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Species diversity reduces parasite infection through cross-generational effects on host abundance

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Abstract. With growing interest in the effects of biodiversity on disease, there is a critical need for studies that empirically identify the mechanisms underlying the diversity–disease relationship. Here, we combined wetland surveys of host community structure with mechanistic experiments involving a multi-host parasite to evaluate competing explanations for the dilution effect. Sampling of 320 wetlands in California indicated that snail host communities were strongly nested, with competent hosts for the trematode *Ribeiroia ondatrae* predominating in low-richness assemblages and unsuitable hosts increasingly present in more diverse communities. Moreover, competent host density was negatively associated with increases in snail species richness. These patterns in host community assembly support a key prerequisite underlying the dilution effect. Results of multigenerational mesocosm experiments designed to mimic field-observed community assemblages allowed us to evaluate the relative importance of host density and diversity in influencing parasite infection success. Increases in snail species richness (from one to four species) had sharply negative effects on the density of infected hosts (~90% reduction). However, this effect was indirect; competition associated with non-host species led to a 95% reduction in host density (susceptible host regulation), owing primarily to a reduction in host reproduction. Among susceptible hosts, there were no differences in infection prevalence as a function of community structure, indicating a lack of support for a direct effect of diversity on infection (encounter reduction). In monospecific conditions, higher initial host densities increased infection among adult hosts; however, compensatory reproduction in the low-density treatments equalized the final number of infected hosts by the next generation, underscoring the relevance of multigenerational studies in understanding the dilution effect. These findings highlight the role of interspecific competition in mediating the relationship between species richness and parasite infection and emphasize the importance of field-informed experimental research in understanding mechanisms underlying the diversity–disease relationship.

Key words: amphibian malformations; biodiversity loss; disease ecology; emerging disease; epidemiology; Ribeiroia ondatrae.

Introduction

A central goal in disease ecology is to identify how changes in community structure influence pathogen transmission and the resulting likelihood of disease (Ostfeld et al. 2008, Tompkins et al. 2011). While increases in species richness have the potential to enhance or diminish infections (Begon 2008), a growing number of studies provide support for the "dilution effect" hypothesis, in which host diversity negatively affects disease incidence (Ezenwa et al. 2006, Brisson et al. 2008, Thieltges et al. 2008, Johnson et al. 2009, Pongsiri et al. 2009, Keesing et al. 2010). A fundamental premise of this hypothesis is that species-poor communities are dominated by highly competent hosts that maintain and transmit infections, whereas diverse communities include species that directly or indirectly inhibit infection success (Ostfeld and Keesing 2000*a*, *b*, Dobson et al. 2006, Keesing et al. 2006). Correlational field data supporting the dilution effect hypothesis have been collected for a range of human, plant and wildlife diseases (Mitchell et al. 2002, 2003, Ezenwa et al. 2006, LoGiudice et al. 2008, Swaddle and Calos 2008).

A continuing challenge in the study of the diversity–disease relationship involves identifying the mechanisms underlying the dilution effect. While several processes through which species richness could influence infection have been advanced (e.g., Keesing et al. 2006), experimental identification of operational mechanisms has lagged behind cross-sectional survey data and theoretical research, often making it unclear how changes in community structure are influencing infection and whether such effects are direct or indirect. Based on a limited number of experimental studies, added species sometimes function as parasite "dead ends," distracting

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infections away from more sensitive species (a form of encounter reduction), whereas in other cases increased richness leads to a decrease in the density of competent hosts (susceptible host regulation; LoGiudice et al. 2003, Johnson et al. 2008, 2009, Clay et al. 2009, Suzan et al. 2009, Thieltges et al. 2009). In a large-scale field experiment with foliar fungal pathogens, for instance, Mitchell et al. (2002) found that while plant species richness reduced infection levels, this result was driven entirely by competition-mediated changes in competent host density.

Broader integration of observational, experimental, and theoretical research is essential for understanding under what conditions diversity will influence infection and at what scale (Pongsiri et al. 2009, Keesing et al. 2010). Beyond a few well-studied examples (e.g., Lyme disease; LoGiudice et al. 2008), most research on the dilution effect in animal disease systems has focused either on field surveys or small-scale experiments, with few efforts to bridge the gap between such approaches. Data from natural communities provide the necessary ecological context for community structure, including the sequence of community assembly and disassembly (Ostfeld and LoGiudice 2003, Bracken et al. 2008). Using simulation models of Lyme disease, for instance, Ostfeld and LoGiudice (2003) showed that random removal of species from a community led to positive correlations between disease and host diversity, whereas species losses that mirrored empirically derived disassembly rules caused a dilution effect. Experiments, in turn, offer a mechanistic perspective that can remain elusive among strictly observational studies. Because transmission is a dynamic process that may depend on factors beyond the local community (Ostfeld et al. 2005, Loss et al. 2009, Johnson and Thieltges 2010), whether a negative relationship between diversity and infection is observed in natural assemblages will depend on parasite input rate and the scale at which communities are examined (e.g., Ostfeld and Keesing 2000*a*, Loss et al. 2009), emphasizing the need for experimental manipulations that control parasite inputs.

