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RESEARCH ARTICLE

# Beyond single host, single parasite interactions: Quantifying competence for complete multi-host, multi-parasite communities

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## Abstract

1. Understanding parasite transmission in communities requires knowledge of each species' capacity to support transmission. This property, 'competence', is a critical currency for modelling transmission under community change and for testing diversity–disease theory. Despite the central role of competence in disease ecology, we lack a clear understanding of the factors that generate competence and drive its variation.
2. We developed novel conceptual and quantitative approaches to systematically quantify competence for a multi-host, multi-parasite community. We applied our framework to an extensive dataset: five amphibian host species exposed to four parasitic trematode species across five ecologically realistic exposure doses. Together, this experimental design captured 20 host–parasite interactions while integrating important information on variation in parasite exposure. Using experimental infection assays, we measured multiple components of the infection process and combined them to produce competence estimates for each interaction.
3. With directly estimated competence values, we asked which components of the infection process best explained variation in competence: barrier resistance (the initial fraction of administered parasites blocked from infecting a host), internal clearance (the fraction of established parasites lost over time) or pre-transmission mortality (the probability of host death prior to transmission). We found that variation in competence among the 20 interactions was best explained by differences in barrier resistance and pre-transmission mortality, underscoring the importance of host resistance and parasite pathogenicity in shaping competence.
4. We also produced dose-integrated estimates of competence that incorporated natural variation in exposure to address questions on the basis and extent of variation in competence. We found strong signals that host species identity shaped competence variation (as opposed to parasite species identity). While variation in infection outcomes across hosts, parasites, individuals and doses was considerable, individual heterogeneity was limited compared to among-species differences. This finding highlights the robustness of our competence

  

estimates and suggests that species-level values may be strong predictors for community-level transmission in natural systems.
5. Competence emerges from distinct underlying processes and can have strong species-level characteristics; thus, this property has great potential for linking mechanisms of infection to epidemiological patterns.

**KEYWORDS**
amphibian, community, competence, disease ecology, diversity–disease, host–parasite interaction, trematode

## 1 | INTRODUCTION

A central goal in disease ecology is understanding how parasites are transmitted within complex communities. This goal has been accentuated by rapid and dramatic alterations to communities, including extinctions and species invasions, alongside a global rise in emerging infectious diseases (Daszak et al., 2000; Johnson et al., 2015). But predicting how parasites will respond to changes in complex networks of free-living species has proven challenging. At the forefront of this challenge is the absence of empirically grounded expectations for how the species comprising a community support parasite transmission (Stewart Merrill & Johnson, 2020). In pursuit of a host, a parasite may encounter one of several species along a spectrum of quality, from dead-end hosts that eliminate parasite fitness to competent hosts that amplify parasite transmission (Dobson et al., 2006). By shaping infection outcomes, the host with whom a parasite interacts has substantial capacity to impact transmission. Quantifying how diverse hosts transmit infection—and understanding where their differences arise from—is therefore a critical step for both forecasting disease in response to changing communities and testing community disease theory.

Competence represents a species' capacity to support transmission and offers a powerful currency for linking community structure to infectious disease. The importance of competence for transmission has been affirmed across multiple modes of inquiry. Theory has demonstrated that competence shapes parasite spread (Cortez & Duffy, 2021; Dobson, 2004; Halsey & Miller, 2020), and this finding has been echoed in experimental and field studies (Johnson et al., 2013; Rosenthal et al., 2021; Strauss et al., 2018). Yet, despite broad recognition that competence is critical for transmission, this property is rarely measured in a consistent and ecologically relevant way. Competence (when measured) has been estimated using host susceptibility (Hall et al., 2009), observed infection (Telfer et al., 2005), xenodiagnosis (Mather et al., 1989) and a range of other proxies—each of which can lead to imprecision and misinterpretation. Host susceptibility, or the probability of patent infection given exposure (Stewart Merrill et al., 2019), is commonly used as a proxy for competence but omits important information on survival of infected hosts and the number of parasite stages produced over the lifetime of an infection. More problematic, however, has been the use of observed infection as a surrogate for competence. This approach assumes that exposure is constant among species, which is almost never the case. Host species vary in habitat use and infection avoidance behaviours (Poirotte et al., 2017; Rohr et al., 2009; Weinstein et al., 2018), and parasites or their vectors can exhibit host choice behaviours (Johnson et al., 2019a). Together, such factors broaden the divide in parasite exposure among potential host species. When observed infection is used as a proxy, misunderstandings can ensue. For instance, two hosts of identical competence may be miscategorized as highly competent and non-competent, when in fact, their only difference is that one routinely encounters the parasite and the other does not. Accurate estimates of competence must therefore measure initial infection success given controlled exposure, as well as the persistence and replication of the parasite to the point of subsequent transmission.

A key goal for advancing disease ecology involves quantifying competence across a suite of host–parasite combinations within a community. Historically, competence values have been obtained for one or a few host species exposed to a common parasite, but this approach prevents us from extrapolating to community-wide transmission. Moreover, the single parasite focus neglects the suite of infective agents that collectively comprise the parasite community, and therefore prevents us from assessing the generality of community transmission mechanisms (Johnson et al., 2015). Indeed, the ongoing focus on single parasites is a core gap in diversity–disease research and continues to fuel controversy regarding the generality of negative diversity–disease relationships (Halsey, 2019; Kilpatrick et al., 2017). Any future reconciliation of diversity–disease hypotheses will therefore require a more inclusive approach that considers complete ecological assemblages, with variation in host and parasite taxa. By producing robust estimates of competence for multi-host, multi-parasite communities, we can powerfully test theory on how ecological communities shape disease.

Our study uses novel conceptual and analytical approaches to measure competence for a community of hosts and parasites. The amphibian host–trematode parasite interactions in our study are tractable and relevant for several reasons. First, the amphibians vary broadly in multiple aspects of infection, including susceptibility and pathology (Johnson & Hartson, 2009), while the trematodes vary in factors like tissue specificity (Schell, 1985) and pathogenicity

(Johnson & Hoverman, 2012). This variation creates the potential for broad variation in competence. Second, the trematodes (in the infection stage our study focuses on) do not replicate within the host—each infective trematode can result in only one transmittable stage, which aids in our quantification of competence (although our approach can be adapted for microparasites that replicate within their hosts; Stewart Merrill & Johnson, 2020). Finally, the interactions under study occur naturally in California pond ecosystems (Moss et al., 2020), such that experimental estimates can provide predictions for real transmission patterns.

