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FUNGAL METABOLITES PROVIDE PRE-EXPOSURE PROTECTION BUT NO POSTEXPOSURE BENEFIT OR HARM AGAINST *BATRACHOCHYTRIUM DENDROBATIDIS*

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ABSTRACT: Disease control tools are needed to mitigate the effect of the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) on amphibian biodiversity loss. In previous experiments, Bd metabolites (i.e., noninfectious chemicals released by Bd) have been shown to induce partial resistance to Bd when administered before live pathogen exposure and therefore have potential as an intervention strategy to curb Bd outbreaks. In the wild, however, amphibians inhabiting Bd-endemic ecosystems may have already been exposed to or infected with Bd before metabolite administration. It is therefore critical to evaluate the efficacy and safety of Bd metabolites applied postexposure to live Bd. We tested whether Bd metabolites administered postexposure would induce resistance, exacerbate infections, or have no effect. The results confirmed that Bd metabolites applied before pathogen exposure significantly reduced infection intensity, but Bd metabolites applied after pathogen exposure neither protected against nor exacerbated infections. These results reveal the importance of timing the application of Bd metabolite early in the transmission season for Bd-endemic ecosystems and emphasize that Bd metabolite prophylaxis may be a useful tool in captive reintroduction campaigns where Bd threatens the success of re-establishing endangered amphibian populations.

Key words: Amphibian decline, chytridiomycosis, conservation, prophylaxis, resistance, wildlife vaccination.

INTRODUCTION

Emerging infectious diseases of wildlife such as chytridiomycosis, Tasmanian devil facial tumor disease, white nose syndrome, and hemorrhagic septicemia are increasingly linked to biodiversity loss (Smith et al. 2009; Fisher et al. 2012; Fereidouni et al. 2019; Scheele et al. 2019). Wildlife populations that have already declined because of habitat destruction, invasive species, pollution, and climate change are especially vulnerable to disease-induced extinction (Smith et al. 2009; McCallum 2012; Fereidouni et al. 2019). Additionally, novel pathogens may threaten otherwise stable populations (McCallum 2012). Disease control interventions are needed to prevent further biodiversity loss and promote the conservation of many wildlife taxa.

Chytridiomycosis is a disease threatening amphibian biodiversity that is caused by the aquatic fungal pathogen *Batrachochytrium dendrobatidis* (Bd; Scheele et al. 2019). This pathogen is a host generalist, infecting amphibians and invertebrates, and has spread globally in recent decades (McMahon et al. 2013; Scheele et al. 2019). The contribution of chytridiomycosis to biodiversity loss is huge, with a connection to at least 90 amphibian species extinctions and the decline of hundreds more (Scheele et al. 2019). There is a pressing need to mitigate Bd-induced declines, and many methods to control Bd (e.g., antifungal treatments, microbiome augmentation, and vaccination) are being explored (McMahon et al. 2014; Knapp et al. 2021; Waddle et al. 2021).

Prophylactic treatments, such as vaccines, enable vulnerable populations to withstand disease outbreaks better and are promising tools to prevent disease-induced extinctions (Barnett and Civitello 2020). Vaccination has been implemented to protect prairie dog populations (Tripp et al. 2017) and has been recently proposed for Amur tigers (Gilbert et al. 2020) and little brown bats (Rocke et al. 2019; Gilbert et al. 2020). Environmentally

distributed vaccines (e.g., oral vaccine baits) are very useful for increasing vaccination coverage in wildlife, given that parenteral vaccines require a catch-vaccinate-release or darting strategy, which may be challenging or impractical (Undurraga et al. 2020). However, environmentally distributed vaccines require the assessment of additional ecological factors, such as host exposure history, to optimize intervention success and ensure that vaccines are safe for target populations, ecological communities, and ecosystems (Barnett and Civitello 2020). Timing vaccine administration according to host life history traits may increase population coverage and is especially effective for hosts, such as amphibians, with short life spans and seasonal population fluctuations (Schreiner et al. 2020). In a scenario where vaccination has no effect on previously exposed hosts, administering vaccines at or immediately after the end of a birth pulse may increase vaccination coverage compared with vaccinating later in the season when the endemic pathogen has had more time to infect the newly born susceptible hosts (Schreiner et al. 2020).

