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Community disassembly and disease: realistic—but not randomized—biodiversity losses enhance parasite transmission

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Debates over the relationship between biodiversity and disease dynamics underscore the need for a more mechanistic understanding of how changes in host community composition influence parasite transmission. Focusing on interactions between larval amphibians and trematode parasites, we experimentally contrasted the effects of host richness and species composition to identify the individual and joint contributions of both parameters on the infection levels of three trematode species. By combining experimental approaches with field surveys from 147 ponds, we further evaluated how richness effects differed between randomized and realistic patterns of species loss (i.e. community disassembly). Our results indicated that community-level changes in infection levels were owing to host species composition, rather than richness. However, when composition patterns mirrored empirical observations along a natural assembly gradient, each added host species reduced infection success by 12–55%. No such effects occurred when assemblages were randomized. Mechanistically, these patterns were due to non-random host species assembly/disassembly: while highly competent species predominated in low diversity systems, less susceptible hosts became progressively more common as richness increased. These findings highlight the potential for combining information on host traits and assembly patterns to forecast diversity-mediated changes in multi-host disease systems.

1. Introduction

Global losses of biodiversity have galvanized efforts to understand how changes in communities affect ecological processes, including transmission of parasites and pathogens (hereafter, 'parasites'). Because most emerging diseases involve multi-host parasites [1,2], determining how shifts in community composition and species diversity alter parasite transmission, persistence and temporal dynamics is a pressing issue for both conceptual and applied research [3,4]. Evidence from a growing number of empirical surveys, modelling studies and experimental manipulations indicate that biodiversity changes can influence infection dynamics through diverse mechanisms. For example, changes in the density, identity or interactions among available host species all have the potential to suppress or enhance infections [5]. In the African savannah, removal of large mammal species results in significantly higher helminth burdens within rodents owing to consequent increases in their density [6].

With the rising interest in understanding the links between host diversity and infection dynamics, a polarizing debate has emerged over whether biodiversity losses will lead to increased pathogen transmission (via the 'dilution effect'; [7]), decreased transmission [8], or whether responses are idiosyncratic

among systems [9]. A key difficulty is that diversity can influence infection through multiple and potentially even opposite pathways. In addition to species richness (number of species), shifts in diversity are frequently accompanied by changes in host density/biomass (i.e. species abundance or evenness) and species composition (i.e. species identity) [6,10]. Field-based correlational studies in isolation are therefore often limited in their capacity to identify underlying mechanisms or quantify their relative importance in altering transmission.

These observations highlight the need for experimental approaches that can disentangle alternative mechanisms. Because natural communities rarely assemble at random [11], quantifying the effects of species richness and composition requires carefully designed experiments with multiple configurations at each richness level [12,13]. Such experiments can also be combined with empirical data to test how the effects of diversity depend on the order in which species are gained or lost from communities (i.e. community assembly and disassembly, respectively) [14,15]. For instance, Bracken *et al*. [15] showed that 'realistic' increases in seaweed diversity that mirrored observed patterns of species assembly led to enhanced nitrogen uptake in experimental communities, whereas randomly composed seaweed assemblages led to no such changes. Thus, even when changes in a response are driven predominantly by species composition (i.e. the identity and abundance of species), diversity losses may nonetheless lead to consistent shifts in a response if richness and composition covary. This information may be especially relevant to forecasting how changes in diversity are likely to influence community or ecosystem-level processes, including those related to infectious diseases [16–19].

Here we integrated approaches from community ecology and biodiversity-ecosystem function (BEF) research to test mechanisms underlying the diversity–disease relationship. Building upon designs from BEF studies [13,20], we used an experimental approach involving five host species and 14 assemblage permutations to disentangle the influence of amphibian host richness and species composition on infection success by three different trematode parasites. Our aim was to test whether observed changes in infection—either at the level of individual hosts or the community as a whole—were owing strictly to (i) host species composition, (ii) host species richness or (iii) a joint outcome of both factors. By combining experimental results with complementary field surveys from 147 ponds (2009–2015) along a richness gradient, we further tested whether the link between richness and infection depended on whether experimental communities were assembled randomly or following empirical patterns. This work aims to help bridge the gap between correlational field surveys and small-scale manipulations by isolating the effects of diversity on multiple parasites, at multiple scales and under alternative assembly scenarios.

