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Parasite transmission in complex communities: Predators and alternative hosts alter pathogenic infections in amphibians

SARAH A. ORLOFSKE,¹ ROBERT C. JADIN, DANIEL L. PRESTON, AND PIETER T. J. JOHNSON

Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado 80309 USA

Abstract. While often studied in isolation, host–parasite interactions are typically embedded within complex communities. Other community members, including predators and alternative hosts, can therefore alter parasite transmission (e.g., the dilution effect), yet few studies have experimentally evaluated more than one such mechanism. Here, we used data from natural wetlands to design experiments investigating how alternative hosts and predators of parasites mediate trematode (*Ribeiroia ondatrae*) infection in a focal amphibian host (*Pseudacris regilla*). In short-term predation bioassays involving mollusks, zooplankton, fish, larval insects, or newts, four of seven tested species removed 62–93% of infectious stages. In transmission experiments, damselfly nymphs (predators) and newt larvae (alternative hosts) reduced infection in *P. regilla* tadpoles by ~50%, whereas mosquitofish (potential predators and alternative hosts) did not significantly influence transmission. Additional bioassays indicated that predators consumed parasites even in the presence of alternative prey. In natural wetlands, newts had similar infection intensities as *P. regilla*, suggesting that they commonly function as alternative hosts despite their unpalatability to downstream hosts, whereas mosquitofish had substantially lower infection intensities and are unlikely to function as hosts. These results underscore the importance of studying host–parasite interactions in complex communities and of broadly linking research on predation, biodiversity loss, and infectious diseases.

Key words: *amphibian decline; amphibian malformations; biodiversity; community ecology; dilution effect; global change; infectious disease; invasive species; Pseudacris regilla; Ribeiroia ondatrae.*

INTRODUCTION

While often studied in isolation, parasites and hosts interact with members of a diverse community that can influence patterns of infection and pathology (Hopper et al. 2008). The hypothesis that community diversity, including host and non-host species, leads to reduced pathogen transmission has been termed the “dilution effect” (Keesing et al. 2006, 2010). Previous research on the dilution effect has focused primarily on vector-borne microparasites (e.g., West Nile virus and the Lyme-causing bacterium, *Borrelia burgdorferi*; Ostfeld and Keesing 2000a, b, Ostfeld and LoGiudice 2003, Ezenwa et al. 2006). In these cases, additional vertebrate host diversity is hypothesized to increase the number of instances in which infected vectors bite low-competency species, thereby lowering the number of successful

transmission events (“encounter reduction”; Keesing et al. 2006).

However, the occurrence of alternative, low-competency hosts may be but one of several pathways through which community members influence parasite transmission to suitable hosts. Because most studies of the dilution effect have been correlational, typically comparing richness to a measure of infection risk, it is often not clear what ecological mechanisms are responsible (Allan et al. 2003, Ezenwa et al. 2006). In particular, predators in a community can alter infections either by consuming hosts (susceptible host regulation) or by preying upon parasite infectious stages (encounter reduction) (Ostfeld and Holt 2004, Johnson et al. 2010). Given the high frequency of predator–parasite links in aquatic food webs, predation upon parasites is likely a common occurrence with the potential to influence transmission dynamics (Lafferty et al. 2008, Johnson et al. 2010). This is especially likely to occur for parasites that depend on free-living stages to move between hosts, which include many trematodes, ces-

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¹ E-mail: sarah.orlofske@colorado.edu

todes, and nematodes (Johnson and Thieltges 2010). Indeed, predation of parasites has been used as a method of biological control for schistosomiasis in humans (Siau et al. 1992) and helminths of livestock (Nichols et al. 2008). In some cases, multiple mechanisms could operate simultaneously (e.g., alternative hosts “distract” parasites away from focal hosts), while predators reduce the abundance of free-living stages. These observations underscore the importance of evaluating the dilution potential of multiple species in nature and assessing how they affect transmission to sensitive hosts.

To evaluate the influence of ecological communities on parasite transmission and infection risk, we focused on interactions between the pathogenic trematode *Ribeiroia ondatrae*, a sensitive amphibian host (*Pseudacris regilla*), and other vertebrate and invertebrate members of the community. Our primary objectives were to: (1) quantify the ability of diverse aquatic species (crustaceans, mollusks, insects, fish, and amphibians) to reduce the abundance of *R. ondatrae* free-living stages (cercariae), and (2) examine the effect of a subset of species on parasite transmission to focal host tadpoles by serving as predators or alternative hosts. To increase the biological relevance of our study, we utilized existing field data to identify aquatic free-living species to use in laboratory trials and compared infection patterns of two vertebrate taxa between experimental and field data. Our study aimed to identify the relative importance of multiple underlying biological mechanisms (i.e., consumption, infection, or both) that can lead to changes in disease risk. Improving our understanding of the individual mechanisms through which species diversity influences disease dynamics has important consequences for human and wildlife health in a period of biodiversity loss.

