Parasite diversity and coinfection determine pathogen infection success and host fitness

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While the importance of changes in host biodiversity for disease risk continues to gain empirical support, the influence of natural variation in parasite diversity on epidemiological outcomes remains largely overlooked. Here, we combined field infection data from 2,191 amphibian hosts representing 158 parasite assemblages with mechanistic experiments to evaluate the influence of parasite richness on both parasite transmission and host fitness. Using a guild of larval trematode parasites (six species) and an amphibian host, our experiments contrasted the effects of parasite richness vs. composition, observed vs. randomized assemblages, and additive vs. replacement designs. Consistent with the dilution effect hypothesis extended to intrahost diversity, increases in parasite richness reduced overall infection success, including infections by the most virulent parasite. However, the effects of parasite richness on host growth and survival were context dependent; pathology increased when parasites were administered additively, even when the presence of the most pathogenic species was held constant, but decreased when added species replaced or reduced virulent species, emphasizing the importance of community composition and assembly. These results were similar or stronger when community structures were weighted by their observed frequencies in nature. The field data also revealed the highly nested structure of parasite assemblages, with virulent species generally occupying basal positions, suggesting that increases in parasite richness and antagonism in nature will decrease virulent infections. Our findings emphasize the importance of parasite biodiversity and coinfection in affecting epidemiological responses and highlight the value of integrating research on biodiversity and community ecology for understanding infectious diseases.

microbiome | parasite competition | emerging infectious disease | ecosystem function | amphibian decline

Ecological research has focused increasingly on the importance of changes in biodiversity, thereby forming a fundamental link between community and ecosystem ecology (1–4). The loss or gain of species into a community, often in association with anthropogenic activities, can have remarkable effects on productivity, carbon storage, nutrient cycling, and species invasions (5–8). More recently, this line of inquiry has been extended to explore the role of biodiversity in affecting parasite transmission (i.e., the dilution effect; ref. 9). Building from historical research on agricultural plant communities (10), a series of recent studies has reported an inverse relationship between host diversity and the risk of disease in humans, plants, birds, amphibians, and corals (9). On the basis of experimental manipulations of host diversity, common mechanisms for this relationship involve changes in susceptible host density, such that higher diversity leads to a concomitant decline in susceptible hosts, or in encounter reduction, with added species interfering with parasite transmission (11, 12).

Despite a growing emphasis on how host diversity affects parasite transmission, few studies have examined the effects of parasite diversity on disease dynamics. Simultaneous infections by multiple parasite species are common in many systems (13, 14), yet how this cryptic component of diversity affects infectivity and host morbidity remains largely conjectural. Theory predicts that, unlike the many weak interactions that characterize free-living communities, parasites within a host have a high likelihood of interacting strongly (15, 16). Indeed, multipathogen interactions are believed to play an influential role in HIV and malaria in humans, colony collapse disorder in honey bees, and emerging infections in coral reefs (17–20). Given that many parasites cause limited pathology within their hosts (21), antagonism among coinfecting parasites could represent a novel mechanism of the dilution effect, particularly if less virulent parasites replace or compete with virulent groups at higher diversity (22, 23). Because most studies on community structure and disease have relied on correlational approaches (11, 12), identifying the "hidden" importance of each dimension of community diversity, including that of parasites themselves, remains challenging (24, 25).

