total PO activity and mesocosm mean growth rate (changes in body size through time) from Siepielski et al. (2020) across all treatments and mesocosms.

Prediction 2: Density dependent immune function along predator and prey density gradients. We examined if the effects of competition (as generated through variation in damselfly cage densities; Siepielski et al. 2020), affected damselfly immune function (total PO activity) among lakes varying in fish and prey densities. Specifically, we were interested in whether there were significant interactions between total PO activity and damselfly, fish, and prey densities among lakes; fish and prey densities were not correlated among lakes (n = 6, r = 0.148, p = 0.780). To examine this, we built a GLM of the form: cage mean total PO activity as the response variable, with damselfly cage density, fish density, prey density, and interactions between damselfly density and fish or prey density as predictors (Table 1). As above, cage mean damselfly protein content was included as a covariate to control for potential differences in size. To quantify if total PO levels were condition-dependent, we again tested for a correlation between cage mean total PO activity and cage mean growth rates from Siepielski et al. (2020) across all treatments and cages. All analyses were performed in R ver 4.0.0 (R Core Team 2020).

Results

Effects of predator consumption, activity, and selection on immune function

There was considerable variation in both individual activity rates and total PO activity among damselflies, although there was no correlation between these factors (Fig. 2e-g). For the single density treatments, where we had individual-level data, there was also no correlation between activity rates and total PO activity (n = 11, r = -0.004, p = 0.99, Fig. S1). There was also no effect of damselfly density (a predator consumptive effect, $F_{1,49}$ = 0.09, p = 0.76), average activity level (a trait mediated effect, $F_{1,49}$ = 0.01, p = 0.99), or selection (the interaction between density and mean activity level, $F_{3,49}$ = 0.02, p = 0.99, Fig. 2a-d) on total PO activity. Likewise, there was no significant correlation between total PO activity and mean growth rates (n = 58, r = 0.14, p = 0.29, Fig. S2a).

Immune function along environmental gradients

Total PO activity varied significantly among lakes (Fig. 3a-f). Though total PO activity was not correlated with average activity rates among lakes (Fig. S3), it was weakly positively correlated with mean growth rates (n = 78, r = 0.23, p = 0.04, Fig. S2b). Larvae from Lake Wilson had the lowest total PO activity (mean = 37.40, s.e. = 3.95), while it was almost three-times higher on average for the larvae in the other five lakes, as those from Bobb Kidd Lake had the highest total PO activity (mean = 106.62, s.e. = 3.49). Tukey post-hoc tests revealed that Lake Wilson larvae had significantly lower total PO activity than larvae from the other lakes ($p < 0.001$ for all tests), but total PO activity did not differ for larvae from the other five lakes ($p > 0.45$ for all tests).

Although total PO activity varied among lakes, we found no evidence for an effect of damselfly density on mean total PO activity (Table 1, Fig. 3a-f). There was also no significant effect of fish density, or an interaction between fish and damselfly densities on mean total PO activity among lakes (Table 1, Fig. 3g). However, we did find that mean total PO activity increased with greater prey density among lakes (Fig. 3h), although this relationship was not density dependent as there was no significant interaction between prey and damselfly densities (Table 1). Graphical inspection revealed that the relationship between mean total PO and prey density was non-linear. Thus, we built an asymptotic regression model of mean total PO on prey density to quantify this association using the nls function (R Core Team 2020). This model was of the form total PO = $a(1\text{-}e^{(\text{-}c*\text{prey/L})})$, where a is the estimated upper total PO limit and c is a parameter describing the proportional increase in total PO with prey/L. This model showed that total PO activity rapidly increased once prey density exceeded about 17/L and then stabilized at an upper limit of about 110 (Δod 485nm/min) (Fig. 3h).

Discussion

Predators frequently generate cascading effects on competitive interactions that affect host resource acquisition (Gurevitch et al. 2000, Chase et al. 2002, Siepielski et al. 2020). Because the ability to mount an immune response is also resource limited and condition-dependent, we

posited that trait- and density-mediated effects of predators on competitive interactions suppressing damselfly resource acquisition would in turn influence total PO. Yet, our results fell contrary to this overall hypothesis. We found no support for the prediction that total PO would vary due to non-consumptive trait-mediated effects on activity rates, or through effects of selection on activity that affects resource acquisition. Similarly, although we expected to observe declines in total PO with increases in damselfly density, we found no relationship between density and total PO. Despite the lack of trait- or density-mediated effects, we did find that total PO increased with prey density among lakes. Taken together, the replicated nature of these results among two independent sets of experiments imply that predation and competition do not constrain a key aspect of immune function.

We found no relationship between total PO levels and differences in activity rates arising through either a non-consumptive trait-mediated effect or an effect of selection. This was surprising as i) our mesocosm experiment showed that differences in activity rates affect damselfly prey acquisition by lowering attack rates and increasing handling times, and ii) damselflies in the field experiment had reduced activity rates as an adaptive response to increasing fish predation (Siepielski et al. 2020). Mlynarek et al. (2015) also found no consistent difference in total PO levels between *Enallagma* species found in lakes with either fish or dragonflies as the top predator. These differences in top predator are noteworthy, as fish select for reduced activity levels (Stoks and McPeek 2003, Strobbe et al. 2011, Siepielski et al. 2020) while dragonflies select for more active damselflies that have higher prey attack rates (McPeek 1997). Thus, collectively, these population-level and comparative findings imply that activity-based adaptations to predators do not constrain this aspect of immune function.

Though we tested for an effect of predators on one key aspect of immune function, we acknowledge that immune function is multi-faceted. Prey responses to predators have been shown to have no effect on some components of immune function, but also increase and simultaneously decrease other components (Rigby and Jokela 2000, Vinterstare et al. 2019). Resource limitation can also differentially affect immune function components. For example, De

Block and Stoks (2008) found that haemocyte levels returned to normal after a period of resource restriction, but total PO and proPO levels remained low into the adult stage for damselflies. However, the PO cascade is not only an important defense against many parasites and pathogens, it is also used in pigment synthesis, egg production, and wound-healing of damaged tissue (reviewed in González-Santoyo and Córdoba-Aguilar 2012). Damselflies engage in both attempted cannibalism (Anholt 1994) and direct interference competition (McPeek et al. 2001), both of which result in wounding that would require PO-facilitated repair. Additionally, defense against parasites in insects often relies on melanization (Siva-Jothy et al. 2005, González-Santoyo and Córdoba-Aguilar 2012). For example, the most common ectoparasite in this system are *Arrenurus* water mites (Smith et al. 2010), and adult damselflies defend themselves from these mites by melanizing their feeding tubes via the PO-cascade (Marmaras et al. 1996). PO responds to experimental parasite challenges much like other immune parameters (Stoks et al. 2006, Gershman 2008, Srygley and Jaronski 2011) and PO levels positively correlate with not only melanization (Zhang et al. 2008), but also with defense against parasites (Fedorka et al. 2013). Thus, as in many studies, our use of total PO is an appropriate, though limited, representation of overall immune function (Gershman 2008, Srygley and Jaronski 2011, Stahlschmidt et al. 2020). Future studies should nevertheless consider a multitude of immune defenses such as haemocyte counts, nitric oxide, and proPO (e.g., Siva-Jothy et al. 2005, Mlynarek et al. 2015).

A key aspect of our mesocosm experiment was that by having predator cues absent it uncoupled changes in activity rates from any other possible non-consumptive effects of predators on immune function. This is important to consider as other studies have found that the mere presence of predators affects immune function (Stoks et al. 2006, Mikolajewski et al. 2008, Duong and McCauley 2016). For example, Duong and McCauley (2016) found that the presence of dragonfly predators did not affect activity levels of their prey, but it did increase prey melanization levels via the PO cascade. However, this is also a strength of our experimental design because unlike previous studies it allowed us to specifically isolate effects of activity

relative to any other confounding effects of plastic responses to predator cues. Notably, however, in the field experiment such cues were present, and higher fish densities should correspond with stronger cues that illicit predator threat responses (Siepielski et al. 2014, Tollrian et al. 2015, Siepielski et al. 2016). Yet there was no correlation between total PO activity and fish densities. Thus, despite inhabiting communities where the risk of predation is greater (Ousterhout et al. 2018), damselflies maintained consistently high total PO, implying that this aspect of immune function is not constrained by predators, and that the lack of finding an effect on total PO is likely not an artifact per se of not having predator cues present (e.g., Stoks et al. 2006).

We predicted that reduced damselfly activity would correspond with lower total PO levels, since lower activity is associated with reduced resource acquisition (Strobbe et al. 2011, Siepielski et al. 2020), and total PO levels are food-limited (Campero et al. 2008, De Block and Stoks 2008). However, resource acquisition is only the first step in resource utilization (McPeek 2004), which is also dictated by an individual's ability to digest and assimilate resources. Consequently, simply acquiring more prey resources through greater foraging rates and reduced competitive effects may not be the limiting step. Predators and conspecifics can also suppress digestive physiology just by being present (McPeek et al. 2001, McPeek 2004). Thus, the lack of an association between activity and total PO may indicate that immune function is more strongly coupled to other aspects of digestive physiology. Indeed, Tye et al. (2020) found no association between larval damselfly (*E. vesperum*) prey consumption rates and immune function, but did find a positive correlation between assimilation efficiency and immune function.

We found that total PO levels increased markedly with increases in prey density, supporting the widely-posited relationship between immune function and resource availability (Siva-Jothy and Thompson 2002, De Block and Stoks 2008, Kristan 2008, Forbes et al. 2016; Budischak et al. 2018, Hite and Cressler 2019). However, this association was non-linear as immune function rapidly increased with prey density, after which there was generally little variation. This pattern implies that some minimal amount of prey is necessary to generate a more robust immune function, but levels above that generate no further benefit. This apparent threshold could be

adaptive, as the PO cascade produces toxic by-products that can harm the host (Dowling and Simmons 2009). Thus, by limiting investment in immune function, despite increasing levels of resource abundance, hosts can protect themselves from self-harm (e.g., melanization of host tissue, Sadd and Siva-Jothy 2006). We do note, however, that prey density was not experimentally manipulated (e.g., Forbes et al. 2016). Total PO levels could be responding to other factors correlated with prey densities that vary among lakes (i.e., prevalence of parasites, temperature, lake productivity), and in addition to spatial variation, damselfly total PO levels are also known to vary over time (Córdoba-Aguilar et al. 2011). The relationship between immune function and resource availability requires further study to determine to what degree individual total PO levels are resource-limited, and if there is some threshold, after which immune function does not increase.

That we found an effect of resources, but no corresponding effect of density-dependence in total PO is counterintuitive. Like many organisms, damselfly growth is resource-limited (Anholt 1990, McPeek 1990, 1998, Siepielski et al. 2010, Ousterhout et al. 2019, Siepielski et al. 2020), and aspects of immune function are resource-limited (this study, Campero et al. 2008, De Block and Stoks 2008, Forbes et al. 2016, Budischak et al. 2018), both of which are often density dependent. Thus, our results are contrary to long-held and widely supported views that immune function, at least for total PO, is density dependent (Wilson and Reeson 1998, Barnes and Siva-Jothy 2000, Wilson et al. 2002, Kong et al. 2018, Murray et al. 2020). Instead, our results support evidence for a lack of density-dependent immune function in other studies (Svensson et al. 2001, Miller and Simpson 2010, Thomas et al. 2010, Piesk et al. 2013). In combination with these other studies, such results highlight the lack of a clear and direct relationship between these two facets (Elliot and Hart 2010).

Although total PO levels did not vary with damselfly density, it may be that growth is prioritized over, and decoupled from, immune function (Stoks et al. 2006, van der Most et al. 2010). This decoupling makes sense, as growth must be prioritized if organisms are to complete their life cycles (Brodin and Johansson 2004, De Block and Stoks 2004, Stoks et al. 2006), while

parasitism rarely leads to the death of the host and represents a lesser cost. Thus, any effects of food-limitation would primarily affect growth as we and others previously found. Indeed, we found no association between total PO activity and growth rates in the mesocosm experiment, and only a weak positive association in the field experiment, where natural resource levels were sufficiently limiting in at least one lake to reduce total PO. However, it may also be that variation among individuals in resource acquisition and energetic investment toward immune function may obscure the ability to detect associations with particular traits at the population level (Reznick et al. 2000, Kortet et al. 2007, Tye et al. 2020) although this seems unlikely (e.g., Fig. S1). Regardless, the lack of a condition-dependent density effect or interaction between damselfly density and prey density implies that competitors, at least over a 10 times range of densities bookending those observed in natural lakes, do not sufficiently limit resource acquisition such that they reduce total PO. Conversely, it may simply be that resources were never sufficiently limiting, either through density dependent effects or through reduced resource acquisition via selection or trait mediated effects imposed by predators. Rather, innate immunity (in the form of total PO) may result from hosts sequestering a given percentage of their energy acquired from available prey, but that is determined by more local resource levels and apparently not subject to the effects of competition.

The ability to simultaneously defend against predators, thwart off competitors, and generate immune responses are but one example of the conflicting demands species face when living in complex communities (Stearns 1992). Although the consequences of these conflicting demands can be promulgated by trait and density-mediated effects, few studies have considered them simultaneously. By investigating how predators exert direct consumptive, indirect trait-mediated, and combined effects though natural selection on a key behavioral trait that mediates resource acquisition underlying immune function, our results suggest that the tradeoffs arising between resource acquisition and predation do not always constrain the key aspect of immune function considered here. Although we only investigated a single aspect of immune function, and future studies should consider alternative components of immunity, our results suggest that predators

and competitors may not constrain immune function if resources are sufficiently abundant. As a result, the potential cascading and constraining effects of predators on immune function are likely population specific (Kortet et al. 2007). Determining under what ecological conditions predators and competitors do and do not constrain immune function will provide critical insight into how species defend themselves against parasites in complex food webs.

