Contributed Paper

Regional Decline of an Iconic Amphibian Associated with Elevation, Land-Use Change, and Invasive Species

PIETER T. J. JOHNSON,*†† VALERIE J. McKENZIE,* ANNA C. PETERSON,* JACOB L. KERBY,† JENNIFER BROWN,† ANDREW R. BLAUSTEIN,‡ AND TINA JACKSON§

*Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, U.S.A.
†Department of Biology, University of South Dakota, Vermillion, SD 57069, U.S.A.
‡Department of Zoology, Oregon State University, Corvallis, OR 97331, U.S.A.
§Colorado Division of Wildlife, Colorado Springs, CO 80907, U.S.A.

Abstract: *Ecological theory predicts that species with restricted geographic ranges will have the highest probability of extinction, but species with extensive distributions and high population densities can also exhibit widespread population losses. In the western United States populations of northern leopard frogs (*Lithobates pipiens*)—historically one of the most widespread frogs in North America—have declined dramatically in abundance and geographic distribution. To assess the status of leopard frogs in Colorado and evaluate causes of decline, we coupled statewide surveys of 196 historically occupied sites with intensive sampling of 274 wetlands stratified by land use. We used an information-theoretic approach to evaluate the contributions of factors at multiple spatial extents in explaining the contemporary distribution of leopard frogs. Our results indicate leopard frogs have declined in Colorado, but this decline was regionally variable. The lowest proportion of occupied wetlands occurred in eastern Colorado (2–28%), coincident with urban development and colonization by non-native bullfrogs (*Lithobates catesbeianus*). Variables at several spatial extents explained observed leopard frog distributional patterns. In low-elevation wetlands introduced fishes, bullfrogs, and urbanization or suburbanization associated negatively with leopard frog occurrence, whereas wetland area was positively associated with occurrence. Leopard frogs were more abundant and widespread west of the Continental Divide, where urban development and bullfrog abundance were low. Although the pathogenic chytrid* Batrachochytrium dendrobatidis *(Bd) was not selected in our best-supported models, the nearly complete extirpation of leopard frogs from montane wetlands could reflect the individual or interactive effects of Bd and climate patterns. Our results highlight the importance of considering multiple, competing hypotheses to explain species declines, particularly when implicated factors operate at different spatial extents.*

Keywords: biodiversity loss, biological invasions, bullfrog, emerging disease, freshwater conservation, landscape ecology, leopard frog, *Lithobates catesbeianus, Lithobates pipiens*

Declinación Regional de un Anfibio Icónico Asociado con la Altitud, el Cambio de Uso de Suelo y Especies Invasoras

Resumen: *La teoría ecológica predice que especies con rangos geográficos restringidos tendrán la mayor probabilidad de extinción, pero especies con distribuciones amplias y densidades poblacionales altas también pueden presentar pérdidas poblaciones extendidas. En el oeste de los Estados Unidos, poblaciones de* Lithobates pipiens*—históricamente una de las ranas más ampliamente distribuidas en Norte América—han declinado*

1

dramáticamente en abundancia y distribución geográfica. Para estimar el estatus de L. pipiens *en Colorado y evaluar las causas de su declinación, combinamos muestreos realizados en 196 sitios ocupados históricamente con el muestreo intensivo en 274 humedales estratificados por uso de suelo. Utilizamos una aproximación de información teórica para evaluar la contribución de factores en extensiones escalares múltiples para explicar la distribución contemporánea de* L. pipiens. *Nuestro resultado indica que* L. pipiens *ha declinado en Colorado, pero esta declinación fue variable regionalmente. La menor proporción de humedales ocupados ocurrió en el este de Colorado (2-28%), coincidiendo con el desarrollo urbano y la colonización por ranas exóticas* (Lithobates catesbeianus). *Variables en varias extensiones espaciales explicaron los patrones de distribución de* L. pipiens *observados. En humedales en altitudes bajas, peces introducidos,* L. catesbeianus, *y urbanización o suburbanización se asociaron negativamente con la ocurrencia de* L. pipiens, *mientras que la superficie del humedal se asoció positivamente con su ocurrencia.* L. pipiens *fueron más abundantes y se distribuyeron más al oeste de la Divisoria Continental, donde el desarrollo urbano y la abundancia de* L. catesbeianus *fueron bajos. Aunque el quitridio patogénico* Batrachochytrium dendrobatidis *(Bd) no fue incluido en nuestros mejores modelos, la casi extirpación completa de* L. pipiens *de humedales montanos podría reflejar los efectos individuales o interactivos de Bd y el cambio climático. Nuestros resultados resaltan la importancia de considerar hipótesis múltiples y contrapuestas para explicar las declinaciones de especies, particularmente cuando los factores implicados operan en extensiones espaciales diferentes.*

