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Batrachochytrium dendrobatidis (Bd) in American bullfrogs: Assessing the role of bullfrogs as a pathogen reservoir in California's Bd-endemic ponds

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Environmental Sciences

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Abstract

Batrachochytrium dendrobatidis (Bd) in American bullfrogs: Assessing the role of bullfrogs as a pathogen reservoir in California's Bd-endemic ponds

By Olivia Milloway

American bullfrogs (*Lithobates catesbeianus*) are listed on the International Union for Conservation of Nature's (IUCN) list of 100 worst invasive species in part because they have facilitated the spread of *Batrachochytrium dendrobatidis* (Bd), an emerging fungal pathogen, around the globe. However, the role of bullfrogs in facilitating Bd persistence as a reservoir species in endemic systems remains unclear. In order to elucidate the relationship between bullfrogs and Bd persistence, I used 11 years of field surveys on 9 ponds in California's East Bay Region as well as to test three assumptions of competent reservoirs: (1) bullfrogs have high infection prevalence and intensity, (2) bullfrogs do not experience mortality from Bd infections, and (3) overwintering bullfrog tadpoles are able to sustain Bd infections and therefore serve as a between-season maintenance host. My results provide some support for assumptions one and three, and tenuous support for assumption two. Together, these findings indicate that bullfrogs might be competent reservoirs of Bd in certain life stages and environments but might not be as competent a Bd reservoir as initially thought. Further study is needed to understand the relationship between invasive species and Bd ecology in order determine if interventions like invasive species removal are effective Bd disease control strategies.

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Acknowledgements

I would first like to thank KM Barnett for bringing me back into the world of amphibians and providing invaluable mentorship both during the field season and throughout development of the project. Learning from her example, I've become a better, more compassionate advocate both for myself and for others in academia and in life. I am very grateful to my advisor, Dr. Civitello, for welcoming me into his lab and enthusiastically supporting the project from the beginning; to Dr. Keogh for answering my many questions about honors requirements; and to Dr. Prokopec for serving on my committee. I would also like to thank Brendan Hobart for mentoring me through exploratory data analysis over the summer of 2021 and Dr. Pieter Johnson and Dain Calhoun at the University of Colorado Boulder for providing access to the data for this project. I'd also like to thank Blue Oak Ranch Reserve, a part of the University of California's Natural Reserve System, for hosting me for the 2021 field season. This project was funded through the Research Experience for Undergraduates (REU) program through the National Science Foundation as well as by the Halle Center for Global Research Undergraduate Global Research Fellowship.

I would also like to thank all of my mentors throughout my time at Emory, especially John Wegner, for his unending support and friendship, and for teaching me the two most important tools I have as an ecologist are curiosity and an open mind. I'm also very grateful to my father, from whom I inherited my love for the natural world, and to my mother, who always reminded me I can do hard things. Finally, I would like to thank the frogs swabbed and collected in this study; without them, none of this work would have been possible.

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Introduction

The global trade of *Lithobates catesbeianu*s (American bullfrog, hereafter “bullfrog”) has facilitated the invasion of bullfrogs outside of their native range (Snow and Witmer 2010), resulting in catastrophic ecosystem consequences and landing the species a spot on the International Union for Conservation of Nature’s (IUCN) list of 100 worst invasive species (Lowe et al. 2000). One deleterious consequence of their invasion has been the spread of *Batrachochytrium dendrobatidis* (Bd) (Garner et al. 2006), an emerging fungal pathogen that has been implicated in the decline of more than 500 amphibian species, including the extinction of 90 (Scheele et al. 2019). Though extensive research has been conducted on the deleterious impacts of bullfrogs and Bd, there is still uncertainty in how bullfrogs contribute to the maintenance of Bd within a disease system in which Bd is already established. Despite this uncertainty, bullfrog culling has been proposed as a means to reduce Bd outbreaks (Garner et al. 2005, Laufer et al. 2018), but further study is needed to elucidate the relationship between invasive species and Bd ecology in order to determine if such interventions are effective and inform conservation strategies across the globe.

Bd attacks amphibians’ keratinized tissue—which includes the epidermis—and causes the disease chytridiomycosis. Symptoms of Bd infection include hyperkeratosis, or the thickening of keratinized tissue (Van Rooij et al. 2015). Once zoospores infect a host, they are able to replicate in the intracellular space in the skin, producing more zoospores that eventually emerge (Rosenblum et al. 2010). Zoospores can then reinfect the individual, or swim through the water to infect other individuals. This disease can be especially lethal for amphibians because their skin is an organ critical to their survival which is responsible for regulating gas exchange with the environment. It is important to note, however, that tadpoles only have keratinized skin on the mouthparts, so only mouthparts can harbor infection (Fig A7).

In recent years, bullfrogs have been considered important contributors to Bd transmission in their introduced range (Schloegel et al. 2012). Bullfrogs are native to the eastern United States (U.S.) but were introduced beyond their native range for farming purposes in the late 1800s (Yap et al. 2018). After their introduction west of the Rocky Mountains, bullfrogs quickly became a widespread invasive species (Snow and Witmer 2010). It was previously thought that the expansion of one of the most important strains of Bd—the Global Pandemic Lineage (Bd-GPL)-—mirrored the expansion of bullfrogs within the U.S. (Yap et al. 2018). However, recent phylogenetic evidence indicates that Bd-GPL has likely been in the American West for centuries and can persist in environments for an extended period of time pre-epidemic, suggesting that the factors leading to Bd outbreaks are more complicated than simply Bd introduction by bullfrogs into susceptible amphibian communities (Rothstein et al. 2021).

Regardless of the relationship between bullfrogs and the introduction of Bd to new areas, it is critical to evaluate the role of bullfrogs as a Bd reservoir in order to predict if mitigation strategies targeting bullfrogs—such as culling—could effectively curb outbreaks in Bd endemic systems. Reservoir hosts are tolerant of infection (i.e., can become infected with a pathogen but have low infection–induced morbidity or mortality), meaning that they can easily transmit a pathogen (Brannelly et al. 2018). Nearly all parasites transmit more rapidly when host density increases. In the absence of a reservoir host population, disease outbreaks can drive down the abundance of susceptible hosts until transmission slows and populations of susceptible hosts may be able to rebound until the next outbreak (Brannelly et al. 2018). But, if pathogens are maintained in reservoirs—in this case, if infected bullfrogs do not die and remain highly infectious—susceptible species are not always able to rebound and are consequently driven to extinction (Blaustein et al. 2005).

