

ORIGINAL ARTICLE

A role for the local environment in driving species-specific parasitism in a multi-host parasite system

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Abstract

1. The extent and magnitude of parasitism often vary among closely related host species and across populations within species. Determining the ecological basis for this species and population-level variation in parasitism is critical for understanding infection dynamics in multi-host-parasite systems. To investigate such ecological underpinnings of variation in parasitism, we studied *Enallagma* damselfly host species and their water mite (*Arrenurus* spp.) ectoparasites in lakes.
2. We first evaluated how host identity and density could shape parasitism. To test the effects of con- and heterospecific host density on parasitism, we used a field experiment with *Enallagma basidens* and *E. signatum*. We found that parasitism did not vary with con- or heterospecific density and was determined by host identity alone, with no spillover effects.
3. We also evaluated the potential role of local adaptation and resource availability in shaping parasitism. To do so, we used *E. signatum* in a reciprocal transplant experiment crossed with a prey resource-level manipulation. This experiment revealed that parasitism declined sharply for one host population in its non-local lake, but not the other source population, with no effects of prey levels. This asymmetry implies that damselflies express enhanced defences against parasitism that are neither population-specific nor dependent on resource abundance, or that mites developed heightened local host specificity.
4. The results of multivariate modeling from an observational study generally supported these experimental findings: neither host density nor resource abundance strongly explained among-population variation in parasitism. Instead, local abiotic conditions (pH) had the strongest relationship with parasitism, with minimal associations with predator density, temperature and a measure of immune function.
5. Collectively, our findings suggest a crucial role for the local environment in shaping host-parasite interactions within multi-host-parasite systems. More generally, these results show that research at the intersection of community ecology and disease ecology is critical for understanding host-parasite dynamics within natural communities.

KEYWORDS

Arrenurus, density-dependence, *Enallagma*, food web, local adaptation

1 | INTRODUCTION

Multiple host species often experience similar parasite pressures (Lafferty et al., 2006; Ostfeld et al., 2018). Yet, despite being attacked by shared parasites, the prevalence (proportion of the host population parasitised) and intensity (number of parasites per infected host) of parasitism often vary considerably, both among closely related host species within the same community (Gobbin et al., 2020; Mlynarek et al., 2015; Worthen & Turner, 2015) and among populations of the same host (Bolnick et al., 2020; Penczykowski et al., 2014; Preisser, 2019). Why do closely related host species that share similar defensive strategies and co-occur in local communities often exhibit such striking variation in the outcomes of their interactions with parasites? Addressing this question is critical for understanding how multi-host-parasite systems function in ecological networks.

Variation in the prevalence and intensity of parasitism among species could indicate that susceptibility to parasitism may be an intrinsic or extrinsic property of individual host species (Krasnov & Poulin, 2010). For example, some species have weaker immune systems (Céspedes et al., 2019) or have greater tolerance to parasitism (Fan et al., 2013). Conversely, such variation may be an extrinsic property, emerging because of local ecological interactions that shape parasitism. For instance, common, geographically widespread species with higher population densities generally support larger parasite populations (Arneberg et al., 1998). When parasite transmission is density-dependent, high host densities increase parasitism levels (Detwiler & Minchella, 2009). Additionally, if the most common species is the most heavily parasitised, it could generate spillover dynamics and increase parasitism in other closely related host species (Chapman et al., 2005). Thus, density-dependent effects of single host species may generate cascading effects on other species which share the same parasites (Lootvoet et al., 2013).

Spatial variation in parasitism among populations of the same host species can reflect how any intrinsic, but especially extrinsic differences within species are further shaped by the local abiotic and biotic conditions which hosts and parasites experience (e.g., Poulin, 2006). Abiotic factors such as temperature, pH, and precipitation often explain spatial variation in prevalence and intensity among host populations (LoScerbo et al., 2020; Preisser, 2019). Likewise, interactions between hosts and competitors and predators can influence population-level variation in parasitism. For example, predators can indirectly increase host susceptibility to parasites by reducing host immune function (e.g., Navarro et al., 2004). Reduced resource acquisition because of competition among hosts also can affect susceptibility to parasites by suppressing host immune function (Raffel et al., 2008). Despite evidence linking parasitism to spatial variation in abiotic and biotic factors, few studies have investigated the joint influence of abiotic and biotic drivers of parasitism among host species and populations.

Additionally, differences in the extent of parasitism among populations of the same host species may reflect the contribution

of local adaptation or plasticity of hosts to parasites (or vice versa; Greischar & Koskella, 2007). Among populations, parasites often differ in their ability to attack hosts (e.g., Worthen & Turner, 2015) and hosts can differ in their ability to mount defences against parasites (e.g., Céspedes et al., 2019; Mesquita et al., 2017). Such local adaptation may be particularly likely when hosts (or parasites) have limited dispersal (Greischar & Koskella, 2007). The potential for local adaptation also makes assessing the role of local environmental conditions challenging. For instance, defence against parasitism varies not only with local infection risk (Joop & Rolff, 2004), but also with local resource abundance (Campero et al., 2008; Hasik et al., 2021), as immune defences are energetically costly (González-Santoyo & Córdoba-Aguilar, 2012). Thus, high levels of parasitism in an area may occur either because a host is in a low-resource environment where mounting defences is difficult, or because its parasites are particularly adept at attacking their hosts.

In order to investigate how and why parasitism varies among host species and populations, we used *Enallagma* damselflies and their ectoparasites, water mites (*Arrenurus* spp.), as our study system. 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 (Figure 1; Appendix S1). This observation provided an opportunity to adopt a focal species approach, and based on this observation, we asked three questions. Firstly, are parasite prevalence and intensity determined by host species or competitor density? We predicted that parasites would parasitise the most common host species due to its high population densities, and, if these parasites are indeed species-specific, we expect that any effects of density on parasitism should be stronger within than between species. To test this prediction, we conducted a field experiment where we manipulated host intra- and interspecific competitor density.