METHODS

Study system

The trematode *Ribeiroia ondatrae* has a complex life cycle involving sequential infection of planorbid snails, larval amphibians or fish, and aquatic birds (Johnson et al. 2004). Free-living cercariae released by infected snails typically locate an amphibian larva, penetrate the developing limb buds, and develop into metacercariae, which can cause limb malformations or mortality (Johnson et al. 1999). The parasite is trophically transmitted to definitive host birds through consumption of infected amphibians. In the western United States, Pacific chorus frogs (*Pseudacris regilla*) are a common host for *R. ondatrae* that frequently exhibit severe pathology (Johnson et al. 1999, 2002), making them an appropriate focal host.

Predation bioassays

We examined the abilities of seven aquatic species to remove *R. ondatrae* cercariae in laboratory trials. These included two vertebrates (California newts [*Taricha*

torosa] and western mosquitofish [*Gambusia affinis*]) and five invertebrates (California clam shrimp [*Cyzicus californicus*], backswimmers [*Notonecta* sp.], damselfly nymphs [*Lestes* sp. and *Enallagma* spp.], dragonfly nymphs [*Anax* sp.], and clams [*Sphaerium* sp.]). Selection of these taxa was based on their co-occurrence with *R. ondatrae* in natural wetlands, their suspected potential to remove parasites via multiple mechanisms (i.e., consumption and successful transmission), and prior knowledge of the ability of related taxa to consume cercariae in other systems (Schotthoefer et al. 2007, Thieltges et al. 2008, Kaplan et al. 2009). In selecting these species, we utilized existing field survey data on the occurrence of mosquitofish, amphibians, and invertebrates from 49 ponds that contained *R. ondatrae* in the San Francisco Bay Area of California, USA (see also Johnson and Buller 2011). Field methods and the frequency of co-occurrence of each of the seven taxa in ponds supporting *R. ondatrae* are provided in Appendix A.

We developed laboratory bioassays to determine the degree to which each of the selected species reduced the abundance of free-living cercariae and the mechanism(s) through which such effects occurred. The body sizes and sample sizes of species used in bioassays are provided in Appendix A: Table A1 and methods for animal collection and maintenance in Appendix B. Each individual was fasted for 24 h prior to assay. We isolated cercariae from field-collected snails by placing them individually in 50-mL centrifuge tubes over night. For each assay, we placed a single individual of each species in 60 mL of water with 30 *R. ondatrae* cercariae for 30 min, after which we removed the individual and counted any remaining cercariae. To assess parasite recovery in the absence of predators or hosts, we included containers with parasites and no other species. Forty-eight hours following trial completion, we necropsied any vertebrate hosts (mosquitofish, chorus frog tadpoles, and newts) to quantify the number of encysted parasites, thereby allowing us to evaluate the roles of infection and consumption as two distinct mechanisms. We also dissected a subset of damselflies ($N = 3$), clam shrimp ($N = 5$), and clams ($N = 5$) 2 d after completing the trials to confirm that metacercariae did not infect invertebrates (Schotthoefer et al. 2007). Lastly, we explored the propensity of two predators to eat cercariae in the presence of alternative prey. Damselfly nymphs (*Enallagma* spp.) and mosquitofish were offered cercariae and *Daphnia middendorffiana* simultaneously at three prey densities. We used a prey selectivity index to quantify preference (see Appendix C; Chesson 1983).