Palabras Clave: dulceacuícola, ecología del paisaje, enfermedad emergente, invasiones biológicas, *Lithobates pipiens*, *Lithobates catesbeianus*, pérdida de biodiversidad

Introduction

Ecological theory predicts that species with small geographic ranges, low densities, and long lifespan will have the greatest probability of declines and extinction (e.g., Cooper et al. 2008). Extinctions of species with extensive geographic distributions and historically high local abundances are therefore unexpected. In some cases the causes of extirpations of populations of species with extensive ranges are readily apparent, for instance, the near-complete loss of the American chestnut (*Castanea dentata*) following infection by introduced chestnut blight (*Cryphonectria parasitica*) (e.g., Anagnostakis 1987). Often, however, multiple factors operating at different spatial or temporal extents interact to cause declines or extirpations, creating uncertainty over how best to mitigate declines and manage remaining populations.

The issue of multifactorial etiologies for species declines is especially relevant to amphibians, which have become one of the most threatened classes of vertebrates worldwide (e.g., Wake & Vredenburg 2008). Habitat loss and alteration are often considered the most important drivers of declines; additional causes include infectious disease, intensive harvesting, pollution, introduced species, and changes in climate (e.g., Pounds et al. 2006; Knapp et al. 2007; Collins & Crump 2009). The relative importance of each cause varies by species, by region, and as a function of the spatial or temporal extent under consideration. In some cases factors operate simultaneously and may interact to amplify negative effects on amphibians (Blaustein & Kiesecker 2002; Price et al. 2005; Boone et al. 2007). For example, land-use change can promote the spread of non-native species, which, in turn, can function as reservoirs for emerging pathogens (Adams et al. 2003; Garner et al. 2006).

The biphasic life cycle of amphibians, which typically involves dependence on aquatic systems for larval development and on terrestrial environments for adult foraging, further underscores the potential for causes of declines to vary through time and among life-history stages (e.g., Blaustein & Kiesecker 2002).

The northern leopard frog (*Lithobates pipiens*) is distributed widely across North America and was historically one of the most abundant anurans in wetland ecosystems (Stebbins & Cohen 1995; Rorabaugh 2005; Smith & Keinath 2007). As a result, it has played an iconic role in the disciplines of physiology, developmental biology, and genetics (Gibbs et al. 1971). Since the 1960s, however, reports of decreases in the abundance and geographic range of leopard frogs have become common in the western portion of its range, where the species is under consideration by the U.S. government for listing as a threatened or endangered distinct population segment (Nichols 2006; Smith & Keinath 2007). Reported declines of the leopard frog have coincided with the spread of introduced fishes and bullfrogs (*Lithobates catesbeianus*), emergence of the pathogenic chytridiomycete *Batrachochytrium dendrobatidis* (Bd), increases in human population density, and greater variability in temperature and precipitation (Hayes & Jennings 1986; Adams & Pearl 2007; Smith & Keinath 2007; Collins & Crump 2009). Nevertheless, the quantitative data necessary to address the roles of hypothesized causes of declines across multiple spatial extents are often unavailable.

We conducted an extensive, multifactorial investigation to determine the current status of northern leopard frogs in Colorado and identify the potential causes of reported declines. To evaluate changes in leopard frog distributions, we surveyed 196 historically occupied sites across northeast, southeast, northwest, and

southwest Colorado, which vary in land use, human population growth, and distributions of non-native invasive species. Concurrently, we sampled 274 wetlands stratified by land-use type in northern Colorado and used an information-theoretic approach to evaluate competing models for explaining the distribution of leopard frogs, including the individual and combined roles of non-native species, disease, land use, water chemistry, and selected interactions among factors. We included in the models factors at extents ranging from local (i.e., within wetland) to landscape (i.e., 1 km surrounding land use) and regional (i.e., location relative to the Continental Divide). Because leopard frogs are distributed widely and use both aquatic and terrestrial environments, our results may help identify threats affecting a wide range of taxa.

