

Parasite richness and abundance within aquatic macroinvertebrates: testing the roles of host- and habitat-level factors

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Abstract. The importance of parasites as both members of biological communities and structuring agents of host communities has been increasingly emphasized. Yet parasites of aquatic macroinvertebrates and the environmental factors regulating their richness and abundance remain poorly studied. Here, we quantified parasite richness and abundance within 12 genera of odonate naiads and opportunistically sampled four additional orders of aquatic macroinvertebrates from 35 freshwater ponds in the San Francisco Bay Area of California, U.S.A. We also tested the relative contributions of host- and habitat-level factors in driving patterns of infection abundance for the most commonly encountered parasite (the trematode *Haematoloechus* sp.) in nymphal damselflies and dragonflies using hierarchical generalized linear mixed models. Over the course of two years, we quantified the presence and intensity of parasites from 1612 individuals. We identified six parasite taxa: two digenetic trematodes, one larval nematode, one larval acanthocephalan, one gregarine, and a mite, for which the highest infection prevalence (39%) occurred in the damselfly genus, *Ishnura* sp. Based on the hierarchical analysis of *Haematoloechus* sp. occurrence, infection prevalence and abundance were associated predominantly with site-level factors, including definitive host (frog) presence, nymphal odonate density, water pH, and conductivity. In addition, host suborder interacted with the presence of fishes, such that damselflies had higher infection rates in sites with fish relative to those without, whereas the opposite was true for dragonfly nymphs. These findings offer insights into the potential interaction between host- and site-level factors in shaping parasite populations within macroinvertebrate taxa.

Key words: *Haematoloechus* sp.; host-habitat interaction; macroinvertebrate; Odonata; parasite.

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INTRODUCTION

For more than 50 yr, pond ecosystems have been an influential study system in community ecology, offering novel insights into the factors affecting biodiversity, ecological interactions, and species composition (Hutchinson 1961, Werner and Hal 1976, Wilbur 1987, Downing and Leibold 2002, Wellborn 2002, Chase et al. 2009, Johnson et al. 2013a). Owing to their discrete

boundaries, numerical abundance, and biological diversity, ponds and their communities of predominantly invertebrate taxa have provided tractable opportunities to test ecological theory (De Meester et al. 2005, Johnson and Paull 2011). Nonetheless, considerably less is known about the ecological roles of parasites and pathogens within these ecosystems, despite estimates that parasites comprise a significant proportion of total biomass and biodiversity in

freshwater communities (Preston et al. 2013). In some of the few cases in which they have been well studied, parasites have important effects on invertebrate host population dynamics, predator–prey interactions, and ecosystem functioning (Hurd and Eisenberg 1990, Kohler and Wiley 1992, 1997, Johnson et al. 2013*b*, Mischler et al. 2016). For instance, Mischler et al. (2016) found that high levels of trematode parasite infections in aquatic snails—which are key grazers in freshwater habitats—altered nitrogen cycling at the ecosystem scale through their effects on host stoichiometry and excretion. Similarly, in stream communities, population crashes of the dominant caddisfly and cascading effects on periphyton abundance were associated with infections by a microsporidian parasite (Kohler and Wiley 1997).

One of the most commonly reported parasite groups in ponds is helminths with complex life cycles, such as digenetic trematodes (Esch et al. 1990*a, b*, Johnson et al. 2013*a*, Preston et al. 2014, Russell et al. 2015). Because their complex life cycles functionally interconnect suites of different species, trematode infections have the potential to affect numerous aspects of host populations and communities (Esch et al. 1990*a, b*, Lafferty et al. 2006). Research from marine systems, for instance, has illustrated how trematodes affect community interactions (Wood et al. 2007, Wood and Lafferty 2015), food web connectivity (Britton 2013, Poulin et al. 2013), and energy flow across an ecosystem (De Vos et al. 2016). Trematodes typically use snails as first intermediate hosts; macroinvertebrates or aquatic vertebrates as second intermediate hosts; and amphibians, fishes, birds, mammals, or reptiles as definitive hosts (Schell 1985, Galaktionov and Dobrovolskij 2013). Transmission among host species involves a combination of direct transmission via the production of free-living infectious stages, such as eggs, miracidia, and cercariae, and trophic transmission in which a host becomes infected by consuming an infected intermediate host (Yamaguti 1971). In light of parasites' ubiquity and potential influences on food webs and ecosystems, incorporation of parasite infection into research programs on even well-studied systems can reveal new insights at scales ranging from local species adaptation to ecosystem-level transformations (Wood and Johnson 2015).

A persistent challenge in parasite ecology involves identifying the relative importance of multi-scale factors in determining the presence and abundance of complex life cycle parasites (Esch et al. 1990*a, b*, Poulin 2011, Johnson et al. 2016). In parallel with research on free-living species, parasite distribution is a function of both variables that shape site-level occurrence (i.e., How likely is the parasite to colonize and establish at a given site?) and more local, host-level effects (i.e., How suitable is a given host for becoming and maintaining infection?). Decomposing the relative influence of each scale of predictors and their potential interactions is essential to accurately understanding the determinants of parasite community composition and infection dynamics across space and time. While individual host attributes such as body size, susceptibility, and age have all been shown to affect parasite infection within hosts (Pacala and Dobson 1988, Arneberg 2002, Calhoun et al. 2018), habitat-level features of the surrounding environment such as site area, temperature, host density, community composition, and habitat connectivity will also affect the capacity of parasites to effectively disperse between hosts and among sites (Krasnov et al. 2005). Teasing apart the effect of each level and their interactions will help better understand and predict changes in both parasite communities and parasite populations. For example, McCaffrey and Johnson (2017) demonstrated that freshwater snail symbiont diversity is driven by both host-level effects, such as snail size and abundance, and habitat-level effects that reflect both transmission and colonization opportunities. At the habitat level, snail symbiont richness correlated positively with wetland size, the proximity of nearby ponds, and diversity of the free-living community, likely reflecting opportunities for colonization and the presence of additional hosts necessary for parasite transmission.