Secondly, is parasitism shaped more by local environmental conditions or host condition (determined by resource limitation) that influences immune defence? Because immune function in damselflies, as in many other organisms, is resource-limited (Campero et al., 2008; Hasik et al., 2021), we predicted that hosts in environments with low prey abundance will have reduced immune function and be less able to resist parasites, resulting in increased parasitism. Alternatively, if other facets of the local environment influence parasitism, then the extent of parasitism should differ in the non-local environment and not be related to resource abundance. To test this idea, we used a reciprocal transplant field experiment, crossed with a manipulation of prey resources, transplanting damselflies between two lakes that naturally varied in prey abundance.

Thirdly, what environmental features influence prevalence and intensity of parasitism among populations of our focal host species? To address this question, we used a detailed observational study. By evaluating both between- and within-species parasitism dynamics, our study provides an opportunity to examine

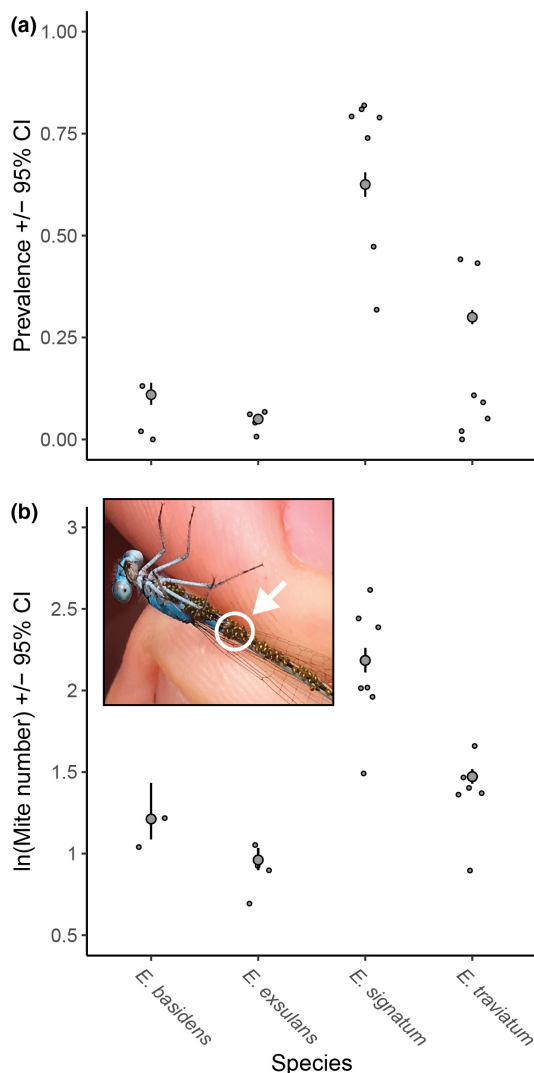


FIGURE 1 Mean parasite prevalence and intensity of infection by mites among *Enallagma* species. Shown are plots of (a) mean prevalence and (b) mean ln(mite number) for *Enallagma* species from the adult parasitism surveys. Large points denote the overall bootstrapped species mean among the surveyed lakes, whereas smaller points denote the bootstrapped mean at individual lakes. Error bars denote bias-corrected and accelerated (BCa) 95% CIs. Inset photograph: *Arrenurus* mites on an *Enallagma* host, with an arrow highlighting a cluster of 17 mites

how these two sources of ecological variation affect a key species interaction.

2 | METHODS

2.1 | Study system

Our studies were conducted in anthropogenic lakes located in the Ozark Highlands and River Valley of Arkansas, U.S.A. (Figure S1). The dominant macrophyte is water willow (*Justicia americana*) and bluegill sunfish (*Lepomis macrochirus*) are the dominant predator of

damselflies (Hasik et al., 2021; Ousterhout et al., 2018; Ousterhout et al., 2019; Siepielski et al., 2020).

Enallagma damselflies are aquatic predators for most of their development, emerging as aerial adults to complete their life cycle. *Enallagma* lay eggs in summer, spend ~11 months as aquatic larvae, and spend ~2–3 weeks as aerial breeding adults. Larvae inhabit plants found in the littoral zones of lakes, feed on smaller invertebrates and are fed upon themselves by larger predators, especially fish, and one another. Local *Enallagma* diversity often is high, with most lakes where fish are the top predator having five or more species (McPeck, 1989, 1990, 1998; Siepielski et al., 2010). Despite their flying prowess, *Enallagma* have very limited dispersal and rarely leave their natal lake, with previous studies suggesting that dispersal of >1 km is infrequent (McPeck, 1989). This dispersal limitation should accentuate local adaptation by reducing gene flow (Bourret et al., 2012; Johansson et al., 2013; Watts et al., 2004), as found for *Enallagma* (Siepielski et al., 2016).

Enallagma damselflies are commonly infected with *Arrenurus* mites – a generalist parasite of odonates (Mlynarek et al., 2013; Worthen & Turner, 2015). Mites attack when larval damselflies transition into flying adults (Forbes & Robb, 2008; Smith et al., 2010), and the number of mites on a single damselfly ranges from one to >400 (e.g., Worthen & Turner, 2015). As in other studies (LoScerbo et al., 2020; Mlynarek et al., 2013, 2015; Worthen & Hart, 2016; Worthen & Turner, 2015), we did not identify mites to species level, as the subgenus *Arrenurus* is a large and diverse group of cryptic species, and it is unknown how many species parasitise *Enallagma*. Like *Enallagma*, *Arrenurus* mites probably are limited in their dispersal, as they rely on the dispersal ability of their hosts (Bohonak et al., 2004).

