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RESEARCH ARTICLE

Identity and density of parasite exposures alter the outcome of coinfections: Implications for management

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Abstract

1. Although research has focused on density-dependent responses of hosts to single-parasite infections, hosts are exposed to numerous parasites simultaneously under natural conditions and if these exposures lead to infections, they can threaten host populations and ecosystem stability. Moreover, spatiotemporal variation in abundance of co-occurring parasites might influence host infection intensity. If interactions are consistent between different coinfecting parasites, then these patterns could give managers another tool to control disease spread and even predict problematic disease emergences.
2. We investigated how parasite density and identity alter within-host coinfection dynamics. To test this, we simultaneously exposed Cuban treefrogs *Osteopilus septentrionalis* as a model amphibian species to all pairwise combinations of three problematic parasites that commonly coinfect amphibians: the fungus *Batrachochytrium dendrobatidis* (Bd), the nematode *Aplectana hamatospicula* and Ranavirus. Hosts were exposed to one parasite at a fixed dose and another parasite at a range of five doses.
3. Higher doses of Bd decreased Ranaviral and *A. hamatospicula* loads, but Bd load was not influenced by the dose of either parasite. Ranaviral load was negatively associated with *A. hamatospicula* dose, but *A. hamatospicula* load was not affected by Ranaviral dose. We found that all the pairwise coinfections were dependent on parasite density and that pairwise interactions were highly asymmetric—strong in one direction and weak in the other—consistent with interactions dominating food webs.
4. *Synthesis and applications*: We also revealed that the exposure dose of *A. hamatospicula* was positively associated with host tolerance to Bd infection and negatively associated with Ranaviral load in hosts. Ranavirus and Bd cause mass die-offs in amphibians, but *A. hamatospicula* does not. Therefore, in systems where these parasites coexist, maintaining or increasing densities of *A. hamatospicula* could reduce the negative effects of Bd and Ranavirus infections. Additionally, if these asymmetric and density-dependent patterns from community ecology are applicable to other amphibian coinfections or coinfections in other systems, this should allow conservation organizations and resource

managers to predict outbreaks and manage host declines associated with deadly parasites by modifying the abundance of coinfecting parasites that might be easier to manage.

KEYWORDS
amphibian, *Batrachochytrium dendrobatidis*, coinfection, conservation, density dependence, dose, nematode, Ranavirus

1 | INTRODUCTION

Coinfections, where hosts are infected with more than one parasite (any organism that lives on or in another organism and benefits at its expense) at a time, are extremely common (Greub et al., 2000; Kim et al., 2003; Pedersen & Fenton, 2007). Additionally, variation in disease progression that can arise from coinfections can significantly affect host health by altering resistance (i.e. ability to reduce the abundance of parasites) or tolerance (i.e. ability to reduce the per capita cost of parasites to maintain health) to parasites. For example, HIV infection reduced resistance to and increased mortality by a *Mycobacterium* that causes tuberculosis in humans (Corbett et al., 2003). Coinfection with helminths improved host resistance and reduced the spread of bovine tuberculosis in African buffalo populations (Ezenwa & Jolles, 2015).

Coinfecting parasites can interact directly or indirectly within hosts (de Roode et al., 2004; Kuris & Lafferty, 1994). Direct competition can occur when coinfecting parasites infect the same host tissue or use the same resources (Bell et al., 2006). Indirect interactions between parasites can occur through the host immune system. Coinfections with macroparasites (multicellular parasites who typically do not replicate within a host) and microparasites (parasites that replicate within their host) can facilitate one another as they activate different arms of the acquired immune system that are challenging to upregulate simultaneously (Berger, 2000; Morel & Oriss, 1998). Conversely, coinfections with two microparasites or two macroparasites could be inhibitive if they upregulate the same arm of the acquired immune system (Johnson et al., 2015; Pedersen & Fenton, 2007). Additionally, hosts use different aspects of innate immunity to combat specific parasites, which can vary in their effectiveness to combat a coinfecting parasite (Fites, 2014; Motran et al., 2018). Infected hosts can exhibit behavioural changes such as increased or reduced food intake to lessen the negative effects of some parasites (Hess et al., 2015; Knutie, Wilkinson, Wu, et al., 2017). Finally, a healthy and more complex host microbiome can increase host resistance to parasites (Becker et al., 2015; Knutie, Wilkinson, Kohl, et al., 2017). These factors and others can cause indirect interactions between coinfecting parasites.

