



Chance or choice? Understanding parasite selection and infection in multi-host communities



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ABSTRACT

Ongoing debate over the relationship between biodiversity and disease risk underscores the need to develop a more mechanistic understanding of how changes in host community composition influence parasite transmission, particularly in complex communities with multiple hosts. A key challenge involves determining how motile parasites select among potential hosts and the degree to which this process shifts with community composition. Focusing on interactions between larval amphibians and the pathogenic trematode *Ribeiroia ondatrae*, we designed a novel, large-volume set of choice chambers to assess how the selectivity of free-swimming infectious parasites varied among five host species and in response to changes in assemblage composition (four different permutations). In a second set of trials, cercariae were allowed to contact and infect hosts, allowing comparison of host-parasite encounter rates (parasite choice) with infection outcomes (successful infections). Cercariae exhibited consistent preferences for specific host species that were independent of the community context; large-bodied amphibians, such as larval bullfrogs (*Rana catesbeiana*), exhibited the highest level of parasite attraction. However, because host attractiveness was decoupled from susceptibility to infection, assemblage composition sharply affected both per-host infection as well as total infection (summed among co-occurring hosts). Species such as the non-native *R. catesbeiana* functioned as epidemiological ‘sinks’ or dilution hosts, attracting a disproportionate fraction of parasites relative to the number that established successfully, whereas *Taricha granulosa* and especially *Pseudacris regilla* supported comparatively more metacercariae relative to cercariae selection. These findings provide a framework for integrating information on parasite preference in combination with more traditional factors such as host competence and density to forecast how changes within complex communities will affect parasite transmission.

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1. Introduction

Recent interest in the relationship between biodiversity and disease risk underscores the importance of understanding patterns of parasite transmission in complex communities comprised of multiple host species (Ostfeld and Keesing, 2013; Johnson et al., 2015a; Halliday et al., 2017). Many parasites can infect multiple host species, even in the same community, yet those hosts often vary in their capacity to become infected and subsequently support or transmit infection (i.e., competence). While some species are highly susceptible and facilitate parasite persistence or spread, others function as epidemiological ‘dead-ends’, distracting infectious stages away from more suitable hosts (Kilpatrick et al., 2006; Keesing et al., 2009; Strauss et al., 2015). This variation

may be especially important for parasites that depend on vectors or use short-lived infectious stages; because each vector or parasite uses only a single (or small number of) hosts, an infection (or bite) of a low-competence host species comes at the direct cost of infecting a more suitable host species. As a result, shifts in biodiversity and community composition can influence the likelihood of a parasite or vector encountering a highly competent host species rather than a low-competence host (Levine et al., 2017). Even in the absence of changes in overall host density, which are traditionally emphasised in epidemiological research, compositional shifts within host communities therefore have the potential to markedly alter transmission and infection load (Civitello et al., 2015). When these changes lead to an increase in transmission in response to higher diversity, it is termed an amplification effect; reciprocally, reductions in infection as diversity increases are referred to as dilution effects (Keesing et al., 2006; Johnson et al., 2015b).

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A key mechanism of diversity-mediated changes in infection involves encounter reduction, when the addition of less susceptible host species reduces the rate of encounter between infectious stages and suitable hosts, regardless of host density (Keesing et al., 2006). Central to understanding whether biodiversity affects transmission is knowledge of the extent to which parasites select hosts. If parasites or vectors selectively infect the hosts that are the most competent, even in the presence of alternative ‘decoy’ hosts, biodiversity changes may have little effect on transmission. Conversely, a disconnect between the attractiveness of host species and their suitability for infection can lead to ‘ecological traps’ for parasites or vectors (Keesing et al., 2009). Although encounter rates are often assumed to be a function of host density or frequency, parasites and vectors can also exhibit non-random or even targeted selection of hosts. Among vectors such as mosquitoes, for instance, host-feeding behaviour may differ dramatically from the availability of hosts in the community (Kilpatrick et al., 2006; Hamer et al., 2009; Simpson et al., 2009). For the mosquito *Culex pipiens* in northeastern USA, Kilpatrick et al. (2006) reported that American robins constituted 43.4% of blood meals, despite accounting for only ~3.7% of avian abundance. Because bird hosts also vary in their competence to support West Nile virus, these effects have the potential to influence community-scale patterns of transmission and amplification.

The challenges of finding a suitable host are particularly acute for parasites that depend on free-living infectious stages to transmit among host individuals or species. These stages are often relatively short-lived and confront physical as well as biological challenges associated with the surrounding environment (Selbach and Poulin, 2018). For instance, motile aquatic trematode (flatworm) infectious stages such as miracidia and cercariae, often have <24 h to infect a host and are vulnerable to predators as well as adverse environmental conditions (Pietrock and Marcogliese, 2003; Thieltges et al., 2008; Johnson et al., 2010). Experimental research has illustrated the capacity of such stages to use physical and chemical cues associated with the host (e.g., vibrations, shadows, organic molecules) or the environment (light, gravity, water currents) to increase the probability of a successful host encounter (Feiler and Haas, 1988; Kalbe et al., 1997, 2000; Haas, 2003). Less well understood, however, is the degree to which infectious stages can reliably differentiate among an entire assemblage of alternative hosts. Most previous work has been conducted with small volumes using host chemical cues, often in pairwise choice experiments, limiting opportunities to evaluate how host attractiveness depends on what other species are present within the community. Given that simple pairwise choice tests may offer little predictive validity in scenarios with more than two host options, as is likely common in natural systems, experiments that can identify how parasites select hosts within more complex choice sets – and whether such preferences are consistent or context-dependent – are a valuable step forward in understanding parasite selection (Luce, 1959; Real, 1991; Schuck-Paim et al., 2004). For instance, research on prey selection by predators has long emphasised the importance of using multiple and varied ‘choice sets’ to experimentally identify prey preference, which frequently varies strongly as a function of the possible choices (Stephens and Krebs, 1986).