2. Material and methods

(a) Study system

Interactions between pond-breeding amphibians and their larval trematode parasites are well suited for addressing questions related to host composition and host richness. Digenetic trematodes have complex life cycles involving sequential transmission among host species embedded in ecological food webs [21]. Trematode-infected snails release free-swimming cercariae that have less than 24 h to find a suitable subsequent host, such as a larval amphibian [22]. Because infection success is strongly influenced by the surrounding community [23], shifts in the identity of host species and their density often have measurable effects on transmission. The resultant infection load—which can be discretely quantified—determines host pathology and transmission potential to downstream hosts. Finally, the small size, well-defined boundaries and a tractable number of taxa facilitate extensive community-level replication, which frequently limits empirical opportunities to test the mechanistic processes underlying the diversity–disease relationship.

(b) Field surveys

To determine the relative frequency of alternative host community compositions, we sampled 147 wetlands between 2009 and 2015. On average, each pond was surveyed over 2–3 years (average of 2.9 years per site). Ponds were selected from among publicly accessible parks, open space reserves and conservation easements within the East Bay region of California (Alameda, Contra Costa and Santa Clara counties). Each pond was visited twice over the course of a summer (May through to August); we used visual encounter surveys, dipnet surveys and habitat-stratified seine hauls to assess what amphibian species were present (see detailed methods in [24]). The five most common lentic-breeding amphibians in this region are Pacific chorus frogs (*Pseudacris regilla*), western toads (*Anaxyrus boreas*) American bullfrogs (*Rana catesbeiana*), California newts (*Taricha torosa*) and rough-skinned newts (*Taricha granulosa*) [25]. California red-legged frogs (*Rana draytonii*) and California tiger salamanders (*Ambystoma californiense*) also occur in the area but are federally protected and were thus excluded from experiments.

For each pond-by-year combination ($n = 426$), we determined the community composition of larval amphibians as this is the stage in which infection by trematode cercariae occurs. Surveys of the same site done within a single year (e.g. early versus late summer) were combined to generate an annual value of species composition, whereas assessments done in different years were treated as separate observations. We thus used 'pond-year' as our unit of observation, although we explored the sensitivity of results to using only the most recent year in which a site was sampled. We calculated the relative frequency of alternative assemblage compositions when one, two or four species were present. Thus, for site-years with exactly two amphibian species, we determined the proportion of observations in which this involved *P. regilla* + *An. boreas*, *P. regilla* + *T. torosa*, *T. torosa* + *Ra. catesbeiana* and so on. The two protected amphibian species—even when present—were not included in these calculations (i.e. data on these species were omitted when characterizing host species composition).

(c) Experimental design and establishment

Using the five most common amphibian species from surveyed ponds (above), we conducted a factorial manipulation of amphibian host richness and host species composition to understand their individual and joint effects on infection. We obtained amphibian egg masses (*P. regilla*, *An. boreas* and *T. torosa*) and allowed them to hatch in the laboratory. After hatching, larvae were raised until early limb development (anurans: [26] stage 30–31; newts: Wong & Liversage [27] stage 2T–4T). For *Ra. catesbeiana* and *T. granulosa*, we collected comparably staged larvae from sites free of trematode infection. Temperature was maintained at 20°C on a 12 : 12 L : D schedule. Amphibians were fed ad libitum a mixture of Tetramin fish food and ground *Spirulina*.

Each microcosm (2.25 l) was filled with 1.8 l of treated water (ultraviolet-sterilized, carbon-filtered and dechlorinated) and stocked with four amphibian larvae, the identities of which depended on the treatment. Specifically, we used one of three levels of amphibian richness (1, 2 or 4 species) and 14 different

