Partitioning the net effect of host diversity on an emerging amphibian pathogen

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The ‘dilution effect’ (DE) hypothesis predicts that diverse host communities will show reduced disease. The underlying causes of pathogen dilution are complex, because they involve non-additive (driven by host interactions and differential habitat use) and additive (controlled by host species composition) mechanisms. Here, we used measures of complementarity and selection traditionally employed in the field of biodiversity–ecosystem function (BEF) to quantify the net effect of host diversity on disease dynamics of the amphibian-killing fungus Batrachochytrium dendrobatidis (Bd). Complementarity occurs when average infection load in diverse host assemblages departs from that of each component species in uniform populations. Selection measures the disproportionate impact of a particular species in diverse assemblages compared with its performance in uniform populations, and therefore has strong additive and non-additive properties. We experimentally infected tropical amphibian species of varying life histories, in single- and multi-host treatments, and measured individual Bd infection loads. Host diversity reduced Bd infection in amphibians through a mechanism analogous to complementarity (sensu BEF), potentially by reducing shared habitat use and transmission among hosts. Additionally, the selection component indicated that one particular terrestrial species showed reduced infection loads in diverse assemblages at the expense of neighbouring aquatic hosts becoming heavily infected. By partitioning components of diversity, our findings underscore the importance of additive and non-additive mechanisms underlying the DE.

1. Introduction

Biodiversity loss is happening at increasingly rapid rates and changing the distribution of organisms around the globe [1]. Biodiversity declines alter several features of communities, including the number of species (species richness), identity of species (species composition), their relative abundances (species evenness) and species interactions [2]. These features, in concert, drive key mechanisms responsible for ecosystem functioning, such as primary production, competition, predation and disease dynamics [3–7].

A number of recent studies have shown that declines in biodiversity can lead to increases in disease risk [7–10], but the underlying mechanisms leading to this pattern, and their generality, are complex and controversial [11–13]. This phenomenon of high host diversity reducing disease, termed the dilution effect (DE), can arise through several potential mechanisms by which diversity affects transmission, including encounter reduction, susceptible host regulation, infected host mortality and recovery augmentation (reviewed in [9]). These mechanisms have in common that both host interactions and the identity of the species...
influence the likelihood of transmission and disease. Encounter reduction—one clear mechanism that lowers transmission rates and thus leads to DE—operates in communities in a couple of ways. First, the proportion of susceptible and immune hosts may change with shifts in diversity, leading to reduced encounter rates between infected and uninfected hosts [9]. Second, diverse host communities may partition niche space more finely due to local adaptation, specialization and competition [14], and may in turn experience reduced encounter rates and transmission among conspecifics, and reduced pathogen spillover across host species [5]. Identifying the drivers of transmission most affected by biodiversity loss and their relative contribution to wildlife diseases has been challenging, despite their importance for effective wildlife management and disease forecasting. Without a clear understanding of these mechanisms, we will not be able to predict general patterns of disease dynamics in nature.

The mechanisms behind diversity–disease relationships are in many ways parallel to other important processes driving ecosystem functioning or performance [15–17]. Studies in the field of biodiversity–ecosystem function (BEF) demonstrate that a decrease in species diversity can reduce primary productivity [16–18] and increase herbivory [19] through a variety of additive and non-additive mechanisms. Within the BEF literature, additive mechanisms are those entirely driven by host composition, such that the ecological response of any species in a diverse assemblage can be predicted by its response in monoculture and its relative abundance in the mixed community [20]. Additive mechanisms also apply to diversity–disease relationships [5], and as defined, a necessary condition is that host species will respond identically to disease in single-host and mixed assemblages. The sampling effect is a common additive mechanism that applies to both BEF and DE [6,16]. It states that highly diverse assemblages have a higher probability of including at least one species with extreme ecological characteristics that can substantially affect ecological responses such as primary productivity [15,21] and/or pathogen transmission.