Broadly, this raises the question: to what extent do parasites select hosts at random (i.e., in proportion to their relative availability) versus preferentially in relation to their suitability for infection (see Sears et al., 2012; Alacid et al., 2016)? This question is directed at identifying the processes underlying encounters between parasites and hosts in natural systems composed of multiple, alternative host species. From an evolutionary standpoint, the degree to which parasites are able to select among alternative hosts will depend on the availability of cues to reliably differentiate host species, the amount of time or energy available for host searching, the

relative fitness costs of infecting a low quality host, and the genetic or physiological mechanisms available for such specificity (Wojdak et al., 2013; Seppälä and Leicht, 2015; Forbes et al., 2017). Previous investigations have found mixed evidence for host selection in relation to host competence. While some studies have reported a positive relationship between host susceptibility and parasite or parasitoid attraction (e.g., Sears et al., 2012; Han et al., 2013), many other parasites select hosts that are of low competence or completely unsuitable (e.g., Driessen et al., 1990; Kimura and Suwito, 2014; Langeloh and Seppälä, 2018) – an outcome with implications for biocontrol applications (e.g., Goldson et al., 1992). The avoidance of already infected individuals by parasites – presumably as a mechanism to reduce intra-host competition – has also been demonstrated across a range of systems including parasitoid wasps, entomopathogenic nematodes, and trematode miracidia (e.g., Grewal et al., 1997; Allan et al., 2009; Ruschioni et al., 2015).

The goals of the current study were to quantify the selectivity of cercariae of the pathogenic trematode, *Ribeiroia ondatrae*, among alternative larval amphibian host species that vary in competence. Previous work has indicated that amphibian host diversity can inhibit *R. ondatrae* transmission and the risk of disease (e.g., Johnson et al., 2013), yet relatively little is known about how parasites select hosts or the degree to which such choices are linked to host competence. Focusing on interactions between larval amphibians and their trematode parasites, we first used experimental choice trials to assess how the selectivity of cercariae for a given host species varied as a function of what other hosts were available using four alternative community permutations. In a subsequent set of trials, cercariae were allowed to contact and infect hosts, thereby providing an opportunity to compare host-parasite encounter rates (parasite choice) with infection outcomes (number of successful infections per host). To help understand the factors influencing parasite attraction, we contrasted among models using host species identity, host biomass, and community composition. Our questions are therefore relevant for predicting whether parasites encounter hosts selectively or in accordance with their density or frequency, as commonly assumed, and for identifying particular species that contribute disproportionately to parasite loss or persistence relative to encounter rate (i.e., dilution or amplification hosts).

2. Materials and methods

2.1. Constructing choice chambers

To test cercariae selection for larval amphibian hosts, we used choice arenas (Carolina Biological Supply, USA; 143054P) including four circular chambers (15.24 cm circumference, 2.5 cm depth) connected to a central acclimation compartment. Removable gates regulate the timing and direction that test organisms placed into the central compartment are allowed to travel (see Fig. 1). To allow water (but not parasites) to move freely through the gates, we drilled a hole (1.016 cm diameter) in each gate and covered it with 11 µm nitex mesh that was attached using silicone. Cercariae of *R. ondatrae* are approximately 800 µm long (including tail) and 200 µm wide (Beaver, 1939; Johnson et al., 2004). This ensured that chemical cues from hosts in each chamber could move into the acclimation compartment and that water levels on each side of the gate were equal. This design was modelled after that used by Sears et al. (2012) with the following modifications: we used much larger chambers to allow for greater host and parasite movement (5 mL versus 1.3 L), we included multiple, alternative configurations of the host community to test whether parasite selection was context-dependent (different ‘choice sets’), and we contrasted the influence of host choice set on parasite choice and parasite