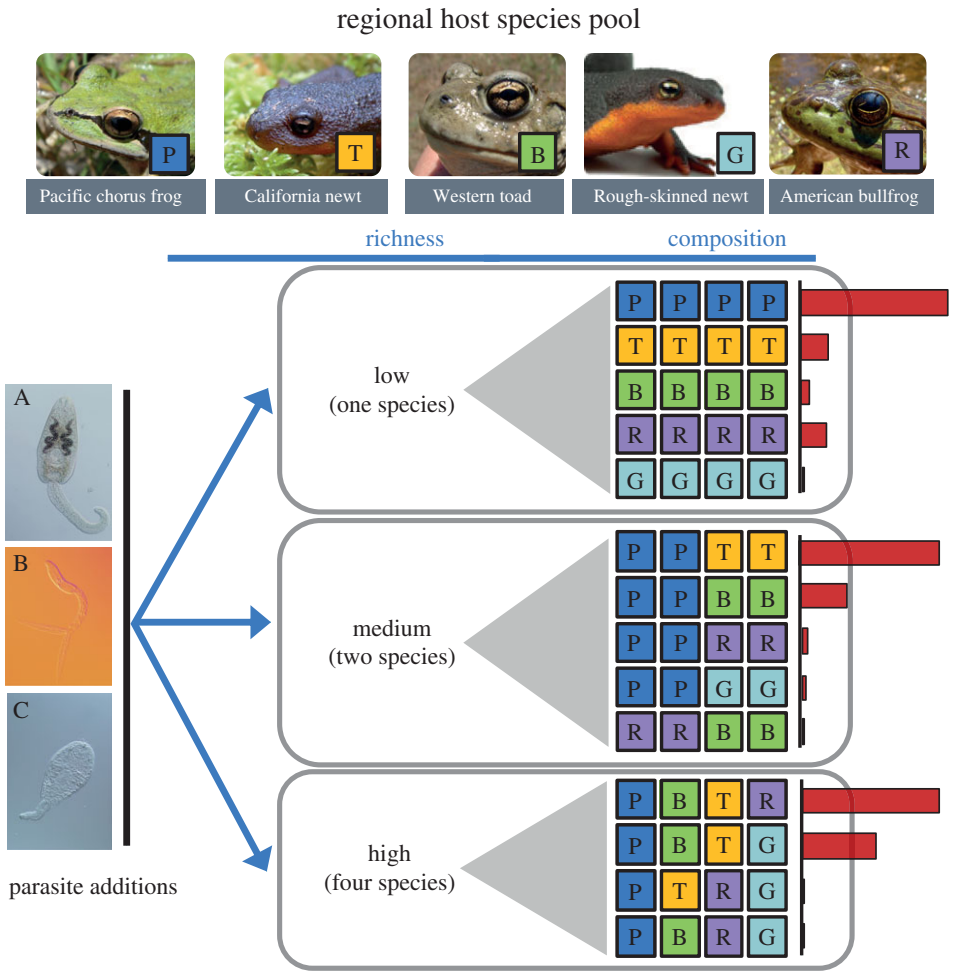

Figure 1. Conceptual diagram of the experimental design and its integration with field survey data on amphibian community composition and richness. Five lentic-breeding amphibian species (top) comprised the regional species pool from which we developed different configurations for each host species richness level, with five combinations of the one-species [monoculture] and two-species conditions, and four combinations of the four-species richness condition. Each microcosm contained exactly four individual hosts such that density was held constant across treatments. The red-coloured bars on the right side of the figure reflect the relative frequency in which assemblage was observed during pond field surveys; bars are scaled to reflect a maximum total of 1.0. Thus, for the one-species assemblages, 68.3% of field observations involved the Pacific chorus frog, 16.7% involved the California newt, 11.7% involved the American bullfrogs and so on. Amphibian species are indicated based on the colour and letter depicted in the squares: Pacific chorus frog (*P. regilla*) [P], California newt (*T. torosa*) [T], western toad (*An. boreas*) [B], rough-skinned newt (*T. granulosa*) [G] and American bullfrog (*Ra. catesbeiana*) [R]. Image credits: D. Preston. Infectious stages (cercariae) of each parasite are illustrated on the left side of the figure: (A) *Ribeiroia ondatrae*, (B) *Alaria marcinae*, and (C) *Cephalogonimus americanus*. (Online version in colour.)

assemblage compositions (figure 1; electronic supplementary material, table S1). Each assemblage was replicated between four and eight times for a total of 79 microcosms and 315 individual amphibian larvae (eight replicates were removed from the analyses because of mortality). The experiment encompassed all possible monocultures (one-species treatments), five of the 10 possible two-species permutations and four of the five combinations for four species. Thus, while amphibian density remained fixed (four larvae per microcosm), both richness and composition varied. Because larvae of different amphibian species differ in size, even when of comparable developmental stages, total host biomass nonetheless varied across treatments. We thus tested the potential effects of host biomass on infection. Average sizes (snout-vent length) and wet masses of each amphibian species are provided in the electronic supplementary material, table S2.

(d) Parasite exposures

We obtained cercariae of three trematode species (*Ribeiroia ondatrae*, *Alaria marcinae* and *Cephalogonimus americanus*) by collecting infected rams horn snails (*Helisoma trivolvis*) and isolating them into sterile 50 ml centrifuge vials. Although each of these trematodes uses rams horn snails and larval amphibians as first and second intermediate hosts, respectively, they vary in pathology, host specificity and definitive host species (e.g. see [28,29]). Parasites were identified using a combination of morphological characteristics and genetic data [30]. For the experiment, we collected cercariae from infected snails within 3 h of peak release [31] and added them to microcosms containing amphibian larvae over a 72 h period. Cercariae dosages were selected to maximize detection while limiting the risk of host mortality (a total of 115 *Ri. ondatrae* cercariae, 100 *Al. marcinae* cercariae or 100 *C. americanus* cercariae) [28,29]. One week after cercariae additions, the number and identity of trematode cysts (metacercariae) were quantified following systematic necropsy.