While additive mechanisms almost certainly play a role in disease dynamics, many important mechanisms for BEF and diversity–disease relationships are non-additive. Non-additive mechanisms occur when the ecological response of a given species in mixed assemblages cannot be predicted by how it responds in uniform populations. A non-additive mechanism commonly identified in the field of BEF arises due to interspecific differences in resource utilization with downstream effects on primary productivity [16]. Non-additive mechanisms also apply to diversity–disease relationships [5], and can result from host interactions and differential habitat use to alter disease response of host species in diverse assemblages. Two non-additive ecological mechanisms that have been widely studied in BEF, facilitation/inhibition and niche partitioning, are jointly referred to as ‘complementarity’ [17]. In facilitation/inhibition, heterospecific neighbours control damage to a particular plant species by attracting or repelling herbivores (e.g. associational susceptibility/resistance) [22]. In niche partitioning, species have complementary habitat use or resource utilization, and thus diversity often has a positive influence on primary productivity [3]. Although complementarity clearly applies to BEF studies on primary productivity, its potential role in DE is not intuitive, because host species do not complement each other in order to obtain higher or lower infection loads. However, parallel processes do exist. For example, differential habitat use among host species can affect disease risk if species diversity causes a reduction in niche overlap, and thereby decreases host contact rates and transmission.

In addition to complementarity, selection is a second component of diversity that can potentially affect diversity–disease relationships [5]. Selection measures the disproportionate impact of one particular species (or guild) in diverse assemblages compared with its performance in uniform populations [21], and therefore it can be driven by both additive and non-additive mechanisms [15,16]. Selection can occur due to one species’s ability to become disproportionately less infected (thus becoming locally dominant over multiple generations) at the expense of neighbouring species becoming heavily infected (and becoming locally extinct) [16]. Therefore, selection does not depend just on species frequencies (the so-called sampling effect), but also on a variety of host species interactions (non-additive mechanisms) [16].

Here, we used an amphibian host–pathogen system to identify mechanisms underlying diversity–disease relationships. We partitioned the net effect of host diversity on pathogen infection loads and measured the relative contribution of ‘complementarity’ and ‘selection’ to disease risk. We experimentally exposed tropical amphibians to a panzootic strain of the chytrid fungus Batrachochytrium dendrobatidis (Bd) in single- and multi-host treatments. This epidermal pathogen has a broad host range among amphibians [23], and is implicated in population declines and species extinctions worldwide [24–27]. We used seven wild-collected tropical amphibian species that fall along a continuum of breeding mode and habitat use, ranging from fully terrestrial to mostly aquatic. Our specific goals were to (i) test whether the mechanisms of complementarity and selection, or a combination of both, drive diversity–disease relationship in our study system, and (ii) identify the contribution of species composition to pathogen dynamics. Our work demonstrates the application of principles of BEF to disease ecology. This perspective offers an accurate and fine-scale measurement of the effects of biodiversity on disease and enhances our mechanistic understanding of diversity–disease outcomes. Both of these goals are increasingly critical with the rapid anthropogenic acceleration of biodiversity loss.

2. Material and methods

(a) Host species

We captured adult anurans of seven locally abundant species in October 2012 from Parque Estadual da Serra do Mar—Núcleo Santa Virginia in the Brazilian Atlantic Forest (−23.35°S, −45.16°W). We assigned a host aquatic index (AI) to each species (adapted from [28]), which quantifies the amount of time spent in aquatic environments summed across different amphibian life stages. Because Bd is a water-borne fungal pathogen, AI also serves as a relative measure of species-specific exposure and transmission probability in natural communities [29,30]. Our seven focal host species ranged from exclusively terrestrial species (i.e. direct developers) occupying forest leaf-litter (AI = 0) Brachycephalus pitangui (PTT) and Ischnocnema parva (PAR); Brachycephalidae), to species breeding in aquatic habitats but occupying the arboreal stratum ((AI = 1) Dendropsophus minutus (MIN), Scinax hayii (HAY) and Hypsiboas biperdantes (BAN)), to species breeding in aquatic habitats and occupying the margins of streams and other bodies of water ((AI = 2) Physalaemus cuvieri (CUV), Leptodactylidae; Hylidae).
Bd infection loads were reduced by 66.5% in multi-host compared with single-host treatments (multi-host: least-square mean, LSM = 61.892 zoospore GE; 1.171 logGE; single-host: 201.208 GE). Nonetheless, because mortality affects total host densities, which was otherwise controlled in our experiment, we repeated the analyses and quantified complementarity, selection and the net host diversity effect while excluding assemblages that experienced mortality.