This system is useful 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 damselfly host species (LoScerbo et al., 2020; Mlynarek et al., 2015) and across environmental gradients (LoScerbo et al., 2020). Parasitism of damselflies by water mites also is known to vary between the sexes (Robb & Forbes, 2016; Subrero et al., 2021), among female morphs (Subrero et al., 2021; Willink & Svensson, 2017), and can vary over the flight season as a consequence of differential immune expression (Nagel et al., 2010; Yourth et al., 2002). Water mite parasitism is associated with fitness costs for damselflies (Forbes & Baker, 1991; Gómez-Llano et al., 2020), and water mites may more heavily parasitise one host species as a result of greater host population density, if they respond to increases in the density of their primary host species, or underlying attributes of the host species (e.g., weaker immune function). Importantly, both damselfly density (McPeck, 1990, 1998; Ousterhout et al., 2019; Siepielski et al., 2011) and defences against parasitism, such as phenoloxidase (PO, a component of damselfly immune function; Hasik et al., 2021; Mlynarek et al., 2015) vary among *Enallagma* populations (Hasik et al., 2021; Mlynarek et al., 2015).

In order to defend themselves from water mites, *Enallagma* encase mite feeding tubes with melanin via the PO cascade (González-Santoyo & Córdoba-Aguilar, 2012), which kills the mite. Indeed, PO levels positively correlate with melanisation and defence against parasites (Butt & Raftos, 2008), making PO an appropriate (though limited) representation of overall immune function (Srygley & Jaronski, 2011). PO in damselflies varies not only with infection risk (Joop & Rolff, 2004), sex (Córdoba-Aguilar et al., 2006; Willink & Svensson, 2017), and female morph (Iserbyt et al., 2012), but also with resource abundance (Campero et al., 2008; Hasik et al., 2021), as immune defences are energetically costly (González-Santoyo & Córdoba-Aguilar, 2012). Thus, the extent of parasitism should vary among species and host populations over environmental gradients that either directly or indirectly shape interactions between damselflies and mites.

Indeed, from parasitism surveys across seven lakes in our study region we found that, although there is considerable variation among species and population, one species, *E. signatum*, was consistently much more heavily parasitised than any other species (Figure 1, Appendix S1). Because *E. signatum* was the most heavily parasitised species, and both parasite prevalence and intensity varied among populations within species (Table S1), we focused on *E. signatum*. To understand potential drivers of the intense parasitism in *E. signatum*, we designed a series of experiments and used an observational study to test relationships between environmental factors and parasitism.

2.2 | Experiment 1: Are parasite prevalence and intensity determined by host species or competitor density?

2.2.1 | Experimental design

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 as larvae (Figure S2; e.g., at the life stage immediately before the period when parasitism would occur, making larval density the critical factor for density-dependent parasitism), we wanted to determine whether the elevated rates of parasitism that we observed were driven by density-dependent responses to heterospecific density or species-specific responses to conspecific density. We also wanted to determine if any density-responses were stronger within than between species, as it could be that high densities of one species increases the risk of parasitism via parasite spillover.

In order to test these ideas, we used a response surface design crossing intra- and interspecific competitor density and host species (full details in the Appendix S1). We used *E. signatum* and *E. basidens*, because *E. signatum* was the most heavily parasitised species and *E. basidens* was rarely parasitised (Figure 1). *Enallagma signatum* also had the highest larval population densities, whereas *E. basidens* had considerably lower density among lakes (Figure S2). By using species

that occurred at naturally low and high densities, this experiment allowed us to determine if the elevated parasitism observed for *E. signatum* was driven by its higher 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, Arkansas, U.S.A. (Figure S1), as this was right before the emergence period for both species (Abbott, 2011). Cages consisted of chicken wire enclosed in mesh netting (0.6 × 1.2 mm mesh), ~1.5 m in height, capped at the bottom by a flat plastic tray (0.25 m diameter, bottom area = 0.05 m²) and tied off at the top. The size of the mesh netting allowed prey and larval parasites to colonise the cages, but excluded damselflies, and the top of the cages extended above the water surface to allow larval damselflies to emerge and transition to their adult stage (Smith et al., 2010). We established three density treatments: single species low density, single species high density, and mixed species high density. Single-species treatments contained either 10 similarly sized late instar larvae (low density, equivalent to 200 larvae/m²) or 20 larvae (high density, equivalent to 400 larvae/m²), whereas the mixed species treatment contained 10 larvae of both species (high density, equivalent to 400 larvae/m²). Importantly, both species, like all *Enallagma* in our study region, do not appear to partition microhabitats in any way and instead come along with one another on the same macrophytes. Cages were positioned side-by-side along a ~10 m span, with treatments randomly applied to cages. We replicated each treatment five times and included an empty cage ($n = 26$ total cages) 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. We then examined damselflies for mites under a dissecting scope to quantify prevalence and intensity.

During this experiment, Bob Kidd Lake experienced an intense storm and multiple cages from all treatments were damaged such that non-experimental larvae entered and subsequently emerged from the cages. These non-experimental larvae included *E. signatum*, *E. basidens*, and other non-target species. Any change in density and species presence may have affected our focal host-parasite dynamics, and though we could not determine how many non-experimental larvae emerged from the cages, they were few in comparison to the total, with two larvae on average entering the 22 cages that were damaged. Importantly, however, despite this contamination we still observed a striking pattern of species-specificity when including the non-target species that contaminated the cages (Figure S3; Table S2) and our interpretations are not affected.

2.2.2 | Data analysis

In order to evaluate the effects of host species and intra- and interspecific 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 log-transformed mite number to meet model

assumptions and constructed a linear model of the form: $\ln(\text{mite number})$ 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. For the analysis of prevalence, we grouped the number of infected and uninfected individuals within a cage to account for differences in the number of individuals between cages. We conducted all analyses in R v4.0.2 (R Core Team, 2020).

2.3 | Experiment 2: Is parasitism shaped more by host condition or local environmental conditions?

2.3.1 | Experimental design

We designed this second experiment to test for an effect of host condition (mediated by the effects of damselfly prey resource abundance on immune function) and source population on parasite prevalence and intensity. Because damselfly condition is resource-limited, with immune function (Hasik et al., 2021) and somatic growth rate increasing with prey abundance (Campero et al., 2008), we wanted to determine if the differing prevalence and intensity of parasitism we observed among *E. signatum* populations (e.g., Figure S4) were due to differences in host condition driven by prey abundance among lakes. Alternatively, we wanted to determine either if parasites differ in their ability to attack hosts or if hosts may differ in their defences against parasites, via local adaptation or plasticity, irrespective of local resource levels.