One possible opportunity for bullfrogs to act as a reservoir of Bd is during their larval stage. Because Bd attacks keratinized tissue, which is only present in the mouthparts of tadpoles, larvae of all species can often maintain Bd infections without experiencing significant infection-induced morbidity and mortality (Briggs et al. 2010). Additionally, bullfrog larvae are much larger than tadpoles of most sympatric species and have an extended larval stage; bullfrog larvae can live for two years, overwintering in their ponds rather than metamorphosing within a single season (Stebbins & McGinnis 2012). Infected bullfrog larvae even have the ability to persist in ponds through the next year even after die-offs of susceptible adults occur (Blaustein et al. 2005). Bullfrog’s protracted larval stage and larger size have been hypothesized to relate to increased Bd prevalence in the species because of tadpole’s potential to maintain Bd infections over the winter months when other amphibians have metamorphosed and migrated out of the ponds (Peterson and McKenzie 2014).

While bullfrogs likely play a role in influencing infection dynamics in anurans within the ponds they inhabit, other factors such as species richness and abiotic environmental characteristics do as well (Peterson and McKenzie 2014). Bullfrogs have also been described to have no greater role in within-season Bd dynamics than other sympatric species in Californian ponds, including *Pseudacris regilla* (Pacific chorus frog, hereafter “chorus frog”), which are thought to be Bd reservoirs in part because of their sheer numbers and pervasiveness throughout the landscape (Wilber et al. 2020). Moreover, there is evidence of Bd–induced mortality in bullfrogs (Gervasi et al. 2013), including within natural and manmade cattle ponds in California’s East Bay Region (C. Briggs, personal communication). These findings incite doubt into whether bullfrogs are truly as important reservoirs of Bd within the California Bd–amphibian disease system as previously thought.

Previous studies of bullfrogs and Bd lead to three assumptions which, taken together, assist in understanding whether bullfrogs are important Bd reservoir hosts. If bullfrogs are highly competent Bd reservoirs, then they are likely to satisfy at least one of the three fundamental assumptions: (1) Bullfrogs have high infection prevalence and intensity, (2) bullfrogs do not experience mortality from Bd infections, and (3) overwintering bullfrog tadpoles are able to sustain Bd infections and therefore serve as a between-season maintenance host. Evaluating these assumptions will better characterize the role of bullfrogs in Bd transmission in the East Bay, California pond systems.

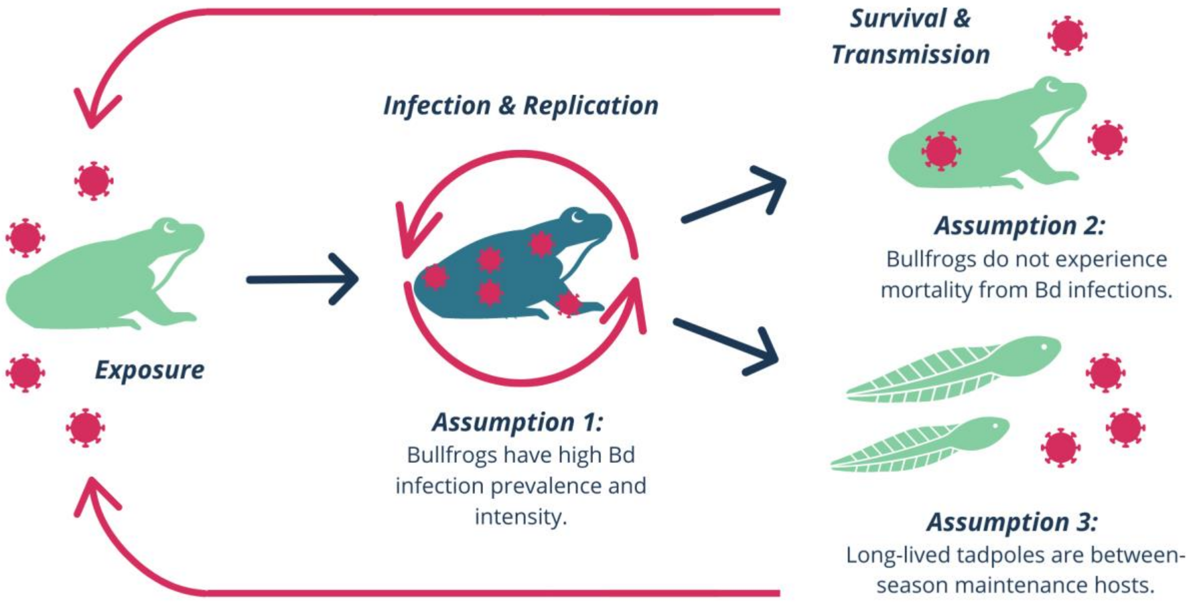

Figure 1(a): Each of these three assumptions of highly competent reservoirs need to be tested in order to assess if bullfrogs are highly competent reservoirs of Bd.

Methods

Field Collection

Over the past decade, field crews have sampled dozens sites in the East Bay Area in California, USA for bullfrog and chorus frog presence and Bd infection status. Sampling sites were either natural or man-made cattle ponds on private and public land in Contra Costa, Almeda, and Santa Clara counties and spanned a ~60 mile north–south gradient. The landscape surrounding the sampling sites were grassland, chaparral, and oak woodlands within the urban sprawl of the East Bay Region (Moss et al. 2021). Sampled sites consisted of a mix of both permanent and vernal ponds and included native amphibians such as *Rana draytonii* (California red-legged frog), *Anaxyrus boreas* (western toad), *Taricha torosa* (California newt), and chorus frogs as well as invasive bullfrogs.