In this study, we tested the relative importance of hypothesized drivers of parasite occurrence and infection abundance with a focus on the effects of host- and habitat-level characteristics. Specifically, we quantified parasite richness and community composition within the macroinvertebrate communities of 35 ponds in California over two years to (1) identify the parasite taxa present in this system, (2) examine patterns in

parasite communities between major host groups and among sites, and (3) test potential drivers of natural variation in parasite presence, infection prevalence, and abundance by one of the most commonly encountered parasites (the trematode *Haematoloechus* sp.) within larval odonates. This genus of trematodes uses freshwater snails in the genera *Gyraulus* sp. and *Physa* sp. as first intermediate hosts, dragonfly and damselfly larvae as second intermediate hosts, and ranid frogs as definitive hosts (Snyder and Janovy 1996, Bolek and Janovy 2007, Novak and Goater 2013). While many trematode species use highly mobile definitive hosts (e.g., birds and mammals) whose visits may be difficult to detect, detections of pond-dwelling ranid frogs are much more reliable and thus offer an opportunity to explore the influence of definitive host presence on intermediate host prevalence and infection load.

By using a hierarchical statistical approach, we aimed to identify the individual and combined influence of factors at the host- and habitat-scale. We expected parasite occurrence at a site to be driven by the presence of both the definitive host, a ranid frog, and the first intermediate host, a freshwater snail (Snyder and Janovy 1996). We also expected high variation in infection prevalence and infection load as a function of host identity alongside concurrent effects of host traits, such as body size and development time. Previous research has shown how host body size can increase the probability of infection by enhancing encounter rate (Arneberg 2002, Kamiya et al. 2014) or parasite accumulation with age (Pacala and Dobson 1988, LaBarbera 1989). We further expected differences as a function of host suborder (Zygoptera vs. Anisoptera), given distinct differences in how damselflies and dragonflies become infected (i.e., cercariae invasion via penetration at the base of the caudal gills for damselflies, whereas cercariae are passively acquired through abdominal water intake in dragonflies; Snyder and Janovy 1996). For the habitat level, we included factors such as pond size, the presence of fishes, the density of odonate and snail hosts, and water pH and conductivity. Fish represent a top predator in many aquatic systems and are known to influence odonate abundance and behavior (Johnson 1991), thereby potentially altering infection patterns. Similarly, host density is often an important

factor influencing infection patterns in a given habitat (Arneberg et al. 1998, Morand and Poulin 1998), while water chemistry can alter the survival and mobility of trematode infectious stages as they seek out hosts (Möller 1978, Pietroock and Marcogliese 2003).

METHODS

Field surveys

In the summers of 2014 and 2015, we sampled 35 ponds distributed across Alameda and Santa Clara counties within the East Bay Region of California. These ponds, many of which were originally constructed to support livestock grazing (Garone 2011), are often managed for mixed use as regional or county parks alongside several privately owned ranches. Each pond was sampled twice between May and July to collect specimens and assess habitat-level characteristics. We used the track function on a handheld global positioning system unit (GPSMAP 642; Garmin, Lenexa, Kansas, U.S.A.) to calculate pond perimeter. pH and conductivity were measured using a water probe (PCSTestr 35; Oakrion, Vernon Hills, Illinois, U.S.A.), which was held 150 mm underwater and 5 m from shore. To determine the abundance of aquatic host taxa, we conducted standardized, –m net sweeps using a D-frame dipnet (30.5 × 35.5 cm) every 10–15 m around the shoreline. The contents of each sweep were transferred to a sorting tray, counted, and identified. We focused on members of the invertebrate order Odonata (dragonflies and damselflies) and opportunistically sampled within the orders Ephemeroptera (mayflies), Hemiptera (true bugs), Coleoptera (beetles), and Malacostraca (amphipods; for a full taxonomic list, see Appendix S1). We collected an additional subset of 10 individuals per taxon per site for further taxonomic identification and parasite examination in the laboratory. The taxonomic keys provided in Merritt et al. (1996) and Thorp and Covich (2009) were used to help facilitate identification of the macroinvertebrates to genus or lowest possible taxonomic level. We determined whether fishes or ranid frogs were present based on visual observation, prior sampling of these sites, and detection during dipnet and seine surveys. Two species of ranid frogs utilize these ponds as breeding sites: the American bullfrog,

Lithobates catesbeianus, and the California red-legged frog, *Rana draytonii*. We classified bullfrog and red-legged status at each site as either being present or absent based on the detection of the adult stage.

Parasite examination

To characterize patterns of parasite taxonomic richness and abundance, we measured each invertebrate host using digital calipers (total length, mm) and carefully examined for ectoparasites using a dissecting microscope at 40–100× magnification (SZX10; Olympus, Shinjuku, Tokyo, Japan). We counted all ectoparasites and placed a subsample on a slide for identification under 200–400× magnification. The host was subsequently dissected by cutting along the lateral sides, removing the internal organs, and examining the compressed tissues between two slides (25 × 75 × 1 mm) under a compound microscope (SZX10, Olympus, Shinjuku, Tokyo, Japan). We used multiple taxonomic keys to identify parasites to lowest taxonomic level; although species-level identifications were generally not possible given that many of the infections constitute larval stages, we identified trematode (Platyhelminth) and gregarine (Apicomplexan) parasites to genus, juvenile acanthocephalans and nematodes to phylum, and mites (Arthropoda) to subclass (Poinar 1975, Schell 1985, Clopton 2002, Thorp and Covich 2009).

