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SYMPOSIUM ARTICLE

A legacy of competitive exclusion: Host demography and amplified disease

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Synopsis Dilution effects arise when increases in species diversity reduce disease risk, and amplification effects arise when the opposite occurs. Despite ample evidence for both phenomena, the mechanisms driving dilution and amplification effects and how they are mediated by environmental factors remain poorly understood. Mechanisms involving demographic rates or stage structure of hosts are particularly lacking in the diversity–disease literature. In Midwestern lakes, *Metschnikowia bicuspidata* parasites infect *Daphnia dentifera* focal hosts in autumn, with epidemics beginning when water is warm (~25°C) and peaking when lakes have cooled (~15°C). Epidemics are smaller in lakes with more *Ceriodaphnia dubia* alternative hosts, which serve as key diluters of disease. However, it is unclear whether seasonal changes in temperature affect their ability to alter host population dynamics and reduce disease. We conducted a mesocosm experiment to test how temperature (15, 20, or 25°C) mediated the effects of these key alternative hosts on density, stage structure, and disease dynamics in focal host populations. The experiment yielded several surprising results. First, focal hosts rapidly outcompeted alternative hosts at all temperatures. By the time parasites were added, alternative hosts had been almost completely excluded. Second, despite diluting disease in the field, initial presence of these alternative hosts *amplified* infection prevalence in the experiment. Third, this amplification arose as a legacy effect, lasting generations after alternative hosts were gone. Our explanation for this legacy amplification effect centers on focal host stage structure and demography. Competition with alternative hosts resulted in focal host populations that were more adult-biased when parasites were added, at all 3 temperatures. Additionally, host densities in these treatments increased more rapidly in the subsequent 10 days, consistent with reduced background death rates. Since adults consume more parasites than juveniles, and since exposed hosts must survive 10 days before producing infectious spores, these initial differences in stage structure and population growth seem to have set disease dynamics along amplified trajectories. These results highlight the need for a broader understanding of the mechanisms that can amplify or dilute disease, including altered host stage structure and mortality of exposed hosts in diverse communities.

Introduction

Higher species diversity can either decrease or increase disease risk, dubbed dilution effects or amplification effects, respectively (Keesing et al. 2006; Ostfeld and Keesing 2012). These ideas originated with Lyme disease in the Eastern United States, where risk of human disease is "diluted" by higher biodiversity of mammals and lizards (Ostfeld and Keesing 2000). Theory for dilution and amplification effects was later generalized (Keesing et al. 2006) and applied broadly to other parasites and pathogens infecting humans (Allan et al. 2009; Johnson et al. 2009; Luis et al. 2018), animals (Hall et al. 2009; Johnson et al. 2013; Venesky et al. 2014), and plants (Mitchell et al. 2002; Rosenthal et al. 2022; Strauss et al. 2024). Although the frequency of dilution versus amplification effects has been vigorously debated (Randolph and Dobson 2012; Rohr et al. 2020), meta-analyses suggest that dilution is more common, at

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least at local spatial scales (Civitello et al. 2015; Halliday and Rohr 2019; Halliday et al. 2020). Active research on diversity–disease relationships seeks to clarify how these patterns vary with parasite transmission mode (Cortez and Duffy 2021; Chen et al. 2022), how they are mediated by changes in focal host density (Rosenthal et al. 2022; Strauss et al. 2024), how they extend to multiple parasite species (Johnson et al. 2024), and whether dilution effects persist at larger spatial scales (Halliday and Rohr 2019; Magnusson et al. 2020; Rohr et al. 2020).

One way to clarify expectations of dilution versus amplification is to ground emergent patterns between diversity and disease in mechanistic species interactions among focal hosts, alternative or non-hosts (hereafter: alternative hosts), and parasites (Keesing et al. 2006). For example, "encounter reduction" occurs when alternative hosts reduce contact between focal hosts and parasites by diverting infectious vectors (LoGiudice et al. 2003), consuming free-living parasites (Johnson et al. 2010), or blocking airborne pathogens (Boudreau 2013). In contrast, "host regulation" occurs when alternative hosts lower focal host density—for example, via interspecific competition—and thereby inhibit density-dependent transmission (Mitchell et al. 2002; Strauss et al. 2018). Species interactions that *increase* disease—in other words, amplification mechanisms—are less well understood, but can arise from higher contact rates between hosts and parasites (Luis et al. 2018), higher density of vectors (Randolph and Dobson 2012), addition of alternative hosts without reductions in focal host density (i.e., additive community assembly) (Johnson et al. 2024), or higher density of focal hosts in diverse communities (Strauss et al. 2024). The strength of these mechanisms can reveal why addition of a species to a community might increase or decrease disease.

Other underappreciated dilution or amplification mechanisms could arise if alternative hosts alter focal host demography beyond changes in density. For example, if certain stages of hosts (e.g., juveniles versus adults) are more epidemiologically important for parasites (e.g., Hite et al. 2016; Shaw et al. 2024), then altered host stage structure in diverse communities could increase or decrease disease. Similarly, elevated death rates of exposed hosts could reduce the likelihood of a parasite completing its lifecycle before host death [i.e., death during a period of latency in hosts or extrinsic incubation in vectors (Childs and Prosper 2020)]. Although dilution or amplification mechanisms grounded in host stage structure or mortality of exposed hosts seem possible, we are unaware of any empirical examples in the diversity–disease literature.