With this system, we quantified competence for a nearly complete host–parasite community, assessing interactions between all non-endangered amphibians and all dominant helminth parasites (constituting 95% of natural infections; Moss et al., 2020). Recognizing that parasite exposure can be highly variable and is essential for determining infection outcomes (Stewart Merrill et al., 2019), we assessed competence along a gradient of ecologically realistic exposure doses. Our experimental design resulted in an extensive dataset consisting of 100 host–parasite–dose treatments examined for 1,176 amphibians. By combining the key factors of exposure dose, host susceptibility and host survival, we generated systematic competence estimates and addressed four primary questions: (a) how does competence vary among 20 host–parasite interactions? (b) what components of the infection process underlie variation in competence? (c) is competence a property of the host species, the parasite species, or a unique outcome of a particular interaction? and (d) how much of total variation in competence is attributable to individual differences (i.e. how robust is competence at the species level)? By addressing these questions, and presenting associated competence estimates, our study provides a common currency for modelling community-level transmission and foundational knowledge on diversity–disease connections.

## 2 | MATERIALS AND METHODS

### 2.1 | Study system

Trematodes are common helminths of freshwater habitats (Preston et al., 2013). As parasites with complex life cycles, trematodes use multiple host species, sequentially, for their development (Schell, 1985). In this study system, these hosts include snail first intermediate hosts, amphibian second intermediate hosts and vertebrate definitive hosts. We focus specifically on interactions between five amphibian intermediate hosts and four trematode parasites, representing a total of 20 host–parasite combinations. These combinations comprise the dominant amphibian and trematode taxa of natural pond communities in the East Bay region of California. The amphibians include: Western toad *Anaxyrus boreas* ('ANBO'), chorus frog *Pseudacris regilla* ('PSRE'), American bullfrog *Rana catesbeiana* ('RACA'), rough-skinned newt *Taricha granulosa* ('TAGR') and California newt *Taricha torosa* ('TATO'). The trematodes include: *Alaria marcinae* ('ALMA'), *Cephalogonimus* sp. ('CESP'), *Echinostoma* spp. ('ECSP') and *Ribeiroia ondatrae* ('RION'). Amphibians used in the study were collected as eggs from wetlands in California and Oregon (Table S1), with the exception of rough-skinned newts, which were collected as adults then bred in the laboratory to produce offspring for experiments. Trematode-infected snails (*Helisoma trivolvis*) were also collected from wetlands in California and Oregon, and infective cercariae for the infection assays were obtained using standard protocols (Johnson et al., 2012). Details regarding the collection and rearing of amphibians and the collection of trematodes are provided in the Supporting Information (Section S1: Extended Methods).

All data were collected with the approval of the University of Colorado's Institutional Animal Care and Use Committee (protocols 1002.021302.01) and in accordance with sampling protocols approved by the California Department of Fish and Wildlife (SC-3683 and SC-10560), the Oregon Department of Fish and Wildlife (014-21), the US Fish and Wildlife Service (TE-181714-4/5), Santa Clara County Parks, East Bay Regional Parks District, East Bay Municipal Utility District, California State Parks and other local landowners.

### 2.2 | A framework for competence

The life cycle of parasites inside their hosts guides our quantification of competence and provides a narrative for decomposing competence into its constituent processes. In the amphibian–trematode system, amphibians become infected during the larval stage. Infective trematode cercariae released from snail first intermediate hosts seek out larval amphibians in the aquatic environment and actively enter the host by penetrating host tissue. Following entry, each cercaria excretes a protective cyst, ultimately forming a metacercaria in the site of infection (with the exception of *A. marcinae* which forms mesocercariae [Schell, 1985]; for simplicity we refer to all internal stages as 'metacercariae'). If it is not cleared by the amphibian's immune response, the metacercaria persists through host metamorphosis and into the host's adulthood. Transmission generally occurs when the infected amphibian is consumed by a definitive host (trophic transmission). Although trematode infection may alter the vulnerability of amphibians to predation, because definitive hosts themselves can vary in competence, trophic transmission does not guarantee perpetuation of the parasite's life cycle. Competence therefore does not include transmissibility from amphibian to definitive host, but merely reflects the capacity of the amphibians to support metacercariae to that point.

Our study adheres to the strict definition and conceptual model of competence for macroparasites with discrete transmission (transmitted in one discrete event rather than continuously) provided in Stewart Merrill and Johnson (2020). Competence refers to successful passage of a parasite through the infection pathway (from exposure to the transmittable state) and is hence decreased by any processes that remove parasites. We consider competence to arise from three principal forms of parasite removal. *Barrier resistance* is the process by which parasites are prevented from entering (infecting) their host (Stewart Merrill et al., 2021). In this system, barrier resistance encompasses

physical, chemical and behavioural barriers that block cercariae during penetration of larval tissue, thereby inhibiting the formation of metacercariae (Stutz et al., 2019). Because cercaria penetration and encystment occur quickly following exposure (LaFonte & Johnson, 2013), barrier resistance is relegated to a short period of time. *Internal clearance* is the process where established parasites inside their host are lost via internal immunological defences (although this loss may also include natural death of parasites in the host; Stewart Merrill et al., 2021). Because metacercariae remain inside the host until transmission to a definitive host, internal clearance can remove metacercariae over the life span of the host (LaFonte & Johnson, 2013; Stutz et al., 2019). *Pre-transmission mortality* emerges from two distinct death processes. First, amphibians can die immediately following exposure, due to stress and pathology associated with cercaria penetration (Schotthoefer, Cole, & Beasley, 2003; Schotthoefer, Koehler, et al., 2003). Second, amphibians can die before they have metamorphosed into adults, due to pathology associated with metacercariae (Holland et al., 2007; Schotthoefer, Cole, & Beasley, 2003; Schotthoefer, Koehler, et al., 2003). We condense these processes into overall 'pre-transmission mortality' that comprises any deaths occurring as a result of the host–parasite interaction (before transmission can occur). Hence, to successfully navigate the infection pathway, trematodes must overcome barrier resistance, evade internal clearance and their host must survive to the point of subsequent transmission. Competence is thus the proportion of administered cercariae that ultimately became 'transmittable' metacercariae.

## 2.3 | Infection assays to quantify competence and its core processes

Our infection assays mirror the infection pathway, such that barrier resistance, internal clearance and pre-transmission mortality can be quantified from host–parasite longitudinal data (Figure 1). To decompose these processes, we exposed individual larval amphibians to 20 trematode cercariae in 500 ml microcosms (Figure 1a; 'exposure'). After approximately 1.5 days, a subset of amphibians from each parasite treatment were necropsied to count the number of metacercariae that formed in host tissue (Figure 1a; 'first necropsy'; metacercariae of these four parasites can be reliably detected after this short time period). Remaining amphibians were maintained for an additional 18.5 days (for a total experiment duration of approximately 20 days) and were euthanized to count persisting metacercariae (Figure 1a; 'second necropsy'). Parasites counted at each time period can be directly related to the infection pathway: $E$ is the number of cercariae to which each host was initially exposed, $M_{1.5}$ is the number of metacercariae detected at 1.5 days, and $M_{20}$ is the number of metacercariae remaining in host tissue after 20 days. Importantly, natural mortality of parasite-exposed hosts did occur (beyond that observed for unexposed controls), and values $M_{1.5}$ and $M_{20}$ contain metacercaria counts from hosts that died (and were subsequently necropsied) before their target day of euthanization. Metacercariae that remained alive and transmittable (due to host survival) at 20 days are represented by $T$ (any metacercariae whose hosts died were given $T = 0$).