In order to disentangle these possibilities, we used a reciprocal transplant experiment crossing host source population and prey supplementation. By moving individuals between lakes with contrasting levels of natural parasitism and naturally low and high resources (prey levels), and crossing this with prey 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, U.S.A., differing in both prey abundance (Ousterhout et al., 2019) and parasite prevalence (Figure S4). Lake Fayetteville had a naturally low level of parasitism (Figure S4) and high prey density (~57 prey items/L), whereas Lake Wilson had a naturally high level of parasitism (Figure S4) and low prey density (~11 prey items/L). Prey density was measured as the mean of six replicate samples with a 6-L box sampler (100- μm mesh); full details on prey sampling can be found in the Appendix S1. Lake Fayetteville and Lake Wilson were separated by ~22 km (Figure S1) and fish lake *Enallagma* damselflies are known to have a limited dispersal ability of <1 km (McPeck, 1989), which should accentuate local adaptation by reducing gene flow (Siepielski et al., 2016). We constructed and established cages in each lake in the same manner noted in Experiment 1.

We established treatments by crossing damselfly source populations among lakes 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/m²). To test for an effect of food supplementation on host condition, we supplemented half of the cages in each experimental lake with equivalent biomass of prey (full details in the Appendix S1). Because experimental cages were constructed such that prey items and parasite larvae were able to colonise them, there is a possibility that supplemental prey also could escape the cages. Logistically there was no way to prevent prey escape while simultaneously allowing for parasites to colonise the cages. Regardless, larval damselflies in these cages were still exposed to a greater abundance of prey, and previous studies have shown that this same kind of food supplementation can increase other facets of damselfly condition. For example, experimentally increasing prey abundance increases damselfly growth rates, which is an important fitness component (that can result in an increase in larval growth rates as seen in McPeck, 1998). Cages were positioned side-by-side along a ~12-m span, with treatments randomly assigned to cages. We replicated each of the four treatment combinations seven times in each experimental lake, and again included an empty cage ($n = 29$ total cages per experimental lake) to check for non-target larvae. We collected adult damselflies every other day for 26 days, as this is when emergences stopped. This also was a sufficient time period to allow for differences in PO to manifest under food supplementation (De Block & Stoks, 2008). We again examined damselflies to quantify cage mean prevalence and intensity.

2.3.2 | Data analysis

In order to evaluate if parasite prevalence was the result of resource-limited differences 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 (see the Appendix S1 for interpretations of interaction effects). To evaluate these effects on parasite intensity, we used the same model structure as above, but used a linear model with $\ln(\text{mite number})$ as the response variable to meet model assumptions. We used cage mean prevalence and intensity values in all statistical analyses, which is the appropriate experimental unit. For the analysis of prevalence, we again grouped the number of infected and uninfected individuals within a cage to account for differences in the number of individuals between cages.

All damselflies that emerged in Lake Wilson were parasitised, resulting in perfect separation of the data (i.e., an independent variable perfectly predicts the dependent variable; Albert & Anderson, 1984), consequently the SE of estimated model parameters may have been inflated. To correct for this we used the R/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 SE 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, whereas 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.

2.4 | Observational study to determine what host and environmental features best explain prevalence and intensity of parasitism

2.4.1 | Study design

We examined the relationships between parasite prevalence and intensity and host immune function (total PO activity) and five environmental factors (fish density, larval density, prey density, pH, and mean air temperature). We focused on these factors because previous studies have suggested that they can either directly or indirectly shape parasitism in this system (e.g., da Silva et al., 2021; Hasik et al., 2021; LoScerbo et al., 2020; Worthen & Turner, 2015). Full details of sampling procedures for fish density, larval density, prey density, and pH have been previously published (Siepielski et al., 2010), and thus we only briefly summarise them here (full details can be found in the Appendix S1). Fish density was taken as the mean of three standardised seine hauls through the macrophyte bed of each lake where fish, damselflies and water mites comingle. Larval density was measured as the mean of two separate sampling events, each of which used 10 standardised dip net sweeps (Crowley & Johnson, 1992; Stoks & McPeck, 2003). Prey density was measured as the mean of six replicate samples with a 6-L box sampler (100- μ m mesh). Samples were sorted in the laboratory, and all captured prey were identified to the lowest possible taxonomic unit (family or genus in most cases) and consisted primarily of *Daphnia*, chironomids, ostracods, and copepods – all organisms regularly consumed by damselflies (e.g., Corbet, 1999; Thompson, 1978). pH was measured with a YSI probe (YSI ProPlus; YSI Inc.). We extracted daily summaries of mean air temperature from the PRISM Climate Group website (<http://prism.oregonstate.edu>) for the period of 16 May to 26 August 2017, spanning the adult flight season in our study, using GPS coordinates for each sampling location.

In order to quantify damselfly innate immune function, we used a standard measure of PO activity (Hasik et al., 2021; 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 of uninfected adult damselflies. In short, we measured total PO activity as the slope of the enzymatic reaction curve over a fixed time interval. Higher total PO activity has been shown to positively correlate with melanisation and defence against mites (Butt & Raftos, 2008; but see Mlynarek et al., 2015). As in previous studies, we used the protein content of each damselfly to control for variation in body size (Hasik et al., 2021; Mlynarek et al., 2015). We measured total PO activity and protein content in duplicate, with means used for analyses (details in the Appendix S1).

2.4.2 | Data analysis

In order to investigate the associations between total PO and environmental factors on both infection prevalence and intensity, we constructed several candidate structural equation models (SEMs; Shipley, 2009, full model details in the Appendix S1). We used SEMs because each SEM allowed us to test a specific hypothesis about the relationships between both the direct and indirect effects of the host and/or environment on parasitism, and differences in model selection between prevalence and intensity may point to differential drivers of these two parameters. We used the comparative fit index and root mean square error of approximation to select the final model (see the Appendix S1), and we log-transformed mite number to meet model assumptions for our SEMs of parasite intensity.