3. Results

Bd infection loads were reduced by 66.5% in multi-host compared with single-host treatments (multi-host: least-square mean, LSM = 61.892 zoospore GE; 1.171 logGE; single-host: 201.208 GE).
Figure 1. Effects of host diversity on Bd infection loads. (a) Average Bd infection loads in single- and multi-host treatments (mean ± 95% s.e.). (b) Net effect of host diversity partitioned into the two components complementarity and selection.

LSM = 184.612 GE; 1.354 logGE; F = 4.434, p < 0.039; figure 1a). We found that this significant reduction in Bd infection loads in diverse host assemblages was driven by the complementarity component (figure 1b). Our measures of selection, however, showed both positive and negative values (of lower intensity) across our mixed host assemblages (figure 1b). Combined, complementarity and selection resulted in a net effect of host diversity reducing Bd infection loads (mean = −0.695 logGE; −0.040, −1.351 CI). These results remained unaltered after assemblages that experienced mortality were omitted from calculations (electronic supplementary material, table S1).

As expected, host species with a high AI carried higher Bd infection loads than terrestrial hosts independent of diversity treatment (F = 11.288, p = 0.001; figure 2). Furthermore, most host species showed a decrease in Bd infection loads in multi-host assemblages (figure 2). Our test for the effect of host identity on the observed DE showed no association between average host AI and strength of complementarity across diverse host assemblages (F = 0.003, p = 0.954). Conversely, host species composition significantly predicted the direction of selection. Specifically, multi-host assemblages where selection was positive were composed of species with lower host AI when compared with assemblages where selection was negative (F = 8.267, p = 0.008; electronic supplementary material, table S2; figure 3). This pattern was strongly influenced by the terrestrial host Brachycephalus pitanga, which showed a decrease in infection loads as neighbouring aquatic host species experienced pathogen amplification (figure 3).

4. Discussion

Our experiment demonstrated that diversity can reduce Bd infection loads in amphibians through a non-additive mechanism that falls under the umbrella of complementarity (sensu BEF). Specifically, lower Bd transmission among host species due to reduced shared habitat use was the likely mechanism leading to the observed DE at the community level. Furthermore, the highly variable selection component in our experiment indicated that the presence of a particular host species in diverse assemblages can disproportionately increase or decrease infection loads in neighbouring hosts through direct association. Therefore, non-additive and additive mechanisms of diversity were tightly linked as drivers of chytridiomycosis in our experimental system.

Previous foundational work attributes the DE to a combination of additive and non-additive mechanisms [10]. Nevertheless, isolating the effect of biodiversity per se from the impact of host species composition is often a challenging task in natural systems due to the correlative nature of field-collected data. In many cases, anthropogenic habitat change is the proximate force selectively removing host species with high degrees of ecological specialization, and thus habitat generalist hosts often become dominant in depauperate communities [44–47]. In the case of Lyme disease, biodiversity loss promotes dominance of the habitat generalist Peromyscus leucopus, a highly competent host of the pathogen Borrelia burgdorferi [44]. For West Nile virus infections, host diversity and community competence are tightly negatively correlated, such that depauperate host communities are dominated by competent reservoirs [46]. Likewise, biodiversity loss increases transmission of the parasitic trematode Ribeiroia ondatrae because highly competent amphibian hosts dominate species-poor communities [47]. These studies found evidence for non-additive mechanisms (shifts in host species interactions) as well as a strong additive mechanism driving DE (numerical dominance of a competent host species). In our study system with randomly assembled host communities, diversity was negatively associated with disease risk when total host density was controlled for. We expect that the DE we observed experimentally would be even stronger in the wild if biodiversity loss in a real system simultaneously leads to changes in density and in community composition (e.g. by favouring superspreaders or species that induce continuous re-infection in neighbouring host species). Even though our experimental study does not perfectly mimic the natural assembly and disassembly of amphibian communities, it provides a quantitative framework for the relative contribution of two important components of diversity to disease dynamics.