Mounting evidence shows that frogs can acquire resistance to Bd following any of these treatments: a live Bd exposure and clearance regime that uses itraconazole or temperatures outside the thermal tolerance of Bd; killed Bd zoospores with Bd metabolites (i.e., water-soluble noninfectious chemicals released by Bd); and Bd metabolites alone (McMahon et al. 2014; Barnett et al. 2021; Waddle et al. 2021; Nordheim et al. 2022), suggesting that Bd vaccination may be effective. Direct comparisons of killed Bd zoospores alone, killed Bd zoospores with Bd metabolites, and Bd metabolites alone have indicated that prophylactic exposure to Bd metabolites may drive equal or better resistance responses than killed Bd zoospores alone (Nordheim et al. 2022). Moreover, Bd metabolites have been found to be effective at inducing resistance across amphibian life stages (tadpoles and adults) and in at least two frog species (Cuban treefrog, *Osteopilus septentrionalis*, and Pacific chorus frog, *Pseudacris regilla*); however, importantly, Bd strain may affect treatment efficacy (Barnett et al. 2021; Nordheim et al. 2022).

For disease control and conservation, success would be maximized if Bd metabolites induced resistance regardless of exposure history and could be used as both a pre-exposure (i.e., Bd metabolites applied before frogs have been exposed to live Bd) prophylaxis and postexposure (i.e., Bd metabolites applied after frogs had been exposed to live Bd) treatment. Previous controlled laboratory studies have shown that prophylactic exposure to Bd metabolites provided protection against subsequent Bd challenge in frogs, but these studies only tested the prophylaxis on Bd-naïve animals (Barnett et al. 2021; Nordheim et al. 2022). In the wild, amphibians inhabiting Bd-endemic ecosystems may have already been infected with Bd before the time of prophylaxis administration. It is possible that Bd metabolites applied postexposure might exacerbate infections by increasing Bd infection probability or intensity, given that some metabolites released by Bd have immunosuppressive properties and are hypothesized to aid zoospore infection establishment (Rollins-Smith et al. 2019). If this were the case, it could be detrimental to broadly administer a Bd metabolite treatment to a Bd-endemic system. Thus, evaluating the effect of a postexposure Bd metabolite treatment is crucial for optimizing treatment efficacy and assessing the safety of a Bd metabolite treatment for use in the wild.

We tested whether Bd metabolites administered pre- or postexposure to live Bd would induce resistance, exacerbate infections, or have no effect.

MATERIALS AND METHODS

Egg collection and tadpole husbandry

Pacific chorus frogs (listed as least concern according to the IUCN Red List [Hammerson and Santos-Barrera 2004]) tadpoles are a well-studied reservoir of Bd (Reeder et al. 2012). We collected Pacific chorus frog egg clutches from Alameda County, California, USA, under permit CA DFW S-193500003-20017-001 and sent them to New London, Connecticut, USA. All laboratory procedures were approved by the Connecticut

College Institutional Care and Use Committee under protocol 236. *Batrachochytrium dendrobatidis* is endemic in Alameda County; however, collected eggs were presumed to be Bd-free because Bd appears not to be associated with amphibian eggs (Bancroft et al. 2011), being found only on keratinized tissues, which eggs lack (Marantelli et al. 2004). Tadpoles were maintained together in low densities (150 tadpoles in a 38-L container) until they reached Gosner stage 25, when they were separated into individual 500-mL plastic containers with 200 mL of artificial spring water (ASW; Cohen et al. 1980). Throughout the entire experiment, tadpoles were maintained in a natural light regime (10:14 h light:dark photoperiod) at 19 C, a temperature well within the thermal tolerance range, both for tadpoles of this species and Bd (Brattstrom 1963; Cohen et al. 2017). We fed the tadpoles fish flakes that are high in plant-based protein every second day. We conducted daily mortality checks and removed fecal matter from containers every 3 d.

