THESIS

TESTING NOVEL METHODS TO ASSESS HOW HABITAT ASSOCIATIONS AND INVASIVE SPECIES INFLUENCE NORTHERN LEOPARD FROG (*LITHOBATES PIPIENS*) PERSISTENCE IN NORTHERN COLORADO

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Waverly B. Davis

Department of Fish, Wildlife, and Conservation Biology

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Master's committee:

Advisor: Larissa Bailey

Ellen Wohl
Dan Preston

ABSTRACT

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Land cover change, invasive species, and disease are among the leading causes of amphibian decline worldwide but are often studied in isolation and over relatively short time scales. Understanding how species respond over time to interacting stressors is essential to inform conservation decisions. The northern leopard frog (*Lithobates pipiens*) is a classic example of an amphibian experiencing declines related to these stressors: populations in the western U.S. are threatened by interactions among land cover change, the invasive American bullfrog (*Lithobates catesbeianus*), and the fungal pathogen *Bd* (*Batrachochytrium dendrobatidis*). In northeastern Colorado, northern leopard frog distribution has declined for decades, while bullfrog populations have expanded. American bullfrogs, native to the eastern U.S., threaten northern leopard frog persistence as a predator, competitor, and asymptomatic carrier of the fungal pathogen *Bd*. Land cover and habitat have changed substantially in northeastern Colorado in recent decades, resulting in natural wetland loss, often through the replacement of ephemeral ponds and wetlands with artificial permanent water bodies that favor invasive species encroachment.

Understanding how northern leopard frog distribution is influenced by interacting stressors over time relies on effective monitoring programs. However, effective monitoring is challenging because declining native and recently arriving invasive taxa are inherently difficult

to detect. Unaccounted for variation in species' detection across space (e.g., locations) or time can bias our understanding of how species distributions (e.g., occurrence, persistence) are influenced by interacting stressors over time. Comparing species' detection probabilities across multiple detection methods enables the optimization of monitoring protocols that can then be used to address hypotheses related to species declines and associated conservation actions.

The first chapter of this thesis compares the efficacy of three amphibian detection methods—eDNA, automated acoustic recorders, and visual encounter surveys— for native northern leopard frogs and invasive bullfrogs. We explored hypotheses related to how environmental (e.g., temperature, pH) and survey-specific (e.g., survey date, search time) predictors influence detection probabilities for each species and method. We collected detection-nondetection information for both amphibian species at 38 potential breeding sites in Colorado's South Platte River Basin and used an occupancy modeling approach to estimate resulting detection probabilities. We hypothesized that detection probabilities for all three methods would vary with environmental factors, some survey-specific considerations, and be associated with species' distinct life histories and habitat preferences. We found that northern leopard frog detection probability was higher for visual encounter surveys and eDNA than for automated acoustic recorders, where detection was lower but peaked in early spring. Northern leopard frog eDNA detection was highest in neutral pH conditions and visual encounter survey detection increased slightly with longer search times. Bullfrog eDNA and automated acoustic detection were higher than that of northern leopard frogs, with comparable visual encounter detection probabilities for both species. Bullfrog eDNA and visual detection increased in warmer water temperatures and acoustic detection increased later in the season. These results aligned with each species' breeding habitat preferences, behaviors, and seasonal emergence. Using our results, we

provided examples of how to combine methods to create optimized monitoring programs for given objectives.

The second chapter of this thesis explores how northern leopard frog persistence is influenced by interactions among habitat features and bullfrog presence. To predict species' responses to interacting threats at a landscape-level, researchers benefit from estimating persistence (or conversely, local extirpation), and colonization. Because the effects of invading species on native species persistence occurs over broad time scales (e.g., decades) at a landscape level, understanding northern leopard frog response to bullfrog invasion benefits from long-term monitoring data, which does not currently exist in our study area. Instead, we conditioned on previous occurrence (detection only) records of northern leopard frogs to estimate persistence by visually resurveying those previously occupied sites. We recorded the contemporary presence of bullfrogs and habitat features (e.g., emergent vegetation, canopy cover, surrounding land cover) to explore hypotheses related to how northern leopard frog persistence was influenced by interactions among bullfrog presence and habitat. We used a two-species static occupancy approach to investigate hypotheses related to bullfrog presence and habitat features. We predicted that bullfrog presence and anthropogenic land cover (urban, agricultural) would interact to decrease northern leopard frog persistence while bullfrog absence and grassland cover would increase persistence probability. We expected multiple stressors (e.g., bullfrogs and low emergent vegetation) would reduce persistence and beneficial habitat features (e.g., high emergent vegetation) would mitigate the negative effects of bullfrog presence. Contrary to expectations, bullfrog presence was associated with northern leopard frog persistence, suggesting bullfrogs may colonize remaining northern leopard frog habitats. Northern leopard frogs were more likely to persist at sites with bullfrogs when surrounding grassland cover was high; thus, grassland mediates the potential negative effects of invasive bullfrogs. Ultimately, long term detection-nondetection information for both the native and invasive species are needed to predict whether northern leopard frogs *will persist* in the future, but our results represent a time-specific description of where they *have persisted*. Our findings suggest that grassland conservation and restoration is an important tool to support the persistence of northern leopard frogs amidst bullfrog encroachment.

Northern leopard frog declines have been pervasive in northeastern Colorado, prompting an urgent need for better informed conservation efforts and monitoring programs. Monitoring methods can be combined in novel ways to achieve desired species' detection probabilities, yielding improved estimates of their distributions/occurrences and better-informed conservation initiatives. Conservation efforts for northern leopard frogs should consider the important role of surrounding grassland for northern leopard frog persistence, especially in areas where bullfrogs co-occur. In this thesis, we provide suggestions on how to improve monitoring for northern leopard frogs and bullfrogs, evaluate how northern leopard frog persistence is influenced by interacting threats, and suggest future directions for management and conservation actions.

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CHAPTER ONE: LIFE HISTORY TRAITS INFLUENCE METHOD-SPECIFIC DETECTION OF NATIVE AND INVASIVE AMPHIBIANS

INTRODUCTION

Conservation efforts rely on efficient and effective monitoring programs to address management objectives and differentiate among *a priori* hypotheses about factors influencing the system of interest. Importantly, for most wild vertebrate species, monitoring programs must address both variation in species' detectability and spatial sampling to achieve unbiased estimates of target state variables (e.g., species distribution). Many researchers have criticized monitoring programs for their failure to state clear objectives and inadequate attention to sources of bias such as variation in detectability (Mattfeldt et al., 2009; Nichols and Williams, 2006; Yoccoz et al., 2001). Imperfect detection occurs when the species is present at the site but goes undetected during the survey, creating uncertainty in whether the species was present and undetected, or truly absent (MacKenzie et al., 2002). Unmodeled variation in species detection across space (e.g., locations) or time can bias monitoring state variables. In a recent review, Bennett et al. (2024) found that only 6% of scientific studies published detection estimates for declining taxa in the U.S., emphasizing the need to estimate detectability among species. Accordingly, guidance on how to maximize detection probability and thus minimize bias in resulting state variables is essential but oftentimes lacking in monitoring programs. Additionally, early detection of invasive species is paramount to successful management or containment during a period when invasive densities are low (Prior et al., 2018). Thus, estimating

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distributional metrics for both declining species and colonizing invasive species is challenging and prone to detection bias.

Commonly used population metrics such as abundance, survival, reproduction, and population growth require identification of unique individuals, which is especially challenging for declining or invasive species. Occupancy is a favored state variable in these cases because it does not require individual identification and can provide insight into spatial and temporal patterns of species occurrence and persistence. Although occupancy can be defined numerous ways depending on research objectives, it commonly denotes the proportion of sites occupied by a focal species. Contemporary occupancy models allow investigators to decouple the state variable (occupancy or species occurrence) from the observational process (i.e., detection probability, given occurrence). Studies that incorporate occupancy estimation and modeling have been used to identify priority areas for conservation initiatives, evaluate management decisions, and understand biases in detection probabilities (Moor et al., 2022; Ribeiro et al., 2022; Steenweg et al., 2019).

Since MacKenzie et al.'s (2002) seminal paper describing occupancy modeling, an occupancy framework has been commonly applied to amphibians and other cryptic species to understand species distributions, evaluate conservation priorities, and investigate detection biases (Bailey et al., 2019; Gould et al., 2012; Petitot et al., 2014). Worldwide, over 40% of amphibian species are imperiled (Luedtke et al., 2023) and northern leopard frogs (*Lithobates pipiens*) are a classic example: historically one of the most widespread frogs in North America, northern leopard frogs have experienced dramatic range reductions in recent decades, specifically in the Intermountain West (Corn and Fogleman, 1984; Johnson et al., 2011; Rogers and Peacock, 2012). Declines have been attributed to habitat alteration (Johnson et al., 2011), disease

(Voordouw et al., 2010), and the invasive American bullfrog (*Lithobates catesbeianus*) (Rogers and Peacock, 2012). In the western part of the northern leopard frog range, invasive bullfrogs threaten leopard frog persistence through their role as a predator and disease reservoir (Ficetola et al., 2007). Conservation concerns have led to regional programs with monitoring initiatives targeting northern leopard frogs and bullfrogs, but these initiatives have been hampered by a lack of clear objectives, sporadic efforts, and different survey approaches with varying levels of success. In recent years, growing support for coordinated conservation actions addressing northern leopard frog declines in the Intermountain West has highlighted a need for better monitoring programs. Many potential conservation decisions rely on knowledge of the locations and local abundance of remaining northern leopard frog populations and recently established invasive bullfrog populations, which are discerned through our ability to detect these species (Bennett et al., 2024). Hence, estimating detectability and incorporating estimates into these decisions is vital to allocate survey effort, improve management outcomes, and evaluate the effectiveness of any chosen conservation action (Bennett et al., 2024).

Our research focused on estimating species-specific detection probability and understanding the factors that influence detection using different survey methods. Specifically, we assessed the efficacy of three detection methods – environmental DNA collection (eDNA), visual encounter surveys (VES) and automated acoustic recorders (AAR) – for two focal amphibians: native northern leopard frogs and invasive bullfrogs. These three survey methods are commonly employed for a variety of amphibian taxa, and each has its own strengths. Benefits of eDNA sampling include its ability to detect low density populations and that it does not require essential species identification skills (Keck et al., 2022; Pilliod et al., 2013). Visual encounter surveys (VES) require observers to search habitat for the species of interest but can identify different life stages, disease, and malformations. Automated acoustic recorders (AAR) passively record amphibian breeding calls and can continuously sample for long periods of time without disturbing potentially fragile habitat (Sugai et al., 2019).

We used detection-nondetection information for both species (northern leopard frogs and bullfrogs) at 38 sites to understand how environmental and survey-specific characteristics affect species- and method-specific detection probabilities (Table 1). We hypothesized that detection probabilities for all three methods would be influenced by environmental factors, some survey-specific considerations, and be associated with species' distinct life histories and habitat preferences. Subsequently, this information can be used to optimize sampling effort for a given study or monitoring objective(s).

MATERIALS AND METHODS

Study System and Species

Our study was conducted in the Colorado portion of the South Platte River Basin, USA (Figure 1). To investigate factors influencing species-specific detection probability, we selected sites likely to contain both northern leopard frogs and bullfrogs. Northern leopard frog populations have been documented in the basin, and American bullfrogs are considered widespread (Johnson et al., 2011; Peterson et al., 2013). We compiled northern leopard frog records from local nonprofits, city, state, and county agencies, and Citizen Science platforms. We randomly selected a subset of 38 sites, including lentic and lotic habitats, with observations from at least three decades, including the most recent decade (2011-2021), rationalizing that these sites likely supported one or both of our target species.

Field Survey Methods

We conducted eDNA and visual encounter surveys (VES) at all selected sites (n=38) and deployed automated acoustic recorders at a subset (n=19), due to equipment limitations. Sites were visited twice in a given year, either in 2022 or 2023. Visits were separated by at least one month and timed to target multiple life stages for each species. During each visit we surveyed up to 500 meters of bank habitat using eDNA and VES (Figure 2).

We used the Smith-Root eDNA sampler system for all eDNA collection, which supports sampling from the bank (Thomas et al., 2018). During each site visit, we collected four eDNA field samples starting at the downstream end of lotic sites to limit potential movement of eDNA to other sampling locations. For lentic sites, collectors began at a pre-selected point and moved clockwise. All samples were evenly spaced throughout the sites using satellite imagery. We filtered up to five liters of water through 47 mm filters and recorded the amount of water filtered (liters). To avoid contamination, we followed a strict protocol developed by Colorado Parks and Wildlife designed to minimize false positive and false negative samples. One person recorded data and the other collected samples with no direct contact between the two. All equipment that contacted site water was thoroughly sanitized, rinsed, and dried between sites using a 10% bleach solution. At each site, we also collected one field negative control of dechlorinated water filtered to the average number of liters collected at the site. If negative control samples tested positive for northern leopard frog or bullfrog DNA, we eliminated all other samples collected on the same survey event where contamination was observed. All eDNA samples were kept on ice in the field and stored at approximately -20 ºC in a sterile freezer for 3 months before being processed individually by Pisces Molecular LLC in Boulder, Colorado, USA (see Supplementary Material S1 for details). After eDNA collection, site water temperature, air temperature, and pH were recorded using the Hanna combo tester. Covariates used to model potential variation in eDNA detection probability included Julian date (Julian), liters of water filtered per sample (Liters), site pH (pH), site water temperature (Wtemp), and air temperature (Atemp; Table 1).

During each site visit we also conducted two independent visual encounter surveys (VES), where each survey was conducted by a separate team of 1-2 observers. Visual encounter surveys began 40 minutes after eDNA sampling, started from the same location, and followed the same trajectory through the streambank or shoreline habitat. The two VES were separated by at least 20 minutes to ensure independence. Observers visually inspected all suitable habitat while conducting dip net sweeps for amphibian larvae. Observers recorded encountered species, life stage, and covariates thought to influence species- and method-specific detection probabilities (Table 1). Species observations were recorded as certain or uncertain and only those certain identifications were included in the occupancy analysis. Covariates used to model potential variation in VES detection probability included time spent searching (SearchT), the number of observers (ObsCount), Julian date (Julian), water temperature (Wtemp), and air temperature (Atemp; Table 1).

Automated acoustic recorders (AAR) were deployed at a subset of sites (n=19). We programmed recorders to sample a 3-minute interval every 27 minutes for the entire season (April – October). Recorders were deployed near ideal frog breeding habitat, based on supporting literature (Pope et al., 2000; Smith and Keinath, 2007). Specifically, we installed units 1 meter above ground on a t-post at unshaded lentic locations with emergent vegetation and ample shallows. At a subset of the larger lentic sites (> 300 meters radius; n=6), we placed an additional recorder to capture vocalizing anurans and moved it bi-weekly to different potential breeding areas within the site. We compared detection probabilities from these ‘roving units’

(ROV) to their stationary counterparts and detection probabilities at sites with two units to those with one. We also explored how AAR detection probabilities varied with Julian date (Julian; Table 1). Acoustic data was sorted and processed using Kaleidoscope Pro (version 5.4.2, Wildlife Acoustics) to extract breeding call detections for each species in each week (see Supplementary Material S2 for details).

Detection Hypotheses: Occupancy Analysis

We hypothesized that detection probabilities for all three methods would be influenced by selected environmental factors with some method-specific considerations (Table 1). We expected that species-specific detection probabilities for all methods would be influenced by Julian date as our two target species have different breeding and developmental life histories. In our study area, northern leopard frogs are early spring breeders and typically emerge to call in April and May whereas bullfrogs usually begin calling in July (Bury and Whelan, 1985; Larson, 2004). Accordingly, we expect acoustic recorder detection probabilities to decline with Julian date for northern leopard frogs but increase with date for bullfrogs (Table 1). As eggs hatch and tadpoles become larger and more active, we expect the detection probabilities for both species to increase with Julian date for visual or eDNA surveys (Table 1) (Buxton et al., 2017). Similarly, both species' activity increases with temperature, thus detection probabilities for both visual and eDNA surveys are expected to increase with water (or air) temperature (Table 1). Because DNA may be less abundant in acidic (<5; Schlichter, 1981), and perhaps extreme basic (> 10; Fominykh 2008), environments due to reduced amphibian tolerance of these conditions, we also expect eDNA detection probabilities to vary with pH (Fominykh, 2008; Schlichter, 1981). Finally, detection probability for each method might be influenced by characteristics specific to each method. If DNA is dispersed in the water column, eDNA detection should increase for samples with a larger volume of filtered water. Visual encounter surveys conducted by two observers may have higher detection probabilities compared to those with only a single observer and longer survey search times may yield higher detection probabilities. Acoustic recorders that are moved throughout the site during the season (roving units) could have higher mean detection probabilities as they sample more than one location within an occupied site. Sites with both stationary and roving units should have higher cumulative detection probabilities than sites with only stationary units.

We used a single-season (static) occupancy approach to test our hypotheses regarding method- and species-specific detection probabilities (MacKenzie et al., 2002). Detection histories were compiled for each species and site and indicated whether the species (northern leopard frog or bullfrog) was detected (1) and not detected (0) during a given survey. Detection histories included four independent visual encounter surveys (2 per visit), eight eDNA samples (4 per visit) and a subset of sites included up to 28 weekly acoustic surveys. We analyzed detection histories for each species separately and fit a series of models to explore how environmental (pH, Wtemp, Atemp) and survey-specific (Julian, Liters, SearchT, ObsCount) covariates influenced species' detection probabilities. Due to sample size, we tested univariate relationships and only considered an additive effect of Julian date and unit type (roving vs. stationary) for AAR detection probability.

We used a secondary candidate set model building strategy to develop and fit our candidate models (Morin et al., 2020). Specifically, we fit models representing hypotheses associated with detection probability for a given method (e.g., p_{VES},), while retaining a constant structure on the detection probability for the other two methods (e.g., p_{eDNA}, or p_{AAR}) and occupancy. Because we selected sites with recent observations of northern leopard frogs and a high likelihood of bullfrogs occurring, we used a constant occupancy structure ($\psi(.)$) for each species in all models. We identified detection covariate(s) with substantial support for each method as those within ΔAICc <5 and included all combinations of supported covariate structures in our final combined model set (Table 2 and Table 3; Supplementary Material S3 and S4). We fit all models in program MARK version 6.2 (White and Burnham, 1999) and used Akaike Information Criterion and associated model weights to evaluate model performance (Anderson and Burnham, 2002).

We report average detection probabilities for each method and species using the constant model structure for each parameter, $p_{VES}(.)$ $p_{eDNA}(.)$ $p_{AAR}(.)$ $\psi(.)$ and coefficient estimates from the best supported model that includes the specified covariate. Additionally, we calculated the cumulative detection probability for each species and method as $p^{*}=1-(1-p)^{n}$ where p^{*} is the probability of detecting the species at least once over n samples/surveys/weeks and p is the probability of detection for a single sample/survey/week. We used resulting values to determine the sampling effort required to detect a species, given the site is occupied, with 90-99% confidence.

RESULTS

All three methods (eDNA, VES, AAR) were compared at 19 sites and northern leopard frogs were detected at 15 of them. AARs detected northern leopard frogs at approximately 50% (n=8) of the known occupied sites, while eDNA and VES surveys detected the species at 87% of sites (n=13), although not always at the same sites. For sites with bullfrog detections (n=8), AARs and VES detected the species at 63% of these sites (n=5), whereas eDNA detected the species at all but one site (n=7).

When considering all sampled sites (n=38) and pooling detections across methods, northern leopard frogs were detected at 24 sites (naïve occupancy = 0.63), while bullfrogs were detected at 18 (naïve occupancy = 0.45). Interestingly, northern leopard frogs and bullfrogs co-occurred at 13 sites (34%), despite bullfrogs being considered a threat to northern leopard frog persistence (Johnson et al., 2011). Overall, eDNA and VES performed similarly for each species: eDNA detected northern leopard frogs at 53% (n=20) and VES at 58% (n=22) of sites. For bullfrogs, eDNA detected the species at 37% (n=14) and VES also at 37% (n=14) of sites.

Of our 76 negative control eDNA samples, two came back positive for bullfrog DNA and none came back positive for northern leopard frog DNA. The two positive control samples were collected during the first week of eDNA collection and on the same day, but at two different sites. All 8 samples from those two eDNA surveys associated with the contaminated controls were removed from analysis.

Species-specific occupancy estimates, from the constant model, $p_{VES}(.)\ p_{eDNA}(.)\ p_{AAR}(.)\ \psi(.)$, were identical to naïve estimates for northern leopard frogs ($\hat{\psi}$= 0.63 (95% Confidence Interval; CI: 0.47 to 0.77)) and bullfrogs ($\hat{\psi}$ = 0.45 (95% CI: 0.30 to 0.60)), indicating cumulative detection probability is close to 1 by using a combination of detection methods at our sampled sites over the course of the season.

eDNA

The average probability of detecting our target species in a single eDNA sample, given the site was occupied, was 0.46 (95% CI: 0.39 to 0.53) for northern leopard frogs and 0.53 (95% CI: 0.45 to 0.62) for bullfrogs (Table 4). eDNA detection probabilities for northern leopard frogs were strongly influenced by environmental conditions and were lowest at occupied sites with slightly basic pH values and highest in neutral conditions (Figure 3A, Table 2, Supplementary Table S3.1). Although detection was highest in neutral ranges as predicted, none of our sites were extremely acidic (pH < 5) or basic (pH > 10). eDNA detection for northern leopard frogs decreased slightly across Julian date, though the effect was imprecise. Neither water nor air temperatures were supported as influencing detection probabilities, despite their known relationship with the emergence and development of tadpoles (Supplementary Table S3.1; Sommers et al., 2018). The amount of water filtered had a slight positive effect on eDNA detection probability, but the effect was imprecise (Supplementary Table S3.1).

In contrast, eDNA detection probabilities for bullfrogs were influenced by water temperature and the amount of water filtered, but not by pH (Table 3, Supplementary Table S3.5). Consistent with our expectation, eDNA detection for bullfrogs increased with warmer water temperatures ($\hat{\beta}$= 0.16, SE = 0.05; Figure 3B) and the amount of water filtered ($\hat{\beta}$= 0.46, SE= 0.15; Figure 3C). For bullfrogs, the probability of detection via eDNA sampling increased by approximately 0.10 for each additional liter of water filtered (Figure 3C).