Here, we combined cross-sectional field surveys with mechanistic experiments to evaluate how snail community structure affected infection by a complex life cycle parasite. We focused on the trematode *Ribeiroia ondatrae*, which moves sequentially among rams horn snails, amphibians, and water birds (Beaver 1939, Johnson et al. 2004), and has gained recent notoriety for its role in causing frog limb deformities (Sessions and Ruth 1990, Johnson et al. 1999, 2010*b*, Rohr et al. 2009). We used field surveys to first evaluate whether aquatic snail communities showed evidence of nestedness, with competent host species predominating in low-diversity communities and a greater fraction of unsuitable hosts in more species-rich systems. This pattern of community assembly is a key condition of the dilution effect hypothesis because it means that species-poor communities will be dominated by suitable hosts, whereas species-rich communities will support more dilution hosts (Ostfeld and Keesing 2000*a*, Ostfeld and LoGiudice 2003). Second, we used observed patterns of snail community structure as a template for designing mesocosm experiments that contrasted the effects of community diversity (number of snail species) and host density (number of suitable host individuals) on parasite infection success. Specifically, we tested the relative importance of hypothesized mechanisms of the dilution effect, including "encounter reduction," in which nonsusceptible snail species distract parasites away from suitable hosts (identified as changes in infection prevalence), and "susceptible host regulation," in which nonsusceptible snails reduce infection indirectly by decreasing the survival or reproduction of suitable hosts (identified as changes in the number of infected hosts). By using seminatural pond mesocosms that mirrored observed snail assemblages and allowed for dynamic fluctuations in community structure, we aimed to evaluate the diversity–disease relationship under ecologically relevant conditions. To the best of our knowledge, this represents the first experimental study to assess the effects of species richness on an animal parasite over multiple generations.

Materials and Methods

Field surveys.—We used field surveys to establish patterns of snail community composition and the order of assembly. Between May and August of 2009–2010, we sampled randomly selected wetlands on properties throughout Santa Clara, San Mateo, Alameda, and Contra Costa counties in the Bay Area of California, USA. During each visit, we estimated snail relative abundance by counting the number of individuals of each species in standardized dipnet sweeps conducted every 15 m around a pond's perimeter (Johnson et al. 2002). We also conducted three to five habitat-stratified seine hauls through each pond to ensure we were not missing any additional species. Snail genera were identified following (Burch 1989) and we used rarefaction analyses (Colwell 2009) to validate the efficacy of our methods in measuring snail species richness (see the Appendix). *Helisoma trivolvis* density (mean number per sweep) was compared against snail species richness using Spearman's ρ nonparametric correlation. We used the nestedness temperature calculator to assess the degree of order in our site-by-species matrix of snail species presences (Atmar and Patterson 1993). Matrix temperature ranges from 0° for a perfectly nested community to 100° for a perfectly random community. This analysis provides an assessment of the degree to which snail communities of low diversity represent subsets of more rich communities, which provides valuable information about community structure and was used to guide design of our experimental assemblages.

Experimental design.—We conducted two interrelated experiments to evaluate the effects of host density and community composition on the infection success of