Host taxa characteristics

To explore the drivers of *Haematoloechus* sp. infections, we collected details on species-level odonate host traits from Corbet (1999) and Corbet et al. (2006), including host size and voltinism (the number of generations per year). We used voltinism as a proxy for development time, with the expectation that bivoltine taxa (more than one generation per year) have higher growth rates than univoltine taxa (single generation per year; Roff 1980, Nygren et al. 2008). We also indicated whether the specific taxon was a damselfly (suborder Zygoptera) or a dragonfly (suborder Anisoptera) as a proxy for host phylogeny.

Analysis

We estimated host specificity for each parasite taxon as the total number of host taxa in which it occurred during the survey and calculated parasite richness both overall for each host taxon

(across all sampled ponds) and for each site specifically (across all sampled host taxa). For *Haematoloechus* sp. infections within odonates, which were among the most commonly observed host-by-parasite combinations, we conducted analyses to assess the relative contributions of habitat characteristics vs. host characteristics in explaining site-level occurrence (whether the parasite occurred in at least one host at a site), infection status of each individual (whether each host was infected), and infection abundance (number of metacercariae per host in sites with *Haematoloechus* sp. present, including uninfected host; Bush et al. 1997). While infection prevalence and infection abundance are inherently correlated with one another, identifying their shared as well as unique predictors can provide valuable information for understanding parasite distributions. For the site-level *Haematoloechus* sp. occurrence model, we included both definitive host status (red legged, bullfrog, or both) and intermediate snail host as fixed effects which all likely affect parasite occurrence at the site. At the individual host level, we included body length (mm) and voltine status (bivoltine and univoltine) as fixed effects to help capture variation in body mass and aquatic development time, which likely affect exposure risk. We also included a fixed effect for suborder due to potential differences in transmission between Zygoptera and Anisoptera: For Anisoptera, *Haematoloechus* sp. is passively drawn into the brachial basket through the anal opening during gas exchange, whereas in Zygoptera, *Haematoloechus* sp. penetrate the host at the base of the caudal gills (Snyder and Janovy 1996).

At the habitat level, we tested for effects of pond perimeter (\log_{10} -transformed in m), snail density, odonate host density (number of the specific odonate taxon detected per dipnet sweep), fish presence, water pH, and conductivity. We included water conductivity and pH to account for variation in the extra-host environment in which *Haematoloechus* sp. cercariae must find a host. Multiple characteristics of water quality can alter the lifespan of cercariae, including ion concentration and pH (Möller 1978, Pietrock and Marcogliese 2003, Paull and Johnson 2014). Finally, we included an interaction between host suborder and fish presence based on the potential for infection risk in damselflies and

dragonflies to be differentially affected by fishes, particularly in light of the variation in their transmission pathways (Johnson 1991, Brown and Robinson 2016). In order to compare effect size across variables, we scaled each continuous explanatory variable by subtracting the mean and dividing by the standard deviation (Gelman and Hill 2007).

To explore factors contributing to parasite occurrence at the site level, we used a Firth's bias reduction logistic regression to account for issues of perfect separation between sites with the definitive host and sites without it (Firth 1993) with the `logistf` package (Heinze et al. 2013) in R (R Core Development Team 2010). To analyze the relative contribution of host-level and habitat-level variables on host-level parasite infection, we used generalized linear mixed models (GLMM) with the `lme4` package (`glmer`, `glmer.nb`; Bates et al. 2014) in R. For this analysis, we only included sites at which ranid frogs (either red-legged frogs or bullfrogs) were detected to prevent issues related to perfect separation (Firth 1993). We fit a binomial model for analyses of parasite infection status using a logit link function and a negative binomial model for parasite abundance using a log link function. Initially, we contrasted models that included random effects only: site (i.e., pond) and host taxonomic identity (i.e., species or lowest taxonomic resolution) using intraclass correlation coefficients (ICC) to determine the proportion of the variance within each group (Gelman and Hill 2007). The ICC score ranges from 0 to 1 with a score of 1 indicating that all captured variation is associated with a particular random effect. Building from this approach, we incorporated a full model with the complete set of fixed effects associated with host and pond characteristics after testing for collinearity among predictors using variance inflation factors (VIF). The VIF scores ranged from 1.05 to 1.74 suggesting a lack of collinearity (O'Brien 2007, the highest correlation between remaining variables ($r = 0.43$) involved odonate host density and host suborder identity; see Appendix S2 for a list of all pairwise correlations among continuous variables). After fitting the full model, we sequentially eliminated the least influential factor, refit the model, and performed a likelihood-ratio test to compare the full and reduced models. We continued removing factors

until the minimum adequate model was achieved (i.e., further removal of terms produced a significantly worse fit based on the likelihood-ratio test). We assessed model assumptions by visually inspecting residual plots (Bolker et al. 2009).