3. Statistical analysis

(a) Community-level infection

To evaluate how community composition and richness influenced the total infection for each parasite species, we summed the number of metacercariae of a particular parasite species among the four hosts from a replicate (total infection load). We used generalized linear mixed models (GLMMs) to test how host species richness, parasite species (as a factor) and their interaction affected parasite count, which was

modelled as an overdispersed Poisson distribution with a log-link function. Assemblage composition was incorporated as a random intercept term. Because counts of parasite infection were overdispersed, we also included an observation-level random effect. An offset term was used initially to account for the differing numbers of cercariae added by parasite species, although this was removed in subsequent analyses for specific parasite species. Our expectation was that, if richness and composition jointly determined infection, richness would significantly influence parasite load even while accounting for host composition as a random effect. Similarly, removal of the host composition random effect should significantly worsen model fit if the composition was responsible for influential variation, over and above richness. Models were implemented using the glmer function within the lme4 package in R [32,33]. Likelihood-ratio tests were used to identify the significance of specific terms by comparing full and reduced versions of nested models.

(b) Randomized versus realistic assemblages

To determine whether the influence of host richness depended on the empirical link between composition and diversity within natural systems, we incorporated a fixed, numeric term to represent the relative frequency in which each assemblage was observed empirically, relative to all observations at that richness value (hereafter, 'community frequency'). For instance, if 80% of four-species communities from field surveys involved *P. regilla*, *An. boreas*, *T. torosa* and *Ra. catesbeiana*, this treatment combination in the experiment would receive a value of 0.8. We then tested the effects of host richness, community frequency and their interaction using the same analytical approach described above (only without the random effect for composition). A significant interaction between community frequency and host richness would indicate that the effects of host richness on infection success depended on the degree to which disassembly patterns mirrored those from nature (i.e. high values of community frequency).

(c) Individual-level infection

To better understand the mechanisms underlying infection changes at the community level, we also tested how host richness and species composition influenced the number of parasites detected per individual host. Changes in total infection observed at the community level (summed among co-occurring individuals) could result from one of two pathways. First, the total infection could change with richness owing entirely to shifts in which host species were included, given that species often vary in competence. This scenario would imply that changes observed at the community level depended strictly upon the identity of host species in an assemblage. Second, the total infection might change if richness causes a shift in the parasite loads for particular host species (i.e. a shift in encounter likelihood). This could occur if changes in host richness are associated with shifts in either the likelihood a species encounters infectious parasites or its infection probability following the encounter. To explore these scenarios, we used GLMMs to test how parasites per individual host (modelled using an overdispersed Poisson distribution) varied by host species identity, host richness and their interaction. As random intercept terms, we included the replicate (because multiple hosts occur within each replicate) and the individual host (observation-level random effect, to account for overdispersion). We used Tukey's pairwise comparisons to evaluate differences in infection load between host species (using the glht function in the multcomp package; [34]). If the interaction between richness and host species identity was significant based on a likelihood-ratio test (LRT), we tested the influence of richness on each host species individually. For two host-by-parasite combinations (*C. americanus* in *Ra. catesbeiana* and *Al. marcinae* in *T. granulosa*), infection loads were low enough to cause convergence problems and were omitted.

Finally, for host species in which infection depended on host richness, we examined whether such changes were associated with the presence of a specific species. Thus, if the response variable was infection per *P. regilla*, we tested how the occurrence of the other host species (*An. boreas*, *Ra. catesbeiana*, *T. torosa* and *T. granulosa*) affected observed load. We did not include additional interaction terms among host species (i.e. the presence/absence of species A × species B × species C × species D) because of the large number of possible combinations and the lack of *a priori* biological hypotheses.

