Effects of land use, invasions and disease on Colorado's Northern leopard frog (*Lithobates pipiens*)

Prepared for the Colorado Division and Wildlife by

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Executive Summary

In the western USA, populations of the northern leopard frog (*Lithobates pipiens*) – historically one of the most widespread frogs in North America – have declined dramatically in abundance and geographic distribution. The reported decline of northern leopard frog populations in Colorado has coincided with habitat loss and alteration, invasion by predatory fishes and the American bullfrog (*Lithobates* [=*Rana*] *catesbeianus*), and emerging infections by the chytridiomycete pathogen, *Batrachochytrium dendrobatidis* (Hayes & Jennings 1986; Adams & Pearl 2007; Smith & Keinath 2007; Collins & Crump 2009). The purpose of our study was to assess the status of leopard frogs in Colorado and evaluate causes of decline. In order to standardize sampling efforts for northern leopard frogs and bullfrogs in Colorado, we developed and validated a sampling protocol for this project that has been incorporated by CDOW state personnel. Once developed, we used this protocol to assess the status of leopard frogs in Colorado and evaluate causes of decline through two main approaches. First, we revisited 196 historically occupied leopard frog sites distributed throughout Colorado. Second, we intensively sampled 220 randomly selected wetlands in northeast Colorado where declines are believed to have been severe. Our results indicated that leopard frogs have declined in Colorado, but that this pattern was regionally variable. For historically occupied sites, we found that leopard frogs still occur at about 50% of the sites west of the Continental Divide, while the majority of historic sites east of the divide, especially in the northeast, no longer support historic populations (1.7-28%). Wetlands east of the Divide were more also more likely to support bullfrogs (~24%) in comparison to western sites (2.2%-4.8%). This pattern was reinforced by our intensive sampling in northeastern Colorado along the Front Range, where leopard frogs occurred at only 5.9% of sites overall while 31.9% of sites supported bullfrog populations. Infections by the pathogenic chytrid *Batrachochytrium dendrobatidis* (*Bd*) occurred throughout the sampling area and were not strongly correlated with leopard frog occurrence; however, the sharply negative relationship between leopard frogs and elevation in the Northeast suggests *Bd* could be a contributing factor in montane declines. Our results indicate that leopard frogs are no longer present in many of the areas where they were once considered common. Several factors including invasive species, land use change and infectious disease, are most likely responsible for this change in distribution. The current findings also indicate areas where leopard frogs persist, suggesting that management action could be effective in reducing declines and maintaining viable populations. We advocate immediate protection of known leopard frog breeding sites along the Front Range in conjunction with continued research to better assess the status and conservation of this declining species in Colorado.

Introduction

Over the last 30 years, growing evidence suggests that the northern leopard frog – historically one of the most widespread and iconic amphibians in North America – has undergone large-scale population declines. This is particularly true in the western portion of its range (Stebbins & Cohen 1995; Rorabaugh 2005; Nichols 2006) where it is under consideration for protection under the US Endangered Species Act (Nichols 2006; Smith & Keinath 2007). The northern leopard frog is now considered to be declining in parts of Arizona, Montana, Wyoming, California, and Colorado (see review by Smith & Keinath 2007). The most severe declines appear to have occurred in the western portions of its range, from California east over the Rocky Mountains. Currently, the northern leopard frog is listed as a species of 'special concern', 'state-endangered', or 'protected' in Idaho, Indiana, Connecticut, Washington, Oregon, Montana, and Colorado. In the Colorado Division of Wildlife's Comprehensive Wildlife Conservation Strategy, leopard frogs are classified as a Tier 1 "species of most concern," with populations considered "low" and "declining" by experts.

Lambert (2006), working with the Colorado Natural Heritage Program (CNHP), established a preliminary database of current and historical northern leopard frog sites in Colorado. Based on regional population trends, he suggested that, for most regions, significant data gaps exist and more extensive sampling is needed to determine the status of leopard frogs in Colorado. Northern leopard frogs are declining or data deficient for all but two of state's regions (Lambert 2006). Declines for lower elevation sites are less well documented than those at higher elevations, but recent surveys suggest that leopard frogs have been extirpated from many sites at lower elevations along Colorado's Front Range (Johnson et al. 2011). Similar information is reflected on The Colorado Herpetofaunal Atlas webpage, which hosts information on the distribution and abundance of amphibian population throughout Colorado including northern leopard frogs and bullfrogs.