Visual Encounter Surveys

The average probability of detecting our target species during a visual encounter survey, given the site was occupied, was comparable for both species: 0.67 (95% CI: 0.56 to 0.77) for northern leopard frogs and 0.65 (95% CI: 0.52 to 0.77) for bullfrogs (Table 4). We found some evidence that VES detection probabilities for northern leopard frogs increased as observers spent longer searching the habitat ($\hat{\beta}$= 0.02, SE= 0.01; Figure 4A; Table 2; Supplementary Table S3.2), though the estimated effect was somewhat imprecise. For example, the detection probability for a single observer searching for 25 minutes was ~ 0.60, but if the observer searched for 50 minutes, detection probability was ~ 0.70 (Figure 4A). Interestingly, the number of observers per survey did not influence northern leopard frog detection probability (Supplementary Table S3.2). Contrary to our expectations, environmental conditions (Wtemp, Atemp) and survey date (Julian) did not influence northern leopard frog VES detection probability (Supplementary Table S3.2).

In contrast, environmental conditions influenced VES detection probability of bullfrogs, but survey-specific covariates did not (Table 3; Supplementary Table S3.6). The probability of visually detecting a bullfrog increased with warmer water temperatures ($\hat{\beta}$= 0.20, SE= 0.09; Figure 4B). In fact, detection probability increased by ~ 0.55 as water temperature increased from 15ºC to 25ºC. Despite our expectations, survey-specific variables (Julian, SearchT, ObsCount) were not well supported as predictors of bullfrog VES detection probability, emphasizing the detection disparities between the two species (Supplementary Table S3.6).

Automated Acoustic Recorders

Our acoustic units targeted two species with asynchronous breeding activity. Non-detections outside northern leopard frog or bullfrog seasonal activity resulted in a low average weekly detection probability at occupied sites: 0.14 (95% CI: 0.11 to 0.19) for northern leopard frogs and 0.22 (95% CI: 0.17 to 0.29) for bullfrogs (Table 4). Therefore, we also report monthly average and cumulative detection probabilities, as they describe the probability of detecting calls during peak breeding. As hypothesized, northern leopard frog acoustic detections decreased throughout the summer, with the highest weekly detection probability in April and then declining throughout the season ($\hat{\beta}$ = -0.016, SE= 0.004; Figure 5A; Supplementary Table S3.3). Deploying AARs during peak breeding windows can improve cumulative detection probabilities considerably over a month. For instance, if AARs sampled for a month starting in early April, the probability of capturing northern leopard frog breeding calls was 0.81 (95% CI: 0.64 to 0.93); however, if the unit sampled at the end of the season during the month of July, detection dropped by approximately half to 0.40 (95% CI: 0.28 to 0.52).

Bullfrog detection probabilities also varied with Julian date, but the weekly probability of detecting a breeding call increased throughout the summer ($\hat{\beta}$ = 0.02, SE= 0.004; Figure 5B;

Supplementary Table S3.7). Again, deploying units during peak bullfrog breeding improved detection estimates over a month of sampling. If units were deployed in the first week of August and sampled for a month, there would be a ~ 0.75 cumulative probability of detection compared to ~ 0.25 if units were deployed for the month of May.

For northern leopard frogs, acoustic units that rotated to different potential breeding pools within a site had slightly lower detection probabilities relative to the stationary units. For example, during peak detection months for northern leopard frogs (April) stationary units had monthly average detection probabilities approximately 0.10 higher than paired roving units. For bullfrogs, roving units had slightly higher detection probabilities compared to stationary units. However, our results were imprecise as our sample size of roving units was small (n=6). Deploying multiple units at a site improved overall estimates of detection, as sites with both roving and stationary units had a higher cumulative detection probability during peak breeding months than sites with only stationary units. For northern leopard frogs, combined multi-unit monthly detection probability for peak calling during the month of April was ~ 0.90 and a single, stationary unit detection was ~ 0.80. For bullfrogs sampled during peak calling periods in October, combined monthly multi-unit detection probability was ~ 0.95 while a single, stationary unit detection was ~ 0.75.

DISCUSSION

We investigated how detection probabilities for each species and method were influenced by survey-specific and environmental covariates for native northern leopard frogs and invasive American bullfrogs. We found detection probabilities varied considerably among methods and species, a result that is consistent with other studies (Moss et al., 2022; Randall et al., 2023; Wikston et al., 2023). Detection probabilities were highest for visual encounter surveys, followed by eDNA and then automated acoustic recorders for both species (Table 4). eDNA and acoustic detection for bullfrogs was higher than for northern leopard frogs and visual encounter survey detection was comparable for both species (Table 4). Differences in detection between the species were predicted by environmental covariates associated with life history traits while those associated with the observational process were less influential (e.g., search time, number of observers) (Knutson et al., 2018).

The availability of eDNA for detection depends on both the quantity of DNA released by the species and environmental factors influencing its degradation rate (Barnes et al., 2014; Yates et al., 2021), thus we anticipate larger quantities of DNA are released in ideal breeding conditions. We found eDNA detection was highest for northern leopard frogs in neutral pH ranges (Figure 3A), representing their habitat preferences (Smith and Keinath, 2007). Unmodeled variables influencing DNA availability or degradation could contribute to lower detection in somewhat basic conditions (pH 8-9.8). This relationship would benefit from further investigation, as mechanisms influencing DNA availability and degradation are interacting and complex (Strickler et al., 2015). For bullfrogs, water temperature was a well-supported predictor of both eDNA and visual encounter survey detection and reflected their preferences for warm aquatic habitats (Bury and Whelan, 1985; Lillywhite, 1970) (Figure 3B). DNA also degrades faster at warmer water temperatures (Strickler et al., 2015), but this decline can be offset by higher amphibian activity levels (Bedwell and Goldberg, 2020; Buxton et al., 2017; De Souza et al., 2016), which increases availability of eDNA. Visual survey detection probability also increased with temperature (Figure 4B). Higher activity of bullfrogs in warmer waters likely increases the probability an observer will notice and record an individual.

In addition to preferring warm temperatures, bullfrogs require permanent sites to breed. Developing tadpoles are much larger than the larvae of other amphibian species in our study area and require multiple seasons to develop. This consistent activity in the water column could contribute to more widely available DNA for detection. We found that filtering more water increased eDNA detection probabilities for bullfrogs (Figure 3C), but the effect was much lower for northern leopard frogs (Supplementary Table S3.1 and S3.5). Filtering more water increases eDNA detection probability for some amphibian populations (Bedwell and Goldberg, 2020; Ficetola et al., 2008; Sepulveda et al., 2019) and we found this could be an effective strategy to increase bullfrog detection as well. In contrast, northern leopard frogs are more terrestrial, have smaller clutch sizes, and smaller tadpoles that metamorphose in late summer, possibly resulting in lower quantities of available eDNA. Collecting additional eDNA samples may be a more effective way of improving overall cumulative eDNA detection for this species. Future research would benefit from exploring optimal eDNA sampling strategies for northern leopard frogs and investigating how eDNA detection varies seasonally for both species with additional sampling dates.

Trends in method-specific detection also highlighted differences in species cryptic or conspicuous behaviors. Challenges in detecting northern leopard frogs visually and acoustically at occupied sites are likely related to their cryptic use of habitat and discrete breeding calls. We found some evidence that longer visual encounter surveys contributed to increased probability of detecting northern leopard frogs (Figure 4A), a trend that is similarly supported for declining congeneric Chiricahua leopard frogs (Hossack et al., 2022). Northern leopard frogs are often hidden in tall grasses and when startled, seek cover quickly. These behaviors may increase necessary search time to detect this cryptic species compared to bullfrogs, which often produce a characteristic and easily identifiable alert call when disturbed. Additionally, northern leopard frog breeding calls are among the most complex of any temperate zone amphibian, composed of low-volume chuckles, grunts, and snores (Larson, 2004). Unlike bullfrogs, northern leopard frogs do not form large choruses, tend to call sporadically, and are easily masked by background noises such as wind (De Solla et al., 2005; Larson, 2004). Bullfrog breeding calls consist of a series of similar croaks that can be heard from a substantial distance and large breeding choruses are common (Capranica, 1968; Guzy et al., 2014). Bullfrog calling behavior creates easily recognizable calls with a cumulative monthly acoustic detection probability near one (i.e., $\hat{p} \approx 1$) during peak breeding. These calling differences produced an overall lower probability of detecting northern leopard frogs acoustically compared to bullfrogs (Table 4, Figure 5). Northern leopard frogs emerge to breed in early spring (Oseen and Wassersug, 2002; Sommers et al., 2018) corresponding to our highest detection probabilities, followed by declining calling activity (Figure 5A). Contrarily, bullfrogs are late-season breeders, and we found acoustic detection probabilities increased throughout the summer (Figure 5B). Thus, acoustic detection probabilities for both species are improved by focusing sampling during their seasonal emergence.

Efficient and effective monitoring programs are essential to inform conservation efforts and determining the effects of management actions. However, many monitoring programs lack clear objectives and the ability to account for imperfect detection. Here, we provide two examples to illustrate how results from our study can inform future monitoring efforts aimed at our two focal species in the Intermountain West. Suppose researchers are interested in exploring factors influencing northern leopard frog colonization and recruitment following a management action (e.g., habitat improvement or translocation). Our results indicate that pairing 2 weeks of acoustic sampling during the expected peak breeding season with 2 visual encounter surveys at study locations would detect both northern leopard frog attempted (e.g., breeding calls) and successful (e.g., young of year emergence) breeding and achieve a cumulative detection probability ≥ 0.90, a sufficient value for most research questions (MacKenzie et al., 2002; Table 5). Alternatively, if researchers were interested in documenting the presence of bullfrogs early in their invasion, or following control actions, our results suggest collecting 7 eDNA samples and filtering 1 liter of water per sample, would achieve a cumulative detection ≥ 0.99. For this objective, achieving high cumulative detection probabilities provides accurate presence and absence information (Table 5). Subsequently, VES and further removal efforts could then be scheduled at sites with positive eDNA detections.

A possible reason there are few conservation success stories, despite innovative monitoring tools and a plethora of research documenting species declines, is due to inadequate attention to imperfect detection. Understanding how management actions influence state variables over time can be clouded by a misunderstanding of the detection process. Our research provides an excellent framework to improve our understanding of detection probabilities for multiple species and methods to inform future conservation efforts. We encourage other investigators to employ a similar approach for their focal species, specifically: 1) identifying and modeling covariates that influence species- and method-specific detection probabilities, 2) determining the optimal sampling efforts required to achieve a desired detection probability, and 3) applying this knowledge to develop sampling strategies to effectively monitor amphibians to inform conservation decisions.

TABLES

Table 1: Hypothesized effects of covariates on method- and species-specific detection probabilities for northern leopard frogs (NLF; *Lithobates pipiens*) and American bullfrogs (BF; *Lithobates catesbeianus*). Survey-specific covariates are those that can be altered by modifying aspects of the survey design. Environmental covariates are intrinsic to the surveyed habitat and cannot be controlled or known in advance of surveying. The observed range of values of each covariate is reported and the expected relationships between covariates and species-specific detection probabilities were positive (+) or negative (-). "Quad" indicates an expected quadratic relationship for detection probability.

Predictor (Model Notation)	**Covariate Type**	**Range**	**Survey Method**	**Expected Effect NLF Detection (p)**	**Expected Effect BF Detection (p)**
Julian Date (Julian)	Survey-specific	5/18 – 9/27	eDNA	+	+
		5/18 – 9/27	VES	+	+
		4/6 – 10/12	AAR	-	+
Water Filtered (Liters)	Survey-specific	0.01 – 5	eDNA	+	+
Number of Observers (ObsCount)	Survey-specific	1 – 2	VES	+	+
Search Time (SearchT) Minutes	Survey-specific	5 – 149	VES	+	+
Presence of Roving Unit (ROV)	Survey-specific	0 or 1	AAR	+	+
pH	Environmental	6.7 – 9.81	eDNA	Quad	Quad
Air Temperature (Atemp) °C	Environmental	8.9 – 34	eDNA	+	+
		8.9 – 34	VES	+	+
Water Temperature (Wtemp) °C	Environmental	12.7 – 33.4	eDNA	+	+
		12.7 – 33.4	VES	+	+

Table 2: Model selection results for northern leopard frog (*Lithobates pipiens*) detection models. Detection parameters are modeled separately for visual encounter surveys (p_{VES}), eDNA (p_{eDNA}), and automated acoustic recorders (p_{AAR}). Model selection results are only shown for the top five models. The constant model, $p_{VES}(.)$ $p_{eDNA}(.)$ p_{AAR} $(.)$ ψ $(.)$, is given for comparison. Occupancy (ψ) was held constant for all models and is not shown in model notation. Model notation along with Akaike InformationCriterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), and deviance (-2*log(L)*) are given for each model.

Model	**AICc**	**Δ AICc**	***w***	***K***	**-2log(L)**
$p_{VES}(.)$ p_{eDNA} (pH^2) p_{AAR} (Julian)	636.07	0	0.31	7	618.33
p_{VES}(SearchT) p_{eDNA} (pH^2) p_{AAR} (Julian)	636.60	0.54	0.23	8	615.64
p_{VES} (.) p_{eDNA} (pH^2) p_{AAR} (Julian+ROV)	637.09	1.02	0.18	8	616.12
p_{VES} (SearchT) p_{eDNA} (pH^2) p_{AAR} (Julian+ROV)	637.86	1.79	0.13	9	613.43
p_{VES} (.) p_{eDNA} (pH) p_{AAR} (Julian)	639.80	3.74	0.05	6	625.09
p_{VES} (.) p_{eDNA} (.) p_{AAR} (.)	658.44	22.38	0.00	4	649.23

Table 3: Model selection results for American bullfrog (*Lithobates catesbeianus*) detection models. Detection parameters are modeled separately for visual encounter surveys (p_{VES}), eDNA (p_{eDNA}), and automated acoustic recorders (p_{AAR}). Model selection results are only shown for the top five models. The constant model, $p_{VES}(.)$ $p_{eDNA}(.)$ p_{AAR} $(.)$ ψ $(.)$, is given for comparison. Occupancy (ψ) was held constant for all models and is not shown in model notation. Model notation along with Akaike InformationCriterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), and deviance (-2*log(L)*) are given for each model.

Model	**AICc**	**ΔAICc**	***w***	***K***	**-2LogL**
p_{VES}(Wtemp) p_{eDNA}(Liters) p_{AAR}(Julian)	490.51	0	0.36	7	472.78
p_{VES}(Wtemp) p_{eDNA}(Wtemp) p_{AAR}(Julian)	491.90	1.39	0.18	7	474.17
p_{VES}(Wtemp) p_{eDNA}(Liters) p_{AAR}(Julian+ROV)	492.87	2.36	0.12	8	471.91
p_{VES} (.) p_{eDNA}(Liters) p_{AAR}(Julian)	493.34	2.83	0.09	6	478.63
p_{VES}(SearchT) p_{eDNA}(Liters) p_{AAR}(Julian)	493.88	3.36	0.07	7	476.14
$p_{VES}(.)p_{eDNA}(.)p_{AAR}(.)$	519.62	29.10	0.00	4	510.40

Table 4: Average detection and occupancy probabilities and 95% confidence intervals are given for each species using the constant model: $p_{VES}(.)p_{eDNA}(.)p_{AAR}(.)\ \psi\ (.)$. Reported detection probabilities correspond to a single eDNA sample (p_{eDNA}), a visual survey conducted by one observer (p_{VES}), and weekly detection probability for a single acoustic unit (p_{AAR}). Average occupancy probabilities ($\widehat{\psi}$) are also reported for each target species: northern leopard frog (*Lithobates pipiens*) and American bullfrog (*Lithobates catesbeianus*).

Average Estimates	**N. Leopard Frog**	**Bullfrog**
$\hat{\boldsymbol{p}}_{eDNA}$	0.46 (0.39, 0.53)	0.53 (0.45, 0.62)
$\hat{\boldsymbol{p}}_{VES}$	0.67 (0.56, 0.77)	0.65 (0.52, 0.77)
$\hat{\boldsymbol{p}}_{AAR}$	0.14 (0.11, 0.19)	0.22 (0.17, 0.29)
$\widehat{\boldsymbol{\psi}}$	0.63 (0.47, 0.77)	0.45 (0.30, 0.60)

Table 5: Potential sampling strategies for northern leopard frog (*Lithobates pipiens*) and American bullfrog (*Lithobates catesbeianus*) to achieve target cumulative detection (p^*). Sampling methods include combinations of eDNA samples, visual encounter surveys (VES), and automated acoustic recorders (AAR). Bolded strategies correspond to examples given in the Discussion.

Method(s)	**Northern Leopard Frog**	**American Bullfrog**
Cumulative Detection (p^*)	$p^* \geq 0.90$	$p^* \geq 0.99$
eDNA	5 samples	**7 samples, 1 liter filtered**
VES	3 surveys	4 surveys at average water temp (~22ºC)
AAR	6 weeks, during expected peak season	8 weeks, during expected peak season, with 2 stationary units deployed
eDNA + VES	2 eDNA samples + 2 VES	4 eDNA samples, + 2 VES 1 liter filtered, 22ºC water
eDNA + AAR	2 eDNA samples + 4 weeks AAR, during expected peak season	4 eDNA samples + 6 weeks AAR, starting 8/1
VES + AAR	**2 VES + 2 weeks AAR, during expected peak season**	3 VES + 4 weeks AAR, during expected peak season

FIGURES

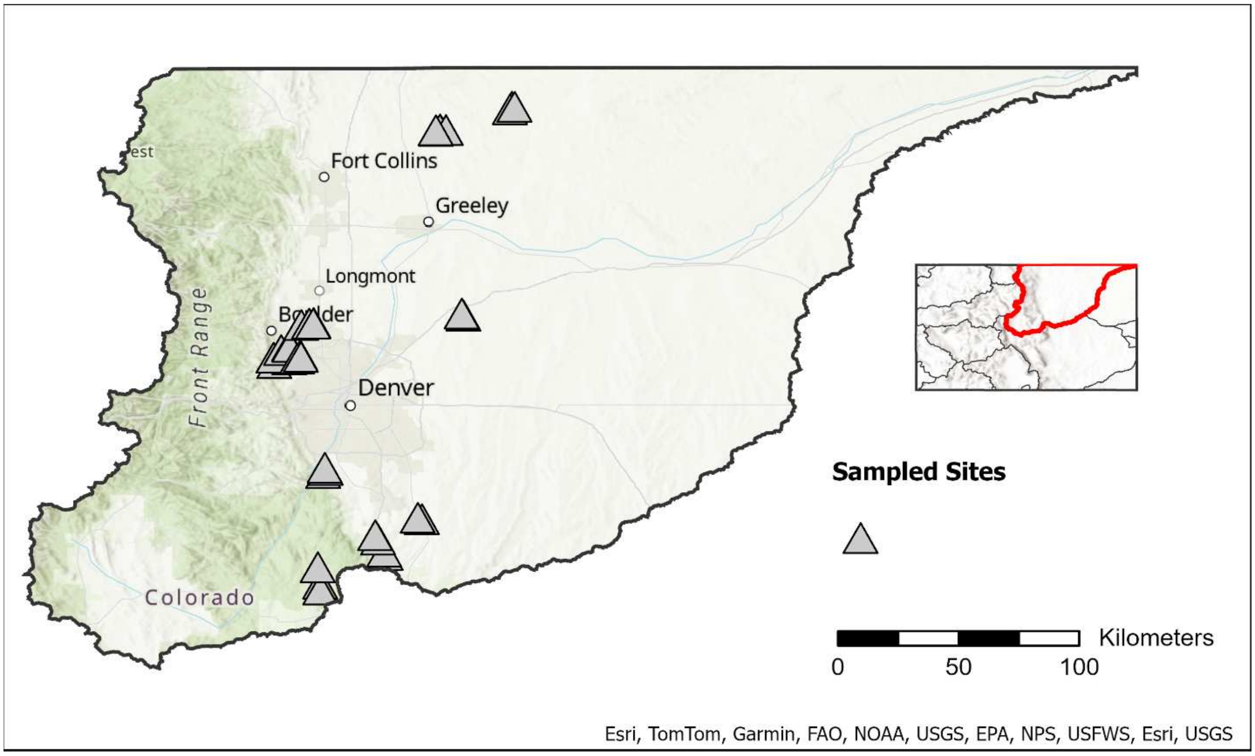

Figure 1: Sampled sites (n=38) in Colorado's South Platte River Basin, USA. The reference map illustrates the placement of the South Platte River Basin within Colorado. Sites were selected based on occurrence data for northern leopard frogs in the past decade (2011-2021) as well as two previous decades.

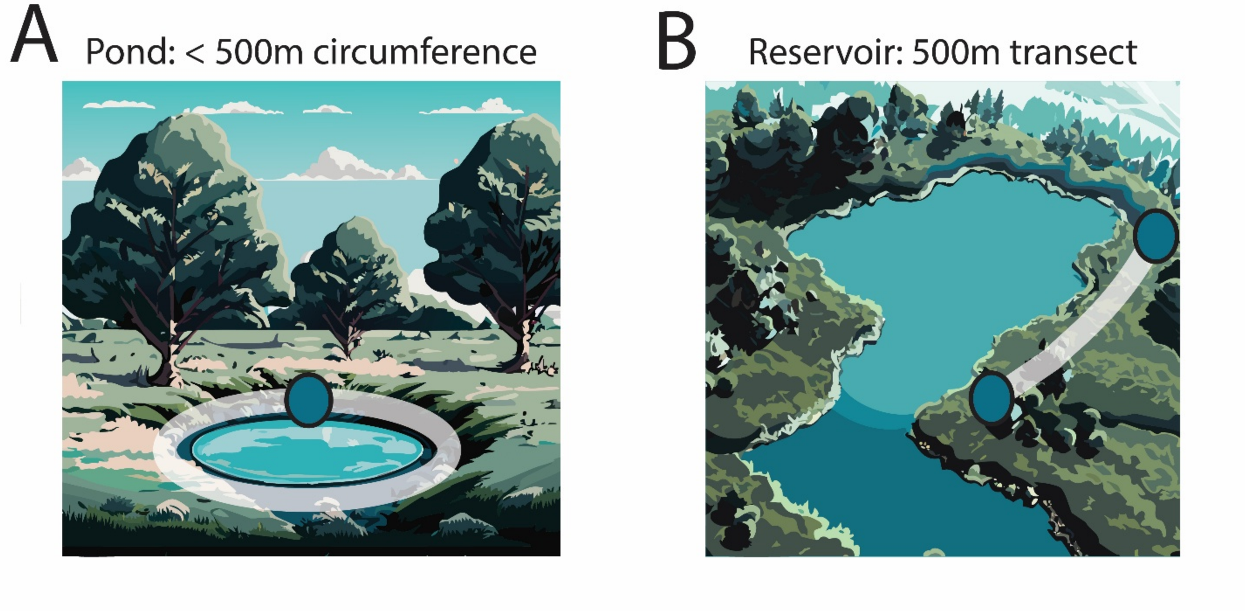

Figure 2: Examples of two sites **(A)** and **(B)** surveyed for northern leopard frogs and bullfrogs. Surveyors searched up to 500 meters of bank at each site for both focal species. At a subset of sites, (n=6), two automated acoustic recording (AAR) units were placed to sample different parts of the acoustic environment. White shading illustrates the surveyed transect during a visit and blue dots represent AAR unit placement. Image was generated using Adobe Illustrator version 28.0 with lines added to generated images using the Text to Vector Graphic tool.