4. Results

(a) Field surveys

Between 2009 and 2015, we surveyed 147 ponds over an average of 2.9 years each. Across surveyed pond-years ($n = 426$), larval amphibian species richness ranged from 0 to 5 with an average ± 1 s.e. of 2.31 ± 0.05 (excluding the endangered species of amphibians). *Pseudacris regilla* was the most commonly encountered species, occurring in 92% of assemblages in which at least one amphibian species was detected ($n = 400$), followed by *T. torosa* (79%), *T. granulosa* (33.3%), *An. boreas* (28.5%) and *Ra. catesbeiana* (12.8%). The relative frequency of different species combinations also varied sharply (electronic supplementary material, table S1). At pond-years with only a single detected host species ($n = 60$), the most commonly detected species was *P. regilla* (68.3%) followed by *T. torosa* (16.7%) (e.g. figure 1). Single-species communities involving other species constituted less than 12% of observations (electronic supplementary material, table S1). Similarly, for assemblages with exactly two species ($n = 137$), the combination of *P. regilla* and *T. torosa* predominated (70.1%), with a secondary peak for *P. regilla* and *An. boreas* (18.3%). When four species co-occurred together ($n = 37$), the most common permutation consisted of *P. regilla*, *An. boreas*, *T. torosa* and *T. granulosa* (62.2% of observations) (electronic supplementary material, figure S1 and table S1). These patterns were broadly comparable if we used only the most recent survey for each pond.

(b) Community-level infection

Among treatments, the total number of metacercariae (summed among hosts in the same microcosm) varied by parasite species and by the experimental treatment. There was a main effect of parasite species (LRT, $\chi^2_2 = 126.21$, $p < 0.000001$); after accounting for differences in the number of cercariae added using an offset term, infection success was greatest for *Ri. ondatrae* (27.2%), followed by *C. americanus* (12.0%) and then *Al. marcinae* (5.9%) (Tukey pairwise comparisons, all $p < 0.00001$). Inclusion of assemblage composition (as a random effect) improved fit relative to the model without this term (LRT, $\chi^2_1 = 130.54$, $p < 0.00001$). For each parasite, variance in infection was greatest in the one-species treatment

(monoculture), reflecting broad differences in amphibian species susceptibility (figure 2).

Host richness had no effect on total parasite infection when all treatment compositions were included (both 'realistic' and 'unrealistic' assemblages) (GLMM: richness = 0.031 ± 0.284, $p = 0.914$), nor was there a richness-by-parasite species interaction. Similarly, when richness and composition were both included as random effects, species richness was associated with zero variance relative to composition (variance components analysis). Importantly, however, richness interacted significantly and strongly with the community frequency variable based on empirical observations (GLMM: richness × frequency = -0.25 ± 0.093, $p = 0.005$). Among commonly observed assemblages (frequency greater than 0.40), richness inhibited infection success by approximately 50% (GLMM: richness = -0.69 ± 0.138, $p < 0.00001$; $n = 45$), whereas no such effect occurred among combinations of species that were rare or unobserved in field surveys (GLMM: richness = 0.159 ± 0.113, $p = 0.156$; $n = 192$) (figure 2). Parasite species identity also interacted with richness (LRT $\chi^2_2 = 16.162$, $p = 0.0003$), such that the magnitude of the effect on infection varied by parasite: richness [*Alaria*] = -0.712 ± 0.176, $p < 0.0001$; richness [*Cephalogonimus*] = -0.778 ± 0.119, $p < 0.00001$; richness [*Ribeiroia*] = -0.121 ± 0.087, $p = 0.165$). Based on the exponentiated values of the coefficients from the Poisson model, each one unit increase in host richness led to a decrease in infection by between 12% and 55% (figure 2).

(c) Individual-level infection

Alongside differences in total infection, we also detected strong variation in parasite load among individuals. While all hosts became infected with each parasite at least once, host species differed considerably in average infection load for each parasite (LRT of the model with host species factor versus intercept-only model: *Ribeiroia* $\chi^2_3 = 261.13$, $p < 0.00001$; *Cephalogonimus* $\chi^2_3 = 196.76$, $p < 0.00001$; *Alaria* $\chi^2_3 = 182.95$, $p < 0.00001$) (figure 3). *Pseudacris regilla* had the highest average infection loads for *Alaria* and *Cephalogonimus*, with 4.72 and 7.53 metacercariae per host, respectively, while the two newt species were the most susceptible to *Ribeiroia* (average yields of 16.9 and 14.9 for *T. torosa* and *T. granulosa*, respectively) (electronic supplementary material, table S3). Based on Tukey's pairwise comparisons (electronic supplementary material, table S3), bullfrogs (*Ra. catesbeiana*) supported the lowest infection values for *Ribeiroia* and *Cephalogonimus*, with less than 1 metacercariae per host, while the *Taricha* species had especially low susceptibility to *Alaria*. Toads (*An. boreas*) had intermediate levels of infection for all three trematodes.