Transmission experiments

Building from the laboratory bioassays, we selected three of the species most effective at removing *R. ondatrae* cercariae to evaluate their effects on parasite transmission to chorus frog tadpoles. We exposed individual tadpoles to 30 cercariae either alone (control) or in the

presence of an individual damselfly (*Enallagma* spp. or *Lestes* sp.), mosquitofish, or newt in 1 L of water. Trials lasted for 1 h with 16 replicates of each treatment (sizes of all species provided in Appendix D). Because these species could be amphibian predators and thus alter the behavior of tadpoles with potential effects on infection (Thiemann and Wassersug 2000, Gunzberger and Travis 2005), we also included effluent treatments in which we added cercariae and 50 mL of water from containers housing damselflies ($N = 8$ replicates), mosquitofish ($N = 8$ replicates), or newt ($N = 16$ replicates). Forty-eight hours after the end of each trial, we quantified metacercariae within chorus frog tadpoles, newts, and mosquitofish to evaluate the relative contributions of predation-mediated, behavior-mediated, and infection-mediated changes in transmission to chorus frogs (see Appendix E for necropsy procedures). To examine whether infection patterns from laboratory transmission trials were consistent with field infection patterns, we collected newts ($N = 46$), mosquitofish ($N = 25$), and chorus frogs ($N = 170$) from 10 sites in California. Specimens were necropsied and examined for *R. ondatrae* metacercariae as described in the previous section.

Statistical analysis

We used generalized linear mixed models (Zuur et al. 2009) to test the effects of each species in the bioassays on the fate of each administered parasite (removed [eaten or transmitted] vs. remaining at the end of 30 min). Specifically, we used the R package lme4 and the Laplace approximation method (R Development Core Team 2008) with a binomially distributed error and logit-link function to evaluate the influence of species (main effect) with individual predator identity as a random effect to account for variation beyond what we could control (i.e., the fate of parasites within the same trial may not be independent due to predator satiation, individual differences among predators, and so on). To further differentiate the relative roles of infection and consumption for vertebrates in the bioassay, we analyzed the fate of removed cercariae (encysted or not) with species as a main effect and individual predator identity as a random effect. To evaluate the roles of predators and alternate hosts on *R. ondatrae* transmission to chorus frogs and total infection, we used ANCOVA with tadpole stage as a covariate and damselflies, mosquitofish, or newts as fixed effects. For field and laboratory data, we used paired *t* tests to compare infections between co-occurring species (e.g., newts or mosquitofish and chorus frogs). Additionally, we conducted an unpaired *t* test on the ratios of infection in newts and chorus frogs to assess whether laboratory infections differed from those in the field.

RESULTS

Predation experiments

We recovered all cercariae in control treatments without predator or alternative host species present

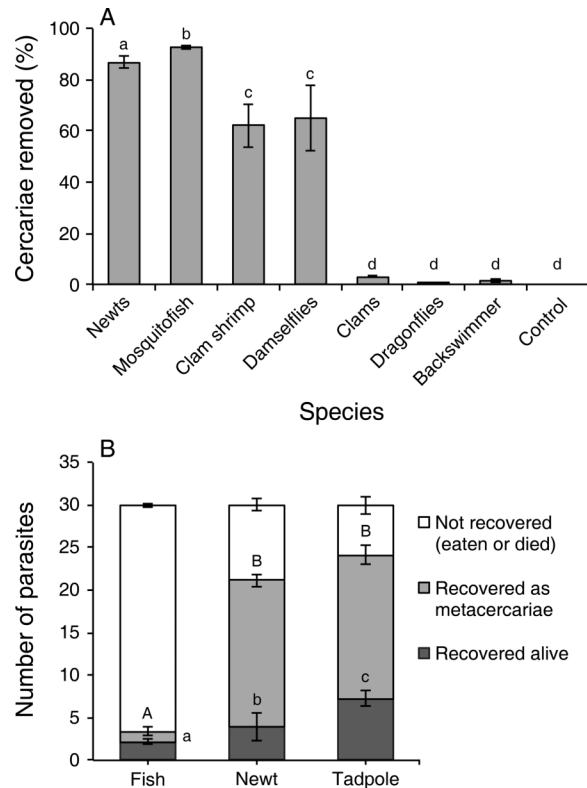


FIG. 1. (A) Mean percentage of 30 *Ribeiroia ondatrae* cercariae removed by a single alternative host or predator. (B) Mean number of *R. ondatrae* cercariae that were recovered alive after a 30-minute trial (lowercase letters), the average number of cercariae recovered as metacercariae (uppercase letters) from each species, and the number eaten or died. Different letters represent statistically significant differences at $P < 0.05$. Error bars represent \pm SE.