Here, we sought to build an empirical bridge between research on the dilution effect and parasite coinfections by experimentally evaluating the effects of parasite community structure on pathogen infection success and host fitness. Using a study system involving six trematode parasite species (Fig. 1) and an amphibian host (*Pseudacris regilla*), we assessed the relative importance of parasite richness and species composition in driving the outcome of host–parasite interactions. We used an experimental approach that included multiple community configurations for most levels of richness, contrasted additive vs. replacement designs, and compared observed and randomized community assemblages (26–28). By incorporating field data on parasite community structures from 134 wetlands and 2,191 hosts, we also evaluated the relationship between parasite richness and parasite abundance in natural assemblages and compared the influence of observed and random community assemblages on the diversity–disease relationship (27, 29). We predicted that cross-reactive immunity within this parasite guild would lead to negative effects on infection success with increasing richness (30, 31). Less clear, however, was how more complex parasite assemblages would affect host pathology; coinfections could reduce parasite infection success yet still enhance pathology (32), emphasizing the need for data on the pathogenicity of each parasite, the order in which they assemble in natural communities, and how their effects combine to influence host fitness. Given the ubiquity of parasite coinfections in natural systems coupled with ongoing changes in ecological communities, including those of parasites, we highlight the conceptual and applied importance of understanding the relationships among parasite diversity, transmission, and disease.

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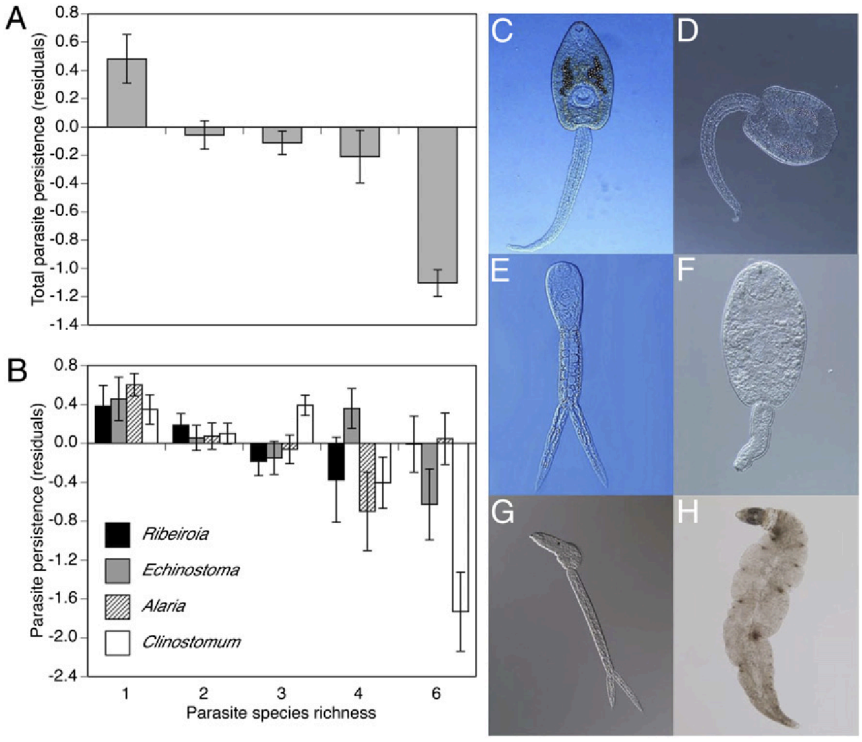

Fig. 1. (*A*) Total parasite persistence and (*B*) persistence of each parasite species in metamorphosed *Pseudacris regilla* exposed to different levels of parasite richness. Persistence values (±1 SE) represent residuals from regression analyses with time to metamorphosis as a covariate. Infectious cercariae of *Ribeiroia ondatrae* (*C*), *Echinostoma trivolvis* (*D*), *Alaria* sp. 2 (*E*), *Cephalogonimus americanus* (*F*), *Clinostomum attenuatum* (*G*), and an undescribed echinostome magnacauda (*H*).