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Figures

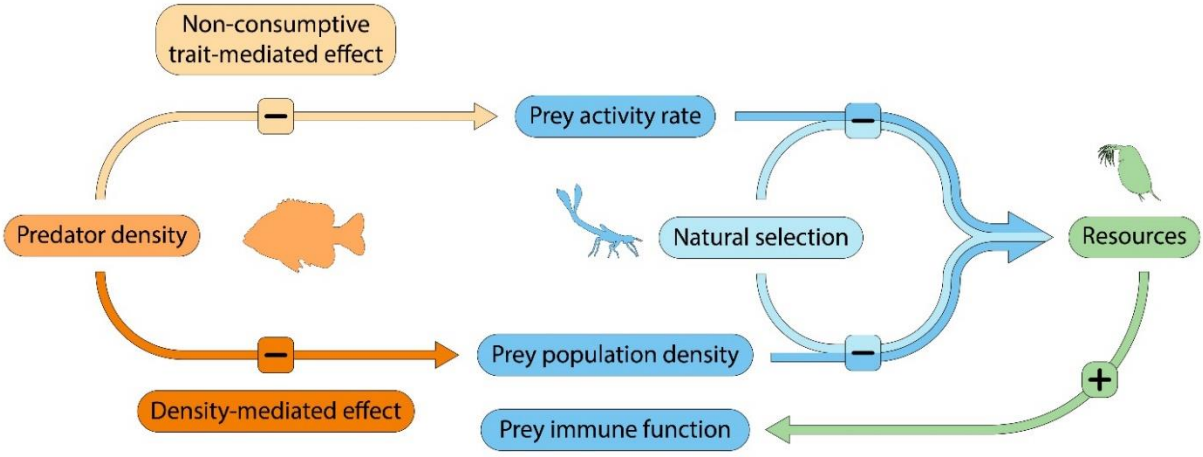

Figure 1 - Conceptual diagram of the hypothesized relationships between predators, prey (host) population density, prey activity rates, resource acquisition rates, and prey immune function. Predators reduce both prey population density (via direct, density-mediated consumptive effects) and prey activity rates (via indirect, non-consumptive trait-mediated effects). Natural selection lowers both the average activity rate and density of a given prey population, as predators disproportionately consume the more active individuals. Predator-mediated selection decreases competition for resources, increasing the per capita resource acquisition rate. Increases in resource acquisition rate then drive increases in prey immune function.

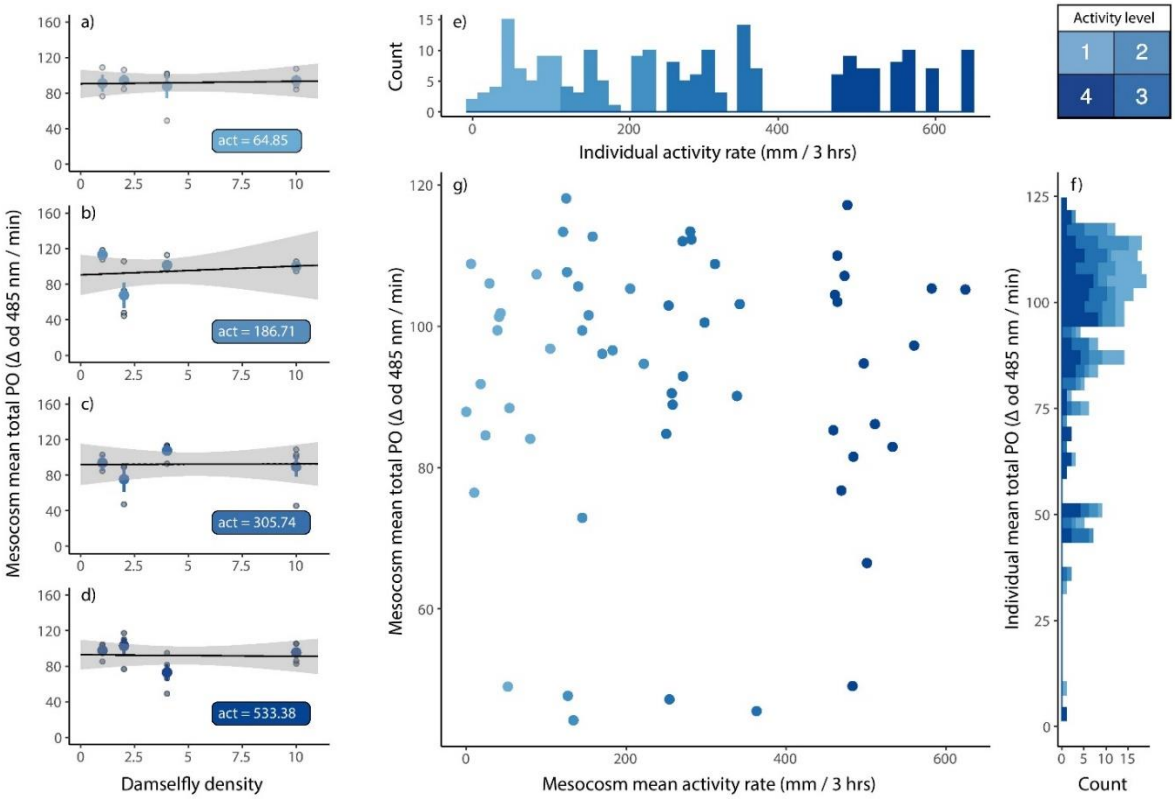

Figure 2 – There was no effect of damselfly density or activity level on damselfly total PO activity. Shown are the linear regressions (grey bands denote 95% CI) between mean total PO activity and larval density for each of the four activity levels, ordered from least active to most active (a-d). Darker points denote density treatment means and one s.e., lighter points denote mean total PO activity from individual mesocosms. Inset values denote average activity rate (mm/3 hrs.) for each activity level. Also shown are histograms of (e) individual damselfly activity rates and (f) individual total PO activity levels from the mesocosms. Points in (g) represent mesocosm mean total PO activity in relation to mesocosm mean activity level (r = 0.0006, df = 56, p = 0.99). Colors represent the activity level treatments.

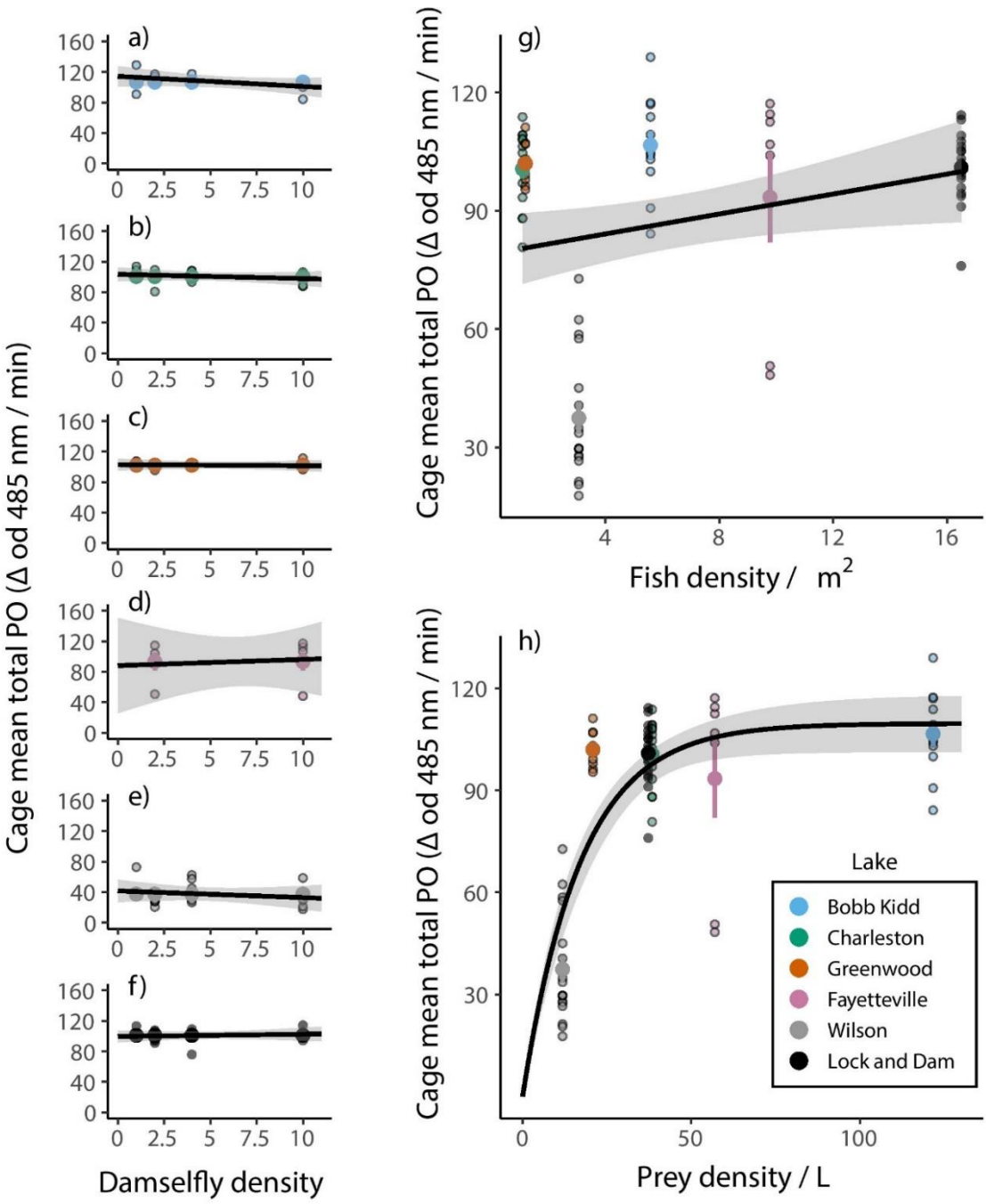

Figure 3 – There was no effect of damselfly density or fish density on damselfly total PO activity, but immune function increased with prey density among lakes. Panels in (a-f) are linear regressions of damselfly mean total PO activity on damselfly density from each of the six lakes. (g) Shows the linear regression of cage mean total PO activity on fish density, and (h) shows the asymptotic regression of cage mean total PO activity on prey density (*a*, estimated upper limit = 109.70, s.e. = 4.19, $t = 26.16$, $p < 0.001$; *c*, proportional change = 0.058, s.e. = 0.007, $t = 8.27$, $p < 0.001$, $r^2 = 61.89$). Grey bands in all panels denote 95% CI, darker points in (a-f) denote density treatment means and one s.e., while darker points in (g) and (f) denote lake means and one s.e. Lighter points in all plots denote mean total PO activity values from individual cages.

Tables

Table 1 – Results from the general linear model of damselfly immune function (total PO activity) in relation to damselfly cage density, fish density, and prey density among lakes.

Term	Estimate	s.e.	*t*	*p* value
Intercept	-34.72	29.44	-1.18	0.24
Prey density	0.35	0.15	2.40	0.02
Fish density	0.32	0.80	0.40	0.69
Cage density	0.24	1.41	0.17	0.86
Prey density x cage density	-0.02	0.02	-0.69	0.49
Fish density x cage density	0.09	0.13	0.66	0.51

Note: The overall model is significant ($F_{5,71}$ = 7.5, p = 3.03e-06) with an r^2 = 38.79.