(Fig. 1A), illustrating the effectiveness of our method in tracking parasites. Among bioassayed species, parasite removal varied significantly, ranging from 0% to 93% (Fig. 1A). Four of the seven tested species removed $>60\%$ of parasites, with the greatest removal by mosquitofish ($Z = 12.221$, $P < 0.0001$), followed by newt larvae ($Z = 10.435$, $P < 0.0001$), damselflies ($Z = 7.458$, $P < 0.0001$), and clam shrimp ($Z = 7.573$, $P < 0.0001$; Fig. 1A). Dragonfly nymphs, backswimmers, and clams had no significant effect on cercarial abundance ($Z < 1.116$, $P > 0.264$; Fig. 1A). We also observed differences in whether effects stemmed from consumption, infection, or both. While damselflies, clam shrimp, and clams showed no evidence of infection at the end of trials, $\sim 57\%$ of administered cercariae were recovered as metacercariae from both chorus frogs and newts, with no significant difference between the two species ($Z = -0.174$, $P = 0.862$; Fig. 1B). Interestingly, however, the total recovery of parasites (sum of leftover cercariae and encysted metacercariae) was significantly lower for newts than chorus frogs ($Z = -2.817$, $P = 0.005$; Fig. 1B). Although we cannot differentiate failed

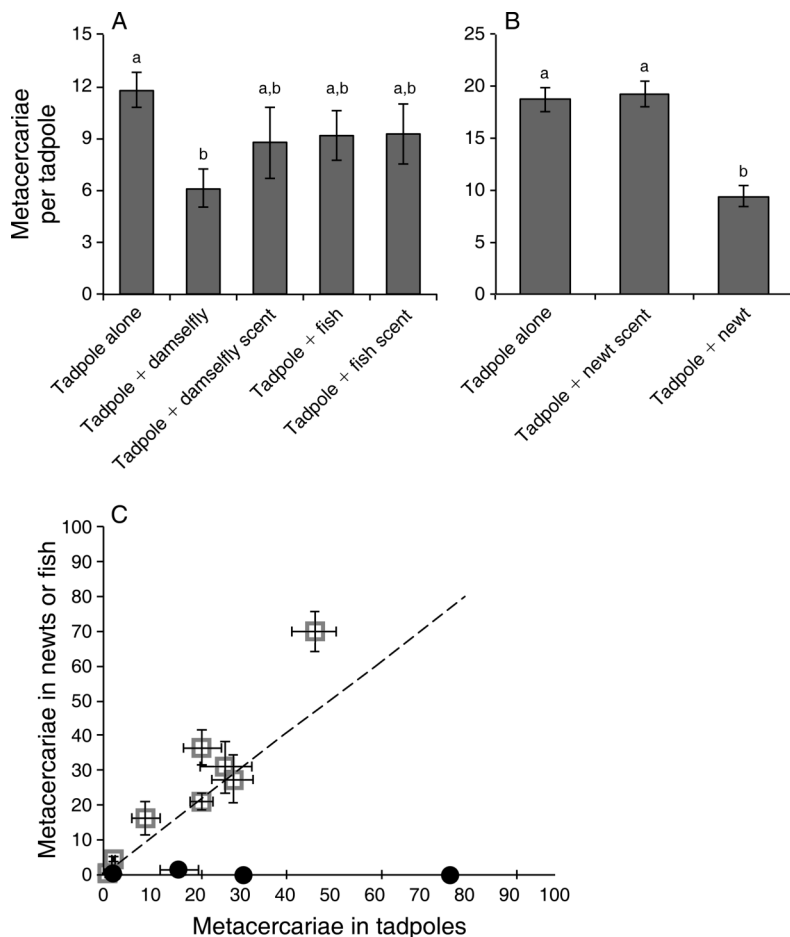


FIG. 2. (A) Mean number of *Ribeiroia ondatrae* metacercariae recovered from Pacific chorus frog (*Pseudacris regilla*) tadpoles following exposure to cercariae alone or in the presence of an individual damselfly nymph or mosquitofish (*Gambusia affinis*), or 50 mL of damselfly effluent or mosquitofish effluent (or “scent,” as 50 mL of water from the container used to maintain the individual predators). (B) Metacercariae recovered from tadpoles following exposure in the presence of an individual California newt (*Taricha torosa*) larvae, or 50 mL of newt effluent, or control with newt absent. Note differences between y-axes. Different letters represent statistically significant differences $P < 0.05$. (C) Mean number of *R. ondatrae* metacercariae recovered from mosquitofish (circles) and California newt larvae (squares) plotted against the number of metacercariae in co-occurring Pacific chorus frog tadpoles collected from 10 sites in the San Francisco Bay Area of California, USA, in 2010. The dashed line represents the 1:1 relationship of equal infection intensity in both species. Error bars represent \pm SE.