Richness also had host species-specific effects on the infection load for each parasite (LRT of model with host species × richness interaction versus host species-only model: *Ribeiroia* $\chi^2_5 = 17.42$, $p < 0.005$; *Cephalogonimus* $\chi^2_4 = 13.93$, $p < 0.008$; *Alaria* $\chi^2_4 = 20.67$, $p < 0.0005$) (figure 3). Specifically, increases in host richness led to a reduction in per-host infection in *P. regilla* for all three parasite species and in *An. boreas* for *Alaria* and *Cephalogonimus* (figure 3). In no case was higher richness associated with increased infection. Based on analyses of which host species were influential in driving observed infection changes with richness, the presence of bullfrog (*Ra. catesbeiana*) larvae emerged as a consistently negative predictor of average infection in both *P. regilla* and *An. boreas*. Across all treatments, for instance, average infection in *P. regilla* decreased by 71% with the addition of *Ra. catesbeiana* (electronic supplementary material, figure S2). There was no additional effect of host richness after accounting for bullfrog presence.

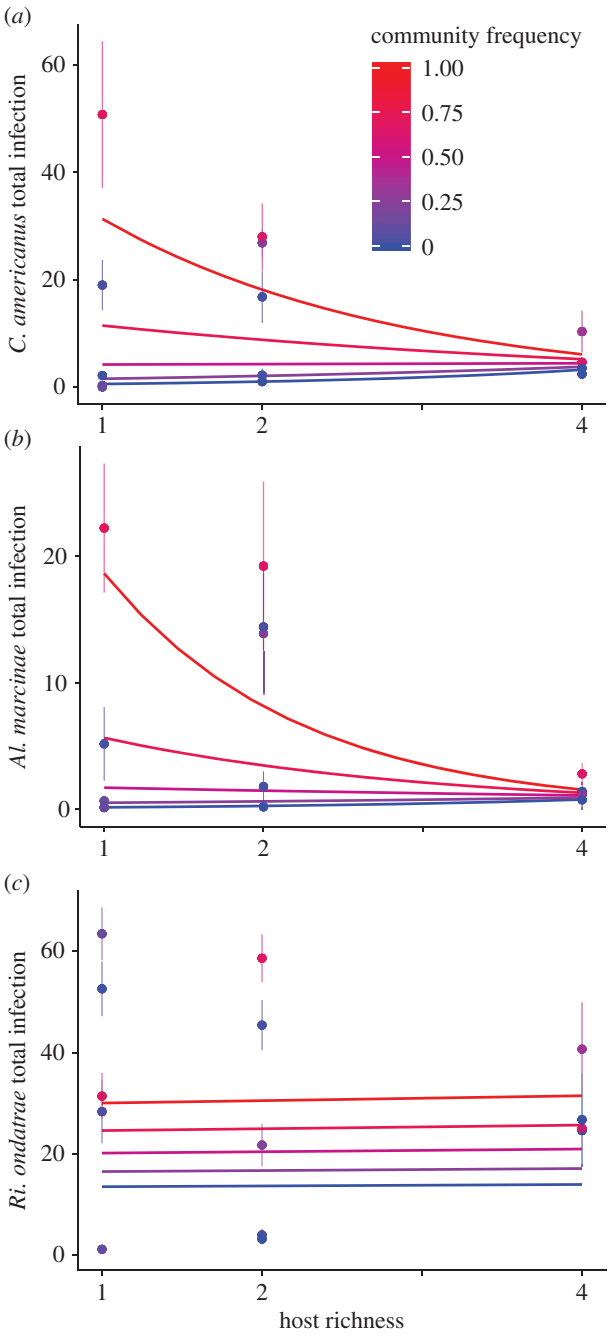

Figure 2. The effect of host richness on total infection success among parasite species and the community weight. For each plot, presented in the mean ± 1 s.e. of total infection success (summed among the four hosts in the same microcosm) as a function of host species richness (1, 2 or 4, *x*-axis). The different lines reflect the 'community frequency' of different assemblage compositions, or how frequently they were observed during field surveys along a natural richness gradient (with 1 as very frequent (red lines) and 0 (blue lines) as never observed). For the trematodes (*a*) *Cephalogonimus americanus* and (*b*) *Alaria marcinae*, increased host community richness led to a significant reduction in infection success when patterns mirrored natural assembly compositions. For (*c*) *Ribeiroia ondatrae*, there was no effect of richness regardless of whether the community was assembled realistically or not. (Online version in colour.)