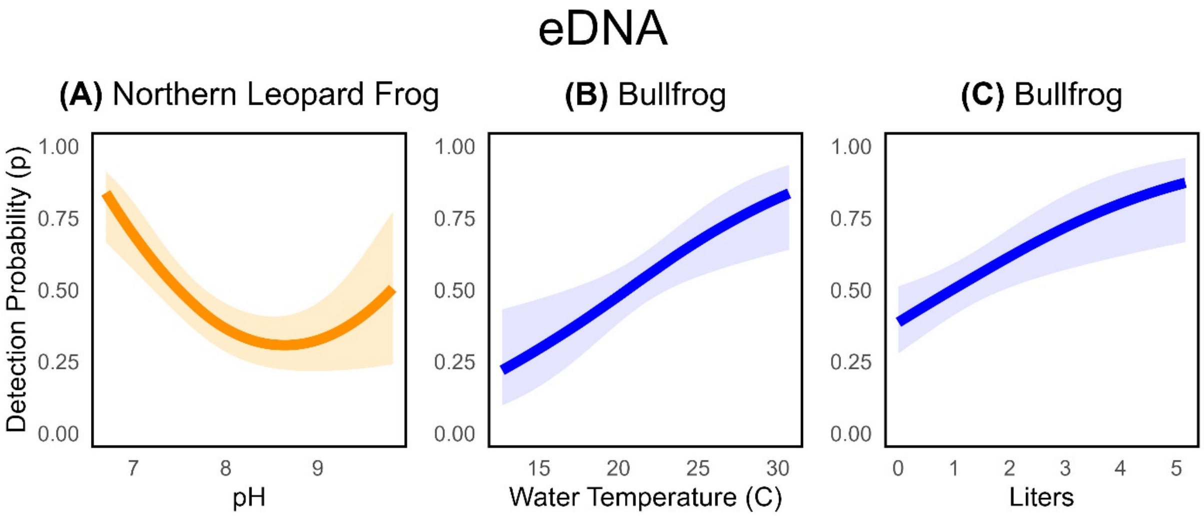

Figure 3: Estimated relationships between eDNA detection probabilities for northern leopard frogs **(A)** and bullfrogs **(B)** and **(C)** and pH ($\hat{\beta}$= -10.64, SE= 3.05), water temperature ($\hat{\beta}$= 0.16, SE= 0.05), and amount of water filtered ($\hat{\beta}$=0.46, SE=0.15). Shaded areas represent 95% confidence intervals associated with detection probability estimates. Relationships and estimates are given for the best-supported model that contains the covariate.

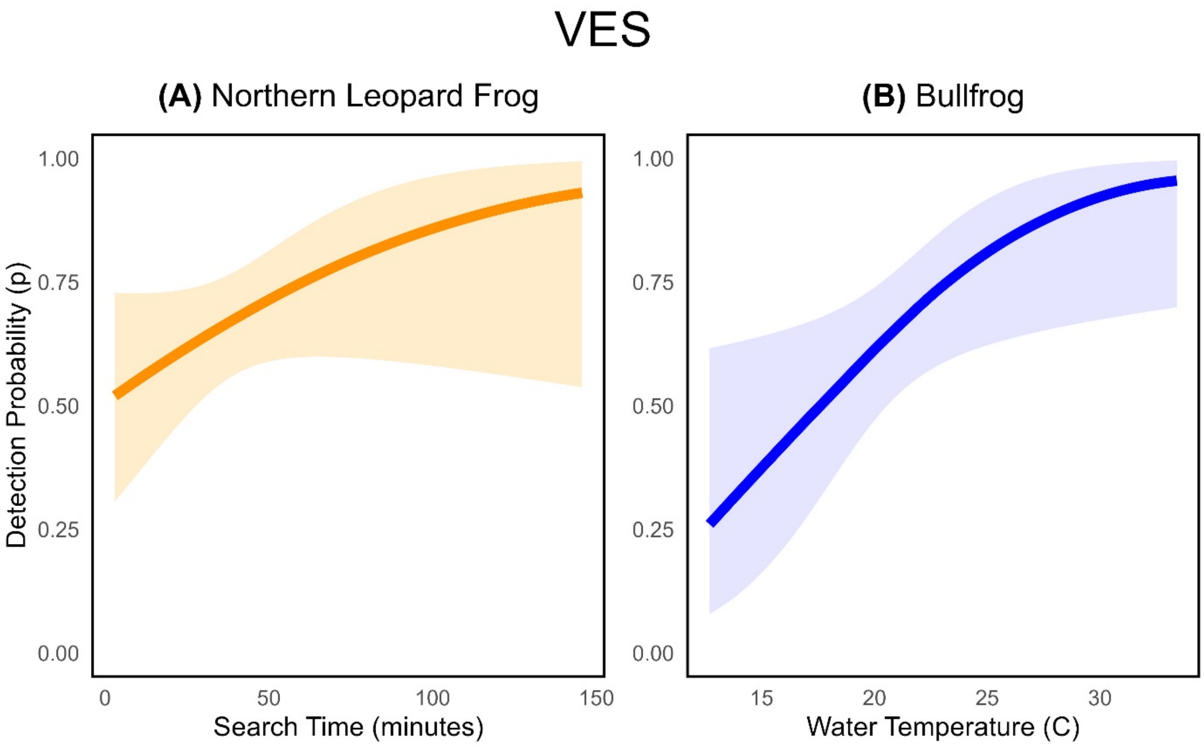

Figure 4: Estimated relationships between visual encounter survey (VES) detection probability for **(A)** northern leopard frogs and search time ($\hat{\beta}$= 0.02, SE= 0.01) and **(B)** bullfrogs and water temperature ($\hat{\beta}$= 20, SE= 0.09). Shaded areas represent 95% confidence intervals associated with detection probability estimates. Relationships and estimates are given for the best-supported model that contains the covariate.

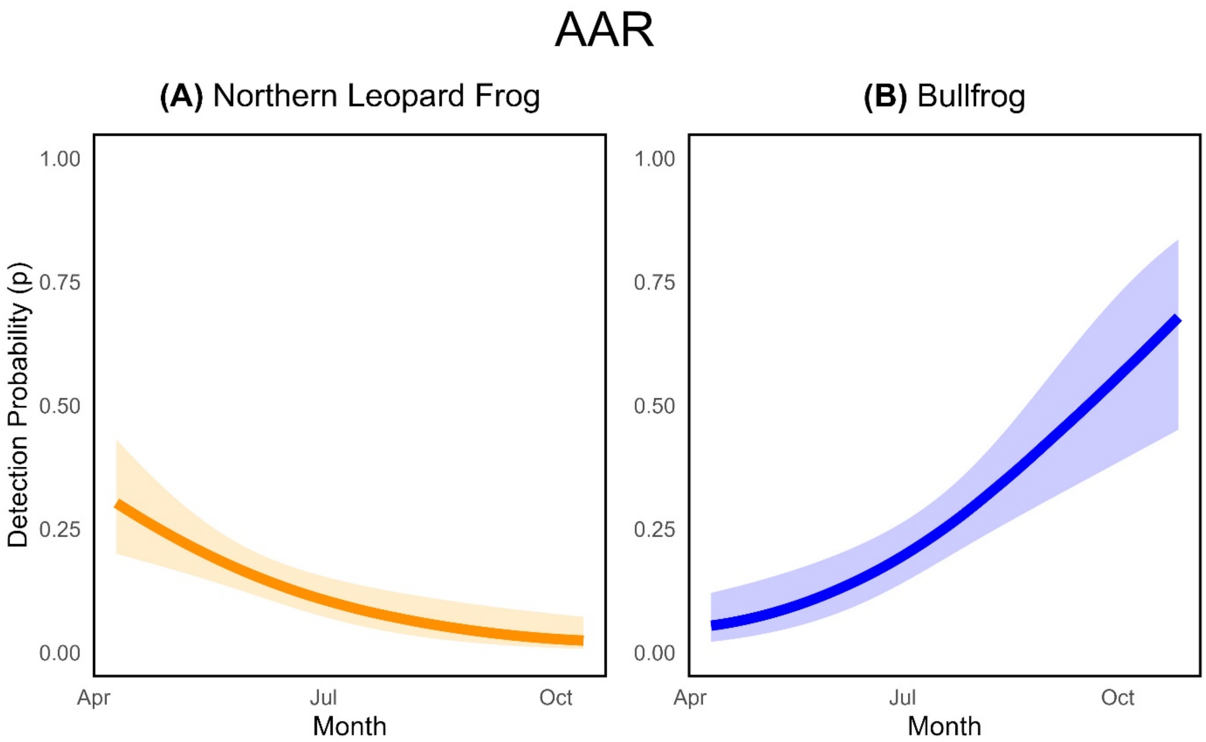

Figure 5: Estimated relationships between detection probability for a single automated acoustic recorder (AAR) and survey date for **(A)** northern leopard frogs ($\hat{\beta}$= -0.016, SE=0.004) and **(B)** bullfrogs ($\hat{\beta}$=0.02, SE=0.004). The probability of detecting the target species, given occurrence, in any single sampled week is depicted on the y-axis. Shaded areas represent 95% confidence intervals associated with detection probability estimates. Relationships are given for the best-supported model that contains the covariate.

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CHAPTER TWO: THE EFFECTS OF HABITAT AND INVASIVE SPECIES ON NORTHERN LEOPARD FROG (*LITHOBATES PIPIENS*) PERSISTENCE: A STATIC PERSPECTIVE ON A DYNAMIC PROCESS

INTRODUCTION

Non-native competitors, habitat change, and disease are major drivers of species' declines worldwide, with successful conservation actions requiring a better understanding of how these threats interact (Craig et al., 2017; Dirzo et al., 2014). Potential interactions among stressors, often operating at different spatiotemporal scales, creates uncertainty in their relative effects on species' declines (Craig et al., 2017; Johnson et al., 2011). To predict species' responses to cumulative threats at a landscape-level, researchers benefit from estimating persistence (or conversely, local extirpation), and colonization (Yackulic et al., 2015). However, the effects of invading species on native species' persistence occur over broad time scales (e.g., decades), creating challenges in discerning their relative effects on native species persistence (Yackulic, 2017). For example, the Barred Owl, a well-known invasive competitor of Northern Spotted Owls, did not exhibit competitive exclusion in the early years following its arrival in the Pacific Northwest, USA(Bailey et al., 2009) but diminished Northern Spotted Owl persistence over decades (Franklin et al., 2021; Rockweit et al., 2023; Yackulic et al., 2019). Additionally, understanding how non-native competitors interact with habitat is crucial when determining species responses to effective conservation actions (Craig et al., 2017). Invaders often benefit from human-modified landscapes (e.g., modified hydroperiod, habitat fragmentation) which may mediate or exacerbate the effects of competitors on native species persistence (Adams, 2000; Didham et al., 2007). Conversely, habitat heterogeneity may reduce effects or promote coexistence of competing species. For example, large and complex habitats decreased the negative effects of competing yellow-billed loons on the persistence of the Pacific loon (Haynes et al., 2014). Finally, the introduction of non-native diseases that often accompany invasive species arrivals further complicates the understanding of system dynamics, as disease prevalence is often unknown for many years following invasion. Imperfect detection is an impediment to understanding native species' responses to multiple stressors, especially when considering invasive pathogens. The disease sampling process typically requires host availability, as diseases are difficult to detect in the environment alone (e.g., Mosher et al., 2018). Host availability and disease prevalence may vary seasonally and between populations, creating additional uncertainty in disease detection. Thus, understanding how the interacting threats of non-native competitors, habitat change, and disease influence species' declines is inherently complicated but essential to effective conservation actions.

Understanding the potential interacting effects of multiple stressors is especially relevant to amphibian conservation. With more than 40% of species vulnerable to extinction, amphibians are considered the most threatened vertebrate taxa (Luedtke et al., 2023). Amphibians are unique in their reliance on terrestrial and aquatic ecosystems to complete their bi-phasic life cycle, exposing them to different threats across distinct life stages (Reid et al., 2019). Additionally, because many amphibian species experience natural population fluctuations, distinguishing human-induced stressors can require long-term datasets, which are rare (Pechmann et al., 1991). Moreover, extensive monitoring is often commenced after initial declines are noted; thus, broad scale occurrence information is rarely known prior to local declines (Noon et al., 2012). Despite the need for more long-term datasets, amphibian declines continue to be pervasive and have been largely attributed to habitat loss (Cordier et al., 2021), non-native species (Ficetola et al., 2007), and disease (Scheele et al., 2019), emphasizing the urgent need for conservation action (e.g., Grant et al., 2023; Scheele et al., 2014).

A classic example of a native amphibian facing these threats is the northern leopard frog (*Lithobates pipiens*), where western U.S. populations have experienced unprecedented declines since the mid-1900s largely attributed to habitat loss and non-native species (competitors and disease) (Baxter 1946; Johnson et al. 2011; Koch and Peterson 1995; Rogers and Peacock, 2012; Werner 2003). Northeastern Colorado has experienced especially concerning declines; a 2011 study found only 1.7% of historic northern leopard frog populations persisted in the region (Johnson et al., 2011). Northeastern Colorado represents a hot spot of land-cover change and wetland loss, as it continues to rapidly develop as a metropolitan area (Johnson et al., 2013; Zou et al., 2024). Additionally, the replacement of ephemeral streams and wetlands with permanent residential or agricultural ponds favors invasive species encroachment (Peterson et al., 2013; Valdez et al., 2021). Native to the eastern United States, the American bullfrog (*Lithobates catesbeianus*) is considered invasive in Colorado and benefits from these permanent water bodies (Peterson et al., 2013), which support its multi-season tadpole development. In addition to their competition with and predation on native taxa, bullfrogs are known carriers of *Batrachochytrium dendrobatidis* (*Bd*), a fungal pathogen implicated in amphibian declines worldwide (Fisher and Garner, 2007; Scheele et al., 2019). Although *Bd* can be difficult to discern as the cause of decline in areas where amphibians are no longer present (Mosher et al., 2017), it is implicated in the loss of northern leopard frog populations at high-elevation wetlands, which have not experienced the same level of habitat loss and invasive species encroachment as lower elevation sites (Johnson et al., 2011).

In this study, we investigated how the effects of land-use change, invasive bullfrogs, and *Bd* influenced northern leopard frog persistence in northeastern Colorado, USA. We sampled 137 sites with previous northern leopard frog occurrence records to investigate individual and combined factors limiting species persistence in the South Platte River Basin (Figure 1; Table 1). The spatial variability of agricultural, urban, and natural land-cover types, along with the continued encroachment of bullfrogs and *Bd* in our study area provided an ideal location to investigate the additive and interactive effects of these threats on northern leopard frog persistence, using a static (single-season) occupancy modeling approach that accounted for imperfect detection. Specifically, we investigated how bullfrogs influenced native northern leopard frog persistence and detection and estimated the current distribution of invasive bullfrogs at sites historically occupied by northern leopard frogs. We hypothesized that the presence of invasive species (bullfrogs, *Bd*), and anthropogenic land-use types (urban or agricultural) would interact to limit northern leopard frog persistence in northeastern Colorado. We also expected that the presence of bullfrogs would reduce the probability of detecting northern leopard frogs at sites where they both occur.

MATERIALS AND METHODS

Study Design

We conducted this study in the Colorado portion of the South Platte River Basin (Figure 1). The basin includes the Rocky Mountains, foothill shrublands, urban areas, and shortgrass prairies. In Colorado, northern leopard frogs can be found breeding in wetlands, ponds, reservoirs, and streams at elevations between 1,000 to 3,500 meters (Hammerson, 1999). Non-native bullfrogs were introduced in the upper South Platte River valley in the early 1900s and have since spread to other parts of the basin (Hammerson, 1999). They occupy a variety of habitats including northern leopard frog breeding sites but are not yet abundant at colder higher elevation sites (Bury and Whelan, 1985; Johnson et al., 2011).

To investigate factors influencing northern leopard frog persistence, we compiled northern leopard frog occurrence records from the Colorado Natural Heritage Program, Colorado Parks and Wildlife databases, and Community Science platforms, and overlayed these records in HUC-12 watersheds. The HUC-12 watershed (hereafter; watershed) level is the smallest watershed unit available in the USGS National Watershed Boundary dataset and is often used for aquatic species distributional studies (Dudgeon et al., 2006; Smith et al., 2020). We defined watersheds as 'recent' if northern leopard frogs were documented in the previous decade (2011-2021) or 'historic' if they were documented pre-2011. We employed a stratified random sampling approach, choosing 25 watersheds with 'recent' and 24 watersheds with 'historic' records of northern leopard frogs (Figure 1). This approach ensured that selected watersheds were spatially and temporally representative of previous northern leopard frog habitat in the basin, allowing us to investigate factors influencing northern leopard frog persistence (Figure 1). Bullfrog distribution is not well documented throughout the basin, but the species is considered widespread (Johnson et al., 2011; Peterson et al., 2013), and we anticipated bullfrogs would be present in many selected watersheds.

Site Selection and Land Cover Covariates

We selected two or three potential amphibian breeding sites within each watershed to survey. We defined sites as one of four habitat types: wetlands, streams, reservoirs, or ponds, according to the number of pools and predominance of lentic or lotic water (Figure 2). Wetlands were composed of many interconnected lentic pools. Ponds and reservoirs consisted of a singular pool, differing in size, and streams were dominated by flowing channels (Figure 2). Because of the differences in sizes, we expected that site type captured variation in hydroperiod, with reservoirs and wetlands representing primarily permanent water bodies and ponds and streams representing semi-permanent or ephemeral sites. We selected sites based on their proximity to existing northern leopard frog records and access permissions. We recorded the date and distance of the most recent northern leopard frog observation within 5 km of a site, representing northern leopard frog dispersal distance (Smith and Keinath, 2007). We included these records and their associated distance variables in a preliminary occupancy analysis to discern whether watershed- or site-level features better explained expected variation in northern leopard frog persistence (see Supplementary Material S3). Additionally, we used the National Land Cover Database (NLCD) to quantify the percentage of terrestrial land cover within a 1-km radial area surrounding each sampled site. Land cover categories were grouped as grassland, forest, agriculture, and urban per Johnson et al., 2011.

Field Surveys

We surveyed 137 potential breeding sites for our focal amphibian species: northern leopard frogs and bullfrogs. We visited sites twice between April and October during a given year, in either 2022 or 2023. We surveyed a 500-meter radial area for wetlands or a 500-meter length of shoreline or bank for ponds, reservoirs, and streams (Figure 2) and assigned these site boundaries using satellite imagery. During each visit, we conducted two visual encounter surveys using independent observers. One observer walked clockwise along the shoreline of a reservoir or pond, or from the downstream to the upstream end of wetlands and streams. The second observer would follow the same path 20 minutes later (Dosch et al. 2008). Observers recorded observations of post-metamorphic frogs while conducting dip net sweeps for larval amphibians. Encountered species were recorded as certain or uncertain identifications, but uncertain identifications were removed from data analysis to limit the potential for false positive detections. Site and survey features hypothesized to influence species occupancy, persistence, or detection were recorded including site type, survey (Julian) date, emergent vegetation, canopy cover, survey search area, search time, elevation, and water temperature (Table 1).

To estimate *Bd* occurrence and prevalence at sampled sites, we swabbed up to ten amphibians. Previous studies found that *Bd* prevalence was ~20% in our study area, suggesting a sample size of ten amphibians would achieve a >80% likelihood of detecting the pathogen at occupied sites (Johnson et al., 2011; Supplementary Table S1.2). We targeted post-metamorphic northern leopard frogs and bullfrogs when present, but we swabbed Woodhouse's toads (*Anaxryus woodhousii*), Great Plains toads (*Anaxyrus cognatus*), and western chorus frogs (*Pseudacris triseriata*) when the focal species were not available (see Supplementary Material S1 for more detail). Captured amphibians were held separately in sterile nets in shady, moist environments until sampling was complete. Individuals were swabbed 25 times on the abdomen followed by 3-5 times on the legs and feet, per standard guidelines (Brem et al. 2007). Any individuals exhibiting stress responses (e.g., increased respiration, prolonged agitation) were immediately released where initially captured. Swabs were stored on ice in the field, then kept in a sterile refrigerator until being processed using qPCR techniques by Pisces Molecular LLC in Boulder, Colorado (see Supplementary Material S1 for details).

Hypotheses

We hypothesized that northern leopard frog persistence could be influenced by univariate, additive, and interactive effects of invasive species (bullfrogs, *Bd*) and habitat at a site, as well as by the surrounding land cover. Bullfrogs are known competitors and predators of northern leopard frogs and other native anurans (Corn and Fogleman, 1984; Hayes and Jennings, 1986), and we expect their occurrence to negatively influence the probability of northern leopard frog persistence at a site. Bullfrogs are known vectors of *Bd* (Daszak et al., 2004) and bullfrog occurrence is likely highly correlated with *Bd* occurrence and prevalence. Similarly, we hypothesized that northern leopard frog persistence will be lower at *Bd* positive sites, although the susceptibility and fitness consequences of northern leopard frogs to *Bd* is debated (see Chatfield et al., 2013; Gahl et al., 2012; Trujillo et al., 2021; Voordouw et al., 2010).

We first describe our hypotheses involving factors that are likely to influence the distribution of bullfrogs in our system. Then, we describe hypothesized univariate, additive, or interactive relationships between bullfrog occurrence and other site-level covariates affecting northern leopard frog persistence (e.g., land cover, site type, etc.). We expect that habitat features and surrounding land cover will influence contemporary bullfrog occupancy probabilities. Bullfrogs require permanent water bodies for multi-season tadpole development and thus we expect their occupancy will vary with site type and be highest in reservoirs and ponds and lower in semi-permanent streams and wetlands (Table 1; Johnson et al., 2011; Peterson et al., 2013). Bullfrogs prefer well-vegetated, open canopy, warm water habitats to breed and are not yet abundant at high elevation sites (Bury and Whelan, 1985; Ficetola et al., 2007). We therefore expect bullfrog occurrence to decline with elevation and canopy cover and increase with average water temperature and emergent vegetation (Table 1). Finally, bullfrogs benefit from human-modified landscapes (Adams, 2000; Adams and Pearl, 2007; Peterson et al., 2013), and we therefore expect their probability of occurrence to be higher in areas with increased agriculture and urban land cover compared to sites dominated by grassland or forest land cover types (Table 1).