As coinfecting parasites interact with one another and the environment of the host, competition principles from community ecology can inform our understanding of coinfections and how they affect host health (Johnson et al., 2015; Pedersen & Fenton, 2007). In community ecology, competition between species may depend on the density of each species, with higher densities creating stronger negative effects on other competing species (Maron et al., 2016; Tarjuelo et al., 2017; Watts & Holekamp, 2008; Young, 2004). Additionally, competitive interactions often affect community stability (Chesson & Kuang, 2008; Mayfield & Levine, 2010; Terborgh, 2012). Although there are many studies on the effects of parasite density or exposure dose on single-species infections (Echaubard et al., 2010; Garner et al., 2009; Khuroo, 1996; Pearman et al., 2004), only two few studies, both conducted on chickens either in eggs or embryo cells in vitro, have examined how parasite density or exposure dose alters coinfections and associated disease progression (Ge et al., 2012; Niczyporuk et al., 2014). Both studies focused on closely related parasite taxa (two viruses in both cases) which are more likely to lead to direct effects or cross-immunity than more distantly related parasites. Nevertheless, these studies generated inconsistent patterns. In one of the studies, a tested virus was used as a prophylactic treatment for a more deadly virus and researchers found no effect of dose (Niczyporuk et al., 2014). The other study found that dose of one parasite affected the dose of another, but the reverse was not true (Ge et al., 2012). Furthermore, to our knowledge no studies have explicitly tested how parasite densities affect within-host competition.

Hosts are often concomitantly exposed to multiple parasites in nature, and due to the spatiotemporal variation in abundance of parasites that a host might encounter, coinfection with multiple parasites at different densities is more likely to occur in the wild than coinfections with identical parasites densities. Our experiment aims to test if competition principles from community ecology can be applied to determine how coinfection with varying doses alters within-host competition and disease in amphibians. To address this aim, we exposed Cuban treefrogs (*Osteopilus septentrionalis)* to pairwise coinfections of the following parasite species: *Batrachochytrium dendrobatidis* (Bd), Ranavirus and *Aplectana hamatospicula*. Importantly, we tested how the exposure dose of each parasite species affected disease progression of coinfections. We use the Cuban treefrog and these parasites as a model system to address these aims, with the goal that patterns seen here could be applicable to other vertebrate species (Du Pasquier et al., 1989).

Amphibians are the most highly threatened vertebrate taxa partly due to the spread of parasites (Wake & Vredenburg, 2008). Bd is a chytrid fungus that is associated with extinctions and population declines of amphibians globally (Berger et al., 1998; Kilpatrick et al., 2010). The infectious zoospores of this parasite enter the skin

of amphibians and limit osmoregulation through the skin, which can cause cardiac arrest (Kilpatrick et al., 2010; Voyles et al., 2009). Ranavirus is a group of viruses that causes amphibian mass mortality globally (Gray et al., 2009; Green et al., 2002). Ranavirus replicates in the internal organs, particularly in the liver and kidneys, causing haemorrhaging (Gantress et al., 2003; Gray et al., 2009). We also tested a macroparasite, the nematode *A. hamatospicula*, which is a common parasite of amphibians in the Southeastern US and Latin America (Ortega et al., 2015; Vhora & Bolek, 2013). *A. hamatospicula* infects the host when juvenile worms penetrate the host skin and migrate to the amphibian gastrointestinal tract where they mature into adults (Knutie, Wilkinson, Wu, et al., 2017). To our knowledge, there are no published accounts of coinfections between *A. hamatospicula* and Bd or Ranavirus. However, there are no field surveys exploring coinfection between *A. hamatospicula* and Bd or Ranavirus. Nevertheless, other parasitic worms, Ranavirus and Bd frequently co-occur in amphibian hosts in the wild (Stutz et al., 2018; Watters et al., 2018). If these coinfecting parasites interact synergistically or additively, wildlife managers might need to change their strategies and limit transmission of the parasite that is easiest to manage. Parasites are often easier to manage if they have longer replication times or lower transmission rates, which limits disease spread between individuals and populations. If transmission rates are already low, management techniques which reduce transmission may be able to completely stop disease spread. If coinfecting parasites interact antagonistically, then managers might be able to promote (increasing habitat or food) the species that is least problematic.

The goals for this study were to test if the exposure dose of parasite alters (1) the load of the coinfecting parasite (i.e. resistance), (2) survival or growth rate of the host and (3) the host's ability to tolerate infections. We hypothesized that the load of an infecting parasite would be affected by the exposure dose of the coinfecting parasite, with higher doses having stronger effects and lower doses having weaker effects consistent with competition principles from community ecology. We also hypothesized that the competitive advantage a parasite has when it is at a high density (i.e. high exposure dose) could be amplified if the coinfecting parasites inhibit one another. Inhibition could occur if coinfecting parasites activate similar acquired immune responses (i.e. are both microparasites) or through direct competition. The competitive advantage a parasite has at high density could be lessened if one parasite is immunosuppressive (e.g. Bd), if hosts compensate in some way for infection (e.g. increasing their nutrient intake), and if hosts combat one infection with mainly local innate immune responses (e.g. antimicrobial peptides combatting Bd). Coinfections could be facilitative if the coinfecting parasites activate a different acquired immune response (i.e. a macro- and a microparasite). We hypothesize that metrics of host health, such as survival and growth, will be reduced when infected with more problematic parasites, such as Bd and Ranavirus, compared to controls or *A. hamatospicula* infected hosts. If coinfections inhibit or facilitate one another, we expect host health to be reduced or increased respectively, compared to single infections. Finally, we hypothesize that coinfection will decrease a host's tolerance (e.g. a host's ability to maintain their health despite high infection loads) when the coinfecting species are facilitative and increase host tolerance when they inhibit one another. Understanding when coinfecting parasites interact synergistically or antagonistically, should allow managers to better understand their management options and thus better control problematic diseases.