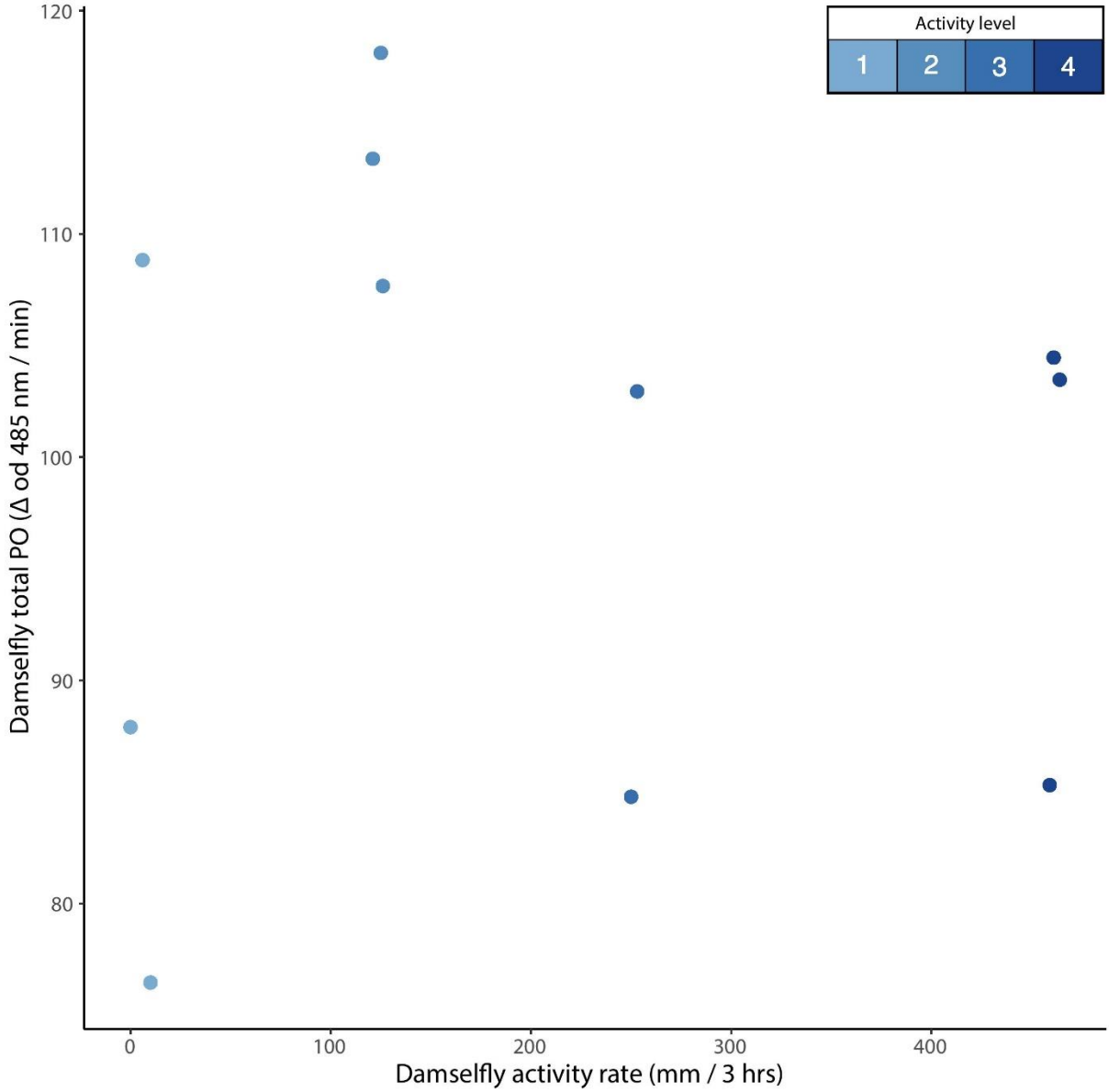

Figure S1 – Damselfly total PO activity from the single density treatments in the mesocosm experiment did not correlate with activity levels (r = -0.004, df = 9, p = 0.99). Points represent individual total PO activity values in relation to activity level. Colors represent the activity level treatments.

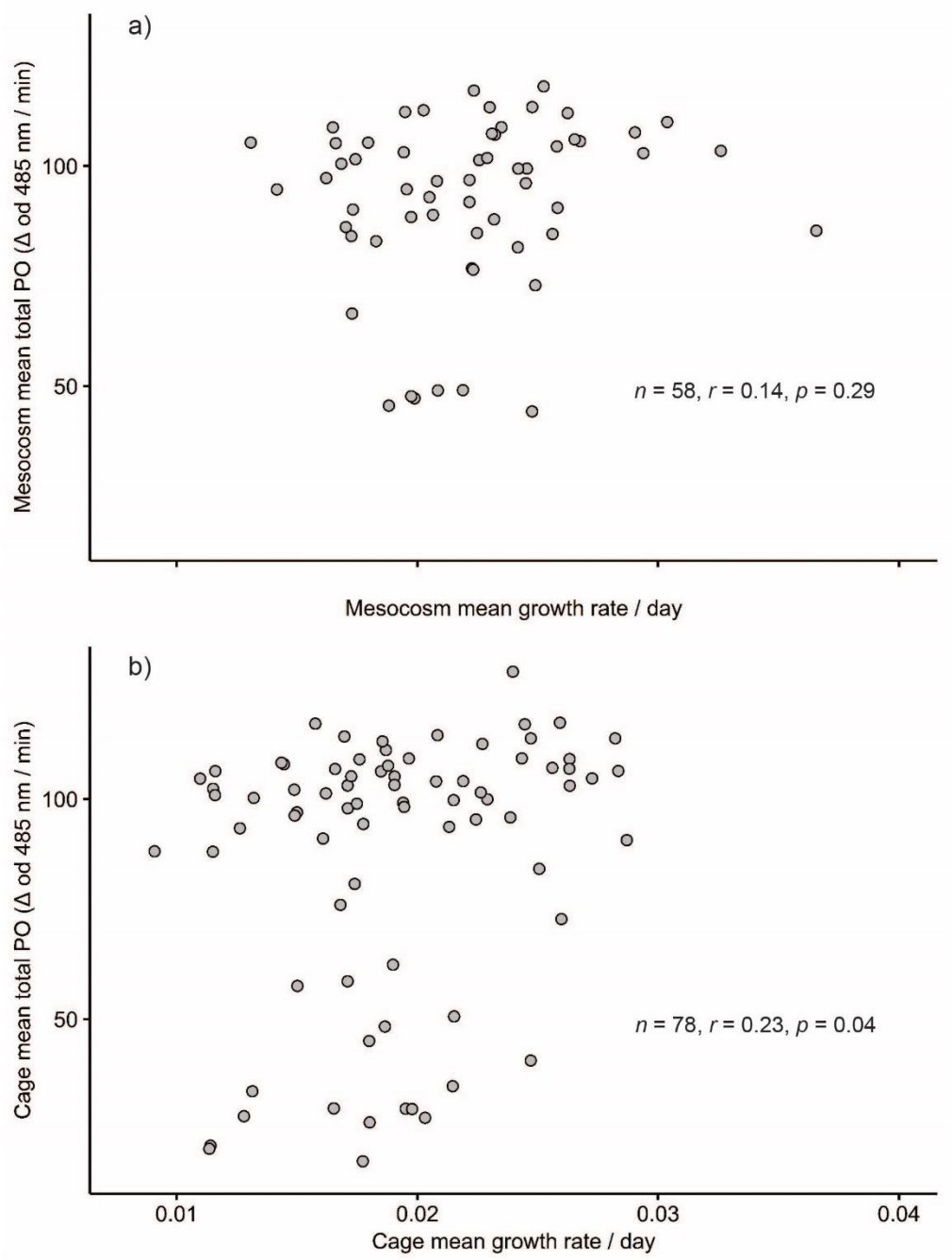

Figure S2 – Damselfly mean total PO activity was weakly and positively associated with mean growth rates in both the mesocosm and field experiment. Points in (a) and (b) represent mesocosm and cage mean total PO activity in relation to mesocosm and cage mean growth rates in the mesocosm and field experiments, respectively.

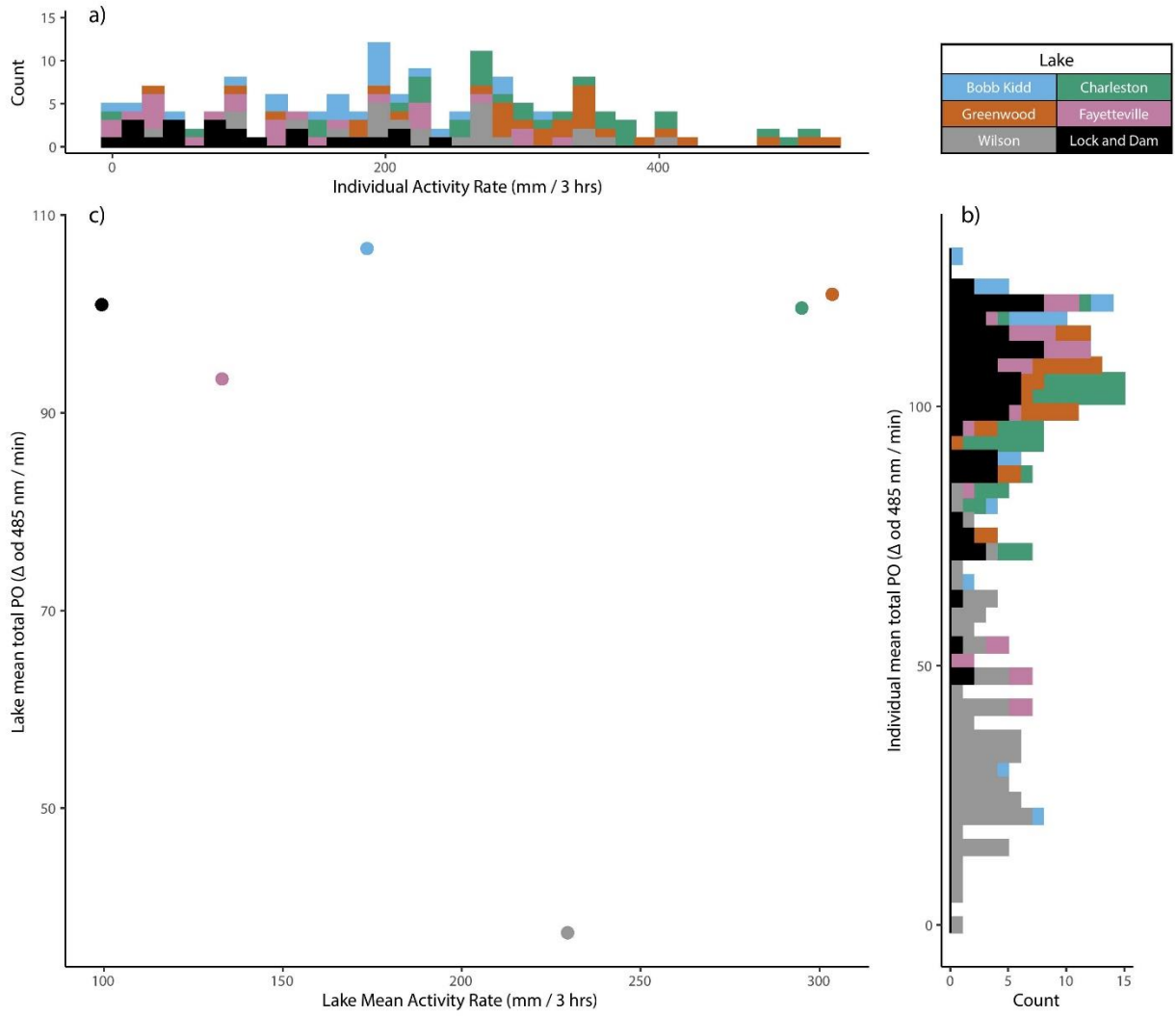

Figure S3 – Damselfly mean total PO activity from the field experiment did not correlate with mean activity levels from each lake (r = -0.20, df = 4, p = 0.85). Shown are histograms of (a) individual activity rates and (b) individual total PO activity levels from the six target lakes. Points in (c) represent lake mean total PO activity in relation to lake mean activity level. Colors represent the lake.

Chapter 3

Home is where the host is: the local environment enhances species-specific parasitism

Adam Z. Hasik & Adam M. Siepielski

Abstract

Although closely related host species frequently co-occur in local communities, they often exhibit marked differences in the prevalence and intensity of parasitism from shared parasites. Such differences in parasitism are likely shaped by the combined effects of spatial variation in abiotic conditions and species interactions. We studied damselfly (*Enallagma* spp.) hosts and their water mite (*Arrenurus* spp.) ectoparasites to determine abiotic and biotic drivers of among species and population variation in parasitism. Specifically, we tested the effects of conspecific and heterospecific density, resource abundance, and potential local adaptation on parasitism using field experiments. We found that parasitism did not vary with conspecific or heterospecific density and was determined by host identity alone, with no evidence for spillover effects. The results of a reciprocal transplant experiment revealed strong asymmetries in the effects of host populations. Parasitism declined sharply for one host population in its non-local lake, but not for the other source population, with no effects of resource abundance. These results imply two possibilities: either damselflies developed enhanced defenses against parasite attack via local adaptation or plasticity, or mites similarly developed heightened local host specificity. The results of structural equation and multivariate models from a detailed observational study supported these experimental findings: neither host density nor resource availability strongly explained among population variation in parasitism. Instead, local abiotic conditions (pH) had the strongest relationship with parasitism, with minimal associations with predator density and a measure of immune function. Our findings reveal how host and environmental factors interact to shape the dynamics of parasitism unfolding within complex ecological communities.

Introduction

Host-parasite interactions are embedded within diverse ecological communities that often contain multiple host species sharing the same parasites (Lafferty et al. 2006, Ostfeld et al. 2018). Yet, despite being attacked by the same parasites, the proportion of host populations parasitized (prevalence) and the number of parasites per host (intensity) often vary considerably among closely related host species within the same community (Mlynarek et al. 2015), and among populations of the same host (Preisser 2019). Here we examine how these two sources of variation combine to explain infection dynamics of a multi-host-parasite system.

Although the extent of parasitism is not fixed across the range of a given host species (Poulin 2006), the repeatability of high or low prevalence and intensity among certain species indicates these attributes can be considered an emergent property of individual host species (Krasnov and Poulin 2010). For example, common species with larger populations generally support larger parasite populations (Arneberg et al. 1998), but it may also be that greater host densities increase parasite encounter rates and subsequent parasite prevalence and intensity (Detwiler and Minchella 2009). Additionally, if the most common species is the most heavily parasitized, it could generate spillover dynamics and increase parasitism in other host species (Chapman et al. 2005). Thus, single host species within systems may generate cascading effects on other species sharing parasites (Lootvoet et al. 2013).