In the absence of bullfrogs, we expect northern leopard frog persistence will be influenced by site characteristics and surrounding land cover. Previous studies have found northern leopard frogs prefer breeding in open canopy, semi-permanent sites with emergent vegetation (Pope et al., 2000; Smith and Keinath, 2007; Zero and Murphy, 2016). We expect northern leopard frog persistence to be highest in open canopy, vegetated ponds and wetlands without co-occurring bullfrogs (Table 1). We expect persistence to be slightly lower in streams due to their less abundant lotic pools and lowest in reservoirs with few shallow breeding areas (Table 1). Previous studies found northern leopard frog occupancy and abundances were highest in habitats dominated by prairie cover (Cassel, 2019; Pope et al., 2000), thus we anticipate that northern leopard frog persistence will increase with increased surrounding grassland (Table 1). Low intensity agriculture can mimic the structural function of grassland (Knutson et al., 2018; Pope et al., 2000), whereas urban sites are substantially more fragmented. Additionally, high elevation sites in our study area have experienced local extirpations (Corn and Fogleman, 1984; Hammerson, 1999; Johnson et al., 2013). We therefore expect persistence will decrease slightly with increasing agricultural land cover and be lowest in urban and high elevation forested land cover types (Table 1).

Lastly, we hypothesize that bullfrog occurrence could have an additive or interactive effect with habitat variables to further decrease northern leopard frog persistence. For example, we might expect northern leopard frog persistence would have an additive relationship between emergent vegetation and bullfrog presence. Specifically, we expect persistence to increase with emergent vegetation at all sites, but persistence would be lower at sites with bullfrogs. Alternatively, the presence of bullfrogs may alter the relationship, making emergent vegetation even more important for northern leopard frog persistence at sites where bullfrogs co-occur, as emergent vegetation provides complex habitats where leopard frogs can avoid bullfrog predation. Similarly, northern leopard frogs may persist in open canopy sites when bullfrogs are absent, but in the presence of bullfrogs, there may be no relationship (benefit) of open canopy cover to leopard frog persistence. We expect northern leopard frog persistence to be highest in wetlands and ponds but lower when bullfrogs are present (i.e., additive relationship between site type and bullfrog occurrence). Another possibility is that bullfrog presence changes the relationship, making ephemeral habitats (e.g., streams or ponds) even more important for northern leopard frog persistence, as these habitats would only support transient bullfrog populations. Additionally, site type and vegetation could interact with bullfrogs. We predict bullfrogs will dramatically lower persistence at permanent sites with lower vegetation compared to well-vegetated ephemeral sites, as these sites would provide less cover from likely abundant bullfrog populations. Finally, we expect an interaction between surrounding land cover and bullfrog presence. Specifically, we predict that when bullfrogs are present, surrounding grassland will be even more important to increasing the probability of northern leopard frog persistence and, conversely, urban and agriculture will substantially reduce persistence probability.

Northern leopard frogs and bullfrogs are detected imperfectly, and we expect detection probabilities will vary according to survey, site, and species-specific factors based on our findings in Chapter 1. Unlike northern leopard frogs, bullfrogs emit a characteristic alert call when startled, thus we expect bullfrog detection probabilities to be high and relatively invariant of many site characteristics (e.g., vegetation, search area, search time). For bullfrogs, we do not expect detection probabilities to be influenced by northern leopard frog co-occurrence (Table 1). We expect reservoirs to support abundant bullfrog populations and have higher detection probabilities than other site types (Table 1; Peterson et al., 2013). Due to their preference for warm water, we expect bullfrog detection to increase with water temperature as abundance and activity increase (Table 1; Bury and Whelan, 1985; Ficetola et al., 2007; Chapter 1). We expect northern leopard frog detection to be lower at larger sites (e.g., wetlands, reservoirs, streams) and sites with dense vegetation compared to smaller sites (e.g., ponds) with less vegetative cover where they are not as obscured (Table 1). For this reason, we also expect longer search times to yield higher detection (Chapter 1). Surveys later in the season may have a higher detection probability for both amphibian species, as emerging post-metamorphic northern leopard frogs are abundant and bullfrogs are active later in the season in Colorado (e.g., July) (Bury et al., 1984; Larson, 2004; Smith and Keinath, 2007, Chapter 1). Additionally, we expect that the probability of northern leopard frog detection will be lower at sites where bullfrog co-occur due to expected decreased northern leopard frog abundances in the presence of a predator/competitor (Table 1)

Data Analysis

Occupancy Analysis

We used a static two-species occupancy framework to test our hypotheses about factors influencing bullfrog occurrence and northern leopard frog persistence at sites that were formerly occupied by northern leopard frogs (MacKenzie et al., 2004; Richmond et al., 2010). Ideally, we could use a multi-species occupancy to test hypotheses about the influence of bullfrogs and *Bd* on northern leopard frog persistence, while accounting for each species' (bullfrogs, *Bd*, northern leopard frog) imperfect detection (Rota et al., 2016). However, these models often require large sample sizes and reasonable detection probabilities for all species (Rota et al., 2016). Our sampling yielded limited information on *Bd* distribution and low detection probabilities (see Supplementary Material S1). Additionally, *Bd* occurrence is likely to be highly correlated with bullfrog occurrence; therefore, we focused our evaluation on our two focal amphibian species.

In our study system, bullfrogs are known competitors and predators of northern leopard frogs (Johnson et al., 2011; Smith and Keinath, 2007) and thus are considered the dominant species (denoted species “A”). Northern leopard frog persistence (i.e., current occurrence) is likely conditional on the presence of bullfrogs, so they are the subordinate species (denoted species “B”). We estimated occupancy probabilities for bullfrogs (ψ^A) as well as persistence of northern leopard frogs at sites where bullfrogs co-occur (ψ^{BA}) and sites where bullfrogs do not co-occur (ψ^{Ba}), while accounting for imperfect detection. To test hypotheses that northern leopard frog persistence is influenced by bullfrog occurrence, we fit model structures where northern leopard frog persistence is different at sites with bullfrogs ($\psi^{BA \neq Ba}$) and structures where persistence is unchanged by bullfrog presence ($\psi^{BA=Ba}$). We also estimated the Species Interaction Factor (SIF), which is defined as a ratio of the probability that both species occupy a site to the product of their independent occurrence probabilities: $(\widehat{SIF}) = \frac{\psi^{AB}}{\psi^A \psi^B}$. If the SIF >1, the species occur together more often than would be expected by chance and values <1 indicate they co-occur less often that would be expected by chance. If species occurrences are independent, the SIF is equal to 1 (MacKenzie et al., 2017; Richmond et al., 2010).

Detection probability parameters are allowed to differ based on the presence or absence of the other species. In the two-species occupancy framework, there are two types of detection parameters: p describes the probability of detecting a species, when the other species is absent (denoted $p^A,\ p^B$), and r describes the probability of detecting a species when both species are present (denoted, r^A, r^{BA}, r^{Ba}). Note, there are two r parameters for the subordinate species: r^{BA} is the probability of detecting northern leopard frogs on a survey when bullfrogs are also detected and r^{Ba} is the probability of detecting northern leopard frogs on a survey when bullfrogs are not detected. We did not expect that visually detecting a bullfrog would change the probability of detecting northern leopard frogs at sites with both species, thus we set these parameters equal ($r^{BA=Ba}$) in all models. We tested hypotheses regarding the effects of bullfrog occurrence on northern leopard frog detection probability by comparing models that included an effect of bullfrog occurrence ($p^B \neq r^{BA=Ba}$) to models where bullfrog occurrence has no effect on northern leopard frog detection probability ($p^A = r^{BA=Ba}$). Additionally, we compared models that included an effect of northern leopard frog occurrence on bullfrog detection probability ($p^A \neq r^A$) to models where bullfrog detection probability was unchanged at sites where northern leopard frog did and did not occur ($p^A = r^A$).

Hypotheses Testing: Model Building and Selection

We calculated pairwise correlation coefficients between all continuous covariates (Supplementary Material S2) and did not include correlated covariates ($|r| > 0.60$; Dormann et al., 2013) in the same parameter structure. We explored relationships between categorical (e.g., site type) and continuous covariates using one-way ANOVAs (Supplementary Material S2) and did not include covariates with p-value <0.05 in the same parameter structure (i.e., via additive or interactive relationships). Additionally, we conducted a preliminary analysis to explore an appropriate structure to model known variation in northern leopard frog persistence as a function of time since the species' last detection (see Supplementary Material S3). We selected the single top performing model structure to include this known variation in the final candidate model set (Table 2, Table S3.1).

We used a secondary candidate set model building strategy to develop and fit models representing our *a priori* hypotheses (Morin et al., 2020). We investigated hypotheses in four sub-model categories: 1) bullfrog occupancy (ψ^A), 2) northern leopard frog persistence (ψ^{BA}, ψ^{Ba}), 3) bullfrog detection (p^A, r^A), and 4) northern leopard frog detection (p^B, r^{BA}, r^{Ba}), while keeping a constant structure on all non-focal parameters (Supplementary Material S4). We expected northern leopard frog persistence and bullfrog occurrence to be influenced by habitat features. We included habitat features expected to influence amphibian occupancy or persistence as covariates using the logit link function (Table 1; Supplementary Material S4). The detection process can be influenced by site and survey-specific covariates. We modeled site and survey-specific covariates on detection parameters using the logit link (Table 1; Supplementary Material S4). The effect of the other species' occurrence on persistence or detection was modeled as a group-level effect with a shared intercept. We considered covariates with substantial support as those better supported than the null model and within ΔAICc <5 of the top model, excluding pretending variables (Arnold, 2010). We included all combinations of supported covariate structures in our final combined model set.

RESULTS

Preliminary analyses

Although we detected amphibians at more than half of our surveyed sites (>70), *Bd* samples were collected at only 28 sites, due to time constraints and seasonal availability of different life stages. We swabbed 157 amphibians for *Bd*: 120 recently metamorphosed and 37 adult or juvenile amphibians. *Bd* detection probability (i.e., prevalence) for recently metamorphosed individuals was very low and imprecise ($\hat{p} = 0.015, \widehat{SE} = 0.019$) compared to adults or juveniles ($\hat{p} = 0.28, \widehat{SE} = 0.13$). Thus, we excluded *Bd* from further analyses (See Supplementary Material S1 for more details).

We estimated pairwise correlation coefficients between two survey-specific variables (survey date and water temperature) and eight continuous habitat variables. We found that elevation and forest cover were positively correlated ($r = 0.86$), but correlations ($|r|$) were < 0.60 for all other continuous variables (Supplementary Material S2). One-way ANOVA results identified significant relationships between site type and search area, water temperature, and grassland (p-values <0.05; see Supplementary S2). Finally, we found that site-level observations of northern leopard frogs (year record) were better supported than watershed type (recent vs. historic). We used site-level observations to model expected variation in northern leopard frog persistence in the final candidate model set (Supplementary Table S3.1).

Occupancy and Persistence

We surveyed for northern leopard frogs and bullfrogs at 137 sites in 2022 or 2023. Northern leopard frogs were detected at 39 sites (naïve persistence = 0.28) and bullfrogs were detected at 44 sites (naïve occupancy = 0.32). Both species were detected at 16 sites (naïve occupancy = 0.12). Using a constant model [$\psi^{A}(.)\psi^{BA}(.)p^{A=rA}(.)p^{B=rBA=Ba}(.)$], average estimates of northern leopard frog persistence ($\hat{\psi}^{B} = 0.29,\ \widehat{SE} = 0.04$), bullfrog occupancy ($\hat{\psi}^{A} = 0.34,\ \widehat{SE} = 0.04$), and species co-occurrence ($\hat{\psi}^{A} * \hat{\psi}^{BA} = 0.10,\ \widehat{SE} = 0.02$), were similar to naïve estimates, indicating that cumulative detection probabilities for both species were close to 1.

Bullfrog occupancy was influenced by surrounding land cover and showed a strong negative relationship with the amount of forest surrounding a site ($\hat{\beta} = -5.99, \widehat{SE} = 2.32$; Figure 3), and a positive relationship to the amount of agriculture in univariate sub-model structures (Supplementary Table S4.1). While not as well supported, bullfrog occurrence was higher in warmer sites and at lower elevations. Surprisingly, bullfrog occurrence showed little variation among site types (Supplementary Table S4.1). Forest cover surrounding a site was the most parsimonious explanation for variation in bullfrog occupancy. All other model structures had

ΔAICc >5; thus, we included this structure for bullfrog occupancy in the final candidate model set (Table 2).

When we ignored the time component to northern leopard frog persistence and focused entirely on habitat and bullfrog effects, we found that an interaction between land cover covariates and bullfrog occurrence best described northern leopard frog persistence (Supplementary Table S4.2). When bullfrogs were present, northern leopard frog persistence increased dramatically with increasing grassland cover (Figure 4). When bullfrogs were absent, northern leopard frog persistence was nearly invariant to grassland cover (Figure 4). The interaction between bullfrogs and grassland cover was 20 times better supported than the additive (ΔAICc = 6.16; evidence ratio = 22.75) or the two univariate relationships (Grassland only: ΔAICc = 7.77; evidence ratio = 46; Bullfrog only: ΔAICc = 19.03; evidence ratio >100), indicating surrounding grassland may mediate the effects of the non-native competitor/predator. We found weak evidence that northern leopard frogs had a higher probability of persisting with co-occurring bullfrogs when agricultural cover was low than in highly agricultural areas without bullfrogs, although the effects were imprecise (Supplementary Table S4.2; Figure S4.1). Contrary to hypotheses, additive and interactive relationships between bullfrogs and urban land cover, emergent vegetation, canopy cover, and site types (wetland, pond, reservoir, stream) were not well-supported as explaining variation in northern leopard frog persistence.

Detection Probabilities

Estimates of species detection probabilities from constant models were relatively high for both species. The probability of detecting a species in a visual encounter survey was 0.63 ($\widehat{SE} = 0.04$) for northern leopard frogs and 0.69 ($\widehat{SE} = 0.04$) for bullfrogs. The probability of detecting bullfrogs was positively influenced by water temperature ($\hat{\beta} = 0.28, \widehat{SE} = 0.05$) and slightly higher at sites where northern leopard frog co-occurred ($\hat{\beta} = 0.99, \widehat{SE} = 0.46$; Figure 5). The model structure that included only water temperature (and not northern leopard frogs) was also well-supported (ΔAICc = 3.16; Supplementary Table S4.3) and both sub-model structures were included in the final candidate model set (Table 2). Site type (wetland, pond, reservoir, stream) and survey date were not well supported predictors of bullfrog detection probabilities (ΔAICc >5; Supplementary Table S4.3).

Surprisingly, northern leopard frog detection probabilities were not lower at sites where bullfrogs co-occurred; the bullfrog effect was often identified as a pretending variable when compared to similar models without a bullfrog effect (Supplementary Table S4.4). Surveys conducted later in the summer did have slightly higher detection probability for both species, but the relationship was better supported for northern leopard frog detection ($\hat{\beta} = 0.009$, $\widehat{SE} = 0.006$) compared to bullfrog detection ($\hat{\beta} = 0.008$, $\widehat{SE} = 0.005$; Supplementary Tables S4.3 and S4.4; Figure 6). Contrary to our expectations, site type (wetland, pond, reservoir, stream), water temperature, emergent vegetation, search time, and search area were not well supported covariates explaining variation in northern leopard frog detection probability; all were less parsimonious than the null model (Supplementary Table 4.4). We therefore included the top ranked models with survey date and the constant structure for northern leopard frog detection in the final candidate model set (Table 2).

Final Candidate Model Set

In the final candidate model set, we incorporated the best supported northern leopard frog time structure for persistence from a preliminary analysis (see Supplementary Material S3) and combinations of supported structures (ΔAICc <5) in each of the four secondary candidate model sets (Supplementary Material S4). The final candidate model set included eight models, representing one structure for bullfrog occupancy and two structures each for northern leopard frog persistence, bullfrog detection probabilities, and northern leopard frog detection probabilities. As expected, northern leopard frog persistence varied among sites based on year of the last known detection; this structure was included in the top four model structures accounting for ~100% of the AICc weight (Table 2). Northern leopard frog persistence was low even for sites where the species had been recently detected (e.g., 2020) but varied with grassland cover and bullfrog presence (Figure 7). For a site completely surrounded by grassland where northern leopard frogs were seen in 2020, there was a 0.98 ($\widehat{SE} = 0.03$) probability the species persisted, if the site was also occupied by bullfrog (Figure 7). This probability dropped to 0.63 ($\widehat{SE} = 0.12$) at sites without bullfrogs, suggesting bullfrogs may be colonizing the areas where northern leopard frogs currently persist (Figure 7). Persistence probability decreased dramatically with time since the last observation in both habitats; for example, if northern leopard frogs were last observed at a site in 1990, or earlier, the species is unlikely to persist today (Figure 7). Using our best supported model, the estimated species interaction factor was greater than 1 ($\widehat{SIF} = 1.34, \widehat{SE} = 0.47$), indicating that bullfrogs and northern leopard frog co-occur more often that would be expected if their distributions were independent. This suggests that the two species may be selecting similarly breeding habitats.

Model uncertainty in the final candidate set was related to the detection probabilities for both species. There was some evidence that northern leopard frog detection probability increased with survey (Julian) date (Figure 6; cumulative weight $\widehat{w} = 0.45$; constant structure $\widehat{w} = 0.55$). Additionally, bullfrog detection probabilities increased with water temperature and may be slightly higher at sites where northern leopard frogs co-occur (Figure 5; cumulative weight $\widehat{w} = 0.49$; equal structure $\widehat{w} = 0.51$).

DISCUSSION

Land cover change, invasive species, and disease are considered among the most important drivers of biodiversity loss and species' declines worldwide (Craig et al., 2017; Didham et al., 2007; Dirzo et al., 2014). However, these factors are often studied independently and over relatively short time scales, resulting in conservation decisions that lack information on interacting species and system dynamics (Didham et al., 2007; Hastings et al., 2018). We used a two-species occupancy model, conditioning on sites previously occupied by the native northern leopard frog, to evaluate how invasive bullfrogs and habitat features influence the species' persistence in northeastern Colorado, an area experiencing dramatic declines. Additionally, we investigated factors influencing current bullfrog occurrence and both species' detection probabilities.

We found the probability of northern leopard frog persistence was alarmingly low. Given the species occurred at a site in 2021, there was only a ~70% probability of persistence just one year later (when we surveyed), when not accounting for additional stressors. Over half of sites (~65%) previously occupied by northern leopard frogs did not support either the native or invasive species. Although these sites have seemingly resisted bullfrog invasion, they no longer support northern leopard frog populations. Contrary to our predictions, northern leopard frog persistence was somewhat higher at sites where bullfrogs co-occurred compared to sites where bullfrogs were absent, though this effect was imprecise. Although bullfrog presence has been negatively associated with leopard frog occurrence (Hossack et al., 2023; Johnson et al., 2011; Hammerson 1999), we present three explanations for this effect using our results. First, we found that ~35% of sites that were previously occupied only by northern leopard frogs are now occupied by bullfrogs, while the native species persists at only ~30% of these same sites.

Bullfrogs may be slowly out-competing northern leopard frogs on a landscape level, resulting in instances of co-occurrence. Our study area has continued to experience a dramatic reduction in ephemeral habitat and replacement with permanent water bodies that facilitate bullfrog invasion into northern leopard frog occupied areas (Hammerson, 1982; Johnson et al., 2013; Peterson et al., 2013). Second, we found a species interaction factor (SIF) value of ~1.5, which indicates that bullfrogs and northern leopard frogs occur more often together than if they were distributed independently. This evidence suggests that bullfrogs may preferentially colonize sites that support northern leopard frogs. Both species require similar breeding site characteristics, such as intermediate pHs and high emergent vegetation cover (Bury and Whelan, 1985; Graves and Anderson, 1987; Smith and Keinath, 2007). Additionally, we found bullfrog detection probability was higher where northern leopard frogs co-occurred, indicating that bullfrog populations are thriving, abundant, and easily detected at these sites. Third, we found that approximately one third of sites where northern leopard frogs have persisted are concurrently occupied by bullfrogs. We believe our study system is in a transient state where interspecific interactions are ongoing on a landscape scale, resulting in species co-occurrence. The fluctuating nature of amphibian populations (Pechmann et al., 1991) and multiple decades required for bullfrog populations to peak (Chang et al., 2022) yield a dynamic density-dependent system. Additionally, native species may delay the negative effects of co-occurrence through behavioral responses to invaders. For instance, in a mesocosm experiment, northern red-legged frogs (*R. aurora aurora*) avoided bullfrog predation by spending more time in terrestrial microhabitats (Pearl et al., 2004). Northern leopard frogs exhibit similar habitat preferences to northern red-legged frogs (Smith and Keinath, 2007), which could prolong their persistence with bullfrogs before declines are detected. In short, ecologically complex systems support transient states and our study area is likely no exception (Hastings et al., 2018).

Investigating interspecific dynamics requires multi-year datasets, which do not exist yet for our study system. We instead modeled native species persistence by conditioning on previous occurrence records, but this approach only provides estimates of a single dynamic parameter (native species' persistence) (e.g., Karanth et al., 2010). To understand complex species' interactions over time and predict potential coexistence at equilibrium, colonization and persistence probabilities are required for both the invasive and native species (Yackulic, 2017; Yackulic et al., 2015). Without these additional parameters, we cannot provide insights into complex interacting effects that manifest over time (Yackulic, 2017). Our findings are consistent with another classic invader system: when invasive Barred Owls started to invade Northern Spotted Owl territories in the Pacific Northwest, no measurable competition was detected by initial static two-species occupancy models (Bailey et al., 2009). In fact, the invader and native species were more likely to co-occur than expected based on independent species occupancy probabilities, similar to our findings. However, Northern Spotted Owl populations and distribution declined over decades as revealed by long-term monitoring data for both species (Franklin et al., 2021; Rockweit et al., 2023; Yackulic et al., 2019). We believe our study system could be experiencing a similar dynamic, highlighting in the need for additional years of detection-nondetection information for both the invasive and native species to predict native species' persistence or exclusion. The dynamic nature of our study system results in an inability to *predict* future northern leopard frog persistence using static (single-season) occupancy data (Karanth et al., 2010; Yackulic et al., 2015). Rather, our results represent a time-specific description of where northern leopard frogs *have* persisted, not where they *will continue* to persist, and where bullfrogs currently occur in habitats previously occupied by northern leopard frogs. We suggest future researchers collect occurrence information on both northern leopard frogs and their associated stressors (bullfrogs, *Bd*, and habitat change) over a longer timer period to better discern interspecific dynamics. Although our detection probability was too low to include *Bd* in our analysis, we suggest researchers prioritize swabbing breeding adults to better discern disease dynamics (see Supplementary S1).