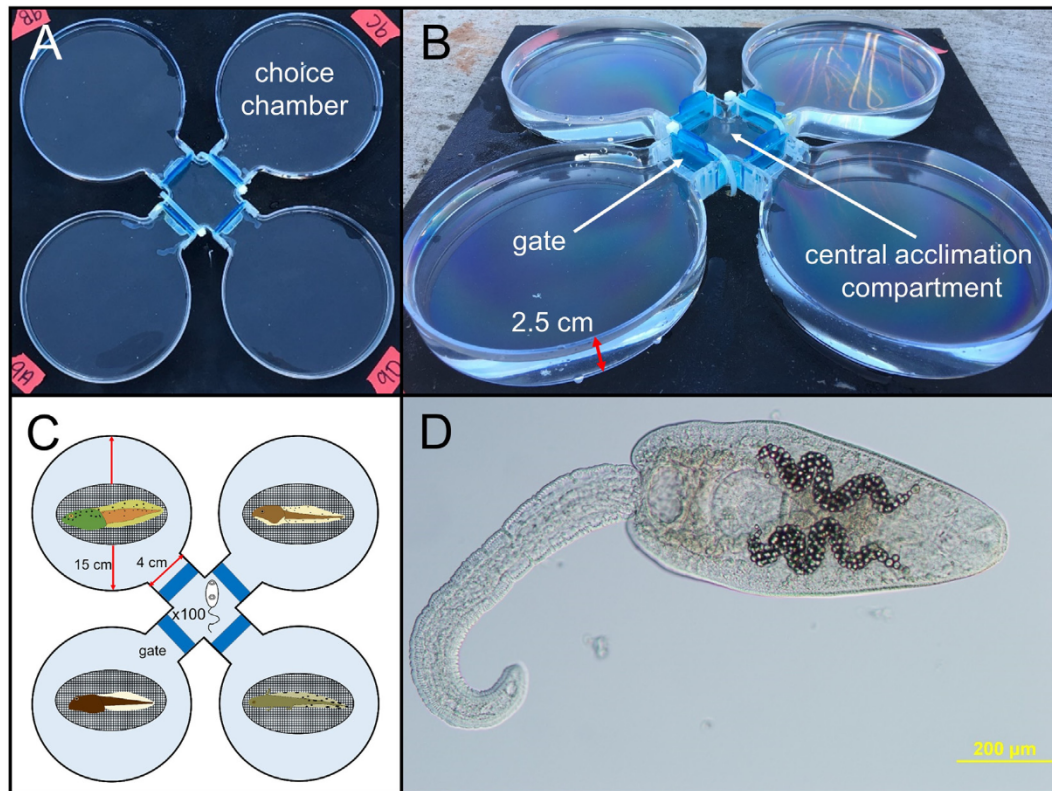


Fig. 1. Experimental setup of host selection trials by cercariae of *Ribeiroia ondatrae*. (A) Photograph and (C) illustration of choice chambers used to evaluate preference for amphibian host species. Into each choice chamber, a single amphibian larva was placed within a cage (14 μ m mesh) that allowed host chemical and physical cues into water but no direct parasite contact. Each trial included larvae of four amphibian species selected from the pool of five species (i.e., *Pseudacris regilla*, *Anaxyrus boreas*, *Rana catesbeiana*, *Taricha torosa*, and/or *Taricha granulosa*). In a second set of trials, the cage mesh was 1000 μ m, allowing cercariae to contact and infect hosts. Procedures were the same with the exception that 75 cercariae were used. (B) A side view of the choice chambers. (D) An isolated cercaria of *R. ondatrae*.

infection using the same experimental design. This further afforded an opportunity to assess how the host choice set affected total infection success (defined as the number of established infections among co-occurring hosts).

2.2. Experimental design and execution

By conducting host selection trials that presented cercariae with the physical and chemical cues of alternative larval amphibian hosts, we measured ‘choice’ as the directional movement of parasites into one of four chambers after 60 min. Into each chamber we placed an individual larva of one of five amphibian species: Pacific chorus frog (*Pseudacris regilla*), California newt (*Taricha torosa*), rough-skinned newt (*Taricha granulosa*), western toad (*Anaxyrus boreas*), and American bullfrog (*Rana catesbeiana*). Collectively, these are the most common wetland-breeding amphibians in the Bay Area region of California, USA, where infections by the trematode *R. ondatrae* are common (Johnson and Wilber, 2017). All of these species are susceptible to infection by the trematode *R. ondatrae*, albeit to varying degrees (Johnson et al., 2013, 2012). *Ribeiroia ondatrae* cercariae were identified morphologically using Schell (1985) and Johnson et al. (2004). Amphibians were either raised from field-collected egg masses (*P. regilla*, *T. torosa*, *A. boreas*) or obtained as larvae from ponds where the trematode *R. ondatrae* does not occur (*T. granulosa*, *R. catesbeiana*).

Four alternative host configurations were comprised by drawing from the pool of five naturally co-occurring amphibians. Thus, while each arena included four amphibian larvae, each of which represented a different species in each chamber, we created four

different alternative combinations from the available species (e.g., species A, B, C, and D versus species A, B, C, and E, and so on). The inclusion of alternative compositions was used to evaluate the degree to which parasite choice was context-dependent. Each combination was replicated six times. Within a given trial, the species of amphibian, its individual identity, and the cardinal direction of the arena were randomly determined (i.e., whether chamber A was facing North, East, West, or South). Each was filled with 1.3 L of treated tapwater (dechlorinated, carbon-filtered, and UV-sterilised; hereafter reported as ‘treated tapwater’) and tested to make sure its orientation was level. Amphibian hosts were placed into cages (6.10 cm diameter) covered with 14 μ m mesh and positioned in the centre of each chamber. Pilot experiments verified that cercariae were unable to navigate through the mesh and infect amphibians. After a 30 min period to allow the establishment of host chemical cues, 100 cercariae of *R. ondatrae* were added into the central acclimation compartment (Fig. 1). Cercariae were collected within 4–5 h of emergence from naturally infected snails, pooled together, and transferred into the central compartment while the gates were closed. Cercariae were allowed 10 min to acclimate before we removed the gates, thereby connecting the acclimation and choice chambers. Trials were conducted for 60 min in the dark (corresponding to the light levels when this parasite is naturally active; Hannon et al., 2017) to give cercariae an opportunity to move toward one of the four included hosts in each trial.

After 1 h, we closed the gates, removed the cages containing amphibian hosts, and used a boom-arm stereo-dissecting microscope (Bausch and Lomb Model 190) to examine the water in each chamber for cercariae (including any in the central acclimation

compartment). Cercariae were counted as they were pipetted into a separate petri dish and the totals were verified under a microscope after all cercariae had been removed. Amphibian hosts were measured (snout-vent length) and massed (g). In a subsequent set of trials ('infection trials'), we repeated the experiment but modified the mesh size of the cages from 14 μm to 1000 μm to allow cercariae to infect the hosts that they had selected. This included two replicates of each four-host species combination used above ($n = 8$ trials). Parasites were administered as described previously, with the exception that 75 rather than 100 cercariae were used to help ensure that no hosts died during the trials (*R. ondatrae* cercariae can be highly pathogenic). After the 1 h selection period, we re-inserted the gates but allowed an additional 5 h for cercariae within the choice chambers to complete the infection process and encyst. Hosts were then transferred to individual 1.0 L containers filled with 500 mL of treated tapwater before being necropsied to quantify encysted metacercariae after 196 h. Hereafter we differentiate these as "infection trials" versus "choice trials". Finally, we conducted five additional infection trials in which all presented hosts were of a single species (i.e., monospecific trials with four individuals of a given species per trial, one trial for each of the five host species) using the same methods described above. These trials were designed to provide complementary information on patterns of infection and its heterogeneity when host species identity was fixed.