Among populations of the same host species, spatial variation in the prevalence and intensity of parasitism can often be attributed to abiotic and biotic conditions within local communities. Abiotic factors such as temperature, pH, and precipitation have been shown to explain spatial variation in prevalence and intensity among host populations (Preisser 2019, LoScerbo et al. 2020). Similarly, interactions between hosts (or parasites) and competitors and predators can influence population-level variation in parasitism. For example, predators can indirectly increase host susceptibility to parasites by reducing immune function (Navarro et al. 2004). Reduced resource acquisition because of intra and interspecific competition among hosts can also affect susceptibility to parasites, with decreased resource availability suppressing host immune function

(Raffel et al. 2008). Additionally, parasitism among populations of the same host species may be further mediated by local adaptation of the host to the parasite (or vice versa, Greischar and Britt 2007). Despite evidence linking parasitism to spatial variation in abiotic factors (LoScerbo et al. 2020), few studies have investigated the joint influence of abiotic and biotic drivers of parasitism among host species and populations.

An appealing system for understanding the combined roles of host and environmental factors in driving parasitism dynamics is that of *Enallagma* damselflies and their water mite (*Arrenurus* spp.) ectoparasites. *Enallagma* damselflies are aquatic predators for the majority of their development, emerging from the water as aerial adults to complete their life cycle when they are attacked by *Arrenurus* mites (Smith et al. 2010), which are generalist parasites on multiple host species (Mlynarek et al. 2013, Worthen and Turner 2015). This system is ideal for testing hypotheses about the role of host and environmental factors in driving parasitism, as water mite prevalence is not only frequently high, but also varies within and among species (Mlynarek et al. 2015, LoScerbo et al. 2020) and across environmental gradients (LoScerbo et al. 2020). Local *Enallagma* diversity is also often high, with most lakes where fish are the top predator having between 5-12 co-occurring species (McPeek 1989, 1990, 1998, Siepielski et al. 2010). Abiotic and biotic environmental factors at both the host species and population level are expected to shape the prevalence and intensity of parasitism in this system. Water mites may selectively parasitize one host species due to greater host population density or underlying attributes of the host species (e.g., weaker immune function). Importantly, both density (McPeek 1990, 1998, Siepielski et al. 2011) and phenoloxidase (PO, a component of damselfly immune function, Mlynarek et al. 2014, 2015) vary among *Enallagma* populations. *Enallagma* defend themselves against mites by melanizing mite feeding tubes via the PO cascade (González-Santoyo and Córdoba-Aguilar 2012), and such immune defenses are predicted to increase in response to elevated risks of parasitic infection (Wilson et al. 2002). Indeed, PO levels positively correlate with melanization and defense against parasites (Butt and Raftos 2008), making PO an appropriate (though limited) representation of overall immune function (Srygley and Jaronski

2011). PO should vary not only with infection risk, but also with resource availability, as immune defenses are energetically costly (González-Santoyo and Córdoba-Aguilar 2012). Thus, damselflies in lakes with low prey availability may be less able to mount an effective immune response, resulting in more intense parasitism. Conversely, parasite populations may vary in their ability to attack their hosts. Thus, the extent of any parasitism should vary among host populations over environmental gradients.

To investigate how parasitism varies among host species and populations across environmental gradients, we first assessed parasite prevalence and intensity of *Enallagma* populations across lakes that varied in abiotic and biotic factors. We observed a striking pattern - one species, *E. signatum*, was overwhelmingly attacked by parasites (Fig. 1). On the basis of this observation, we conducted two experiments and an observational study to ask three questions. First, are parasite prevalence and intensity determined by host species or competitor density? We predicted that parasites would preferentially parasitize the most common host species due to its high population densities, and that any effects of density should be stronger within than between species. Second, is parasitism shaped more by local environmental conditions or host condition (shaped by resource limitation) that influences immune defense? Because immune function is resource-limited (Forbes et al. 2016), we predicted that hosts in environments with low prey availability will have a reduced immune function (in the form of PO) and be less able to resist parasites, resulting in increased parasite prevalence and intensity. Alternatively, if other facets of the local environment influence parasitism then parasitism should increase or decrease in the non-local environment. Third, we used structural equation modelling and multivariate statistical approaches to ask what environmental features influence prevalence and intensity of parasitism among host populations? By addressing these questions, our work establishes a link between species and population level processes shaping disease dynamics in ecological communities.

Materials and Methods

Parasitism surveys

During summer of 2017, we visited seven lakes (Appendix S1: Table S1) to estimate parasite prevalence and intensity. Because species vary in when they are active throughout the day, we sampled each lake at three different time points per sampling round three times a week. Sampling occurred every other week from late May through early August (n = 141 sampling rounds), with each visit totaling six person-hours of sampling. This duration covered the entire flight season when damselflies emerge and are parasitized. We collected adults using aerial sweep-nets and stored them in 70% ethanol. On each sampling day, we stored a subset of non-parasitized damselflies in a -80ºC freezer for PO assays (Mlynarek et al. 2015). Because our methods standardized sampling effort throughout the surveys, we estimated species total densities across the breeding season on encountering rate, calculated as the number of individuals caught per sampling period summed over the breeding season. Encountering rate has been used previously as a measure of density in adult damselflies (Gosden and Svensson 2009).

Mite Counts

We examined damselflies for mites under a dissecting scope (Leica MZ7.5, Leica Microsystems). We also checked for "scars" - melanized spots on the exoskeleton where the mite feeding tube was located, which indicated successful parasitism by a single mite (Smith et al. 2010). We therefore scored infection intensity per individual as the sum of intact mites and mite scars. We scored prevalence of infection by dividing the number of infected hosts of a given species by the total number collected at a lake.

Quantification of total PO activity

To quantify damselfly innate immune function we used a standard measure of PO activity (Mlynarek et al. 2015) and measured the amount of total PO (i.e., PO measured in the absence of an immune challenge) present in the haemolymph. In short, we measured total PO activity as the

slope of the enzymatic reaction curve over a fixed time interval. Higher total PO activity translates to increased ability to resist attack by mites. We used the protein content of each damselfly to control for variation in body size. We measured total PO activity and protein content in duplicate, with means used for analyses (details in Appendix S2)

Focal species approach

We encountered seven *Enallagma* species during our surveys, however, *E. civile, E. geminatum,* and *E. vesperum* were rarely encountered (Appendix S1: Table S1). Therefore, we only included the four most common species for statistical comparisons (Fig. 1). These surveys revealed that *E. signatum* was the most heavily parasitized species, with mean prevalence (logistic regression model: $\chi^2 = 1825.40$, $p < 0.0001$, $df = 3$, pseudo-$R^2 = 68.52$) and intensity (poisson regression model: $\chi^2 = 3565.90$, $p < 0.0001$, $df = 3$, pseudo-$R^2 = 34.61$) significantly higher than the other three species (Fig. 1). Previous sampling of *Enallagma* larval densities (details in Appendix S2) revealed that *E. signatum* was the most abundant species in most lakes (Appendix S1: Fig. S1), yet *E. signatum* was not the most abundant species during adult surveys (Fig. 1). Because *E. signatum* continues activity well into the night, when it was not possible to safely survey, we suspect our adult density estimates are underestimated. Regardless, because *E. signatum* was the most heavily parasitized species, and the extent of parasitism varied among populations (Appendix S1: Table S2), we focused on *E. signatum*. To understand potential drivers of the intense parasitism in *E. signatum*, we designed a series of experiments and utilized an observational study to test relationships between environmental factors and parasitism.

Are parasite prevalence and intensity determined by host species or competitor density?

We designed this first experiment to investigate the effects of intra and interspecific competitor density and host species on parasite prevalence and intensity. Because *E. signatum* naturally occurred at high densities, we wanted to determine if the elevated rates of parasitism we observed were driven by density-dependent responses to competitor density or were instead a

species-specific effect. We also wanted to determine if any density-responses were stronger comparing intra to interspecific densities, as it could be that high densities of one species increases the risk of parasitism via pathogen spillover. To do so, we used a response surface design crossing intra and interspecific competitor density and host species (details in Appendix S2). We used *E. signatum* and *E. basidens*, because *E. signatum* was the most heavily parasitized species, while *E. basidens* was rarely parasitized (Fig. 1). *E. signatum* also had the highest larval population densities, whereas *E. basidens* was considerably lower in density among lakes (Appendix S1: Fig. S1). By using naturally low- and high-density species, this experiment allowed us to determine if the elevated parasitism observed for *E. signatum* was a product of its higher population densities, or if it was a species-specific effect, independent of density.

In May 2019 we established cages in the littoral zone of Bob Kidd Lake in northwest Arkansas, USA, as this was right before the start of the flight season for both species (Abbott 2011). The top of the cages extended above the water surface to allow larval damselflies to emerge and transition to their adult stage when parasitism occurs (Smith et al. 2010). Treatments for each species were low density (10 larvae/0.05m^2) and high density (20 larvae/0.05m^2), in addition to a treatment containing a mixture of both species (10 larvae of both *E. signatum* and *E. basidens*, for a total of 20 larvae/0.05m^2). We replicated each treatment five times (n = 25 total cages), and also included an empty cage to check for intrusion by non-target hosts. We collected adult damselflies every other day for a period of 38 days, as this is when emergence ceased.

Is parasitism shaped more by host condition or local environmental conditions?

We designed this second experiment to test for an effect of host condition (mediated by the effects of prey resource abundance on immune function) and source population on parasite prevalence and intensity. Because damselfly condition is resource-limited, with immune function and growth rate increasing with food supply (Campero et al. 2008), we wanted to determine if the differing rates of parasitism we observed were due to natural differences in host condition driven by prey availability among lakes. To do so, we used a reciprocal transplant experiment

crossing host source population and prey supplementation. Among populations, parasites may also differ in their ability to attack hosts, or hosts may differ in their defenses against parasites, via local adaptation or plasticity. By moving individuals between lakes with contrasting levels of parasitism and naturally low and high resources and crossing this with food supplementation, this design allowed us to understand how local conditions (as captured by the transplant) or resource levels (as captured by manipulating resources) shaped parasitism.

In May 2020 we established cages in the littoral zones of two lakes (Fayetteville and Wilson) in northwest Arkansas, USA differing in both prey availability (Ousterhout et al. 2019) and parasite prevalence and intensity (Appendix S1: Fig. S2). Lake Fayetteville had a naturally low level of parasitism (Appendix S1: Fig. S2) and high prey density (~57 prey items/L), whereas Lake Wilson had a naturally high level of parasitism (Appendix S1: Fig. S2) and low prey density (~11 prey items/L). We constructed and established cages in each lake in the same manner noted in the above experiment. We established treatments in each experimental cage by crossing damselfly source population with prey supplementation in a fully factorial design. To test for an effect of host source population, we stocked cages in each experimental lake with either 10 local or non-local individuals (equivalent to 200 larvae/m^2). To test for an effect of food supplementation on host condition via its possible effects on aspects of immune function like total PO, we supplemented half of the cages in each experimental lake with equivalent amounts of prey (full details in Appendix S2). We replicated each of the four treatment combinations seven times in each experimental lake (n = 28 total cages per experimental lake), and again included an empty cage to check for non-target larvae. We collected adult damselflies every other day for 26 days, as this is when emergences stopped.

What environmental features influence prevalence and intensity of parasitism?

We examined the relationships between parasite prevalence and intensity and host immune function (total PO activity) and four environmental factors (fish density, larval density, prey density, and pH). Full details of environmental sampling have been previously published

(Ousterhout et al. 2019), thus we only briefly summarize them here. Fish density was measured as the mean of three standardized seine hauls through the macrophyte bed of each lake; larval density was measured as the mean of two separate sampling events, each of which used 10 standardized dip net sweeps; prey density was measured as the mean of six replicate samples with a 6L box sampler, and pH was measured with a YSI probe (YSI ProPlus, YSI Inc.).

We focused on this set of environmental factors because we had a priori predictions based on our general understanding of how these factors could shape parasitism (see *Introduction*). We expected fish density to affect parasitism dynamics, as they are intra-guild predators of *Enallagma* damselflies and compete for the same resources. Increases in fish density could result in lower prey availability for damselflies and limit their resource-limited immune defense (González-Santoyo and Córdoba-Aguilar 2012). Thus, parasite prevalence and intensity should increase with fish predator density. We predicted that parasitism would increase as a function of host density, because larger host populations support larger parasite populations (Arneberg et al. 1998). Because investment into immune function requires host organisms to acquire energy (González-Santoyo and Córdoba-Aguilar 2012), we predicted that parasitism should increase as resources become limited. We expected parasitism to increase as a function of pH, as water mite parasitism rates are known to increase with pH (LoScerbo et al. 2020). We also predicted that parasitism should increase as immune function (total PO activity) decreases, as hosts are less able to defend themselves.

Statistical analysis

Parasitism as a species-specific or density-dependent effect

To evaluate the effects of host species and intra and inter-specific density on prevalence, we constructed a logistic regression of the form: proportion infected as the response variable with host species, density treatment, and their interaction as predictors. For parasite intensity, we constructed a linear model of the form: ln(mite number) as the response variable with host species, density treatment, and their interaction as predictors. We used cage mean prevalence and

intensity values in all analyses, which is the appropriate experimental unit. We conducted all analyses in R v4.0.2 (R Core Team, 2020).

Parasitism as a function of host condition or local environment

To evaluate if parasite prevalence was due to resource-limited differences in immune function or because of local lake conditions we used a logistic regression of the form: proportion infected as the response variable with experimental lake, source population, prey supplementation, and all two- and three-way interactions as predictors. To evaluate these same effects on parasite intensity, we used the same model structure as above, but used a linear model with ln(mite number) as the response variable. We used cage mean prevalence and intensity values in all statistical analyses, which is the appropriate experimental unit.