Methods

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 5000 amphibian records, including >800 observations of leopard frogs 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. We prioritized records that could be linked to an extant wetland. Thus, our approach was conservative because we excluded cases in which 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 surveyed for leopard frogs in any life stage (Heyer et al. 1994; Dodd 2009). During visual-encounter surveys, we recorded the number and identity of observed amphibians within 3 m of each wetland's shoreline. We used habitat-stratified dipnet sweeps 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 [NLCD] 2001) with spatial information on the location of aquatic ecosystems (National Wetlands Inventory and Colorado Division of Wildlife's Natural Diversity Information Source [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 northwestern Colorado (Moffat, Rio Blanco, Garfield, Routt, and Rio Grande counties). We linked these data with information (NDIS, NLCD) on hydrologic regime, surface area, ownership, elevation, and surrounding land use. Published and anecdotal reports suggest leopard frogs along the Front Range have declined over the last 40 years (e.g., Hammerson 1982; Corn & Fogleman 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 radial area around the perimeter of each wetland in the following land use categories: forest (sum of deciduous, evergreen, and mixed forests), grassland (sum of grassland or herbaceous and shrub or scrub areas), agriculture (sum of cultivated crops and pasture or hay areas), and urban or 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 surface area because these tend to support native amphibians and facilitate detection of amphibians when the species are present (Merrell 1977; Smith & Keinath 2007). We surveyed wetlands on public (60%) and private (40%) lands. A three- to four-person field crew used standardized methods to sample wetlands once in early summer (May–June) and once in late summer (July–August) (Heyer et al. 1994; Dodd 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 × 1.8 m seine net size). We estimated pond area by walking the pond perimeter with a handheld geographic positioning system (60CSx, Garmin, Olathe, Kansas; accuracy = ± 2° or <10 m) and measured conductivity (μS/cm) with a multimeter (Yellow Springs Instruments, Yellow Springs, Ohio). We sterilized equipment between sites to avoid inadvertent transport of free-living and parasitic organisms.

We tested for the presence of Bd by nonlethally swabbing the skin of captured amphibians and analyzing the swabs with a polymerase chain reaction (PCR) assay (Annis et al. 2004; Lips et al. 2006). Because the frequency of Bd infection is highly variable, we aimed to swab at least 20 amphibians to achieve a reasonable likelihood of detection at a site (sample size required to detect 30% prevalence with 95% confidence and 10% accepted error = 19). We handled amphibians carefully and released them quickly to limit stress. We prioritized swabbing

bullfrogs and leopard frogs when they were present, 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 pooled batches of swabs for each wetland.

Analyses and Hypothesis Development

We used an information-theoretic approach to select the best-supported model from a set of competing models designed to explain the distribution and breeding of northern leopard frogs in Colorado (Burnham & Anderson 2002; Mazerolle 2006). On the basis of previous research, we expected leopard frog distributions to be affected by variables at several spatial extents. At the extent of the wetland, we hypothesized that non-native species (bullfrogs and fishes) and predators (crayfishes [*Orconectes virilis* and *O. immunis*]) would affect leopard frog occurrence negatively (Hammerson 1982; Kiesecker et al. 2001; Knapp et al. 2007; Witte et al. 2008). Similarly, we expected Bd to be associated with low leopard frog occurrence, especially at higher elevations (Carey et al. 1999; Muths et al. 2003; Skerrat et al. 2007). We hypothesized that conductivity, as a proxy for road-salt runoff, affects leopard frogs negatively (Sanzo & Hecnar 2006). At the landscape or among-wetland extent, we expected leopard frogs to associate negatively with urbanization but positively with grassland area (Cushman 2005; Price et al. 2005; Simon et al. 2009). At the regional extent, we expected location relative to the Continental Divide (east vs. west of the divide) and elevation to be associated with the number of detections of leopard frogs because most reported declines have occurred in Colorado's Front Range, especially in montane areas (e.g., Hammerson 1982; Corn & Fogleman 1989; Carey et al. 1999). We expected elevation to serve as a proxy for climate, as evidenced by its strong correlation with both maximum temperature ($r = -0.90$, $n = 274$) and precipitation ($r = 0.88$, $n = 274$) among our study sites (averaged between 1971 and 2000 with estimates from PRISM [2004]). Finally, we included selected interactions suggested by previous research, including fish × bullfrogs, bullfrogs × Bd, and Bd × elevation (Adams et al. 2003; Garner et al. 2006; Pounds et al. 2006).

We transformed predictor variables to the logarithm (surface area, elevation, conductivity) or arcsine square root (proportion of land-use types) and tested whether predictor variables were collinear. We removed forest because it was highly correlated with agriculture $\rho = -0.52$) and elevation ($\rho = 0.93$). Because our analyses included variables measured at multiple spatial extents, we tested for spatial autocorrelation by fitting generalized linear models with all predictors and testing the residuals for autocorrelation with Moran's *I* (Rhodes et al. 2009).

