LETTER

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Biodiversity decreases disease through predictable changes in host community competence

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Accelerating rates of species extinctions and disease emergence underscore the importance of understanding how changes in biodiversity affect disease outcomes[1–3]. Over the past decade, a growing number of studies have reported negative correlations between host biodiversity and disease risk[4–8], prompting suggestions that biodiversity conservation could promote human and wildlife health[9,10]. Yet the generality of the diversity–disease linkage remains conjectural[11–13], in part because empirical evidence of a relationship between host competence (the ability to maintain and transmit infections) and the order in which communities assemble has proven elusive. Here we integrate high-resolution field data with multi-scale experiments to show that host diversity inhibits transmission of the virulent pathogen *Ribeiroia ondatrae* and reduces amphibian disease as a result of consistent linkages among species richness, host composition and community competence. Surveys of 345 wetlands indicated that community composition changed nonrandomly with species richness, such that highly competent hosts dominated in species-poor assemblages whereas more resistant species became progressively more common in diverse assemblages. As a result, amphibian species richness strongly moderated pathogen transmission and disease pathology among 24,215 examined hosts, with a 78.4% decline in realized transmission in richer assemblages. Laboratory and mesocosm manipulations revealed an approximately 50% decrease in pathogen transmission and host pathology across a realistic diversity gradient while controlling for host density, helping to establish mechanisms underlying the diversity–disease relationship and their consequences for host fitness. By revealing a consistent link between species richness and community competence, these findings highlight the influence of biodiversity on infection risk and emphasize the benefit of a community-based approach to understanding infectious diseases.

Worldwide, ecological systems continue to undergo dramatic changes in biodiversity that affect a range of community and ecosystem processes[1,3]. Recently, biodiversity changes have also been linked to shifts in disease risk for humans and wildlife[9,10,14]. Because many pathogens infect multiple host species that vary in their competence[2,15], host community composition can acutely influence disease outcomes. If more diverse assemblages support a greater fraction of low-competency hosts, biodiversity losses have the potential to increase disease risk ('dilution effect')[16]. Although support for this hypothesis has accumulated for a growing list of human, plant and wildlife diseases[4,5,7,8,17–19], uncertainty persists over the generality of such patterns and their underlying mechanisms[11–13].

The outcome of biodiversity changes for infectious disease risk ultimately depends on the specific order in which species are added to or lost from a community relative to their competence for supporting infection[20]. If increases in diversity lead predictably to the addition of low-competency species—thereby lowering the community's capacity to support infection ('community competence')—a dilution effect will occur. In contrast, the addition of high competency hosts with increasing richness can cause an amplification effect[16,20]. Although ecologists have long-recognized that communities are nonrandomly structured[21], the influence of naturally occurring patterns of community assembly and disassembly has only recently been more widely incorporated into studies of biodiversity loss[22]. Aside from simulation-based models[20], however, this concept has received surprisingly little attention in disease research, and how community host competence and species richness co-vary in natural systems remains largely unexplored[7].

Here we combined high-resolution sampling of replicate host assemblages with multi-scale experiments to test the effects of host diversity on pathogen transmission and evaluate the role of functional changes in community competence in driving this pattern. We focused on interactions between pond-breeding amphibians and the multi-host trematode *Ribeiroia ondatrae*, which causes mortality and severe malformations[23]. Pond systems are well suited to address questions involving assembly because they provide replicate assemblages and support an experimentally tractable gradient of host richness (here, 1 to 6 species). Over three years, we sampled amphibian richness and composition within 345 wetlands across a 758,100 hectare region in California, USA (Supplementary Fig. 1) and assessed pathology (malformations) in 24,215 hosts. Experimentally derived estimates of host competence were combined with information on species composition to test whether increases in host richness lead predictably to decreases in community competence. Because predictions of the dilution effect often involve changes in transmission[16], or the dynamic ability of pathogens to move between hosts, simple comparisons of host richness and infection prevalence or intensity may fail to adequately test this framework. We therefore derived empirical estimates of realized transmission (see Supplementary Methods) by quantitatively linking information on infection from 17,516 snails, which function as intermediate hosts, to *R. ondatrae* load within 4,520 amphibians. We complemented field surveys with experiments that directly tested for diversity-driven changes in pathogen transmission within realistic host assemblages.