2 | MATERIALS AND METHODS

2.1 | Animal husbandry and parasite culture

Cuban treefrog tadpoles *Osteopilus septentrionalis* were collected from kiddy pools filled with water (140 L) in the University of South Florida Botanical Gardens (Tampa, FL, USA) in August 2016. Amphibians were collected under permit LSSC-15-00014 issued by the Florida Fish and Wildlife Conservation Commission. All animal husbandry throughout the experiment was carried out according to IUCAC protocol #W IS00002203. *A. hamatospicula* were collected from the gastrointestinal tract of euthanized Cuban treefrogs from Flatwoods Wilderness Park (Tampa, FL, USA). Identical Petri dishes, but with gastrointestinal content from uninfected frogs were used as a sham treatment. Ranavirus (FV3) was cultured in fathead minnow *Pimephales promelas* cells and maintained at −80°C in minimal essential medium (MEM). MEM without Ranavirus was used as a sham. Bd (SRS-JEL 212 strain) was grown in a 1% tryptone solution. SRS-JEL212 was chosen as it was isolated from the Southwestern US, where the experimental frogs were collected. Additionally, this strain successfully infects Cuban treefrogs. Identical plates, but with a sterile 1% tryptone solution were used as a sham. See supplemental methods for more details.

2.2 | Experimental design

To examine how the dose of parasite exposures affects host–parasite dynamics, we exposed a total of 174 frogs to one of 28 total parasite treatments. Treatments included exposure to Ranavirus, Bd, or *A. hamatospicula* alone, simultaneous infections with all pairwise combinations (six total pairwise combinations) of these parasites at a range of densities (all $n = 6$), and controls (no exposure; $n = 12$). In each of the pairwise coinfection treatments hosts were exposed to one parasite at an intermediate density and the second parasite at one of a range of densities (one of four potential densities) in all possible combinations (Table 1).

2.3 | Parasite exposures

Two days before parasite exposure, individuals were moved to a 17°C environmental chamber because Cuban treefrogs can clear Bd at higher temperatures (Cohen et al., 2017; McMahon et al., 2014). In the coinfection treatments, the host was exposed to one parasite

TABLE 1 Hosts were coexposed to one parasite at a fixed density and a second parasite over a range of densities shown below

	Fixed dose	Changing dose	*n*
Controls	None	None	12
Single Infections	Bd 10^4	None	6
	Rv 10^4	None	6
	Ah - 30	None	6
Coinfections	Bd 10^4	Rv 10^3	6
	Bd 10^4	Rv 10^4	6
	Bd 10^4	Rv 4.5×10^4	6
	Bd 10^4	Rv 10^5	6
	Bd 10^4	Ah 15	6
	Bd 10^4	Ah 30	6
	Bd 10^4	Ah 45	6
	Bd 10^4	Ah 60	6
	Rv 10^4	Bd 10^3	6
	Rv 10^4	Bd - 10^4	6
	Rv 10^4	Bd 4.5×10^4	6
	Rv 10^4	Bd 10^5	6
	Rv 10^4	Ah 15	6
	Rv 10^4	Ah 30	6
	Rv 10^4	Ah 45	6
	Rv 10^4	Ah 60	6
	Ah 30	Rv 10^3	6
	Ah 30	Rv 10^4	6
	Ah 30	Rv 4.5×10^4	6
	Ah 30	Rv 10^5	6
	Ah 30	Bd 10^3	6
	Ah 30	Bd 10^4	6
	Ah 30	Bd 4.5×10^4	6
	Ah 30	Bd 10^5	6

Notes: Ranavirus (Rv) doses are in PFUs. *Batrachochytrium dendrobatidis* doses (Bd) are in zoospores. And *Aplectana hamatospicula* doses (Ah) are counts of J3 larvae. The numbers in the '*n*' column indicates the number of hosts tested in each treatment group.