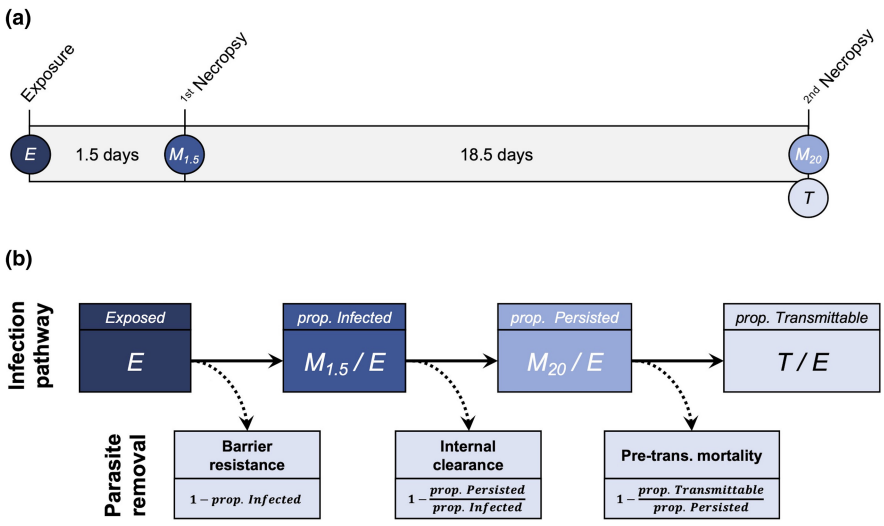


**FIGURE 1** Infection assays to quantify competence and its core processes. (a) Schematic of the infection assay. Amphibian larvae were exposed to trematode cercariae ('exposure'). A subset of amphibians were necropsied ~1.5 days later ('first necropsy') to quantify number of cercariae that infected host tissue and developed into metacercariae. Remaining amphibians were necropsied at 20 days post-exposure ('second necropsy') to quantify number of metacercariae that persisted through the duration of the experiment. $E$ represents cercariae a host was exposed to; $M_{1.5}$ represents metacercariae detected in host tissue after 1.5 days (including from hosts that died naturally); $M_{20}$ represents metacercariae remaining in host tissue after 20 days (including from hosts that died naturally); $T$ represents metacercariae that remained alive and transmittable (due to host survival) through the full 20 days. (b) Tracking trematodes through the infection pathway to quantify the three forms of parasite removal. By calculating the proportion of initially administered trematodes ($E$) that infected, persisted and were transmittable, we could also quantify the proportion that did not achieve those stages owing to barrier resistance, internal clearance or pre-transmission mortality

For each host–parasite interaction, the average values of $E$, $M_{1.5}$, $M_{20}$ and $T$ demonstrate how parasite numbers are reduced over time (Figure 1b; top row). The proportion of parasites that successfully infected the host is $M_{1.5}/E$ (Figure 1b; 'prop. Infected'). The proportion that both infected and persisted through amphibian development is $M_{20}/E$ (Figure 1b; 'prop. Persisted'). Finally, the proportion of parasites that were able to infect, persist and survive is $T/E$ (Figure 1b; 'prop. Transmittable'). Because competence is the successful passage of a parasite through the infection pathway, $T/E$ represents our direct estimate of competence for individual hosts over the time span of the assay (20 days).

Considering how parasites are lost from each transition provides the three probabilities of parasite removal (Figure 1b; bottom row). Barrier resistance is the proportion of administered parasites that did not infect the host at exposure (1 − prop. Infected). Internal clearance is the proportion of infected parasites that did not persist through the amphibian's development $\left(1-\frac{\text{prop. Persisted}}{\text{prop. Infected}}\right)$. Pre-transmission mortality is the proportion of persisting parasites that died because their hosts succumbed to mortality $\left(1-\frac{\text{prop. Transmittable}}{\text{prop. Persisting}}\right)$. We quantified these probabilities for each of the 20 interactions. We note that in some cases a specific probability could not be quantified. For instance, if a host species was not infected by any trematodes following exposure (prop. Infected = 0), we could not estimate its internal clearance or pre-transmission mortality probabilities (Supporting Information Section S2: Additional Statistical Details; see Table S3 for sample sizes).

## 2.4 | A dose-integrated estimate of competence

Because host exposure to parasites may be highly variable, we further examined competence as a function of exposure dose. For each host–parasite combination, we inoculated amphibians at one of five ecologically realistic dose treatments: 0 (control), 20, 40, 100 or 200 cercariae (Johnson et al., 2013). Hosts were necropsied 20 days following exposure (or at the time of natural mortality) to count encysted metacercariae.

The three forms of parasite removal (see Section 2.2) cluster into those owing to *host susceptibility* (barrier resistance and internal clearance) and *host survival* (pre-transmission mortality). We could examine these separate processes by regressing metacercariae per host on exposure dose—which provides an indication of how host susceptibility changes with exposure (Figure 2a)—and by regressing host survival (1 or 0) on exposure dose to generate a host survivorship curve (Figure 2b). Because competence is the outcome of both processes, multiplying the two curves and integrating the area under the curve provides a *dose-integrated estimate of competence* (Figure 2c). This estimate serves as a standard metric for comparing competence among interactions that takes dose dependence into account.

To model host susceptibility as a function of dose, we fit generalized linear mixed models for each host–parasite interaction (function 'glmer' in 'LME4'; Bates et al., 2015) assessing the effects of exposure dose on each cercaria's success or failure to become a persisting metacercaria (0, 1). The individual parasite served as the unit of replication, such that our susceptibility models used successes ($M_{20}$) versus failures ($E-M_{20}$) as the response variable. Because infection data were over-dispersed, we included host ID as an observation-level random effect to achieve a dispersion parameter of approximately one. For host survival, we fit generalized linear models for each host–parasite interaction ('GLM'; R Core Team, 2014) assessing the effects of exposure dose on amphibian survival (0, 1), with each amphibian individual serving as the unit of replication. For both susceptibility and survival, we modelled the residuals with binomial error distributions. The survival models also included Firth's bias correction ('brglmFit' in 'BRGLM2'; Kosmidis, 2020) to deal with cases in which all hosts (or almost all) survived or died as a function of parasite exposure, which could result in complete separation problems. For each host–parasite interaction, we multiplied the fitted host susceptibility curve (Figure 2a) and survival curve (Figure 2b) to produce a smooth fit of how their product—competence—changed over different exposure doses. The area under each resulting curve was then calculated and scaled to the per cent of total area occupied to arrive at our dose-integrated estimate of competence (Figure 2c). We provide a guide to calculating dose-integrated competence in the Supporting Information (Section S3: How to measure dose-integrated competence).