Results

Parasite Infection Success. Experimental increases in parasite species richness caused a reduction in overall infection success within amphibian hosts ($F_{2,\ 225} = 22.61$, $P < 0.0001$; $\lg_2$(parasite richness), $B = -0.0479$, $P < 0.0001$; time postexposure: $P < 0.0001$; $n = 228$). Infection success (summed among species) decreased from an average of 40% in the monocultures to 34 and 23% in the four- and six-species treatments, respectively (Fig. 1*A*). This effect appeared linear on the basis of polynomial contrasts in ANOVA. Restricting the data to the factorial portion of the experiment with four of the most common parasites did not change the negative effect of parasite richness ($F_{2,\ 213} = 17.02$, $P < 0.0001$; $\lg_2$(parasite richness), $B = -0.039$, $P < 0.0002$; time postexposure: $P < 0.0001$; $n = 216$). Although no parasite interactions were detected, inclusion of parasite identity provided an improvement in model fit relative to using richness alone [decrease in Akaike Information Criterion (AIC) from −378.4 to −396.98; *SI Text*]. For richness levels with multiple community structures, a linear mixed effects model indicated that parasite richness also reduced overall infection success when composition was included as a random effect ($B = -0.039$, $t = -2.23$, $P < 0.05$; *SI Text*). Realized parasite richness correlated strongly with richness treatments ($r = 0.94$), indicating the effectiveness of the experimental manipulations in achieving the desired gradients in parasite composition.

Parasite richness consistently reduced infection success when common parasites were analyzed individually (*Ribeiroia* $B = -0.038$, $P = 0.0413$; *Echinostoma* $B = -0.0713$, $P = 0.0284$; *Alaria* $B = -0.0516$, $P = 0.0161$; and *Clinostomum* $B = -0.128$, $P < 0.0001$) (Fig. 1*B*). Thus, experimental increases in parasite richness functioned to decrease infection success, including infections by the most pathogenic species (e.g., *Riberioia*). For the factorial portion of the experiment, parasite richness negatively affected persistence of two species (*Ribeiroia*: richness $B = -0.0578$, $P = 0.0113$; *Alaria*: richness $B = -0.087$, $P = 0.001$), had a marginally negative effect on *Echinostoma* ($P = 0.07$), and no effect on *Clinostomum*. Analyzing the data with parasite composition (rather than parasite richness) provided a comparable fit for changes in *Ribeiroia* infection (~1 ΔAIC), whereas composition (main effects only) had a lower AIC (−93) relative to the richness model (−87) for *Alaria* infection.

Host Pathology. Parasite species richness increased host mortality in the additive experiment ($\chi^2 = 59.3$, df = 1, $P < 0.0001$; $n = 289$). Whereas survival was 100% among controls and averaged 98% in the monocultures, only 30% of hosts in the all-parasite treatment survived through metamorphosis (Fig. 2*A*). This pattern persisted regardless of whether we examined the entire dataset, treatments that included only the most pathogenic trematode *Ribeiroia* ($\chi^2 = 34.59$, df = 1, $P < 0.0001$; $n = 140$), or the factorial experiment ($\chi^2 = 20.23$, df = 1, $P = 0.0014$; $n = 239$). However, parasite species composition strongly influenced mortality, and only *Ribeiroia* had a significant main effect on mortality ($\chi^2 = 9.76$, df = 1, $P = 0.0018$). There was also an interaction between *Echinostoma* and *Clinostomum* ($\chi^2 = 4.53$, $P = 0.0334$), such that *Echinostoma* increased mortality when *Clinostomum* was present ($\chi^2 = 5.65$, $P = 0.0174$).

Results from the additional treatments with *Ribeiroia*, *Alaria*, and *Echinostoma* further emphasized the importance of parasite composition in determining host survival. Total cercarial exposure increased host mortality (Firth corrected $\chi^2 = 62.27$, df = 5, $P < 0.0001$; $n = 134$), but this effect depended on the identity of the parasite species (Fig. 2). Host survival decreased monotonically with progressive increases in *Ribeiroia* exposure, from 100 to 0% ($\chi^2 = 41.72$, $P < 0.0001$; $n = 65$), whereas exposure dosage had no significant effect for *Alaria* or *Echinostoma* (Fig. 2*B*). Examining the experimental results in a substitutive (rather than additive) manner indicated that both richness (one vs. four species) and community composition influenced mortality when