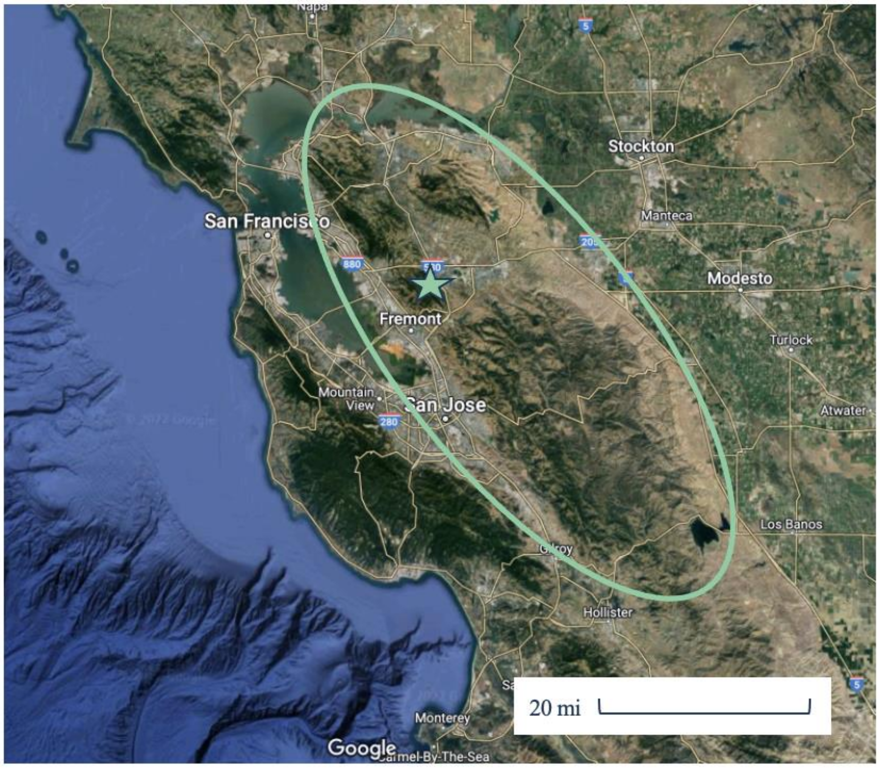

Figure 1(b): Map of the East Bay Region highlighting location of sampled ponds. Star denotes location of PRPND009 and PRPND010.

In October of 2019, larval, metamorph, and adult bullfrogs were collected and swabbed at two ponds (i.e., PRPND009 and PRPND010) in order to quantify infection prevalence and intensity within and among life stages. Larvae were classified as either first-year larvae (L1) or second-year larvae (L2). Additionally, chorus frogs were surveyed between 4-6 times at 10 ponds during 2017, including the two ponds in which bullfrogs were also opportunistically sampled in 2019 (PRPND009 and PRPND010). Routine bullfrog surveys were completed from 2009-2020 at eight ponds in which each pond was sampled two or three times during the months of May–August of each year. During the first visit, the ponds were sampled using dipnets (1.4 mm mesh and 2600 cm^2 net area) to obtain estimates of larval amphibian densities. Dipnet sweeps were performed approximately every 10 meters around the pond, with each sweep sampling about 0.5 m^2 of the pond (Wilber et al. 2020). The number of amphibians in each dipnet and their respective life stages were recorded as well as the number of total dipnets taken (Joseph et al. 2016). During the second visit, 10–12 bullfrog metamorphs were collected using transect methods and sent in coolers to the Johnson laboratory at the University of Colorado Boulder for swabbing and dissection. Standard methods for swabbing were followed and additional metamorphic bullfrogs at each pond were captured, swabbed, and released (Hyatt et al. 2007). If adequate bullfrog metamorphs could not be caught during the day, a third survey was conducted at night, as bullfrogs are often easier to catch after dark. For analysis, field and laboratory swabbing data were pooled for total prevalence. All animals were handled with University of Colorado Boulder IACUC-approved methods and with appropriate state and federal permits.

Bd Detection and Quantification

Metamorph and adult frogs (both bullfrogs and chorus frogs) were swabbed 10 times on each of seven surfaces (ventral surface, under the chin, right hind limb, left hind limb, right forelimb, left forelimb, cloaca) for a total of 70 swabs. If no limbs were present on larvae, swabbing focused on the ventral surfaces and mouthparts while keeping the total number of swabs (70) consistent. Collected animals sent to the laboratory were swabbed using an identical protocol. The tips of the swabs were snapped off into sterile microcentrifuge tubes, stored in standard freezer, and sent to the Briggs laboratory (University of California Santa Barbara) for quantitative polymerase chain reaction (qPCR). Swabs were analyzed using real-time TaqMan assay (Boyle et al. 2004). The assays were conducted using an Applied Biosystems Prism 7700 Sequence Detection System. Each reaction was conducted in triplicate, containing 12.5 μL 2 x Taqman Master Mix, PCR primers at a concentration of 900 nM, the MGB probe at 250 nM and 5 μL of DNA. For each individual, Bd zoospore load was calculated (Boyle et al. 2004). Samples with estimated zoospore loads >1 were considered infected and <1 were considered uninfected. For the 2010-2020 survey data, mean Bd load and Bd prevalence at each pond were aggregated to a site-year level for plotting and summarizing.

Data Visualization and Analysis

To investigate Assumption 1 (bullfrogs have high infection prevalence and intensity), I compared the 2019 bullfrog and 2017 chorus frog, a known reservoir of Bd (Wilber et al. 2020), infection prevalence and intensity data from PRPND009 and PRPND010. I tested for differences in prevalence between species using a binomial generalized linear model (package: glmmTMB, function: glmmTMB) with pond as a random effect. I calculated prevalence confidence intervals using the Wilson Score interval (Brown et al. 2001) and used a likelihood ratio test (package: stats, function: anova) to evaluate significance. I tested for differences in infection intensity with a zero-inflated negative binomial generalized linear model (package: glmmTMB, function: glmmTMB) using species as the predictor for infection intensity and pond as a random effect. I chose the zero-inflated negative binomial GLM model to compare infection intensities because this model analyzes infection intensity while controlling for a right-skewed distribution. In other words, the zero-inflated model controls for a high number of uninfected individuals with an infection intensity of 0. If bullfrogs are similarly competent reservoirs of Bd as compared to chorus frogs, I expect there to be no significant difference between infection prevalence and intensity in chorus frogs and bullfrogs.