The reported decline of northern leopard frog populations in Colorado has coincided with habitat loss and alteration, invasion by predatory fishes and the American bullfrog (*Lithobates* [=*Rana*] *catesbeianus*), and emerging infections by the chytridiomycete pathogen, *Batrachochytrium dendrobatidis* (hereafter '*Bd'*) (Hayes & Jennings 1986; Adams & Pearl 2007; Smith & Keinath 2007; Collins & Crump 2009). The introduction and spread of bullfrogs has contributed to large-scale declines of native amphibians in western North America, South America, Europe, and Australia (Moyle 1973; Adams 1999; Kats & Ferrer 2003; Daszak et al. 2004). The fast-growing tadpoles often out-compete native species (Kupferberg 1997; Kiesecker et al. 2001), while the large adults are generalist predators that consume a broad diversity of native species (Doubledee et al. 2003). Unlike native ranids in the West, bullfrogs overwinter as tadpoles their first year and therefore require permanent wetlands (Hammerson 1999). Permanent wetlands are historically uncommon in the arid climate of Colorado, and most native amphibians are adapted to breeding in temporary wetlands with metamorphosis occurring in a single season. This suggests that the bullfrog invasion is likely aided by continued human alteration of wetlands from temporary to permanent for agricultural and urban uses (Adams 1999).

Similarly, emergence of the pathogen *Bd* has led to amphibian population losses worldwide and is considered one of the greatest threats to global amphibian diversity (Johnson 2006; Lips et al. 2006; Skerrat et al. 2007). Along the Rocky Mountains in Colorado, boreal toads (*Bufo boreas*) have suffered severe declines in association with *Bd* infections and are now endangered within Colorado (Carey 1993; Muths et al. 2003). Northern leopard frogs are also sensitive to *Bd* and may be experiencing similar declines, but insufficient data exist to adequately address this question (Longcore et al. 2007). Despite the individual importance of bullfrogs and *Bd* in depressing native amphibian populations, growing evidence also suggests these threats may not always be independent. While often infected by *Bd*, bullfrogs are relatively insensitive to chytridiomycosis (Garner et al. 2006) and may act as reservoir hosts, helping to transport *Bd* to new environments in which it infects more sensitive native amphibian species. In this manner, the spread of non-native bullfrogs could accelerate the spread of *Bd* and exacerbate the severity of declines.

Objectives

We undertook a series of projects designed to assess the current status of Colorado's Northern leopard frogs (*Lithobates pipiens*) and to evaluate hypothesized causes of their declines. Our approach involved three inter-related components: (1) development and validation of a protocol for use in a statewide sampling effort to evaluate the distribution of leopard frogs in Colorado; (2) use of this protocol to both re-sample sites known historically to support *L. pipiens* as well as sample a random selection of sites with an unknown history and (3) examination of the relationships among *L. pipiens*, bullfrogs, and *Bd* infection. Our goals were to provide information for developing conservation management strategies for leopard frogs in the form of four primary products. First, the development and validation of a sampling protocol for use by CDOW personnel was intended to provide a standardized methodology that can be implemented at the statewide scale to broadly assess the status of leopard frogs. Second, the use of this protocol to (a) re-sample sites known historically to support *L. pipiens* and (b) to broadly sample a random selection of wetlands stratified by land use type was intended to provide a quantitative assessment of both the geographic patterns and severity of suspected declines. Finally, our sampling effort was designed to generate information on the likely causes responsible for leopard frog declines, including the relative roles of bullfrogs, *Bd* and their collective interaction, which is necessary for management and restoration.

Approach

Protocol development

We compiled background information on northern leopard frog identification, distribution, habitat, life history, and status. In order to standardize the site assessment protocol, we included detailed instructions and data collection forms. We also assembled user friendly guides to common amphibian species in Colorado along with a Gosner stage chart for proper identification of amphibian species and stages encountered in the field (Dosch *et al*., 2008). A similar form of this protocol has been field-validated by project personnel during research efforts over previous summers.

Statewide surveys of historically occupied sites

We compiled historical records of northern leopard frog occurrences from the Colorado Natural Heritage Program (Lambert 2006), the Colorado Herpetofaunal Atlas (http://ndis.nrel.colostate.edu/herpatlas/coherpatlas/), University of Colorado Museum, City of Boulder Open Space and Mountain Parks, primary literature, and museums. Collectively, we compiled nearly 5,000 amphibian records, including >800 leopard frog observations dating back to the early 1900s. We defined "historical" as pre-2000 (1908-1999) and focused our surveys on accounts involving leopard frog breeding, as indicated by observations of egg masses, tadpoles, or aggregations (>10 individuals) of recently metamorphosed frogs. Highest sampling priority was assigned to records that could be linked to an extant wetland, making our approach conservative by excluding cases where a wetland had been destroyed. When leopard frog activity was observed at a given wetland in multiple years, we recorded the year of last observation.