The results of this analysis suggested that a nonspatial model was adequate (Moran's *I* standard deviate approximately -0.003, $p > 0.5$). We confirmed this result by comparing results of a nonspatial global model with a generalized linear mixed model (GLMM) that included a spatially autocorrelated error structure ("glmmPQL" in the R-package MASS; R Core Development Team 2008). We used GLMM rather than autologistic models because the latter tend to produce biased estimates (Dormann et al. 2007). We used Monte Carlo simulations to compare the estimated range parameter in the spatial model against the null hypothesis that the range parameter was zero, which confirmed that a spatial model was unnecessary ($n = 500$ simulations, $p = 0.12$).

Building on our hypotheses, we developed 33 candidate models to explain the occurrence of leopard frogs and their breeding activity, as indicated by the presence of egg masses or larvae. These included a null model (intercept only), a global model with all predictor variables, 10 single-variable models, and 21 models with combinations of wetland, landscape, and regional predictors (Supporting Information). We included pond surface area as a covariate in all models that were not null models. Because we were only able to assess Bd at 135 of the 274 sampled sites, often due to sites with no or few amphibians, we performed one set of analyses with Bd as a predictor variable ($n = 135$ wetlands) and a second set of analyses without Bd with data for all wetlands ($n = 274$). For the latter, we eliminated all models and interactions involving Bd, which reduced the number of models to 26.

We used multivariate logistic regression analyses to fit candidate models and ranked them according to Akaike's information criterion (AIC) (Burnham & Anderson 2002). Because our response variables included many zero values (<20% of sampled sites supported leopard frogs), we used the complementary log-log (cloglog) link rather than the logit link (Zuur et al. 2009). We considered models with $\Delta AIC \leq 2.0$ as supported by the data and used model averaging to calculate regression coefficients, standard errors, and 95% confidence intervals (Burnham & Anderson 2002). We ranked each variable included in our best-supported models as strongly supported if its model-averaged confidence intervals did not include zero or its cumulative Akaike weight was >0.7, moderately supported if at least one of its confidence intervals in a best-supported model did not include zero or its cumulative Akaike weight was >0.7, and not supported if neither criterion was met (Marchetti et al. 2004).

Results

Between May 2007 and August 2009, we sampled 196 sites where leopard frogs occurred historically (northwest 47 sites; northeast, 65; southeast, 62; southwest 21 (Fig. 1). Twenty-one of these wetlands, all in eastern
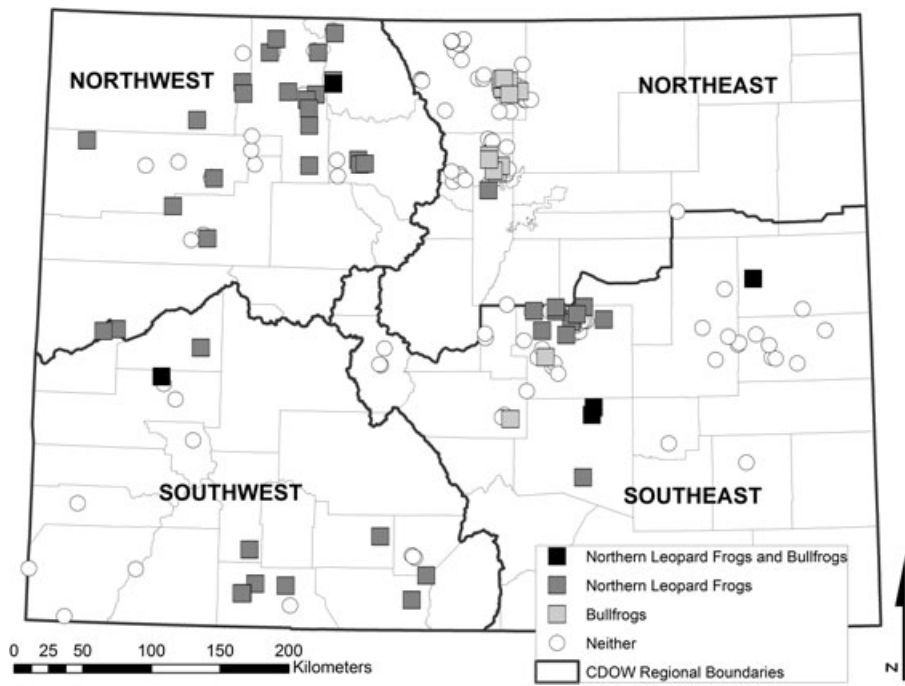
*Figure 1. Contemporary presence and absence locations of northern leopard frogs (*Lithobates pipiens*) and bullfrogs (*Lithobates catesbeianus*) in wetlands in Colorado (U.S.A.) that supported leopard frogs historically (*n = 196*). All sites were surveyed between 2007 and 2009. Dark lines are Colorado Division of Wildlife regions.*