Because I cannot directly measure bullfrog mortality based on the data that has been collected, in order to investigate Assumption 2 (bullfrogs do not experience mortality from Bd infections) I compared metamorph bullfrog infection intensity data from the field to infection intensity demonstrated to cause Bd–induced metamorph bullfrog mortality in controlled experimental settings (Gervasi et al. 2013). Additionally, Bd mortality is generally dependent on high infection loads and it is often thought to occur during metamorphosis when the amphibian immune system is most vulnerable (Rollins-Smith 1998). To investigate potential Bd-induced mortality through metamorphosis, I used a zero-inflated negative binomial GLMM (package: glmmTMB, function: glmmTMB) to test for differences in infection intensity with species and life stage as predictors of infection intensity and pond as a random effect. Because tadpoles carry over infections through metamorphosis (McMahon and Rohr 2015), in the absence of Bd-induced mortality during metamorphosis, infection intensity distributions would remain roughly the same between the two life stages. Therefore, if bullfrogs are more tolerant hosts of Bd than chorus frogs (i.e., experience less Bd-induced mortality), I expect that bullfrogs would have a smaller change in mean infection intensity between larva and metamorph stages than that of chorus frogs. Statistically, this would appear as a species-by-life stage interaction within the model. To better interpret the results of the model, I extracted the mean infection intensity estimates from the model by species for each life stage using the emmeans package.

Finally, in order to test Assumption 3 (overwintering bullfrog tadpoles are able to sustain Bd infections and therefore serve as a between-season maintenance host), I plotted Bd infection prevalence and intensity across life stages (L1, L2, metamorph, adult) in PRPND009 and PRPND010. I tested for differences in prevalence between year 1 larva (L1) and year 2 larva (L2) using a binomial generalized linear model (package: glmmTMB, function: glmmTMB) with pond as a random effect. I calculated prevalence confidence intervals using the Wilson Score interval (Brown et al. 2001) and used a likelihood ratio test (package: stats, function: anova) to evaluate significance. I tested for differences in infection intensity with a zero-inflated negative binomial generalized linear model (package: glmmTMB, function: glmmTMB) using life stage (L1 vs. L2) as the predictor for infection intensity and pond as a random effect. If overwintering bullfrog tadpoles are able to sustain Bd infections, I expect there to be no significant difference in infection intensities among L1 and L2 bullfrogs.

Assumption	Test	Data Used
1	Binomial generalized linear model with pond as a random effect; ANOVA to evaluate significance	PRPND009 and PRPND010 bullfrog (2019) and chorus frog (2017) infection prevalence, all life stages
1	Zero-inflated negative binomial generalized linear model with pond as a random effect and species as the predictor of infection intensity	PRPND009 and PRPND010 bullfrog (2019) and chorus frog (2017) infection intensity, all life stages
2	Compared infection intensities in bullfrog metamorphs to loads shown to cause mortality in experimental settings	2009-2020 bullfrog infection intensity from eight ponds, metamorphs only (adults excluded)
2	Zero-inflated negative binomial generalized linear model with pond as a random effect and life stage as the predictor of infection intensity	PRPND009 and PRPND010 bullfrog (2019) and chorus frog (2017) infection prevalence, larvae and metamorphs
3	Binomial generalized linear model with pond as a random effect; ANOVA to evaluate significance	PRPND009 and PRPND010 bullfrog infection prevalence (2019), all life stages
3	Zero-inflated negative binomial generalized linear model with pond as a random effect and species as the predictor of infection intensity	PRPND009 and PRPND010 bullfrog infection intensity (2019), all life stages

Table 1: Summary of each test and dataset used to address each assumption.

Results

While 2 of the 10 ponds routinely sampled for bullfrogs (BARN and BASSGCP) had near-zero average Bd loads throughout the 11-year study period with only one infected bullfrog at each pond, there was a high degree of variability in Bd infection intensities across the other 8 ponds with sample sizes ranging from 2 to 28 individuals (Fig A8).

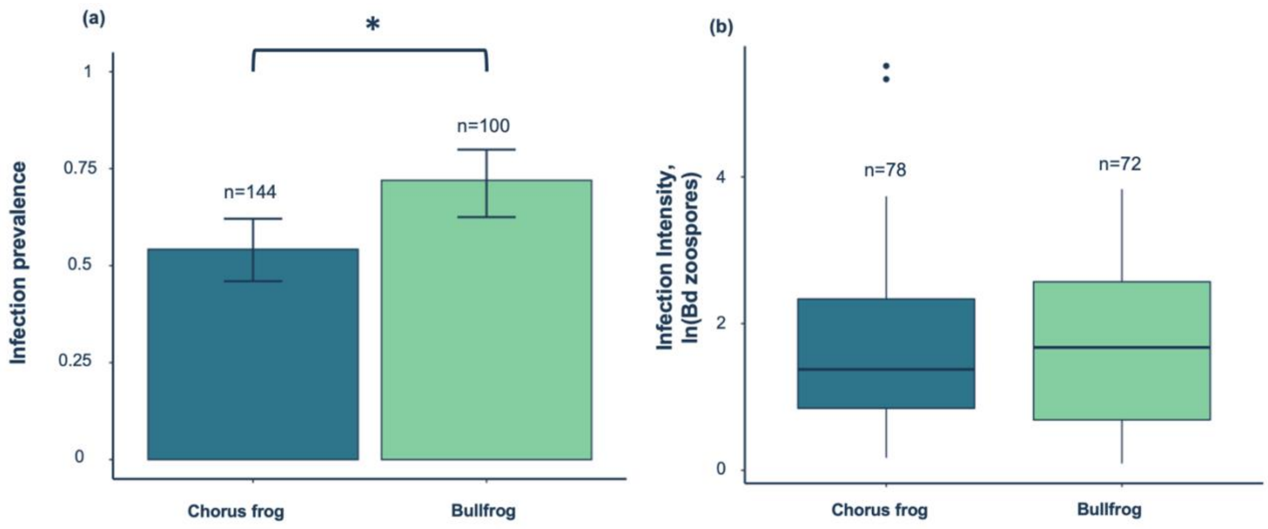

Figure 2: (a) Chorus frogs had significantly lower Bd infection prevalence compared to bullfrogs. (b) There was no significant difference between chorus frog and bullfrog infection intensities.