In addition to characterizing novel dilution and amplification mechanisms, another frontier in diversity–disease research is to delineate how the density, stage structure, and per capita traits of multiple species jointly vary along environmental gradients. In the motivating case of Lyme disease, habitat fragmentation shifts host community composition in ways that elevate community competence (Ostfeld and Keesing 2000; LoGiudice et al. 2003). In alpine communities, warmer temperature favors plants with faster-paced life histories which also suffer more disease (Halliday et al. 2023). In lakes, shallower refuges intensify fish predation on zooplankton, shifting community composition and inhibiting transmission (Strauss et al. 2016). In each of these examples, environmental gradients shape disease outcomes by altering relative densities of focal and alternative hosts. Fewer studies have explored how abiotic gradients concurrently mediate host stage structure or intraspecific variation in relevant per-capita traits. In one intertidal example, warmer temperature magnified encounter reduction by increasing the rate at which oysters and barnacles consumed free-living parasites of mussels (Goedknegt et al. 2015). Other examples of environmental gradients driving intraspecific variation in traits that cause dilution or amplification—potentially coinciding with shifts in community composition—are extremely rare. Nevertheless, delineating such effects could lead to insightful predictions about how biodiversity–disease relationships might shift under conditions of environmental change.

Here, we conducted a mesocosm experiment to test how temperature mediated the effects of a key alternative host on population and disease dynamics in focal hosts, using a zooplankton-fungus model system. The experiment yielded several surprising results. First, focal hosts rapidly outcompeted alternative hosts at all temperatures, resulting in all mesocosms being dominated by the focal host at time of parasite exposure. Second, initial presence of alternative hosts (i.e., before parasites were added) altered disease dynamics in focal hosts, even several generations after alternative hosts had been competitively excluded. Thus, we detected a legacy effect of diversity on disease. Third, infection prevalence in focal hosts was *higher* in communities that began with alternative hosts—especially at intermediate temperatures. Our explanation for this unexpected legacy amplification effect is grounded in host stage structure and demography. In short, interspecific competition resulted in adult-biased focal host populations on the day of initial parasite exposure. Moreover, these populations grew faster (suggesting reduced background mortality rates) over the subsequent 10 days, coinciding with the latent period for these infections (Stewart Merrill and Cáceres 2018). Together, these changes in stage structure and demographic rates seem to have unleashed larger epidemics in communities that initially contained alternative hosts, at all

3 temperatures. These results introduce 2 novel amplification mechanisms to the diversity–disease literature: altered stage structure and mortality of exposed hosts in diverse communities.

Methods

Natural history of the study system

The focal host, *Daphnia dentifera*, is a dominant cladoceran grazer in many Midwestern lakes (Tessier and Welser 1991). Its populations often suffer autumnal outbreaks of the virulent fungus *Metschnikowia bicuspidata*, with peak infection prevalence sometimes exceeding 50% (Hall et al. 2010; Strauss et al. 2016). No other cladoceran species are infected nearly as frequently, making *D. dentifera* the focal host. Epidemics typically begin in August, when temperature in the epilimnion is near 25°C. Epidemics peak when water has cooled to ∼15°C and end in late November as lakes cool further (Shocket et al. 2018). Hosts ingest parasite spores while filter-feeding for algae (Hall et al. 2007). Ten days after exposure, if infections have not been cleared by hosts' physical and immune defenses, parasites reach the terminal ascus stage (Stewart Merrill and Cáceres 2018). Infected hosts that die after this stage release infectious asci back into the environment; however, hosts that die sooner are much less likely to release infectious spores (Stewart Merrill and Cáceres 2018). Infection prevalence is typically 2–3 times higher in adults than juveniles (Hite et al. 2017), mostly because juveniles that consume spores mature into adults before terminal infections become apparent. Exposure rates are also higher for adults, because adults consume more spores via faster feeding rates (Hite et al. 2017). Then again, juveniles are more susceptible per spore consumed (Hite et al. 2017), due to weaker physical defenses and immune responses (Stewart Merrill et al. 2019).

Epidemics are typically smaller in lakes where alternative hosts (i.e., other cladocerans) are more common (Hall et al. 2009; Hall et al. 2010). Lakes with more *Ceriodaphnia dubia* in particular have lower infection prevalence in focal host populations, making *C. dubia* a key diluter of disease in the field (Hall et al. 2010; Strauss et al. 2016). *Ceriodaphnia dubia* consume parasites but rarely become infected (a form of encounter reduction) and also compete with hosts for shared resources (host regulation) (Strauss et al. 2015). Additionally, when *C. dubia* do become infected, they produce far fewer spores than focal hosts (Auld et al. 2017). When *D. dentifera* focal hosts are weaker competitors, *C. dubia* (hereafter: alternative hosts) reach higher densities and drive stronger dilution effects; when these alternative hosts are rarer, they exert weaker effects on disease (Strauss et al. 2018).

We broadly hypothesized that warmer temperature would strengthen dilution effects in this system. Although growth rates of both focal and alternative hosts increase with temperature from 15 to 25°C, this increase is steeper for *Ceriodaphnia*, potentially due to their smaller body size (Kooijman 2000). Competition should therefore favor *C. dubia* alternative hosts at the onset of epidemics when water is warmer, but *D. dentifera* focal hosts should gain a competitive advantage as water cools into the fall. Thus, warmer temperature should strengthen host regulation. Warmer temperature should also strengthen encounter reduction, since filter-feeding rate of ectothermic grazers generally increases with temperature (Goedknegt et al. 2015; Shocket et al. 2018).

Mesocosm experiment

We established a multi-generational mesocosm experiment to test how a relevant range of temperature (15–25°C) altered the effects of *C. dubia* alternative hosts on population and disease dynamics in *D. dentifera* focal hosts. We manipulated temperature (15, 20, or 25°C) and community composition (focal hosts alone, alternative hosts alone, or both together) in a full factorial design, replicating each treatment combination 4x. Each experimental unit (60 L polyethylene bucket) was filled with 90% tap water (passed through activated carbon) and 10% filtered lake water (1 µm Pall A/E). Mesocosms were nested in thermostatically-controlled water baths (1.3 × 3 m) and heated or cooled using water heaters and chillers. All mesocosms were inoculated with initial doses of phosphorus (20 μg/L P as K_2HPO_4) and nitrogen (300 μg/L N as $NaNO_3$) and a high-quality algal food for hosts (*Ankistrodemsus falcatus*). Algal growth was stimulated with LED grow lights (16:8 h light:dark) and weekly replacement of N and P, assuming a 5% daily loss rate (Strauss et al. 2015). Algae grew alone for one week before zooplankton were added to the mesocosms.