Colorado, 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 $\chi^2 = 37.21$, df = 3, $p < 0.0001$). Leopard frogs were most likely to persist in historically occupied wetlands from the northwest (47.6%) and southwest (52.2%), including numerous breeding sites. East of the Continental Divide, leopard frogs occupied a smaller proportion of wetlands in southeastern Colorado (28%) than in either of the western regions (southwest $\chi^2 = 4.8$, df = 1, $p = 0.048$; northwest $\chi^2 = 10.14$, df = 1, $p = 0.002$). In northeastern Colorado, leopard frogs occurred in only 1 (1.7%) historically occupied wetland, which was a significantly lower proportion than observed in the southeast ($\chi^2 = 13.9$, df = 1, $p < 0.0001$), the northwest ($\chi^2 = 40.6$, df = 1, $p < 0.0001$), or the southwest ($\chi^2 = 31.1$, df = 1, $p < 0.0001$). Wetlands east of the divide were also more likely to support bullfrogs ($\chi^2 = 6.22$, df = 1, $p = 0.013$) (24.1% of northeastern sites contained bullfrogs; 23.8%, southeastern sites; 2.2%, northwestern sites [1 wetland]; 4.8%, southwestern sites) (Fig. 2).

In our randomized survey we sampled 220 wetlands in the northeast (56 agricultural, 61 forested, 50 grassland, and 53 urban or suburban) and 54 in the northwest (14 agricultural, 30 forested, 10 grassland, and 1 suburban) (Fig. 3). 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 was obtained from amphibians at four of 36 tested sites (11.1%), including two samples from leopard

frogs, one from tiger salamanders, and one from western chorus frogs that tested positive. Along the Front Range, leopard frogs occurred at 13 (5.9%) wetlands, and there was breeding in five wetlands (2.3%) ($n = 220$). At sites with metamorphic leopard frogs, the number of individuals observed during visual encounter surveys averaged 9.8 (SE 3.6) along the Front Range and 455.0 (332.8) ($n = 27$) in the northwest (t test on $\lg_{10}$-transformed abundances
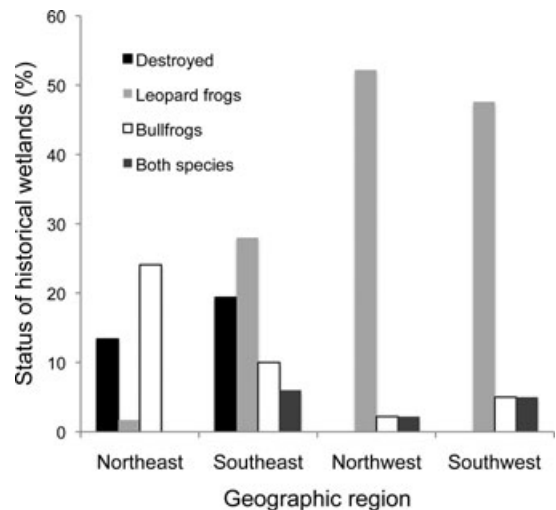