Bd culture and Bd metabolite treatment preparation

We produced stock Bd culture and Bd metabolite treatments as previously described (McMahon et al. 2019). We used Bd isolate JEL 270 (isolated from California) for both the Bd metabolite treatments and live challenges). In brief, we cultured Bd in 1% tryptone broth and then inoculated 1% tryptone agar plates with 3 mL of the Bd isolate. Plates were maintained at 19 C for 2 wk, after which we flooded the plates with ASW for approximately 3 min, to suspend the zoospores and zoosporangia, then homogenized the liquid across all plates to create a Bd-positive (Bd+) stock consisting of ASW, Bd, and Bd metabolites. We determined the concentration of zoospores in the Bd+ stock by analyzing a 10-µL aliquot on a hemocytometer and averaging the number of zoospores from the four field of view quadrats, methods standard in the field. We then diluted the concentration to 400 zoospores/mL with ASW (we refer to the concentration of this Bd metabolite filtrate as 400 zoospores-removed/mL in reference to this prefiltration concentration). This diluted Bd+ stock was filtered through a 1.2-µm filter (GE Whatman Laboratory Products, CITY, STATE, COUNTRY) to remove zoospores and zoosporangia, creating the Bd metabolite treatment. We verified that no zoospores or zoosporangia remained in the Bd metabolite treatment by the same light microscopy approach that was used for calculating the concentration of zoospores in the Bd+ stock explained above. All Bd metabolite aliquots were maintained in a laboratory-grade –20 C freezer, and the amount needed for each day was brought to room temperature before each dosing event.

Study design

We conducted a 24-d infection experiment with three treatments: a pre-exposure prophylaxis, a postexposure prophylaxis, and an ASW control. The experiment began with 30 tadpoles per treatment; all tadpoles that died did so before the live Bd challenge and were excluded from analysis. Every second day from the start of the experiment until the day of live Bd exposure (i.e., days 1, 3, 5, 7, 9, and 11), tadpoles in the pre-exposure treatment were dosed topically with 1 mL of Bd metabolites (the solution was diluted into the 200 mL of ASW in the tadpole's housing container, for a final treatment dose of 2 zoospores-removed/mL), and tadpoles in the other treatments were dosed with 1 mL ASW. On day 12, we challenged all tadpoles with 1 mL of live Bd (4×10^5 zoospores/mL), which was diluted into the 200-mL housing containers for a final exposure dose of 2,000 live zoospores/mL. To reduce water fouling, a minimal water change was performed to remove fecal matter on the day after live Bd challenge. Starting on day 13, on every second day (i.e., days 13, 15, 17, 19, and 21), tadpoles in the postexposure treatment were dosed topically with 1 mL of Bd metabolites as described for pre-exposure treatment, while tadpoles in the other treatments received 1 mL of ASW.

Molecular detection of Bd

On the 24th day of the experiment, all tadpoles were euthanized with an overdose of MS 222 (10 g/L of ASW) buffered with sodium bicarbonate as needed to maintain a neutral pH (Leary et al. 2020), and mouthparts were dissected for molecular detection of Bd. We quantified the Bd load in number of genome equivalents from each tadpole by quantitative PCR (see Boyle et al. 2004) with plasmid standards designed to target Bd/Bsal (Pisces Molecular, CITY, STATE, COUNTRY). We screened for, and confirmed lack of, inhibition in every sample with TaqMan Exogenous Internal Control Reagents (Applied Biosystems, Foster City, California, USA).