RESULTS

Parasite survey

In total, we sampled and dissected 1801 macroinvertebrates representing 22 genera and five orders of pond-dwelling insects and crustaceans. We observed six different parasite taxa among examined hosts, including two digenetic trematodes (*Halipegus* sp. and *Haematoloechus*, sp.), one apicomplexan gregarine (*Hoplorhyncus* sp.), one larval nematode, one larval acanthocephalan, and an ectoparasitic mite (Acari; Fig. 1). Patterns of parasite richness differed considerably among host taxa. Among the five orders of dissected arthropods, only three supported parasitic infections (Odonata, Coleoptera, and the amphipod order, Malacostraca), with Odonata harboring the majority of parasite diversity (five of six parasite taxa). Coleoptera and Malacostraca were infected with only a single parasite taxon, *Hoplorhyncus* sp., and the larval acanthocephalan, respectively (Table 1). Amphipod infections by larval acanthocephalans occurred at two of the five sites (40%) with a mean infection prevalence [± 1 standard error (SE)] of $4.60 \pm 4.40\%$. Odonate infections occurred at 29 of 35 sites (82.86%) with prevalence values ranging from 0 to 75% (mean ± 1 SE = $19.9 \pm 3.22\%$). *Halipegus* sp. occurred only in Anisoptera while *Haematoloechus* sp. occurred in eight of nine Anisoptera genera and two of five Zygoptera genera (Table 2). *Hoplorhyncus* sp. occurred in two orders, Coleoptera and Odonata, while larval nematodes were detected in both suborders of Odonata.

Occurrence, infection prevalence, and abundance of *Haematoloechus* sp

Site-level occurrence of *Haematoloechus* sp. was associated with the presence of ranid frog definitive hosts, for which infection was detected at 12 of the 27 sites supporting ranid frogs and 0 of 8 sites that did not (Firth correction logistic regression, ranid frog status: 3.45 ± 1.52 , $P < 0.001$).

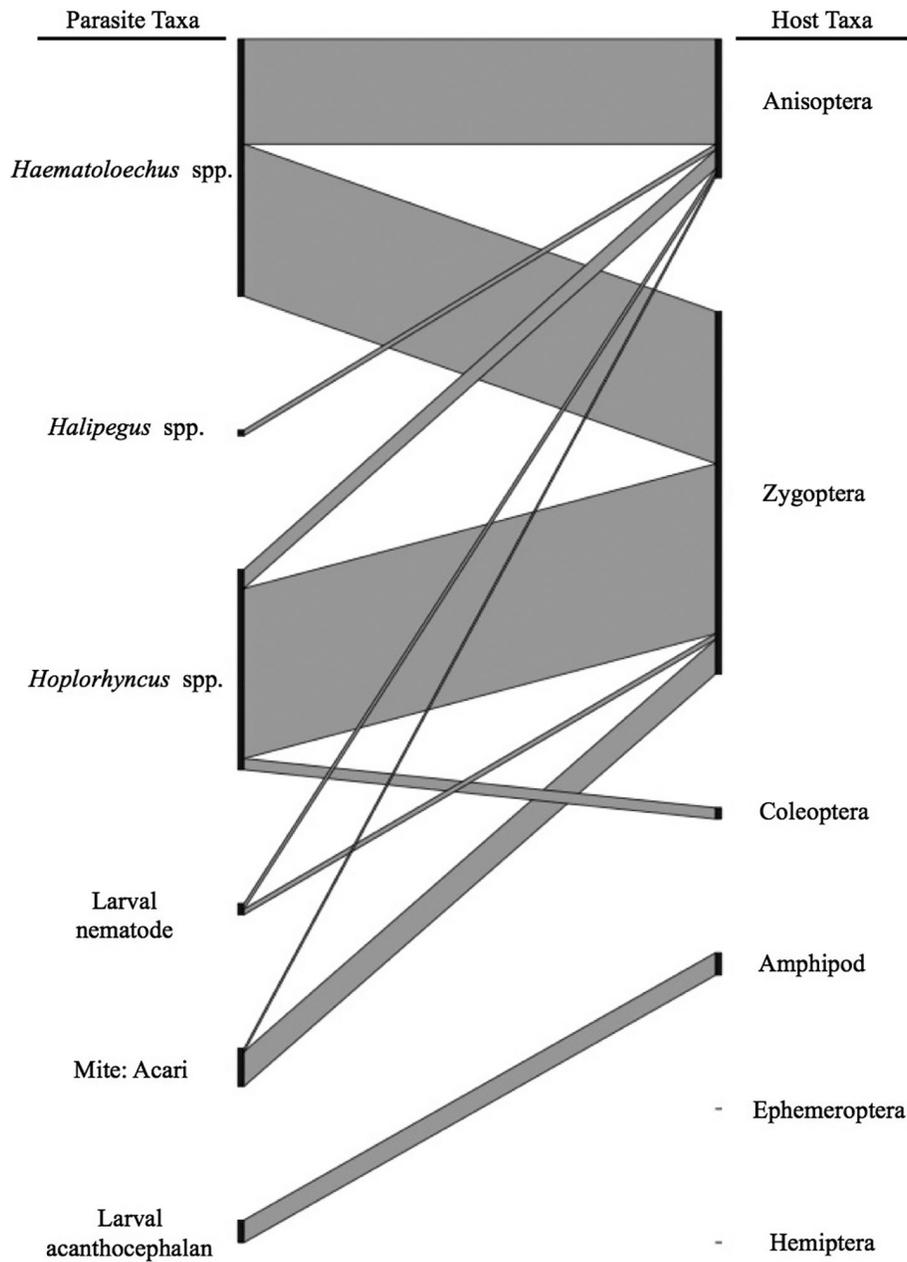


Fig. 1. Bipartite graph with parasite taxa on left and host taxa on right side. Lines indicate host utilized by each parasite taxa. The size of the connecting lines corresponds to the total number of infections between the host and parasite.

The identity of the ranid frog species (red-legged vs. bullfrog) did not influence the presence of *Haematoloechus* sp., nor did the density of the first intermediate host, a freshwater snail.