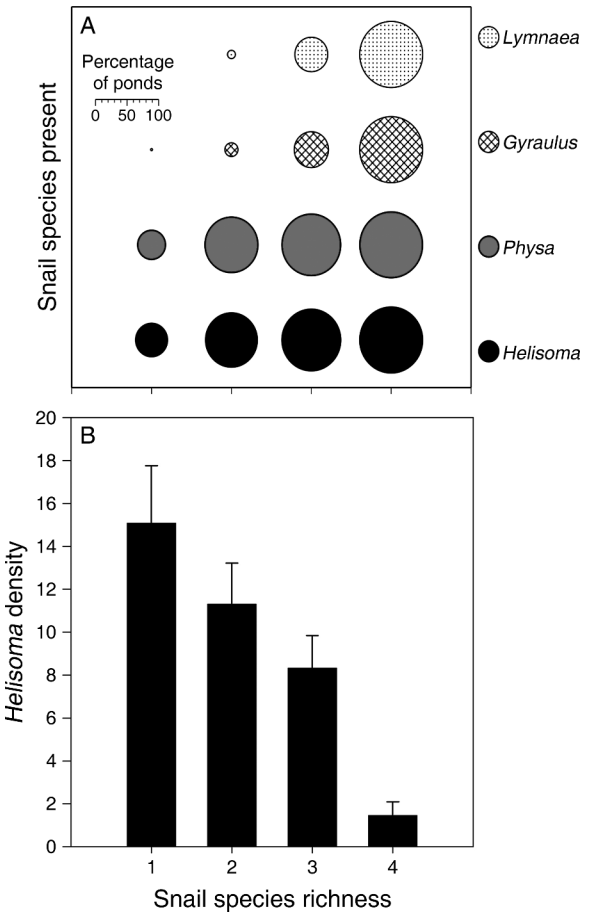

FIG. 1. (A) Snail species community compositions among 320 sampled wetlands; the number of wetlands with 1, 2, 3, or 4 species were 130, 96, 45, and 6, respectively. Snails were not detected in 43 wetlands. For a given level of species richness, the percentage of ponds containing each species is represented by the size of the circle. (B) Relationship between *Helisoma* density (mean number per sweep + SE) and snail species richness within wetlands that supported *Helisoma* ($n = 159$ wetlands).

Ribeiroia ondatrae (hereafter *Ribeiroia*) into snail hosts. The first (diversity experiment) was a 2×3 factorial experiment manipulating *Ribeiroia* egg input (yes vs. no) and snail species richness (one, two, or four species). Snail community composition and the order of assembly were derived from field data, such that *Helisoma trivolvis* (hereafer *Helisoma*) was present in all communities, *Physa acuta* (hereafter *Physa*) was added in the two-species community, and *Gyraulus* sp. and *Lymnaea* sp. (hereafter *Gyraulus* and *Lymnaea*) were added to the four-species communities (Fig. 1A). Because only *Helisoma* is susceptible to *Ribeiroia* (Johnson et al. 2004), with other species functioning as competitors and potential decoys, we used a fixed initial density of *Helisoma* (50 per mesocosm) and added equal numbers of other species in an additive design (i.e., 50, 100, and 200 snails per mesocosm for the one-, two-, and four-species treatments, respectively). While these species varied in biomass, dilution potential is influenced by many factors, including phylogeny, shape, and excreted effluent (e.g., Upatham and Sturrock 1973, Combes and Mone 1987), and the use of consistent densities allowed us to assess the per capita effect of non-host snails on *Ribeiroia* infection success.

To complement and enhance this design, we conducted an additional experiment that evaluated the effects of three levels of *Helisoma* density on infection success, independent of any other snail species (density experiment). The total number of snails mirrored those in the diversity experiment (50, 100, and 200 snails per mesocosm), but treatments included only *Helisoma* that were exposed to *Ribeiroia*. The maximum initial snail density in the mesocosms was less than half of the highest density observed in our field surveys. Collectively, this design has the advantages of (1) assessing the effect of host density alone on parasite infection success and (2) offering direct comparisons with the mixed species treatments, which had comparable initial snail densities but different community compositions (i.e., monospecific vs. heterospecific).

Experimental setup and execution.—Outdoor mesocosms (378 L) were established in an open field at the Hopland Research and Extension Center in Hopland, California, using standard methods (e.g., Semlitsch and Boone 2009). On 19 April 2010, mesocosms were filled with well water and 6 kg of silica sand and seeded with 30 g of rabbit chow, 30 mL of pond sediment, and 150 mL of concentrated zooplankton from local water bodies. We covered mesocosms with mesh lids (1 mm) to prevent unintended immigration or emigration and submerged a sheet of plexiglass (30×45 cm) in each mesocosm to provide a standardized surface for enumerating snail egg masses. Each treatment was replicated five times for a total of 40 mesocosms (30 for the diversity experiment + 10 for the density experiment, with one treatment shared between experiments). After 32 days, we randomly assigned mesocosms to a condition and added field-collected snails of the appropriate species (mean size ± 1 SE: *Helisoma* = 9.37 ± 0.23 mm, *Physa* = 6.60 ± 0.08 mm; *Gyraulus* = 5.13 ± 0.04 mm; *Lymnaea* = 17.75 ± 0.32 mm). A subset of *Helisoma* ($n = 99$ snails) was dissected to ensure preexisting trematode infections were rare (none were detected). Over the course of three addition events (26 May, 31 May, and 11 June), we added ~16 000 embryonated eggs of *Ribeiroia* obtained from surrogate rat hosts (see the Appendix; Johnson et al. 2007). Mesocosms in the parasite-absent condition received equivalent amounts of filtered feces from uninfected rats.