Among naturally occurring assemblages, amphibian species composition changed consistently along a gradient of species richness, leading to a nested pattern in which low-diversity communities formed near-perfect subsets of more diverse assemblages (matrix temperature = 20.47°; $P < 0.001$; Fig. 1a). Estimates of host competence correlated positively with field-based measures of both a species' occurrence and its abundance when present, leading to variation in each species' contribution to 'community competence' (see Methods and Fig. 1). Accordingly, the most-competent amphibian host (*Pseudacris regilla*) was also the most common species with progressive decreases in the fraction of highly competent hosts in more diverse assemblages (Fig. 2a). Integrating host species' competence with their relative abundance, we estimated a 35.7% decrease in community competence over the observed richness gradient (generalized linear model (GLM) $F_{1,\ 286} = 7.72$, $P = 0.006$, Fig. 2b).

The nested structure of host assemblages led to strong differences in realized transmission and disease pathology with changes in host richness. Among host populations, mean infection load explained ~75% of variation in malformation frequency (GLM, $t = 24.46$, $P < 0.0001$;

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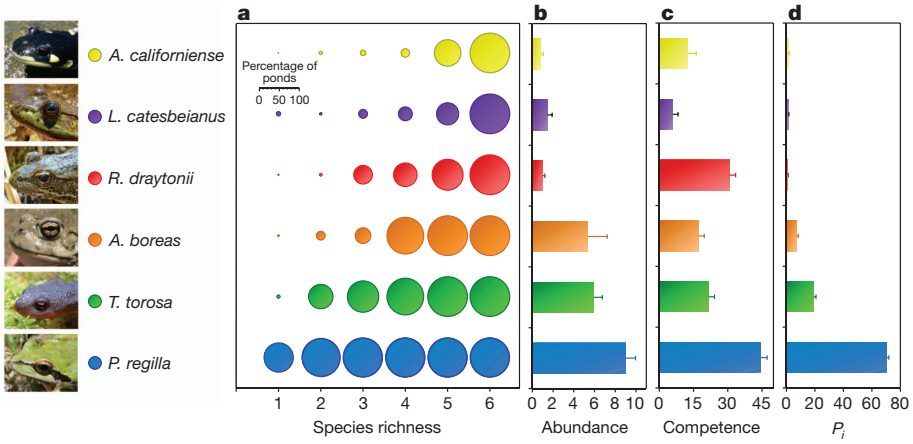

Figure 1 | Consistent linkages among species richness, community composition and the traits of individual amphibian host species across 345 sampled wetlands. **a**, The percentage of wetlands supporting each species is represented by the size of the circle within each richness level (nested structure; observed matrix temperature = 20.47°; average null model matrix temperature = 69.13°; $P < 0.001$). The host species were *Ambystoma californiense, Lithobates catesbeianus* (also known as *Rana catesbeiana*), *Rana draytonii*, *Anaxyrus boreas*, *Taricha torosa* and *Pseudacris regilla*. **b**–**d**, Shown for each host species is the mean abundance (number m^{-2}) when present (**b**), its laboratory-measured competence for supporting *R. ondatrae* (**c**), and an index of each host species' contribution to community competence (P_i, which combines the fraction of wetlands occupied by a host, its relative abundance when present, and host competence—scaled between 0 and 100%) (**d**). All error bars represent standard error (s.e.). Species occurrence, abundance, competency and body size (not shown) all loaded strongly ($>|0.87|$) on a single principal component (eigenvalue = 3.5, 89% of variation). Images were provided by G. Nafis (*A. californiense*) and D. Preston (all others).