For analyses of parasite infection presence within hosts, the intraclass correlation coefficient

analysis indicated that the majority of captured variation was associated with the habitat-level random effect (0.75), with a score of 0.25 for the host genus random effect. Correspondingly, the best-fitting model for *Haematoloechus* sp. prevalence included two host-level predictors (body

Table 1. List of parasite taxa identified in a survey of 18 macroinvertebrate host taxa across 35 ponds sampled in 2014 and 2015.

Parasite taxon	Sites with infection	Site-level infection prevalence	Infection abundance
<i>Haematoloechus</i> sp.			
Odonata-Anisoptera	11/35	9.42 ± 2.88	6.76 ± 1.72
Odonata-Zygoptera	11/35	13.64 ± 4.47	10.62 ± 3.06
Coleoptera	1/13	0.07	1.00 ± 0.00
<i>Halipegus</i> sp.			
Odonata-Anisoptera	4/35	0.49 ± 0.27	2.31 ± 0.72
<i>Hoplorhyncus</i> sp.			
Odonata-Anisoptera	6/35	1.68 ± 0.07	9.75 ± 7.27
Odonata-Zygoptera	21/35	15.19 ± 3.92	4.27 ± 0.47
Coleoptera	2/13	11.53 ± 6.39	6.00 ± 5.00
Larval nematode			
Odonata-Anisoptera	3/35	0.48 ± 0.03	2.33 ± 1.33
Odonata-Zygoptera	3/35	0.54 ± 0.04	1.00 ± 0.00
Mite: Acari			
Odonata-Anisoptera	2/35	0.25 ± 0.01	19.50 ± 18.50
Odonata-Zygoptera	10/35	3.11 ± 1.15	2.40 ± 0.54
Larval acanthocephalan			
Malacostraca	2/8	0.04 ± 0.29	1.00 ± 0.00

Notes: Below each parasite taxon, we identify the different host(s) utilized with corresponding number of sites where the parasite was found, site-level infection prevalence of the host [mean host infection prevalence at ponds where the parasite was observed ± standard error (SE)] and infection abundance of individuals at sites where the parasite was observed in at least one host (mean ± SE).

length and suborder), four site-level predictors (host density, pH, conductivity, and fish presence), and the targeted interaction between fish presence and host suborder (for full summary of the models run, see Table 3). The marginal R^2 value was 0.17 (fixed effects only), while the conditional R^2 was 0.75 (fixed and random effects). Body length and host density positively

Table 2. Parasite infection prevalence (mean ± standard error) of four parasite taxa across all sites and years infecting Odonata hosts.

Genus	Percentage of sites occupied	No. dissected	Infection prevalence (%)				
			Total infection	<i>Haematoloechus</i> sp.	<i>Halipegus</i> sp.	<i>Hoplorhyncus</i> sp.	Larval nematode
Aeshnidae							
<i>Aeshna</i>	46	64	19 ± 8	11 ± 6	1 ± 1	1 ± 1	6 ± 6
<i>Anax</i>	80	267	10 ± 4	8 ± 4	0 ± 0	0 ± 0	0 ± 0
Coenagrionidae							
<i>Coenagrion</i>	31	53	30 ± 12	11 ± 9	0 ± 0	16 ± 9	0 ± 0
<i>Ischnura</i>	100	408	39 ± 6	16 ± 5	0 ± 0	21 ± 4	1 ± 0
<i>Telebasis</i>	09	6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lestidae							
<i>Archilestes</i>	06	3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Lestes</i>	40	100	7 ± 7	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Libellulidae							
<i>Erythemis</i>	43	125	18 ± 6	12 ± 6	4 ± 3	0 ± 0	0 ± 0
<i>Erythrodiplax</i>	06	10	6 ± 6	6 ± 6	0 ± 0	0 ± 0	0 ± 0
<i>Pachydiplax</i>	57	132	38 ± 8	26 ± 8	2 ± 2	9 ± 6	0 ± 0
<i>Planthemis</i>	46	78	3 ± 2	0 ± 0	0 ± 0	1 ± 1	0 ± 0
<i>Sympetrum</i>	77	261	13 ± 4	10 ± 3	1 ± 1	2 ± 1	1 ± 1
<i>Tramea</i>	29	105	23 ± 9	21 ± 9	0 ± 0	1 ± 1	0 ± 0

Note: Hosts are identified to genus with proportion of sites occupied by each genus and total number dissected.

predicted infection status, each leading to around a 60% increase in infection probability with a one standard deviation unit increase in average host body length or average host density, respectively (body length: 0.60 ± 0.11 , $P < 0.001$; host density: 0.52 ± 0.24 , $P = 0.05$). Increased values of pH and conductivity were also associated with higher infection probability (pH: 0.32 ± 0.11 , $P = 0.02$; conductivity: 1.76 ± 0.52 , $P < 0.001$). Hosts in the suborder Zygoptera and those at sites with fishes were both associated with lower probabilities of infection. However, there was a strong, positive interaction between fish presence and the suborder Zygoptera (interaction

coefficient: 4.93 ± 0.59 , $P < 0.001$; Fig. 2). This effect resulted from a higher overall infection prevalence of the damselfly suborder Zygoptera relative to Anisoptera when fish were present but the opposite pattern when fish were absent (Fig. 2).

Patterns of parasite infection abundance (i.e., metacercariae per odonate nymph) were similar to parasite infection prevalence: Once again, the habitat-level random effect for parasite infection abundance had the highest intraclass correlation score (0.74), with host-level identity exhibiting an ICC score of 0.26. The best-fitting model was also similar to that for prevalence, with two host-level

Table 3. Model outputs for all models ran for both infection prevalence and infection abundance analysis.