Focal and alternative hosts were reared under standardized conditions prior to the experiment (20°C; 60 L^{-1}; fed 1.0 mgC/L *A. falcatus* daily). A single genotype of each species was used to avoid any temperature-dependent differences in clonal selection. The focal host genotype ("Standard Clone") was the strong competitor that drove *dilution failure* in Strauss et al. (2015); the alternative host genotype had not been used in a prior experiment. After several generations, populations of 200 hosts were established for each experimental unit and gradually acclimated to experimental conditions (1.5–2°C per day) before being added to the mesocosms (day 0). We used an additive design, so each species started at the same densities in competition treatments and

single-species treatments, with total cladoceran density starting twice as high in competition treatments. We did not sample on day 0, but estimated that initial densities were ~4 L^{-1} for each host and assumed an initial 50/50 split between juveniles and adults. Mesocosms were sampled weekly (sieving 1 L through 153 μm mesh) for 3 weeks. Then, on day 21, a low dose of *M. bicuspidata* parasites (5 spores/mL) was added to all mesocosms. All parasites were recently reared (<6-weeks old) in the same focal host genotype used in the experiment. Note that since parasites were added to all mesocosms, we cannot assess impacts of parasites on focal host population dynamics, or the impacts of alternative hosts on these dynamics in the absence of disease. After parasite addition, mesocosms were sampled twice weekly for 7 additional weeks until the end of the experiment (day 66). In each sample, focal hosts were counted and classified by demographic stage (juvenile or adult) and infection status (visibly infected or not). Alternative hosts were counted and classified only by infection status. Infection prevalence was calculated for each species as the proportion of hosts that were infected, and stage structure was summarized as the proportion of focal hosts that were adults.

Statistical analyses

We used a combination of general additive mixed models (GAMMs), generalized linear models (GLMs), and linear mixed models (LMMs) to ask how community composition affected host population dynamics, disease dynamics, and stage structure. Four mesocosms were excluded from the analysis because they were invaded by non-target species. All analyses were conducted in R version 4.3.1 (R Core Team 2021). GAMMs were fit using the mgcv package (Wood 2023) to ask whether presence of one host altered time series dynamics of the other. Separate GAMMs were fit for focal host density, alternative host density, focal host infection prevalence, and focal host stage structure (i.e., proportion adult). We assumed negative binomial distributions for the density responses and binomial distributions for infection prevalence and proportion adult, weighted by the total number of focal hosts observed in each sample. All GAMMs included smooth terms for time (*t* effects), factors for presence of the other species (community; *C* effects), factor-smooth interactions to assess whether presence of the other species altered the time series (*C* × *t* effects), and random error smooth terms to account for repeated measures from each replicate mesocosm. Separate GAMMs were fit for each temperature. Due to our small sample sizes, we used restricted maximum likelihood to help avoid overfitting the GAMMs (Wood 2023).

We fit GLMs to assess whether presence of the other host altered integrated or cumulative metrics over the entire time series. GLMs also revealed the directions of effects, which were obscured in the GAMMs by factor-smooth interactions. Integrated density was calculated as the area under the curve of densities over time, and cumulative infection prevalence and proportion adult were calculated as the proportion of all focal hosts observed in a mesocosm over its entire time series that were infected or adults, respectively. We assumed Gaussian distributions for integrated densities and binomial responses for cumulative prevalence and proportion adult, weighted by the cumulative number of focal hosts observed. All GLMs included presence of the other host as a factor.

Finally, because initial presence of alternative hosts consistently elevated infection prevalence in focal host populations at all 3 temperatures (see Results), we investigated 2 potential explanations for this unexpected outcome. Specifically, we tested whether initial presence of alternative hosts altered either (1) stage structure of the focal host population on the day that parasites were added, or (2) changes in population growth over the subsequent 10 days. Altered stage structure on the day of parasite exposure could have affected which hosts (e.g., juveniles or adults) consumed the initial dose of parasites. Variation in subsequent population growth could indicate differences in either birth rates or death rates while this initial dose of parasites matured inside hosts (i.e., the latency period). In particular, elevated death rates could have reduced the likelihood of exposed hosts surviving until these parasites reached the infectious ascus stage, 10 days later. We used GLMs to ask whether presence of the alternative host affected stage structure of the focal host on day 21. We fit LMMs with package nlme (Pinheiro and Bates 2000) to ask whether alternative hosts affected population growth of focal hosts in the 10 days after parasite addition. These LMMs included random intercepts for each replicate mesocosm, allowed variance to increase exponentially with sampling day to account for the observed heteroskedasticity, and were re-centered so that community effects (*C* effects) indicated any differences on the day of parasite addition.

Results

In general, focal hosts outcompeted alternative hosts regardless of temperature (Fig. S1 in Appendix), and adult focal hosts were more likely to be infected than juveniles or alternative hosts. Of the 36,026 total zooplankton observed in competition treatments, 7.86% were alternative hosts and 92.14% were focal hosts. Of these focal hosts, 53.68% were juveniles and 46.01% were adults,

with stage structure fluctuating in all treatments over time. Depending on temperature and time, the mean percent of adults ranged from 12 to 76%. A tiny fraction of focal hosts (0.32%) were males, which are produced by asexually-reproducing females under stressful conditions. Of the 24,921 focal hosts observed in focal-only treatments, 3.77% were infected. After dividing this focal host population into demographic groups, infection prevalence was much higher in adults (8.07%) than juveniles (0.34%). Of the 62,551 alternative hosts observed in alternative-only treatments, only 0.36% were infected. Thus, in single-host treatments, infection prevalence was similarly low for alternative hosts and juvenile focal hosts, and much higher for focal host adults. Importantly, although we designed the experiment to assess the effects of temperature, impacts of alternative hosts were qualitatively similar at 15, 20, and 25°C. Therefore, we focus our results on a more intriguing set of patterns that emerged between host demography and disease at all 3 temperatures. Despite our small sample size at the mesocosm scale, several significant effects emerged.

