Contributed Paper

Epidemiologic Analysis of Factors Associated with Local Disappearances of Native Ranid Frogs in Arizona

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Abstract: *We examined factors that may independently or synergistically contribute to amphibian population declines. We used epidemiologic case-control methodology to sample and analyze a large database developed and maintained by the Arizona Game and Fish Department that describes historical and currently known ranid frog localities in Arizona, U.S.A. Sites with historical documentation of target ranid species* ($n = 324$) *were evaluated to identify locations where frogs had disappeared during the study period (case sites) and locations where frog populations persisted (control sites). Between 1986 and 2003, 117 (36%) of the 324 sites became case sites, of which 105 were used in the analyses. An equal number of control sites were sampled to control for the effects of time. Risk factors, or predictor variables, were defined from environmental data summarized during site surveys and geographic information system data layers. We evaluated risk factors with univariate and multifactorial logistic-regression analyses to derive odds ratios (OR). Odds for local population disappearance were significantly related to 4 factors in the multifactorial model. Disappearance of frog populations increased with increasing elevation (OR = 2.7 for every 500 m,* $p < 0.01$*). Sites where disappearances occurred were 4.3 times more likely to have other nearby sites that also experienced disappearances (OR = 4.3,* $p < 0.01$*), whereas the odds of disappearance were 6.7 times less (OR = 0.15,* $p < 0.01$*) when there was a source population nearby. Sites with disappearances were 2.6 times more likely to have introduced crayfish than were control sites (OR = 2.6,* $p = 0.04$*). The identification of factors associated with frog disappearances increases understanding of declines occurring in natural populations and aids in conservation efforts to reestablish and protect native ranids by identifying and prioritizing implicated threats.*

Keywords: amphibian decline, case–control methods, frog decline, risk factor analysis, wildlife epidemiology

Análisis Epidemiológico de Factores Asociados con Desapariciones Locales de Ranas Nativas en Arizona

Resumen: *Examinamos los factores que pueden contribuir independiente o sinérgicamente a la declinación de poblaciones de anfibios. Utilizamos una metodología epidemiológica de control de casos para muestrear y analizar una base de datos desarrollada y mantenida por el Departamento de Caza y Pesca de Arizona que describe las localidades históricas y actuales de ranas en Arizona, E. U. A. Los sitios con documentación histórica de las especies de ránidos* ($n = 324$) *fueron evaluados para identificar localidades donde las ranas desaparecieron durante el período de estudio (sitios caso) y localidades donde las poblaciones de ranas persistieron (sitios control). Entre 1986 y 2003, 36% (117) de los 324 sitios se volvieron sitios caso, de los*

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cuales 105 fueron utilizados en los análisis. El mismo número de sitios control fueron muestreados para controlar los efectos del tiempo. Los factores de riesgo, o variables predictivas, fueron definidos a partir de datos ambientales obtenidos de los muestreos en los sitios y de capas de datos de un sistema información geográfica. Evaluamos los factores de riesgo con análisis de regresión logística univariada y multivariada para derivar proporciones de probabilidades (PP). Las probabilidad para la desaparición de una población local estuvo relacionada significativamente con 4 factores en el modelo multifactorial. La desaparición de poblaciones de ranas incrementó con la elevación (PP = 2.7 por cada 500 m, $p < 0.01$*). Los sitios donde ocurrieron las desapariciones fueron 4.3 veces más propensos a estar cerca de otros sitios donde ocurrieron desapariciones (PP = 4.3,* $p < 0.01$*), mientras que la probabilidad de desaparición fue 6.7 veces menos (PP = 0.15,* $p < 0.01$*) cuando había una población fuente cercana. Los sitios con desapariciones fueron 2.6 veces más propensos a tener langostinos introducidos que los sitios control (PP = 2.6,* $p = 0.04$*). La identificación de factores asociados con la desaparición de ranas incrementa el conocimiento de las declinaciones de poblaciones naturales y ayuda a los esfuerzos de conservación para el reestablecimiento y la protección de ránidos nativos mediante la identificación y priorización de las amenazas implicadas.*

Palabras Clave: análisis de factores de riesgo, declinación de anfibios, declinación de ranas, epidemiología de vida silvestre, métodos de control de casos