Northern leopard frog persistence and bullfrog occurrence were also influenced by surrounding land cover. Previous studies have found northern leopard frog occupancy and abundances increased in grassland environments (Cassel, 2019; Knutson et al., 2004) and their dispersal was limited by row crops and roads (Bartelt and Klaver, 2017; Bouchard et al., 2009). Our results support these findings, with agricultural cover reducing northern leopard frog persistence, and grassland cover improving persistence, at sites where bullfrogs co-occur. Conversely, bullfrog occupancy increased with surrounding agriculture and decreased with natural land cover (Figure 3; Table S4.1). Forested sites typical in high elevation colder habitats continue to resist bullfrog invasion. Conversely, anthropogenic land conversion has been widely supported as benefitting invasive species and disadvantaging native species populations (e.g., Didham et al., 2007; Sievers et al., 2018). Our study area has many reservoirs and permanent ponds that can support robust bullfrog populations. These sites may simultaneously reduce northern leopard frog persistence and function as source populations for the continued dispersal of bullfrogs, complicating invasive species management strategies (Peterson et al., 2013).

Modifying habitat to promote native and/or disadvantage invasive species persistence is often overlooked as an invasive species management tool, with early detection and eradication typically prioritized (Adams and Pearl, 2007). However, modifying habitat can be an immensely successful management tool, especially when paired with invasive species removal. For instance, removing invasive ant colonies did not benefit the native ant species until the habitat disturbance facilitating invasion was mitigated (King and Tschinkel, 2006). Our results suggest that improving grassland cover will benefit northern leopard frog persistence, amidst bullfrog encroachment. In addition to breeding ponds, northern leopard frogs use grassy meadows for summer foraging (Smith and Keinath, 2007). Surrounding grassland may support habitat partitioning in the presence of bullfrogs as northern leopard frogs can disperse into adjacent grassland post-breeding (Smith and Keinath, 2007). Conservation decisionmakers would benefit from knowledge on which habitat features, in addition to grassland, are important for northern leopard frog persistence and whether habitat improvements could be used as a complementary management tool with bullfrog control. Specifically, comparing northern leopard frog persistence across combined and isolated treatments of habitat restoration and bullfrog control— compared to untreated sites—would provide valuable insights to guide conservation efforts.

Successful conservation actions to reduce or reverse the impacts of invasive species require an improved understanding of how multiple interacting factors influence native species decline (or persistence). However, understanding the ecological complexity of species' responses to multiple interacting factors when a system is not at equilibrium is challenging and requires monitoring of the system over time (e.g., decades). Our study provides initial steps in that process and valuable information to guide future monitoring and management efforts. Conditioning on sites believed to be historically occupied by native northern leopard frogs, bullfrogs occur at ~35% of sites, while the native species persist at only ~30% of these same sites. Forested sites, typically found in the higher elevation regions of the basin, continue to resist bullfrog invasion. Northern leopard frog persistence when bullfrogs are present is higher in sites found primarily in grassland landscapes. Grassland restoration in areas with low grassland cover could be an effective management tool to increase northern leopard frog persistence amidst bullfrog co-occurrence. Additional research will be required to better understand species dynamics in all habitat types, with and without active management. Our research provides an excellent baseline understanding of where northern leopard frogs have persisted and future guidance on how we can better understand complex interacting threats in dynamic systems.

TABLES

Table 1: Hypothesized univariate effects of covariates on northern leopard frog (NLF; *Lithobates pipiens*) persistence probability $(\psi^{B\cdot})$, American bullfrog (BF; *Lithobates catesbeianus*) occupancy probability (ψ^{A}), and both species' detection probabilities $(p^{B},\ r^{B\cdot},\ p^{A},\ r^{A\cdot})$. The observed ranges and categories for each covariate is reported and the expected relationships to each parameter: (+) positive, (-) negative, or (0) when the covariate was modeled for comparison purposes, but hypothesized effects were expected to be close to zero. For categorical covariates, expected relationships are ranked: increase (>) or decrease (<) across categories. Categories expected to have similar effects are denoted as equal (=) to each other. Blank cells are those where there was no associated *a priori* hypothesis with the estimated parameter.

Predictor (Model Notation)	**Range or Categories**	**Expected Effect NLF Persistence** $(\psi^{B\cdot})$	**Expected Effect BF Occupancy** (ψ^{A})	**Expected Effect NLF Detection** $(p^{B}\&\ r^{B\cdot})$	**Expected Effect BF Detection** $(p^{A}\&\ r^{A\cdot})$
Bullfrog occurrence (BF)	present/absent	-		-	
NLF occurrence (NLF)	present/absent				0
Site type (site.type)	pond, wetland, reservoir, stream	pond>wetland> stream>reservoir	reservoir=pond> wetland=stream	pond>stream> reservoir> wetland	reservoir> pond> wetland= stream
% Emergent Vegetation (veg)	0-100%	+	+	-	0
% Canopy cover (canopy)	0-100%	-	-		
Elevation (m) (elev)	1240-3010	-	-		
Land cover (land.cover)	0-100%	grass>ag> urb>forest	ag = urb> grass>forest		
Survey Julian date (date)	130-277			+	+
Search time (min) (search.time)	5-145			+	0
Search area (m^2) (search.area)	175-250,000			-	0
Water temperature (°C) (wtemp)	7.2-37.9	0	+	+	+

Watershed type (recent vs. historic)	recent/historic	recent > historic	0		
Year of last NLF observation (year record)	1915-2021	+			
Distance of observation to site (m) (distance)	0-5,000	-			

Table 2: Final candidate model set. Model parameters include northern leopard frog (*Lithobates pipiens*) persistence given bullfrogs are present (ψ^{BA}) or absent (ψ^{Ba}) and American bullfrog (*Lithobates catesbeianus*) occupancy (ψ^{A}). Northern leopard frog detection probability does not vary with the presence of bullfrogs ($p^B = r^{BA=Ba}$) and bullfrog detection probability is either affected ($p^A \neq r^A$) or unaffected ($p^A = r^A$) by northern leopard frog presence. The null model [ψ^A(.)$\psi^{BA=Ba}$ (.)$p^A = r^A$ (.)$p^B = r^{BA=Ba}$(.)] is given for comparison. We report model notation along with Akaike Information Criterion for small sample sizes (AICc), ΔAICc, model weights (w), number of parameters (K), and deviance (-2log(L)) for each model.

Model	**AICc**	$\Delta AICc$	w	K	**-2log(L)**
ψ^A (forest) $\psi^{BA\neq Ba}$(year record +BF*grassland) $p^A = r^A$(wtemp) $p^B = r^{BA=Ba}$(.)	610.68	0	0.29	10	588.93
ψ^A(forest) $\psi^{BA\neq Ba}$(year record +BF*grassland) $p^A \neq r^A$(NLF + wtemp) $p^B = r^{BA=Ba}$(.)	610.85	0.17	0.27	11	586.74
ψ^A(forest) $\psi^{BA\neq Ba}$(year record +BF*grassland) $p^A \neq r^A$(NLF + wtemp) $p^B = r^{BA=Ba}$ (date)	611.19	0.51	0.22	12	584.67
ψ^A(forest) $\psi^{BA\neq Ba}$(year record +BF*grassland) $p^A = r^A$ (wtemp) $p^B = r^{BA=Ba}$ (date)	611.22	0.54	0.22	11	587.11
ψ^A(forest) $\psi^{BA\neq Ba}$ (BF*grassland) $p^A = r^A$ (wtemp) $p^B = r^{BA=Ba}$ (.)	657.41	46.73	0	9	638
ψ^A(forest) $\psi^{BA\neq Ba}$ (BF*grassland) $p^A = r^A$ (wtemp) $p^B = r^{BA=Ba}$ (date)	657.42	46.74	0	10	635.67
ψ^A(forest) $\psi^{BA\neq Ba}$ (BF*grassland) $p^A \neq r^A$ (NLF + wtemp) $p^B = r^{BA=Ba}$ (.)	657.77	47.09	0	10	636.02
ψ^A(forest) $\psi^{BA\neq Ba}$ (BF*grassland) $p^A \neq r^A$ (NLF + wtemp) $p^B = r^{BA=Ba}$ (date)	657.77	47.09	0	11	633.66
ψ^A(.)$\psi^{BA=Ba}$ (.)$p^A = r^A$ (.)$p^B = r^{BA=Ba}$(.)	724.60	113.92	0	4	716.3

FIGURES

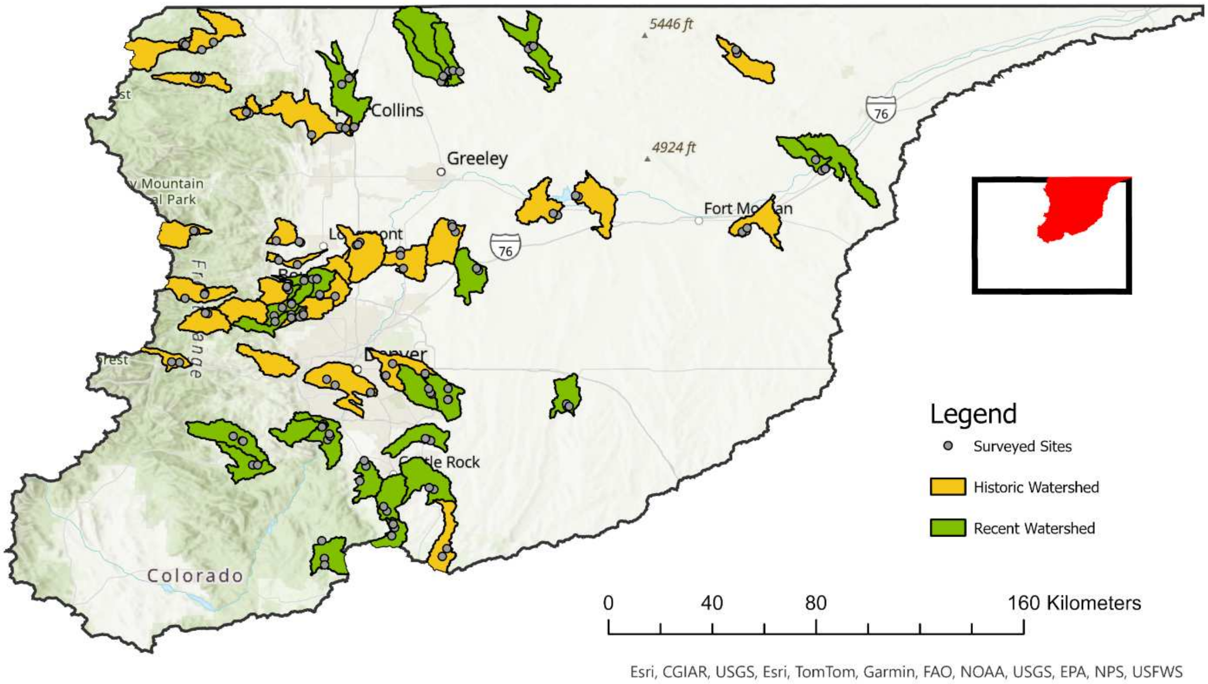
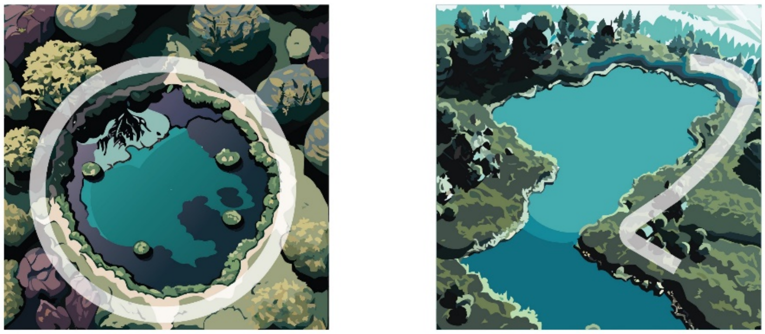


Figure 1: Map of sampled watersheds and sites within the South Platte River Basin, Colorado, USA. The reference map illustrates the placement of the South Platte River Basin within the state of Colorado. Historic watersheds (HUC12) are represented in yellow and recent watersheds in green. Up to three sites were surveyed within each HUC12 watershed and are represented by gray dots. Watershed boundaries (HUC6 and HUC12) and state outlines were downloaded from the USGS Watershed Boundary Dataset and the National Boundary Dataset via the National Map Downloader (https://apps.nationalmap.gov/downloader/) accessed April 2025. Basemap imagery is from Esri's World Topographic Map (© Esri, TomTom, Garmin, FAO, NOAA, USGS, EPA, NPS, USFWS, Esri, USGS).

Pond: < 500m circumference

Reservoir: 500m transect



Wetland: search within 500m radius

Stream: 500m transect

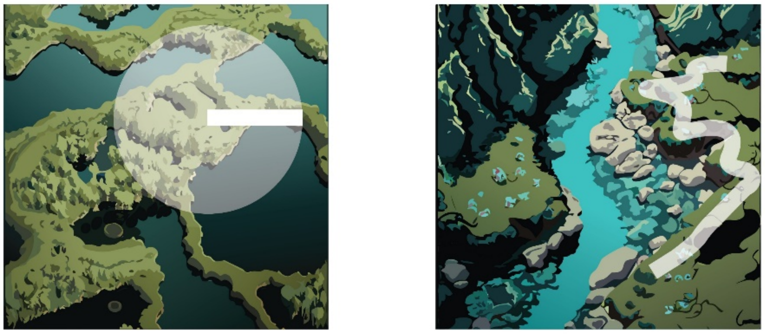


Figure 2: Four site types were surveyed for northern leopard frogs and bullfrogs. Ponds were a lentic pool <500 meters circumference and reservoirs >500 meters. Wetlands were composed of many lentic pools and streams predominated by flowing channels. Surveyed transects included up to 500 meters of bank or within a 500-meter radius at each site for both focal species. White shading illustrates the surveyed transects/areas. Image was generated using Adobe Illustrator version 28.0 with lines added to generated images using the Text to Vector Graphic tool.

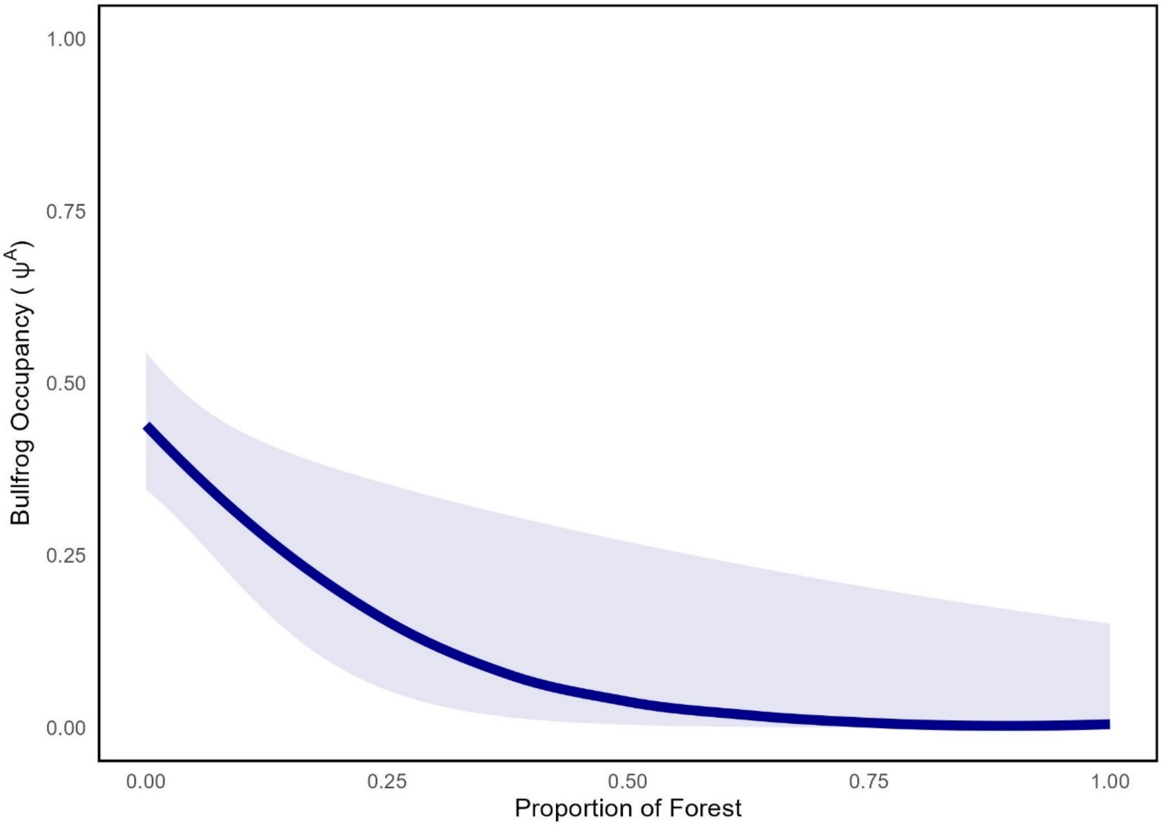


Figure 3: Estimated relationships between American bullfrog (*Lithobates catesbeianus*) occupancy probability and the proportion of forest cover within 1km surrounding the site ($\hat{\beta} = -5.99, \widehat{SE} = 2.32$). Shaded areas represent 95% confidence intervals associated with occupancy estimates. Relationships and estimates are given for the best-supported sub-model that contains the covariate (Supplementary Table S4.1).

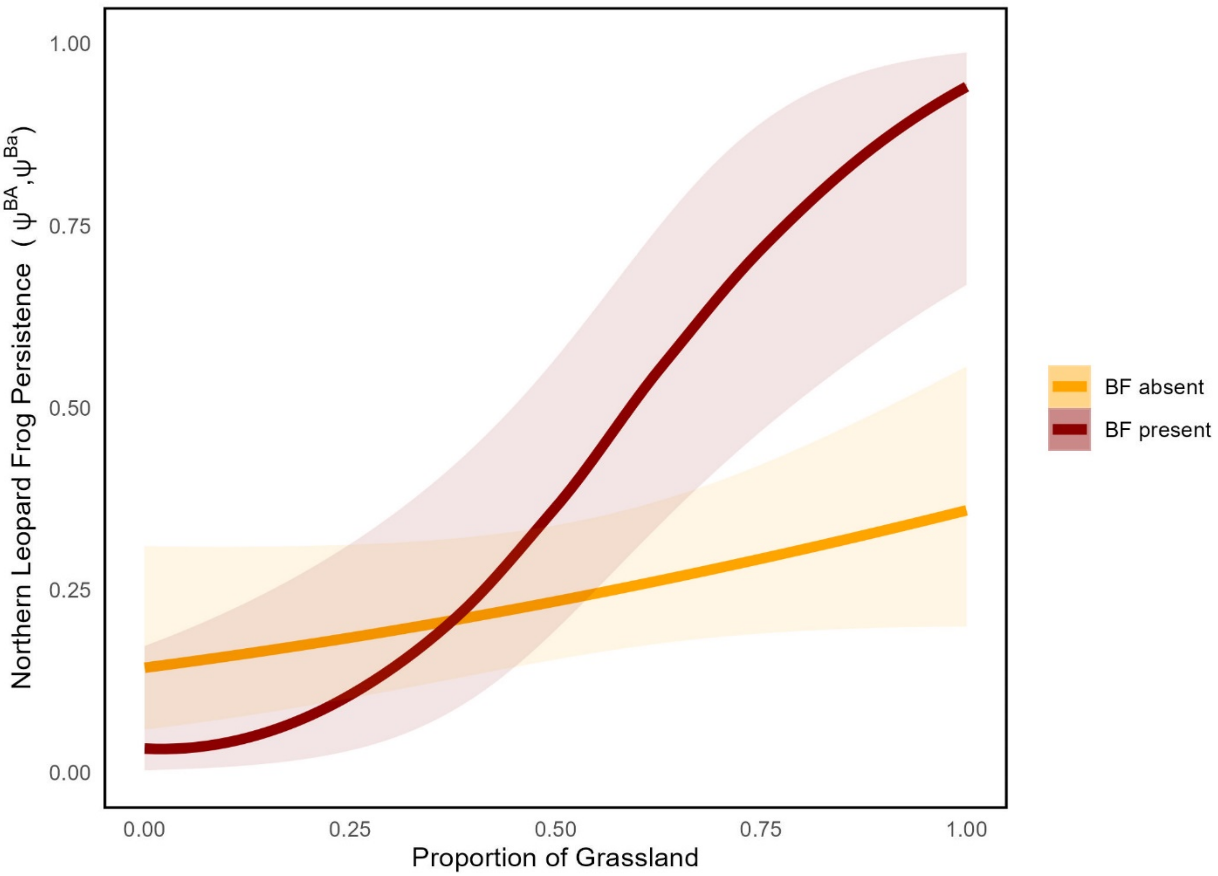


Figure 4: Estimated northern leopard frog persistence as an interactive function of grassland cover and bullfrogs (BF) occurrence. Estimates are given for the best supported sub-model structure (Supplementary Table S4.2).