Model type	Variables	No. of variables	Log likelihood	Akaike's information criterion (AIC)	Δ AIC
Infection Prevalence	Conductivity + pH + Fish status + Host size + Host suborder + Host voltine status + Host density + Pond perimeter + Snail density + Host suborder: Fish status	10	-355.1	36.3	5
Infection Prevalence	Conductivity + pH + Fish status + Host size + Host suborder + Host voltine status + Host density + Pond perimeter + Host suborder: Fish status	9	-355.3	734.5	3.2
Infection Prevalence	Conductivity + pH + Fish status + Host size + Host suborder + Host voltine status + Host density + Host suborder: Fish status	8	-355.4	732.8	1.5
Infection Prevalence	Conductivity + pH + Fish status + Host size + Host suborder + Host density + Host suborder: Fish status	7	-355.7	731.3	0
Infection Prevalence	Conductivity + Fish status + Host size + Host suborder + Host density + Host suborder: Fish status	6	-360.0	738.0	6.7
Infection Prevalence	Conductivity + Fish status + Host size + Host suborder + Host suborder: Fish status	5	-362.5	741.0	9.7
Infection Prevalence	Conductivity + Fish status + Host size + Host suborder	4	-419.6	853.2	121.9
Infection Prevalence	Intercept only	1	-444.3	894.5	163.2
Infection Abundance	Conductivity + pH + Fish status + Host size + Host suborder + Host voltine status + Host density + Pond perimeter + Snail density + Host suborder: Fish status	10	-1763.0	3553.9	4.6
Infection Abundance	Conductivity + pH + Fish status + Host size + Host suborder + Host voltine status + Host density + Pond perimeter + Host suborder: Fish status	9	-1763.9	3551.1	2.2
Infection Abundance	Conductivity + pH + Fish status + Host size + Host suborder + Host voltine status + Host density + Host suborder: Fish status	8	-1763.4	3550.8	1.5
Infection Abundance	Conductivity + pH + Fish status + Host size + Host suborder + Host density + Host suborder: Fish status	7	-1763.8	3549.6	1.2
Infection Abundance	Conductivity + pH + Fish status + Host size + Host suborder + Host suborder: Fish status	6	-1763.7	3549.3	0
Infection Abundance	Conductivity + Fish status + Host size + Host suborder + Host suborder: Fish status	5	-1769.9	3557.7	8.4
Infection Abundance	Conductivity + Fish status + Host size + Host suborder	4	-1824.6	3665.3	116
Infection Abundance	Intercept only	1	-1874.7	3757.3	208

Notes: For each model, we included a random intercept term for site nested in park identity and host genus identification. Bolded text indicates best-fitting model for both infection prevalence and abundance.

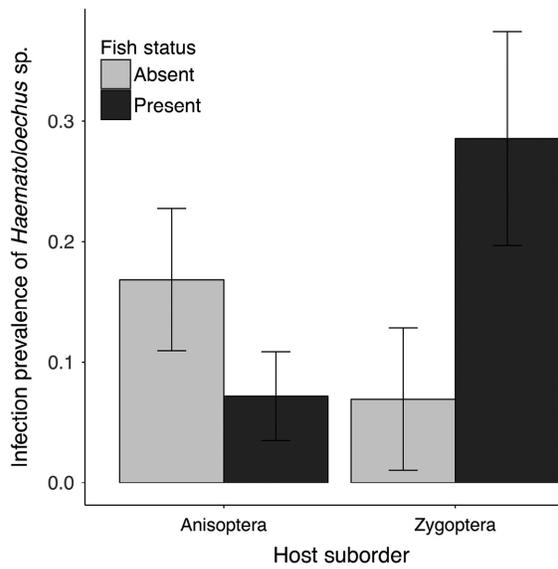


Fig. 2. The effect of the presence of fish on mean infection prevalence of *Haematoloechus* sp. for the two suborders of Odonata, Anisoptera (Dragonflies), and Zygoptera (Damselflies). Height of bars represents mean prevalence \pm standard error (from generalized linear mixed models: Zygoptera; fish interaction estimated coefficient: 4.93 ± 0.59 , $P < 0.001$).

covariates (body length and suborder), three habitat-level covariates (pH, conductivity, and fish presence), and the interaction between fish and host suborder (for full summary of the models, see Table 3). An increase in host body length was associated with an increase in parasite abundance (scaled coefficient: 0.16 ± 0.02 , $P < 0.001$). Increases in both water conductivity and pH were associated with an increase parasite abundance (scaled conductivity coefficient: 1.03 ± 0.28 , $P > 0.001$; pH coefficient: 0.40 ± 0.13 , $P = 0.001$). While dragonfly taxa tended to have higher infection abundances compared to damselflies (Zygoptera: -2.50 ± 1.09 , $P = 0.02$), the presence of fish diminished this effect (Zygoptera-by-fish interaction: 4.78 ± 0.47 , $P < 0.001$; Fig. 3), such that damselflies had higher infection abundances than dragonflies in sites with fishes, whereas this pattern was reversed at sites without fishes.