Data analysis

We conducted all statistical analyses in R statistical software, version 4.0.3 (R Core Team 2020). We verified the proportional hazards assumption (P=0.58, package: survival, function: cox.zph) and used the Cox proportional hazards model (package: KMsurv, function: coxph) with treatment as the predictor to assess mortality. We used a binomial generalized linear model (GLM) on binary infection status to assess treatment effects on probability of Bd infection (package:

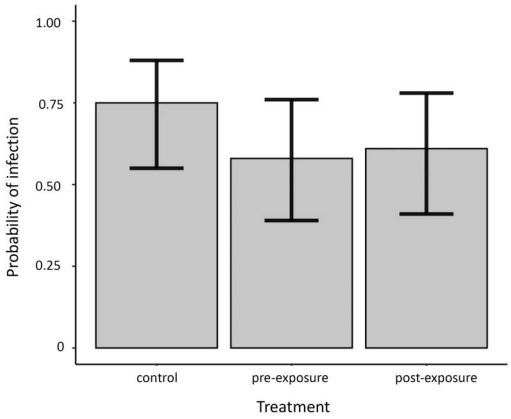

FIGURE 1. Probability of *Batrachochytrium dendrobatidis* (Bd) infection for Pacific chorus frog (*Pseudacris regilla*) tadpoles treated with (a) control treatment: artificial spring water before and after a live Bd challenge; (b) pre-exposure treatment: Bd metabolites before a live Bd challenge; (c) postexposure treatment: Bd metabolites after a live Bd challenge. Probability of infection among treatments was not significantly different. The bars on the plot represent 95% confidence intervals.

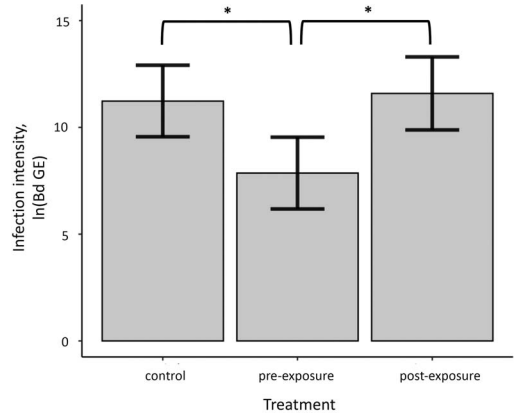

FIGURE 2. Estimated mean infection intensity (i.e., *Batrachochytrium dendrobatidis* [Bd] genome equivalents of infected individuals) for Pacific chorus frog (*Pseudacris regilla*) tadpoles treated with (a) control treatment: artificial spring water before and after a live Bd challenge; (b) pre-exposure treatment: Bd metabolites before a live Bd challenge; (c) postexposure treatment: Bd metabolites after a live Bd challenge. Tadpoles in the pre-exposure treatment had significantly lower mean infection intensities than tadpoles in the control and postexposure treatments. An asterisk (*) denotes a significant difference. The bars on the plot represent 95% confidence intervals.

glmmTMB, function: glmmTMB), and we calculated confidence intervals for the probability of infection by the Wilson Score interval (Brown et al. 2001). In both cases, we used likelihood ratio tests (package: stats, function: anova) to evaluate significance. We found no effect of treatment on probability of infection; therefore, we tested for differences in infection intensity with a zero-inflated negative binomial GLM (package: glmmTMB, function: glmmTMB) with treatment as the predictor for infection intensity. Given the similarity in probability of infection among treatments, we fit a common intercept for the zero-inflation component of the model. Furthermore, we conducted pairwise post hoc tests by rerunning the zero-inflated negative binomial glmmTMB models across all treatment combinations and using Bonferroni corrections for multiple testing (corrected α=0.017). We extracted the mean infection intensity estimates for each treatment from the model with the emmeans package (function: emmeans).

RESULTS

Overall, 94% of tadpoles survived the entire experiment. Mortality was not significantly different (Cox proportional-hazards model: P=0.8) or probability of Bd infection across treatments (binomial GLM): P=0.42; Fig. 1). From the zero-inflated models we found that tadpoles treated with the pre-exposure Bd metabolite treatment exhibited a 97% reduction in infection intensities compared with the control treatment (GLM P=0.003) and a 98% reduction in infection intensities compared with the postexposure treatment (GLM P=0.002; Fig. 2). Additionally, we found no effect of the postexposure Bd metabolite prophylactic treatment on infection intensity compared with the control group (P=0.77).