I found a significant difference (binomial GLMM; p=0.014) in infection prevalence between bullfrog Bd prevalence (62.5%) in 2019 and chorus frog Bd prevalence (46%) in 2017 (Figure 2a). However, there was no significant difference in infection intensities across chorus frogs and bullfrogs (zero-inflated negative binomial GLM; p=0.096). Moreover, I detected a marginally significant (zero-inflated negative binomial GLM; p=0.057) life stage by species interaction; on average, infection intensity decreased by 46% in the transition from tadpoles to metamorphs in bullfrogs, whereas infection intensity decreased by 49% in the same life stage transition in chorus frogs.

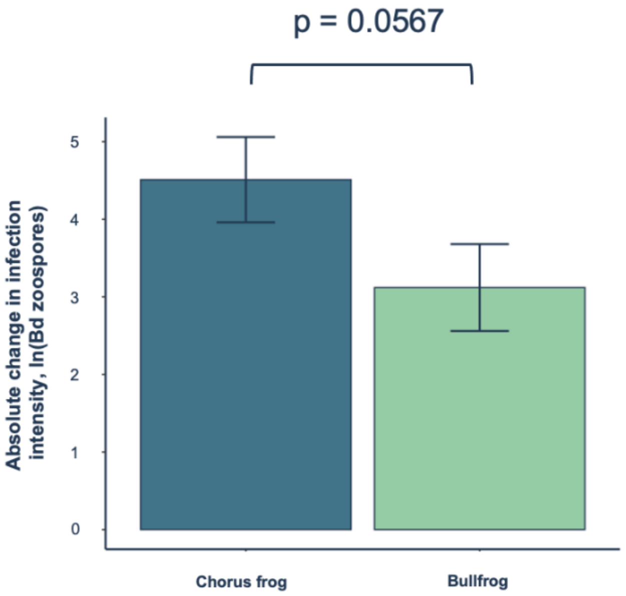

Figure 3: Estimated mean bullfrog infection intensity changed less between larvae and metamorphs than that of chorus frogs.

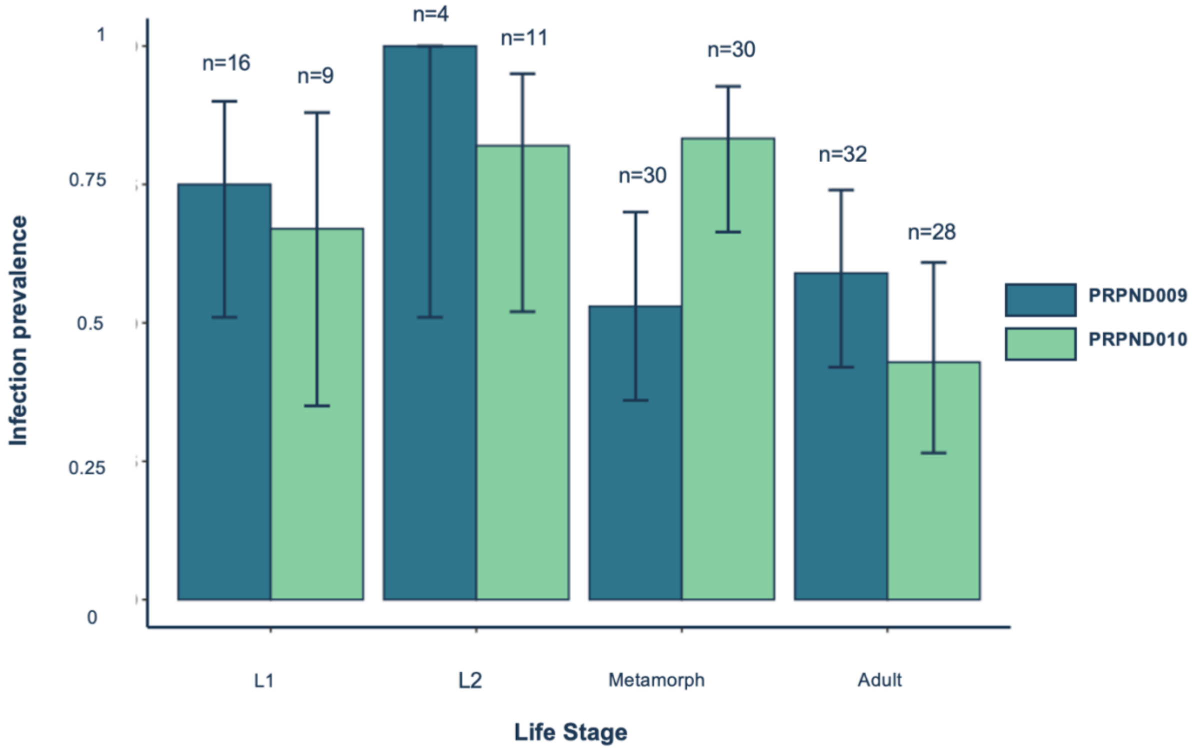

Figure 4: Bullfrog infection prevalence among each life stage in PRPND009 and PRPND010. Though there is little variation in prevalence between L1 and L2, larval bullfrogs seem to have a higher Bd prevalence than metamorphs and adults.