After 10 weeks, we removed all snails and examined *Helisoma* for *Ribeiroia* infection. All adult snails of each species were measured (shell length, mm) and we used length–dry-mass regressions to estimate biomass (see the Appendix). Given the large number of small snails, however, we calculated the number of juveniles by first

weighing their aggregate dry mass and then using the mass of three randomly selected subsamples of 10 individuals to extrapolate the total number of individuals of each species. To evaluate *Ribeiroia* infection, we dissected an average of 60 *Helisoma* snails (range: 17 to 166 depending on abundance) under a stereo-dissecting microscope to detect trematode rediae and mature cercariae. Snails of all sizes are susceptible to *Ribeiroia* (Huizinga 1973; Redmond et al., *in press*), and infections are detectable by dissection <3 weeks following exposure, such that our experimental timeline was suitable to quantify infection prevalence (see Johnson et al. 2004, 2007). We divided *Helisoma* into adults (>10 mm), which represented individuals added initially, and juveniles (<10 mm), the majority of which hatched in the mesocosms and remained reproductively immature. Three weeks into the experiment (14 June), we counted the number of *Helisoma* egg masses on the plexiglass sheets placed in each mesocosm. We measured total dissolved nitrogen, phosphorus, and organic carbon at the beginning and end of the experiment. Phytoplankton fluorescence (a relative measure of phytoplankton abundance) was measured at the end of the experiment using a fluorometer (Turner Designs Instruments, Sunnyvale, California, USA).

Mesocosm analysis.—We used a regression-based approach (Cottingham et al. 2005) to evaluate the effects of host density (three levels), snail species richness (three levels), and *Ribeiroia* exposure (two levels) on final *Helisoma* density (total vs. adults), the number and percentage of infected snails (total vs. adults), reproduction (egg masses and mass of juvenile snails), and snail size. Upon reaching maturity, *Ribeiroia* causes complete castration of infected snails (Johnson et al. 2004), and we hypothesized that infection could influence host population dynamics directly. Data were transformed prior to analyses using the logarithmic and arcsine-square-root functions to meet statistical assumptions. Because our experimental design was not fully factorial, notably omitting unparasitized treatments in the density experiment, the subset of data used varied by analysis (i.e., the effects of *Ribeiroia* and *Ribeiroia* x richness were analyzed only in the diversity experiment). For analyses in which responses were nested within mesocosms, such as infection status or size of each snail, we used generalized linear mixed effects models in which mesocosm was treated as a random effect (lme and lme4 in library lmer; R Core Development Team 2008).

Results

Field sampling

Among the 320 sampled wetlands, snail species representing four genera (*Helisoma*, *Physa*, *Lymnaea*, and *Gyraulus*) occurred at 277 sites. Snail species richness averaged 1.5 (range: 0–4 species) with *Helisoma* and *Physa* as the most common snails, occurring at 70% and 68% of ponds containing snails, respectively (Fig. 1A). For monospecific communities, *Helisoma* was the only species present in 51% of ponds while *Physa* was the only species present in 45% of ponds. *Lymnaea* and *Gyraulus* were found in <3% of monospecific communities. *Helisoma* also occurred in 82% and 96% of two- and three-species communities, respectively (Fig. 1A). Snail communities showed significant nestedness with an observed matrix temperature of 28.88° (P ($T < 28.88°$) $= 3.17 \times 10^{-9}$ [SD $= -5.81$]), such that low-diversity communities represented a near-perfectly nested subset of richer communities. Among sites in which they were present, snail species also varied in mean abundance: *Helisoma* (11.14 ± 1.18 per sweep), *Physa* (5.65 ± 0.93 per sweep), *Gyraulus* (4.38 ± 1.26 per sweep), and *Lymnaea* (1.61 ± 0.67 per sweep). While *Helisoma*, which is the only suitable host for *Ribeiroia* infection, was the most abundant snail species overall, its density correlated negatively with snail species richness ($\rho = 0.19$, $P = 0.019$; Fig. 1B).

Mesocosm experiments

Host population dynamics.—Both *Helisoma* density and species richness had strong effects on host population dynamics. Initial density had a positive effect on final adult density ($F_{3,36} = 21.55$, $P < 0.0001$), but no effects on final *Helisoma* density (adults + juveniles) or biomass owing to compensatory reproductive output in the low-density treatment (final density $F_{3,36} = 2.98$, $P = 0.093$; final dry mass $F_{3,36} = 0.03$, $P = 0.867$; Fig. 2A; Appendix: Table A1). Correspondingly, initial density had a negative influence on the biomass of juvenile snails ($F_{3,36} = 5.17$, $P = 0.029$; Fig. 2A), egg mass production ($F_{3,36} = 11.355$, $P = 0.0018$; Fig. 2D), and adult size ($F_{3,35} = 30.04$, $P < 0.0001$; Appendix: Table A1). In the diversity experiment, species richness had sharply negative effects on all measures of *Helisoma* population dynamics, including final adult density ($F_{3,36} = 10.0$, $P = 0.0032$), final total density ($F_{3,36} = 178.98$, $P < 0.0001$), adult size ($F_{3,36} = 39.45$, $P < 0.0001$), aggregate biomass ($F_{3,36} = 207.18$, $P < 0.0001$; Fig. 2B), and egg mass production ($F_{3,35} = 29.69$, $P < 0.0001$; Fig. 2E; Appendix: Table A2). In monospecific treatments, the final *Helisoma* dry mass (adults + juveniles) was nearly fivefold greater than in the four species treatments (Fig. 2).