DISCUSSION

Studies of macroinvertebrates in pond ecosystems have historically played an important role

in community ecology (McPeck 1997, Benke and Huryn 2010, Chase 2010). However, the parasites of these organisms as well as the factors that influence infection risk have rarely been considered, despite evidence that such parasites can be both abundant and have strong effects on the community (Kohler and Wiley 1997, Vance and Peckarsky 1997, MacNeil et al. 2003). By surveying macroinvertebrate taxa from replicate ponds for parasite infection, we identified six distinct taxa, including metacercariae of two digenetic trematodes, a gregarine, a larval nematode, an acanthocephalan, and an ectoparasitic mite. The majority of sampled sites supported at least one parasite taxon, although patterns of parasite richness and abundance varied widely among examined host taxa. It should be noted that our estimates of parasite richness and prevalence are likely underestimates of “true” richness due to the subsampling methods employed, the challenges of detecting cryptic infections, and the shortage of morphological traits to definitively

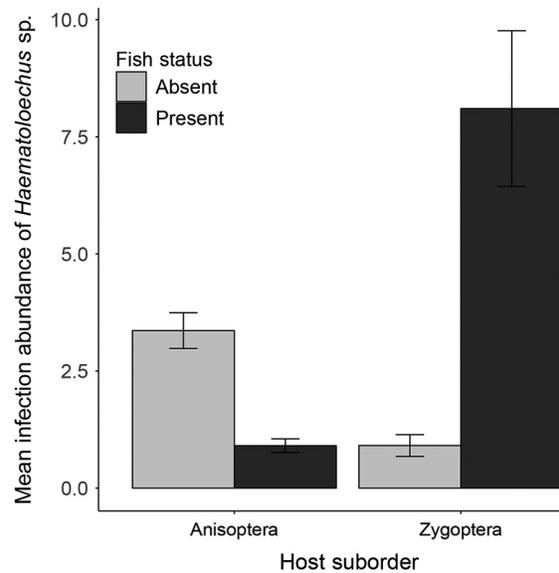


Fig. 3. The relationship between the presence of fish on mean infection abundance of *Haematoloechus* sp. for the two suborders of Odonata, Anisoptera (dragonflies), and Zygoptera (damselflies). Height of bars represents mean *Haematoloechus* sp. abundance \pm standard error (from generalized linear mixed models: Zygoptera: fish interaction estimated coefficient: 4.78 ± 0.47 , $P < 0.001$).

separate parasites into species, particularly for larval stages.

Despite the range of macroinvertebrate diversity sampled in the current study, the majority of infections (>95%) and parasite taxa (83%) occurred among hosts from the order Odonata, which are among the most common taxa in small pond ecosystems (Corbet 1980). One of the most surprising findings was the low overall diversity and abundance of macroparasites detected among the non-odonate invertebrate taxa, including groups such as Ephemeroptera and Coleoptera. Mayflies (Ephemeroptera) have previously been found to be important hosts for both mermithid nematodes and digenetic trematodes in stream ecosystems (Esch et al. 1986, Vance and Peckarsky 1997). Similarly, aquatic Coleoptera showed a low level of infection prevalence and harbored only a single gregarine parasite taxon, despite their relative ubiquity among sampled ponds. Whether this pattern stemmed from a lower sampling effort and sample size for these groups or reflects a truly depauperate parasite fauna in these pond invertebrates will require additional investigation, ideally incorporating surveys for a broader range of parasites (including microparasites) and molecular methods for differentiating among morphologically similar larval stages of different species. The artificial nature of the sampled pond environments—most of which were constructed for cattle grazing or water storage—could contribute to a less well-established parasite community. It is worth noting, however, that even the odonates supported fewer parasite taxa than detected in other vertebrate and invertebrate hosts surveyed from these and other similar pond ecosystems. For instance, studies with amphibian, fish, and snail hosts from pond communities have detected anywhere from 12 to 23 macroparasite taxa, compared to the five taxa from odonates in the current study (Muzzall et al. 2001, Pulis et al. 2011, Johnson et al. 2016, McCaffrey and Johnson 2017, Calhoun et al. 2018).

In a review of aquatic macroinvertebrate parasites, Kohler (2008) commented on the overall rarity of parasitological surveys among macroinvertebrate hosts, with those that have been performed tending to focus on a particular host–parasite interaction or specific class of host taxa.

Past parasitological studies focusing on particular taxa have nonetheless revealed a high diversity of both parasites and potential hosts across these groups of organisms. These include multiple species of trematodes (Prakash and Pande 1970, Mariluan et al. 2012, Stigge and Bolek 2016), gregarines (Locklin and Vodopich 2010), acanthocephalans (Lagrue et al. 2007), and arthropod parasites (Rolff et al. 2000), including a parasitoid wasp (Jackson 1966). The macroinvertebrate host–parasite interactions studied have also illustrated the importance and often complex roles of parasites in altering host population structure (Kohler and Wiley 1997, Vance and Peckarsky 1997, Chen et al. 2015). Infections can influence macroinvertebrate hosts through castration (Lafferty and Kuris 2009), increased mortality (Kohler and Wiley 1992), or alteration in behavior (Bethel and Holmes 1977), in some cases with cascading effects through the community (Kohler and Wiley 1997). The few community-wide surveys that have been conducted highlight the hidden diversity of parasites that use macroinvertebrates as hosts; for instance, Grabner (2017) performed a broad parasitological survey of six orders of stream-dwelling arthropods and identified 26 unique parasites utilizing these taxa as hosts in a given community, emphasizing the continued need for more assessments of parasites infecting aquatic macroinvertebrates and their ecological roles.