At each wetland, we determined whether leopard frogs occurred in any life stage using standardized visual encounter surveys and dipnet surveys (Heyer et al. 1994; Dodd et al. 2009). Visual encounter surveys, during which the number and identity of observed amphibians were recorded, were conducted within 3 m of each wetland's shoreline. Habitat-stratified dipnet sweeps were used to detect larval stages. If leopard frogs were not detected during an initial visit in early summer, sites were sampled again in mid- to late summer when leopard frogs metamorphose (e.g., Hammerson 1999).

Randomized surveys in northern Colorado

We coupled land cover data (National Land Cover Data 2001) with spatial information on aquatic ecosystems (National Wetlands Inventory and Colorado Division of Wildlife's NDIS 1:24,000 water bodies) for regions on either side of the Continental Divide: Colorado's Front Range, including the north-central counties of Boulder, Jefferson, Larimer, Douglas, Broomfield, and Adams, and a five county region in northwest Colorado (Moffat, Rio Blanco, Garfield, Routt, and Rio Grande). We linked these data with information on hydrologic regime, surface area, ownership, elevation, and surrounding land use. Published and anecdotal reports suggest that leopard frogs along the Front Range have declined over the last 40 years (e.g., Hammerson 1982; Corn & Fogelman 1984), concurrent with the spread of bullfrogs and extensive land use changes. In contrast, robust populations of leopard frogs are believed to be present in wetlands in northwestern Colorado, where bullfrogs are absent or rare. The prevalence of *Bd* in these two areas remains largely unexplored.

We quantified the percentage of area within a 1-km buffer around the perimeter of each wetland in the following land use categories: forest (sum of deciduous, evergreen, and mixed forests), grassland (sum of grassland/herbaceous and shrub/scrub areas), agriculture (sum of cultivated crops and pasture/hay areas), and urban/suburban (sum of low, medium and high intensity development). We randomly selected systems in the uppermost quartile of different land use types with a focus on lentic wetlands between 0.05 and 2 ha in surface area as these (a) tend to support native amphibians and (b) facilitate detection of amphibians when the species are present (Merrell 1977; Smith & Keinath 2007). We surveyed wetlands on public (60%) and private (40%) lands. Wetlands were sampled once in early summer (May-June) and once in late summer (July-August) with a 3 to 4 person field crew using standardized methods (Heyer et al. 1994; Dodd et al. 2009). Visual encounter surveys followed the protocol outlined above. To quantify the abundance of larval amphibians and predators (e.g., fishes and aquatic insects), we performed 1-m dipnet sweeps every 15 m around the pond perimeter and 2-4 seine hauls (1.2 x 1.8 m seine net size). We estimated pond area by walking the pond perimeter with a handheld GPS (Garmin 60CSx, accuracy = ± 2 deg or <10 m) and measured conductivity ($\mu S\ cm^{-1}$) using a YSI multimeter (Yellow Springs Instruments). We rigorously sterilized equipment between sites to avoid inadvertent transport of free-living and parasitic organisms.

We tested amphibians for *Bd* using non-lethal swabs followed by a PCR assay (Annis et al. 2004; Lips et al. 2006). Because the frequency of *Bd* infection is highly variable, inspection of a large number of individuals (>20) was targeted to achieve a reasonable likelihood of detection (sample size required to detect 30% prevalence with 95% confidence and 10% accepted error = 19). Amphibians were handled carefully and released quickly to limit stress. Priority was given to swabbing bullfrogs and leopard frogs, but other species (e.g., Woodhouse's toads [*Anaxyrus woodhousii*], tiger salamanders [*Ambystoma tigrinum*], chorus frogs [*Pseudacris triseriata*]) were swabbed if leopard frogs were absent. Because our goal was detecting *Bd* presence rather than estimating prevalence, we batch-pooled species swabs for each wetland.