2.3. Analysis

For the choice trials, our primary response variable was the number of cercariae found in each chamber, which we modelled as an overdispersed Poisson distribution (Elston et al., 2001; Harrison, 2014). This approach involves inclusion of a random intercept term for each observation to accommodate the "extra" variation beyond what would be expected with a Poisson, and is thus functionally similar to a negative binomial distribution with a shape parameter. To test whether parasite choice differed from host availability (i.e., from the null expectation of 0.25 for each of the four hosts), we included fixed effects for the species identity of the host in each chamber (as a factor), host body mass (z-score transformed by species so as not to be confounded with species identity), and the assemblage composition (i.e., the identity of the community configuration, as a factor). We also included an interaction between species identity and assemblage composition to assess whether cercariae selection for a species depended on what other hosts were included. Trial was added as a random intercept term to account for the non-independence of chambers within the same trial. To account for differences in the number of parasites per trial because some cercariae remained in the central compartment even after 60 min, we used an offset term to adjust observed parasite counts to these modified totals (as the log of the number of cercariae added minus any that failed to make a choice), although removing the offset did not appreciably alter the influence of included variables.

After fitting the full model, we used likelihood-ratio tests (LRT) to remove non-significant terms (i.e., $P > 0.05$) and identify the minimum adequate model using the anova function. As alternative models, we also tested the explanatory power of (i) host biomass (without z-score transformation) instead of species identity, and (ii) whether bullfrogs were present in the trial (yes or no) instead of assemblage composition, using LRT to compare these against the original models described in Section 2.2. For trials involving parasite infection rather than parasite choice, we used a similar analytical approach in which the response variable was the number of established metacercariae within a host. The total number of detected metacercariae (summed among all four co-occurring hosts in a trial) was used as the offset, thereby focusing the analy-

sis on the fraction of successful parasites found in each host species (see Fig. 2). The fixed and random effects were identical to those discussed above with the aim of assessing whether infection varied non-randomly among hosts or assemblage composition. Because individual hosts were not re-used between the choice and infection trials, we were unable to test the role of individual level heterogeneity (see Sears et al., 2012). Finally, we evaluated how assemblage composition affected the total number of metacercariae summed among co-occurring hosts in the same treatment (with an offset for the number of administered cercariae minus any that remained after the trial). All models were constructed using the glmer function from the lme4 package (Bates, D., Maechler, M., Bolker, B., Walker, S., 2013. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5) in the R computing environment (R Core Team, 2014. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. RDC TeamR: A language and environment for statistical computing. Vienna, Austria. <http://www.R-project.org>).

To test how cercariae choice covaried with infection success, we subsequently combined the two experiments in a single analysis: parasites per chamber was the response (which involved cercariae for the choice trials and metacercariae for the infection trials), with fixed effects of the trial type ('infection' or 'choice', mesh size of 1000 μm or 14 μm , respectively), host species identity (at the chamber level), assemblage composition (at the trial level), and targeted interactions (trial type $\times$ species and trial type $\times$ assemblage composition). The same offsets listed above were used here. Our primary interest was in whether the effect of host species differed in magnitude or direction between the choice trials and infection trials (i.e., the trial type $\times$ species interaction). Stated another way, were the hosts selectively favoured by cercariae also the most susceptible hosts with the highest infection loads? If host species were comparably susceptible, the fraction of successful infections in a given species should broadly mirror patterns of cercarial choice given the consistency in experimental apparatus; if, however, host attractiveness covaried positively or negatively with susceptibility, we expected sharp differences in both the fraction of successful infections associated with a particular host species as well as the total infection load (summed among all hosts) (see alternative hypothesised outcomes in Fig. 2).

3. Results

Among the cercarial choice trials, we recovered an average ± 1 SE of $91.6\% \pm 0.2\%$ of the administered cercariae. In most cases, $\sim 75\%$ of cercariae were found in the choice chambers with a host species; in two trials, however, 65% and 80% of cercariae remained in the central acclimation compartment. These trials were omitted from the analysis, leaving a total sample size of 21 trials and 84 hosts. Cercarial selection among choice chambers was strongly influenced by host species identity (LRT against intercept-only model; $\chi^2 = 26.85$, $\text{df} = 4$, $P < 0.0001$), with no added influence of host body mass (z-scored within species) or community composition (LRT results against model with species identity only, all $P > 0.05$). Based on pairwise contrasts using the Tukey-Kramer method, cercariae were significantly more attracted to bullfrog (*R. catesbeiana*) larvae compared with all other species, regardless of the overall community composition (all $P < 0.005$) (Fig. 3A). None of the other pairwise differences among species were significantly different. After accounting for the random effects, the selection of cercariae for each host species (i.e., the exponentiated coefficients from a zero-intercept model (95% confidence intervals (CI)) were: *R. catesbeiana* (0.409 (0.308–0.542)), *A. boreas* (0.205 (0.151–0.275)), *P. regilla* (0.169 (0.130–0.220)), *T. granulosa* (0.137 (0.099–0.187)) and *T. torosa*