Fig. 3a), which affected up to 90% of hosts in some populations (Supplementary Discussion). However, amphibian species richness strongly moderated transmission between snails and amphibians ($R^2 = 0.48$, $F_{3,\ 132} = 40.33$, richness × infected snail density $P < 0.0001$), with a 78.4% reduction in realized transmission and a 52.6% decrease in pathology in more diverse assemblages (Fig. 3b and Supplementary Discussion). Thus, infected snail density positively predicted amphibian infection, but infection success between snails and amphibians decreased with progressive increases in amphibian richness (Fig. 3b). The best-supported models included main effects and interactions between infected snail density and either amphibian richness (ΔAkaike's information criterion (AIC) = 0, $w_i = 0.76$) or the occurrence of each host species (ΔAIC = 4.08, $w_i = 0.10$) (Supplementary Discussion). Results were robust to whether we analysed data using individual hosts nested within wetlands or averaged among hosts from each site (Supplementary Discussion). Among wetlands for which we obtained infection data from all amphibian host species, community competence and its interaction with infected snail density explained 89% of the variance in total *R. ondatrae* load (summed among all host species) ($R^2 = 0.89, F_{3,\ 21} = 57.93, P < 0.0001$). This model was a better fit to the data than those with amphibian richness, host density, or the occurrence of particular host species (ΔAIC = 10.7).

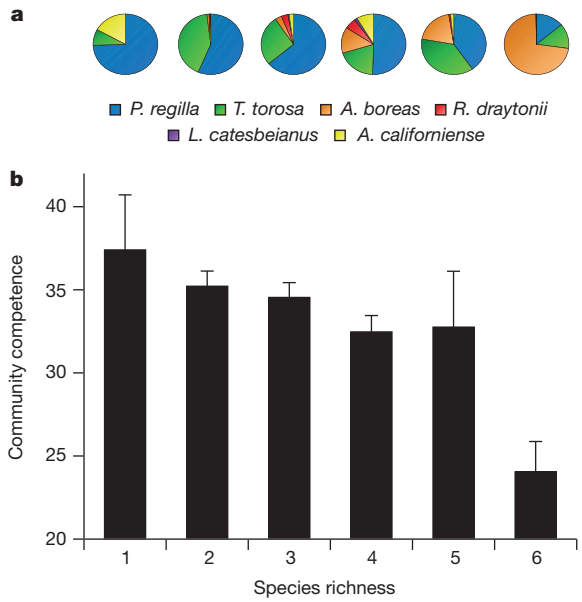

Figure 2 | Influence of amphibian species richness on the capacity of communities to support parasite infection in naturally occurring wetlands. **a**, **b**, Relative abundance of each host species (**a**) and mean community competence (**b**) (+1 s.e.) as a function of amphibian species richness. Community competence integrates information on each host species' competence and relative abundance within the community, providing a metric of the potential for communities at each richness level to support infection (see Supplementary Methods). This measure scales between the lowest (*L. catesbeianus* = 1) and highest (*P. regilla* = 44.34) observed competence values (c_{min} and c_{max}).

Experimental manipulations reinforced field observations and provided insight into the relative effects of host density and composition on transmission. In laboratory manipulations, increases in host richness from one to three species caused a 64% reduction in transmission (GLM, richness: -9.648 ± 2.265, $P < 0.0001$; Fig. 4a). This effect was not entirely attributable to changes in the density of the most competent host, as infection decreased by 28% even when *P. regilla* density was fixed and other species were introduced additively. In outdoor mesocosms designed to mimic natural assemblages, both total infection (summed among host individuals and species) and per capita infections in *P. regilla* decreased by approximately 50% between the lowest (one species) and highest richness (four species) treatments (total: GLM $F_{2,\ 22} = 18.19$, $P < 0.0001$; *P. regilla*: Generalized linear mixed model (GLMM) $t = -3.6761$, $P = 0.0013$; Fig. 4b and Supplementary Discussion). Correspondingly, increases in richness caused a decline in disease pathology (Fig. 4c).