By partitioning the net effect of host diversity, we quantified the relative contribution of complementarity and selection. The main mechanism leading the observed community-level DE falls under the umbrella of complementarity (sensu BEF) [16], where diversity per se led to lower infection loads in diverse assemblages. Niche theory predicts that species in diverse assemblages will compete for resources such as space, and thus benefit from reduced overlap in habitat use [14]. Therefore, lower niche overlap can cause both host encounter reduction and decreased exposure to the aquatic pathogen reservoirs such as Bd (reviewed in [9]), thus having a potential impact on both density- and frequency-dependent Bd transmission [48,49]. A second potential mechanism by which complementarity can lead to DE is inhibition through associational resistance among particular host species [22]. However, our community-level measures of Bd infection loads in diverse assemblages were not strongly driven by a particular combination of species,
as we did not find an association between host composition and the intensity of complementarity.

Even though host species composition did not explain the intensity and direction of complementarity, we found it to be important in explaining selection. Specifically, we found positive values for selection in assemblages containing both terrestrial and aquatic hosts, and negative values in assemblages dominated by aquatic hosts (figure 3). This seems counterintuitive because terrestrial host species carry lower infection loads while in single-host treatments, and thus cannot be directly responsible for positive selection. However, the presence of the terrestrial aposomatic pumpkin toadlet (*Brachycephalus pitanga*) may indirectly cause a disproportionate increase in infection loads of one or few neighbouring aquatic host species (figure 3). *Brachycephalus pitanga* secretes tetrodotoxin (a potent neurotoxin) from its skin, and it is possible that this species deterred more susceptible aquatic hosts from the dry terrestrial habitat, thus disproportionately increasing their exposure to *Bd* in the aquatic environment. Alternatively, pumpkin toadlets may compromise the ability of neighbouring hosts to fight infections through chemical interference [50] in a way similar to allelopathy in plants. According to our results, *B. pitanga* could potentially increase the likelihood of local extinction in aquatic hosts and, over multiple generations, become the dominant host species. We must highlight, however, that this final outcome of selection was not captured by our short-term experiment, as competitive exclusion could not take place during the length of our study. Nevertheless, the multiple aspects of host species interactions highlight the importance of measuring both complementarity and selection, allowing us to propose further hypotheses about potential mechanisms for species- and community-level processes leading to pathogen dilution or amplification.

Our results, combined with previous empirical laboratory studies [51,52], support the DE in amphibian–*Bd* systems. By contrast, our previous field-based empirical studies found strong support for *Bd* amplification [53]. Using field-collected data, we reported a positive relationship between amphibian species richness and *Bd* infection, after accounting for the effects of land cover and climate [53]. Two habitat generalist amphibians from Costa Rica (the rain frog, *Craugastor fitzingeri*) and Australia (the stony creek frog, *Litoria lesueuri*) showed higher *Bd* occurrence, prevalence and infection loads in diverse communities. We hypothesized that natural species-rich communities are more likely to include competent hosts for *Bd* than depauperate ones, increasing pathogen transmission. For instance, natural species-rich communities include a higher proportion of stream-dwelling specialists [54,55] that often carry higher *Bd* infection intensities in the wild [25,29]. Most host species in these diverse natural communities may have had a higher likelihood of suffering *Bd* spillover from the highly infected stream dwellers, such as species of *Atelopus* in Central America [25] and *Triturus viridescens* in Australia [56]. Combined, these findings illustrate that studies investigating diversity–disease relationships will show contrasting results when non-additive and additive effects of diversity are not quantified independently. Because observational field studies cannot fully disentangle the impact of species interactions from additive effects, laboratory-controlled experiments will continue to be important to understand mechanisms of species interactions driving wildlife diseases.