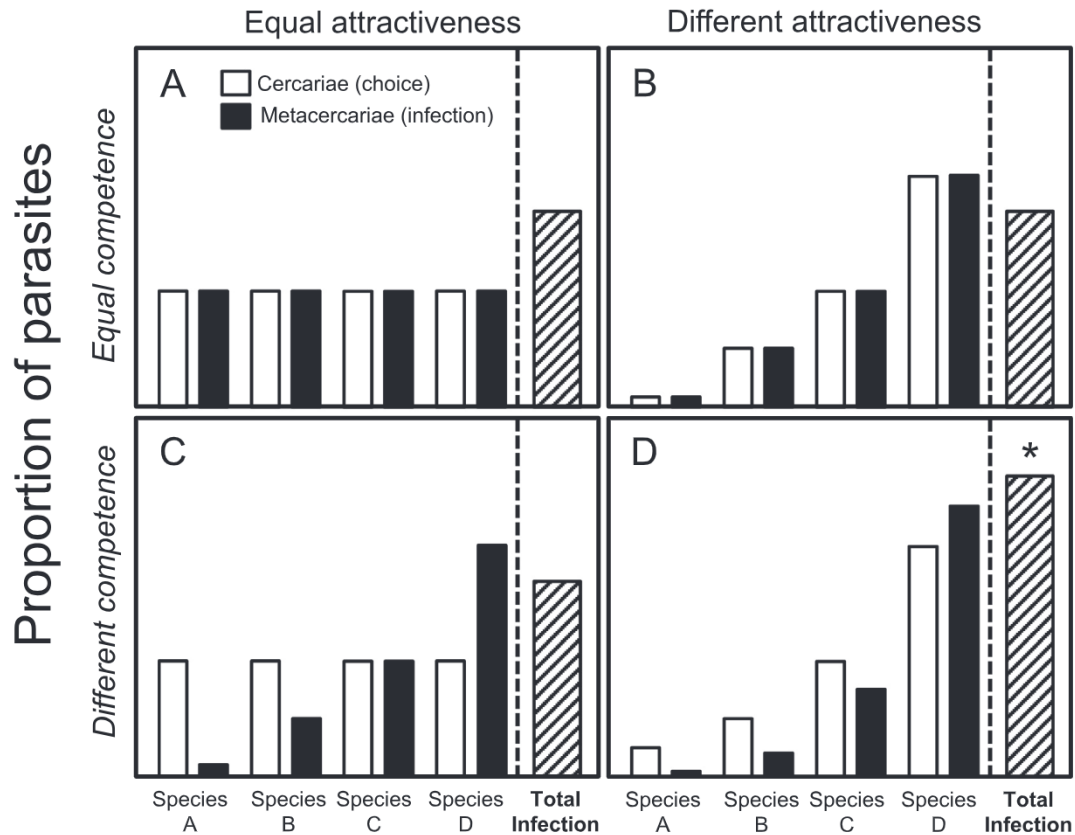


Fig. 2. Hypothesised outcomes of trials as a function of whether hosts vary in attractiveness to parasites ('no' in A and C; 'yes' in B and D) or in competence ('no' in A and B; 'yes' in C and D). Depicted are the proportions of cercariae selecting each host (white bars) and, of those cercariae establishing as metacercariae, the proportion found in each host (dark bars). (A) If parasites have no host preference, the fraction ending up in each choice chamber will be 0.25 (although this fraction could be determined by relative biomass rather than frequency). If there are differences among host species in attractiveness to cercariae (B), or competence to support infection (C), the number of established parasites per host will differ but the grand total (summed among co-occurring hosts) will be identical. If both attractiveness and competence differ, this can either amplify or mitigate effects on infection. When these traits correlate positively, as shown in D, the variance in infection among hosts as well as the total infection will be maximised (indicated by "*"). When traits correlate negatively, the most attractive hosts will be the least competent and total infection will be minimised (not shown).

(0.191 (0.141–0.257)). If cercarial selections for hosts were random based on host availability, these values should each be ~ 0.25 (i.e., parasites have a one in four chance of moving into a specific chamber). Because host species varied in body size, however, we also built a model with the log-transformed values of biomass instead of host species identity, which provided a better-fitting model (delta Akaike information criterion (AIC) = -5 ; log(host-mass): 0.25 ± 0.045 , $P < 0.00001$). This effect was driven by the large-bodied bullfrog larvae; analysing the single assemblage without bullfrogs yielded no effects of host biomass ($P > 0.5$, $n = 20$ observations from five trials).

In the infection trials, host species identity significantly influenced the fraction of detected metacercariae per host (LRT against intercept-only model; $\chi^2 = 14.114$, $df = 4$, $P < 0.01$). On average ± 1 SE, $27.59\% \pm 5.16\%$ of the 75 administered cercariae were recovered as metacercariae (summed among all hosts), although this fraction ranged among assemblages from ~ 7 to 68%. An additional 2.6% were found as leftover cercariae that failed to infect a host within the exposure period (and remained in the acclimation chamber). The highest infection loads were observed in *P. regilla* (7.12 ± 1.14 metacercariae per host (range: 3–14 metacercariae)), followed by *T. granulosa* (5.17 ± 3.17) (range: 1–21), *T. torosa* (4.83 ± 4.09) (range: 0–17), *R. catesbeiana* (1.67 ± 1.01) (range: 0–5), with the lowest loads in *A. boreas* (1.33 ± 0.60) (range: 0–5). Host species identity was also a significant predictor of the fraction of established metacercariae per amphibian (LRT against intercept-only model; $\chi^2 = 18.68$, $df = 4$, $P < 0.001$), for which

P. regilla supported a greater fraction than *A. boreas* and a marginally greater fraction than *R. catesbeiana* (Tukey-Kramer, $P < 0.001$ and $P = 0.053$, respectively). There was also an interaction between bullfrog presence and host species identity, such that the fraction of metacercariae in *P. regilla* was nearly three times greater in assemblages with bullfrogs than those without (LRT of bullfrog $\times$ species model against species identity only; $\chi^2 = 13.31$, $df = 4$, $P < 0.01$; see Fig. 3B). Bullfrog presence also led to a significant reduction in infection per host (bullfrog presence: -1.351 ± 0.366 , $P < 0.0005$; Fig. 4A) and total infection summed among hosts in the same trial (Poisson GLM: bullfrog presence: -1.143 ± 0.1722 , $P < 0.0001$; Fig. 4B). In the five monospecific trials, average infection loads were: *P. regilla* (mean ± 1 SE = 12.8 ± 5.04), *A. boreas* (7.5 ± 2.4), *T. torosa* (6.0 ± 4.02), *T. granulosa* (5.0 ± 1.68), and *R. catesbeiana* (2.25 ± 0.048), supporting the relatively high and low competence of *P. regilla* and *R. catesbeiana*, respectively.