at an intermediate, fixed dose and simultaneously to the other parasite at a dose that ranged from low to high for each of the tested parasites (Echaubard et al., 2010; Garner et al., 2009; Gervasi et al., 2013; Hoverman et al., 2010; Knutie, Shea, Kupselaitis, et al., 2017; Knutie, Wilkinson, Wu, et al., 2017). For the microparasites Bd and Ranavirus, doses ranged from 10^3 to 10^5 zoospores or plaque forming units (PFU), respectively, and the intermediate dose was 10^4. For the macroparasite *A. hamatospicula*, doses ranged from 15 to 60 J3 larvae and the intermediate dose was 30 (Table 1). Doses were applied to frogs held in Petri dishes (25 × 100 mm) for 24 h. For Bd and *A. hamatospicula* exposures, 1 ml of DI water containing Bd zoospores or *A. hamatospicula* larvae was pipetted directly onto the backs of the hosts. This simulates exposure through water or soil contact (Kilpatrick et al., 2010; Roznik et al., 2021). For Ranavirus exposures, a 69 µl aliquot of MEM with Ranavirus was applied directly into the host's mouth to mimic faecal-oral or cannibalistic transmission that is common in natural settings (Hoverman et al., 2010). All hosts also received sham exposures for parasites to which they were not exposed. For example, hosts coexposed to Bd and *A. hamatospicula* also received the Ranavirus sham treatment. Control individuals received sham treatments for all three parasites.

2.4 | Assessing parasite load and host health

To assess loads of Ranavirus, hosts were swabbed five times around the mouth and cloaca on day 4 after exposure. To assess Bd load, hosts were swabbed five times from hip to toe on both rear legs on day 16 after exposure. These time points were chosen to measure load differences as they represent times where each parasite would have had time to establish, but not create high levels of mortality (Gray et al., 2009; Knutie, Wilkinson, Wu, et al., 2017; Voyles et al., 2009). Swabs were stored at −80°C for later processing. DNA from each swab was extracted using a Qiagen DNEasy Blood & Tissue Kit and analysed using quantitative polymerase chain reaction (qPCR; Boyle et al., 2004, Picco et al., 2007). *Aplectana hamatospicula* loads were assessed by counting adult worms in amphibian gastrointestinal tracts when they experienced mortality or at the end of the experiment.

To measure growth, frogs were weighed weekly for 4 weeks. Individuals were also checked twice daily for mortality. If mortality occurred, frogs were weighed and swabbed and/or dissected, depending on their treatment group. All surviving frogs were euthanized and dissected 28 days after initial parasite exposure.

2.5 | Statistical analyses

All analyses were run with R version 3.6.1 (R Core Team, 2019). Plots were created using the VISREG package and *visreg* function (Breheny & Burchett, 2019). The survival plot was created using the SURVMINER package and *ggsurvplot* function (Kassambara et al., 2019).

To test how the interaction between the identity of a coinfecting parasite and its dose altered the load of Ranavirus or Bd, we conducted a generalized linear model with a negative binomial error distribution. For the *A. hamatospicula* load model, we used a binomial error distribution, and the dependent variable was defined as the proportion of larvae that successfully reached maturity (i.e. using the *cbind* function on 'successes' and 'failures'). The independent variables were dose, identity of the changing-dose parasite, and their interaction. Dose was expressed as a log 10-transformed proportion of the highest possible dose hosts were exposed to for each parasite. This allowed us to make comparisons across doses even though the exposure dose varied widely for macro- and microparasites.

To test how the interaction between the identity of a coinfecting parasite and its dose affect host weight, a generalized linear model was run with growth rate as the dependent variable.

Growth rates were calculated as the final mass minus the initial mass divided by weeks spent alive (g/wk). To address changes in host survival, we conducted a survival analysis using the SURVIVAL package and the *coxph* function (Therneau & Lumley, 2019), with host survival as the dependent variable. The identity of the fixed-dose parasite, the identity of the changing-dose parasite and the dose of the changing-dose parasite were used as interacting independent variables in both host health analyses. To test how these same factors affected tolerance (measured as host growth rate or survival given a parasite burden), the above-described models were rerun, but the interacting predictor variables were the identity of the changing-dose parasite, exposure dose and parasite load of the fixed-dose parasite. For all analyses Tukey post-hoc tests were run to compare among parasite identities when the variables were significant (MULTCOMP package and *glht* function; Hothorn, 2010). Table S1 outlines all above-described analyses and error distributions.

3 | RESULTS

Analyses revealed a significant negative association between the dose of the coinfecting parasite and the load of Ranavirus for coinfections with both Bd and *A. hamatospicula* ($p = 0.04$; Figure 1a). For *A. hamatospicula* load, there was an interaction between the identity and the dose of the changing-dose parasite ($p < 0.001$). Dose of Bd was associated negatively with *A. hamatospicula* ($p < 0.001$), whereas dose of Ranavirus did not affect *A. hamatospicula* load ($p = 0.22$; Figure 1b). Neither the identity of the coinfecting parasite ($p = 0.86$) nor its dose ($p = 0.92$) affected Bd load in coinfected hosts. See supplemental tables (Tables S2–S4) for complete statistical output from all models.