## 2.5 | Analyses

### 2.5.1 | What processes underlie variation in competence?

Probabilities for barrier resistance, internal clearance and pre-transmission mortality were estimated for each host–parasite interaction from longitudinal data collected at the 20 cercariae exposure dose (see Figure 1 and Section 2.3). We used general linear models ('LM'; R Core Team, 2014) with Gaussian error distributions to evaluate how these three probabilities explained variation in average competence among the 20 interactions. Our models independently assessed the effects of each process (predictor) on competence (response; measured as average $T/E$), with the host–parasite interaction as the unit of replication. Because competence values are proportions, we arcsine-transformed them prior to fitting models. The three models represent alternate hypotheses for the processes shaping competence, for which we compared their fits using $R^2$.

### 2.5.2 | Is competence a property of the host species or parasite species?

Competence may largely reflect host traits, parasite traits, or may be an emergent property of a particular interaction. Because our study design included multiple parasite species exposed to each host and multiple host possibilities provided for each parasite, we could determine whether competence displayed consistency or variability in either of these contexts. As with the dose-integrated estimates of competence, we integrated the area under the fitted susceptibility and survival curves and scaled the area to per cent

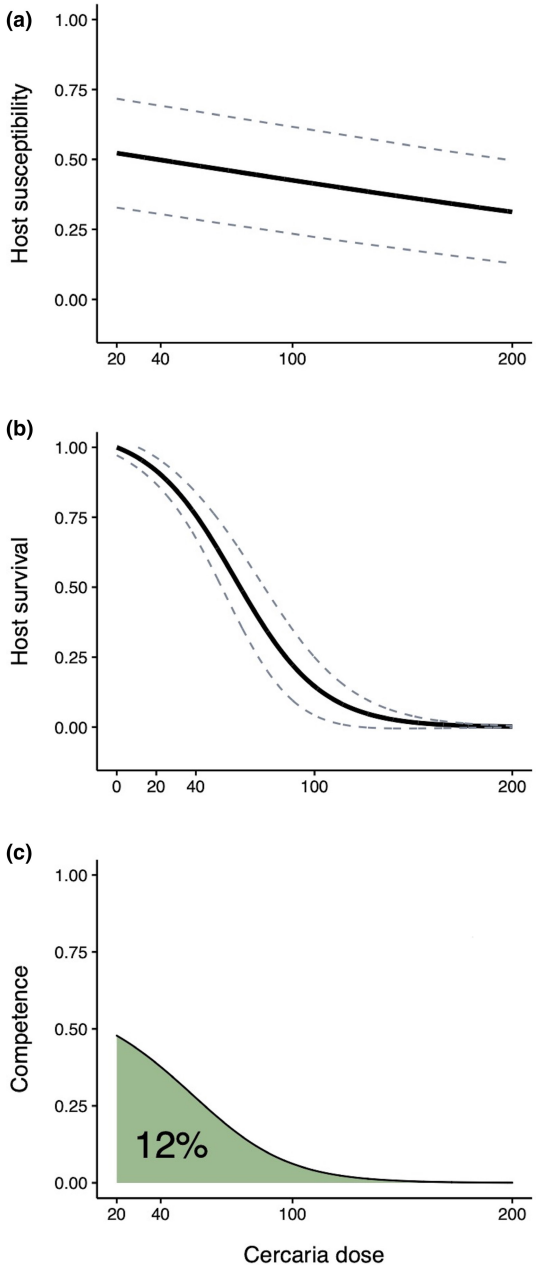


**FIGURE 2** A dose-integrated estimate of competence. We examined host susceptibility (a) and survival (b) as a function of parasite exposure dose. The product of these two curves provides an association between exposure dose and competence (c). We calculated the area under the competence curve to arrive at a dose-integrated measure of competence (green shading), which facilitated comparisons among hosts, parasites and interactions. The area under the curve was scaled to a percentage, where perfect competence could be achieved by maintaining 100% competence (1.0 on the *y*-axis) across all doses. Depicted data are from chorus frogs *Pseudacris regilla* exposed to *Ribeiroia ondatrae*; the dose-integrated estimate of competence is 12%. Dashed lines represented standard error

of total area occupied (as in Figure 2). These scaled areas represented dose-integrated measures of susceptibility and survival for each host–parasite interaction. We then used ANOVA with Gaussian error distributions to independently test for effects of host species or parasite species on dose-integrated susceptibility, survival and more encompassing competence. We arcsine-transformed each of these values prior to inclusion in the models. Although our central question was whether host or parasite explained more variation in the dose-integrated values, we also ran post-hoc pairwise comparisons (Tukey's HSD) to examine if any particular hosts or parasites had significantly high or low values. Finally, we examined potential associations between dose-integrated susceptibility and survival using Pearson's product–moment correlation test.

### 2.5.3 | How robust is competence?

Our dose-integrated measures of competence provided standard, mean values of competence for each of the host–parasite interactions. These values represent predictions for which hosts should lead to successful transmission of a given parasite, given contact. However, individual heterogeneity of hosts and parasites may be strong and may produce broad variation within an interaction. To test the reliability of our interaction-level estimates, we partitioned their variance among host–parasite interactions and individual hosts. Using the dissection data, we ran a generalized linear mixed model with a Poisson error distribution (function 'glmer' in 'LME4'; Bates et al., 2015) that included host–parasite interaction as a random effect (20 levels, representing all combinations of hosts and parasites) and amphibian individual as an observation-level random effect. We did not include individuals sacrificed at 1.5 days or individuals at the zero exposure dose (because controls were not relevant to the question; $N = 764$). The only fixed effect in our model was the intercept. Our response variable included the number of transmittable metacercariae ($T$) with log-transformed exposure dose ($E$) incorporated as an offset term. Variance was then attributed to each random effect by dividing the random effect's variance by the total variance (Crawley, 2013; Reeves et al., 2013).

We visually explored scales of variation to complement the variance partitioning analysis. For each trematode parasite, we quantified total variance in the proportion of transmittable metacercariae (variance of $T/E$ observed among all individuals of all host species) and within-species variance in the proportion of transmittable metacercariae (variance of $T/E$ among individuals of a given host, quantified separately for each host species). We then plotted the within-host variances with an overlaid line representing the total variance. Variance values below the line represent lower individual variation than total variation, and values above the line represent greater individual variation than total variation. All analyses were run in R (R Core Team, 2014) and plots were produced with GGPLOT2 (Wickham, 2009).

## 3 | RESULTS

We exposed 1,176 amphibians representing five host species to four trematode species for a total of 96 host–parasite–dose treatments

(four treatments were not run due to limited host availability; Table S2). Competence values varied considerably across individuals, doses and interactions, from 0 (no transmittable parasites) to 1 (100% of administered parasites were transmittable). This variation arose from differences in both host susceptibility and pre-transmission mortality, underscoring the importance of host traits and parasite pathogenicity in shaping competence. The two newt species (*Taricha* spp.) showed markedly low susceptibility, with almost no infections occurring for three of the four parasites. While some parasites caused high levels of pre-transmission mortality (for instance, *R. ondatrae* killed most Western toads), other parasites had minimal effects on host survival even at the highest doses.