Results

Protocol

As part of our efforts we developed a northern leopard frog sampling protocol for Colorado. The goal of this protocol was to provide a standardized methodology that is practical, reliable, and accurate to allow Colorado Division of Wildlife personnel to conduct a systematic survey of northern leopard frogs in Colorado. The protocol was designed for CDOW staff to efficiently sample a wetland regardless of their previous experience with amphibians. This protocol has been field-validated in the Front Range and found to have high estimated probabilities of leopard frog detection, particularly in wetlands <5 ha in surface area. It is designed to estimate the likelihood of species occurrence based on repeated sampling efforts and known uncertainties (occupancy modeling, MacKenzie et al. 2002). A secondary objective was to determine the extent of the bullfrog invasion and the spread of *Batrachochytrium dendrobatidis* (*Bd*). With this protocol, the status of other amphibian species, including invasive bullfrogs, can be determined with minimal additional effort. Included in this protocol are guidelines for *Bd* swabbing (see attached protocol, Dosch, K. L., P. T. J Johnson, and V. J. McKenzie. 2008. Northern Leopard Frog (*Lithobates [=Rana] pipiens*) Sampling Protocol for Colorado).

Statewide surveys of historically occupied sites

Between May 2007 and August 2009, we sampled 196 historical leopard frog sites across Colorado, including 47 in the Northwest, 65 in the Northeast, 62 in the Southeast, and 21 in the Southwest (Fig. 1). Twenty-one of these wetlands, all in eastern regions, either no longer existed or no longer contained water. Among extant wetlands, the year-of-last observation was positively associated with contemporary leopard frog occurrence (Spearman rho = 0.34, $P < 0.0001$), but we observed substantial differences in the percentage of inhabited sites among regions (Pearson chi-square = 37.21, df = 3, $P < 0.0001$). Leopard frogs were most common in the Northwest (52.2% of historical wetlands) and the Southwest (47.6%), including many breeding sites. East of the Continental Divide, leopard frogs occurred at 28% of southeastern historical sites and in only one (1.7%) wetland in the Northeast. Wetlands east of the Divide were also more likely to support bullfrogs (Pearson chi-square = 6.22, df = 1, $P = 0.013$), including 24.1% of Northeast sites and 23.8% of Southeast sites, relative to a single wetland in the Northwest (2.2%) and Southwest (4.8%) regions west of the Divide (Fig. 2).

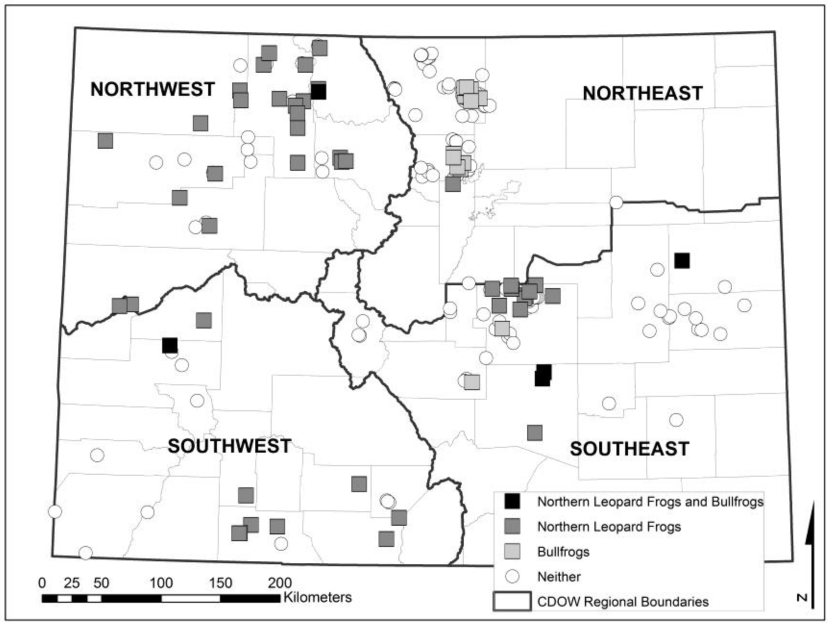

Figure 1. Distribution and current status of northern leopard frogs (*Lithobates pipiens*) in wetlands in Colorado, USA, that historically supported the species (n=196). All sites were surveyed between 2007 and 2009. Dark lines correspond with Colorado Division of Wildlife regions.