In the diversity experiment, *Ribeiroia* exposure also had negative effects on final adult *Helisoma* density ($F_{3,36} = 15.19$, $P = 0.0004$), aggregate biomass ($F_{3,36} = 29.97$, $P < 0.0001$) and the biomass of juvenile snails ($F_{3,36} = 8.95$, $P = 0.005$; Fig. 2C; Appendix: Table A2). Total *Helisoma* biomass was >50% higher in treatments without *Ribeiroia* (Fig. 2C). There were no significant interactions between *Ribeiroia* exposure and snail species richness on any response variable (all $P > 0.05$). Within mesocosms exposed to *Ribeiroia*, infected snails were significantly larger than uninfected snails (among individuals >10 mm, linear mixed-effects model fit by restricted maximum likelihood [REML], infection $t = 6.07$, df $= 1733$, $P < 0.0001$; Fig. A3). While

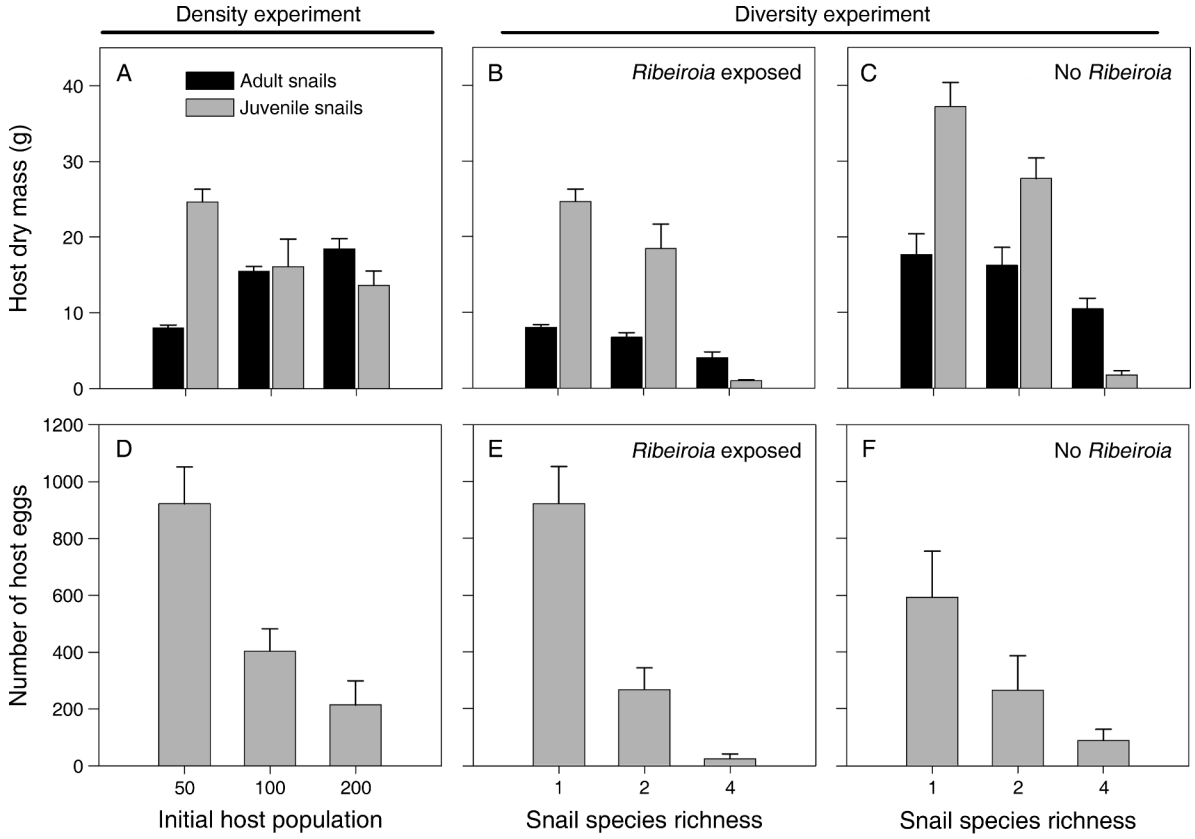

FIG. 2. Effects of density and diversity on snail host population dynamics. Patterns of final host dry mass (*Helisoma* adults and juveniles) as a function of (A) initial host density and (B, C) snail species richness. (D–F) Total number of *Helisoma* eggs counted on 14 June as a function of (D) initial host density and (E, F) snail species richness. Panels C and F illustrate treatments without *Ribeiroia* exposure. Error bars represent +SE.

dissolved organic carbon and total dissolved nitrogen decreased significantly over time (repeated-measures MANOVA, time $F_{3,30} = 128.812$, $P < 0.0001$), none of the water chemistry response variables related significantly to host density, species richness, or *Ribeiroia* (all multivariate $P > 0.05$; Appendix: Tables A3 and A4).