### 3.1 | Barrier resistance and pre-transmission mortality underlie species-level differences in competence

Among the interactions examined, barrier resistance and pre-transmission mortality both explained much of the variation in competence measured at the 20 cercaria dose. The relationship between barrier resistance (see Figure 1b) and competence (measured with average *T/E*) was negative (est = −0.808, $p = 0.0002$; $R^2 = 0.561$; $N = 19$; Figure 3a). That is, amphibian species that inhibited the most metacercariae by 1.5 days post-exposure also had the lowest competence. In this assessment there was one outlier. All western toads died when exposed to *R. ondatrae* resulting in no transmittable metacercariae (zero competence; red triangle in Figure 3a). We therefore reran the analysis with this outlier removed and arrived at the same qualitative result, albeit with a better model fit (more normal residuals) and a higher $R^2$ value (est = −1.053, $p < 0.0001$; $R^2 = 0.897$). There was no relationship between internal clearance (loss of metacercariae from day 1.5 to day 20; Figure 1b) and competence (est = −0.093, $p = 0.731$, $R^2 = 0.011$; $N = 13$; Figure 3b). Pre-transmission mortality and competence were also negatively associated (likely due to high pre-transmission mortality in *A. boreas* and *R. ondatrae*; est = −0.448, $p = 0.008$, $R^2 = 0.454$; $N = 14$; Figure 3c), although the barrier resistance models (with and without the outlier) fit better (i.e. had higher $R^2$ values). Together, barrier resistance by the host and pre-transmission mortality, likely driven by parasite pathogenicity, shape competence.

### 3.2 | Competence is more reflective of host species differences than parasite species differences

Dose-integrated susceptibility varied among hosts ($F = 5.246$, $p = 0.008$; Figure 4a). Conversely, parasite species did not significantly vary—that is, a given host had fairly consistent levels of susceptibility regardless of the parasite with which it interacted ($F = 1.672$, $p = 0.213$; Figure 4a). Dose-integrated survival did not vary among hosts ($F = 1.938$, $p = 0.156$; Figure 4b), but did vary among parasites

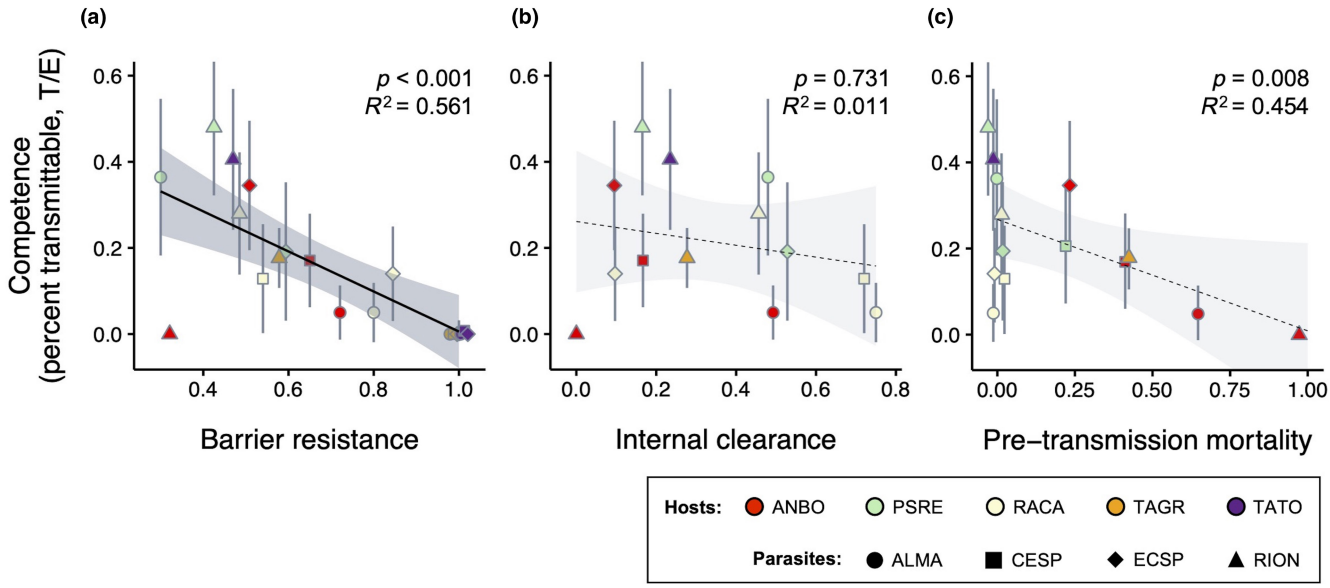


FIGURE 3 Barrier resistance and pre-transmission mortality underlie species-level differences in competence. We evaluated relationships between competence (measured as 'proportion transmittable', or *T/E*) and each form of parasite removal (a: Barrier resistance [$N = 19$], b: Internal clearance [$N = 13$], and c: Pre-transmission mortality [$N = 14$]) to identify which processes explained variation in competence. Barrier resistance (a) best explained variation in competence, with the highest $R^2$ value. Each point represents a host–parasite interaction measured at the standard 20 cercaria dose; colours denote amphibians and shapes denote trematodes. Standard error of the regression is indicated with shading, and error bars represent standard error calculated for proportional data. Slight jitter was added to visualize overlapping points. Host acronyms: *Anaxyrus boreas* (ANBO), *Pseudacris regilla* (PSRE), *Rana catesbeiana* (RACA), *Taricha granulosa* (TAGR) and *Taricha torosa* (TATO). Parasite acronyms: *Alaria marcinae* (ALMA), *Cephalogonimus* sp. (CESP), *Echinostoma* spp. (ECSP) and *Ribeiroia ondatrae* (RION)