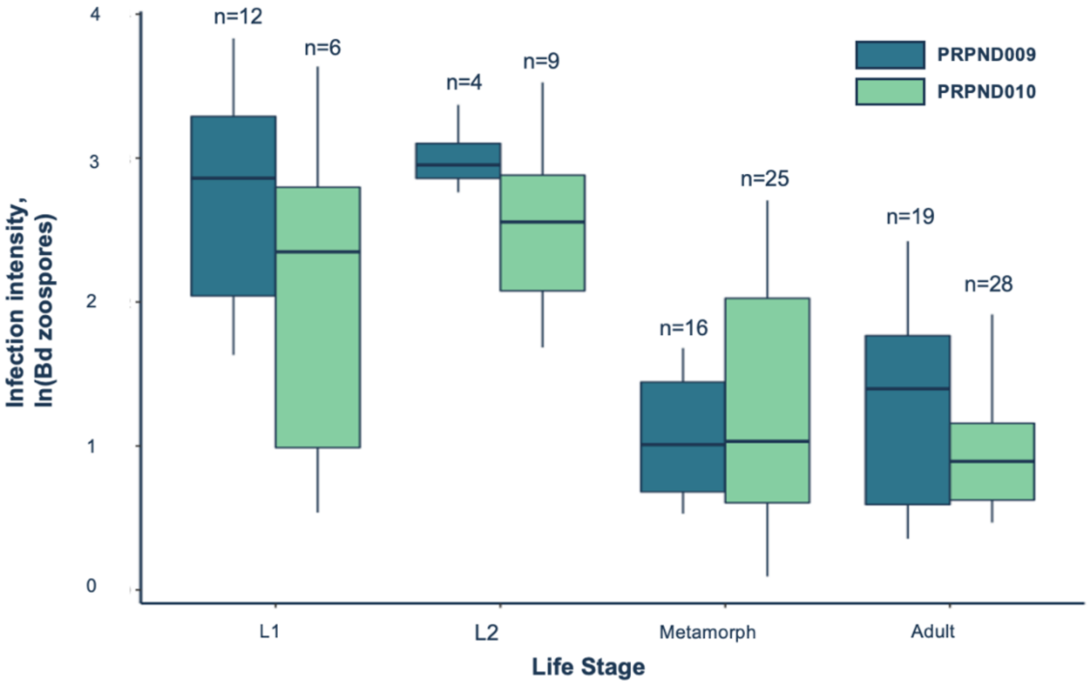

Figure 6: Log-transformed Bd load of bullfrogs across each life stage in ponds PRPND009 and PRPND010. L1 indicates first-year larvae while L2 indicates second-year larvae.

I found no significant difference in infection prevalence (binomial GLM; p = 0.385) or infection intensity (zero-inflated negative binomial GLM; p = 0.642) between year 1 bullfrog larva (L1) and year 2 bullfrog larva (L2).

Discussion

Assumption 1

To investigate Assumption 1, that bullfrogs have high infection prevalence and intensity, I compared bullfrog infection prevalence and intensity in ponds PRPND009 and PRPND010 in 2019 to infection prevalence and intensity in the same two ponds in 2017 to a known Bd reservoir, chorus frogs. While chorus frogs had significantly lower infection prevalence than bullfrogs, there was no significant difference in intensities between the two species despite the existence of two highly infected chorus frogs (estimated zoospore load of 300,000+). It was unexpected that there was no difference, considering the biomass differences of bullfrogs and chorus frogs; chorus frogs can reach a maximum of 2 inches whereas bullfrogs can measure up to 8 inches long (Stebbins and McGinnis 2012). Therefore, bullfrogs have a much larger available surface of keratinized tissue for Bd to infect, reproduce within, and shed zoospores from. In addition, because bullfrogs are much larger than chorus frogs, the distance covered per swab stroke is much larger (i.e., bullfrog legs are much longer than chorus frog legs) even though the number and placement of swabs between species is kept the same. Furthermore, because of their smaller size, chorus frogs are often more plentiful in ponds compared to bullfrogs.

It is important to note, however, that the year is confounded by species in my dataset. Research has shown that environmental variables, such as drought, also influence Bd prevalence.

Because year differs between bullfrog and chorus frog data, the strength of the model in elucidating the influence of species differences on infection prevalence and intensity is limited. In future work, measuring both bullfrog and chorus frog within the same year and pond will control for stochastic environmental and climactic differences. Future studies should additionally aim to quantify bullfrog and chorus frog density in ponds. If infection intensities are not significantly different but chorus frogs are significantly more plentiful (or vice versa), then they could be acting as highly prevalent, highly infected reservoirs of Bd to infect both bullfrogs and other sympatric amphibians. Based on these findings, bullfrogs tentatively meet Assumption 1; while their infection prevalence is significantly greater and intensity not significantly different from that of a known Bd reservoir, chorus frogs, the strength of these inferences is limited because of the differing years in the species observations.

Assumption 2

To investigate Assumption 2 (bullfrogs do not experience mortality from Bd infections), I examined mean Bd infection intensity in bullfrog metamorphs from 2010-2020 (Fig A7). In at least three ponds throughout the study period, average Bd infection intensity far exceeds levels documented to cause mortality in bullfrog metamorphs *in vivo* which ranged from 3.4-123.5 genome equivalents (Gervasi et al. 2013). The average infection loads documented in the field could support a more recent study (Eskew et al. 2015) which argues that the Bd loads documented by Gervasi et al. are too low to cause mortality in bullfrogs, and that higher infection intensities are needed to induce mortality. However, strain differences in genome equivalents as well as virulence might hamper our ability to interpret these differences. In addition, our analysis only focuses on Bd loads shown to induce mortality in metamorphic bullfrogs. Based on our understanding of how Bd is distributed among life stages it is possible that thresholds to induce mortality in adults would be different. Regardless of the threshold, these data do not rule out the possibility that the high Bd loads in bullfrog metamorphs observed in the field are approaching a threshold infection intensity above which disease-induced mortality occurs, supporting the anecdotal evidence of Bd–induced bullfrog mortality within this system (C. Briggs, personal communication). Further *in situ* studies should investigate Bd loads that induce mortality in field settings in a variety of environmental conditions rather than in a stable lab setting as Bd is known to affect hosts differently in different environmental conditions (e.g., temperature).

To further test Assumption 2, I analyzed Bd loads by life stage at PRPND009 and PRPND010 during the 2019 opportunistic survey for bullfrogs and the 2017 survey of chorus frogs in the same ponds to compare. The model showed that there was a marginally significant difference in infection intensity transitions between life stages (larvae and metamorph) in bullfrogs and chorus frogs, with chorus frogs having a slightly larger absolute change in mean infection intensity from larva to metamorph (Fig 3). This statistical inference may suggest that bullfrogs are experiencing less Bd-induced mortality as they undergo metamorphosis compared to chorus frogs, meaning that they are potentially more resistant to Bd-induced mortality, which would support Assumption 2. However, infection distributions show that there are more highly infected bullfrog larvae (both L1 and L2) than there are metamorphs, and considering that Bd mortality is infection intensity-dependent, the larvae with higher loads are potentially disappearing from the metamorph population because they are experiencing mortality as they undergo metamorphosis (Fig A9a).