infection from consumption, metacercariae were frequently recovered from the oral cavity of newts, suggesting that consumption may have been responsible for the difference. For mosquitofish, $<5\%$ of removed cercariae were recovered as metacercariae, suggesting that the primary mechanism was consumption ($Z = -9.007$, $P < 0.0001$; Fig. 1B). This notion is further supported by direct observations of mosquitofish consuming cercariae and the low number of cercariae tails recovered at the end of the trials (1.3 ± 0.1 [mean \pm SE]), which are indicative of attempted infection. Finally, damselflies and mosquitofish in the alternative prey trials still consumed cercariae in the presence of *Daphnia*; mosquitofish consumed all available prey (cercariae and *Daphnia*), while damselflies ate $\sim 33\%$ of

each prey type in proportions similar to their availability (see Appendix C for detailed results).

Transmission experiments

Damselflies reduced transmission of *R. ondatrae* to tadpoles by $\sim 50\%$ compared to trials with tadpoles alone ($F_{4,59} = 2.67$, $P = 0.041$; Tukey post hoc, $P = 0.015$; Fig. 2A). There was no difference in tadpole infection between treatments with damselflies and those with damselfly effluent (Tukey post hoc, $P = 0.735$). Mosquitofish did not influence transmission relative to controls (Tukey post hoc, $P = 0.564$) or their effluent (Tukey post hoc, $P = 1.000$). We recovered an average of 0.4 ± 0.2 metacercariae per fish and 9.2 ± 1.4 in tadpoles, indicating a strong preference of *R. ondatrae* to infect tadpoles in the presence of both species. Newts

also reduced transmission by ~50% relative to tadpoles alone or with newt effluent ($F_{3,47} = 25.99$, $P < 0.0001$; tadpole stage [covariate], $P = 0.0003$; Tukey post hoc, $P < 0.0001$; Fig. 2B). However, newts exhibited comparable infections relative to cohabitating tadpoles ($P = 0.094$), and the sum of metacercariae in both newts and tadpoles did not differ significantly from control tadpoles ($P = 0.851$). Trials with damselflies and fish were analyzed separately from trials with newts because there was a significant difference between infection intensity in the controls between the two experiments ($t_{31} = 4.43$, $P = 0.0001$; Fig. 2A and B), likely arising from variation between years.

Average *R. ondatrae* infection in mosquitofish collected from natural wetlands ranged from 0.2–1.2 metacercariae per fish compared with 2.3–76.6 metacercariae per chorus frog from the same wetland, representing a 193% average difference between the species (paired *t* test, $t_3 = 3.88$, $P = 0.030$; Fig. 2C). However, newt infection intensity did not differ from co-occurring chorus frogs ($t_8 = -0.86$, $P = 0.417$; Fig. 2C), with an overall average of 23.0 ± 2.4 metacercariae per newt and 17.7 ± 5.2 per tadpole across sites. In addition, the ratios of infection between the laboratory trials and the field data were comparable ($t_{24} = 0.80$, $P = 0.429$).

DISCUSSION

Our results indicate that diverse members of ecological communities consume or interfere with the movement of pathogenic trematode cercariae to sensitive amphibian hosts. By carefully tracking the fate of each administered parasite in laboratory trials, we were able to experimentally assess multiple mechanisms of the dilution effect, including predation on infectious stages, the role of alternative hosts, and the combination of the two. Through laboratory bioassays informed by field data, we identified a range of vertebrate and invertebrate predators with varying abilities to remove cercariae. Several feeding modes were effective for consuming cercariae, including active (mosquitofish), ambush (damselfly larvae), and filter feeding (clam shrimp) (see also Schotthoefler et al. 2007, Thieltges et al. 2008, Kaplan et al. 2009). Subsequent bioassays with mosquitofish and damselflies showed that both predators consumed cercariae when offered *Daphnia middendorffiana* as alternative prey, suggesting that cercariae are likely to be consumed even in complex communities with multiple prey species. Because we tracked the fate of each parasite to determine whether it was removed (consumed or died), became encysted, or remained free-swimming at the end of trials, our bioassays allowed clear differentiation among potential dilution mechanisms.