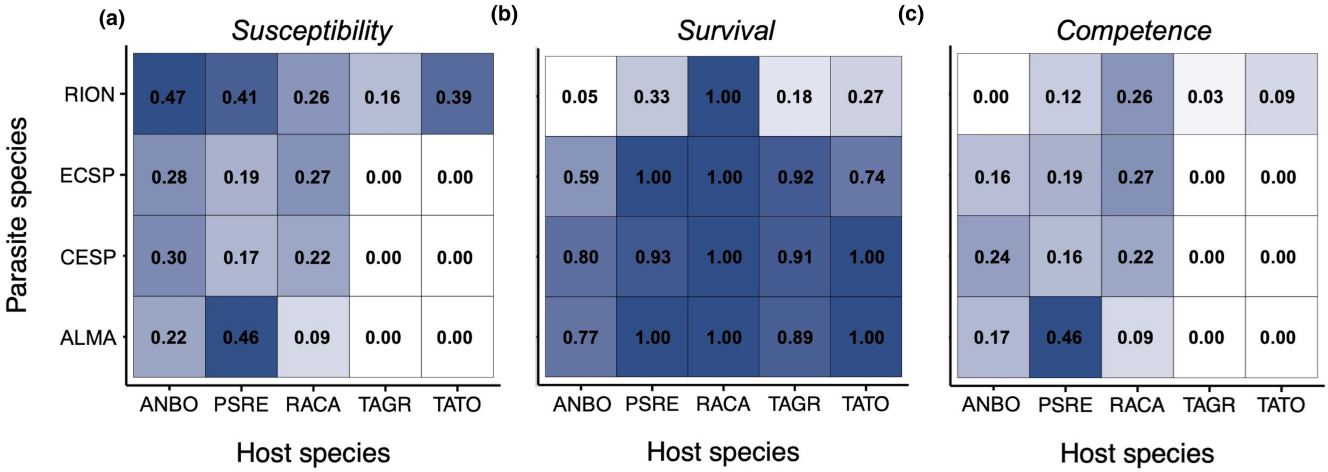


**FIGURE 4** Competence is more reflective of amphibian species differences than trematode species differences. We generated dose-integrated measures of host susceptibility, survival and competence (see Figure 2) and evaluated the extent to which variation in these values was explained by host species versus parasite species. Shading represents the dose-integrated value, with white indicating '0', and increasing saturation representing increasing values. (a) Dose-integrated susceptibility varied among hosts but not parasites. The newts (*T. granulosa* [TAGR] and *T. torosa* [TATO]) exhibited lower levels of susceptibility than the other amphibians (owing to lack of susceptibility to *Echinostoma* spp. [ECSP], *Cephalogonimus* sp. [CESP], and *A. marcinae* [ALMA]). (b) Dose-integrated survival did not vary among hosts but did vary among parasites. This pattern was driven by *R. ondatrae* (RION), which caused low survival at the highest doses in four of five amphibians. (c) Dose-integrated competence varied among hosts but not parasites, with the two newt species having particularly low competence. Host acronyms: *Anaxyrus boreas* (ANBO), *Pseudacris regilla* (PSRE), *Rana catesbeiana* (RACA), *Taricha granulosa* (TAGR) and *Taricha torosa* (TATO)

($F = 4.929$, $p = 0.013$; Figure 4b), with *R. ondatrae* causing higher levels of host mortality than *A. marcinae* ($p = 0.019$) and *C. americanus* ($p = 0.027$). Finally, dose-integrated competence varied among amphibian hosts ($F = 7.377$, $p = 0.002$; Figure 4c), but not among trematode parasites ($F = 0.005$, $p = 0.999$; Figure 4c). Dose-integrated competence was particularly low in the two newt species: *T. granulosa* and *T. torosa* had lower competence than *P. regilla* (both $p < 0.02$) and *R. catesbeiana* (both $p < 0.03$). Dose-integrated survival and susceptibility were inversely associated ($r = -0.49$, $p = 0.025$), suggesting that interactions with the highest metacercariae intensities were the most likely to lead to pre-transmission mortality.

### 3.3 | Competence estimates for 20 bipartite interactions

Our study provides competence estimates for 20 bipartite host–parasite interactions (Figure 5a). Extending these estimates to our understanding of transmission in natural communities requires robust values, and we found strong support for the reliability of these interaction-level estimates. We examined the number of transmittable metacercariae for 764 amphibians and found that, of the full variance in competence, 85% could be attributed to the host–parasite interaction (variance = 13.668), where only 15% was attributed to the individual host (and/or to error; variance = 2.425).

Visualizing total variance relative to within-species variance in *T/E* indicated that some host–parasite interactions had no individual-level variation, such as interactions between newts (*Taricha* spp.) and the trematodes *Cephalogonimus* sp., *A. marcinae*. and *Echinostoma* spp. (Figure 5b; orange and purple bars). The greatest individual-level variance was present in interactions between *R. ondatrae* and chorus frogs (Figure 5b, green bars), for which individual-level variance was 1.5 times higher than total variance. Ultimately, the majority of interactions (80%) exhibited lower individual-level variance than total variance (Figure 5b), mirroring the results of the variance partitioning analysis and supporting the reliability of our interaction-level competence values.

## 4 | DISCUSSION

Predicting parasite spread in complex communities requires knowledge of each species' capacity to transmit infection, which represents one of the most outstanding challenges in disease ecology. We present comprehensive data on competence for a nearly complete host–parasite assemblage. Our estimates of competence encompass five amphibians exposed to four trematodes and integrate information from the full infection process. To arrive at these estimates, we experimentally measured initial infection, persistence of encysted parasite stages and pre-transmission mortality along a gradient of exposure doses. By decomposing competence into constituent parts, we found that barrier resistance (prevention of parasite entry) and pre-transmission mortality (premature death of the host) are both core processes shaping variation in competence. While more generic host susceptibility appeared to be largely host-driven, host survival appeared to be parasite-driven, potentially as a consequence of parasite pathogenicity. However, their product—competence—more greatly reflected host differences. Amphibian taxa may therefore