Host densities: Times series of densities reiterated the result that focal hosts universally outcompeted alternative hosts. Dynamics were qualitatively similar at 20 (Fig. 1), 15, and 25°C (all 3 shown in Fig. S2 in the Appendix). Alternative host dynamics were significantly affected by presence of focal hosts ($P < 0.001$ for $C \times t$ effect in GAMMs Fig. 1A), and the GLM confirmed that focal hosts reduced integrated alternative host density ($P < 0.01$; Fig. 1B). Alternative hosts did not alter population dynamics or integrated density of focal hosts at any temperature (all $P > 0.1$; Fig. 1 and Fig. S2), although it is possible that differences could have emerged with larger sample sizes.

Infection prevalence: Initial presence of alternative hosts consistently elevated infection prevalence in focal host populations. These results were qualitatively similar at 20°C (Fig. 2) and the other 2 temperatures (15 and 25°C shown in Fig. S3). Although alternative hosts became rare in competition treatments (Fig. 1A and Fig. S2), their initial presence significantly altered disease dynamics in focal host populations ($C \times t$: $P < 0.001$; Fig. 2A). Moreover, the GLMs showed that initial presence of alternative hosts significantly *elevated* cumulative infection prevalence of focal hosts. This amplification effect was largest in magnitude at 20°C ($P < 0.001$; Figs. 2B and 4H) but was also significant at 15°C ($P < 0.01$; Fig. 4G) and 25°C ($P < 0.05$; Fig. 4I). Infection prevalence was universally low for alternative hosts, and presence of focal hosts did not alter their cumulative infection prevalence (all $P > 0.1$; Fig. 4J–L).

Stage structure: One potential explanation for the surprising but consistent amplification of disease involves stage structure. At 20°C, stage structure varied significantly for focal host populations over time, with populations sometimes more adult-biased and sometimes more juvenile-biased ($P < 0.001$ for t effect in GAMM; Fig. 3A). This pattern was qualitatively similar at 15 and 25°C (Fig. S4). Although alternative hosts were rapidly outcompeted (Fig. 1), their initial presence altered these stage structure dynamics (all $C \times t$: $P < 0.001$; Fig. 3A and Fig. S4). Initial presence of alternative hosts also significantly increased the cumulative proportion of focal hosts that were adults over the entire time series at 20°C ($P < 0.001$; Fig. 3B), but not at 15 or 25°C (both $P > 0.1$; Fig. S4).

Potential explanations for the legacy amplification effect: Two potential explanations for the legacy amplification effect invoke (1) altered host stage structure on the day of parasite addition, and (2) elevated background death rates for exposed focal hosts in monocultures compared to competition treatments over the subsequent 10 days. Focal host populations were more adult-biased on day 21 (when parasites were added to the mesocosms) in treatments where alternative hosts were initially present. This effect was significant at 15°C ($P < 0.01$; Fig. 4G), 20°C ($P < 0.001$; Fig. 4H), and 25°C ($P < 0.001$; Fig. 4I). Focal host population density on day 21 was similar in treatments with or without alternative hosts at all temperatures (all $P > 0.1$; Fig. 4D–F), but tended to increase more steeply over the subsequent 10 days in treatments that initially included alternative hosts. This pattern is consistent with higher background mortality rates (or lower birth rates) when focal hosts were alone. Population growth was significantly slower in this timeframe for focal hosts growing alone at 20°C ($P < 0.01$ for $C \times t$ effect in LMM; Fig. 5B) and 25°C ($P < 0.05$; Fig. 5C) and also qualitatively slower at 15°C ($P > 0.1$; Fig. 5A). Higher mortality when focal hosts were alone and juvenile-biased on day 21 would be consistent with shorter starvation times for juveniles versus adults (Tessier et al. 1983). Although our interpretation is based on correlations, these transient differences in stage structure (Fig. 4G–I) and demographic rates (Fig. 5) seem to have set stage structure dynamics (Fig. 3) and disease dynamics (Fig. 2) along different trajectories for the remainder of the experiment (3–6 host generations).

Discussion

Adding a species to an ecological community can either increase or decrease disease risk for a focal host species. Dilution mechanisms describe how the new species interactions can reduce disease, while amplification mechanisms describe how they can increase it