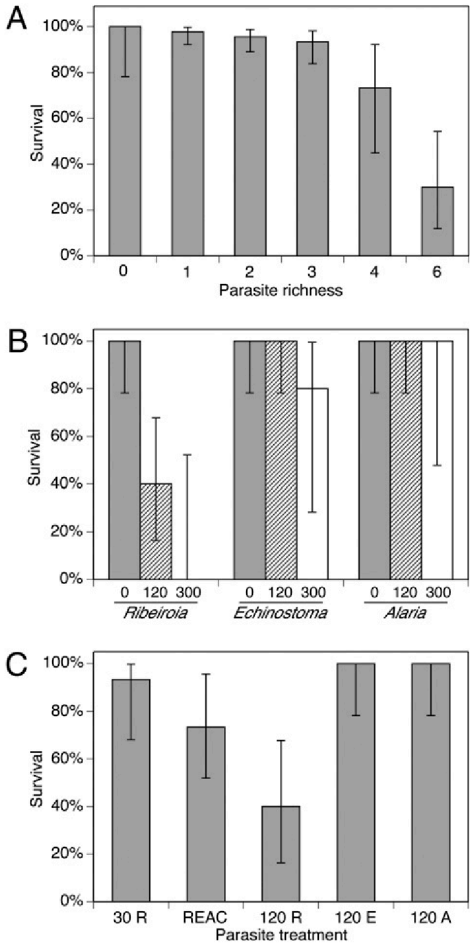

Fig. 2. Survival of *Pseudacris regilla* exposed to (*A*) different levels of parasite richness, (*B*) increasing dosages of three parasites (*Ribeiroia*, *Echinostoma*, and *Alaria*), and (*C*) parasite treatments representing an additive vs. substitutive design. Parasite exposure dosage interacted with parasite identity to determine mortality (Firth-adjusted $\chi^2 = 62.27$, df = 5, $P < 0.0001$; exposure $\chi^2 = 10.67$, $P = 0.0011$, *Ribeiroia* $\chi^2 = 12.23$, $P = 0.0005$, exposure* *Ribeiroia* $\chi^2 = 9.32$, $P = 0.0023$). In *C*, tadpoles were exposed to differing dosages (30 or 120) and combinations of *Ribeiroia* (R) *Echinostoma* (E), *Alaria* (A), and *Clinostomum* (C). The REAC treatment received 30 of each parasite. Data represent proportional survival within each treatment with 95% binomial confidence intervals.

exposure was held constant at 120 cercariae ($\chi^2 = 22.92$, df = 3, $P < 0.0001$; $n = 60$). *Ribeiroia* caused substantially more mortality (60%) than the other monospecific treatments (0%), whereas the mixed species treatment (30 parasites of each of four species) had intermediate mortality (27%, $P = 0.045$; Fig. 2*C*). Thus, substitutive increases in richness reduced mortality only when the added species replaced or reduced the abundance of the most pathogenic species.

Parasite richness reduced host length and mass while delaying host development time [lg_{10}(snout-vent length): $F_{1,266} = 18.74$, $P < 0.0001$; lg_{10}(mass): $F_{1,259} = 5.94$, $P = 0.0154$; time: $F_{1,266} = 5.27$, $P = 0.023$] (Fig. S1), but these effects were sensitive to which data were included. If only treatments with *Ribeiroia* were selected, these patterns persisted for host size ($F_{1,118} = 15.33$, $P = 0.0002$) and development time ($F_{1,118} = 13.24$, $P = 0.0004$), but not for host mass ($F_{1,112} = 2.69$, $P = 0.1034$). In the factorial experiment, richness affected only development time ($F_{1,109} = 5.87$, $P = 0.0171$). Although parasite species richness also increased the frequency of severe malformations ($\chi^2 = 13.48$, df = 1, $P = 0.0002$; $n = 262$), this result owed to the higher likelihood that diverse treatments included *Ribeiroia* (Fig. S2 and *SI Text*).