For both the fixed- and changing-dose parasites, growth was significantly higher for control individuals than for hosts infected with *A. hamatospicula* (fixed dose: $p = 0.02$, changing dose: $p = 0.01$), Bd (both: $p < 0.001$) and Ranavirus (fixed dose: $p = 0.01$, changing dose: $p < 0.01$; Figure 2). Additionally, exposure to Bd at a fixed dose caused a significant decrease in host growth rate (g/week) when compared to exposure to *A. hamatospicula* ($p < 0.001$) or Ranavirus ($p < 0.001$). Dose of coinfecting parasite did not significantly predict weight gain across all tested parasites ($p = 0.19$), but dose of Bd was significantly, negatively associated with host growth ($p < 0.005$). The identity of the fixed-dose parasite was the only significant predictor of host survival ($p < 0.001$), with exposure to Bd significantly reducing host survival relative to Ranavirus ($p < 0.001$), *A. hamatospicula* ($p < 0.001$) and control individuals ($p < 0.001$; Figure 3).

We found no significant effects of dose ($p = 0.9$) or identity of the coinfecting parasite ($p = 0.17$) on host tolerance to Ranavirus. We also found no effects of dose ($p = 0.49$) or the identity of the coinfecting parasite ($p = 0.88$) on host tolerance to *A. hamatospicula*. Host tolerance (defined as less negative slopes between parasite load and weight gain) to Bd was positively associated with the dose of *A. hamatospicula*, but negatively associated with the dose of Ranavirus (Figure 4). We found no significant effects of host tolerance when using host survival as a metric for tolerance.

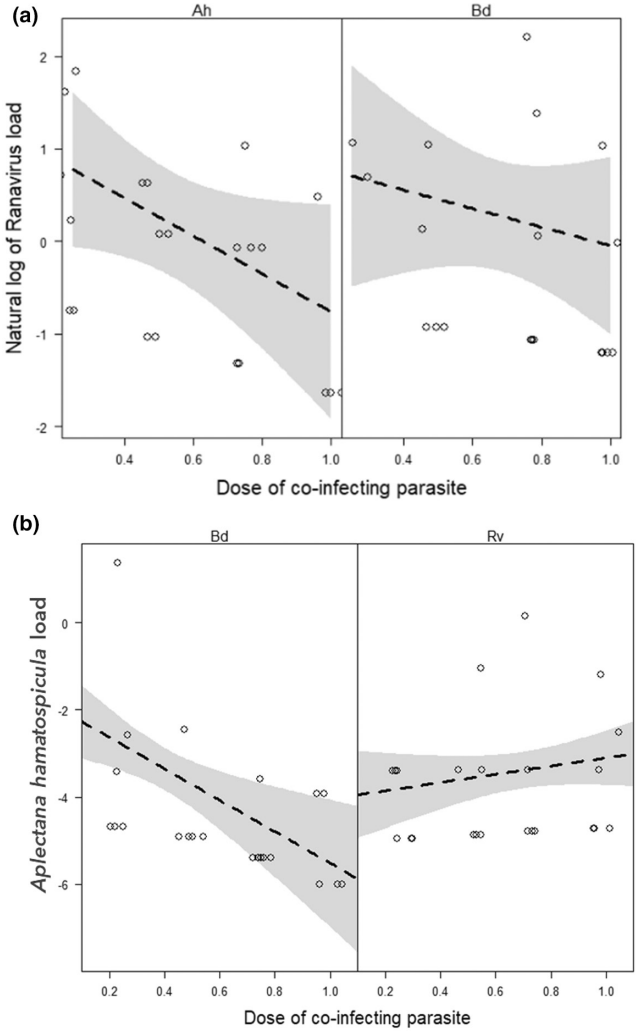

FIGURE 1 (a) Dose of *Aplectana hamatospicula* (Ah) and *Batrachochytrium dendrobatidis* (Bd) was significantly negatively associated with load of Ranavirus (Rv). (b) The dose of Bd was negatively associated with *A. hamatospicula* load, whereas the dose of Ranavirus was not significantly associated with *A. hamatospicula* load. Dose of coinfecting parasite is provided as a proportion of the maximum dose. Shown are conditional plots displaying the expected value (dashed line), a confidence interval for the expected value (grey band) and partial residuals (points).