By integrating information on community structure, host competence and pathogen infection from a large number of replicate assemblages, this study provides empirical evidence of a functional link between biodiversity and pathogen transmission. Our findings help to strengthen the conceptual foundation underlying the dilution effect by demonstrating that strongly nested assemblages can lead to a predictable relationship between host richness and community competence, in which low diversity assemblages support a greater proportion of highly competent host species[6]. Combined results of experiments and field-based measurements of realized transmission

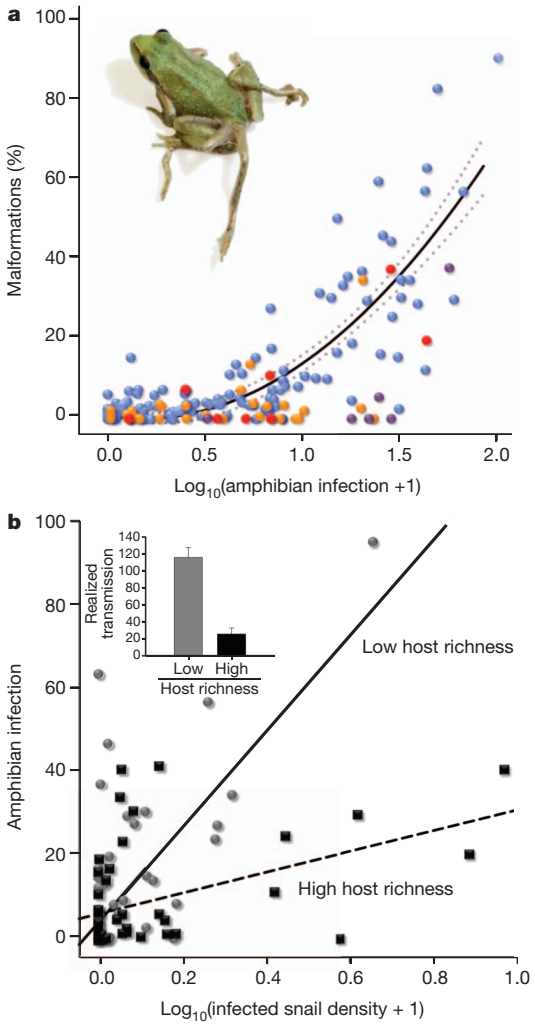

Figure 3 | Effects of parasite infection on amphibian malformations and of host richness on parasite transmission. **a**, Relationship between *R. ondatrae* infection and malformation frequency in anuran host populations. Malformation data were assessed through inspections of 24,215 amphibians, whereas infection data were derived from 4,520 necropsied hosts (see Fig. 1 for host colour scheme). Dotted lines represent 95% confidence (GLM with quasibinomial distribution, $\log_{10}$ (*R. ondatrae* +1), $t = 24.46$, $P < 0.0001$; pseudo-$R^2 = 74.74$, $n = 291$ species × wetland combinations). **b**, Effects of amphibian species richness on realized *R. ondatrae* transmission between snail intermediate hosts and amphibians. The *y* axis displays *R. ondatrae* load (mean per *P. regilla*) and the *x*-axis displays the $\log_{10}$ (density of infected snails +1). Amphibian richness interacted significantly with infected snail density to determine *R. ondatrae* load, with decreases in the transmission coefficient between low richness (1–3 species; grey circles and solid line) and high richness (4–6 species; black squares and dashed line) ($R^2 = 0.48$, $F_{3,\ 132} = 40.3314$, $P < 0.0001$, $n = 136$ site visits; 1,798 necropsied amphibians and 11,041 dissected snails). Inset depicts the realized transmission coefficients (+1 s.e.) in low and high richness assemblages. Image provided by D. Herasimtschuk.