3 | RESULTS

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

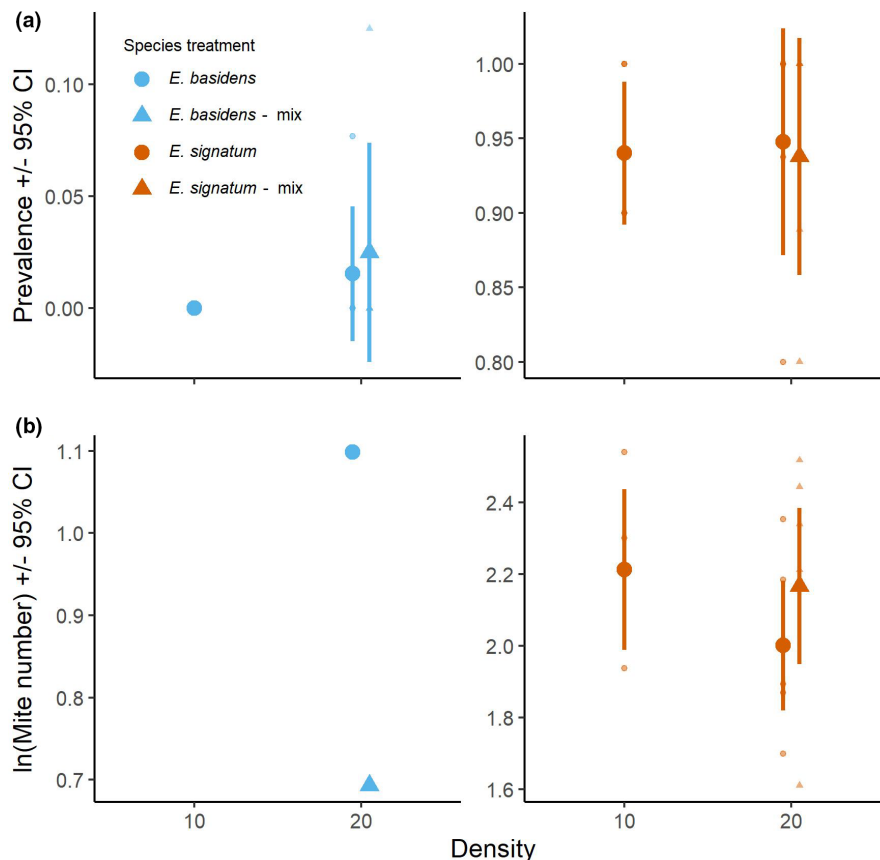
The models of prevalence (pseudo- $R^2 = 86.03$) and intensity ($R^2 = 64.94$) explained substantial variation in these measures of parasitism. For neither analysis did we find a significant interaction between host species and density (prevalence: $\chi^2_2 = 1.12$, $p = 0.57$; intensity: $F_{1,12} = 0.17$, $p = 0.18$), and therefore we present results from the models of main effects only. Most damselflies that emerged were parasitised, but this depended on host species ($\chi^2_1 = 260.61$, $p < 0.0001$), as only two *E. basidens* were parasitised, with *E. signatum* prevalence > 0.90 across density treatments (Figure 2a). Prevalence did not differ with either con- or heterospecific density ($\chi^2_2 = 0.12$, $p = 0.94$; Figure 2a).

Parasitism intensity was on average ~6.5-fold greater in *E. signatum* than in *E. basidens* (*E. signatum* cage mean intensity = 9.80 ± 0.77 [SE] mites per damselfly; *E. basidens* cage mean intensity = 1.5 ± 0.5 [SE] mites per damselfly, $F_{1,13} = 2.47$, $p = 0.0002$; Figure 2b), although again this was a species-specific effect with no differences among con- or heterospecific density treatments ($F_{2,13} = 0.10$, $p = 0.59$; Figure 2b).

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

The model of prevalence explained considerable variation in this measure of parasitism (pseudo- $R^2 = 43.92$). There were no significant interactions between any of the predictors (experimental lake $\times$ source population: $\chi^2_1 = 0$, $p = 1$; experimental lake $\times$ prey supplementation: $\chi^2_1 = 0$, $p = 1$; source population $\times$ prey supplementation: $\chi^2_1 = 0.36$, $p = 0.55$; experimental lake $\times$ source population $\times$ prey supplementation: $\chi^2_1 = 0$, $p = 1$), and therefore we report the results from the model of main effects only. Infection prevalence was on average ~10% lower in Lake Fayetteville than Wilson, with all damselflies that emerged from Lake Wilson parasitised (effect of

FIGURE 2 Results from the experiment manipulating host species, conspecific densities, and heterospecific (mix) densities. Shown are plots of (a) mean prevalence and (b) intensity of infection by mites, with separate subplots for each species (denoted by colour). Note that in (b) no point is shown for a density of 10 for *E. basidens*, because no mites were present at that host density. Also 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 95% CIs) and small points represent the mean prevalence of each replicate cage ($n = 5$ cages/treatment). Large points in (b) represent the mean $\ln(\text{mite number})$ (error bars denote 95% CIs) and small points represent the mean $\ln(\text{mite number})$ of each replicate cage



experimental lake: $\chi^2_1 = 33.92$, $p < 0.0001$; Figure 3a). We also found a significant effect of host source population ($\chi^2_1 = 11.53$, $p = 0.001$), as the non-local damselflies in Lake Fayetteville had a parasite prevalence ~5% lower than local damselflies (Tukey *post hoc* test, $z = -3.10$, $p = 0.002$). There was no effect of prey supplementation on prevalence ($\chi^2_1 = 3.29$, $p = 0.07$).

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, whereas they ranged from 0.81 to 0.97 in Lake Fayetteville. These predicted probabilities of infection from the lasso regression were almost identical to the observed prevalence value point estimates (Figure 3a), and thus the results of the logistic regression were robust to the perfect separation of the data.