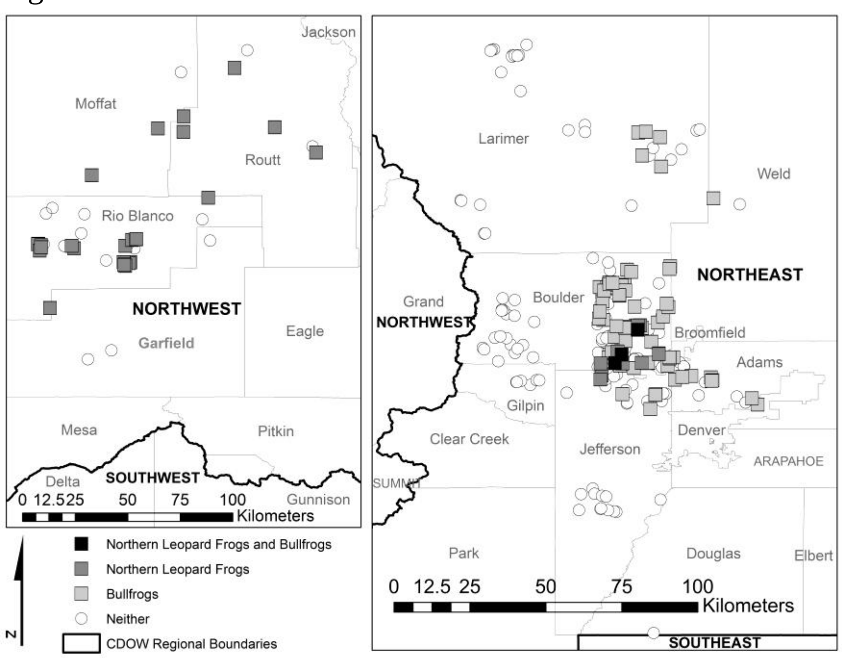

Figure 2. Results of the historical site resurvey as a function of geographic region in Colorado. Depicted is the percentage of sites in each region that were destroyed, supported bullfrogs, or continued to support leopard frogs.

Randomized surveys in northern Colorado

For our randomized survey, we sampled 220 wetlands in the Northeast (56 agricultural, 61 forested, 50 grassland, and 53 urban/suburban sites and 54 in the Northwest (14 agricultural, 30 forested, 10 grassland and 1 suburban site) (Fig. 2). West of the Divide, 50% of sampled wetlands supported leopard frogs, and breeding activity was observed at 33.3%. Bullfrogs were not detected in western Colorado, but *Bd*-positive results were obtained from 4 of 36 tested sites (11.1%), including two detections from leopard frogs, one from tiger salamanders, and one from western chorus frogs. Along the Front Range, leopard frogs occurred at 13 (5.9%) wetlands with breeding at 5 (2.3%) (n=220). At sites with metamorphic leopard frogs, the number of individuals observed during visual encounter surveys along the Front Range averaged 9.8 ± 3.6 SE relative to 455.0 ± 332.8 (n = 27) in the Northwest (t-test on lg_{10}-transformed abundances [equal variances not assumed], t=-3.143, P=0.026; n=10). We recorded bullfrogs at 70 Front Range sites (31.9%) with breeding at 37 (16.9%). Bullfrogs and leopard frogs co-occurred in three wetlands, none of which supported breeding by leopard frogs. *Bd* was detected at 18 of 99 sites (18.2%), including two records from pooled leopard frog samples, four from bullfrogs, four from western chorus frogs, five from tiger salamanders, and three from Woodhouse's toads. Crayfish were more common east of the Divide (42.9% of eastern sites vs. 22.2% of western sites), whereas fish were widespread in both regions (55% of eastern and 45.4% of western sites).

Discussion

Over the last 30 years, growing evidence suggests that the geographic distribution and abundance of the northern leopard frog – historically one of the most widespread and iconic amphibians in North America – has declined substantially in the western USA (Stebbins & Cohen 1995; Rorabaugh 2005; Nichols 2006). Resurveys of 196 historically occupied sites provide compelling evidence that northern leopard frogs are declining in Colorado but suggest the patterns of decline are regionally variable. West of the Continental Divide, approximately 50% of historical sites still support northern leopard frogs, including many active breeding sites with high population densities. East of the Divide, however, we observed leopard frogs at 28% of historical sites in the Southeast and from only one (1.7%) in the Northeast, suggesting that previous, locally documented accounts of leopard frog losses along the Front Range are widespread (Hammerson 1982; Corn & Fogelman 1984). This stands in sharp contrast to historical accounts of the population density of leopard frogs in this region. As qualitatively stated by Ellis and Henderson (1915), "The Leopard Frog has been reported as very abundant near all of the ponds and lakes in eastern Colorado by numerous correspondents (p. 258)." The two eastern regions also exhibited more obvious signs of urban/suburban development and habitat alteration (Fishman & Roberts 2001), including loss or destruction of >20% of historically occupied wetlands and a larger proportion of sites that support invasive bullfrogs (Fig. 3) (Livo et al. 1998).