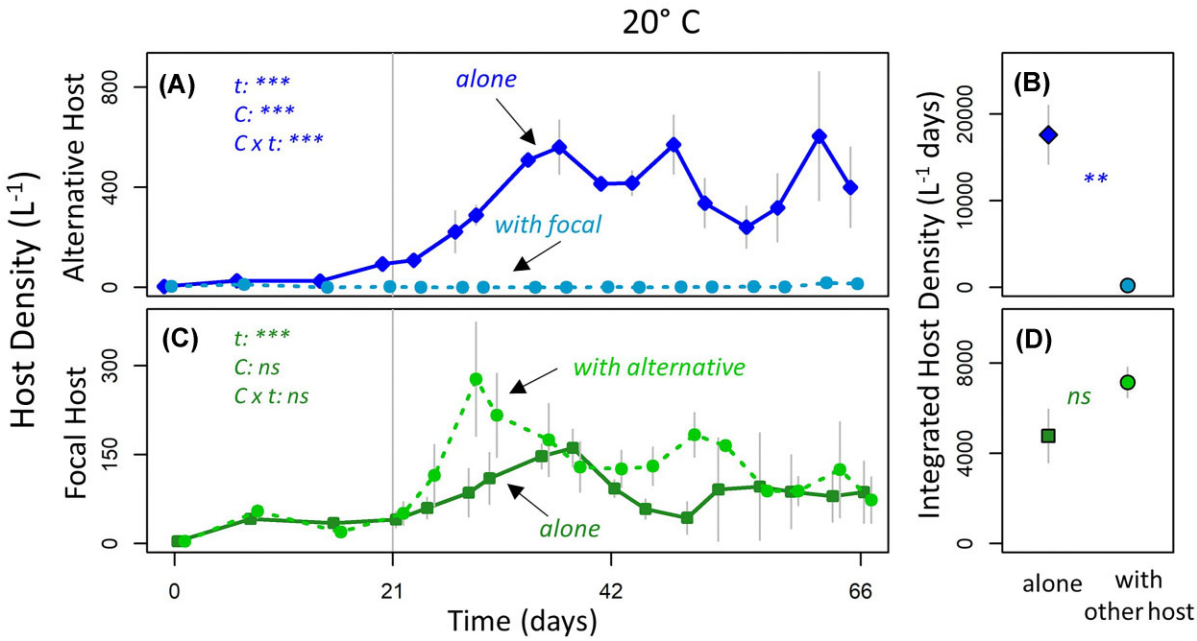

Fig. 1 *Population dynamics of focal and alternative hosts at 20°C.* Focal hosts (*D. dentifera*) rapidly outcompeted alternative hosts (*C. dubia*). **(A)** Population dynamics of alternative hosts was significantly different in the presence (dashed light blue; circles) or absence (solid dark blue; diamonds) of focal hosts. **(B)** Specifically, presence of focal hosts strongly reduced the integrated density of alternative hosts (i.e., area under the curve of the time series). **(C)** In contrast, focal host dynamics were not significantly affected by the initial presence (dashed light green; circles) or absence (solid dark green; squares) of alternative hosts. **(D)** Similarly, focal host density integrated over the entire experiment was not significantly affected by presence of alternative hosts. Results at 20°C are shown here; results at 15 and 25°C are qualitatively similar and shown in the Appendix (Fig. S2). Error bars are standard errors of means among replicate mesocosms. Asterisks indicate significance of GAMMs (time series) or GLMs (integrated densities): $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Vertical gray line at day 21 indicates the addition of parasites.

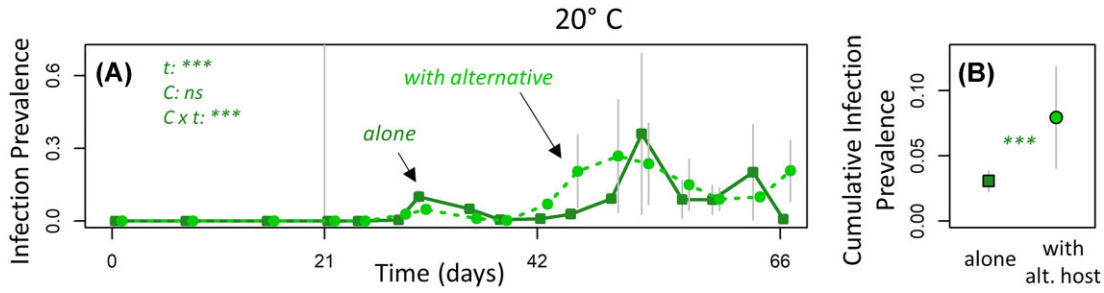

Fig. 2 *Disease dynamics of focal hosts at 20°C.* Although alternative hosts were rapidly outcompeted (Fig. 1), their initial presence affected—and even elevated—infection prevalence in focal host populations. **(A)** Disease dynamics in focal hosts were significantly altered by initial presence of alternative hosts (dashed light green line with circles versus solid dark green line with squares). **(B)** Specifically, initial presence of alternative hosts elevated the cumulative infection prevalence in focal hosts, calculated as the proportion of focal hosts that were observed infected in a mesocosm over the entire duration of the experiment. Results at 20°C are shown here; results at 15 and 25°C are qualitatively similar and shown in the Appendix (Fig. S3). Infections in the alternative host population were extremely rare at all temperatures, with or without focal hosts (Fig. S3). Error bars are standard errors of means among replicate mesocosms. Asterisks indicate significance of GAMMs (time series) or GLMs (integrated densities): $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Vertical gray line at day 21 indicates the addition of parasites.

(Keesing et al. 2006; Strauss et al. 2018). Here, we intended to test how a field-relevant thermal gradient altered the effects of key alternative hosts (*C. dubia*) on infection dynamics of a virulent fungus (*M. bicuspidata*) in populations of a focal host (*D. dentifera*). The mesocosm experiment yielded several unexpected outcomes, which were consistent at all 3 temperatures (15, 20, and 25°C). First, focal hosts rapidly outcompeted alternative hosts. This result was surprising, because warmer temperature was predicted to favor smaller-bodied alternative hosts (Kooijman 2000). Second, despite being excluded, the initial presence of alternative hosts al-