For intensity, there was no significant three-way interaction between experimental lake, host source, and prey supplementation ($F_{1,48} = 0.44$, $p = 0.51$), nor was there a significant interaction between host source and prey supplementation ($F_{1,48} = 0.40$, $p = 0.53$). However, the interaction between experimental lake and source population was significant ($F_{1,48} = 19.02$, $p < 0.0001$), indicating that the intensity of parasitism changed when hosts were moved to non-local lakes. Therefore, we conducted analyses by experimental lake. The overall model for Lake Fayetteville was significant ($F_{3,24} = 10.98$, $p < 0.0001$, $R^2 = 52.58$), 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 2.3-fold more parasites than non-local damselflies (local damselfly cage mean intensity = 29.1 ± 2.35 [SE] mites per damselfly; non-local damselfly cage mean intensity = 12.5 ± 1.51

[SE] mites per damselfly, Tukey *post hoc* test, $t = -4.08$, $p = 0.0004$; Figure 3b). However, there were no effects of prey supplementation ($F_{1,24} = 1.41$, $p = 0.25$), nor was there an interaction between host source and prey supplementation ($F_{1,24} = 0.23$, $p = 0.64$).

3.3 | What environmental features best explain prevalence and intensity of parasitism?

Among SEMs, model 4 (parasitism = direct host + direct environment) had the most support for prevalence, whereas model 5 (parasitism = direct environment) had the most support for intensity. This indicates that prevalence was best predicted by the combined direct effects of host and environmental factors, whereas intensity was best predicted by the direct effects of the environment alone. Prevalence decreased with fish density, yet slightly increased with prey density, mean PO, and mean protein (all $p < 0.02$, magnitude of all effect sizes < 0.29 ; Figure 4a). Infection intensity slightly increased with prey density ($p = 0.01$, effect size = 0.12; Figure 4b), slightly decreased with mean temperature ($p = 0.01$, effect size = -0.11 ; Figure 4b), and strongly increased with pH ($p < 0.0001$, effect size = 0.39; Figure 4b).

4 | DISCUSSION

Determining the ecological factors explaining the extent to which the occurrence and intensity of parasitism varies among species and

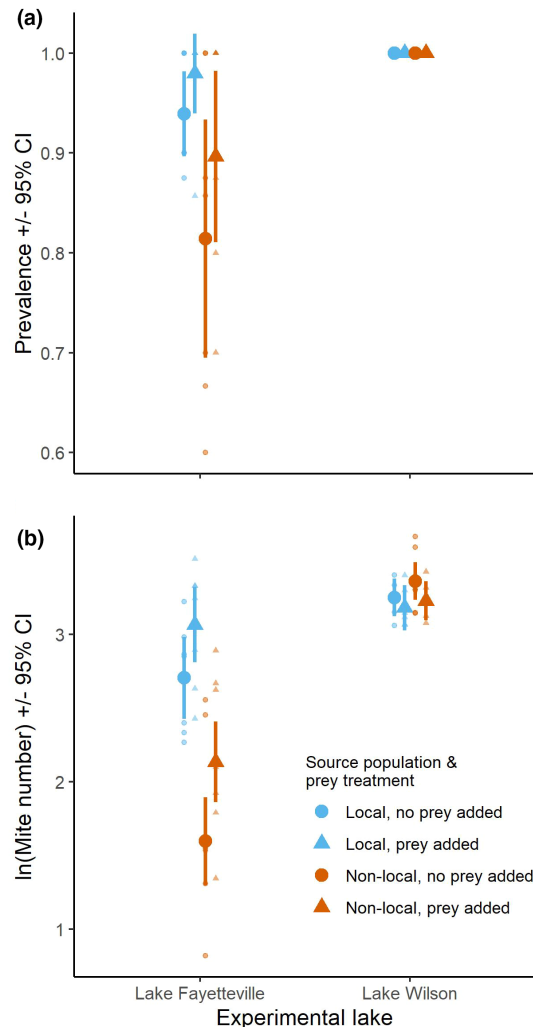


FIGURE 3 Results from the reciprocal transplant experiment manipulating damselfly source population (local or non-local) and prey supplementation (prey added or no prey added). Large points in (a) represent the mean prevalence of infection by mites (error bars denote 95% CIs) and small points represent the mean prevalence of replicate cages ($n = 7$ cages/treatment). Large points in (b) represent mean $\ln(\text{mite number})$ (error bars denote 95% CIs) and small points represent mean $\ln(\text{mite number})$ of replicate cages

populations often proceeds by focusing on either abiotic or biotic factors underlying infection dynamics. Yet, the cumulative effects of these factors probably are what drives infection dynamics, highlighting the need for integrative studies based on observations in wild populations. Despite multiple species of *Enallagma* co-occurring in lakes, we observed a striking pattern whereby one species, *E. signatum*, was overwhelmingly infected by parasites. Our focal species approach, centred on *E. signatum*, found no support for the prediction that parasitism was density-dependent, or was shaped by resource levels affecting host condition. However, we did find some evidence for local adaptation mediating parasitism, suggesting a role for local environmental conditions. Consistent with this latter finding, we also found that parasite prevalence and intensity among lakes were predicted by environmental factors. Collectively, these