**Figure 2.** Average *Bd* infection load across single- and multi-host treatments (least-square mean ± s.e.). Species abbreviations: *B. pitanga*, PIT; *I. parva*, PAR; *D. minutus*, MIN; *H. bandeirantes*, BAN; *S. hayii*, HAY; *H. phyllodes*, PHY; *P. cuvieri*, CUV. Colours represent host AI ranging from fully terrestrial (AI = 0) to mostly aquatic (AI = 2).

**Figure 3.** Selection component across 25 unique and equally diverse host assemblages. Bars represent average host AI (± 95% s.e.) for assemblages experiencing positive and negative selection (graph jittering added to avoid overlapping data points). Assemblages containing the aposematic pumpkin toadlet (*Brachycephalus pitanga*) are highlighted with an asterisk. Colours represent host AI ranging from fully terrestrial (AI = 0) to mostly aquatic (AI = 2).
Global biodiversity is declining sharply, due in large part to anthropogenic habitat change and emerging diseases [4,57,58]. Therefore, understanding the mechanisms by which biodiversity alters disease dynamics can considerably advance the field of disease ecology and has important implications for conservation of natural populations. Our results indicate that shifts in host interactions and habitat use—both mechanisms of complementarity—can drive DE. In our study system, dilution was probably driven by interactions in diverse assemblages that reduced host contact rates and Bt transmission. Partitioning the net effect of host diversity on disease across several unique communities, rather than relying on the correlational effects of host species richness, evenness and composition, will allow us to identify specific mechanisms of diversity–disease relationships and test for their generality across host communities. Finally, our study shows that the application of methods from BEF can facilitate new avenues in experimental design and data analysis, with important theoretical implications for the field of disease ecology, and practical implications for understanding and predicting wildlife epidemics.

Ethics statement. Research permits were provided by Instituto Chico Mendes da Conservação da Biodiversidade–Brazil (Permits 29964-3, 17242-3), Instituto Florestal do Estado de São Paulo (Permit 260108-010479/2012), Universidade Estadual Paulista (UNESP) Comissão de Ética no Uso de Animal (Permit 7180), US Fish & Wildlife Services (Permit 2013MI133729) and the Cornell University Institutional Animal Care and Use Committee (Protocol 2010-0069).


Acknowledgments. We thank A. Agrawal, S. McArd, S. Cook-Patton and B. Dalziel for feedback on experimental design and analyses; A. Agrawal, M. F. K. Becker, S. Claffin, S. McArd, S. Cook-Patton, two anonymous reviewers, and Zamudio and Leibold laboratory members for feedback on the manuscript; and R. Martins, M. Aguiar Passos, A. B. C. Lima, J. Ruggeri, D. Genari and T. A. Pires for field assistance.

Funding statement. Our work was funded by grants from the National Science Foundation (DEB-1209382 to C.G.B.; DEB-0542848 to K.R.Z.), Atkinson Center for a Sustainable Future (to C.G.B.), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Fulbright (grant 2157-08 to C.G.B.), Department of Ecology and Evolutionary Biology at Cornell University (to C.G.B.), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2011/51694-7 to L.F.T., 2012/ 01460-0 to C.L., 2008/50928-1 to C.F.B.H.) and Conselho Nacional de Desenvolvimento Científico e Tecnológico–CNPq (BTS 312695/2014-3 to C.G.B., L.F.T. and C.F.B.H.).

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