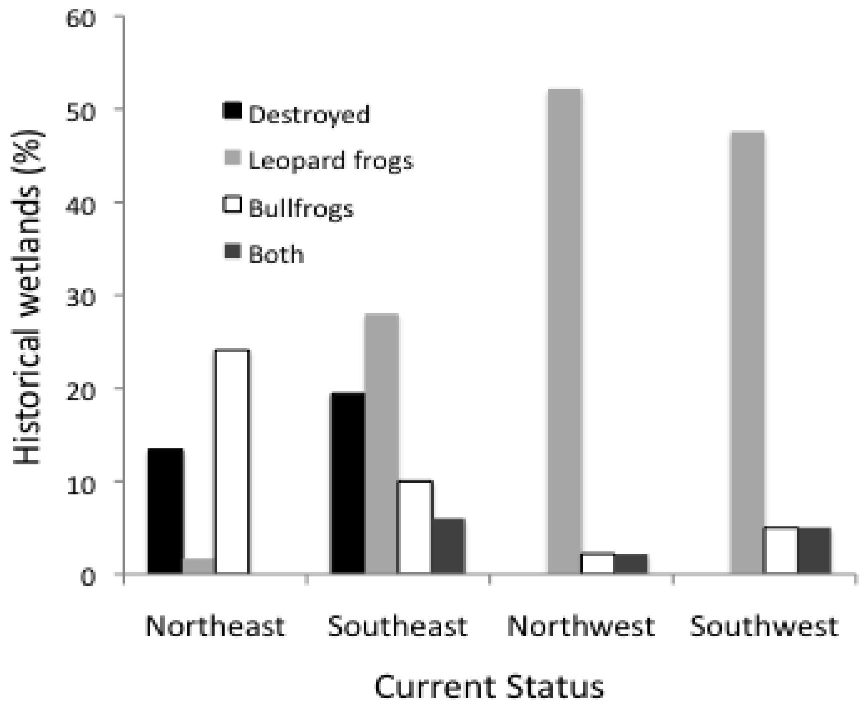

Figure 3. Distribution of the 274 wetlands sampled in the Northwest (A) and Northeast (B) portions of Colorado. Sites were stratified by land use type. Symbols denote whether each site supported native leopard frogs, invasive bullfrogs, both species, or neither.

Our results are broadly consistent with surveys of historically occupied leopard frog sites in California, Oregon, Washington, Montana, Arizona, Alberta and British Columbia, all of which have reported a low frequency (0-20%) of leopard frog occurrence at historically occupied sites upon resurvey (e.g., Hammerson 1982; Corn & Fogelman 1984; Clarkson & Rorabaugh 1989; Leonard et al. 1999; Maxell 2000; Werner 2003; Wilson et al. 2008). Throughout its range, which includes 39 states and provinces, the northern leopard frog has a status of 'declining' in 14, of 'unknown' in 22, and of 'stable' in only 3 (Smith & Keinath 2007). One challenge inherent in resurveys, however, is defining the expected occurrence at historically occupied sites, particularly for groups such as amphibians that experience variable recruitment and might not use the same wetland each year (Pounds et al. 1997; Skelly et al. 2003). Resurveys that do not detect leopard frogs or detect them from a small percentage of historical sites (<20%) suggest a decline, but cases in which leopard frogs are observed at 50% of historical sites, as in western Colorado, are more ambiguous to interpret in the absence of null models that provide estimates of expected values. Skelly et al. (2003) showed that, even when conducted over multiple years, resurveys based on presence data can suggest a 30% decline even when none exists (see also Alford & Richards 1999). Thus, while declines in eastern Colorado and especially along the Front Range are evident, we refrain from qualifying the status of northern leopard frogs in western Colorado until more data are available (but see Mourning 1997 and Lambert 2006).

Intensive surveys of wetlands in northern Colorado reinforced the finding that leopard frogs have declined in this region while highlighting the effects of factors at multiple spatial scales in driving this pattern. While ~50% of wetlands in the Northwest supported northern leopard frogs, including nearly half with breeding, only 6% of sites in the Front Range supported leopard frogs. At the level of wetlands, the presence of bullfrogs and fishes were associated negatively with leopard frog occurrence, consistent with previous work linking these invaders to declines in western ranids (Kiesecker et al. 2001; Adams & Pearl 2007; Knapp et al. 2007). Although these frog species co-occur in portions of their native ranges (e.g., northeastern USA), bullfrogs often consume and compete with leopard frogs in the West, particularly as permanent wetlands that allow the completion of bullfrogs' two-year larval development have become more widespread (Livo et al. 1998; Adams et al. 2003; Rorabaugh 2005). We detected fishes in 55% of Front Range wetlands and 45% of northwestern sites. Despite the frequent co-occurrence of fishes and bullfrogs (25%), their interaction did not explain significant variance in leopard frog occurrence (see Adams et al. 2003).