Figure 2. Results of surveys of sites where leopard frogs occurred historically as a function of geographic region in Colorado. Bars indicate the proportion of wetlands that either continue to support leopard frogs, now support bullfrogs (without leopard frogs), support both bullfrogs and leopard frogs, or no longer exist (i.e., the wetland is no longer present).
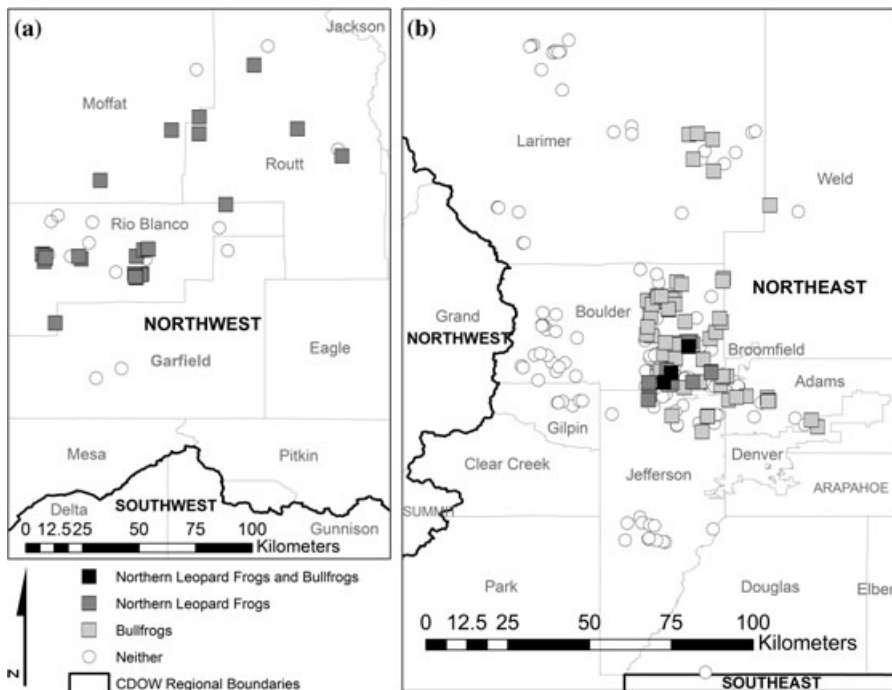
Figure 3. Distribution of the 274 wetlands sampled in (a) northwestern and (b) northeastern Colorado in which northern leopard frogs, bullfrogs, both species, or neither species were detected.

[equal variances not assumed], $t = -3.143$, $p = 0.026$; $n = 10$). We recorded bullfrogs at 70 Front Range sites (31.9%), and there was breeding at 37 (16.9%) sites. Bullfrogs and leopard frogs co-occurred in 3 wetlands, none of which supported breeding of leopard frogs. Bd was detected at 18 of 99 sites (18.2%) (two positive tests 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 and 22.2% of western sites), whereas fish were widespread in both regions (55% of eastern and 45.4% of western sites).

Predictor variables at all three spatial extents were strongly associated with the probability of leopard frog occurrence and breeding. We performed our initial analyses on data from only those sites where we sampled Bd; however, because models containing Bd were not included among the 10 best supported models, we reran the analysis with the full data set. The best models in both analyses were very similar. For leopard frog occurrence, the models with the most support included an effect of region (western wetlands more likely to support leopard frogs than eastern wetlands), a positive association between grassland area and occurrence, and negative associations between occurrence and suburban area, bullfrogs, and fish (models 24 and 25; Table 1). Model 24 also included a negative association between elevation and occurrence and positive associations between occurrence and agricultural area and crayfish. No other models were within 2 ∆AIC of the best-supported model (Table 1). Surface area and region appeared to be associated with occurrence. The cumulative AIC weights

for these variables approached 1 and 95% CIs did not include zero. Additionally, grassland area, suburban area, and bullfrogs explained some leopard frog occurrences (Table 2 & Fig. 4).

Models for leopard frog breeding yielded similar results, although a larger number of models were within 2 ∆AIC of the best-supported model (Table 1). In the model with the most support, occurrences of breeding leopard frog were significantly associated with region and negatively associated with elevation, bullfrogs, fish, and crayfish (model 22, Table 1). Model 23, which was within 0.65 ∆AIC, did not include elevation or crayfish. Three other models were within 2 ∆AIC units (models 3, 12, and 25). In general, region and surface area were strongly supported; elevation, bullfrogs, and fish, each of which had negative coefficients, were moderately supported (Fig. 4). Because no sites supported bullfrogs and breeding leopard frogs together, however, we used Firth's correction to generate parameter estimates for bullfrog presence rather than maximum likelihood methods (Table 2) (Heinze & Schemper 2002).

Discussion

Growing evidence suggests that the geographic distribution and abundance of the northern leopard frog—historically one of the most widespread amphibians in North America—has declined substantially in the western United States (Stebbins & Cohen 1995; Rorabaugh 2005; Nichols 2006). Our resurveys of 196 historically occupied sites suggest that northern leopard frogs are declining in Colorado but that the patterns of decline vary regionally.

Table 1. Best-supported information-theoretic models for leopard frog occurrence and breeding.