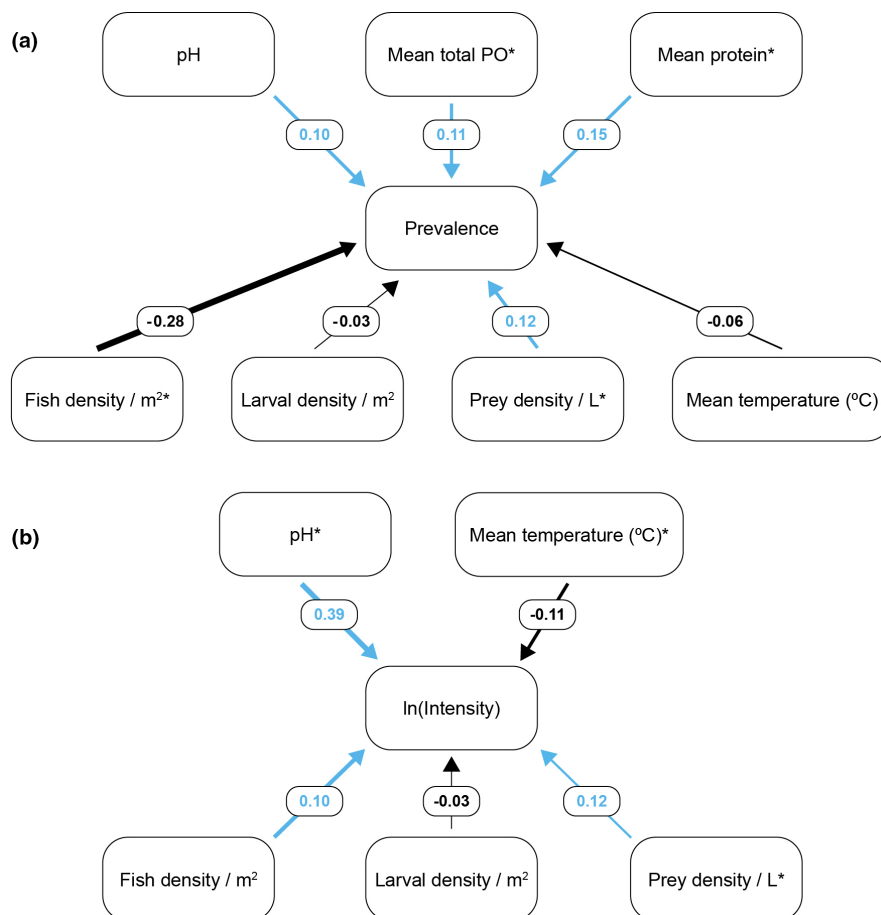
results imply that accounting for both host and environmental factors is necessary to understand how parasitism unfolds within complex ecological communities.

We found no relationship between either prevalence or intensity and host conspecific or heterospecific density among either the rarest or most common host species. This was surprising, as infection risk often increases with host density (Arneberg et al., 1998; Côté & Poulin, 1995; Gunton & Pöyry, 2016). The host densities used in our experiments were well within the range of densities of larval damselflies observed across their geographical distributions (McPeck, 1998; Siepielski et al., 2016), and thus they should have generated strong effects. Alternatively, it may have been that the densities we used exceeded a local host density asymptote beyond which infection does not continue to increase. We also acknowledge that as a result of the contamination of some larvae in some of the cages from the storm, this study could not rigorously capture the effects in a purely experimental way. However, our observational study also found no association between infection and considerable variation in damselfly densities among lakes.

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 which they experienced was not simply a consequence of 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 (e.g., Worthen & Turner, 2015), yet are consistent with patterns seen in other systems. For example, Woodcock & Verbergen (2008) found that parasitism of the leaf-mining weevil (*Rhynchaenus fagi*) by its hymenopteran parasitoid (*Pnigalio longulus*) did not increase with weevil density. Interestingly, *P. longulus* parasite prevalence was greater at the woodland edge where hosts and parasitoids were exposed to differing environmental conditions (Woodcock & Vanbergen, 2008). This pattern is consistent with our findings suggesting that parasitism is more dependent on local environmental conditions than host density.

That mites appear to target one host, *E. signatum*, was also surprising. Previous studies found that multiple *Enallagma* species co-occurring with *E. signatum* have varied but comparable levels of parasitism (Mlynarek et al., 2015; Worthen & Turner, 2015). Additionally, although we did not identify mites to species, *Arrenurus* are thought to be generalists, with single mite species parasitising several host species and multiple mite species parasitising a single host species (Mlynarek et al., 2013). However, 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 (Chapman et al., 2005; Lootvoet et al., 2013; Miller et al., 2018). Although spill-over effects from other odonate species (e.g., dragonflies) is a possibility, it is unlikely as they tend to harbour fewer mites than *Enallagma* damselflies (e.g., Worthen & Turner, 2015). One explanation is that mites in our study lakes are more host-specific than in other locations. Why this could be so

FIGURE 4 SEM plots representing the relationships between host and environment factors and (a) parasite prevalence and (b) parasite intensity. Solid lines represent standardised path coefficients of each predictor of (a) mite prevalence and (b) $\ln(\text{intensity})$, respectively, the values of which can be interpreted as effect sizes. Blue lines denote positive relationships, whereas black lines 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)



remains a mystery, although it is possible that different mite species may be present in the different lakes.

A second explanation is that *E. signatum* is the most competent (i.e., vulnerable) host for local mites. Our study locations are along the leading western edge for the geographic distribution of *E. signatum* and many *Enallagma* species (Abbott & Broglie, 2005), and because *E. signatum* is near its range limit it may be more vulnerable to parasitism. Briers (2003) found a similar pattern in freshwater snails (*Lymnaea stagnalis*), whereby the prevalence of trematode parasites was ~5-fold greater at the range limit than the range centre. Similar differences can be seen in comparisons of *E. signatum* parasite prevalence between this study and studies further from the leading edge of the species range. The average prevalence among the populations surveyed in this study (0.68) is >2-fold greater than what has been found in more northern (~0.25; Mlynarek et al., 2015) and eastern (0.26; Worthen & Turner, 2015) populations of *E. signatum*. However, the other three most common species from our surveys (*E. basidens*, *E. exulans* and *E. traviatum*) also are near the western edge of their geographical distribution and should therefore serve as equally competent hosts for local *Arrenurus* mites. Why *Arrenurus* mites target *E. signatum* requires further study to understand the mechanisms behind such species-specific parasitism.