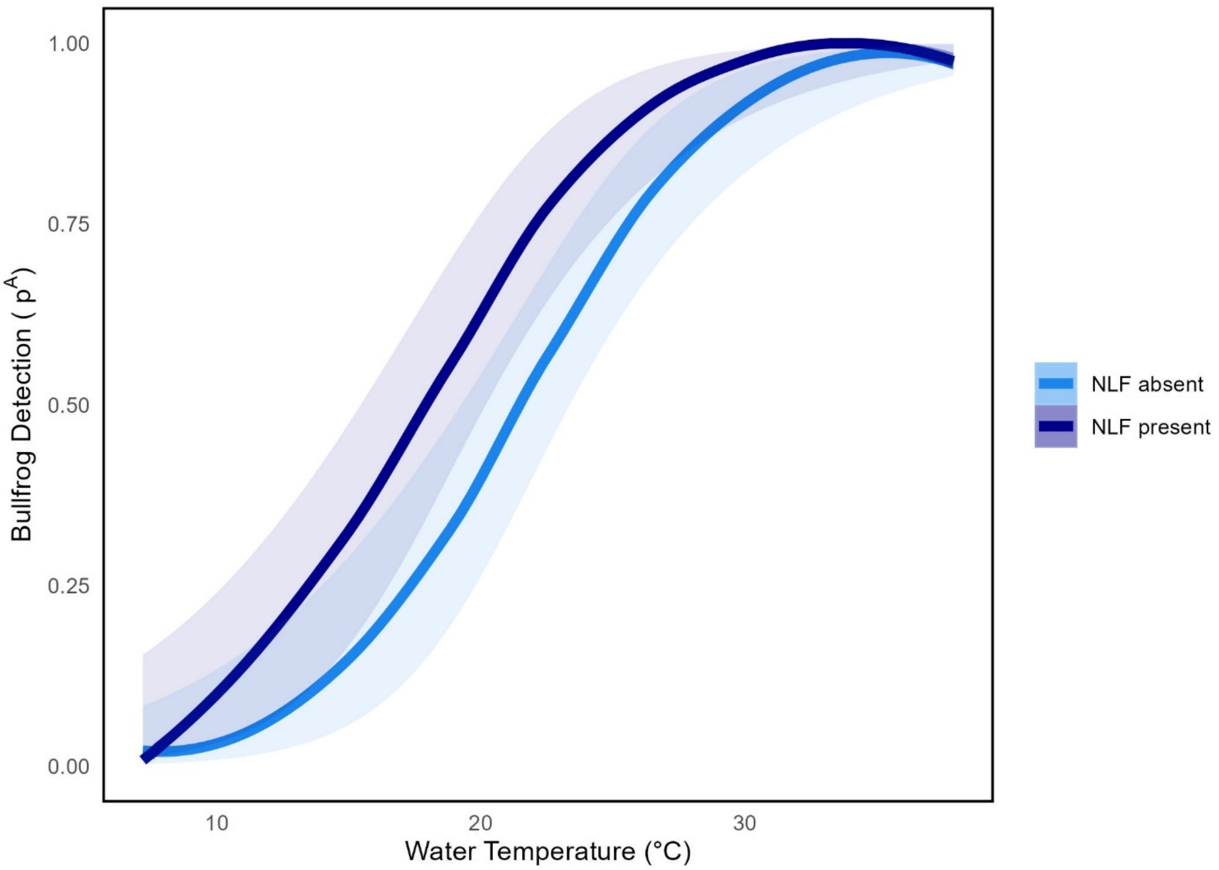


Figure 5: Estimated relationship between American bullfrog (*Lithobates catesbeianus*) detection probability and water temperature. Detection probability increased with water temperature $\hat{\beta} = 0.28, \widehat{SE} = 0.05$) and at sites where northern leopard frogs (NLF) co-occurred ($\hat{\beta} = 0.99, \widehat{SE} = 0.46$). Shaded areas represent 95% confidence intervals associated with detection probability estimates. Relationships and estimates are given for the best-supported sub-model that contains the covariate (Supplementary Table S4.3).

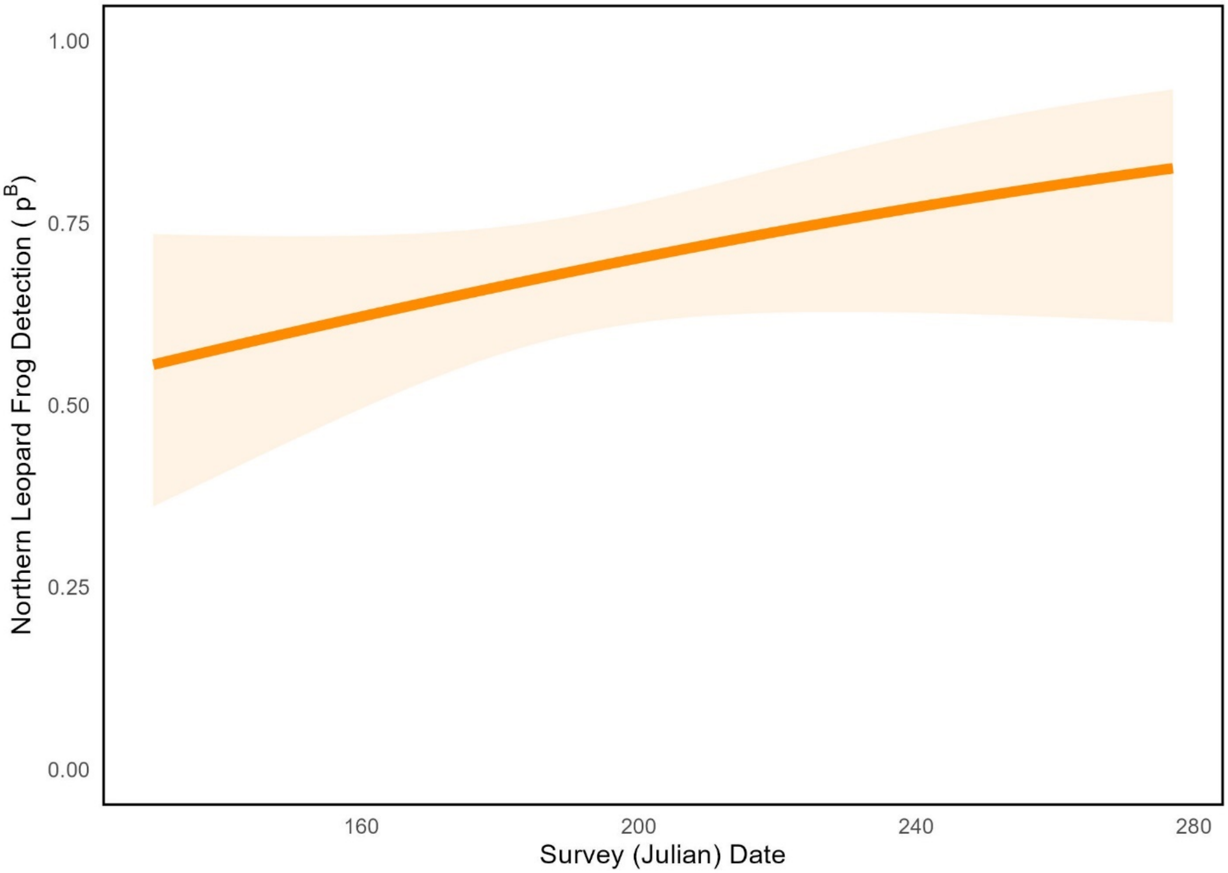


Figure 6: Estimated relationship between northern leopard frog (*Lithobates pipiens*) detection probability and survey date, expressed as Julian date. Detection probability increased slightly with surveys conducted later in the season ($\hat{\beta} = 0.009, \widehat{SE} = 0.006$). Shaded areas represent 95% confidence intervals associated with detection probability estimates. Relationships and estimates are given for the best-supported sub-model that contains the covariate (Supplementary Table S4.4).

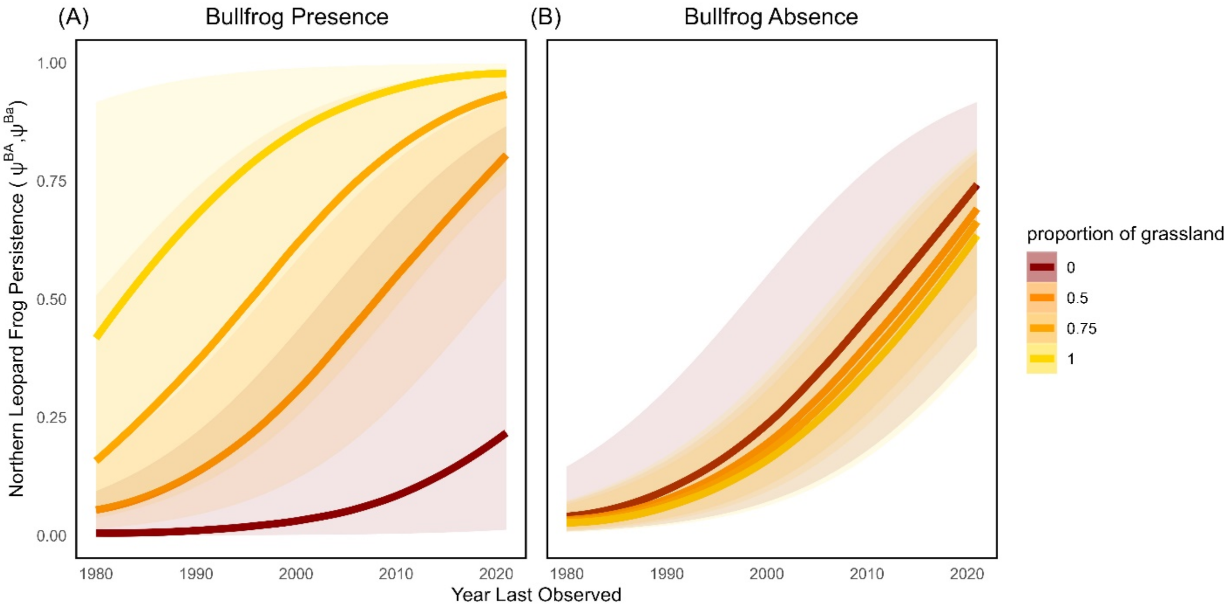


Figure 7: Northern leopard frog (*Lithobates pipiens*) persistence is expressed as a function of the year they were last observed at a given site where American bullfrogs (*Lithobates catesbeianus*) are concurrently **(A)** present or **(B)** absent and with varying proportions of surrounding grassland within 1km of the site. Shaded areas represent 95% confidence intervals associated with persistence probability estimates. Relationships and estimates are given for the best-supported final candidate model that contains the covariate (Table 2).

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APPENDICES

CHAPTER ONE

S1 eDNA Sample Processing

All filters were tested for both northern leopard frog and bullfrog DNA using mitochondrial DNA with quantitative PCR assays targeting specific genes or sequence regions. For bullfrogs, the Cyt B gene was targeted based on methods developed by Veldhoen et al., 2016. For northern leopard frogs, the qPCR assay required a newly designed forward primer and probe to target local northern leopard frog populations, due to base mismatches in these sequence regions for the Colorado northern leopard frog haplotype compared to the Canadian haplotype (Beauclerc et al., 2019). To detect inhibition, the qPCR master mix included a PCR inhibitor resistant Taq polymerase and a VIC-labeled internal positive control using verified gene segments from both target species. All samples were tested in triplicate using an Agilent AriaMx real-time instrument, with at least two positive replicates required to designate a sample positive for target DNA. If negative control samples tested positive for northern leopard frog or bullfrog DNA, we eliminated all other samples collected on the same survey event where contamination was observed.

S2 Classifier Construction and Acoustic Processing

Northern leopard frogs and bullfrogs have different advertisement calls, with northern leopard frog calls consisting of a “snore”, “grunt” and “chuckle” between 900 to 1500 Hz and lasting anywhere from 5-15 seconds (Larson, 2004). Bullfrogs calls are a series of simple croaks between 200-2000Hz and lasting for 6.6 seconds and separated by 30 seconds (Bates et al., 2010;

Suggs and Simmons, 2005). Both species have distinct advertisement calls that cannot be easily misidentified with any other vocalizing species (including non-amphibians) in our study area.

Automated acoustic recorders continuously sampled between April and October, so we sub-divided sampling intervals into weeks: 28 sampling weeks in 2022 and 22 sampling weeks in 2023. Some units malfunctioned and recorded only a portion of the sampling season. Any missing weeks were entered as missing surveys in our occupancy analysis. We used Kaleidoscope Pro version 5.4.2 to process and sort full-spectrum acoustic data into breeding call clusters for northern leopard frogs and bullfrogs.

Signal parameters for Kaleidoscope Pro were developed using 2021 pilot data to target northern leopard frog advertisement calls, of which the same settings adequately captured bullfrog advertisement calls. The minimum length of detection was set to 0.1 seconds and maximum length to 18 seconds because if set lower, it would cut out the northern leopard frog advertisement call and produce more clusters than necessary. Additionally, bullfrogs have 30 second gaps between calls, so this setting would separate bullfrog calls (Larson, 2004; Suggs and Simmons, 2005). The maximum inter-syllable gap was set to 0.6 and determined by trial and error so the classifier would recognize the three distinct parts of the northern leopard frog call (chuckle, grunt, snore). The frequency range was set between 600 to 1000 Hz. The FFT window was set to 10.67 ms to increase the detection rate for low amplitude and frequency calls while including the complete “chuckle, grunt, and snore” of the northern leopard frog call. All other settings were left as default settings.

Kaleidoscope uses hidden Markov models to identify and group similar acoustic signatures into a specified category. This category is referred to as a cluster. We labeled two clusters: “NLF” and “BF” for northern leopard frog and bullfrog breeding calls. We sorted cluster-confirmed species breeding calls by date and visually and audially confirmed detection or non-detection for each species in each weekly interval. All other acoustic data (e.g., cows, wind, road noise) were automatically sorted into separate individual clusters by the program.

Certain clusters can be labeled and saved in a classifier, which can be used to sort additional acoustic data. Classifiers for northern leopard frog and bullfrog breeding calls must be built using a large amount of calling data. We used acoustic data from robust populations of northern leopard frogs and bullfrogs to create individual classifiers for each species' breeding call. We then tested classifiers on additional datasets and manually confirmed a very low number of false negatives with our bullfrog classifier and a high number of false negatives with our northern leopard frog classifier. Our bullfrog classifier performed well for our purposes and no additional changes were made. For northern leopard frogs, we used the poorly performing classifier to process acoustic data from additional sites with robust populations. We then visually and audially confirmed breeding calls throughout the entire dataset (inside and outside of the NLF cluster). We extracted those raw audio files with manually confirmed breeding calls to build a folder of 2,807 confirmed advertisement call files, which were then used to train a new classifier. We re-ran our new classifier with the same initial site to assess improvement and we found the classifier's performance was sufficient and the number of false negatives was greatly reduced. For our purposes, we ensured classifiers detected at least one advertisement call, if present, in a weekly interval.

Once classifiers were completed, we processed all other acoustically monitored sites with both classifiers: northern leopard frog and bullfrog. We visually and audially confirmed and labeled all positive breeding calls within the NLF or BF cluster on a weekly basis and recorded detection (1) or non-detection (0) of a breeding call for each week the unit was actively recording. Detection histories and their corresponding mid-week dates were then used in our analysis. For analyses quantifying breeding activity, phenology, or requiring a false negative rate of zero (e.g., early colonizing species), better performing classifiers may be necessary.

S3: Secondary Candidate Set Approach to Model Building and Selection

We used a single-season (static) occupancy approach to test our hypotheses regarding method- and species-specific detection probabilities (MacKenzie et al., 2002). We used a secondary candidate set model building strategy to develop and fit our candidate models (Morin et al., 2020). Specifically, we fit models representing hypotheses associated with detection probability for a given method (e.g., p_{VES},), while retaining a constant structure on the detection probability for the other two methods (e.g., p_{eDNA}, or p_{AAR}). Occupancy (ψ) was held constant for all models and is not shown in model notation. We analyzed detection histories for northern leopard frogs and bullfrogs separately and fit a series of models to explore how environmental (pH, Wtemp, Atemp) and survey-specific (Julian, Liters, SearchT, ObsCount) covariates influenced species' detection probabilities. See Supplementary Material S4 for a formal description of the model(s) and MacKenzie et al., 2017.

Due to sample size, we tested univariate relationships and only considered an additive effect of Julian Date and unit type (roving vs. stationary) for AAR detection probability. We identified detection covariate(s) with substantial support for each method as those within ΔAICc <5 (bolded below) and included all combinations of supported covariate structures in our final combined model set. We fit all models in program MARK version 6.2 (White and Burnham, 1999) and used Akaike Information Criterion and associated model weights to evaluate model performance (Anderson and Burnham, 2002). Models with pretending variables were not included in the final model set (Arnold 2010).

Table S3.1: eDNA detection probability was (p_{eDNA}) modeled as a function of Julian date (Julian), pH, liters of water filtered per eDNA sample (Liters), and water and air temperature (WTemp and ATemp, respectively). pH^2 denotes a model with a quadratic relationship. Occupancy and detection probabilities for visual encounter surveys and automated acoustic recorders were held constant for all models and are not shown in model notation. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), deviance (-2*log(L)*) and estimated covariate effects, $\hat{\beta}$, and associated standard errors (SE) are given for each model.

Model	**AICc**	**ΔAICc**	***w***	***K***	**-2log(L)**	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
$p_{eDNA}(pH^2)$	**647.91**	**0**	**0.87**	**6**	**633.20**	**-10.64; 0.62***	**3.05; 0.19***
$p_{eDNA}(pH)$	**651.84**	**3.92**	**0.12**	**5**	**639.96**	**-0.65**	**0.22**
$p_{eDNA}(.)$	658.44	10.53	0.00	4	649.23		
p_{eDNA}(Julian)	658.88	10.97	0.00	5	647.01	-0.006	0.004
p_{eDNA}(Atemp)[a]	659.99	12.08	0.00	5	648.12	-0.03	0.02
p_{eDNA}(Liters)[a]	660.41	12.49	0.00	5	648.53	0.11	0.13
p_{eDNA}(Wtemp)[a]	661.08	13.16	0.00	5	649.20	-0.005	0.03

[a] denotes pretending variable (Arnold 2010)

* denotes the estimate of the quadratic term and associated standard error

Table S3.2: Visual encounter survey detection probability was (p_{VES}) modeled as a function of Julian date (Julian), search time (SearchT), the number of observers (ObsCount), and water and air temperature (WTemp and ATemp, respectively). Occupancy and detection probabilities for eDNA and automated acoustic recorders were held constant for all models and are not shown in model notation. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), deviance (-2*log(L)*) and estimated covariate effects, $\hat{\beta}$, and associated standard errors (SE) are given for each model.

Model	**AICc**	**ΔAICc**	***w***	***K***	**-2log(L)**	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
p_{VES}(SearchT)	**658.43**	**0**	**0.29**	**5**	**646.55**	**0.02**	**0.01**
$p_{VES}(.)$	**658.44**	**0.02**	**0.29**	**4**	**649.23**		
p_{VES}(Julian)[a]	659.98	1.55	0.14	5	648.10	0.007	0.007
p_{VES}(ObsCount)[a]	660.27	1.84	0.12	5	648.39	0.94	1.12
p_{VES}(Wtemp)[a]	660.94	2.52	0.08	5	649.07	-0.02	0.05
p_{VES}(Atemp)[a]	661.09	2.66	0.08	5	649.22	-0.005	0.04

[a] denotes pretending variable (Arnold 2010)

Table S3.3: Automated acoustic recorder detection probability was (p_{AAR}) modeled as a function of Julian date (Julian) and/or Roving units (ROV). Occupancy and detection probabilities for eDNA and visual encounter surveys were held constant for all models and are not shown in model notation. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), deviance (-2*log(L)*) and estimated covariate effects, $\hat{\beta}$, and associated standard errors (SE) are given for each model.

Model	**AICc**	**ΔAICc**	***w***	***K***	**-2log(L)**	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
p_{AAR}(Julian)	**646.23**	**0**	**0.58**	**5**	**634.36**	**-0.016**	**0.004**
p_{AAR}(Julian+ROV)	**646.86**	**0.63**	**0.42**	**6**	**632.15**	**-0.016;** **-0.77***	**0.004;** **0.56***
p_{AAR}(.)	658.44	12.21	0.00	4	649.23		
p_{AAR}(ROV)	659.73	13.50	0.00	5	647.86	-0.61	0.55

* denotes the estimate of the additive term and associated standard error

Table S3.4: Northern leopard frog model selection results are depicted as combinations of the top-supported covariates ($\Delta AIC > 5$) from method-specific models (Table S3.1 – S3.3). Occupancy was held constant in all models and is not depicted in model notation. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), and deviance (-2*log(L)*).

Model	**AICc**	**ΔAICc**	***w***	***K***	**-2log(L)**
p_{VES}(.) p_{eDNA}(pH^2) p_{AAR}(Julian)	636.07	0.00	0.31	7	618.33
p_{VES}(SearchT) p_{eDNA}(pH^2) p_{AAR}(Julian)	636.60	0.54	0.24	8	615.64
p_{VES}(.) p_{eDNA}(pH^2) p_{AAR}(Julian+ROV)	637.09	1.02	0.18	8	616.12
p_{VES}(SearchT) p_{eDNA}(pH^2) p_{AAR}(Julian+ROV)	637.86	1.79	0.13	9	613.43
p_{VES}(.) p_{eDNA}(pH) p_{AAR}(Julian)	639.80	3.74	0.05	6	625.09
p_{VES}(SearchT) p_{eDNA}(pH) p_{AAR}(Julian)	640.06	3.99	0.04	7	622.32
p_{VES}(.) p_{eDNA}(pH) p_{AAR}(Julian+ROV)	640.62	4.55	0.03	7	622.88
p_{VES}(SearchT) p_{eDNA}(pH) p_{AAR}(Julian+ROV)	641.08	5.01	0.03	8	620.11
p_{VES}(.) p_{eDNA}(.) p_{AAR}(.)	658.44	22.38	0.00	4	649.23

Bullfrog

Table S3.5: eDNA detection probability was (p_{eDNA}) modeled as a function of Julian date (Julian), pH, liters of water filtered per eDNA sample (Liters), and water and air temperature (WTemp and ATemp, respectively). pH^2 denotes a model with a quadratic relationship. Occupancy and detection probabilities for visual encounter surveys and automated acoustic recorders were held constant for all models and are not shown in model notation. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), deviance ($-2log(L)$) and estimated covariate effects, $\hat{\beta}$, and associated standard errors (SE) are given for each model.