Based on these findings, it is still unclear whether or not bullfrogs meet Assumption 2, either through Bd-induced mortality in adulthood or through metamorphosis. In order to more accurately estimate if Bd-induced mortality is occurring throughout metamorphosis, future studies should indirectly estimate bullfrog mortality by comparing the distribution of infection intensities between larvae and metamorphs using a negative binomial model to test for truncation. Because truncated distributions contains fewer highly infected individuals than non-truncated distributions, this analysis would determine if, in each life stage, highly infected individuals are being removed from the population through mortality and therefore not being detected through sampling methods. As the data currently stands, this analysis is not feasible because the model assumes that while distribution of infection intensities might change across life stages, mean infection intensity does not. Though this might be the case, we currently do not know if mean infection intensity stays the same across life stages, which is a key assumption within the model.

Assumption 3

In investigating Assumption 3 (overwintering bullfrog tadpoles are able to sustain Bd infections and therefore serve as a between-season maintenance host), I found that both L1 and L2 have similarly high infection intensities, indicating that larvae could act as between-season maintenance hosts.

Regardless of the reason larvae are able to maintain significantly higher Bd loads than metamorphs or adults, both L1 and L2 have similarly high infection intensities, indicating that larvae could act as between-season maintenance hosts. In other words, as infected L1 larvae overwinter in ponds, they could introduce Bd to naïve tadpoles. Although L2 loads are slightly higher than L1 loads, this difference is non-significant, and it is therefore difficult to draw any conclusion on whether or not Bd loads are aggregating in the overwintering period between larvae's first and second years. Further studies that track within and between-season larval loads in bullfrogs using mark-recapture methods will be able to determine if Bd loads are higher in L2 larvae than in L1 larvae. Based on these findings, bullfrogs meet Assumption 3 and are potentially important in maintaining infections in ponds between seasons.

Conclusions

This study used 11 years of annual Bd sampling data from 9 ponds in California's Bay Area (2010-2020) as well as a 2019 intensive opportunistic survey of bullfrogs in two ponds (PRPND009 and PRPND010) and a 2017 survey of chorus frogs in the same ponds in order to directly and indirectly analyze the role of bullfrogs in Bd transmission. Specifically, I tested whether bullfrogs meet the three following assumptions of competent reservoirs: (1) bullfrogs have high infection prevalence and intensity, (2) bullfrogs do not experience mortality from Bd infections, and (3) overwintering bullfrog tadpoles are able to sustain Bd infections and therefore serve as a between-season maintenance host.

The results partially support Assumption 1 in that bullfrog infection prevalence was significantly greater than chorus frog infection prevalence, though there was no significant difference in infection intensity which was unexpected considering the large biomass differences between bullfrogs and chorus frogs. Bullfrog Bd loads encountered in the field surpassed Bd loads shown to induce mortality in experimental settings in bullfrogs, supporting anecdotal evidence that bullfrogs experienced Bd-induced mortality in the field setting and refuting Assumption 2. However, my results suggest that Bd-induced mortality may be occurring less in bullfrogs than in chorus frogs during metamorphosis. Finally, high infection intensities in both first- and second-year larvae indicate that larvae could act as an overwintering maintenance host of Bd, supporting Assumption 3.

Bd infection status is not static, though, and transmission can occur between species. Because of the reproductive cycle of Bd, zoospores released into the environment can both reinfect the individual, leading to few individuals with high loads (i.e., parasite aggregation) or swim through the pond to infect other individuals. Because of these transmission dynamics, in ponds where both bullfrogs and chorus frogs exist and experience infection, both species could be infecting and reinfecting each other from season to season. Therefore, understanding Bd infection throughout time in bullfrogs is an important step to understanding the synergistic effects of both species living within the same systems.

Some facets of Bd's disease ecology in bullfrogs—such as potential to be a between-season maintenance host—indicate that, in this system, bullfrogs might be serving as a competent reservoir in this specific mechanism of transmission. But together, the findings of my thesis provide mixed support for the idea that bullfrogs are highly competent reservoirs of Bd, calling into question whether culling bullfrogs would be an effective strategy to reduce Bd outbreaks. Understanding the role bullfrogs play as Bd reservoirs is just one crucial step towards informing effective, targeted, and cost-efficient conservation strategies.

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Appendix

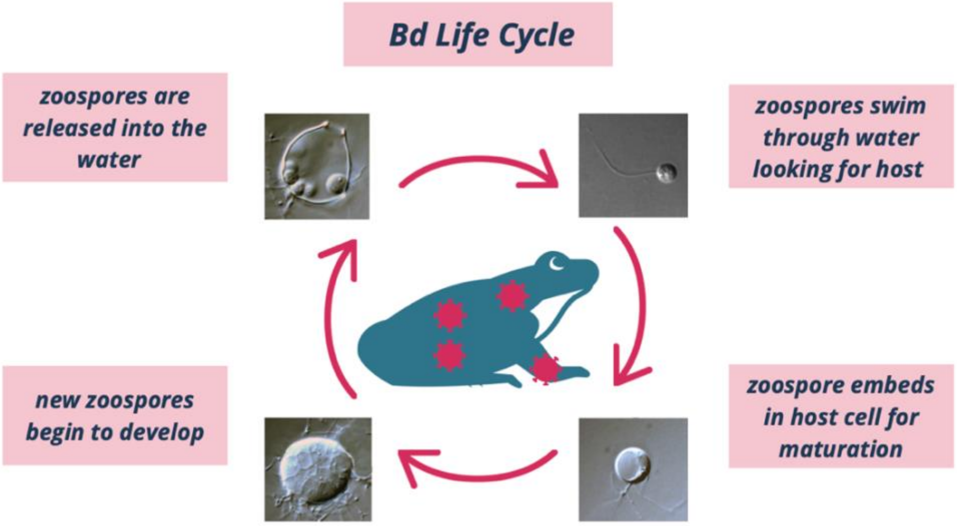

Figure 7: Simplified lifecycle of Bd. Photos taken from Rosenblum et al. 2010.