4 | DISCUSSION

Hosts are frequently coinfected and there is spatiotemporal variation in the densities of parasites that they encounter. We found that every set of pairwise coinfections was density dependent. Additionally, each was highly asymmetric and weak in one of the two directions. This is consistent with other ecological interactions, such as the asymmetric and weak interactions that dominate food webs (McCann et al., 1998; Paine, 1992) and mutualistic

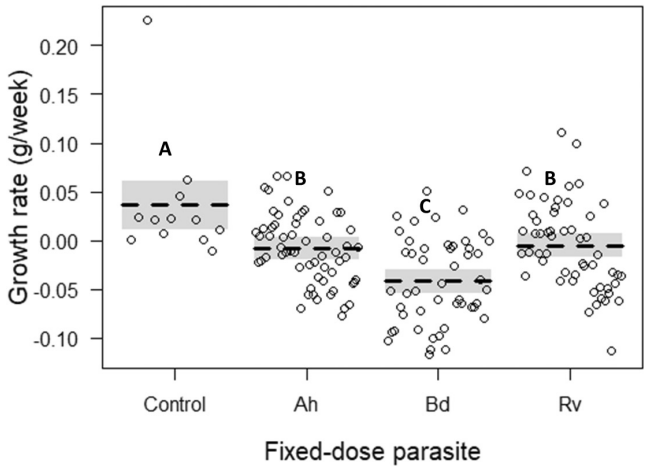

FIGURE 2 All three parasites reduced *Osteopilus septentrionalis* growth rate (grams of growth per week) relative to hosts that were not infected, but *Batrachochytrium dendrobatidis* (Bd) was the most virulent of the parasites, reducing host growth significantly more than *Aplectana hamatospicula* (Ah) or Ranavirus (Rv). Shown are conditional plots displaying the expected value (dashed line), a confidence interval for the expected value (grey band) and partial residuals (points). Treatments with different letters are significantly different from one another.

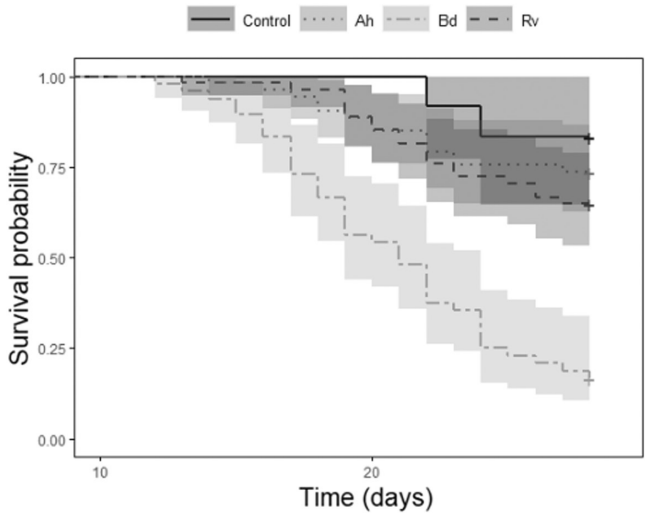

FIGURE 3 Hosts exposed to *Batrachochytrium dendrobatidis* (Bd) had significantly reduced survival relative to hosts exposed to *Aplectana hamatospicula* (Ah), Ranavirus (Rv) or no parasite, regardless of the identity or dose of the coinfecting parasite. Survival curves shown as median survival (line) with the associated 95% confidence interval (grey band).

networks (Bascompte et al., 2006). In keeping with competition principles from community ecology, we found that the density of a coinfecting parasite has the potential to alter the loads of the coinfecting parasite and host tolerance to these infections. Understanding how density affects antagonistic or synergistic interactions between coinfecting parasites should allow amphibian conservation organizations and the wildlife and park managers to make informed decisions about when to remove or maintain parasites.

The dose of Bd negatively impacted the load of *A. hamatospicula* (Figure 1b), but the dose of *A. hamatospicula* did not alter the load of Bd. Higher doses of *A. hamatospicula* lowered viral load (Figure 1a), but higher doses of Ranavirus trended positively with *A. hamatospicula* loads (Figure 1b). Finally, higher doses of Bd decreased viral load (Figure 1a), but higher doses of Ranavirus did not impact Bd load. Every pairwise parasite comparison was significantly affected by exposure dose, with negative density-dependent interactions between the coinfecting parasites. However, the strength of these interactions was highly asymmetric and weak in one of the two directions. We found no evidence that these load patterns are driven by trade-offs with the acquired immune system, as these trade-offs should generate either inhibition or facilitations in both directions (Berger, 2000; Morel & Oriss, 1998). Only Bd and *A. hamatospicula* had the potential for direct interactions, when juvenile *A. hamatospicula* and Bd infect the same organ, skin. Thus, the decrease in *A. hamatospicula* load at higher Bd doses could be driven by upregulation of innate immune responses (i.e. macrophages) in the skin that can combat both Bd (Fites, 2014) and helminth infections (Motran et al., 2018). This could be especially true if macrophages are more effective against *A. hamatospicula* than Bd. The patterns we saw in the other two pairwise parasite comparisons we tested were likely driven by other aspects of host immunity and nonimmune factors. For example, Ranavirus could have been inhibited by *A. hamatospicula* if hosts respond to higher exposure doses by increasing their food intake (Knutie, Wilkinson, Wu, et al., 2017). If hosts increase their food intake, there would be more available resources to mount immune responses against Ranaviral infections, such viral cellular and interferon responses (Grayfer et al., 2012, 2014; Wendel et al., 2017). In the Bd and Ranavirus coinfection treatment, Bd could have inhibited Ranavirus by slowing host growth (Figure 2). Ranavirus grows best in haematopoietic tissues and therefore, if Bd infection slowed host growth, this could have slowed viral replication (Chinchar, 2002).