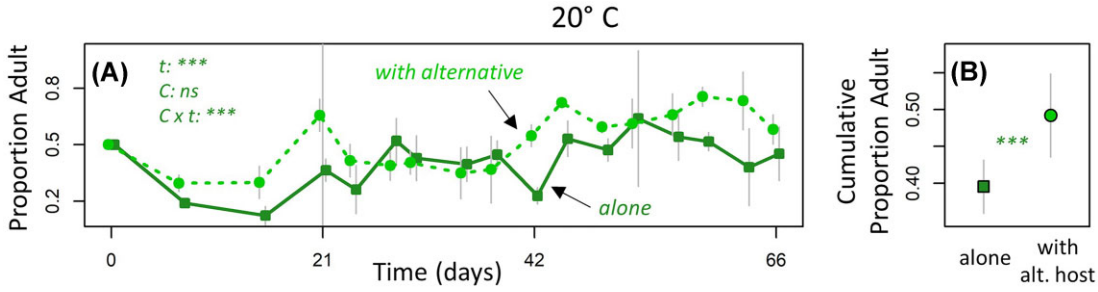

Fig. 3 *Stage structure dynamics of focal hosts at 20°C.* The proportion of focal hosts in the adult stage varied over time, likely both responding to and contributing to disease dynamics (Fig. 2). **(A)** Initial presence of alternative hosts altered stage structure dynamics of focal hosts (dashed light green line with circles versus solid dark green line with squares) and **(B)** increased the cumulative proportion of adults over the course of the experiment. Results at 20°C are shown here; time series results at 15 and 25°C are qualitatively similar and shown in the Appendix, although cumulative effects are only significantly different at 20°C (Fig. S4). Importantly, at all temperatures, initial presence of alternative hosts resulted in focal host populations that were transiently more adult-biased when parasites were added on day 21 (gray vertical lines; see Fig. 4). Error bars are standard errors of means among replicate mesocosms. Asterisks indicate significance of GAMMs (time series) or GLMs (integrated densities): $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Vertical gray line at day 21 indicates the addition of parasites.

tered focal host disease dynamics for the duration of the experiment. Third, infection prevalence was *higher* in communities that initially contained alternative hosts. At face value, this result was surprising because these alternative hosts *reduced* disease in other experiments, mathematical models (Strauss et al. 2015), and the field (Strauss et al. 2024). Previous work also demonstrated that these alternative hosts were unable to reduce disease when they were outcompeted (Strauss et al. 2018). Here, although they were outcompeted before parasites were added, their initial presence somehow elevated disease. Our explanation for this legacy amplification effect involves relationships among species diversity, host demography, and infectious disease (discussed below). In short: amplification appears to have arisen from (1) varied epidemiological importance of juveniles and adults, (2) altered host stage structure in the presence of a competitor, (3) a lag between host exposure and parasite release (i.e., latency), and (4) potential increases in longevity of exposed hosts in more diverse communities. These criteria could be easily met in other host–parasite systems, suggesting that host demography and stage structure could play an underappreciated role in diversity–disease relationships more broadly.

Alternative hosts clearly altered stage structure dynamics in focal host populations. General theory for stage-structured consumer-resource dynamics is well-grounded in interactions between *Daphnia* consumers and their algal resources (Tessier et al. 1983; McCauley et al. 1996; de Roos et al. 2007). Resources can regulate the maturation rate of juveniles, the fecundity of adults, and mortality rates of both stages, driving resource-centric feedbacks that alter population dynamics of both hosts (De Roos et al. 2003; McCauley et al. 2008) and their parasites (Hite et al. 2015; Hite and de Roos 2023). Here, it is unsurprising that *Ceriodaphnia* altered stage structure of *Daphnia,* because these hosts compete for resources. Competition appears to have increased juvenile mortality of focal hosts, imposed a juvenile bottleneck, and skewed focal host populations toward adults in the first 3 weeks of the experiment. Clearly, host populations were more adult-biased when parasites were added. Elevated population growth over the next 10 days likely arose as a pulse of reproduction from the adult-biased populations, enabled by relaxed resource competition as alternative hosts were excluded. Importantly, these demographic changes corresponded with amplified disease. Exposure rates are higher for adults due to their faster feeding rates (Hite et al. 2017), and exposed hosts must survive 10 days before producing infectious spores (Stewart Merrill and Cáceres 2018). Thus, we hypothesize that the initial dose of parasites became concentrated in adults, that more of these exposed adults survived to transmit new infections, and these different initial conditions amplified disease for several generations. Future parameterized models could clarify how well these hypothesized feedbacks recapitulate the dynamics observed in the mesocosms at each temperature.

Dilution or amplification mechanisms centered on host stage structure and mortality seem potentially relevant for a wide variety of host–parasite systems. The stage structure mechanism we propose requires that (1) stages of hosts vary in epidemiological importance, and (2) other species alter focal host stage structure. Host stage and age are important for amphibian chytrid fungus (Hite et al. 2016), snails that transmit schistosomes (Daoust et al. 2010; Shaw et al. 2024), plant pathogens (Panter and Jones 2002), and human childhood diseases (Agur et al. 1993), with juveniles frequently evolv-