Model	AICc	ΔAICc	*w*	*K*	-2log(L)	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
p_{eDNA}(Liters)	**511.18**	**0**	**0.63**	**5**	**499.30**	**0.46**	**0.15**
p_{eDNA}(Wtemp)	**512.62**	**1.44**	**0.31**	**5**	**500.74**	**0.16**	**0.05**
$p_{eDNA}(pH^2)$	516.88	5.71	0.04	6	502.17	13.73; -0.84*	3.29; 0.20*
p_{eDNA} (.)	519.62	8.44	0.01	4	510.40		
p_{eDNA}(Julian)[a]	520.50	9.33	0.01	5	508.63	0.006	0.005
p_{eDNA}(pH)	522.26	11.08	0.00	5	510.38	0.04	0.26
p_{eDNA}(Atemp)	522.27	11.09	0.00	5	510.39	0.003	0.03

[a] denotes pretending variable (Arnold 2010)

* denotes the estimate of the quadratic term and associated standard error

Table S3.6: Visual encounter survey detection probability was (p_{VES}) modeled as a function of Julian date (Julian), search time (SearchT), the number of observers (ObsCount), and water and air temperature (WTemp and ATemp, respectively). Occupancy and detection probabilities for eDNA and automated acoustic recorders were held constant for all models and are not shown in model notation. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), deviance ($-2log(L)$) and estimated covariate effects, $\hat{\beta}$, and associated standard errors (SE) are given for each model

Model	AICc	ΔAICc	*w*	*K*	-2log(L)	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
p_{VES}(Wtemp)	**516.40**	**0**	**0.63**	**5**	**504.53**	**0.20**	**0.09**
p_{VES}(.)	**519.62**	**3.21**	**0.13**	**4**	**510.40**		
p_{VES}(SearchT)	519.79	3.39	0.12	5	507.92	0.02	0.01
p_{VES}(ObsCount)[a]	521.43	5.03	0.05	5	509.56	0.93	1.09
p_{VES}(Atemp)[a]	522.15	5.75	0.04	5	510.27	0.02	0.05
p_{VES}(Julian)[a]	522.17	5.77	0.04	5	510.29	-0.003	0.008

Table S3.7: Automated acoustic recorder detection probability was (p_{AAR}) modeled as a function of Julian date (Julian) and/or Roving units (ROV). Occupancy and detection probabilities for eDNA and visual encounter surveys were held constant for all models and are not shown in model notation. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), deviance (-2*log(L)*) and estimated covariate effects, $\hat{\beta},$ and associated standard errors (SE) are given for each model.

Model	AICc	ΔAICc	*w*	*K*	-2log(L)	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
p_{AAR}(Julian)	**501.61**	**0**	**0.73**	**5**	**489.74**	**0.02**	**0.004**
p_{AAR}(Julian+ROV)	**503.57**	**1.96**	**0.27**	**6**	**488.86**	**0.02; 0.46***	**0.004; 0.48***
p_{AAR}(.)	519.62	18.00	0.00	4	510.40		
p_{AAR}(ROV)[a]	522.23	20.62	0.00	5	510.36	0.09	0.45

[a] denotes pretending variable (Arnold 2010)

* denotes the estimate of the additive term and associated standard error

Table S3.8: Bullfrog model selection results are depicted as combinations of the top-supported covariates $(\Delta AIC > 5)$ from method-specific models (Table S3.5 – S3.7). Occupancy was held constant in all models and is not depicted in model notation. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (*w*), number of parameters (K), and deviance (-2*log(L)*)

Model	AICc	Δ AICc	*w*	*K*	-2log(L)
p_{VES}(Wtemp) p_{eDNA}(Liters) p_{AAR}(Julian)	490.51	0.00	0.36	7	472.78
p_{VES}(Wtemp) p_{eDNA}(Wtemp) p_{AAR}(Julian)	491.90	1.39	0.18	7	474.17
p_{VES}(Wtemp) p_{eDNA}(Liters) p_{AAR}(Julian+ROV)	492.87	2.36	0.11	8	471.91
p_{VES}(.) p_{eDNA}(Liters) p_{AAR}(Julian)	493.34	2.83	0.09	6	478.63
p_{VES}(SearchT) p_{eDNA}(Liters) p_{AAR}(Julian)	493.88	3.36	0.07	7	476.14
p_{VES}(Wtemp) p_{eDNA}(Wtemp) p_{AAR}(Julian+ROV)	494.26	3.74	0.05	8	473.29
p_{VES}(.) p_{eDNA}(Wtemp) p_{AAR}(Julian)	494.79	4.27	0.04	6	480.08
p_{VES}(SearchT) p_{eDNA}(Wtemp) p_{AAR}(Julian)	495.33	4.82	0.03	7	477.60
p_{VES}(.) p_{eDNA}(Liters) p_{AAR}(Julian+ROV)	495.49	4.98	0.03	7	477.76
p_{VES}(SearchT) p_{eDNA}(Liters) p_{AAR}(Julian+ROV)	496.23	5.72	0.02	8	475.27
p_{VES}(.) p_{eDNA}(Wtemp) p_{AAR}(Julian+ROV)	496.94	6.42	0.01	7	479.20
p_{VES}(SearchT) p_{eDNA}(Wtemp) p_{AAR}(Julian+ROV)	497.69	7.18	0.01	8	476.72
p_{VES}(.) p_{eDNA}(.) p_{AAR}(.)	519.62	29.10	0.00	4	510.40

S4: State Space Model Formulation

Occupancy models allow investigators to decouple the state variable (occupancy or species occurrence) from the observational process (i.e., detection probability, given the species occurrence). These two processes can be described in the following manner, which is consistent with presentations in MacKenzie et al. 2017. For simplicity, we describe the constant model and the most parameterized model in our candidate model set for northern leopard frog (Table S3.4)

I. Constant Model: denoted $p_{VES}(.)$ $p_{eDNA}(.)$ $p_{AAR}(.)$

The presence or absence of northern leopard frogs at unit i was modeled as a Bernoulli random variable, z_i, where the probability the species occurred is ψ:

$$z_i \sim Bernoulli\ (\psi)$$

The outcome of the jth survey using method m is also a Bernoulli random variable, y_{imj}:

$$y_{imj} \sim Bernoulli\ (z_i * p_m)$$

Where p_m is the probability of detection for a given survey using method m, given the species occurs at unit i.

II. Most parameterized model: denoted p_{VES}(SearchT) p_{eDNA}(pH^2) p_{AAR}(Julian+ROV)

We expanded on the constant model above by allowing method-specific detection probabilities, p_m to vary as a function of covariates, using the logit link function.

For example, the probability of detecting northern leopard frogs at unit i during visual encounter survey j was expressed as:

$$logit(p_{i,VES,j}) = \beta_{0\,VES} + \beta_{1\,VES} * SearchT_{ij}$$

Similarly, the probability of detecting northern leopard frogs at unit *i* with eDNA survey *j* was expressed as:

$$logit(p_{i,eDNA,j}) = \beta_{0\,eDNA} + \beta_{1\,eDNA} * pH_{i,j} + \beta_{2\,eDNA} * pH_{ij}^2$$

Finally, the probability of detecting northern leopard frogs at unit *i* with an AAR survey *j* was expressed as:

$$logit(p_{i,AAR,j}) = \beta_{0\,AAR} + \beta_{1\,AAR} * Julian_{ij} + \beta_{2\,AAR} * ROV_{ij}$$

where $\beta_{0\,VES}$, $\beta_{0\,eDNA}$ and $\beta_{0\,AAR}$ are the intercepts for each method and $\beta_{1\,VES}$, $\beta_{1\,eDNA}$, $\beta_{2\,eDNA}$, $\beta_{1\,AAR}$, and $\beta_{2\,AAR}$ are the coefficients associated with method-specific covariates ($SearchT$, pH, $Julian$, and ROV).

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CHAPTER TWO

S1: Preliminary *Bd* Analyses

Bd Sample Processing

Each *Batrachochytrium dendrobatidis (Bd)* swab from a given amphibian was stored in a sample tube with 70% ethanol and these samples were processed by Pisces Molecular LLC located in Boulder, Colorado, USA. Liquid in these tubes was pipetted to mix the sample and then transferred to a microcentrifuge tube where it was spun at ~16,000 x G for three minutes. Supernatant was drawn off and lysis buffer added, with any present pellet resuspended by vortexing. Afterwards, 10 µg of carrier DNA was added to the lysis buffer and spin-column DNA purification procedure was used to extract total DNA from the pooled sample. This DNA was then assayed for the presence of *Batrachochytrium dendrobatidis (Bd)* ribosomal RNA Intervening Transcribed Sequence (ITS) region with a 45 cycle PCR amplification procedure. PCR runs included positive DNA and no DNA. The positive DNA used DNA prepped from a plasmid constructed at Pisces Molecular that contained *Bd* ribosomal RNA from the ITS region. The plasmid DNA used to generate a standard curve was serially diluted ten-fold from 1.57×10^6 to 1.57×10^0 molecules/µL. For the no DNA PCR run, water (H_2O) was used in place of template DNA and reactions were uncapped while adding sample DNA, which served as a control for detecting contaminated DNA in PCR reagents or any carryover DNA from sample preparation. Results from PCR runs were used to assign the swabbed amphibian associated with each processed sample as positive (1) or negative (0) for *Bd*.

Covariates and Single Species Occupancy Analysis

We used qPCR results to create detection histories for *Bd* at each of the 28 sampled sites. These detection histories included the detection (1) or non-detection (0) of *Bd* from each amphibian swabbed at the site (up to ten per site) and covariates that included the amphibian's associated life stage and species group. We grouped life stage as metamorphs (recently metamorphosed amphibians) and adults/juveniles. We grouped species as frog (*Pseudacris, Lithobates*) or toad (*Spea, Anaxyrus*), according to their life histories. We then analyzed *Bd* detection histories using a single-species occupancy modelling approach (MacKenzie et al. 2006) in Program MARK (White and Burnham, 1999) to explore the occurrence of *Bd* in our study area and how *Bd* detection varied with life stage and species swabbed. We explored univariate, additive, and interactive relationships between life stage and species swabbed (Table S1.1). Because we investigated site-level detection probability, resulting detection estimates describe the *prevalence* of *Bd* at a site.

Results

We swabbed 157 individuals across 28 sites. We swabbed 16 western chorus frogs (*P. triseriata*), 27 great plains toads (*A. cognatus*), 49 Woodhouse's toads (*A. woodhousii*), one plains spadefoot toad (*S. bomifrons*), 58 northern leopard frogs (*L. pipiens*) and 6 American bullfrogs (*L. catesbeianus*). At least one swab tested positive for *Bd* at 5 of our 28 sites (naïve site occupancy = 0.18). Results from the constant model ($\hat{\psi} = 0.58, \widehat{SE} = 0.31$), did not agree with naïve occupancy, indicating a low cumulative prevalence (i.e., detection probability). In total, 8 individual amphibians tested positive for *Bd*, and all but one of them were an adult or juvenile. The model with age was best supported, followed by the species only model (Table S1.1). Additive and interactive effects were less-well supported, though all models were within ΔAICc <5 (Table S1.1). Model results showed that metamorph *Bd* prevalence was exceptionally low and imprecise ($\hat{p} = 0.015, \widehat{SE} = 0.019$) compared to adult prevalence, which was higher but still imprecise ($\hat{p} = 0.28, \widehat{SE} = 0.13$). We found *Bd* prevalence for toads was higher ($\hat{p} =$

$0.56, \widehat{SE} = 0.23$) than for frogs ($\hat{p} = 0.25, \widehat{SE} = 0.11$), but both estimates were imprecise, likely due to our small sample size of positive swabs. The cumulative probability of detecting *Bd* at least once within a sample of 10 metamorph individuals ($p^* = 1 - (1 - 0.015)^{10}$) was only 0.14. Thus, we decided that *Bd* prevalence was too low to include *Bd* as an additional species in a multi-species model.

Recently metamorphosed amphibians are considered the life stage most susceptible to *Bd*, as their immune systems are underdeveloped (Humphries et al., 2022). Despite this knowledge, there have been few studies explicitly investigating how *Bd* dynamics vary with life stage (Humphries et al., 2022). In our study area, metamorphosis aligns with warm temperatures and drier conditions. Because *Bd* growth and reproduction is limited by temperature (thermal maximum ≥ 26ºC) and desiccation (Sheets et al., 2021; Van Rooij et al., 2015), *Bd* prevalence is typically lower in later season months (Erdmann et al., 2018).

Although *Bd* prevalence was too low to include *Bd* as a threat implicated in northern leopard frog declines, we can recommend improved sampling methods. In our study area researchers should consider preferentially swabbing adult and juvenile amphibians in early spring months. In later summer months when prevalence is likely low, larger sample sizes may be necessary to estimate *Bd* prevalence with precision (Table S1.2).

Table S1.1: *Bd* (Batrachochytrium dendrobatidis) occurrence ψ was modeled as a function of recently metamorphosed age class (age) and toad species (species). Coefficient estimates are associated with the effects of metamorphs or toads on *Bd* detection, and compared to adults/juveniles or frogs. Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (w), number of parameters (K), deviance (-2log(L)) and estimated covariate effects, $\hat{\beta}$, and associated standard errors $\widehat{SE}(\hat{\beta})$ are given for each model. A * denotes the estimate of the interactive term and + denotes the estimate of the additive term.

Model	$AICc$	$\Delta AICc$	w	*K*	*-2log(L)*	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
$\psi(.)p$(age)	53.52	0	0.44	3	46.52	-3.21	1.61
$\psi(.)p$(species)	55.02	1.5	0.21	2	50.54	1.30	1.07
$\psi(.)p$(age*species)	55.95	2.43	0.13	5	43.22	-1.95 1.25+ -20.01*	1.22 1.03+ 10244.75*
$\psi(.)p$(age+species)[a]	55.95	2.44	0.13	4	46.21	-3.42 0.53+	1.23 0.96+
$\psi(.)p(.)$	56.48	2.96	0.1	2	52		

[a]denotes a pretending variable (Arnold 2010).

Table S1.2: Cumulative detection probability ($p^* = 1 - (1 - prevalence)^{\#adults\ swabbed}$) of *Bd* swabs given an expected site prevalence and sample size at that site. Cumulative detection probability is the probability of at least one amphibian testing positive for *Bd* among the sampled individuals and considering a site prevalence value.

	# Adults Swabbed				
Prevalence	**10**	**20**	**30**	**40**	**50**
0.1	0.651322	0.878423	0.957609	0.985219	0.994846
0.2	0.892626	0.988471	0.998762	0.999867	0.999986
0.3	0.971752	0.999202	0.999977	0.999999	1
0.4	0.993953	0.999963	1	1	1
0.5	0.999023	0.999999	1	1	1
0.6	0.999895	1	1	1	1
0.7	0.999994	1	1	1	1
0.8	1	1	1	1	1

S2: Correlation and ANOVA Analyses

We calculated pairwise correlation coefficients between all continuous covariates and did not include correlated covariates ($|r| > 0.60$; Dormann et al., 2013) in the same parameter structure.

We recorded two survey-specific variables used to investigate variation in detection probabilities:

survey (Julian) date and water temperature, which had a correlation coefficient <0.60 (*r* = -0.14). We present a table of results from pairwise correlations for site-specific variables that do not change between survey occasions. Additionally, we explored relationships between categorical (e.g., site type) and continuous covariates using one-way ANOVAs and did not include covariates with p-value <0.05 in the same parameter structure (i.e., via additive or interactive relationships). We present a table of one-way ANOVA regression p-values for categorical and continuous variables with p-values >0.05.

Table S2.1: Pearson pairwise correlation coefficient for site-specific variables: average water temperature (ºC), search area (m^2), elevation (m), proportion of surrounding forest, grassland (grass), agricultural (ag), and urban (urb) land cover within 1 km, and percentage of average canopy cover (canopy) and emergent vegetation (veg). Bolded values correspond to those with a correlation coefficient $|r|$ >0.60.

	Wtemp	**Search area**	**Elev**	**Forest**	**Grass**	**Ag**	**Urb**	**Canopy**	**Veg**
Wtemp	1	-0.10	-0.53	-0.47	0.25	0.11	0.02	-0.37	0.01
Search area		1	0.16	0.19	-0.19	-0.11	0.09	0.13	0.15
Elev			1	**0.86**	0.31	-0.41	-0.18	0.16	-0.03
Forest				1	-0.50	-0.32	-0.23	0.11	-0.05
Grass					1	-0.29	-0.41	-0.20	0.11
Ag						1	-0.06	-0.03	0.08
Urb							1	0.19	-0.13
Canopy								1	-0.03
Veg									1

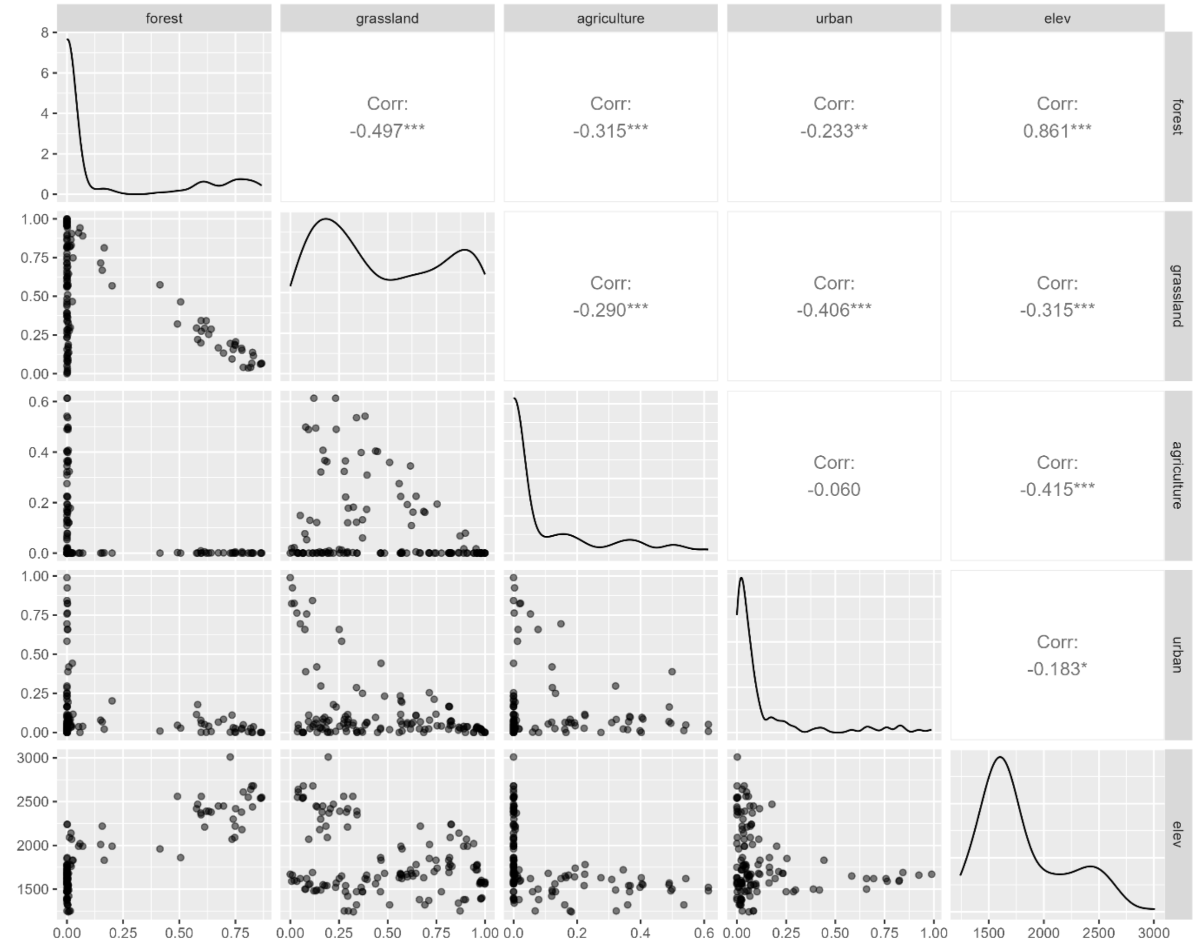

Figure S2.1: Pearson pairwise correlation metrics, scatterplot matrices, and frequency density lines at their corresponding scales. Variables include the proportion of forest, grassland, agriculture, and urban cover within 1 km of a site and elevation (m). Diagonal panels show frequency density lines for each variable and scatterplots show paired variable distributions. Variable ranges are shown on the axes below the column and to the left of the row. The panel above and right shows Pearson correlations (Corr) between variables. Although land cover types do not have a correlation coefficient >|0.6|; Dormann et al., 2013, they are additively correlated as evidenced by their paired scatterplot distributions.

Table S2.2: Results from one-way ANOVA modeling each continuous response variable (Covariate) as a function of site type. A * indicates statistically significant relationships with p-values <0.05.

Covariate	**p-value**
search.area	1.92×10^{-9}*
wtemp	0.00012*
veg	0.371
ag	0.446
forest	0.0691
urban	0.676
grassland	7.03×10^{-5}*

S3: Preliminary Northern Leopard Frog Persistence Analyses

In our study design, we selected sites in recent and historic HUC-12 watersheds. We modeled likely variation in northern leopard frog persistence among sites associated with the time and location of last known northern leopard frog. Specifically, we considered models northern leopard frog persistence ($\psi^{BA=Ba}$) varied based on: (1) the year of the closest record (year record) to an individual site, (2) the distance (m) to the closest record,(3) both year and distance of the closest record, or (4) different among historic and recent HUC-12 watersheds. We include a constant persistence structure for comparison. We retained the most parsimonious time structure for Northern leopard frog persistence in the final candidate set.

Table S3.1: Northern leopard frog persistence $\psi^{BA=Ba}$ was modeled as a function sub watershed type (recent vs. historic), the year of the previous observation at a site (year record), and the distance of that associated record to the site in meters (distance). Model notation along with Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (w), number of parameters (K), deviance (-2log(L)) and estimated covariate effects, $\hat{\beta}$, and associated standard errors $\widehat{SE}(\hat{\beta})$ are given for each model. Bolded models are those included in the final candidate model set.