At the landscape level, our surveys highlighted the negative association of urban/suburban land use and the positive association of grassland area with leopard frog occurrence. Over the last 30 years, human population growth in the Front Range has exceeded that in most other parts of the United States (Fishman & Roberts 2001). In eastern Colorado, much of the native grasslands that were converted previously for agriculture are now being transformed into urban and suburban communities (Fishman & Roberts 2001). Expanding urbanization likely affects amphibians by changing the characteristics of breeding ponds and by altering the quality of upland habitats for frog dispersal, foraging, and overwintering (Blomquist & Hunter 2009; Simon et al. 2009). Wetlands within grassland areas, most of which were semi-permanent and did not harbor fish or bullfrogs, were more likely to support leopard frogs. Although we do not have data on pesticides, which are another mechanism through which land use can affect pond-breeding amphibians (Simon et al. 2009), the proportion of agricultural area around a wetland generally had a weakly positive relation with leopard frog occurrence, likely because the pasture and hay agriculture in this region are structurally similar to native grasslands (Guerry & Hunter 2002).

Finally, our results revealed effects of geographic region (east or west of the Continental Divide) and elevation. The exact mechanism underlying this pattern remains unclear, and could result from variance in regional variables such as climate or from historical legacy. For instance, elevation correlates strongly with both average precipitation and maximum temperature, each of which can influence amphibian breeding and development (Berven 1990). Moreover, even though ostensibly suitable wetlands for leopard frogs occur in northeastern Colorado, the combined effects of habitat fragmentation and non-native species invasions might prevent colonization by remnant leopard frog populations. If breeding individuals do not aggregate at the same sites, demographic and environmental stochasticity may push populations beyond a threshold where recovery is unlikely (Melbourne & Hastings 2008).

Although considerable recent attention has focused on the role of *Batrachochytrium dendrobatidis* (*Bd*) in causing amphibian population extirpations, our data provided equivocal support for *Bd* as a contributing factor in leopard frog declines. The dramatic and persistent loss of leopard frogs from high elevation wetlands in northeastern Colorado (0 in 58 sites above 2200 m) is consistent with a *Bd*-related etiology (Lips et al. 2006; Muths et al. 2003), particularly given that urban/suburban development and bullfrogs are limited in montane areas. Correspondingly, several of the best-supported models in our analysis included a negative coefficient for elevation, potentially indicative of a direct or indirect effect of climate on amphibians or their infections (see also Witte et al. 2008). At lower elevations, however, and for sites with *Bd* data (n=135), we did not find direct or indirect support that *Bd* was associated with leopard frog occurrences. We caution, however, that a brief, one-time survey such as this may not be ideally suited for detecting a disease-driven etiology. Many sampled sites (32.8%) supported no amphibians, precluding the use of proxy species to test for *Bd* occurrence and illustrating the larger problem represented by the "ghost of disease past." Without long-term data, we cannot exclude the possibility that *Bd* caused declines decades earlier only to disappear or leave remaining hosts tolerant of infection. Nevertheless, even if this were the case, we argue that the factors identified in our models are the most salient for informing the current management and future recovery of leopard frogs in Colorado.

One of the greatest challenges in conservation biology involves disentangling the relative contributions of multiple factors in the decline or disappearance of a species (e.g., Blaustein & Kiesecker 2002; Price et al. 2005; Boone et al. 2007). For declines of geographically widespread species, such as northern leopard frogs, multiple causes are likely to be the rule. Our results emphasize the importance of managing threatened species at multiple spatial scales. Because specific threats can vary in severity with spatial extent and among regions, a single management strategy is unlikely to be effective in conserving a species. Declines in widely distributed species thus provide an opportunity to assess the influence of contributing factors. For example, our data indicate that while *Bd* infection may influence the occurrence of montane amphibians (Carey et al. 1999; Muths et al. 2003; Lips et al. 2006), the combined effects of land use change and invasive species are implicated as important factors affecting leopard frog populations at lower elevations. Considering that leopard frog populations are likely declining in at least 14 US States and Canadian provinces, our findings may help inform management of this species and other declining amphibians.