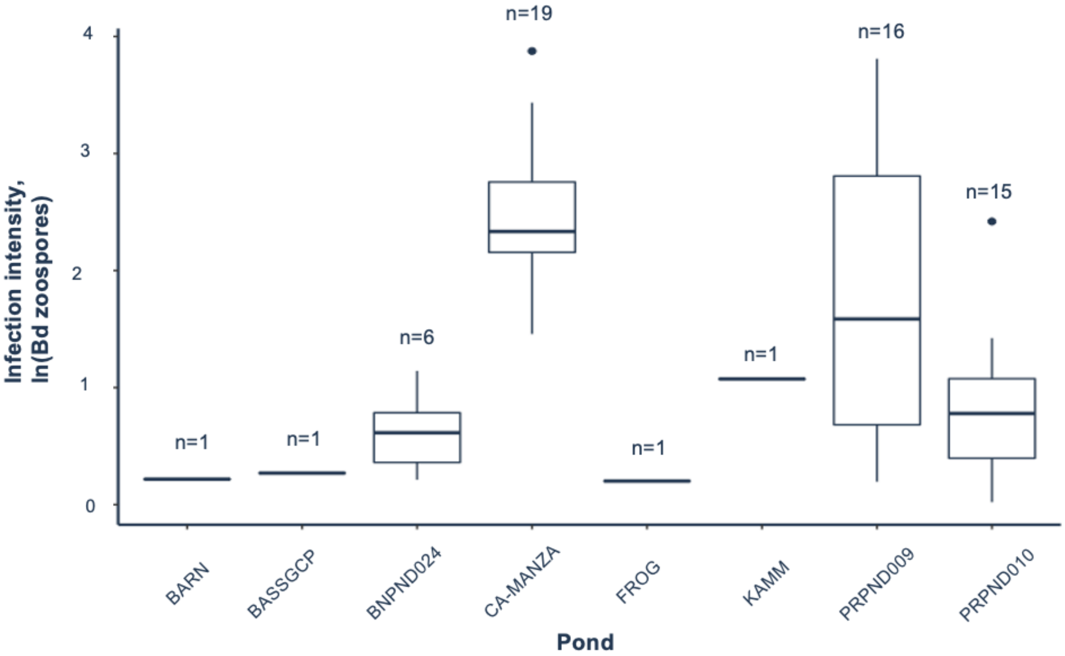

Figure 8: Log-transformed average Bd infection intensity (zoospore load) from 2010-2020.

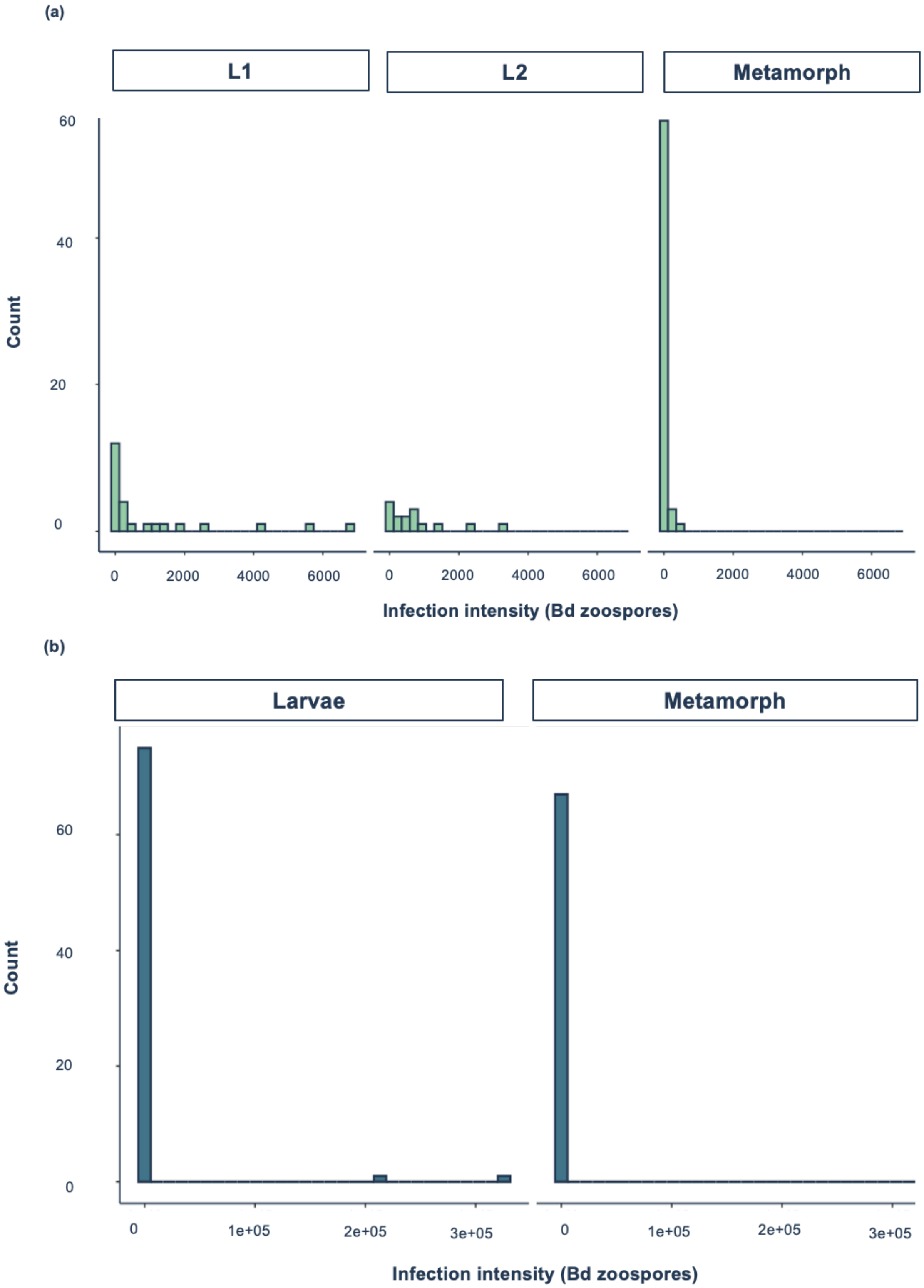

Figure 9: (a) Histogram of Bd infection intensity by life stage in bullfrogs from the opportunistic 2019 survey of PRPND009 and PRPND010. The overwhelming majority of metamorphs have Bd loads of 0 whereas L1 and L2 have loads up to 6,000+ zoospores. (b) Histogram of Bd infection intensities by life stage in chorus frogs across 10 ponds in 2017. Most individuals have loads of 0 while a few highly infected individuals have loads up to 300,000.