All damselflies that emerged in Lake Wilson were parasitized, resulting in perfect separation of the data (i.e., an independent variable perfectly predicts the dependent variable, Albert and Anderson 1984), meaning the standard error of estimated model parameters may have been inflated. To correct for this and evaluate potential influences on our estimates of effects on parasite prevalence, we used the *glmnet* package (Friedman et al. 2010) to conduct a lasso regression (Tibshirani 1996) using the same prevalence model outlined above. There is no consensus for obtaining standard error estimates of lasso estimates (Kyung et al. 2010), instead the lasso regression returns non-zero coefficients for predictors that are likely to influence the outcome of the model, while coefficients of all other predictors are shrunk to zero. As such, we also report the lasso regression-estimated predicted probability of infection for all non-zero predictors.

Parasitism across environmental gradients

To investigate the relative influence of host immune function (total PO activity) and environmental factors (predator density, competitor density, prey availability, and pH) on both infection prevalence and intensity, we constructed several candidate structural equation models

(SEMs, Shipley 2009, full model details in Appendix S3). Each SEM tested a specific hypothesis about the relationships between the direct and indirect effects of the host and/or environment on parasitism. We used the comparative fit index and root mean square error of approximation to select the final model (see Appendix S3).

To further investigate the influence of host and environmental factors on parasite prevalence and intensity we also constructed logistic and linear regression models, respectively. To understand which variables predicted parasite prevalence we constructed a logistic regression of the form: proportion infected as the response variable with larval density, prey density, pH, mean total PO, and mean protein as predictors. To examine intensity we used the same model structure as above, but due to data overdispersion used a negative binomial poisson regression with ln(mite number) as the response variable. We did not include fish density as a predictor in either model to avoid collinearity, as it was highly correlated with pH (r = -0.72).

Results

Are parasite prevalence and intensity determined by host species or competitor density?

The overall models on prevalence (χ^2 = 261.93, df = 3, p < 0.0001, pseudo-R^2 = 86.03) and intensity ($F_{3,26}$ = 213, p < 0.0001, R^2 = 95.64) were both significant and explained considerable variation in these measures of parasitism. For both analyses, we did not find a significant interaction between host species and density (p > 0.40 in both cases), therefore we present results from the models of main effects only. Most damselflies that emerged from the cages were parasitized, but this depended on host species (p < 0.0001), as only two *E. basidens* were parasitized, with *E. signatum* prevalence > 0.90 across density treatments (Fig. 2a). Prevalence did not differ with either con- or heterospecific density (p = 0.94, Fig. 2a).

Parasitism intensity was on average ~ 300 times greater in *E. signatum* than in *E. basidens* (*E. signatum* mean intensity = 9.20 +/- 0.74 [SE] mites per damselfly, *E. basidens* mean intensity = 0.03 +/- 0.02 [SE] mites per damselfly, p < 0.0001, Fig. 2b), though again this was solely a species-specific effect with no differences among con- or heterospecific density treatments (p =

0.46, Fig. 2b). The results for prevalence and intensity were consistent when we included non-target damselflies that contaminated the cages (Appendix S1: Table S3, Fig. S3).

Is parasitism shaped more by host condition or local environmental conditions?

The overall model on prevalence was significant (χ^2 = 44.48, *df* = 3, $p < 0.0001$, pseudo-R^2 = 43.92). There were no significant interactions between any of the predictors ($p > 0.54$ in all cases), therefore we report the results from models of main effects only. All damselflies that emerged from Lake Wilson were parasitized, yet infection prevalence was on average ~10% lower in Lake Fayetteville than Wilson (effect of experimental lake: $p < 0.0001$, Fig. 3a). We also found a significant effect of host source population ($p = 0.001$), as the non-local damselflies had a parasite prevalence ~5% lower than local damselflies (Tukey post-hoc test, $p = 0.002$). There was no effect of prey supplementation on prevalence ($p = 0.07$).

All main effects from the lasso model were influential, but all interaction terms were shrunk to zero. The predicted probabilities of infection for all treatments at Lake Wilson were ~1, while they ranged from 0.81 - 0.97 in Lake Fayetteville. These predicted probabilities of infection from the lasso regression were almost identical to the observed prevalence value point estimates (Fig. 3a), thus the results of the logistic regression were robust to the perfect separation of the data. For intensity, there was no significant interaction between host source and prey supplementation ($p = 0.58$), nor a significant three-way interaction between experimental lake, host source, and prey supplementation ($p = 0.57$). However, the interaction between experimental lake and source population was significant ($p = 0.0002$), therefore we conducted analyses by experimental lake. The overall model for Lake Fayetteville was significant ($F_{3,24} = 10.05$, $p = 0.0002$, $R^2 = 50.14$), but the overall model for Lake Wilson was not ($F_{3,24} = 2.02$, $p = 0.14$, $R^2 = 10.21$). In Lake Fayetteville, local damselflies had approximately three times more parasites than non-local damselflies (local damselfly mean intensity = 28 +/- 0.97 [SE] mites per damselfly, non-local damselfly mean intensity = 10.5 +/- 1.50 [SE] mites per damselfly, Tukey post-hoc test, $p < 0.0001$, Fig. 3b). However, there were no effects of prey supplementation, nor was there an

interaction between host source and prey supplementation (both $p > 0.20$). In Lake Wilson, we found no effect of source population, prey supplementation, nor a significant interaction between host source and supplementation (all $p > 0.13$, Fig. 3b), indicating parasitism levels were similar for all damselflies, regardless of source population or prey supplementation.

What environmental features influence prevalence and intensity of parasitism?

Among SEMs, model 4 (parasitism = direct host + direct environment) had the most support, indicating that both prevalence and intensity were best predicted by the combined direct effects of host and environmental factors. Prevalence decreased with fish density, yet slightly increased with prey density, mean PO, and mean protein (all $p < 0.04$, magnitude of all effect sizes < 0.30, Fig. 4a). Infection intensity slightly increased with pH, prey density, and mean protein (all $p < 0.05$, magnitude of all effect sizes < 0.26, Fig. 4b).

The logistic regression of parasite prevalence ($\chi^2 = 192.70$, $df = 5$, $p < 0.0001$, pseudo-$R^2 = 83.06$) and negative binomial poisson regression of parasite intensity ($\chi^2 = 342.29$, $df = 5$, $p < 0.0001$, pseudo-$R^2 = 9.19$) were both significant. For prevalence, infection increased with pH and PO ($p < 0.0001$ and $p = 0.0002$, respectively, Appendix S1: Table S4). This relationship was strongest for pH, as prevalence at the upper limit of pH was almost three times higher than the lower limit (Fig. 5c). Infection intensity increased ~four-fold over the range of pH ($p < 0.0001$), with minimal increases with greater prey density ($p = 0.02$) and mean PO ($p < 0.0001$; Fig. 5, Appendix S1: Table S5).

Discussion

Determining the extent to which parasitism varies among species and populations provides an opportunity to identify the factors underlying infection dynamics. Despite multiple species of *Enallagma* co-occurring with one another, we observed a striking pattern whereby one species, *E. signatum*, was overwhelmingly attacked by parasites. On the basis of this observation, we took a focal species approach to examine (i) if parasitism was a species-specific effect or was

instead driven by host density, and (ii) whether parasitism was shaped more by local environmental conditions or host condition via resource limitation. We found no support for the prediction that parasitism was a density-dependent process or was shaped by resource levels affecting host condition, although we did find evidence for local adaptation mediating parasitism. Our results instead showed that parasite prevalence was predicted by the combined effects of fish density, host immune function, and pH. Parasite intensity, however, was predicted by prey density, host immune function, host size, and pH. These results imply that accounting for how host and environmental factors interact is necessary to understand how the dynamics of parasitism unfold within complex ecological communities.

We found no relationship between either prevalence or intensity and host conspecific or heterospecific density among either the rare or common host species. This was surprising, as the risk of infection often increases with host density (Gunton and Poyry 2016). Because we found no evidence for positive density-dependence in prevalence or intensity of infection for *E. signatum*, this implies that the high incidence of parasitism they experienced was not simply due to their high natural densities. Likewise, the low incidence of infection in *E. basidens* was not explained by its low natural densities. These results stand in contrast to observational studies in odonates that have reported increases in parasitism with increases in adult abundance (Worthen and Turner 2015). Additionally, increases in *E. signatum* density did not increase *E. basidens* parasitism, and vice-versa, highlighting the degree of species-specificity and lack of any spill-over effects often seen in other systems (Chapman et al. 2005, Lootvoet et al. 2013).
That mites appear to target *E. signatum* was also surprising. Previous studies of *Enallagma* have found that multiple species co-occurring with *E. signatum* have varied but comparable levels of parasitism (Mlynarek et al. 2015, Worthen and Turner 2015). Additionally, *Arrenurus* are thought to be generalists, with single mite species parasitizing several host species and multiple mite species parasitizing a single host species (Mlynarek et al. 2013). One obvious explanation is that mites in our study lakes are more host-specific than in other locations. Why remains a mystery. Notably, most previous work in this system has all been at higher latitude lakes

(Mlynarek et al. 2013, Mlynarek et al. 2015) and this may provide a clue. For example, Krasnov et al. (2008) showed that fleas at higher latitudes had a broader range of host species than those at lower latitudes, a pattern attributed to the increase in niche breadth (degree of host species specificity) with latitude.

Our reciprocal transplant experiment revealed strong asymmetries in the effects of host population, as parasitism declined for one host population in its non-local lake, but not for the other source population. Specifically, we found that individuals transplanted from Lake Wilson to Lake Fayetteville were less parasitized than local damselflies, but the converse was not found. We suggest two possible explanations for this pattern. On the one hand, damselflies may have developed enhanced defenses against parasite attack via local adaptation or plasticity. If so, the asymmetry we detected makes sense, because damselflies from Lake Wilson naturally experienced high levels of parasitism and so should develop enhanced defenses that would confer an advantage in Lake Fayetteville where parasitism was generally lower. Consistent with this idea, total PO and parasite prevalence were positively correlated, reflecting a possible adaptive or plastic response to more intense parasitism. Alternatively, or in tandem, it may be that mites have developed enhanced local host specificity via local adaptation/plasticity. That is, mites from Lake Fayetteville may simply not recognize non-local damselfly hosts. In some ways, this potential population-level host specificity then mirrors what we observed among different species of damselflies. Though we did not transplant mites in our experiment, the asymmetry we detected also supports previous experimental evidence that parasite local adaptation is relatively uncommon (Greischar and Britt 2007). Regardless of the precise nature of this relationship, our results show evidence of local adaptation/plasticity in host-parasite interactions. In combination with our experiment manipulating density, these results also suggest that local adaptation/plasticity have stronger effects than density in explaining parasitism dynamics.

We expected that resource-limited hosts would have reduced immune function (e.g., Campero et al. 2008) and thus elevated parasitism. However, in contrast to this idea we found that both parasite prevalence and intensity increased slightly with prey density. This was evident

in both our observational study and transplant experiment. One possibility is that higher prey densities improve mite condition, as *Arrenurus* mites are predatory adults after leaving their damselfly hosts and so they compete for the same prey base (Smith et al. 2010). High prey densities could therefore improve the ability of these intraguild parasites to attack damselflies. Although we found no evidence for resource-limited total PO affecting parasite prevalence, other aspects of damselfly immune function (e.g., nitric oxide, haemocyte counts, proPO, Siva-Jothy et al. 2005) could be responding to reduced resource levels.

We found a positive association between pH and both parasite prevalence and intensity. These results support previous findings relating pH to the intensity of water mite parasitism (LoScerbo et al. 2020), and compliment them by adding analyses of prevalence. Though we found that fish density, total PO, host size, prey density, and pH were all significant predictors of parasitism, the magnitude of the effect of pH on both prevalence and intensity indicate that the local abiotic environment may be the most important mediator of infection. The local abiotic environment is known to alter host-parasite interactions in myriad host-parasite systems (Wolinska and King 2009). For example, Laine (2004) found that powdery mildew (*Podosphaera plantaginis*) caused more damage to their ribwort plantain hosts (*Plantago lanceolata*) during periods of drought, and parasite prevalence in this system increases as the abiotic environment becomes more favorable for powdery mildew (Penczykowski et al. 2014). The parasites in our study may have similarly benefitted from increases in pH, as mite survival and fecundity are both reduced as water becomes more acidic (Edwards 2004). Conversely, damselfly hosts may be in worse condition in high-pH environments. Experimental studies are necessary to disentangle the nature of the relationship between abiotic factors and mite parasitism.

By considering the effects of the local environment on host-parasite interactions, our results support previous evidence that the occurrence and intensity of parasitism is shaped by both the local environment (Wolinska and King 2009) and likely by local adaptation (Greischar and Britt 2007, Johnson et al. 2020). Indeed, despite striking evidence for species-specificity (this study,

Krasnov and Poulin 2010), our results revealed that such elevated parasitism is not because of the effects of host condition or population density, but instead may be driven more by the effects of local adaptation/plasticity in addition to variation in the abiotic environment. The combined results from this study therefore stress the need to consider alternative hypotheses focusing on the role of the local environment in shaping parasitism among host species and populations. Further research at the intersection of community ecology and disease ecology will thus be critical to understanding host-parasite dynamics within the complex network of species interactions that make up food webs.