Specific Recommendations

We recommend several areas for both continued research as well as immediate conservation action. With regard to continued research, we recommend that further work focus on the causes of declines to better evaluate the mechanistic drivers of decline and the ways in which they interact (e.g., Smith and Keinath 2007). As evident from the current findings, drivers of decline may differ across elevations as well as across land use types, and our research in Colorado has notably omitted potentially important contributing factors such as other pathogens (e.g., raniviruses and iridoviruses), pesticide contamination, and drought. Thus far, almost no research has focused on habitat use by metamorphic, subadult and adult *L. pipiens*, which should be considered a priority toward advancing our understanding of leopard frog conservation. Such information is vital in determining which areas and how much area needs to be protected to increase leopard frog persistence, particularly with respect to overwintering sites (which often differ from breeding sites). Additionally, there is no current understanding regarding the population genetics of leopard frogs on either side of the Rocky Mountains. These data are necessary to understand how many distinct lineages are represented so that if future re-stocking projects are considered, the correct genetic populations are preserved as best as possible. Further, if some populations demonstrate resistance to *Bd*, that information will be valuable in any consideration of reintroductions. Lastly, we advocate for continued surveys to determine the current distributions of viable leopard frog populations and the likely consequences of leopard frog losses on other species, including both prey and predator groups. Ideally, these surveys should involve more extensive communication and standardization of sampling methods among the many interested agencies and conservation groups, which is currently limited.

In terms of immediate actions, we suggest several strategies to improve the situation for leopard frogs in areas of decline. Protection of existing breeding sites and potentially suitable breeding wetlands that neighbor such sites is the main priority. These areas need to be sheltered from ongoing expansions of agriculture, intensive grazing, suburban development, and recreation. Given the small number of such sites identified along the Front Range (all of which are concentrated in southern Boulder County and northern Broomfield and Jefferson counties) and the fact that these sites occur primarily on publicly-held land, this action should undertaken without delay and ought to include specific monitoring plans to assess the population trends in such wetlands. Removal of bullfrogs and non-native fishes (e.g., carp and bass) will also be critical in areas where they threaten to overtake current or potential leopard frog habitats, especially in wetlands that neighbor viable breeding sites. Successful removal of invaders from small pond habitats such as these has been extensively undertaken in parts of California and offers a valuable template for future efforts in Colorado (J. Alvarez, pers. comm.). Such actions will likely facilitate the expansion of leopard frog breeding beyond currently used wetlands. We further recommend that, where possible, small agricultural and suburban ponds be dried down in the fall to mimic the natural hydroperiod of Colorado's ephemeral wetlands. This strategy will allow native frogs to complete their larval development but prevent bullfrog tadpoles from overwintering, thereby preventing invaders from recolonizing after removal. Unlike native frogs, bullfrog tadpoles require more than one year to metamorphose. Lastly, regulation of cattle grazing regimes in leopard frog breeding sites is important, particularly during times of year when leopard frogs are moving to or from a breeding wetland. During late summer, when metamorphs are emerging from wetlands, extensive cattle trampling can cause high mortality as well as destroy emergent vegetation that provides protective refugia (Smith & Keinath 2007). This could be avoided either by restricting the times of year when cattle access a pond or by restricting the amount of shoreline accessible to cattle. Collectively, these actions will help promote not only the persistence and expansion of northern leopard frogs but also a diversity of aquatic taxa, further serving to enhance the viability of other native amphibians before they experience similar declines.

Outreach

We have conducted a number of efforts to promote amphibian conservation education here in Colorado. These have included cooperative meetings with Boulder Open Space and Mountain Parks, USGS and Colorado State University. At a public level, PTJJ has presented on the leopard frog research at the Boulder Ecosymposium (Boulder, CO) and at the Water Institute (Fort Collins, CO). Project personnel also gave presentations on this research at the Guild of Rocky Mountain Ecologists and Evolutionary Biologists (CU Mountain Research Station, CO) and the Southwest Partners in Amphibians and Reptile Conservation (Fort Collins, CO).

Part of our approach to promote amphibian conservation education has been to interact with local students at all levels. Shelley Todd, a local teacher at Erie high school in the St. Vrain Valley School District, participated in this research in 2009 as part of a high school science outreach program. She incorporated her experience in the field into her high school curriculum, including classroom lab work and field collections, which allowed her students to learn and practice the Colorado Model Science Standards (Colorado Department of Education, 2009). Elden Holldorf, an undergraduate at CU and also a member of the 2009 sampling crew, used his experience in the field during this project to develop his own independent research project. Finally, in 2008, the Johnson Lab at CU coordinated an outreach effort for the first official "Save the Frogs Day" (April 28th, 2008), which included talks at local schools, a museum exhibit at the University of Colorado Museum, and a joint presentation by PTJJ and Cynthia Carey that was open to the public.

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