In comparing the trials involving cercariae choice (choice trials) versus established infections (infection trials), we detected a trial type-by-species identity interaction (LRT of trial $\times$ species model against main effects only; $\chi^2 = 29.97$, $df = 5$, $P < 0.0001$). Thus, the fraction of parasites in a given amphibian species varied strongly between the choice trials (cercariae) and the infection trials (metacercariae) (see Fig. 5). The greatest disparities were for *R. catesbeiana* and *A. boreas*, each of which exhibited low levels of successful infections relative to the fraction of cercariae attracted. For instance, while *R. catesbeiana* attracted $44.6\% \pm 3.7\%$ of

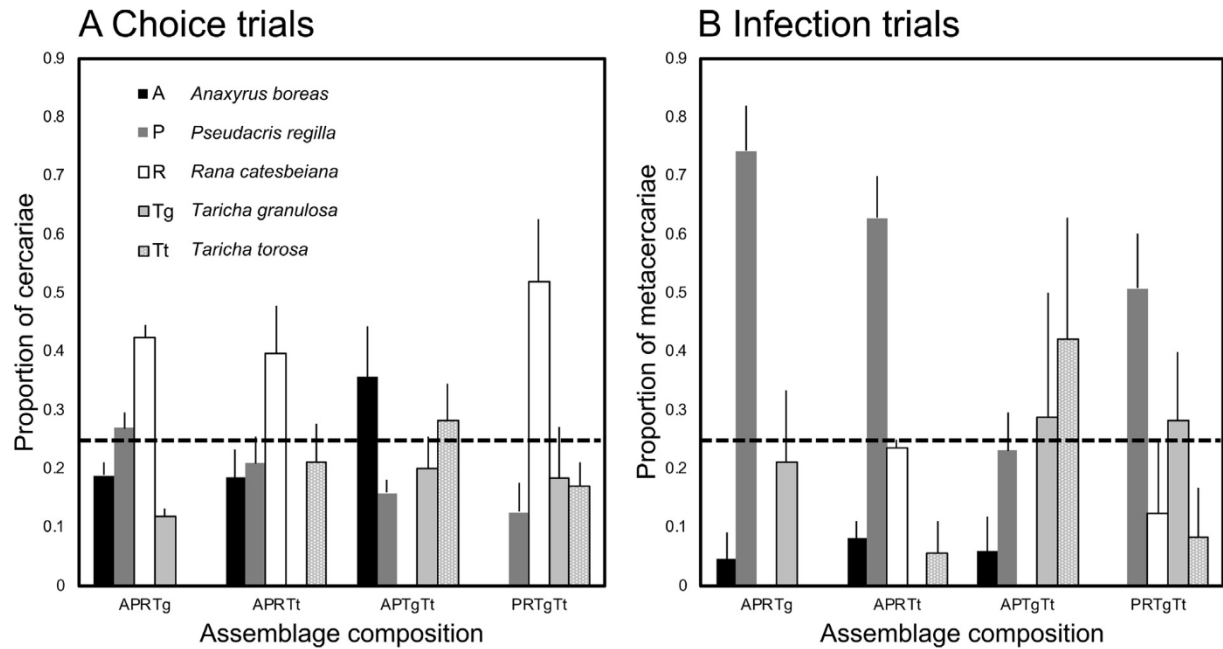


Fig. 3. Proportion of parasites (+1 SE) as a function of host species identity and assemblage composition for (A) the choice trials involving cercariae, and (B) the infection trials involving established metacercariae. For A, the depicted value is the fraction of administered cercariae detected within the choice chamber of a specific amphibian host (A, *Anaxyrus boreas*; P, *Pseudacris regilla*; R, *Rana catesbeiana*; Tg, *Taricha granulosa*; Tt, *Taricha torosa*). Cercariae that failed to make a choice (remained in the central compartment after 1 h) were omitted. Similarly, B represents the average proportion of metacercariae in a given host species from among the total number of infections summed across all four individual amphibians). For both types of trials, alternative combinations of host species (the assemblage composition) are included on the x-axis. Because only four species were included per trial, one of the five species is omitted from each assemblage composition (i.e., which appears as a blank). The expected proportion of parasites per host (cercariae or metacercariae) from a frequency perspective would be 0.25 (the dashed line), although this fraction could alternatively be determined based on relative biomass (see Results).

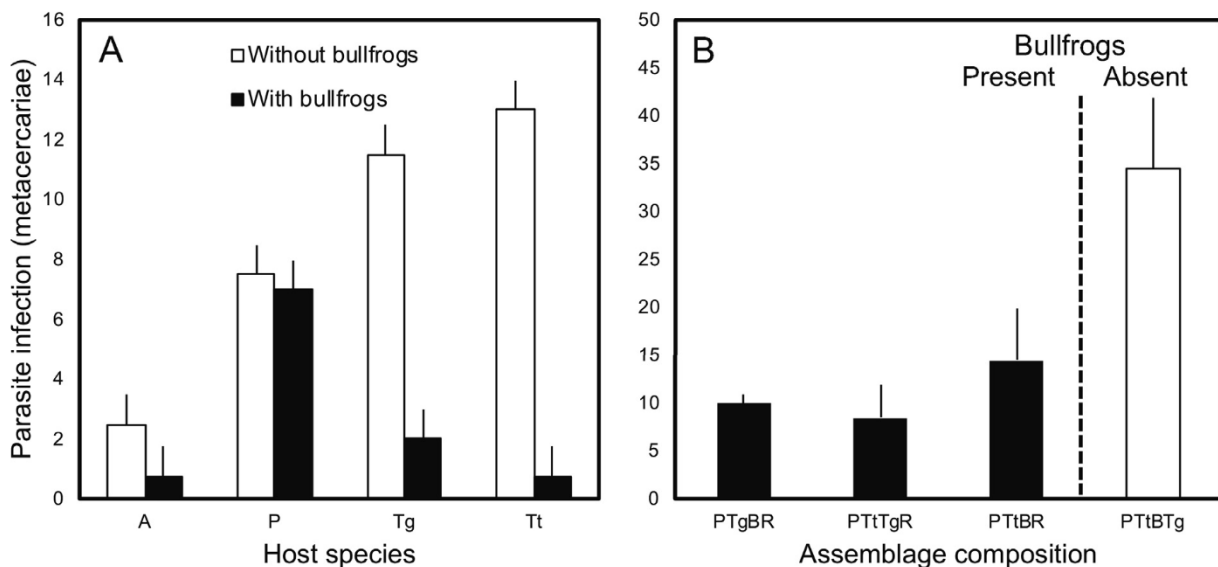


Fig. 4. Effects of bullfrog presence on (A) the average number of metacercariae per host in each of the other amphibian species, and (B) total infection (summed among all four co-occurring individuals in the same trial including bullfrog larvae). With the exception of *Pseudacris regilla* (P), bullfrog presence decreased infection for each amphibian species. Inclusion of bullfrog larvae (dark bars) also reduced the total number of successful *Ribeiroia ondatrae* compared with assemblages in which they were absent (white bars). Error bars represent +1 SE. For A, host species codes are: A, *Anaxyrus boreas*; P, *Pseudacris regilla*; R, *Rana catesbeiana*; Tg, *Taricha granulosa*; Tt, *Taricha torosa*. In B, the host species within assemblages were abbreviated as for A.