Model	$AICc$	$\Delta AICc$	*w*	*K*	*-2log(L)*	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
$\psi^{BA=Ba}$ (year record)	**659.97**	**0**	**0.54**	**5**	**649.51**	**0.10**	**0.01**
$\psi^{BA=Ba}$(year record + distance)	660.32	0.35	0.45	6	647.67	0.10, -0.0004	0.01, 0.0003
$\psi^{BA=Ba}$ (recent vs. historic)	668.13	8.16	0.01	5	657.67	4.44	1.04
$\psi^{BA=Ba}$ (distance)	716.57	56.60	0	5	706.12	-0.0008	0.0003
$\psi^{BA=Ba}$(.)	724.6	64.63	0	4	716.3		

S4: Secondary Candidate Set Approach to Model Building and Selection

We used a secondary candidate set model building strategy to develop and fit models representing our *a priori* hypotheses (Morin et al., 2020).). In our study system, bullfrogs are known competitors and predators of northern leopard frogs (Johnson et al. 2011; Smith and Keinath 2007) and thus are considered the dominate species (denoted species "A"). Northern leopard frog persistence (i.e., current occurrence) is likely conditional on the presence of bullfrogs, so this species is the subordinate species (denoted species "B"). We investigated hypotheses in four sub-model categories: 1) bullfrog occupancy (Ψ^{A}), 2) bullfrog detection (p^{A}, r^{A}), 3) northern leopard frog persistence (Ψ^{BA}, Ψ^{Ba}), and 4) northern leopard frog detection (p^{B}, r^{BA}, r^{Ba}), while keeping a constant structure on all non-focal parameters. We fit models representing hypotheses associated with bullfrog occupancy, northern leopard frog persistence, and both species' detection. We investigated how habitat features influenced bullfrog occupancy probabilities and how site and survey factors and northern leopard frog co-occurrence influenced bullfrog detection probabilities. We investigated univariate, additive, and interactive effects of bullfrogs and habitat features on northern leopard frog persistence. Finally, we determined how site and survey factors and bullfrog co-occurrence influenced northern leopard frog detection probabilities. We identified covariate(s) with substantial support for each method as those better supported than the null and within ΔAICc <5 and used Akaike Information Criterion and associated model weights to evaluate model performance (Anderson and Burnham, 2002). Models with pretending variables were not included in the final model set (Arnold 2010)

Bullfrog Occupancy Models ψ^A

Bullfrog occupancy ψ^A was modeled as a function of the proportion of forest, agriculture (ag), urban, and grassland cover within 1km of a site, elevation (elev), average water temperature (avg wtemp), watershed type (huc.type), average emergent vegetation (veg), average canopy cover (canopy) and site type (pond, wetland, reservoir, or stream). Watershed type (recent or historic) was included as a covariate for bullfrog occupancy to ensure site selection originally intended to capture variation in northern leopard frog persistence did not capture unknown variation in bullfrog occurrence. For models with site.type, estimated effects are relative to stream sites and include pond (p), wetland (w) and reservoir (r) effects.

Table S4.1: Model selection metrics including Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (w), number of parameters (K), deviance (-2log(L)) and estimated covariate effects, $\hat{\beta}$, and associated standard errors $\widehat{SE}(\hat{\beta})$are given for each model. Bolded models are those included in the final candidate model set. A + denotes the estimate of the additive term.

Model	$AICc$	$\Delta AICc$	w	K	*-2log(L)*	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
ψ^A(forest)	**702.02**	**0**	**0.99**	**5**	**691.56**	**-5.99**	**2.33**
ψ^A (elev)	711.91	9.89	0.01	5	701.45	-0.002	0.0007
ψ^A (ag)	719.68	17.66	0	5	709.22	3.06	1.18
ψ^A (urban)	722.27	20.25	0	5	711.81	1.82	0.89
ψ^A (avg wtemp)	722.97	20.95	0	5	712.51	0.09	0.04
ψ^A (.)	724.6	22.58	0	4	716.3		
ψ^A (huc.type)	725.49	23.47	0	5	715.03	0.42	0.38
ψ^A(grassland)	726.01	23.99	0	5	715.56[a]	-0.49	0.58
ψ^A (veg)	726.12	24.1	0	5	715.67[a]	0.13	0.17
ψ^A (canopy)	726.73	24.71	0	5	716.28[a]	0.02	0.16
ψ^A (site.type)	729.62	27.59	0	7	714.75	p: -0.41 w: -0.53 r: 0.08	p: 0.44 w: 0.66 r: 0.55
ψ^A (site.type + veg)	731.03	29.01	0	8	713.9	p: -0.38 w: -0.62 r: 0.13 0.16[+]	p: 0.44 w: 0.67 r: 0.55 0.17[+]

[a] denotes a pretending variable (Arnold 2010).

Northern Leopard Frog Persistence Models ψ^{BA} and ψ^{Ba}

Northern leopard frog persistence ψ^{BA} and ψ^{Ba} was modeled as a function of univariate, additive (+) or interactive (*) effects of: bullfrog occurrence (BF), the proportion of forest, agriculture (ag), urban, and grassland cover within 1km of a site, elevation (elev), average emergent vegetation (veg), average canopy cover (canopy), and site type (pond, wetland, reservoir, stream). For models with site.type, estimated effects are relative to stream sites and include pond (p), wetland (w) and reservoir (r) effects.

Table S4.2: Model selection metrics include Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (w), number of parameters (K), deviance (-2log(L)) and estimated covariate effects, $\hat{\beta}$, and associated standard errors $\widehat{SE}(\hat{\beta})$ are given for each model. Bolded models are those included in the final candidate model set. A + denotes the estimate of the additive term and a * denotes the estimate of the interactive term.

Model	$AICc$	$\Delta AICc$	*w*	*K*	*-2log(L)*	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
$\psi^{BA\neq Ba}$ (BF * grass)	**705.41**	**0**	**0.92**	**7**	**690.55**	**-1.9** **1.21^{+}** **5.02^{*}**	**1.21** **0.76^{+}** **2.00^{*}**
$\psi^{BA\neq Ba}$ (BF + grass)	711.57	6.16	0.04	6	698.93	0.88 2.45^{+}	0.46 0.68^{+}
$\psi^{BA=Ba}$ (grass)	713.18	7.77	0.02	5	702.73	2.25	0.64
$\psi^{BA\neq Ba}$ (BF * ag)	714.2	8.79	0.01	7	699.33	1.49 -2.09^{+} -7.96^{*}	0.52 2.24^{+} 4.58^{*}
$\psi^{BA\neq Ba}$ (BF + ag)	715.44	10.02	0.01	6	702.79	1.07 -5.63^{+}	0.46 2.09^{+}
$\psi^{BA=Ba}$ (ag)	718.83	13.41	0	5	708.37	-4.40	1.88
$\psi^{BA\neq Ba}$ (BF * site type + avg veg)	719.17	13.76	0	13	690.21	3.62 veg: 0.68^{+} p: 1.19^{+} w: -0.13^{+} r: -19.07^{+} veg: -0.82^{*} p: -3.21^{*} w: -1.00^{*} r: 17.62^{*}	1.31 veg: 0.30^{+} p: 0.65^{+} w: 0.93^{+} r: 1064^{+} veg: 0.48^{*} p: 1.13^{*} w: 1.67^{*} r: 1064^{*}
$\psi^{BA\neq Ba}$ (BF * site type)	721	15.58	0	11	696.88	1.73 p: 0.89^{+} w: 0.25^{+} r: -21.36^{+} p: -2.89^{*} w: -1.59^{*} r: 19.88^{*}	0.66 p: 0.56^{+} w: 0.83^{+} r: 991^{+} p: 1.09^{*} w: 1.54^{*} r: 991^{*}
$\psi^{BA\neq Ba}$ (BF * urb)	721.16	15.75	0	7	706.29	1.41 -0.30^{+} -5.93^{*}	0.55 1.38^{+} 3.80^{*}
$\psi^{BA\neq Ba}$ (BF * avg veg)	722.23	16.82	0	7	707.37	2.20 0.57^{+} -0.72^{*}	1.00 0.24^{+} 0.41^{*}
$\psi^{BA\neq Ba}$ (BF + urb)	722.61	17.19	0	6	709.96	0.83 -2.29^{+}	1.44 0.17^{+}
$\psi^{BA=Ba}$ (avg veg)	723.07	17.66	0	5	712.62	0.33	0.18
{$\psi^{BA\neq Ba}$ (BF + avg veg)	723.23	17.82	0	6	710.59	0.60 0.33^{+}	0.42 0.18^{+}
$\psi^{BA\neq Ba}$ (BF + site type + avg veg)	723.97	18.55	0	9	704.55	0.68 veg: 0.34^{+} p: -0.02^{+}	0.43 veg: 0.19^{+} p: 0.44^{+}

						w: -0.59^{+} r: -1.6^{+}	w: 0.68^{+} r: 0.82^{+}
$\psi^{BA=Ba}$ (urb)	724.08	18.66	0	5	713.62	-1.77	1.22
$\psi^{BA=Ba}$ (site.type + avg veg)	724.14	18.73	0	8	707.02	0.34 p: -0.07^{+} w: -0.65^{+} r: -1.55^{+}	0.19 p: 0.44^{+} w: 0.67^{+} r: 0.81^{+}
{$\psi^{BA\neq Ba}$ (BF)	724.44	19.03	0	5	713.98	0.63	0.41
$\psi^{BA=Ba}$ (forest)	724.46	19.05	0	5	714.01	-1.06	0.73
$\psi^{BA=Ba}$ (.)	724.6	19.18	0	4	716.3		
$\psi^{BA\neq Ba}$ (BF + site type)	725.01	19.6	0	8	707.89	0.69 p: -0.07^{+} w: -0.39^{+} r: -1.66^{+}	0.43 p: 0.44^{+} w: 0.66^{+} r: 0.81^{+}
$\psi^{BA=Ba}$ (site type)	725.38	19.97	0	7	710.52	p: -0.13^{+} w: -0.45^{+} r: -1.62^{+}	p: 0.43^{+} w: 0.65^{+} r: 0.80^{+}
$\psi^{BA\neq Ba}$ (BF + canopy)	726.47	21.06	0	6	713.82	0.63 0.07^{+}	0.41 0.17^{+}
$\psi^{BA=Ba}$ (canopy)[a]	726.59	21.17	0	5	716.13	0.07	0.16
$\psi^{BA=Ba}$ (elev)[a]	726.64	21.23	0	5	716.18	0.0002	0.0005
$\psi^{BA\neq Ba}$ (BF*canopy)	728.59	23.18	0	7	713.72	0.45 0.04^{+} 0.13*	0.69 0.19^{+} 0.42*

[a] denotes a pretending variable

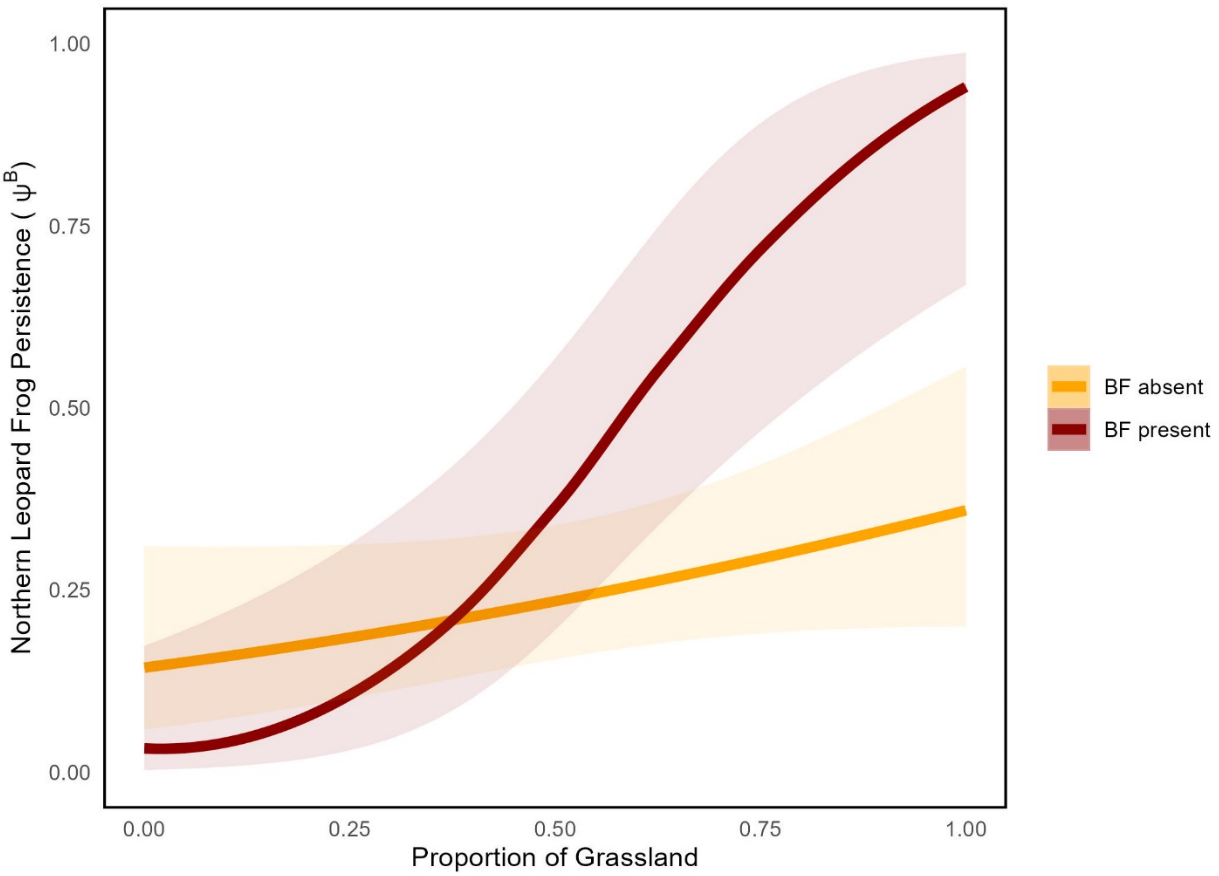

Figure S4.1: Estimated northern leopard frog persistence as an interactive function of agricultural cover and bullfrogs (BF) occurrence. Estimates are given for the interactive sub-model structure (Supplementary Table S4).

Bullfrog Detection Models p^A *and* r^A

Bullfrog detection p^A and r^A was modeled as a function of northern leopard frog occurrence (NLF), survey-specific water temperature (avg wtemp), survey date (date), and site type (pond, wetland, reservoir, or stream). For models with site.type, estimated effects are relative to stream sites and include pond (p), wetland (w) and reservoir (r) effects.

Table S4.3: Model selection metrics include Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (w), number of parameters (K), deviance (-2log(L)) and estimated covariate effects, $\hat{\beta}$, and associated standard errors $\widehat{SE}(\hat{\beta})$ are given for each model. Bolded models are those included in the final candidate model set. A + denotes the estimate of the additive term and a * denotes the estimate of the interactive term.

Model	$AICc$	$\Delta AICc$	w	K	*-2log(L)*	$\hat{\beta}$	$\widehat{SE}(\hat{\beta})$
$p^A \neq r^A$(NLF+wtemp)	**691.86**	**0**	**0.83**	**6**	**679.22**	**0.99, 0.28**	**0.46, 0.05**
$p^A = r^A$(wtemp)	**695.02**	**3.16**	**0.17**	**5**	**684.56**	**0.25**	**0.05**
$p^A = r^A$(site.type)	723.88	32.02	0	7	709.01	p: -0.46 w: -1.27 r: 0.61	p: 0.42 w: 0.72 r: 0.51
$p^A = r^A$(date)	724.32	32.46	0	5	713.86	0.008	0.005
$p^A = r^A$(.)	724.6	32.74	0	4	716.3		
$p^A \neq r^A$(NLF+site.type)	726.11	34.24	0	8	708.98	-0.07 p: -0.49$^+$ w: -1.29$^+$ r: 0.58$^+$	0.42 p: 0.48$^+$ w: 0.73$^+$ r: 0.53$^+$
$p^A \neq r^A$(NLF+date)	726.34	34.48	0	6	713.7	-0.15 0.009$^+$	0.38 0.006$^+$
$p^A \neq r^A$(NLF)[a]	726.73	34.87	0	5	716.27	0.05	0.36

[a] denotes a pretending variable

Northern Leopard Frog Detection Models p^B , r^{BA} *and* r^{Ba}

Northern leopard frog detection, p^B , r^{BA}, r^{Ba}, was modeled as univariate, additive (+) or interactive (*) functions of bullfrog co-occurrence (BF), average emergent vegetation (veg), average canopy cover (canopy), search time, search (survey) area (search area), survey date (date), and site type (pond, wetland, reservoir, stream). For models with site.type, estimated effects are relative to stream sites and include pond (p), wetland (w) and reservoir (r) effects.

Table S4.4: Model selection metrics include Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (w), number of parameters (K), deviance (-2log(L)) and estimated covariate effects, $\hat{\beta}$, and associated standard errors $\widehat{SE}(\hat{\beta})$ are given for each model. Bolded models are those included in the final candidate model set. A + denotes the estimate of the additive term and a * denotes the estimate of the interactive term.

Model	***AICc***	***ΔAICc***	***w***	***K***	***-2log(L)***	$\hat{\boldsymbol{\beta}}$	$\widehat{\boldsymbol{SE}}(\hat{\boldsymbol{\beta}})$
$p^{B} = r^{BA=Ba}$ (date)	**724.39**	**0**	**0.17**	**5**	**713.94**	**0.009**	**0.006**
$p^{B} = r^{BA=Ba}$ (.)	**724.6**	**0.2**	**0.15**	**4**	**716.3**		
$p^{B} = r^{BA=Ba}$ (wtemp)	724.96	0.57	0.13	5	714.5	0.06	0.04
$p^{B} = r^{BA=Ba}$ (search time)[a]	725.88	1.48	0.08	5	715.42	0.008	0.009
$p^{B} = r^{BA=Ba}$ (veg)[a]	725.98	1.58	0.08	5	715.52	0.17	0.20
$p^{B} \neq r^{BA=Ba}$ (BF + date)	726.5	2.11	0.06	6	713.85	-0.12 0.009^{+}	0.41 0.006^{+}
$p^{B} = r^{BA=Ba}$ (search area)[a]	726.67	2.28	0.05	5	716.21	$-0.25e^{-5}$	$0.96e^{-5}$
$p^{B} \neq r^{BA=Ba}$ (BF)[a]	726.75	2.36	0.05	5	716.29	0.01	0.40
$p^{B} \neq r^{BA=Ba}$ (BF + wtemp)	727.13	2.74	0.04	6	714.49	0.05 0.59^{+}	0.40 0.04^{+}
$p^{B} = r^{BA=Ba}$ (site type)	727.79	3.4	0.03	7	712.92	p: -0.68 w: 0.28 r: 0.03	p: 0.42 w: 0.72 r: 0.94
$p^{B} \neq r^{BA=Ba}$ (BF + veg)[a]	728	3.61	0.03	6	715.36	0.18 0.21^{+}	0.45 0.23^{+}
$p^{B} \neq r^{BA=Ba}$ (BF + search time)[a]	728.05	3.66	0.03	6	715.41	-0.04 0.008^{+}	0.40 0.009^{+}
$p^{B} \neq r^{BA=Ba}$ (BF * wtemp)	728.3	3.91	0.02	7	713.44	-1.88 0.02^{+} 0.09^{*}	1.91 0.06^{+} 0.09^{*}
$p^{B} \neq r^{BA=Ba}$ (BF + search area)[a]	728.86	4.46	0.02	6	716.21	-0.01 $0.27e^{-5+}$	0.41 $0.10e^{-4+}$
$p^{B} \neq r^{BA=Ba}$ BF + site type)	729.2	4.81	0.02	8	712.08	-0.45 p: -0.90^{+} w: 0.13^{+} r: 0.22^{+}	0.50 p: 0.50^{+} w:0.74^{+} r: $0.9^{+}1$
$p^{B} \neq r^{BA=Ba}$ (BF * veg)	729.63	5.23	0.01	7	714.76	-0.65 0.02^{+} 0.34^{*}	1.10 0.30^{+} 0.43^{*}
$p^{B} = r^{BA=Ba}$ (site type + veg)	729.91	5.52	0.01	8	712.79	p: -0.63 w: 0.24 r: 0.15 0.08^{+}	p: 0.44 w: 0.73 r: 0.98 0.22^{+}
$p^{B} \neq r^{BA=Ba}$ (BF + site type + veg)	731.49	7.1	0	9	712.08	-0.47 p: -0.92^{+} w: 0.13^{+} r: 0.21^{+} -0.01^{+}	0.55 p: 0.56^{+} w:0.75^{+} r: 0.95^{+} 0.24^{+}

[a] denotes a pretending variable

Final Candidate Model Set

Final candidate model selection results for northern leopard frog (*Lithobates pipiens*) persistence given bullfrogs are present or absent ($\psi^{BA\neq Ba}$), American bullfrog (*Lithobates catesbeianus*) occupancy (ψ^{A}), and both species' detection. Northern leopard frog detection probability was not different if bullfrogs were absent or presence ($p^{B} = r^{BA=Ba}$) and bullfrog detection probability was either affected ($p^{A} \neq r^{A}$) or unaffected ($p^{A} = r^{A}$) by northern leopard frog presence. The null model [$\psi^{A}(.), \psi^{BA=Ba}(.), p^{A} = r^{A}(.), p^{B} = r^{BA=Ba}(.)$] is given for comparison.

Table S4.5: Model selection metrics include Akaike Information Criterion for small sample sizes (AICc), ΔAICc, model weights (w), number of parameters (K), and deviance (-2log(L)) are given for each model.

Model	**AICc**	$\boldsymbol{\Delta AICc}$	$\boldsymbol{w}$	$\boldsymbol{K}$	**-2log(L)**
ψ^{A} (forest) $\psi^{BA\neq Ba}$(year record +BF*grassland) $p^{A} = r^{A}$(wtemp) $p^{B} = r^{BA=Ba}$(.)	610.68	0	0.29	10	588.93
ψ^{A}(forest) $\psi^{BA\neq Ba}$(year record +BF*grassland) $p^{A} \neq r^{A}$(NLF+wtemp) $p^{B} = r^{BA=Ba}$(.)	610.85	0.17	0.27	11	586.74
ψ^{A}(forest) $\psi^{BA\neq Ba}$(year record+BF*grassland) $p^{A} \neq r^{A}$(wtemp) $p^{B} = r^{BA=Ba}$ (date)	611.19	0.51	0.22	12	584.67
ψ^{A}(forest) $\psi^{BA\neq Ba}$(year record +BF*grassland) $p^{A} = r^{A}$ (wtemp) $p^{B} = r^{BA=Ba}$ (date)	611.22	0.54	0.22	11	587.11
ψ^{A}(forest) $\psi^{BA\neq Ba}$ (BF*grassland) $p^{A} = r^{A}$ (wtemp) $p^{B} = r^{BA=Ba}$ (.)	657.41	46.73	0	9	638
ψ^{A}(forest) $\psi^{BA\neq Ba}$ (BF*grassland) $p^{A} = r^{A}$ (wtemp) $p^{B} = r^{BA=Ba}$ (date)	657.42	46.74	0	10	635.67
ψ^{A}(forest) $\psi^{BA\neq Ba}$ (BF*grassland) $p^{A} \neq r^{A}$ (wtemp) $p^{B} = r^{BA=Ba}$ (.)	657.77	47.09	0	10	636.02
ψ^{A}(forest) $\psi^{BA\neq Ba}$ (BF*grassland) $p^{A} \neq r^{A}$ (wtemp) $p^{B} = r^{BA=Ba}$ (date)}	657.77	47.09	0	11	633.66
$\psi^{A}(.)\psi^{BA=Ba}(.)p^{A} = r^{A}(.)p^{B} = r^{BA=Ba}(.)$	724.6	113.92	0	4	716.3

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