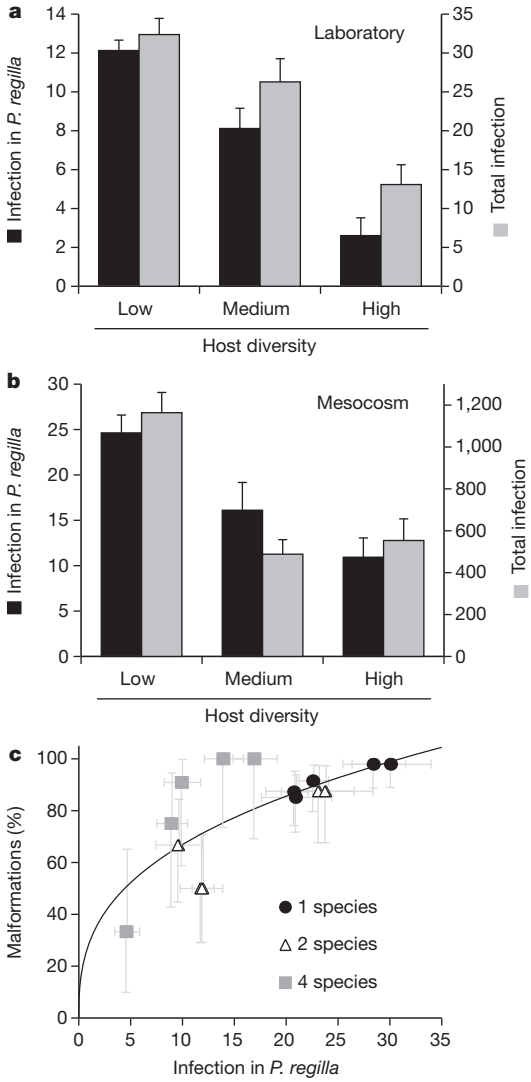

Figure 4 | Experimental effects of host diversity on parasite transmission and host pathology. **a**, **b**, Effects of host richness on per capita *R. ondatrae* infection in *P. regilla* (black bars; mean +1 s.e.) and total infection across all host species (grey bars; mean +1 s.e.) in laboratory (**a**) and mesocosm (**b**) experiments. Low, medium and high richness represent one, two and three species, respectively, in the laboratory experiment, and one, two and four species, respectively, in the mesocosm experiment. **c**, Relationship between average *R. ondatrae* infection (± 1 s.e.) and malformation frequency (±95% confidence interval) in *P. regilla* among mesocosm host richness treatments (GLMM with binomial distribution, effect of richness $Z = -2.71$, $P = 0.029$).

indicate that this relationship leads directly to a reduction in infection success at higher host richness, even after controlling for changes in host density. Nonrandom structuring of ecological communities has proven similarly important for explaining the relationship between biodiversity and ecosystem functioning[22], but has rarely been demonstrated for studies of infectious disease in a community context[20,24]. One possible explanation for the negative relationship between a host's competence and its assembly order is that defences are costly and may incur trade-offs with resource investment in reproduction or dispersal[25]. Indeed, studies in both eco-immunology and conservation support linkages between a species life history traits (for example, 'pace of life') and its vulnerability to infection or extinction, respectively[26,27].

These findings also advocate care in specifying what epidemiological outcomes are associated with diversity changes. Here, host diversity interacted strongly with infected snail density to determine amphibian infection, indicating that the richness of hosts moderated the realized transmission success (that is, β') of parasites in moving between snails and amphibians, rather than through alternative mechanisms such as controlling the abundance of available hosts[16]. For many multi-host and vector-borne infections, the density of vectors, intermediate stages and susceptible hosts will vary spatially and temporally due to a range of environmental factors (for example, climate, resources or habitat), independent of local diversity. This emphasizes the importance of field-based estimates of infection pressure and suggests that studies relying on static comparisons of host richness and infection prevalence or intensity may fail to capture the dynamic effects of diversity on transmission. Finally, it is important to note that the effects of diversity will also depend on the specific response metric under consideration. For instance, host diversity and parasite diversity will often correlate

positively due to enhanced colonization opportunities[28], but parasite richness is not equivalent to disease risk, particularly when parasites vary in virulence and relative abundance[29].