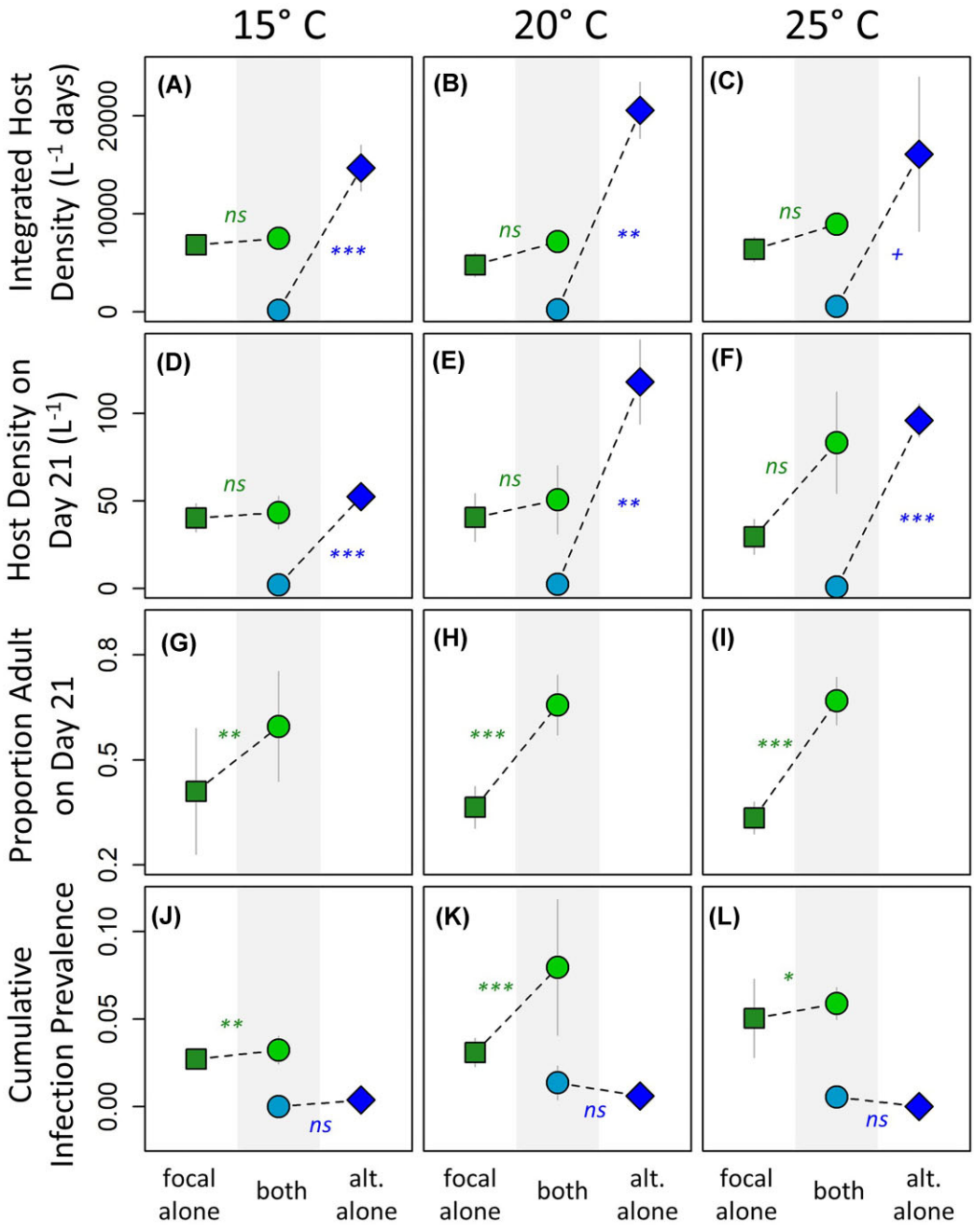

Fig. 4 *Suggestive correlations between stage structure and disease at all 3 temperatures.* Initial presence of alternative hosts altered 2 demographic properties of focal host populations that coincided with amplified disease: adult-biased populations on the day when parasites were added (shown here), and accelerated population growth over the subsequent 10 days (Fig. 5). These patters were remarkably consistent at 15°C (left column), 20°C (center column), and 25°C (right column). **(A–C)** Integrated density of alternative hosts (blue; alone [diamonds] or in compeition [circles]) was strongly reduced by presence of focal hosts at all temperatures. In contrast, integrated density of focal hosts (green; alone [squares] or in compeition [circles]) was qualitatively but not significantly elevated by initial presence of alternative hosts. **(D–F)** Very similarly patterns in density had already emerged by day 21, when parasites were added. **(G–I)** Although densities of focal hosts were similar, stage structure of focal hosts was significantly affected by alternative hosts. Specifically, initial presence of alternative hosts resulted in focal host populations that were significantly more adult-biased on the day of parasite addition, at all 3 temperatures. **(J–L)** Finally, these changes in stage structure corresponded with changes in disease. Specifically, at all 3 temperatures, cumulative infection prevalence in focal hosts was significantly higher with the initial presence of alternative hosts (and stronger stage structure bias toward adults on day 21). Cumulative infection prevalence in alternative hosts was universally low. Error bars are standard errors of means among replicate mesocosms. Asterisks indicate significance of GLMs: $^{+}P < 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

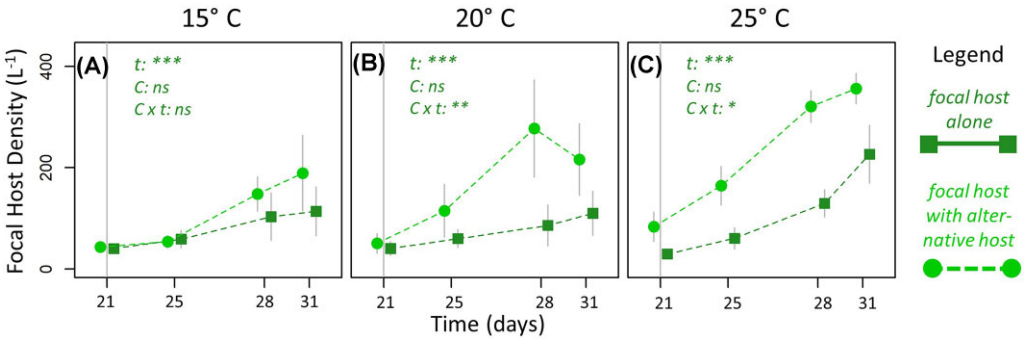

Fig. 5 *Potential differences in focal host mortality in the 10 days after exposure to parasites.* In addition to altering focal host stage structure on the day of parasite addition (Fig. 4), initial presence of alternative hosts (light green circles versus dark green squares) also accelerated population growth of focal hosts over the subsequent 10 days. Accelerated population growth indicates either reduced background death rates, elevated birth rates, or likely both. Background death rates are potentially important, because if exposed hosts die before 10 days, then the parasites they consumed cannot be transmitted. Population growth rate of focal hosts was **(A)** qualitatively elevated at 15°C, **(B)** significantly elevated at 20°C, and **(C)** significantly elevated at 25°C by initial presence of alternative hosts (dashed versus solid lines). Error bars are standard errors of means among replicate mesocosms. Asterisks indicate significance of LMMs: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Data are re-centered so that community effects (C) indicate differences on day 21 and community by time effects ($C \times t$) indicate population growth over the subsequent 10 days.