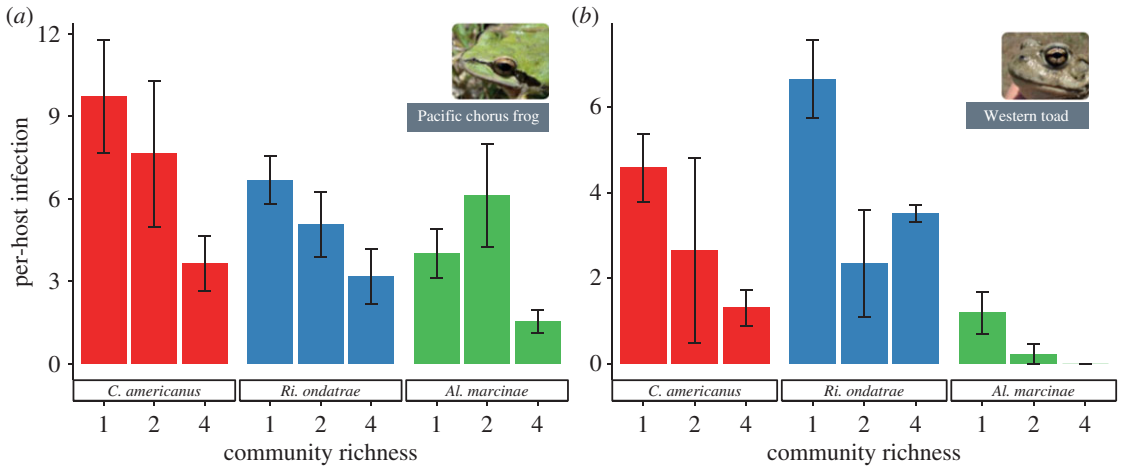

Figure 3. Effects of host richness on per-host infection loads in (*a*) Pacific chorus frogs and (*b*) western toads. Presented is the mean parasite load per host ± 1 s.e. Host richness caused a decrease in per-host infection by *Alaria marcinae* (both host species), *Cephalogonimus americanus* (both species) and *Ribeiroia ondatrae* (chorus frogs only). (Online version in colour.)

5. Discussion

Quantifying how host diversity influences pathogen transmission and disease dynamics remains a core challenge in disease ecology. As illustrated by research on biodiversity and ecosystem function, carefully designed experiments are needed to differentiate the influences of species richness from those of species composition [20,35], which are often confounded in correlational field surveys. By incorporating multiple configurations of host community composition at each richness level, including assemblages that were common or rare in the field, the results of our controlled experiments indicated that community-level changes in infection success were driven primarily by host species composition. While all three parasite species were able to establish in each host, amphibian species varied substantially in their competence as hosts; in the single-species treatments, per-host parasite loads varied by 100-fold among species. As a result, the identity of host species included in assemblages strongly influenced the total number of successful parasites, with no additional influence of species richness after accounting for host species composition. This stands in contrast to many experimental studies focusing on biodiversity and ecosystem functioning (e.g. [35]). In an analysis of grassland diversity manipulations, for instance, Hector *et al*. [36] reported that richness and composition each explained comparable amounts of variation in above-ground biomass production.

Alongside efforts to decouple the relative influence of the numbers and types of species, it is equally important to consider the order in which species are lost or gained in natural communities (disassembly and assembly, respectively), which is rarely random (e.g. [37,38]). Although the use of randomized species combinations offers valuable mechanistic insights [39], incorporation of realistic patterns in community assembly/disassembly is essential for identifying the effects of current and forecasted biodiversity changes [40]. By sampling 147 wetlands over a 6-year period, we found that one or two compositions typically accounted for 65–100% of field observations for each richness level, with many potential combinations either rare or unobserved. This pattern, which has rarely been examined in wildlife disease systems (but see [41]), is probably the result of both variation in the frequency of each species across the landscape as well as the tendency of amphibian communities to exhibit consistently non-random patterns of assembly [24,42]. When field data were used to incorporate the relative frequency of experimental assemblages, species richness decreased overall parasite transmission by approximately 50% in realistic community configurations, whereas there was no such effect among less-common or unobserved species combinations.

The degree to which host richness inhibited parasite infection success varied directly as a function of which host species were most susceptible to infection. When the most suitable host species predominated in species-poor assemblages but were progressively replaced or 'diluted' by lower-competence species in richer communities, infection success decreased with increases in diversity. For both *Al. marcinae* and *C. americanus*, the Pacific chorus frog (*P. regilla*)—which was the most common amphibian among surveyed ponds—was also the most competent host, while the much rarer bullfrog (*Ra. catesbeiana*) was among the least susceptible. Somewhat surprisingly, however, the effect was weakest for *Ri. ondatrae*, for which our previous experiments and field studies have consistently found an inverse relationship between infection success and amphibian host richness [24]. In the current study, this pattern was driven by the high levels of *Ri. ondatrae* infection within the two newt species (*T. torosa* and *T. granulosa*), which have previously exhibited lower susceptibility relative to *P. regilla* (e.g. [28,29]). This discrepancy could stem from differences in the body size or developmental stages of hosts among experiments (here, for instance, the larval *T. granulosa* were 30% larger in mass than the chorus frog larvae), but further research will be needed to assess the relative influence of intraspecific and interspecific variation in host susceptibility.