Field Data. Parasite species richness in *P. regilla* hosts from California wetlands averaged 2.26 ± 0.085 (range of 0–5 species), with all but four ponds supporting at least one of the parasite species used in the experiments. Rarefaction analyses indicated that our sampling design was effective in detecting all parasite species, with estimated and observed richness converging for all examined sites (Fig. S3, Table S2 and *SI Text*). The mean abundance of parasites per host increased monotonically with lg_2(parasite richness) ($F_{1,151} = 21.25$, $B = 0.286$, $P < 0.0001$), supporting an additive (unsaturated) parasite community structure (Fig. 3*A*). The parasite communities of *P. regilla* were also highly nested (observed matrix temperature = 9.44°, P ($T < 9.44°$) = $2.31e^{-29}$ [-11.43σ]) (Fig. 3*B*). Within each level of richness, the observed frequency of parasite community compositions varied (Fig. 3*B*); *Echinostoma* was the most common taxon (present in 91% of ponds), followed by *Ribeiroia* (62%), *Cephalogonimus* (35%), *Alaria* (32%), and *Clinostomum* (6%). Incorporating the information on observed community structures into a weighted regression analysis indicated that nonrandom losses of parasite species led to similar or stronger effects of richness on parasite persistence relative to unweighted communities (*SI Text*). Weighted regression tended to amplify the effects of richness on host mortality and days to metamorphosis,

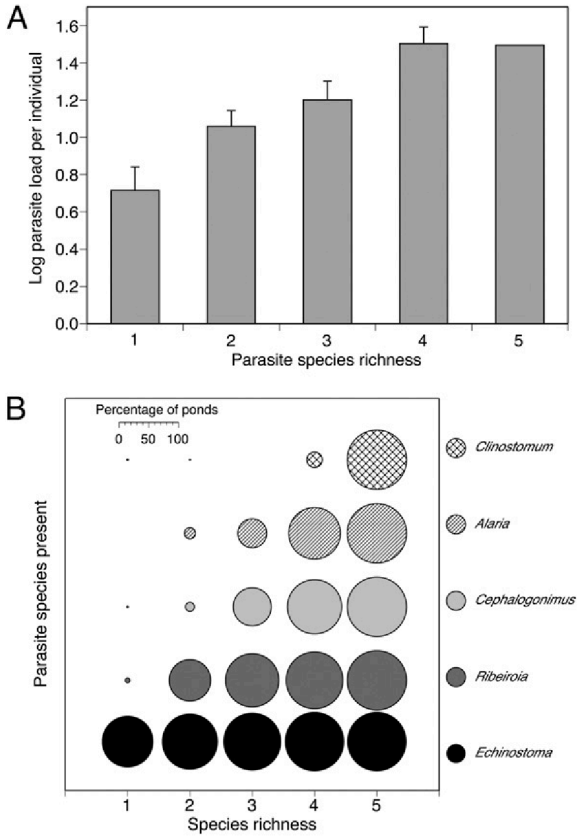

Fig. 3. (*A*) Relationship between mean parasite load (+1 SE) in recently metamorphosed *Pseudacris regilla* and parasite species richness at the wetland. (*B*) Parasite community compositions from recently metamorphosed *P. regilla* representing 134 sampled wetlands; the number of wetlands with one through five parasite species were 38, 53, 39, 23, and 1, respectively. For a given level of parasite richness, the percentage of ponds containing each species is represented by the size of the circle. The parasite communities were highly nested with an observed matrix temperature of 9.44 °C.

whereas richness did not affect host size or mass regardless of whether we used weighted or unweighted regression.