cercariae in the choice trials (of the cercariae that moved out of the acclimation chamber), they supported only $12.0\% \pm 6.8\%$ of established metacercariae in the infection trials (of the total metacercariae summed among hosts within the same trial). In contrast, *P. regilla* supported $52.7\% \pm 10.9\%$ of metacercariae despite attracting only $19.1\% \pm 3.1\%$ of cercariae. Rough-skinned newt larvae

(*T. granulosa*) also supported a disproportionately higher fraction of metacercariae relative to their cercarial selection values, whereas *T. torosa* larvae yielded similar fractions for both sets of trials (Fig. 5). There were no additional effects of assemblage composition, bullfrog presence, or their interactions with either trial type or host species identity.

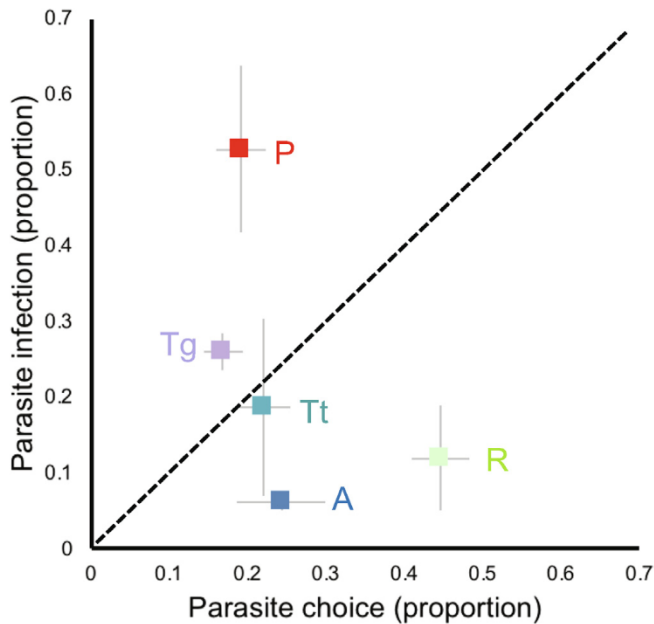


Fig. 5. Disparities between parasite choice and successful infection. Presented is each host species' attractiveness to cercariae (averaged across all assemblage compositions ± 1 SE) on the x-axis versus the fraction of total metacercariae it supported (from among the summed total of metacercariae per trial, average ± 1 SE). The 1:1 dashed line reflects the scenario in which competence is equivalent among species. Species above the reference line support more parasites than would be expected based on parasite choice (e.g., potential amplification hosts), whereas those below the line support fewer established infections relative to their attractiveness to cercariae (e.g., potential dilution hosts). Species codes are: A, *Anaxyrus boreas*; P, *Pseudacris regilla*; R, *Rana catesbeiana*; Tg *Taricha granulosa*; Tt, *Taricha torosa*.

4. Discussion

An ongoing challenge in disease ecology is to understand the factors that regulate parasite transmission in complex ecological communities (Johnson et al., 2015a). Because many parasites infect multiple, alternative host species, which themselves often vary in competence, predicting how shifts in host community composition affect the outcome of infections and disease risk remains especially difficult (Ostfeld and Keesing, 2012). If, alongside differences in susceptibility, host species also vary in attractiveness to infectious parasite stages, predictions made on the basis of host availability and competence alone will be insufficient to characterise community-scale transmission patterns (Kilpatrick et al., 2006; Rizzoli et al., 2015). Using the trematode, *R. ondatrae*, we found that attractiveness varied substantially among host species but was not altered by the host assemblage (i.e., parasite choice was not context-dependent). When presented with larvae of four alternative amphibian species, cercariae exhibited a consistent selective preference towards bullfrog tadpoles, which was between 2.1 and 2.5-fold greater than any other species. In addition, larval toads were selected 20% more than chorus frogs and 50% more than rough-skinned newts, whereas California newts and toads were comparably attractive to cercariae. The experiment in which parasites were able to infect hosts (rather than only choosing among them) illustrated the strong influence of both individual host species identity and the overall assemblage composition in determining infection. For instance, by attracting a high fraction of infectious cercariae but supporting relatively few successful infections, bullfrog larvae tended to reduce observed levels of infection in other species and overall (when summed among all individuals in the same trial).

Mechanistically, such differences in host attractiveness could be driven by body size or chemical and behavioural cues. Previous research has shown that trematode cercariae use a combination of chemical and physical signals to find appropriate hosts, including turbulence, vibrations, shadows, temperature, geotaxis, light, and time of day (Haas, 2003; Sukhdeo and Sukhdeo, 2004). While infectious stages such as miracidia often use macromolecular glycoproteins to accurately locate hosts (Haas et al., 1995), the importance of chemical cues for cercariae – which are generally less host-specific – remains less clear. Cercariae of trematodes that infect slow-moving second intermediate hosts (e.g., snails) have been shown to use small molecular weight cues such as peptides or amino acids to help locate hosts (Fried and King, 1989; Haas et al., 1995); for species that infect more mobile hosts (e.g., amphibians, fishes, birds, mammals), however, there is less evidence for chemo-attraction (Haas, 1992, 1994, 2003; Kolářová et al., 2013). Because the current study used active hosts embedded within relatively large chambers, multiple physical and chemical cues likely contributed to observed patterns. Our findings are consistent with variation driven by host mass; larvae of bullfrogs are considerably larger than those of the other amphibian species used here, despite being of similar developmental stages. Replacing host species identity with individual body mass was statistically supported ($\Delta AIC = -5$). However, this effect was primarily driven by bullfrogs: among trials without bullfrog larvae, neither body mass nor species identity accounted for significant variation in parasite choice (although these analyses included a substantial reduction in statistical power and a much narrower range of host sizes).