At least two factors may help explain the changes in infection success observed under realistic—but not random—patterns of community assembly. From an evolutionary standpoint, parasites could be adapted to maximize infection within commonly encountered hosts [43]. Host species likely to be encountered in both species-poor and species richness assemblages may thus represent preferred habitat patches for parasites (i.e. a nested pattern). Alternatively, or additionally, common host taxa have been hypothesized to undergo a trade-off between colonization ability and immunological

defences [44,45], such that the most widespread host taxa may also be the least protected against infection [46]. However, if changes were owing entirely to variation in susceptibility among the constituent host species, experimental changes in richness ought to affect infection only at the community level (summed among hosts), not at the scale of individual hosts. Yet increased richness led to decreases in parasite load per individual for both *P. regilla* and *An. boreas*. Although we cannot rule out the possibility that these species became more resistant, the more likely explanation is that species richness reduced the likelihood of their encounters with infectious parasites relative to monocultures, especially given that host resistance would be expected to decrease with more competition [47]. Correspondingly, the addition of bullfrog larvae specifically—which are considerably larger than other species even when of a similar developmental stage—were found to be the primary driver of reduced infections in *P. regilla* and *An. boreas*. A recent study in the same system showed that cercariae of *Ri. ondatrae* preferentially select *Ra. catesbeiana* larvae in choice trials, despite its overall low susceptibility to infection [48]. Similar 'identity effects' for particular species in diluting infection among focal hosts has also been demonstrated for other disease systems (e.g. [49]).

Debate surrounding the dilution effect hypothesis has often centred around whether observed changes in infection levels stem from shifts in host diversity or in host species composition. From a strictly mechanistic standpoint, the current findings emphasize the importance of species composition over richness, especially at the community scale. Equally important, however, the incorporation of field surveys highlighted the necessity of determining the degree to which species composition in natural systems changes predictably and consistently along a richness gradient, as seen here. This principle has been well illustrated in studies of BEF (e.g. [15]). With respect to disease research, Ostfeld & LoGuidice [17] were among the first to consider the influence of community disassembly 'rules' on infection patterns. Using simulations parametrized from empirical measurements of 13 vertebrate host species for the vector-borne Lyme disease system in the northeastern USA, the authors found that the proportion of infected ticks depended on how host species were removed from simulated communities: infection increased under realistic patterns of species loss but decreased with randomized disassembly. Similarly, LaCroix *et al*. [41] found that consistent shifts in plant species composition in grassland communities involving the relative dominance of highly susceptible species explained the observed decrease in viral infections with increased species diversity. Thus, even when diversity itself does not mechanistically drive infection outcomes, a tight coupling between diversity and species composition can nonetheless lead to consistent shifts in infection or pathology across diversity gradients.

These results contribute to a growing body of experimental studies highlighting the importance of host species composition in driving parasite transmission within complex communities (see reviews by [2,5]). Experimental studies performed across a range of scales and host–parasite systems have demonstrated the potential for heterospecific assemblages to alter infection success through changes in the availability of suitable hosts (i.e. susceptible host regulation), the likelihood a parasite encounters them (i.e. encounter regulation) or the suitability of hosts following exposure (i.e. competence regulation) [18,19,50]. These changes can lead to increases or decreases in infection in response to shifts in diversity. While most studies of the host diversity–disease dynamics relationship have involved either correlational assessments of infection and some measure of diversity in natural systems or small-scale experimental manipulations, enhanced integration between these approaches holds enormous promise for developing a more mechanistic framework. Although the responses of pathogens are likely to vary, predicting transmission success along gradients in species richness may be feasible when infection success is primarily the product of host identity and relative abundance (or biomass), rather than a non-additive outcome of diversity. Further experimental assessments of host species competence (intraspecific and interspecific) combined with more empirical data on the link between host species' competence and their order of assembly in natural environments could thus be used to more effectively forecast how species losses (and introductions) will alter disease dynamics.

Ethics. The data collection from animals was approved by the Institutional Animal Care and Use Committee at the University of Colorado.

Data accessibility. Data (data and metadata associated with this article) are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.5649t65 [51].

Authors' contributions. P.T.J.J., D.M.C., T.M. and J.K. designed the study; P.T.J.J., D.M.C., T.R. and T.M. collected the data; P.T.J.J. and T.M. analysed the data and all authors helped write the manuscript.

Competing interests. We have no competing interests.

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