Discussion

By combining field data on parasite community assembly with controlled experiments involving interactions among six parasite species and an amphibian host, this study extends research on the dilution effect to intrahost communities. While biomedical research has traditionally focused on isolated interactions between a single host and a single parasite, growing evidence indicates that interactions among coinfecting parasites can affect host pathology, parasite transmission, and virulence evolution (33). Our work with a guild of amphibian parasites illustrates the importance of this cryptic component of biodiversity in affecting both parasite infection success and host pathology. Higher parasite richness consistently reduced the infection success of parasites within hosts, including those that cause pathogenic disease and host mortality. Concurrently, increases in parasite richness caused either increases or decreases in host pathology as a function of community composition and whether species were added substitutively or additively.

Experimental manipulation of parasite community structure revealed that total infection success decreased consistently with increases in parasite species richness, even when community composition was treated as a random effect. In the six-species treatment, parasite infection success was ∼42% lower than in monospecific treatments, and this pattern applied generally to each of the four parasite species studied in detail. Thus, increases in parasite richness had similarly negative effects on the infection success of highly pathogenic and relatively benign parasites alike, suggesting this effect was unlikely to be the result of including or excluding particular species. Using the field data to weight parasite community structures according to their frequency in natural wetlands, we found that this pattern was similar or slightly stronger in observed as opposed to randomly constructed assemblages. Considering that our experiments involved a group of functionally similar and taxonomically related parasites (larval trematodes), many of which localize to different infection sites within the host, the most likely explanation for the decrease in persistence involves cross-reactive immunity (apparent competition) (31, 34) rather than the direct antagonism reported in some free-living microbial communities (4). Previous studies examining pairwise coinfections indicate that antagonistic interactions between similar types of parasites may be common (35), but this study serves to extend this finding to a parasite assemblage. Importantly, however, the outcome of intrahost interactions will likely depend strongly on the types of parasites within a given host (36). Intrahost competition between strains of a particular pathogen, for instance, can lead to increased virulence (37), whereas micro- and macroparasites elicit different immune responses (32), emphasizing the need to extend this approach to a broader range of parasitic communities.

Parasite species richness and coinfection also influenced host fitness. Increases in parasite richness led to a decrease in host survival and growth, particularly in the highest species' richness treatment. However, unlike the results for parasite infection success, this pattern depended strongly on the composition of the parasite assemblage and whether the experiment followed an additive- or replacement-based design (27, 28). Parasite species composition generally and the presence of the most pathogenic species *Ribeiroia* specifically had a disproportionate effect on host fitness, indicating that the addition of specific parasites to the community tended to drive host responses. These results, which illustrate that not all parasites are "pathogens," are consistent with the "sampling effect" from the biodiversity–ecosystem function literature (38, 39): more diverse assemblages had a greater chance of including the most virulent species, contributing to the negative relationship between parasite diversity and host fitness. However, rather than being an experimental artifact, these results emphasize the important distinction between disease risk, which is often a function of the most virulent pathogen, and parasite diversity, which can include many species with few detectable effects on host fitness (21). Here, larger parasites that actively penetrate amphibian hosts, such as *Ribeiroia*, caused the most tissue damage and host death (40). Interestingly, however, increased parasite richness still caused reductions in host survival and development even when controlling for *Ribeiroia* presence, suggesting an additional effect of richness (e.g., a "complementarity effect") (41). The mechanism underlying this synergistic result is not immediately clear, but could involve saturation of immune defenses with increased infections (42).

These results indicate that the influence of parasite richness on host pathology will depend strongly on whether parasite communities assemble in an additive or substitutive manner. When total parasite exposure remained constant and richness determined the number of species that comprised the total (i.e., a replacement design) (43, 44), increases in diversity led to an increase in host fitness only when pathogenic species were replaced by less-virulent infections. A shift from a monoculture of *Ribeiroia* to a mixed assemblage of four parasites, for instance, each with the same total number of parasites, led to a 40% increase in host survival. This model is consistent with a resource-limited, competition-dominated scenario of community structure in which hosts are saturated with infection (39). When parasite communities were assembled additively, however, with concurrent increases in total parasite load and richness, higher parasite diversity had negative or neutral effects on host fitness. Data from the field surveys and previous parasitological research also support an unsaturated assembly pattern (30, 45, 46), but more research is needed to examine this issue in a broader range of disease systems (31).