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Figures

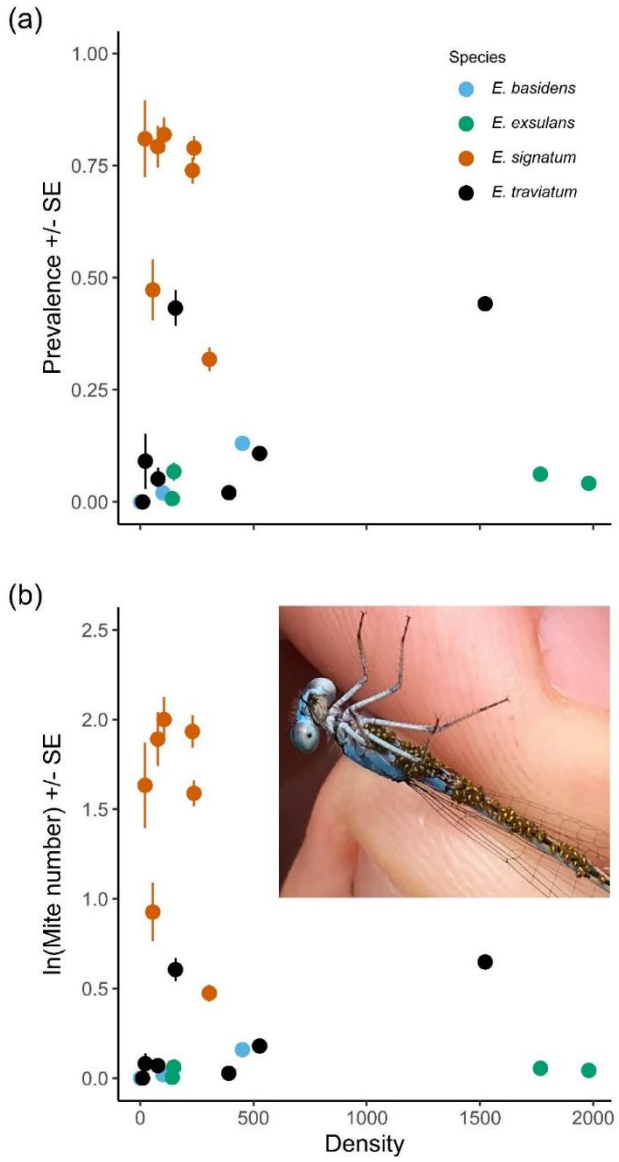

Figure 1 – Mean parasite prevalence and intensity of infection by mites among *Enallagma* species and populations across seven lakes varying in density. Shown are plots of mean prevalence (a) and mean ln(mite number) (b) for *Enallagma* species from the parasitism surveys in relation to total adult density. Error bars denote 1 SE. Inset photograph shows *Arrenurus* mites on an *Enallagma* host.

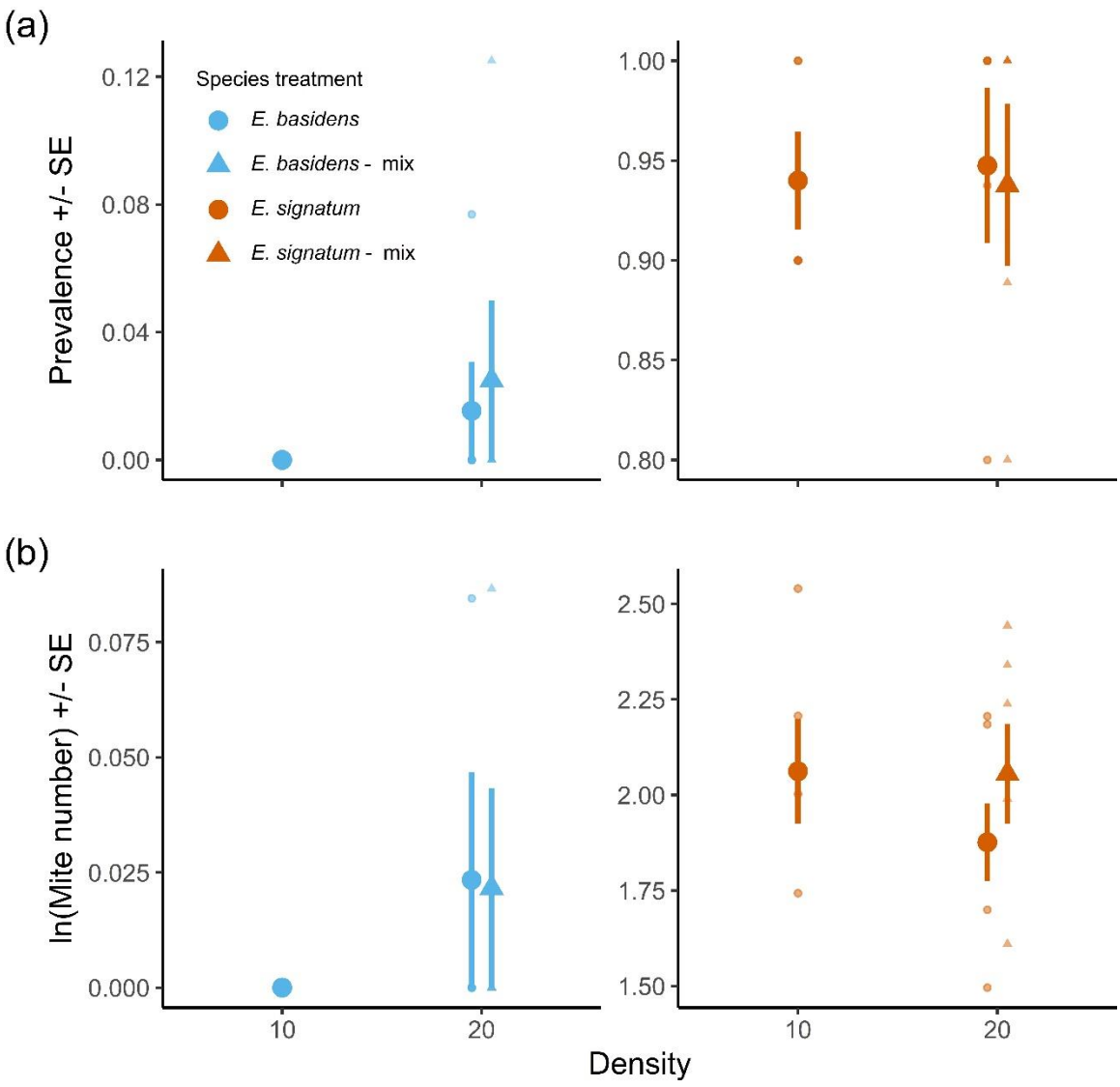

Figure 2 - Results from the experiment manipulating host species, conspecific densities, and heterospecific densities in Bob Kidd Lake. Shown are plots of mean prevalence (a) and intensity (b) of infection by mites, with separate subplots for each species. Note the considerable difference in scale on the y axis between species. Large points in (a) represent the mean prevalence of infection, error bars denote 1 SE, while small points represent the mean prevalence of each replicate cage (n = 5 cages/treatment). Large points in (b) represent the mean ln(mite number), error bars denote 1 SE, while small points represent the mean ln(mite number) of each replicate cage.

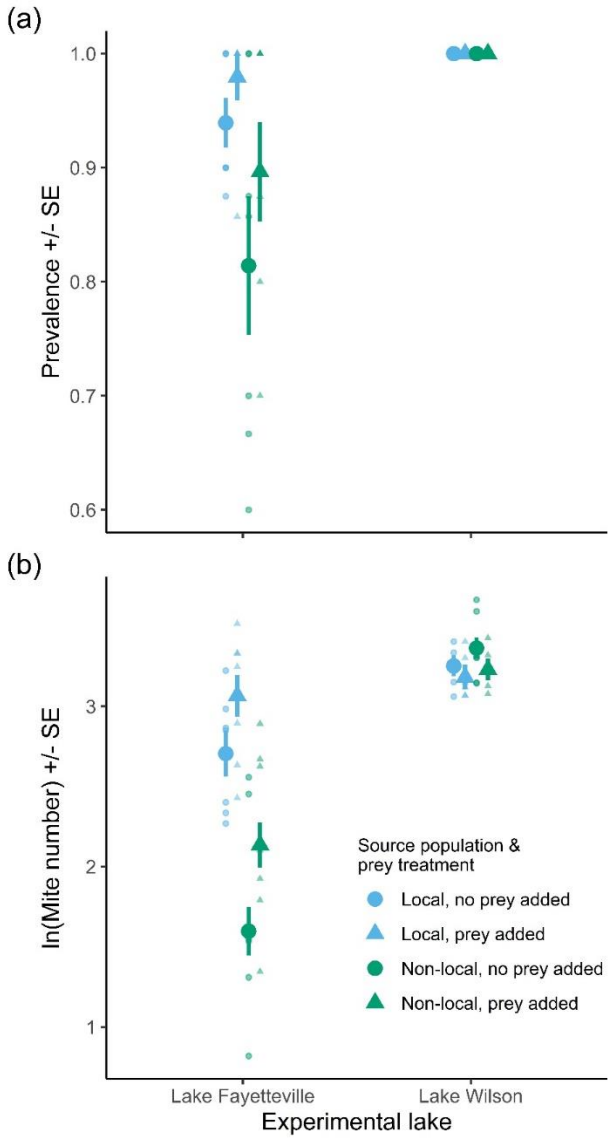

Figure 3 - Results from the reciprocal transplant experiment manipulating damselfly source and experimental lake in relation to food supplementation. Large points in (a) represent the mean prevalence of infection by mites, error bars denote 1 SE, while small points represent the mean prevalence of replicate cages (n = 7 cages/treatment). Large points in (b) represent mean ln(mite number), error bars denote 1 SE, while small points represent mean ln(mite number) of replicate cages.

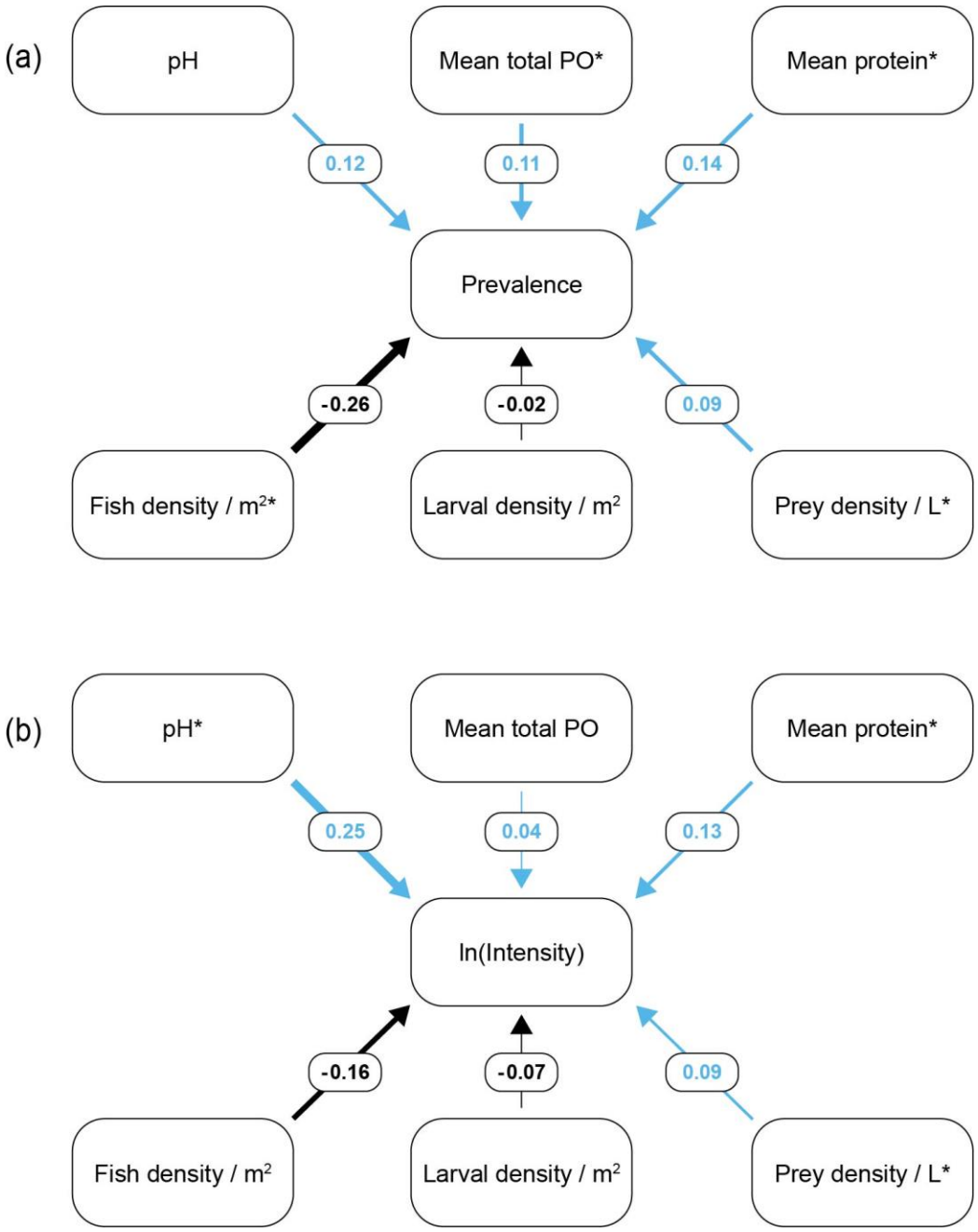

Figure 4 - SEM plots representing the relationships between host- and environment factors and parasite prevalence (a) and parasite intensity (b). Solid lines represent standardized path coefficients of each predictor of mite prevalence and ln(intensity) in (a) and (b), respectively, the values of which can be interpreted as effect sizes. Blue lines denote positive relationships, while black denote negative relationships. Significant predictors ($p < 0.05$) are denoted with (*), and the thickness of the lines represent the strength of the effects (thicker lines represent stronger effects).