Host tolerance to Bd was significantly affected by an interaction between identity and dose of the coinfecting parasites. Tolerance (defined as less negative slopes between parasite load and weight gain) of Bd was associated positively with the dose of *A. hamatospicula* but negatively with the dose of Ranavirus (Figure 4). This suggests that hosts are intolerant of Bd and Ranavirus coinfection, especially at high doses. Mounting immune responses to combat these multiple deadly and rapidly replicating microparasites is costly and could limit resources that the host can allocate to other demanding processes such as growth, leading to the observed patterns (Gray et al., 2009; Green et al., 2002; Kilpatrick et al., 2010; Lochmiller & Deerenberg, 2000). However, the negative effects on host growth caused by higher loads of Bd were lessened by higher doses of *A. hamatospicula*, suggesting that hosts are more tolerant to macro- and microparasite coinfections than micro- and microparasite coinfections. Additionally, we found that exposure to Bd significantly decreased host growth and survival relative to controls and other tested parasites (Figures 2 and 3).

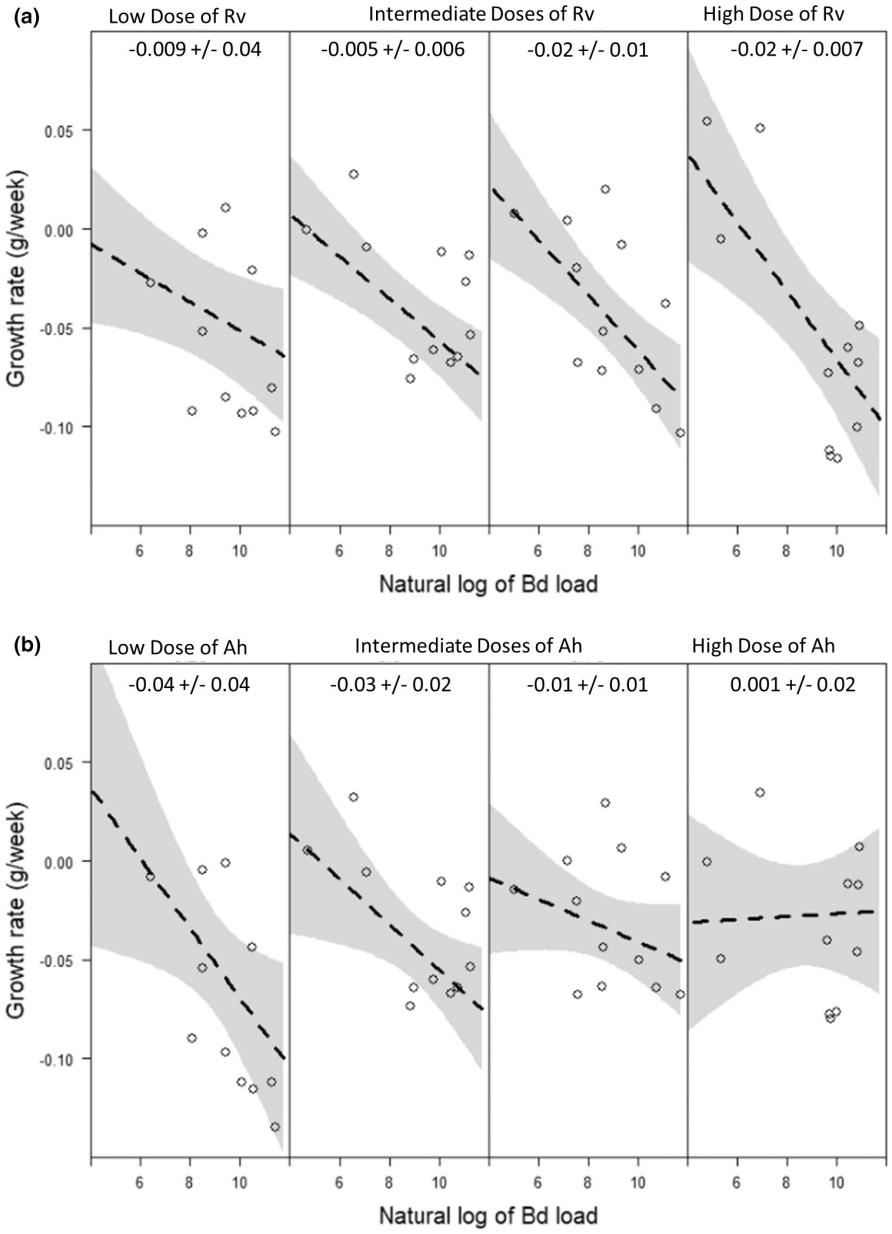

FIGURE 4 Tolerance (defined as less negative slopes between parasite load and growth rate) of *Batrachochytrium dendrobatidis* (Bd) was associated negatively with the dose of Ranavirus (Rv, a), but positively with the dose of *Aplectana hamatospicula* (Ah, b), because the slopes between growth and Bd load become more and less negative with increasing doses of Ranavirus and *A. hamatopsicula* respectively. Shown are the conditional plots displaying the expected value (dashed line), a confidence interval for the expected value (grey band) and partial residuals (points). Slopes and standard error are shown on panels.

This suggests that Cuban treefrogs are particularly susceptible to Bd infection and that competition plays a role in host tolerance and therefore host health.

We found that high doses of a common macroparasite, *A. hamatospicula*, decreased Ranaviral loads and increased host tolerance to Bd infection. Thus, we propose that maintaining or even increasing *A. hamatospicula*—or other reasonably innocuous gastrointestinal macroparasites—could be beneficial for hosts coinfected with more deadly microparasites, such as Bd and Ranavirus. This could be done through food supplementation of the host, a management technique that would be particularly effective when food resources are low, such as in the dry season. Frogs with freely available food can maintain higher loads of *A. hamatospicula* with fewer negative health effects. Additionally, *A. hamatospicula* also has an easier time establishing in the gut when hosts have free access to food. Finally, infected frogs with high resources release more faeces, with which to spread the infectious larvae (Knutie, Wilkinson, Wu, et al., 2017). Therefore, in Cuban treefrog hosts, we show that higher available host