ing greater susceptibility than adults (Ashby and Bruns 2018). Thus, the first requirement is generally met. The second question remains: How commonly do interspecific interactions alter host stage structure? Importantly, if such effects are general, they could either amplify—as occurred here—or dilute disease, depending on which demographic stage is favored in diverse communities, and which stage is more important for disease. The second mechanism (altered mortality of exposed hosts) requires (3) a lag between exposure and infectiousness (i.e., latency), and (4) altered risk of mortality in more diverse communities. This first requirement is generally met: It is represented by the "E" (i.e., Exposed) class in general SEIR models (Keeling et al. 2007) and known as the "extrinsic incubation period" for vectors (Childs and Prosper 2020). Less clear is how frequently interspecific interactions alter mortality rates of exposed hosts. Note that this mortality mechanism differs from mechanisms about host density [host regulation or augmentation (Strauss et al. 2024)], because equally dense populations can arise from relatively fast or slow demographic rates. Future research is needed to explore how frequently the second criteria of both mechanisms is met in other study systems, and therefore how frequently dilution or amplification effects are likely to arise from altered stage structure or host mortality.

Perhaps our most intriguing result is that initial inclusion of *Ceriodaphnia* alternative hosts elevated infection prevalence in *Daphnia* focal hosts later in the experiment, when these alternative hosts were previously shown to reduce disease (Strauss et al. 2015; Strauss et al. 2016; Strauss et al. 2018). In this system, dilution occurs via encounter reduction (*Ceriodaphnia* consume spores while rarely getting infected) and host regulation (*Ceriodaphnia* lower *Daphnia* density through resource competition). Interspecific competitive ability varies among host genotypes, and dilution effects are stronger when alternative hosts reach higher relative abundance (Strauss et al. 2018). For this experiment, it appears that we unintentionally selected a genotype of alternative host that was a universally weak competitor. Given how rare they became, it is unsurprising that alternative hosts did not reduce disease via encounter reduction or host regulation. A silver lining of their competitive exclusion is that in the absence of strong dilution mechanisms, we were able to observe the amplification effects that likely arose from altered host mortality and stage structure. Similar results of disease amplification emerged from another zooplankton experiment with a different species of alternative host (Dallas et al. 2016). These outcomes are a reminder that multiple dilution and amplification mechanisms can operate simultaneously, with overall biodiversity–disease relationships reflecting their net effect. Simultaneous dilution and amplification mechanisms were detected in communities of rodents that transmit hantavirus (Luis et al. 2018) and communities amphibians and trematodes (Johnson et al. 2024), and the same species that diluted disease in one year of a plant biodiversity experiment amplified disease 20 years later (Strauss et al. 2024). The biodiversity–disease literature would benefit from other studies that partition multiple dilution and amplification mechanisms that may frequently overshadow or counteract one another.

Our study has several limitations which suggest intriguing directions for future research. The most con-

spicuous weakness of our study is that our proposed amplification mechanisms are based on correlations. Initial presence of alternative hosts did alter host stage structure and did amplify disease, but we cannot test causality of amplification mechanisms with this experimental design. Other experiments could test these mechanisms directly. For example, a future mesocosm experiment could track stage structure cycles in focal hosts, manipulate whether exposure to parasites occurs when populations are adult-biased or juvenile biased, and follow disease dynamics for several additional generations. A similar experiment could evaluate the importance of mortality rates by adding parasites when host populations were increasing versus decreasing. A second limitation of our study is that we could not estimate death rates directly. However, future experiments could record the number of eggs per adult female and calculate death rates as in Duffy and Hall (2008). Finally, it is possible that the observed amplification effects arose for reasons other than altered stage structure or mortality. Two testable alternatives are that focal hosts elevated their feeding rate (and hence exposure) in the presence of alternative hosts, or that they *reduced* their feeding rate, which could have in turn reduced their resource acquisition and immune function. Either change could have conceivably promoted disease. Presence of conspecific and heterospecific zooplankton tends to reduce per-capita feeding rates in *Daphnia* (Hargrave et al. 2011; Civitello et al. 2013), so the latter seems more likely of these two alternative explanations. Future experiments could evaluate these possibilities by measuring effects of *Ceriodaphnia* and *Ceriodaphnia*-conditioned water on *Daphnia* foraging rates, *Daphnia* immune responses, and their probability of infection per spore consumed.

We initially designed an experiment to assess how temperature altered the effects of alternative hosts on focal host disease dynamics. Effects of alternative hosts were qualitatively similar at all 3 temperatures. Surprisingly, they were consistently excluded, and yet their initial presence consistently elevated disease. Although we designed an experiment about the effects of species addition on disease, the experiment paradoxically became one about species loss. Loss of species from natural communities often causes increases in disease severity (Halliday et al. 2020), implying that the loss of diluter taxa intensifies transmission among the remaining hosts. Our data suggests a subtly different intriguing alternative: losses of alternative species can be associated with changes in focal host demography, and these changes in focal host demography can in turn alter disease dynamics. The legacy amplification effects that we detected in this zooplankton study system likely arose from some combination of altered host mortality and stage structure in communities that initially contained alternative hosts. This research introduces these 2 potentially general mechanisms of dilution or amplification and highlights the importance of host demography for relationships between diversity and disease.

Author contributions

D.C.S. and A.T.S. designed the experiment. D.C.S., K.S., E.F.L., and J.T. conducted the experiment. A.T.S. led analyses and wrote the first draft of the manuscript, and all authors contributed to revisions.

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Supplementary data

Supplementary data available at *ICB* online.

Supplementary analyses and figures are presented in the Appendix entitled, "Legacy Amplification Appendix."

Data availability

All data and code will be made available upon acceptance for publication.

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