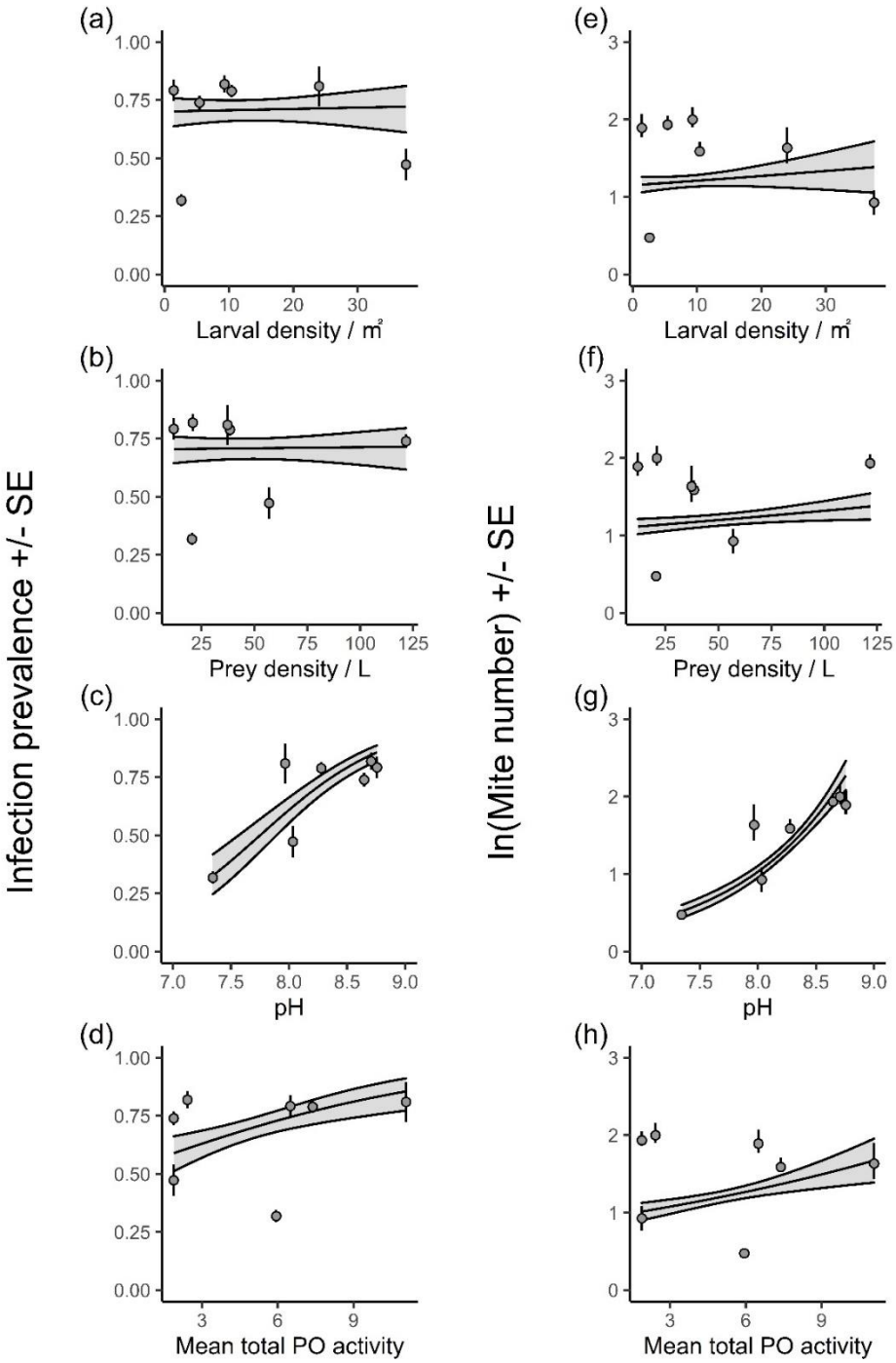

Figure 5 - Relationships between mite prevalence and intensity across environmental gradients. Shown are mite prevalence regressed on larval density (a), prey/L (b), pH (c), and mean total PO activity (d), and mite intensity regressed on larval density (e), prey/L (f), pH (g), and mean total PO activity (h). Lines represent the predicted relationship between the various factors and mite prevalence (a-d) or ln(mite number) (e-h), shaded regions are the 95% CI's, points represent the mean prevalence (a-d) or mean ln(mite number) (e-h) at a given lake, error bars denote 1 SE.

Appendices

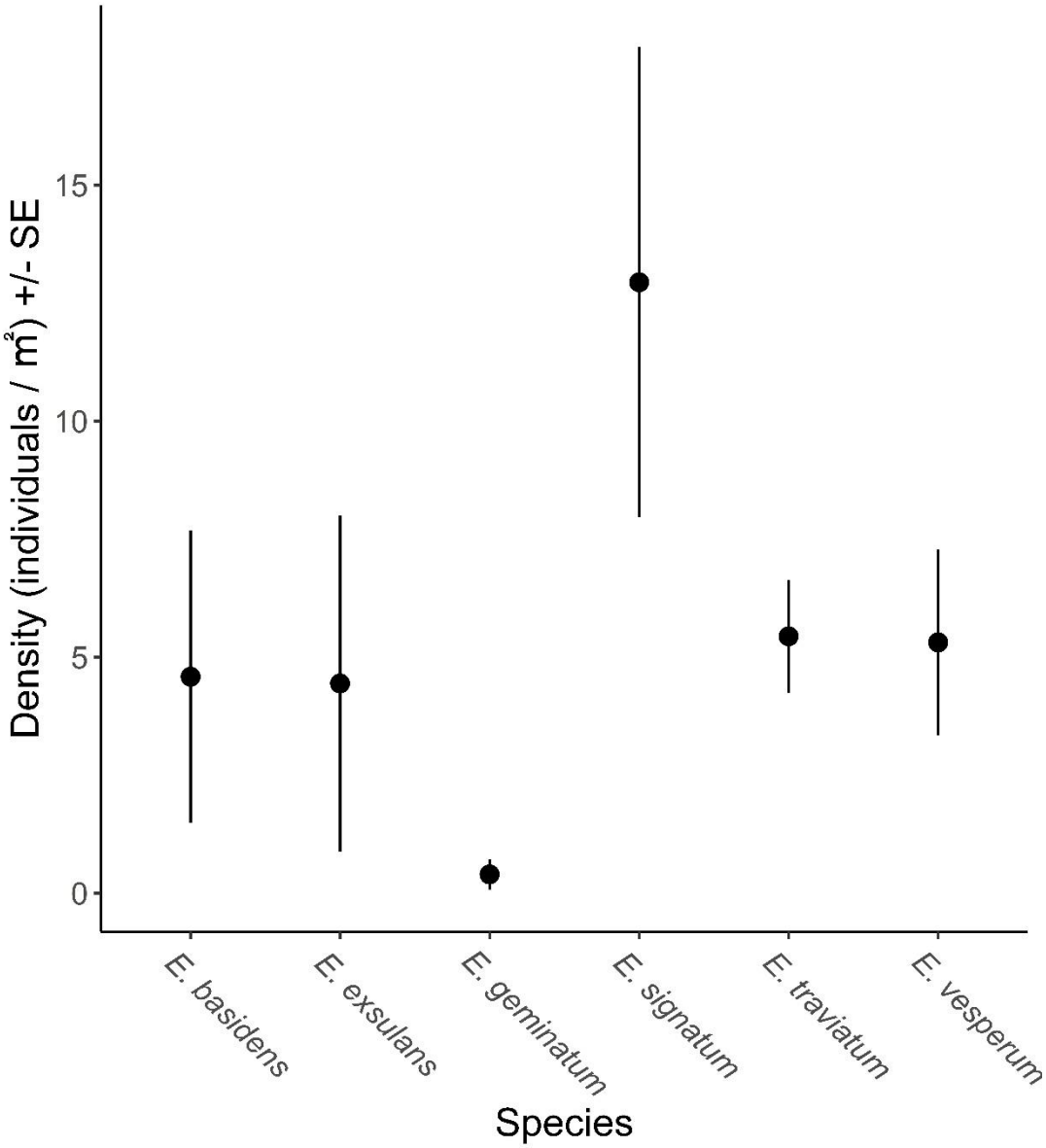

Figure S1 – Estimated larval density among *Enallagma* species across seven lakes. Points represent the mean larval density of each species averaged over lakes, and error bars denote 1 SE.

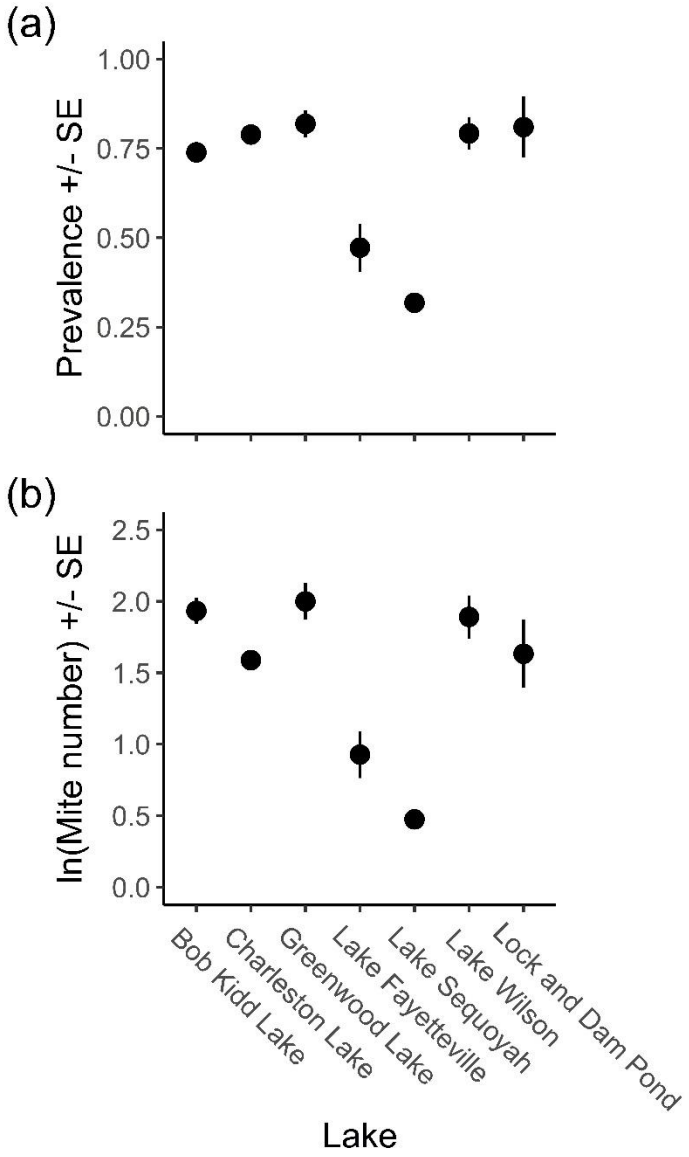

Figure S2 – Mean prevalence (a) and intensity (b) of infection of *E. signatum* by mites across seven lakes. Points in (a) represent the mean prevalence of infection, points in (b) represent the mean ln(mite number), and error bars denote 1 SE.