Based on a further examination of the drivers of *Haematoloechus* sp. infection prevalence and abundance within odonates, our analyses indicated that patterns of infection associated positively with variables at both the site and host levels. The majority of observed variation was associated with habitat-level differences, as indicated by the ICC comparing random effects for pond vs. host taxon. The presence of ranid frogs, which are the necessary definitive hosts for this trematode, was a necessary prerequisite to detecting infections among the co-occurring odonate naiads. *Haematoloechus* sp. only occurred at ponds with at least one species of ranid frog present, although the identity of the frog species (bullfrogs vs. red-legged frogs) did not seem to be important. For both infection prevalence and abundance, habitat-level groupings accounted for 74–75% of the variation, relative to 25–26% for the host-level grouping. In the analyses of

specific covariates, the best-fitting model included two covariates at the host level: host body length and host suborder. These findings are consistent with previous research linking parasite abundance and larger host body size (Kuris 1990, Poulin et al. 2003, Kamiya et al. 2014, Calhoun et al. 2015, Johnson et al. 2016). Larger-bodied hosts represent bigger targets for free-living infectious stages (e.g., cercariae), potentially increasing the likelihood of cercariae encounter in the water column (Arneberg 2002). In addition, host size is often correlated with age, for which there may be a positive relationship between probability of infection and duration of exposure (Morand and Poulin 1998, Lindenfors et al. 2007, Calhoun et al. 2015).

At the site level, four covariates were retained in the best-fitting model, with host density, conductivity, and pH all having significant positive effects on infection status. For many parasites, an increase in host density can enhance parasite transmission (Arneberg et al. 1998, Altizer et al. 2003, Kamiya et al. 2014), while water quality has been shown to play an important role in parasite infections in freshwater ecosystems (Anderson and Sukhdeo 2010). Changes in both pH and conductivity have the ability to alter both the survival of the free-living infectious stage which is often susceptible to the abiotic environment (Karvonen et al. 2003).

Intriguingly, we also found evidence for an interaction between the presence of introduced fishes and the odonate suborder (Zygoptera vs. Anisoptera). In sites with fishes, damselflies (Zygoptera) were both more likely to be infected and supported higher abundances relative to dragonflies (Anisoptera) found in fish sites; however, in ponds without fishes, this pattern was reversed (Fig. 2). For instance, dragonfly nymphs present in fish sites reduced the probability of infection by roughly 28%, whereas damselfly nymphs present in fish sites increased their probability of infection by eightfold compared to sites without fish. One possible hypothesis to explain this result is that fish alter either the species composition or relative abundance of odonates, such that only the most susceptible taxa persist with fishes. In our survey, however, the majority of odonate taxa occurred in both fish and fishless sites, with no differences in taxa presence or specific host density as a function of fish presence (see Appendix S3). We also saw a

similar number of host taxa between fish and fishless ponds, with fishless ponds supporting an average of 3.97 ± 0.43 odonate genera and fish ponds containing 4.90 ± 0.34 (Poisson-distributed generalized linear model (GLM), $P = 0.11$).

An alternative mechanism could stem from the effects of fish on host behavior and microhabitat use and the resulting consequences on transmission (Robinson and Wellborn 1987, Johnson 1991, Stoks and McPeck 2003). Previous research suggests that fish often cause odonates to become less active and/or move closer to nearshore habitats to avoid consumption (Johnson 1991). However, the differential pathways through which the two odonate suborders become infected could create contrasting responses in infection in the presence of predator fish. For damselflies, *Haematoloechus* sp. cercariae actively attach to the lateral caudal gill and migrate to the posterior end of the host before penetrating the host (Bolek and Janovy 2007). For dragonflies, in contrast, cercariae use a more passive transmission pathway, in which they are sucked into the host's abdomen during gas exchange and subsequently encyst on the brachial basket (Snyder and Janovy 1996, Wetzel and Esch 1996). Thus, the presence of fish—by lowering dragonfly larvae activity and gas exchange—could reduce infections by lowering the probability that infectious cercariae are drawn into the brachial basket. For damselflies, the presence of fishes might either increase or have no effect on infection, depending on the relative influence of host movement on the ability of *Haematoloechus* sp. cercariae to attach and penetrate the host. If fish also lead to a shift in the habitat use of the odonate or snail host taxa to warmer, nearshore habitats, this could affect the rate of cercariae release by infected snails and the rate of host–parasite encounter (McCarthy 1999, Poulin 2006). However, whether such an effect would be similar for both Zygoptera and Anisoptera is unclear. In the absence of experimental data, we suggest these potential mechanisms for explaining the observed interaction between fishes and odonate suborder remain conjectural and warrant additional investigation.

Infections within macroinvertebrates generally and odonates specifically have the potential to affect aquatic community structure and influence energy exchange with the surrounding terrestrial

ecosystem. Because many odonates are key predators during their aquatic nymphal phase (Thompson 1978, Johansson 1993, Corbet 1999), infections that affect their abundance, population dynamics, or even foraging strategies could have broad-ranging effects on other members of the aquatic community, including in some cases vertebrates such as amphibian larvae (Skelly 1994). Recent work has found that when infected with a species of *Haematoloechus* sp., nymphal host in the suborder Anisoptera experience elevated foraging rates compared to similar aged uninfected taxa as well as a slower growth rate (Leaphart and Zelmer 2017). Thus, *Haematoloechus* sp. infections could have indirect effects on the odonate prey abundances in these systems. High levels of parasite infection could also affect additional components of the Odonate life cycle, such as the likelihood of metamorphosis or breeding success (Forbes 1991, Marden and Cobb 2004). Aquatic macroinvertebrates that metamorphose into terrestrial adults (such as Odonata) represent a key link between the aquatic and terrestrial environments. Emergence of adult insects, which can number up to $20,000 \text{ m}^{-2}\cdot\text{yr}^{-1}$ (Baxter et al. 2005), often provides key resources to bats, birds, and small mammals (Nakano and Murakami 2001, Murakami and Nakano 2002, Baxter et al. 2005). Increased examination of the parasites of aquatic macroinvertebrates could therefore shed light on an important contributing influence of macroinvertebrate population and community dynamics as well as their inputs to terrestrial ecosystems (Kohler and Wiley 1997, Kohler 2008, Grabner 2017).

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