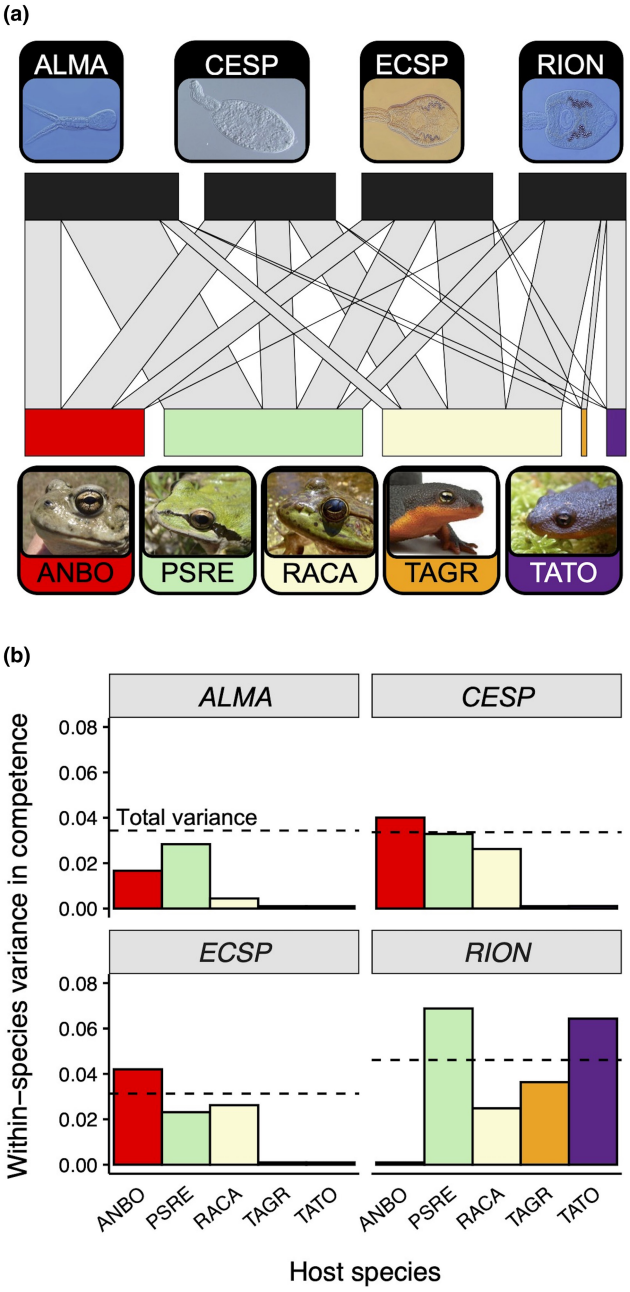


**FIGURE 5** Competence estimates for 20 bipartite interactions. (a) Bipartite network representing the competence of all experimentally measured interactions. The thickness of grey connecting lines denotes dose-integrated competence estimates (the thinnest black lines indicate zero competence; values are those presented in Figure 3c). The width of host bars represents a host's cumulative competence for all parasites, and the width of parasite bars represents a parasite's cumulative competence for all hosts. Newts (TAGR and TATO) show low or zero competence for most trematodes, while chorus frogs (PSRE) exhibit consistently high competence for all trematodes. (b) We found strong support for the robustness of interaction-level competence estimates. To visualize variance at both scales, we plotted individual variance (coloured bars) against total variance (dashed line) estimated for each parasite. Values above the dashed line represent interactions for which individual variation is greater than total variation, and values below the dashed line represent interactions where individual variance is lower than among-host variance. The majority of interactions (80%) possess limited individual variance relative total variance. Host acronyms: *Anaxyrus boreas* (ANBO), *Pseudacris regilla* (PSRE), *Rana catesbeiana* (RACA), *Taricha granulosa* (TAGR) and *Taricha torosa* (TATO). Parasite acronyms: *Alaria marcinae* (ALMA), *Cephalogonimus* sp. (CESP), *Echinostoma* spp. (ECSP) and *Ribeiroia ondatrae* (RION)

have general responses to trematodes that shape their capacity to transmit infection. Competence varied broadly among the host–parasite interactions and was generally robust (possessing limited individual variation). Our measures of competence may therefore be strong predictors of host–parasite interactions in natural systems.

Barrier resistance and pre-transmission mortality both explained variation in competence among the 20 host–parasite interactions. The first mechanism, barrier resistance, represents an amphibian's ability to prevent the establishment of infection. The transition from infective cercariae to encysted metacercariae is rapid (Schell, 1985), and the strong relationship between barrier resistance and competence suggests that the first 36 hr of the amphibian–trematode interaction are critical for shaping the future of the infection. While the importance of barrier resistance hints at physiological mechanisms at play (e.g. innate defences such as skin permeability [Varga et al., 2019], eosinophilic granulocytes [Belden & Kiesecker, 2005], and natural killer cells [Carey et al., 1999]), it also provides solid ground for interpreting field patterns. Negative diversity–disease associations have been observed in amphibians (Johnson et al., 2013) and our experimental results suggest that transmission reduction (where the addition of a new species reduces the average probability of transmission between infective stages and susceptible hosts; Keesing et al., 2006) may be generating these associations. Pre-transmission mortality also explained variation in competence (but this relationship was weaker than that for barrier resistance). This form of parasite loss is often overlooked or unreported and can be especially challenging to observe in field studies (Wilber et al., 2020). But quantifying this attribute is critical, as it can explain a species' low transmission potential. For instance, owing to complete pre-transmission mortality, we observed zero competence in Western toads exposed to *R. ondatrae*. If mortality is not considered, any absence of infections from this interaction would likely be attributed to lack of exposure or limited susceptibility. Our results, however, suggest that mortality is the more likely culprit, with infected individuals dying before their infections can be observed. Pre-transmission mortality likely results from parasite pathogenicity, especially in cases where parasites negatively impact the function of critical host tissues. That pre-transmission mortality (in addition to barrier resistance) shapes competence reinforces that competence is an outcome of both host and parasite traits.

The joint contribution of host and parasite to infection outcomes was echoed in our dose-integrated estimates. Dose-integrated host susceptibility (the outcome of barrier resistance and internal clearance) was largely explained by amphibian identity, while dose-integrated host survival (the inverse of pre-transmission mortality)

was better explained by parasite identity. These two values were also inversely associated; that is, hosts with the highest susceptibility also experienced the lowest survival. If hosts that experience heavy infection loads are more likely to succumb to pre-transmission mortality, the spread of parasites will be reduced. Thus, understanding the link between susceptibility and survival is critical for modelling transmission in communities. Given that host susceptibility and pre-transmission mortality act on competence in opposite directions, a consideration of susceptibility alone will likely overestimate a species' competence.

Although dose-integrated susceptibility and survival emerged from host and parasite differences, respectively, their product—competence—was best explained by the amphibian host. Dose-integrated competence (a metric that accounts for variation in exposure) varied broadly among the 20 host–parasite interactions. On the low end, newts (*Taricha* spp.) had competence values ranging from 0 to 0.09, and on the high end, competence reached its maximum value of 0.46 in chorus frogs exposed to *A. marcinae*. That this extensive variation reflected host differences, as opposed to parasite differences, elicits a dual consideration of phylogeny and immunity. The four trematodes in our study are phylogenetically diverse (representing four families; Gibson et al., 2002; Bray et al., 2008) and vary in life-history attributes, including body size (Orlofske et al., 2015) and tissue specificity (Schell, 1985). Such phylogenetic and life-history differences are also present in the five amphibians, which comprise four families and vary in time to metamorphosis, maximum body size and reproductive output (Johnson et al., 2012). A diverse multi-host, multi-parasite assemblage might imply that competence should be specific to a particular interaction; however, vertebrate innate responses can operate in a less specific manner. Generic anti-helminth responses (e.g. natural antibodies) are some of the first responders to helminth infections (Grogan et al., 2018), and we observed that the initial 36 hr of the interaction strongly shaped competence. It may be that the use of generic anti-helminth immunity during this critical early window resulted in similar infection outcomes for a given host species, regardless of the parasite with which it interacted. In addition, newts may have further broadened host differences because they possess tetrodotoxin, a toxic compound with known negative effects on trematode cercariae (Calhoun et al., 2017). A more diverse suite of infections (e.g. viral, bacterial and helminth) may have increased the contribution of parasite identity to competence and addressing broad parasite assemblages remains an important priority for diversity–disease research.

The differences in competence that we observed among hosts raise the important question of whether competence is driven by host life history, and what that means for transmission across the landscape. Diversity–disease theory proposes that slow pace-of-life species invest more in immune defence, and are therefore less competent, while fast pace-of-life species shunt energy from immunity to growth and reproduction, elevating their competence (Joseph et al., 2013). These life-history attributes are also likely to correspond with species demographics, including abundance, spatial distribution and probability of extinction/colonization (Cardillo et al., 2008). A rigorous empirical connection between competence, life history and demography is therefore an exciting next step for understanding disease during community assembly and disassembly.