By revealing the strongly nested structure of parasite communities within amphibian hosts, the field surveys provide additional evidence of an intrahost dilution effect. Specifically, *Echinostoma* and *Ribeiroia*, which are often reported as the most virulent parasites (47) (although *Echinostoma* caused little pathology in this study), were the most common parasites with other species added to communities as richness progressively increased. These findings parallel a key criterion of the "dilution effect" for host diversity, in which the most competent hosts need to be basally nested components of more diverse assemblages (48, 49). Combining these observations with our experimental findings suggest that, because of antagonism among coinfecting species and the low average level of pathogenicity in this group of parasites, parasite diversity can also function as a mechanism of the dilution effect by reducing the success of virulent species. Whereas such reductions may have only weak effects on individual host fitness, likely because parasite-mediated damage occurs during the initial infection stage (40), competitive effects among parasites could nonetheless cause population-level reductions in pathogen transmission. In this system, for instance, a decrease in parasite persistence in intermediate amphibian hosts will likely reduce transmission to vertebrate definitive hosts (often birds or mammals), ultimately lowering parasite abundance—a pattern that will be further enhanced if parasites also behave antagonistically within other hosts in the life cycle (50).

Continued integration among research on community ecology, the dilution effect, and pathogen coinfections has conceptual as well as applied importance for addressing issues related to ecosystem services, species loss and invasions, and emerging infectious diseases (9, 25, 48). Given that natural communities are composed of a diverse assemblage of parasitic and nonparasitic microorganisms, including heminths, viruses, bacteria, and fungi, there is a growing need to explore the outcome of interactions among these cryptic species across a range of systems and conditions. For instance, experimental studies have demonstrated that skin and soil microbial communities can reduce colonization

by pathogens of amphibians and plants, respectively (7, 22). In humans, endosymbiotic gut bacteria can help to control or prevent colonization by pathogenic species such as *Clostridium difficile* (23), whereas the endosymbiont community of ticks (*Dermacentor andersoni*) helps to limit the distribution of Rocky Mountain spotted fever (*Rickettsia rickettsii*) (51, 52). These observations suggest that the loss of parasite and microbial biodiversity, for which few quantitative estimates are currently available (53), may play an important yet historically overlooked role in understanding disease emergence.

Materials and Methods

Overview of Approach. Our experiments evaluated the influence of parasite species richness and parasite community composition on two main responses: host pathology, including survival, malformations, size-at and time-to metamorphosis, and parasite infection success (transmission), measured as the proportion of parasites detected at the end of the experiment. Individual amphibian hosts (*P. regilla*) were exposed to infectious cercariae representing one, two, three, four, or six trematode species, which encompassed realistic patterns of parasite richness in amphibian host populations (Fig. 3*B*). This translated into a fully factorial experiment for four of the common parasite species, which facilitated comparisons of parasite richness and composition while keeping the design tractable. This experiment used an additive design, such that the total number of administered parasites increased directly with richness; however, we conducted complementary treatments involving varying dosages of the three most common parasites to compare additive and replacement designs (43, 44) (*SI Text*). Finally, we used data from field surveys to weight experimental assemblages by their observed frequency in nature to contrast the effects of realistic and randomized community structures.