Host infection.—*Helisoma* initial density and snail species richness had opposing effects on *Ribeiroia* infection (Fig. 3). Increases in initial *Helisoma* density led to significant increases in the number of infected adults ($F_{2,25} = 4.51$, $P = 0.045$), but had no effect on the total number of infected snails ($F_{2,25} = 1.47$, $P = 0.239$; Fig. 2A and 2C). This effect was driven by the large number of hatchling snails that became infected within the low-density conditions, which served to equalize the higher infection of adult snails in the high-density treatments. Species richness had strongly negative effects on total *Helisoma* infected and the number of infected adults (total infected $F_{2,25} = 79.86$, $P < 0.0001$; adults infected $F_{2,25} = 7.69$, $P = 0.011$; Fig. 3B, D). On average, mesocosms in the monospecific condition had 14 times more infected snails than those in heterospecific treatments by the end of the experiment (Fig. 3D). This effect was driven by the dramatic differences in reproduction as a function of snail community structure, such that the majority (~95%) of infected snails in monospecific conditions were juveniles (Fig. 3B and 3D). Correspondingly, when final host density was included as a covariate, the effects of richness on infection were no longer significant (richness $F_{2,24} = 3.28$, $P = 0.084$; final density $F_{2,24} = 27.73$, $P < 0.0001$). The finding that richness influenced infection through changes in host density was reinforced by our analysis of infection prevalence. While initial host density had a sharply negative effect on infection prevalence, species richness had no effect on the likelihood a given snail was infected (generalized linear mixed models [GLMM] fit with Laplace approximation, initial density $z = -3.742$, $P = 0.0002$; richness $z = -0.69$, $P = 0.49$).

DISCUSSION

Interest in how changes in community structure influence parasite transmission in multi-species assemblages continues to grow (e.g., Keesing et al. 2010), but a shortage of integrated observational and experimental studies has hindered efforts to understand the underlying mechanisms. By combining cross-sectional survey data of California wetlands with experimental meso-