DISCUSSION

We found that Bd metabolites were effective as a pre-exposure prophylaxis but did not reduce or increase Bd loads when applied postexposure. Given that Bd metabolites do not induce resistance when applied postexposure, our results indicate that a Bd metabolite intervention should occur early in the transmission season, before a considerable amount of the population has already been exposed to Bd. This work highlights that timing of

prophylaxis exposure is an important factor for optimizing disease control interventions, especially when the pathogen is endemic (Schreiner et al. 2020). These empirical data are the first step to understanding the importance of prophylaxis timing for Bd metabolite prophylaxis; in the future, modeling various timing scenarios for Bd metabolite administration would be useful to determine optimal intervention strategies.

Although some Bd metabolites have been thought to facilitate infection establishment (Rollins-Smith et al. 2019), our experiment found that Bd metabolites did not increase probability of infection or infection intensity in tadpoles when applied postexposure. Protection regardless of exposure history would be ideal from a management perspective, but our findings do suggest that field administration of the treatment is unlikely to be detrimental to hosts that are already infected.

Our findings also suggest that Bd metabolite prophylaxis may be beneficial as a proactive measure to curb Bd epidemics and reduce the ability of Bd to expand into new populations. Given that Bd-induced mortality is associated with high infection loads (Voyles et al. 2009), by reducing infection intensity, Bd metabolite pre-exposure treatment may also decrease Bd-induced mortality. For the benefit of animal welfare, our experiment ended in a shorter timeframe than Bd-induced mortality is expected to occur, but a future study should directly assess the effect of Bd metabolite prophylaxis on infection-induced mortality. Furthermore, Bd metabolite prophylaxis reduces onward transmission by decreasing zoospore loads; transmission modeling studies should investigate if, under certain conditions, this effect is sufficient enough to generate herd immunity. Environmental persistence of Bd has been a barrier to successful reintroduction of endangered amphibians susceptible to chytridiomycosis (Hammond et al. 2021); Bd metabolite prophylaxis might serve as a powerful tool to remedy this challenge. For example, Bd metabolites could be used to treat captive-bred, Bd-naïve amphibians before their release into Bd-endemic systems, providing the reintroduced amphibians with some protection against Bd to facilitate their successful establishment.

Our experiment lasted only 11 d after live Bd exposure because previous studies (Barnett et al. 2021) have shown that Bd resistance can develop within a short timeframe and we were looking to conserve resources and mitigate animal suffering. However, it is possible that there could be a lag period in mounting the immune response that exceeds 11 d (e.g., it takes adult African clawed frog, *Xenopus laevis*, 1 mo to clear *Ranavirus* (FV3) infections; Gantress et al. 2003) and that Bd metabolites applied postexposure might facilitate faster clearance of Bd in a delayed response that we were unable to detect. Additionally, the effect of combined Bd metabolite pre- and postexposure treatment remains unknown, and it is possible that a postexposure Bd metabolite treatment might boost the resistance response in tadpoles that had already received a pre-exposure Bd metabolite treatment. More work is needed to investigate these possibilities.

Immune defenses may vary greatly by life stage in amphibians because of reorganization of the immune system during metamorphosis (Gantress et al. 2003; Humphries et al. 2022). Although Bd metabolites have been effective at inducing resistance when applied pre-Bd exposure in both tadpoles (Nordheim et al. 2022) and adults (Barnett et al. 2021), it is possible that adult frogs or frogs undergoing metamorphosis may respond differently than tadpoles to postexposure Bd metabolite treatment. Although metamorphs are more likely to succumb to Bd-induced mortality than are tadpoles (Rachowicz al. 2006), metamorphs' immune systems are more mature than that of tadpoles, exemplified by their increased expression of major histocompatibility complex classes I and II and presence of antimicrobial peptides (Humphries et al. 2022). Given the maturity of their immune system, metamorphs and adult frogs may be able to acquire resistance by postexposure treatment with Bd metabolites even though this study found postexposure prophylaxis was ineffective in tadpoles.