Building on bioassay results, our transmission experiments showed that predators and alternative hosts reduced *R. ondatrae* transmission to sensitive tadpole hosts by as much as 50%. The presence of newts did not reduce the cumulative recovery of administered parasites

(summed among hosts), but altered their fate, diverting infection from chorus frogs to newts, which are less-suitable hosts (see next paragraph). Damselflies consumed a substantial fraction of administered parasites and thus prevented them from infecting tadpole hosts. Because chorus frogs are highly sensitive to *R. ondatrae* infection, often exhibiting mortality or developmental malformations (Johnson et al. 1999, 2002, Goodman and Johnson 2011), such changes in transmission could have important effects on host fitness in nature. Surprisingly, mosquitofish, which had strong effects in the bioassay trials, did not significantly alter transmission when paired with a tadpole. This result may stem from methodological differences between the bioassay and transmission trials; the transmission trials used a large container volume but the same number of cercariae, such that the lower cercarial density could explain observed patterns if fish predation is density dependent (Murdoch et al. 1975). Alternatively or additionally, parasites could have infected the tadpole before fish were able to consume them, effectively nullifying the role of predation. Predator effluent did not alter parasite transmission, although predators were not fed tadpoles prior to effluent collection, which can influence behavioral responses (Relyea and Werner 1999).

Our laboratory results have important implications for natural communities. Data from natural wetlands indicate that many of the examined species commonly co-occur with *R. ondatrae*, and thus, have the potential to influence transmission. In particular, members in this community may influence disease because *R. ondatrae* is known to cause mortality and malformations in a variety of amphibian species (Johnson et al. 2002). Our field data from California wetlands indicated that newts commonly co-occur with chorus frogs and often support similar infection levels. Interestingly, however, *R. ondatrae* infecting newts might often be unsuccessful at reaching a definitive host because of the potent tetrodotoxin that prevents birds from preying on adult newts, thus inhibiting parasite transmission after the newt's larval stage (Brodie 1968, Mobley and Stidham 2000, Gunzberger and Travis 2005). Despite this, cercariae in laboratory trials showed no preference for chorus frog tadpoles over newt larvae. This is in contrast with the pattern of mosquitofish, which, despite co-occurring with chorus frogs and *R. ondatrae*, rarely became infected in the laboratory or the field, indicating a strong preference of the parasite for amphibians. Although fish can also mount an immune response against invading *Ribeiroia* cercariae (Huizinga and Nadakavukaren 1997), our laboratory experiments indicated clearly that parasites were preferentially infecting tadpoles rather than encysting on fish and being subsequently eliminated by immune activation. More research is needed to assess the relative importance of diversity, and of different dilution mechanisms, on transmission in natural communities.

The mechanism by which predators reduce parasite transmission helps to link the dilution effect and a growing interest in predation and disease dynamics. Predators have been considered important mediators of disease through their effects on susceptible host regulation or preferential removal of infected hosts (Packer et al. 2003, Ostfeld and Holt 2004, Johnson et al. 2006), or consumption of insect vectors (Moore et al. 2009). Our study further indicates that predation can alter transmission through removal of infective stages, consistent with previous studies that have identified predation as an important mechanism of the dilution effect (Hopper et al. 2008, Thieltges et al. 2008, Prinz et al. 2009, Johnson et al. 2010). Predator–parasite links in food webs, which are common in many systems, can have important consequences for predator diets and resource flow (Kuris et al. 2008, Lafferty et al. 2008), such that quantifying the consumption of parasites in nature presents a promising future research direction (Kaplan et al. 2009). Taken together, these results demonstrate that, alongside the host community, predator community composition can strongly influence patterns of infection. Given widespread increases in diseases of humans and wildlife concurrent with rapid losses in biodiversity (Daszak et al. 2000), investigating changes in community composition has important consequences for understanding pathogenic infections in diverse taxa (Keesing et al. 2010).

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SUPPLEMENTAL MATERIAL

Appendix A

Methods for field surveys of aquatic species including target host amphibians, alternatives, alternative hosts, and predators (*Ecological Archives* E093-107-A1).

Appendix B

Methods for collection and maintenance of amphibians, fish, and aquatic invertebrates used in laboratory experiments (*Ecological Archives* E093-107-A2).

Appendix C

Methods and results of alternative prey bioassays conducted in the laboratory with damselfly and fish predators (*Ecological Archives* E093-107-A3).

Appendix D

Data on the numbers, sizes, and stages of species used in laboratory transmission trials (*Ecological Archives* E093-107-A4).

Appendix E

Methods used for euthanasia and necropsy of vertebrates collected from the field and used in laboratory experiments (*Ecological Archives* E093-107-A5).