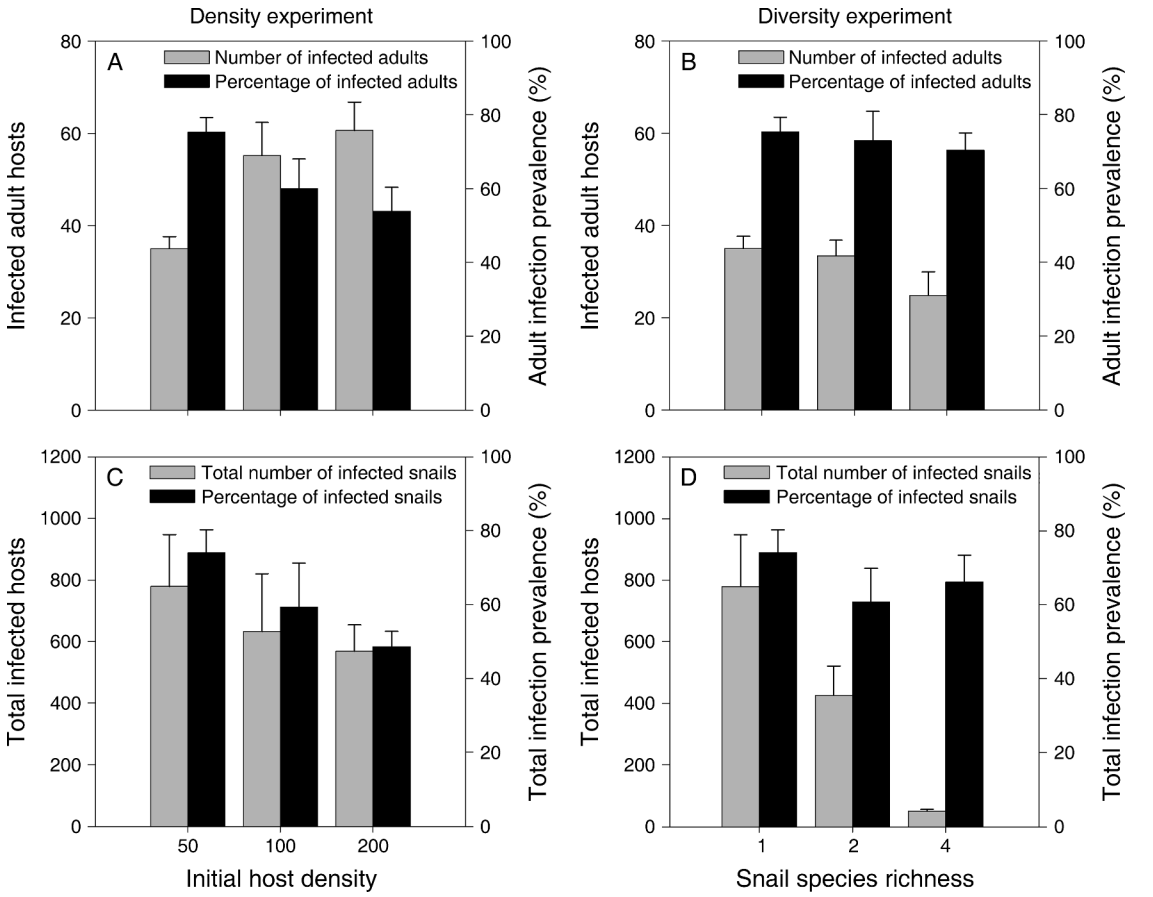

FIG. 3. Effects of density and diversity on snail host infection patterns. (A, B) Influence of (A) initial host density and (B) snail species richness on the number (light bars) and percentage (dark bars) of adult *Helisoma* with *Ribeiroia* infection. (C, D) Influence of (C) initial host density and (D) snail species richness on the number (light bars) and percentage (dark bars) of total (adult + juvenile) *Helisoma* infected with *Ribeiroia*. Error bars represent +SE.

cosm studies, our results provide insights into the relative importance of hypothesized mechanisms underlying the dilution effect for this host-parasite system. Survey data from 320 ponds in California revealed that freshwater snail communities exhibited a nested structure, with low richness communities forming a near-perfect subset of more diverse communities. Importantly, *Helisoma trivolvis*, which is a necessary intermediate host for *Ribeiroia* infection, was the most common and the most abundant snail species (although the non-host *Physa* sp. was nearly as common), and occurred in 70% of all wetlands with snails. Unsuitable host species were detected with increasing frequency in more species-rich assemblages, which also correlated with decreases in *Helisoma* density. These data provide support for an important yet rarely tested prerequisite of the dilution effect: high competency hosts predominate in species-poor communities with an increasing fraction of low competency species in more species-rich systems (Ostfeld and Keesing 2000*a*). This nested pattern is essential because it suggests that species are added or lost to a community in a predictable sequence with non-hosts occurring most commonly in more diverse communities. Ostfeld and Keesing (2000*a*) suggested that, for vector-borne parasites, highly competent hosts are among the most common species in a community due to evolutionary pressure on parasites to adapt to locally abundant and geographically widespread species that function as stable habitats for parasite persistence. Although this pattern has rarely been explored for non-vector-borne infections, our results suggest that this phenomenon occurs in other disease systems as well.

Building from field observations, our mesocosm experiments evaluated the effects of changes in host density and snail species richness on *Ribeiroia* infection in *Helisoma trivolvis*. These mechanisms are particularly important to evaluate experimentally because infection patterns for complex life cycle parasites in nature depend on the amount of parasite input (i.e., from definitive hosts), which is difficult to measure accurately in the field but can be controlled experimentally. Findings from our study revealed a strongly negative effect of species richness on parasite infection success, such that high-diversity communities supported 88–93% fewer

infected snails relative to monospecific conditions by the end of the experiment. However, this outcome was the result of a cross-generational reduction in the availability of susceptible hosts (susceptible host regulation), rather than the direct role of non-host species as parasitic "dead ends" (encounter reduction sensu Keesing et al. 2006). Competition associated with the addition of non-host species caused a 95–97% decrease in the number of host eggs and juvenile snails produced during the experiment, leading to a concomitant decline in the number of hosts available to *Ribeiroia*. These findings agree well with the patterns observed in our field sampling, in which *Helisoma* density decreased by 90% as snail species richness increased from 1 to 4 species (Fig. 1B), and with previous studies demonstrating the poor competitive ability of *Helisoma* against physid and lymnaeid snail species (Brown 1982, Osenberg 1989, Chase et al. 2001, Chase 2003). Had encounter reduction played an important role in driving our experimental results, we would have expected a decrease in the percentage of infected hosts, particularly among adult *Helisoma*; instead, infection prevalence in *Helisoma* remained relatively constant regardless of snail species richness, even while the number of infected snails varied by an order of magnitude (Fig. 3).

The role of susceptible host density in controlling the abundance of infected hosts was reinforced by the results of a complementary experiment that manipulated host density, independent of any other species. Among adult hosts, increases in initial density led to a monotonic decrease in the percentage of infected snails but an increase in the total number of infected individuals. This is because increases in host density enhanced the likelihood that each added parasite found a suitable host before expiring, thereby increasing the total number of infected snails, but such changes in density also "diluted" the total number of administered free-living parasite stages across a larger host population, leading to decreases in the percentage of infected adults. Another possibility is that in conditions with low *Helisoma* density, multiple miracidia were likely to colonize the same host, essentially reducing the effective dose of parasite input. Collectively, these outcomes differ from what often occurs with directly transmitted parasites, in which increases in host density lead to concomitant increases in infection prevalence owing to greater contact rates (e.g., Mitchell et al. 2002, Greer et al. 2008).