Three published studies (this study, Barnett et al. 2021, and Nordheim et al. 2022) now show that Bd metabolites are effective at significantly reducing Bd infection intensity when applied pre-exposure. The consistent reproducibility of this result indicates that Bd metabolite prophylaxis may be a useful tool against Bd-induced biodiversity declines. To be effective, Bd metabolite prophylaxis in Bd-endemic ecosystems should be applied early in the transmission season or in conjunction with influxes of new susceptible hosts, whether reproduction pulses or reintroductions. Further work needed includes evaluation of the safety of Bd metabolites to nontarget wildlife, testing of the efficacy of Bd metabolite prophylaxis in a field setting, and investigation of the potential for Bd metabolite prophylaxis to work synergistically with other Bd mitigation strategies.

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LITERATURE CITED

Barnett KM, Civitello DJ. 2020. Ecological and evolutionary challenges for wildlife vaccination. *Trends Parasitol* 36:970–978.

Barnett KM, Detmering SE, McMahon TA, Civitello DJ. 2021. Asymmetric cross-strain protection for amphibians exposed to a fungal-metabolite prophylactic treatment. *Biol Lett* 17:20210207.

Boyle D, Boyle D, Olsen V, Morgan J, Hyatt A. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis Aquat Organ* 60:141–148.

Brattstrom BH. 1963. A preliminary review of the thermal requirements of amphibians. *Ecology* 44:238–255.

Brown LD, Cai TT, DasGupta A. 2001. Interval estimation for a binomial proportion. *Stat Sci* 16: 101–117.

Cohen JM, Venesky MD, Sauer EL, Civitello DJ, McMahon TA, Roznik EA, Rohr JR. 2017. The thermal mismatch hypothesis explains host susceptibility to an emerging infectious disease. *Ecol Lett* 20: 184–193.

Cohen LM, Neimark H, Eveland LK. 1980. *Schistosoma mansoni*: Response of cercariae to a thermal gradient. *J Parasitol* 66:362.

Fereidouni S, Freimanis GL, Orynbayev M, Ribeca P, Flannery J, King DP, Zuther S, Beer M, Höper D, et al. 2019. Mass die-off of Saiga antelopes, Kazakhstan, 2015. *Emerg Infect Dis* 25:1169–1176.

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484:186–194.

Gantress J, Maniero GD, Cohen N, Robert J. 2003. Development and characterization of a model system to study amphibian immune responses to iridoviruses. *Virology* 311:254–262.

Gilbert M, Sulikhan N, Uphyrkina O, Goncharuk M, Kerley L, Castro EH, Reeve R, Seimon T, McAloose D, et al. 2020. Distemper, extinction, and vaccination of the Amur tiger. *Proc Natl Acad Sci U S A* 117: 31954–31962.

Hammerson G, Santos-Barrera G. 2004. *Pseudacris regilla*. In: *The International Union for Conservation of Nature red list of threatened species*. https://www.iucnredlist.org/species/166731785/53961380. Accessed March 2023.

Hammond TT, Curtis MJ, Jacobs LE, Gaffney PM, Clancy MM, Swaisgood RR, Shier DM. 2021. Overwinter behavior, movement, and survival in a recently reintroduced, endangered amphibian, *Rana muscosa*. *J Nat Conserv* 64:126086.

Humphries JE, Lanctôt CM, Robert J, McCallum HI, Newell DA, Grogan LF. 2022. Do immune system changes at metamorphosis predict vulnerability to chytridiomycosis? An update. *Dev Comp Immunol* 136:104510.

Knapp RA, Joseph MB, Smith TC, Hegeman EE, Vredenburg VT, Erdman JE, Boiano DM, Jani AJ, Briggs CJ. 2021. Effectiveness of antifungal treatments during chytridiomycosis epizootics in populations of an endangered frog. preprint, Ecology. http://biorxiv.org/lookup/doi/10.1101/2021.06.13.448228. Accessed January 2022.

Leary S, Underwood W, Anthony R, Cartner S, Greenacre C, Gwaltney-Brant S, McCrackin MA, Meyer R, Miller D, et al. 2020. *AVMA guidelines for the euthanasia of animals: 2020 edition*. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf. Accessed March 2023.