Our results lend mechanistic insight into how host diversity can reduce disease risk through predictable co-variation between species-level traits and community assembly. In light of mounting evidence that higher biodiversity can buffer against pathogen exposure in human, wildlife and plant disease systems[4,6,8,10,17–19], preserving functional diversity—including both genetic diversity and community richness—has the potential to ameliorate pathogen transmission and offer a novel, cost-effective approach to disease management.

METHODS SUMMARY

Field surveys. Between 2009 and 2011, we estimated the abundance and species composition of amphibians among 345 wetlands in California, USA (Supplementary Fig. 1). At sites with *Planorbella trivolvis* (also known as *Helisoma trivolvis*), the requisite snail host for *R. ondatrae*, we conducted a second visit to quantify malformation frequency and infection load (Supplementary Methods, 678 total site visits). *R. ondatrae*-infected snail density was estimated as the product of snail density and infection prevalence. We used bootstrapping and rarefaction analyses to validate parasite abundance and amphibian richness estimates (Supplementary Figs 3 and 4) and calculated nestedness using the nestedness temperature calculator (Supplementary Methods).

Experimental studies. In 2 litre containers, we tested how the number of amphibian hosts and host species richness affected *R. ondatrae* infection success. To increase ecological realism, we conducted a 2×3 factorial experiment manipulating infection and amphibian host diversity within outdoor mesocosms. Both sets of experiments compared additive and substitutive designs (Supplementary Methods).

Laboratory measures of host competence. We measured host competence by exposing individuals of each host species to *R. ondatrae* cercariae during early limb development and quantifying the proportion of encysted parasites remaining immediately following metamorphosis (see Supplementary Methods and ref. 23).

Full Methods and any associated references are available in the online version of the paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions P.T.J.J. designed the study, D.L.P., K.L.D.R. and P.T.J.J. collected the data, P.T.J.J. and J.T.H. analysed the data, and all authors wrote the manuscript.

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METHODS

Field surveys and parasite assessment. Over 3 years, we used standardized methods to survey amphibian abundance and species composition in 345 wetlands from across a 758,100 hectare region of California, USA (Supplementary Fig. 1 and Supplementary Methods). At sites with *P. trivolvis*, the requisite snail host for *R. ondatrae*, we conducted a second late-summer visit to quantify malformations and collect amphibians for necropsy (678 total site visits). *R. ondatrae*-infected snail density was estimated by multiplying snail density by infection prevalence (Supplementary Methods). To quantify *R. ondatrae* infection in amphibians, we necropsied 10–15 recently metamorphosed *P. regilla* per site[30,31]. We focused on *P. regilla* because this amphibian host is widespread, locally abundant and sensitive to parasite-induced malformations, thereby offering a consistent basis for cross-site comparisons. At a subset of sites ($n = 25$), however, we necropsied all co-occurring amphibian species and used these data to generate estimates of total *R. ondatrae* abundance, or the product of each host's relative abundance and its average *R. ondatrae* infection intensity. We used bootstrapping and rarefaction analyses to validate the efficacy of parasite abundance and amphibian richness estimates (Supplementary Figs 2 and 3).

Estimates of host competence. We estimated host competence, or the ability of *R. ondatrae* to infect and persist within each host species, of the six amphibian species present at our field sites by exposing larvae to *R. ondatrae* cercariae in 1.5 litre containers[32]. Cercariae were obtained from wild-caught *P. trivolvis* and administered to amphibians within 4 h of release. Host species were collected as eggs and allowed to develop to stage 28 (anurans[33]) or stage 2T (newts[34]) before exposure. For the two endangered species under federal protection, we used closely related congeners as surrogates (*Rana aurora* and *Ambystoma tigrinum*)[35,36]. Hosts were necropsied at metamorphosis to quantify metacercariae, and we calculated competence as the percentage of parasites detected relative to the number administered[16]. For field analyses, we built upon this approach by calculating 'community competence' (p) for the amphibian assemblage at each wetland (modified from[37]): $p = \sum_{i=1}^{n} c_i S_i \Big/ \sum_{i=1}^{n} S_i$ where c_i is the competence of species i and S_i is its abundance (which becomes relative abundance when divided by the total number of amphibian larvae captured).