However, a key difference between the density and diversity experiments was that, within monospecific communities, the ending biomass of hosts converged by the end of the experiment, despite initial differences in stocked densities (i.e., the populations moved to carrying capacity; Fig. 2A). As a result of compensatory reproduction in the low-density conditions and an increased number of juvenile snails that became infected with *Ribeiroia*, there was no significant difference in the final number of infected snails (adults and juveniles) as a function of initial density. In contrast, in the multi-species assemblages that contained non-host snails, no such compensatory reproduction by host snails occurred, and high-diversity communities produced ~90% fewer infected snails by the experiment's conclusion. This was likely because, over the time scale of our experiment, non-host snails consumed resources that would otherwise have facilitated growth of the susceptible host population. Because the number of infected snails should be proportional to cercarial production, particularly as infections reach maturity, these findings are relevant for understanding how snail (intermediate host) community structure influences the risk of infection and pathology in second intermediate hosts, such as amphibians, wherein *Ribeiroia* infection causes mortality and severe limb malformations (Johnson et al. 1999, 2010*b*, Rohr et al. 2009).

While parasitism and competition each had negative effects on host population growth, we did not detect any interactions between infection and species richness. In several previous studies, authors have reported indirect benefits associated with the addition of low competency hosts in the form of reduced infection within susceptible hosts (apparent or parasite-mediated facilitation; Johnson et al. 2008, 2009, Hall et al. 2009). *Ribeiroia* exposure, which causes castration in patently infected snails (Johnson et al. 2004), reduced *Helisoma* abundance and biomass relative to snail communities in the unexposed treatments (Fig. 2). Infected snails were also consistently larger than uninfected adults (Fig. 3A), which could reflect either their larger size as a target for host-seeking miracidia or parasite-induced increases in host growth among infected (and castrated) individuals (Kuris and Lafferty 1994). However, interspecific competition resulting from the addition of non-host snails caused a more substantial reduction in host population reproduction relative to infection, affording little opportunity for parasite-mediated facilitation in this system. Collectively, such findings underscore the importance of experiments that incorporate the multigenerational outcomes of species interactions to assess mechanisms underlying the diversity–disease relationship.

Our results emphasize the important role of susceptible host regulation as a mechanism underlying the dilution effect (Elton 1958, Mitchell et al. 2002, Begon 2008), which has rarely been demonstrated for non-vector borne diseases of animals. Counter to our hypotheses, however, we observed no effect of diversity on infection prevalence, despite a rich history of such "decoy effects" in parasitological research on snails and trematodes (Chernin 1968, Upatham and Sturrock 1973, Thieltges et al. 2008, Johnson et al. 2009). These observations were also unlikely to be explained by other hypothesized mechanisms of the dilution effect, such as changes in the recovery or mortality of infected individuals, given that host density accounted for much of the variation in host infection and there was little mortality among adult hosts. The lack of an identified role for encounter reduction could owe to the high levels

of parasite eggs added, which caused ~70% of adult snails to become infected and may have saturated any decoy potential, or to the use of large-scale mesocosms, which provided greater habitat diversity and more ecological realism relative to small-scale laboratory experiments (but see Laracuente et al. 1979, Thieltges et al. 2009). It is also possible that other species not tested in these experiments, such as invertebrate predators, could influence transmission in natural environments (e.g., Thieltges et al. 2008, Johnson et al. 2010*a*). We suggest that the mechanisms of the dilution effect observed here will most likely influence infection success when (1) susceptible hosts are a nested component of both species-poor and species-rich communities and (2) subsequently added species reduce the survival or reproduction of hosts through processes such as competition or predation (see also Knops et al. 1999, Mitchell et al. 2002).

Taken together, the current findings reiterate the importance of linking field and experimental data to investigate the influence of community structure on parasite infection. Cross-sectional field surveys provide a necessary context for designing ecologically relevant experiments and allow comparisons with experimental data, while realistic manipulations offer mechanistic insights into how observed structures influence dynamic processes such as transmission. Our results also emphasize the longer-term effects of species richness on the abundance of susceptible hosts, which led to dramatic decreases in the number of infected hosts only through effects on the second generation. By quantifying the role of susceptible host regulation in parasite infection success, the current study provides empirical support for one of the theoretical mechanisms responsible for the dilution effect (Keesing et al. 2006). In light of ongoing changes in ecological communities, including the loss of native species and the translocation of invaders, a broader understanding of the effects of community structure on pathogen transmission has both applied and theoretical importance (Keesing et al. 2010).

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Supplemental Material

Appendix

Field sampling methods, rarefaction curves, and detailed information on experimental parasite additions, the final size/counts of host snails, and mesocosm water chemistry (*Ecological Archives* E093-006-A1).