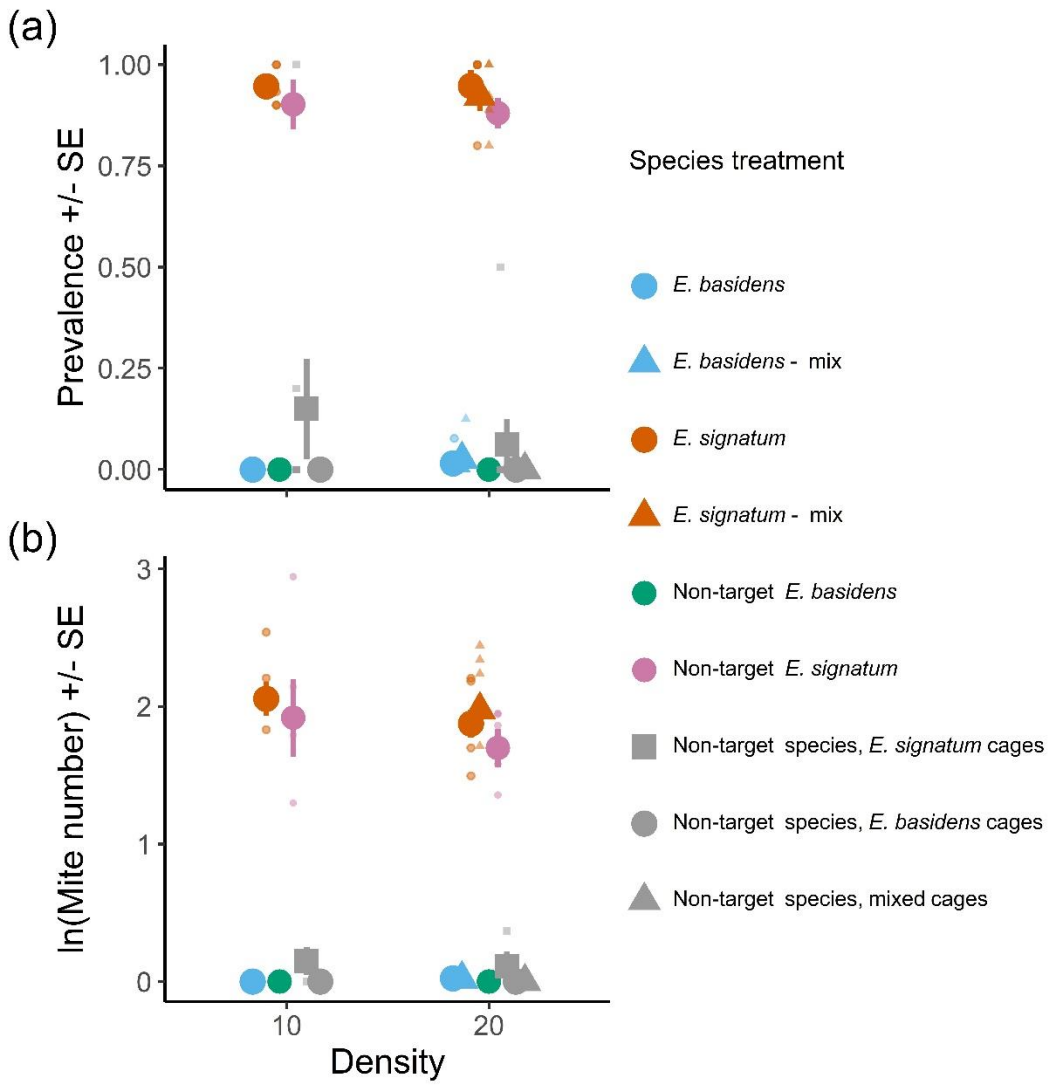

Figure S3 – Results from the experiment manipulating host species, conspecific densities, and heterospecific densities in Bob Kidd Lake when including data from non-target *Enallagma* and *Ischnura* damselflies. Shown are plots of mean prevalence (a) and intensity (b) of infection by mites. Large points in (a) represent the mean prevalence of infection, error bars denote 1 SE, while small points represent the mean prevalence of each replicate cage (n = 5 cages/treatment). Large points in (b) represent the mean ln(mite number), error bars denote 1 SE, while small points represent the mean ln(mite number) of each replicate cage. See also Table S3.

Table S1 – Summary information from the 2017 parasitism surveys for all of the *Enallagma* species at the seven lakes in northwest Arkansas, USA. Sample sizes (*n*) denote the number of adult individuals of a given species captured at each lake.

Lake	Coordinates	Species	*n*
Bob Kidd Lake	35.972245, -94.358390	*E. basidens*	100
		E. exsulans	148
		E. geminatum	4
		E. signatum	230
		E. traviatum	391
Charleston Lake	35.284338, -94.049691	*E. basidens*	1
		E. civile	1
		E. signatum	237
		E. traviatum	1523
Greenwood Lake	36.133058, -94.139463	*E. exsulans*	7
		E. geminatum	1
		E. signatum	105
		E. traviatum	527
Lake Fayetteville	35.186664, -94.242276	*E. basidens*	4
		E. exsulans	1767
		E. geminatum	1
		E. signatum	55
		E. traviatum	10
Lake Sequoyah	36.063459, -94.069764	*E. exsulans*	1981
		E. geminatum	32
		E. signatum	305
		E. traviatum	78

Table S1. (Cont.)

Lake	Coordinates	Species	*n*
Lake Wilson	36.001250, -94.135147	*E. basidens*	451
		E. civile	1
		E. exsulans	141
		E. signatum	77
		E. traviatum	155
		E. vesperum	4
Lock and Dam Pond	35.346022, -94.299038	*E. civile*	2
		E. geminatum	1
		E. signatum	21
		E. traviatum	22

Table S2 – Results from the logistic regression model of parasite prevalence and poisson regression model of parasite intensity across lakes.

Prevalence

Term	χ^2	*df*	*P* value
Species	477.87	3	<2.2e-16
Lake	15.12	3	0.002
Species x Lake	200.85	13	<2.2e-16

Intensity

Term	χ^2	*df*	*P* value
Species	1021.01	3	<2.2e-16
Lake	18.26	3	0.0004
Species x Lake	447.81	13	<2.2e-16

Table S3 – Results from the logistic regression model of parasite prevalence and linear regression model of parasite intensity from the response surface experiment in Bob Kidd Lake when including non-target damselflies. Factors with significant *P*-values ($P < 0.05$) are bolded.

Prevalence

Term	χ^2	*df*	*P* value
Density treatment	0.52	2	0.77
Host species	**348.04**	**2**	**<2e-16**

Intensity

Term	*F*	*df*	*P* value
Intercept	0.01	1	0.58
Density treatment	0.05	2	0.47
Host species	**41.99**	**2**	**<2e-16**

Note: The overall models on prevalence ($\chi^2 = 348.44$, $df = 4$, $p < 2.2e\text{-}16$, pseudo-$R^2 = 87.79$) and intensity ($F_{4,43} = 343.9$, $p < 2.2e\text{-}16$, $R^2 = 96.69$) were both significant.

Table S4 – Results from the logistic regression model of parasite prevalence in relation to larval density, prey density, pH, mean total PO, and mean protein among lakes as depicted in Figure 5 (a-d) of the main text. Factors with significant *P*-values ($P < 0.05$) are bolded.

Term	Estimate	SE	*Z* value	*P* value
Intercept	**-16.92**	**3.98**	**-4.25**	**2.12e-05**
Larval density	0.003	0.009	0.30	0.77
Prey density	0.001	0.003	0.17	0.86
pH	**1.78**	**0.18**	**9.88**	**< 2e-16**
Mean total PO	**0.15**	**0.04**	**3.72**	**0.0002**
Mean protein	2.62	5.39	0.49	0.63

Table S5 – Results from the negative binomial poisson regression model of parasite intensity in relation to larval density, prey density, pH, mean total PO, and mean protein among lakes as depicted in Figure 5 (e-h) of the main text. Factors with significant *P*-values ($P < 0.05$) are bolded.

Term	Estimate	SE	*Z* value	*P* value
Intercept	**-10.02**	**1.30**	**-7.72**	**1.16e-14**
Larval density	-0.005	0.004	1.20	0.23
Prey density	**0.002**	**0.001**	**2.42**	**0.02**
pH	**1.05**	**0.08**	**13.60**	**< 2e-16**
Mean total PO	**0.06**	**0.01**	**3.91**	**9.11e-05**
Mean protein	1.53	1.67	0.92	0.36

Conclusions

Host-parasite interactions are ubiquitous in nature, yet they are also embedded within complex and varied networks of other species interactions composing food webs. Despite attacking organisms at all levels of the food web, effects of parasites on hosts are often thought to be minimal. As such, parasites are subsequently excluded from most experimental designs and theoretical frameworks. In this dissertation, I challenged multiple common assumptions about the dynamics of host-parasite interactions and the role of parasites within complex food webs. Specifically, my results showed that parasites generally have a large and negative effect on the outcome of host species interactions, though the individual effects ranged from beneficial to deleterious. Additionally, my work showed that neither parasitism nor host immune function always increases as a function of host population density. These results were surprising for two reasons. First, host population density is often cited as an important mediator of parasitism dynamics in ecological communities, as larger host populations not only support larger parasite populations, but parasites are also more likely to encounter a host as population density increases. Second, hosts are predicted to increase investment into immune defenses as host population density increases, which is an adaptive response to the elevated risk of parasitism associated with high host population densities. By challenging these common notions, this dissertation has expanded the framework under which we consider parasites and their integral place in the food web.

Chapter 1 involved the construction of the field's most extensive database of the effects of parasites on host species interactions to understand how parasitism mediates other species interactions. I had four primary questions. First, how does parasitism affect the outcome of intra- and interspecific species interactions? Second, within species interactions, do effects of parasites vary between macro- and microparasites? Third, how does parasitism affect fitness components of hosts, and do these effects vary among macro- and microparasites? Finally, do effects of parasites on species interactions vary latitudinally? To address these questions, I used a phylogenetically informed meta-analysis to analyze 658 effect sizes from 178 studies. Overall, I

found that parasites had a large and detrimental overall effect on the outcome of species interactions. Among species interactions, parasites were consistently detrimental to reproductive interactions of their hosts, but effects of parasites on both predation and competition were varied and ranged from beneficial to deleterious. Macro- and microparasites had similar effects on both the species interactions and fitness components of their hosts. Additionally, both accounting for the shared evolutionary histories of hosts and parasites and including viral parasites, which are excluded from the tree of life, did not qualitatively change my results. I also found that parasites had deleterious effects on species interactions both near the equator and at higher latitudes, though these effects had much uncertainty associated with them. My results emphasize the importance of parasites within ecological networks and have implications for future studies of species interactions. Namely, these results imply that any study that fails to account for effects of parasitism on species interactions is likely to over- or underestimate the magnitude of the effects of a focal species interaction such as competition or predation. A second important result is that parasites occasionally "benefit" their hosts. That is, despite suffering the effects of a parasitic attack, host organisms experience a concurrent reduction in predation or competition. My meta-analysis has therefore shown that direct fitness costs of parasites can be overturned when also considering how parasitism indirectly affects the outcome of interactions that a host has with other organisms, which may ultimately determine host fitness. No species exists in an ecological vacuum, as the interactions making up the structure of food webs are complex. Accounting for this complexity and potential for feedbacks to emerge among species interactions is necessary to develop a more complete understanding of how communities are structured.

In Chapter 2 I used a mesocosm and field experiment to investigate if cascading effects of predators constrained a key component of *Enallagma* immune function, as measured by total phenoloxidase (PO). Because the ability to mount an immune response is resource-limited and depends on the ability of the host to acquire sufficient resources, I hypothesized that trait- and density-mediated effects of predators suppressing damselfly resource acquisition would in turn influence total PO. Contrary to my expectations, I found no support in the mesocosm or field

experiment for the prediction that total PO activity would vary due to either non-consumptive trait-mediated effects or selection on damselfly foraging activity underlying resource acquisition. Similarly, although I expected to observe declines in total PO activity with increases in damselfly density, I also found no relationship between density and total PO activity in either experiment. The replicated nature of these results across two independent sets of experiments indicated that total PO did not vary with either damselfly density (a direct effect) or as a function of damselfly activity (a trait-mediated, indirect effect). Despite the lack of trait- and density-mediated effects, my field experiment did show that total PO increased with natural increases in prey density, implying some degree of resource limitation for this aspect of damselfly immune function. The need to simultaneously defend against predators, foil competitors, acquire resources, and generate immune responses are an example of the manifold demands facing species living in complex communities. Though these demands can be exacerbated by the additional influences of trait and density-mediated effects, few studies have considered them simultaneously. My results have shown that, despite the direct consumptive, indirect trait-mediated, and combined effects of predators via natural selection on damselfly hosts, the tradeoffs between resource acquisition and predation do not always constrain a critical component of immune function. This suggests that predators and competitors do not necessarily act to mediate host immune defenses, provided that resources are sufficiently abundant. Further work to determine under what ecological conditions predators and competitors do and do not constrain immune function is necessary to understand how species defend themselves against parasites in complex food webs.

In Chapter 3 I used two field experiments and an observational study to investigate the abiotic and biotic drivers of parasitism. I found that one species, *E. signatum*, was overwhelmingly parasitized despite co-occurring with multiple species of *Enallagma*. Due to this striking pattern of species-specificity, I took a focal species approach to test if parasitism was indeed species-specific, mediated by host density, dependent on resource levels affecting host condition (immune function), or better predicted by local environmental conditions. I found no

evidence that parasite prevalence or intensity were density-dependent or shaped by resource levels. Parasitism in *Enallagma* was instead species-specific and best predicted by the combined effects of host, biotic, and abiotic factors. Specifically, I found that parasite prevalence was predicted by fish density, host immune function, host size, and pH, while parasite intensity was predicted by prey density, host immune function, host size, and pH. Additionally, the reciprocal transplant experiment showed that non-local damselflies had reduced parasitism in one of the experimental lakes. While this effect could have been due to either adaptative evolution or a plastic response of the hosts to the parasites, or the parasites to the hosts, I have provided evidence for a role of the local environment in shaping host-parasite interactions. These results support previous evidence that parasitism is not only shaped by local abiotic and biotic factors, but further mediated by local adaptation and/or plasticity. Accounting for host, abiotic, and biotic factors (and how they interact) is therefore necessary to understand how parasitism varies within complex ecological communities.

The combined results of this dissertation provide a compelling case that parasites are not only a key player in food webs, but by not accounting for them and their effects on their hosts we risk over- or underestimating any effects of a given species interaction. Additionally, interactions within food webs have potential to influence and shape parasitism, both through effects on parasitism or by mediating host immune function. Thus, the results from this dissertation support previous calls for the inclusion of parasites in the experimental designs and theoretical frameworks of ecological studies, providing evidence for a role of parasites and their population and community level effects. Food webs are composed of multifaceted networks of species interactions, with individual organisms connected to numerous others in a complicated and intricate weave. However, by recognizing parasites and accounting for their diverse effects on host organisms and their species interactions, as well as the effects of the local environment on host-parasite relationships, we can further our understanding of how trophic dynamics operate within complex ecological communities.