Laboratory and mesocosm experiments. In laboratory experiments (2 litres), we tested how the number of hosts (1, 2 or 3 *P. regilla*) and host community composition (1, 2 or 3 species) affected both the infection per host and the total number of successful parasites. For the diversity portion, each replicate contained three individuals representing one species (three treatments involving three individuals of *P. regilla*, *Rana cascadae*, or *L. catesbeianus*), two species (two treatments involving two *P. regilla* and either one *R. cascadae* or one *L. catesbeianus*) and three species (one individual of each species) ($n = 15$ replicates per treatment). Over 5 days, we added 60 *R. ondatrae* cercariae to each container; hosts were euthanized, measured and necropsied after 48 h. To increase ecological realism, we conducted a 2×3 factorial experiment manipulating *R. ondatrae* infection (yes or no) and host diversity (1, 2 or 4 host species) within outdoor mesocosms (Supplementary Methods). Diversity treatments contained one species (60 *P. regilla*), two species (30 *P. regilla* and 30 *A. boreas*), or four species (15 *P. regilla*, 15 *A. boreas*, 15 *T. torosa* and 6 *L. catesbeianus*) (substitutive design). The design was informed by field data such that the four most common species (85% of observations) were included in the high diversity treatment, and the most ubiquitous species (*P. regilla*) was the focal host in the one species treatment. In the density experiment, mesocosms contained 15, 30 or 60 *P. regilla*. All treatments were replicated five times. Over 8 days, we added 5,324 cercariae ($\pm$162 s.e.) to each mesocosm (Supplementary Methods) and collected amphibian hosts as they reached stage 42 (*P. regilla* and *A. boreas*) or after 40 days (*L. catesbeianus* and *T. torosa*).

Field analyses. We used the program Aninhado[38] to test for nestedness using the nested temperature calculator[39] and compared the observed metric score to 1,000 permutations of a null model. To evaluate the link between *R. ondatrae* infection and malformations, we used a generalized linear model with a quasibinomial response and included a fixed effect for species identity[40,41] (Supplementary Methods). Among sites that supported *R. ondatrae*, we tested how host species richness affected parasite transmission by incorporating information on infected snail density ($\log_{10}$-transformed). We expected the density of infected snails to positively predict amphibian infection (realized transmission) and sought to identify how host diversity moderated this relationship. This analysis was performed both at the site level, in which infection in frogs was averaged, and at the individual host level, in which frogs were nested within site using a generalized linear mixed model with a negative binomial distribution. Alongside infected snail density, which was included in all models as an a priori measure of parasite infection pressure, we included measurements of host diversity (richness, Shannon diversity and evenness)[42], the presence of each host species, and the density of amphibian larvae ($\log_{10}$-transformed). Because we expected host species richness or composition to function as moderators of transmission success, we included an interaction term between infected snail density and each predictor. We assessed among models using Akaike's information criterion and model weights[43]. For wetlands with necropsy data from all amphibian species ($n = 25$), we repeated this procedure with community competence and its interaction with infected snail density.

Laboratory and mesocosm analyses. To test how host density and species richness affected per capita infections, we used generalized linear mixed models in which hosts were nested within replicates and parasite counts were modelled using a negative binomial distribution[40,41]. This approach was also used to test how assemblage structure affected malformations and host development. To test effects on total amphibian infection (summed among host species), we used generalized linear models with Gaussian or Poisson distributions. By comparing the diversity and density treatments, we also contrasted the effects of diversity between additive and substitutive designs (we compared the effects of changes in species composition both when the total number of hosts was held constant (substitutive) and when the abundance of the focal host was held constant and other species were added sequentially (additive)).

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