Marantelli G, Berger L, Speare R, Keegan L. 2004. Distribution of the amphibian chytrid *Batrachochytrium dendrobatidis* and keratin during tadpole development. *Pac Conserv Biol* 10:173–179.

McCallum H. 2012. Disease and the dynamics of extinction. *Philos Trans R Soc Lond B Biol Sci* 367: 2828–2839.

McMahon T, Laggan N, Hill M. 2019. Metabolites produced by *Batrachochytrium dendrobatidis* alter development in tadpoles, but not growth or mortality. *Dis Aquat Organ* 135:251–255.

McMahon TA, Brannelly LA, Chatfield MWH, Johnson PTJ, Joseph MB, McKenzie VJ, Richards-Zawacki CL, Venesky MD, Rohr JR. 2013. Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proc Natl Acad Sci U S A* 110:210–215.

McMahon TA, Sears BF, Venesky MD, Bessler SM, Brown JM, Deutsch K, Halstead NT, Lentz G, Tenouri N, et al. 2014. Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. *Nature* 511:224–227.

Nordheim CL, Detmering SE, Civitello DJ, Johnson PTJ, Rohr JR, McMahon TA. 2022. Metabolites from the fungal pathogen *Batrachochytrium dendrobatidis* (bd) reduce Bd load in Cuban treefrog tadpoles. *J Appl Ecol* 59:2398–2403.

R Core Team. 2020. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed March 2023.

Rachowicz LJ, Knapp RA, Morgan JAT, Stice MJ, Vredenburg VT, Parker JM, Briggs CJ. 2006. Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* 87:1671–1683.

Reeder NMM, Pessier AP, Vredenburg VT. 2012. A reservoir species for the emerging amphibian pathogen *Batrachochytrium dendrobatidis* thrives in a landscape decimated by disease. *PLoS ONE* 7: e33567.

Rocke TE, Kingstad-Bakke B, Wüthrich M, Stading B, Abbott RC, Isidoro-Ayza M, Dobson HE, dos Santos Dias L, Galles K, et al. 2019. Virally-vectored vaccine candidates against white-nose syndrome induce antifungal immune response in little brown bats (*Myotis lucifugus*). *Sci Rep* 9:6788.

Rollins-Smith LA, Ruzzini AC, Fites JS, Reinert LK, Hall EM, Joosse BA, Ravikumar VI, Huebner MI, Aka A, et al. 2019. Metabolites involved in immune evasion by *Batrachochytrium dendrobatidis* include the polyamine spermidine. *Infect Immun* 87:1–13.

Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, Acevedo AA, Burrowes PA, Carvalho T, et al. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363:1459–1463.

Schreiner CL, Nuismer SL, Basinski AJ. 2020. When to vaccinate a fluctuating wildlife population: Is timing everything? *J Appl Ecol* 57:307–319.

Smith KF, Acevedo-Whitehouse K, Pedersen AB. 2009. The role of infectious diseases in biological conservation. *Anim Conserv* 12:1–12.

Tripp DW, Rocke TE, Runge JP, Abbott RC, Miller MW. 2017. Burrow dusting or oral vaccination prevents plague-associated prairie dog colony collapse. *EcoHealth* 14:451–462.

Undurraga EA, Millien MF, Allel K, Etheart MD, Cleaton J, Ross Y, Wallace RM, Crowdis K, Medley A, et al. 2020. Costs and effectiveness of alternative dog vaccination strategies to improve dog population coverage in rural and urban settings during a rabies outbreak. *Vaccine* 38:6162–6173.

Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, Cook D, Webb R, Alford RA, et al. 2009. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* 326:582–585.

Waddle AW, Rivera R, Rice H, Keenan EC, Rezaei G, Levy JE, Vasquez YS, Sai M, Hill J, et al. 2021. Amphibian resistance to chytridiomycosis increases following low-virulence chytrid fungal infection or drug-mediated clearance. *J Appl Ecol* 58:2053–2064.

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