Another avenue requiring further study would be investigating differences among the sexes, as males and females vary in both parasitism (Robb & Forbes, 2016; Subrero et al., 2021) and immune

function (Córdoba-Aguilar et al., 2006; Willink & Svensson, 2017). Furthermore, *E. basidens* females have multiple morphs (Fincke et al., 2005), and female morphs are known to experience varying degrees of parasitism (Subrero et al., 2021; Willink & Svensson, 2017). We did not investigate sex differences or morph effects for two reasons. Firstly, the majority of our observational data came from males. The only females that we collected were those that were in tandem with a male, and mated damselflies are known to have fewer mites than single damselflies (Honkavaara et al., 2009), and thus these data may have been biased. Secondly, although *E. basidens* females are polymorphic, *E. signatum* females are monomorphic (Fincke et al., 2005), precluding our ability to make comparisons between the two. Our study was limited to males, yet future studies should include both sex and female morph as explanatory factors to gain a more complete understanding of parasitism in this system.

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. We found that individuals transplanted from Lake Wilson to Lake Fayetteville were less parasitised 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 defences against parasitism via local adaptation or plasticity. Importantly, local adaptation is not uncommon in damselflies (Siepielski et al., 2016). If so, the asymmetry we detected

makes sense, because damselflies from Lake Wilson naturally experienced high levels of parasitism and so should develop enhanced defences that would confer an advantage in Lake Fayetteville where parasitism was generally lower. This is possible, as mean total PO levels for adults from Lake Wilson are ~3.5-fold higher than those from Lake Fayetteville. Whether these defences represent host local adaptation or plastic responses to the higher risk of infection is presently unknown; however, defences such as PO-induced melanisation are heritable (Koella & Boëte, 2002). Regardless, these results indicate that mite defences in the damselflies from Lake Wilson are not parasite-population specific, because they also are effective against non-local parasites.

Alternatively, or in tandem with local adaptation/plastic responses of the host, it may be that mites have developed enhanced local host specificity through local adaptation or plasticity. Mites from Lake Fayetteville may simply not recognise non-local damselfly hosts as well. Why a generalist parasite might lose the ability to recognise and attack a particular host is not clear, as this would seem a costly tradeoff (Fournet et al., 2013), especially for a species such as *E. signatum* which has a broad geographical distribution. Greater host specificity should occur when there are costs associated with infecting a broader suite of hosts. That is, selection for mechanisms adapted to a larger range of host species is likely to be costly (reviewed in Combes, 1997). However, the effects of such a tradeoff may be offset here given the limited damselfly dispersal that typifies this system (McPeck, 1989). In some ways, this potential population-level host specificity mirrors what we observed among different species of damselflies. Much like how mites seem to target *E. signatum*, they also may target particular populations. Such population-level host specificity may be the pre-cursor towards species-level specificity, thus representing an important link between local ecological and evolutionary dynamics of host-parasite systems.

We expected that resource-limited hosts would have reduced immune function (e.g., Campero et al., 2008; Hasik et al., 2021) and thus elevated parasitism. However, our observational study and transplant experiment showed that both parasite prevalence and intensity increased slightly with prey density. Although we found no evidence for resource-limited total PO limiting 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. Alternatively, greater prey densities also may improve mite condition since adult mites are voracious predators after leaving their damselfly hosts and consume invertebrate prey that also are the same prey base as damselflies (Smith et al., 2010; Vasquez et al., 2020; Vasquez et al., 2021). This could explain why the most important driver of parasitism in this system appears to be the local environment, as high prey densities could increase mite fitness, which could subsequently increase parasitism in *Enallagma*. We are not aware of other examples whereby across life stages a host's parasite is also its resource competitor (e.g., "intraguild parasitism"), which poses a challenge to understand this unique intersection of parasitism and competition within food webs.

We found a positive association between pH and parasite intensity, supporting previous findings relating abiotic factors to odonate water mite parasitism (LoScerbo et al., 2020). Although we found that fish density, total PO, host size, prey density, mean temperature, and pH were all significant predictors of parasitism, the magnitude of the effect of pH on intensity suggests that the local abiotic environment may be the most important mediator of infection among those factors considered here. The effect of pH on parasite intensity is probably due to the effects of low pH reducing mite hatching success and survival (Edwards, 2004; Rousch et al., 1997). The local abiotic environment frequently alters host-parasite interactions in myriad host-parasite systems (Wolinska & 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 that system increases as the abiotic environment becomes more favourable for powdery mildew (Penczykowski et al., 2014). The parasites in our study likewise may have benefitted from increases in pH. Conversely, damselfly hosts may be in worse condition in high-pH environments, even though odonates can tolerate large changes in pH (Hudson & Berrill, 1986). Although experimental studies are necessary to disentangle the nature of the relationship between abiotic factors and mite parasitism, the results of our study suggest a key role for the local environment in explaining the ecological outcome of host-parasite interactions.

5 | CONCLUSIONS

Despite evidence linking parasitism to spatial variation in abiotic and biotic factors, few studies have investigated the joint influence of abiotic and biotic drivers of parasitism among host species and populations. Our results show that this kind of integrative study is critical for determining the ecological factors that shape parasitism. Indeed, despite striking evidence for species-specificity (this study, Krasnov & Poulin, 2010), our results revealed that elevated parasitism is not because of the widely observed effects of host condition or population density, but instead may be driven more by the effects of local adaptation/plasticity in addition to key abiotic factors. These results support previous studies which have also shown that the occurrence and intensity of parasitism is shaped by both the local environment (Wolinska & King, 2009) and probably by local adaptation (Greischar & Koskella, 2007; Johnson et al., 2021). The combined results from this study therefore stress the importance of considering multiple working hypotheses in shaping parasitism among host species and populations. More generally, further research at the intersection of community ecology and disease ecology will be critical for understanding host-parasite dynamics within the complex network of species interactions that make up food webs.

AUTHOR CONTRIBUTIONS

AMS and AZH designed the study, AZH collected the data and wrote the initial draft, and both authors analysed the data and contributed substantially to revisions.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Data Dryad at <https://doi.org/10.5061/dryad.d7wm37q15>.

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