While competence arose from host species differences, it was also robust to individual variation. In partitioning variance in total competence (measured for each individual as $T/E$), we found that less than 15% of variance could be attributed to the individual. Such robust values enable accurate predictions of transmission and are particularly important for addressing diversity–disease theory (Stewart Merrill & Johnson, 2020). Thus, our reported values may serve as predictions for host–parasite interactions in natural systems and can be used to parameterize community-level transmission models. Of notable interest, again, were the two newt species, who possessed almost no individual-level variation. When exposed to the full range of parasites and doses, only two California newts and four rough-skinned newts were ultimately infected with transmittable metacercariae (at an average of 1.5 transmittable metacercariae per infected individual), supporting field observations of limited to no infection in these species (Johnson et al., 2019b). Taken together, their limited individual heterogeneity and dramatically low competence indicate that newts may be diluter hosts, consistently removing cercariae they contact. In contrast, chorus frogs exposed to *R. ondatrae* exhibited the highest individual-level variation, which was approximately 1.5 times that of total variation. Addressing the basis of chorus frog heterogeneity, whether owing to genetics, stage or condition, will be imperative moving forward. Chorus frogs exhibit the highest cumulative competence and the highest levels of infection in the field (Johnson et al., 2019b). Unlike newts, chorus frogs are likely amplifier species, whose presence helps to sustain parasite transmission. Tackling the drivers and extent of chorus frog variation will provide valuable insight into why disease dynamics may vary among fixed assemblages, as well as the importance of super-spreading individuals.

We generated ecologically relevant competence estimates using new conceptual and quantitative approaches. Breaking down, then reassembling, the infection process can yield insight into the drivers and outcomes of host–parasite interactions. Such reductionist approaches have been successfully employed for some diseases. In a rodent–*Mycoplasma* system, Garrido et al. (2021) quantified probability of initial infection, pathogen intensity, infection duration and probability of clearance (among other attributes) for three gerbil species to establish their ecological roles as pathogen diluters or amplifiers. By taking the reductionist view, the authors found that gerbil clearance traits best explained a dilution effect observed in the field (Kedem et al., 2014). In seminal studies of West Nile virus, Komar et al. (2003) similarly quantified probability of initial infection, daily infectiousness and viremia duration in 25 bird species to arrive at estimates of their competence. These estimates then seeded predictions for numerous subsequent studies, and in some cases, explained patterns of transmission across avian communities (Allan et al., 2009; Ezenwa et al., 2006; Kilpatrick et al., 2006). The success of these studies serves as a reminder that distilling diverse infection processes into their core components can be valuable for testing epidemiological theory (Becker et al., 2020; Downs

et al., 2019; Stewart Merrill & Johnson, 2020). Our approach accomplished this with a focus on both generality (to unite theory across systems) and neglected aspects of the infection process (to embed ecological reality). To measure competence, we addressed the standard steps of initial infection and parasite persistence (LaFonte & Johnson, 2013; Stutz et al., 2019; Stewart Merrill et al., 2019) but were novel in our incorporation of pre-transmission mortality and in our integration of processes over varying exposure doses. With the elements of competence and its emergent outcomes in hand, we gained insight into why species vary in competence, and acquired functional trait values that can be used to predict transmission in communities. Importantly, our system provided a somewhat simple avenue for quantifying competence: because the parasites do not replicate within the host (each infective stage can only become one transmittable stage), we did not need to measure parasite output resulting from infection (as one would for a continuously shedding virus, for example). Opportunities remain to adapt our conceptual and quantitative approaches for parasites and pathogens that produce multiple transmittable stages, over discrete or continuous time periods (Stewart Merrill & Johnson, 2020).

Translating laboratory estimates to functional traits in natural environments is inherently challenging, due to the limited ecological reality offered by controlled artificial settings. Although we strived to minimize such effects, laboratory constraints likely impacted our estimates of competence to some degree. Of note is that we ran our infection assay for 20 days, while amphibians may be able to clear metacercariae over longer time-scales. Bullfrogs, in particular, spend 2 years in the larval stage prior to metamorphosis (Treanor & Nicola, 1972), so extended windows of clearance may be important for shaping competence in this species. Another caveat is that we exposed amphibians to cercariae singly (only one parasite species was administered in a given exposure) and in one discrete time period. Exposure is almost certainly continuous in the field, with possibilities for co-infection by separate parasite species. Both of these factors open the opportunity for prior infections to shape the outcome of future infections by stimulating or suppressing the host immune response (Hoverman et al., 2012). Finally, although our competence values showed a high degree of robustness in the laboratory, natural environments include myriad ecological factors that can amplify individual-level variation. A necessary next step for our study is ground-truthing competence values with natural patterns of infection (while controlling for exposure). While laboratory-field comparisons establish the reliability of competence estimates, they also provide rich ground for further investigation. Any instance where a host species' infection level deviates dramatically from a competence-driven expectation will inspire new questions on within-species variation of both host and parasite.

## 5 | CONCLUSIONS

By incorporating information on host–parasite interactions and their outcomes, competence can bridge organismal processes to community-level patterns. But building this bridge requires operational and ecologically relevant ingredients. We must know: (a) how many parasites can enter and persist within a host; (b) whether a host will survive to the point of transmission; and (c) how infection outcomes vary over natural exposure gradients. When put together, the resulting value—competence—can be used as a functional trait for predicting transmission. More specifically, competence values and density estimates for each host in a community can be combined to compute 'community competence' (an average value used as a predictor in statistical transmission models; Allan et al., 2009; Johnson et al., 2013), and competence values for each species can also parameterize mathematical models. We have provided competence values for a nearly complete ecological assemblage and identified core processes underlying their variation, as well as the biological scales at which variation emerges. Our study can help catalyse new directions for experimental and observational research. For instance, the importance of barrier resistance for competence motivates examining which immunological processes are up-regulated early in the interaction and are therefore responsible for producing amphibian-level differences. In addition, the contrasting competence estimates of newts and chorus frogs motivate examining their roles as diluters and amplifiers during periods of trematode transmission. Our competence values sit at the nexus of host–parasite process and epidemiological pattern; continued study of this critical property will refine our understanding of how community composition and parasite transmission are inherently linked.

### AUTHORS' CONTRIBUTIONS

P.T.J.J. conceived of and designed the experiment; D.M.C. and P.T.J.J. ran the experiments and collected and entered the data; T.E.S.M. developed the conceptual model and analysed the data with assistance from P.T.J.J.; T.E.S.M. produced the first draft of the manuscript, and P.T.J.J. and D.M.C. provided revisions to the manuscript.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.3j9kd51mj (Stewart Merrill et al., 2022).

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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