Parasite Exposures. We field-collected egg masses of Pacific chorus frogs (*P. regilla*), allowed them to hatch in the laboratory, and maintained larvae individually in 1.0-L containers. Once hosts reached early limb development (ref. 54, stage 26), we randomly assigned them to an experimental treatment: no parasites (control) or to 30 cercariae of one parasite species ($n = 6$ treatments), two species ($n = 6$), three species ($n = 4$), four species ($n = 1$), or all six parasites ($n = 1$) (Table S1). Included parasites were *Ribeiroia ondatrae*, *Echinostoma trivolvis*, *Alaria* sp. 2 (55), *Cephalogonimus americanus*, *Clinostomum attenuatum*, and an undescribed echinostome magnacauda (Fig. 1 *C–H*). We obtained cercariae of each parasite by collecting snails (*Helisoma trivolvis*) from sites in California and Oregon (magnacauda only), isolating snails into 50-mL vials for 12–24 h, and administering pooled cercariae to larval amphibians over 14 d (*SI Text*). To evaluate the pathology associated with the common parasites and contrast replacement vs. additive experimental designs (sensu refs. 43, 44), we included complementary treatments that involved exposing hosts to multiple dosages (0, 120, or 300 cercariae) of *Ribeiroia*, *Echinostoma*, or *Alaria* and recorded host survival over 18 d (*SI Text*).

Field Data. We examined parasite infections in 2,191 metamorphosing *P. regilla* from 134 wetlands in the East Bay region of California between 2009 and 2010 (158 total site visits) to quantify patterns of parasite community structure and assembly in natural systems. At wetlands supporting the appropriate snails (*H. trivolvis*), we necropsied a random subset of *P. regilla* hosts (10–60 per site) to determine the identity, richness, and abundance of larval trematodes (*SI Text*). We verified the adequacy of this sampling program to detect parasite richness using sampled-based rarefaction curves and richness estimators (*SI Text*, Table S2, Fig. S3). From these data, we determined the relative frequency of each parasite community permutation and quantified the relationship between mean parasite abundance (averaged across all hosts examined) and parasite species richness.

Analysis of Infection Success. We analyzed the influence of experimental treatments on the abundance of each parasite species detected upon necropsy (lg_{10} transformed) and the total proportion of parasites detected (summed among species, arcsin-square-root transformed). We used species richness (lg_2 transformed) as our primary explanatory variable in a replicated-regression approach (56). To contrast community composition and richness, we analyzed the effect of each parasite and their interactions on infection success, restricting the analysis to the factorial portion of the experiment. We compared the AIC values of models with richness alone vs. models that included parasite identity. For treatments with multiple community compositions at each level of richness (i.e., one, two, and three species), we also used linear mixed effects models to nest different community compositions within richness (57) (*SI Text*). Days postexposure was included as a covariate because of the established effects of time on parasite persistence (e.g., ref. 58).

Analysis of Host Pathology. We examined the effects of parasite species richness on host survival and malformations using generalized linear models with untransformed values of richness (although results did not change if we used lg_2 richness and excluded controls). To evaluate the role of parasite composition, we reanalyzed the effects of parasite richness while controlling for *Ribeiroia* presence, which was the most pathogenic parasite and expected to contribute to an obvious sampling effect. We also analyzed the factorial portion of the experiment iteratively using either richness or parasite presence as predictors and compared AIC values. We used the additional, short-term treatments to (*i*) test the role of parasite identity, exposure dosage (0, 120, or 300), and their interaction on host survival and (*ii*) contrast the effects of replacement and additive designs by comparing treatments with a total exposure of 120 parasites. Among animals that survived to forelimb emergence (stage 42), we tested the influence of parasite richness on time to metamorphosis, length at metamorphosis, and mass at metamorphosis using general linear models, first on the entire experiment (contrasting treatments with and without *Ribeiroia*), and subsequently on the factorial portion of the experiment.

Analysis of Field Data. We used linear regression to examine the influence of parasite richness at a site (lg_2 transformed) on the mean abundance of parasites per frog (summed among parasite species, lg_{10} transformed). Additionally, we recorded the relative frequency of different parasite community structures and used the nestedness temperature calculator to assess whether low diversity communities represented subsets of more rich communities (59). This information was used to weight the experimental community structures, such that commonly observed communities received a higher regression weight relative to more rare communities (60). By comparing these results with the unweighted regressions, we evaluated the effects of parasite species loss between realistic and randomized assemblages, respectively (27). This analysis was performed only up through four species communities as these were well represented in nature.

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