resources could increase *A. hamatospicula* loads in individuals and intra- and interspecific transmission within an environment. Further work is needed to assess if high doses of other macroparasites (i.e. not *A. hamatospicula*) benefit hosts coinfected with Bd or Ranavirus and if these patterns hold for other amphibian life stages and amphibian species in need of conservation. However, it is well understood that while amphibians are often coinfected, Ranavirus and Bd cause more mass mortality than macroparasites (but see *Ribeiroia ondatrae*; Johnson et al., 1999, Green et al., 2002, Skerratt et al., 2007, Kilpatrick et al., 2010). Therefore, if macroparasites generally reduce the impacts of Bd or Ranavirus then similar management techniques could be used across a range of amphibian species. This study addresses density with simultaneous infections, while

many natural infections occur sequentially. If earlier infections allow densities of one parasite to increase before the second parasite infects, then competition patterns seen here may still be applicable. However, these priority effects can play significant roles in coinfections (de Roode et al., 2005; Devevey et al., 2015; Hoverman et al., 2013) and have been studied separately in this system (Ramsay & Rohr, 2021).

The highly asymmetric but significant effects of exposure dose that we saw in this model amphibian system are consistent with other ecological interactions, such as interactions in food webs and in plant–pollinator networks, which often show asymmetric interactions that are weak in one direction (Bascompte et al., 2006; McCann et al., 1998; Paine, 1992). Therefore, as parasites in this system seem to show similar interaction patterns and outcomes as other natural enemy interactions, we suggest that competition principles from community ecology may be useful in predicting the outcome of coinfections in other systems. Many wildlife parasites cause minimal detrimental effects to animal or plant health (Acosta et al., 2020; Rahman et al., 2018), while a few cause massive die-offs that can necessitate management (Bernard et al., 2020; Perry et al., 2022). We suggest that a better understanding of less problematic parasites, such whether they are inhibitory or facilitative to deadly parasites and whether there are techniques that can be used to increase or decrease their natural densities, could give managers another avenue to manage parasites that cause mass mortality.

AUTHOR CONTRIBUTIONS

Chloe Ramsay and Jason Rohr conceived the ideas and designed methodology; Chloe Ramsay collected and analysed the data and led the writing of the manuscript. Chloe Ramsay and Jason Rohr contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

Jason Rohr is an Associate Editor of the Journal of Applied Ecology, but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.j3tx95xjq (Ramsay & Rohr, 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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