Response variable	Model[a]	Category[b]	k[c]	Pseudo R^{2c}	AIC[c]	∆AIC[c]	w$_i$[c]
Leopard frog occurrence							
	25	region + landscape + local	8	38.85	150.93	0	0.401
	24	region + landscape + local	10	40.32	151.73	0.8	0.269
	20	region + landscape	7	37.04	152.99	2.06	0.143
	21	region + landscape	5	35.53	153.16	2.23	0.131
Leopard frog breeding							
	23	region + local	5	30.65	115.39	0	0.284
	22	region + local	7	32.51	116.04	0.65	0.206
	25	region + landscape + local	8	33.55	116.40	1.01	0.172
	3	region	3	27.28	116.51	1.12	0.163
	12	region	4	27.82	117.26	1.87	0.112

[a] *Model numbers correspond to those in Supporting Information, which indicates what predictor variables are included, and are in order of increasing AIC value. All models here are within 3 ∆AIC.*

[b] *Types of predictor variables included (regional, landscape, and local extent). Specific predictor variables included wetland surface area (a priori obvious predictor), region (east or west of the Continental Divide) and elevation (regional variables), the amount of grassland, agriculture, and suburban area around a wetland (landscape-level variables), conductivity and the presence of bullfrogs, fishes, and crayfishes (wetland-level variables), and selected interactions (see Supporting Information).*

[c] *For each model, k is the number of parameters, pseudo-R^2 is the difference between the null deviance and the residual deviance divided by 100, AIC is Akaike's information criterion, ∆AIC is the difference in AIC units between the lowest ranked model and the model under examination, and w$_i$ is the Akaike weight.*

West of the Continental Divide, approximately 50% of historical sites still supported 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 site (1.7%) in the northeast, which suggests that previous, locally documented losses of leopard frogs along the Front Range are widespread (Hammerson 1982; Corn & Fogleman 1984). This stands in sharp contrast to historical accounts of the high density of leopard frogs in this region. Ellis and Henderson (1915) stated that leopard frogs were "reported as very abundant near all of the ponds and lakes in eastern Colorado by numerous correspondents." The two eastern regions have more urban and suburban development and habitat alteration than observed in western Colorado (Fishman & Roberts 2001), including loss or destruction of >20% of historically occupied wetlands and a higher proportion of sites in which bullfrogs occurred (Fig. 3) (Livo et al. 1998).

Our results are broadly consistent with surveys in California, Oregon, Washington, Montana, Arizona, Alberta, and British Columbia, all of which reported a low frequency (0–20%) of leopard frog occurrence at historically occupied sites (e.g., Hammerson 1982; Corn & Fogleman 1984; Clarkson & Rorabaugh 1989; Leonard et al. 1999; Maxell 2000; Werner 2003; Wilson et al. 2008).

Table 2. Model-averaged coefficients and unconditional standard errors for the best-supported variables in models of leopard frog presence and breeding.

| Response variable	Predictor[a]	Model-averaged coefficient[b]	Weighted unconditional SE[b]	Cumulative Akaike weight[c]
Leopard frog presence	surface area[d]	2.165	0.649	1
	region[d]	2.962	2.961	1
	elevation[d]	−7.985	4.904	0.467
	SSG[d]	1.320	0.705	0.998
	suburban[d]	−2.961	1.359	0.998
	bullfrogs	−0.975	0.715	0.725
	fish	−0.689	0.427	0.725
Leopard frog breeding	surface area	1.216	0.681	1
	region[d]	2.746	2.746	0.999
	elevation[d]	−4.569	3.324	0.358
	bullfrogs	−1.774[e]	1.432[e]	0.700
	fish	−0.601	0.497	0.700
	crayfish	−0.303	0.570	0.239

[a] *Predictor variables include wetland surface area (ha), region (position east or west of Continental Divide [categorical]), elevation, shrub-scrub-grassland (SSG) area within 1 km surrounding a wetland, urban or suburban area within 1 km surrounding a wetland, and the presence or absence of bullfrogs, fishes, or crayfishes (binomial variables).*

[b] *Parameter coefficients and standard errors calculated with multimodel averaging.*

[c] *Measure of variable importance and ranges from 0 (lowest) to 1 (highest).*

[d] *Predictor variables with 95% confidence intervals that do not include zero.*