Our second objective was to compare parasite preference for specific host species with infection outcomes – simply stated, did parasites select the available host in which infection success and persistence were maximised? Our results indicated that bullfrog larvae functioned as epidemiological 'sinks': although they attracted 44.6% of cercariae, they supported only 12% of metacercariae. In contrast, rough-skinned newts, and especially chorus frogs, supported disproportionately high infections relative to cercarial attraction (Fig. 4). Previous species-specific estimates of amphibian host competence for *R. ondatrae* and several other trematode species have similarly shown a gradient in infection that correlates negatively with body size and positively with occurrence across natural landscapes (Johnson et al., 2012, 2013; Johnson and Wilber, 2017). Such patterns could reflect local adaptation by parasites to commonly encountered hosts or lower defenses on the part of the host (e.g., due to functional tradeoffs with dispersal capability). In contrast to chorus frogs, for instance, non-native bullfrogs are larger bodied, less prevalent across the landscape, and have had a shorter evolutionary history with the parasites in this region.

These results reinforce previous research illustrating the potential for certain species to function as 'decoys' (e.g., Thieltges et al., 2008; Johnson et al., 2009). While parasites should be under selective pressure to choose the most suitable host species, their capacity to do so is likely influenced by the time and energy available for searching, the risks associated with searching (e.g., predation), the efficacy of host defenses, and the reliability of cues for differentiating among species (Forbes et al., 2017). Because most cercariae survive <24 h, they are under high pressure to find and infect a host quickly, even in cases where it might not be the optimal species (Wojdak et al., 2013). Here, low competence hosts such as bullfrogs likely diverted cercariae away from more suitable species and thereby reduced the total number of encysted parasites available to be transmitted to downstream hosts (such as predatory birds in the case of *R. ondatrae*). Assemblages that included larval bullfrogs had 67% fewer total metacercariae, likely due to this species' role as an ecological 'trap' (Keesing et al., 2009). This suggests that,

in the study of how biodiversity affects infection success (i.e., the dilution effect), quantification of host attractiveness to parasites or vectors is an important consideration alongside competence; depending on how host attractiveness correlates with host competence, such patterns could offset or enhance any inhibitory effects of biodiversity changes on transmission (Alacid et al., 2016). For multi-host parasites such as *R. ondatrae*, however, quantifying the net effect of diversity on transmission requires additional information on subsequent hosts in the life cycle, such as patterns of predation by avian definitive hosts on different amphibian intermediate hosts.

Similarly, Langeloh and Seppälä (2018) reported a disconnect between hosts' attractiveness to cercariae and their suitability for infection. In experimental exposures, larger snails were both more attractive to echinostome cercariae but also exhibited lower susceptibility, likely due to higher metabolic activity and immune activity. In a study of dinoflagellate host communities, Alacid et al. (2016) found that although infectious parasites encounter potential hosts at random, strong differences in host species susceptibility ultimately determine community level patterns of infection. As an interesting contrast to our findings, Sears et al. (2012) reported a marginally significant positive correlation between amphibian host species' attractiveness to the cercariae of a plagiophilic trematode and its competence to support infection. Among four wetland-breeding amphibian species in Florida, cercariae showed the greatest preference for small bodied toad larvae (*Anaxyrus terrestris*) and a much lower attraction toward large-bodied *Lithobates sphenoccephalus* tadpoles, which stands in contrast to our results with bullfrog larvae. This difference could be due to the fact that bullfrogs are non-native in the region of California on which our study was based, in parallel to Sears et al. (2012) finding that cercarial selection was also low for larvae of the invasive Cuban treefrog (*Osteopilus septentrionalis*), or alternatively may stem from differences in the choice arenas used in the respective experiments. For instance, the much larger volume in the current study (1.3 L) relative to that of Sears et al. (2012) (5 mL) may have differentially emphasised the influence of signals related to host movement.

Experimental estimates of parasite or vector host preferences have the potential to help facilitate more mechanistic models for forecasting transmission changes within complex communities. Host use by parasites is ultimately the product of their encounter likelihood, attractiveness to infectious stages, and compatibility for establishment (Combes, 2001), yet the relative importance of variation in host attractiveness is comparatively understudied. Direct estimates of host species-specific preference values by parasitic infectious stages or vectors can be used to 'unpack' the composite transmission coefficient (often represented as β) inherent to many disease models, especially if such preferences are consistently associated with measurable host traits. For instance, Simpson et al. (2009) conducted outdoor experimental trials to quantify the innate feeding preferences of the West Nile virus mosquito vector, *C. pipiens*, for American robins over house sparrows and European starlings, while subsequent research indicated that such preferences are associated with bird species size and plumage colour (Yan et al., 2017). While this principle has been emphasised for vector-transmitted infections through incorporation of an added term for vector feeding preference (LoGiudice et al., 2003; Kilpatrick et al., 2006; Hamer et al., 2009; Simpson et al., 2009), quantifying the selectivity of parasitic free-living stages across a broad range of alternative host species has remained more elusive (but see Haas, 2003). Our data suggest this may be accomplished by using host species-specific preference terms (as determined experimentally), or by making parasite choice a function of relative host biomass (rather than relative frequency or density), although further work with more hosts of varying body sizes and densities

will be needed to validate this outcome. We also highlight the importance of comparative studies that contrast parasites with different levels of host specificity, the lifespan of infectious stages, and activity patterns with the aim of developing a more predictive framework.

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