[e] *Parameter coefficients and standard errors using Firth's correction for separation.*
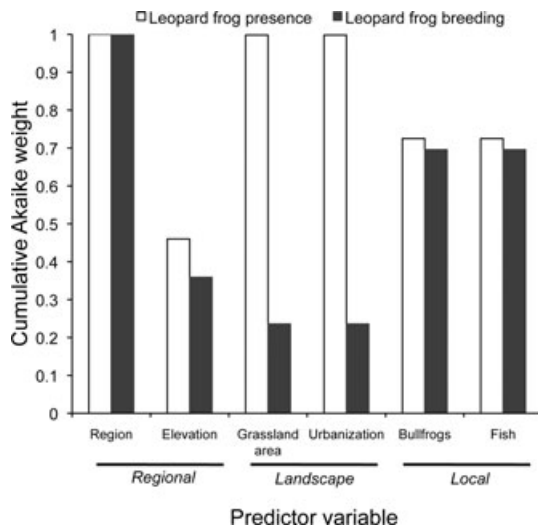
Figure 4. Cumulative Akaike weights of predictor variables included in multivariate logistical regression models for leopard frog presence and leopard frog breeding as a function of spatial extent. Akaike weights are a measure of variable importance and are scaled between 0 (least important) and 1 (most important).

Throughout its range, the status of northern leopard frogs is declining in 14 of 39 states or provinces, unknown in 22, and stable in three (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 have 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. Skelly et al. (2003) showed that, even when conducted over multiple years, resurveys that are based on presence data can suggest a 30% decline when none exists (see also Alford & Richards 1999). Thus, although declines in eastern Colorado, especially along the Front Range, are evident, we cannot qualify the status of northern leopard frogs in western Colorado (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 extents in influencing this pattern. Although approximately 50% of wetlands in the northwest supported northern leopard frogs and nearly half supported breeding, only 6% of sites in the Front Range supported leopard frogs. In wetlands the presence of bull-frogs and non-native fishes were associated negatively with leopard frog occurrence, which is consistent with previous work linking these invasive species 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 United States), bullfrogs often consume and compete with leopard frogs in the western United States, particularly because permanent wetlands that allow the completion of bullfrogs' 2-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 bull-frogs (25%), their interaction did not explain significant variance in leopard frog occurrence (Adams et al. 2003).

At the landscape level our results revealed a negative association of urban and suburban land use and a 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 changes the characteristics of breeding ponds and alters 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 semipermanent and did not harbor fish or bullfrogs, were more likely to support leopard frogs. Although we did 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 pastures and hay fields in this region are structurally similar to native grasslands (Guerry & Hunter 2002).

Finally, our models consistently showed that geographic region (east or west of the Continental Divide) and elevation influenced the likelihood of detecting northern leopard frogs. This result could be due to differences in regional variables such as climate or to unmeasured differences in these regions' histories. 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 presence of non-native species may prevent colonization by remnant leopard frog populations. Although incorporation of a spatial model did not change our

results, Front Range leopard frogs were nevertheless restricted to a relatively small geographic area (Fig. 3). If breeding individuals do not aggregate at the same sites, demographic and environmental stochasticity may push over a threshold beyond which recovery is unlikely (Melbourne & Hastings 2008).

Although considerable recent attention has focused on the influence of Bd on extirpations of amphibian populations, our results show equivocal support for Bd as a contributing factor in leopard frog declines. The dramatic loss of leopard frogs from high-elevation wetlands in northeastern Colorado (0 of 58 sites above 2200 m) is consistent with a Bd-related etiology (Lips et al. 2006; Muths et al. 2003), particularly given that urban and 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 (Witte et al. 2008). At lower elevations, however, and for sites from which we obtained Bd data ($n = 135$), Bd was not associated with leopard frog occurrences. We caution, however, that a brief, one-time survey such as this may not detect 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, we argue that the factors identified in our models (i.e., land-use patterns and invasive species occurrences) are the most salient for informing current management and future recovery plans of leopard frogs in Colorado.

One of the greatest challenges in conservation biology involves disentangling the relative contributions of multiple factors in the decline of a species, particularly when causes interact or vary spatially and temporally in importance (e.g., Blaustein & Kiesecker 2002; Price et al. 2005; Boone et al. 2007). For declines of geographically widespread species, such as northern leopard frogs, multifactorial causes are likely to be the rule rather than the exception.. Because specific threats can vary in severity as a function of spatial extent, 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 multiple contributing factors. For example, our data indicate that although 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 likely affect leopard frog populations at lower elevations. Considering that leopard frog populations are declining in at least 13 U.S. states and one Canadian province (Smith & Keinath 2007), our findings may help inform management of this species and other declining amphibians.

Supporting Information

A table with the candidate models used to predict the distribution and breeding of northern leopard frogs is available as part of the online article (Appendix S1). The author is responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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