Introduction

Amphibian decline and extinction is a global environmental issue with large population losses reported throughout the world (Stuart et al. 2004). Hypotheses for recent declines include introduction of non-native species, commercial overexploitation, land-use alterations, global climate change, increased chemical use and pollution, and emerging infectious pathogens (Collins & Storfer 2003; Stuart et al. 2004; Lips et al. 2006). Although many declines can be linked to habitat destruction, others are characterized by widespread, large-scale population losses occurring in pristine habitat (Stuart et al. 2004). Similar patterns of decline have occurred in the western United States (e.g., Carey 1993; Drost & Fellers 1996). In Arizona all 7 species of native ranid frogs (i.e., *Rana blairi*, *R. chiricahuensis*, *R. onca*, *R. pipiens*, *R. subaquavocalis*, *R. tarahumarae*, and *R. yavapaiensis*) have declined throughout their ranges (Clarkson & Rorabaugh 1989; Sredl et al. 1997*b*). Hypothesized causal processes for Arizona frogs are analogs to the global concerns and include introduction of predator and competitor species, drought, habitat alteration, pollution, and infectious disease (Jennings & Hayes 1994; Sredl et al. 1997*a*; Bradley et al. 2002).

Previously, most researchers examined only the effects of a single factor on amphibian mortality. Nevertheless, the observed dramatic declines more likely result from many factors working in complex, synergistic ways (Kiesecker et al. 2001; Blaustein & Kiesecker 2002). Few large data sources exist that allow multifactorial analyses of the many factors potentially associated with declines in naturally occurring populations. The Arizona Game and Fish Department (AGFD) created and maintains a large, statewide database on the present and historical distribution of native ranid frogs. This database includes over 2000 sites that have been surveyed over the course of a century to describe the distribution of native ranid frogs. Many surveys spanned time points when frogs disappeared and never returned to the site. These data provide a unique opportunity to investigate patterns in frog declines by evaluating detailed data from field populations.

Quantitative epidemiologic methods have been developed to deal with the special challenges of studying naturally occurring diseases (or any outcome of interest, such as frog disappearances) in free-living populations. Specific study designs, sampling strategies, and analytic techniques can be used to aid in minimizing effects of biases such as disproportionate sampling, misclassification, confounding, and interaction (Rothman & Greenland 1998; Thrusfield 2007). This approach has been applied mainly to research on humans and domestic animals, but provides a framework for studying factors underlying health and disease in wildlife populations (e.g., Hungerford et al. 1999; Brown et al. 2003). We used epidemiologic methods to determine the degree to which different variables (potential risk factors) contributed independently and/or synergistically to predict frog disappearances in Arizona. Identifying these factors is important for managing Arizona frogs and for providing a foundation for future studies of amphibian declines.

Methods

Source Data and Study Design

The AGFD database describes native ranid frog localities throughout the state of Arizona from 1891 to present. Historical ranid frog distributions were determined from published and unpublished literature and museum records. To determine recent ranid-frog distributions, AGFD biologists conducted visual encounter surveys (Crump & Scott 1994) during multiple visits to sites with suitable riparian habitat. The intensity of surveys conducted by AGFD increased during the 1990s because attempts were made to identify new localities and revisit sites where frog populations had been documented previously. By 2003 many of the sites had been surveyed multiple times with surveys spanning several years, depending on existing conservation need. The survey methodology is described in detail in Sredl et al. (1997*b*). Briefly, biologists recorded numbers of target species (*R. blairi*, *R. chiricahuensis*, *R. pipiens*, *R. subaquavocalis*, *R. tarahumarae*, and *R. yavapaiensis*), local site characteristics (e.g., water type, vegetation, terrain characteristics) environmental data, Universal Transverse Mercator (UTM) coordinates, and survey conditions (e.g., time, date, weather) during each visit. To maximize chance of species detection, biologists conducted most surveys between dawn and dusk from late March through early November when Arizona native ranids are most active. The number of sites visited and the number of surveys per site differed among years, depending on conservation initiatives, site location, budget, and environmental conditions. This variation in sampling effort and distribution across time and space introduced potential biases that preclude simple analysis of the entire data set. To minimize effects of these sampling biases, we used an epidemiologic case–control study design (Rothman & Greenland 1998) and controlled for time-related heterogeneity by analyzing data from sites within the AGFD study base that had similar sampling distributions through time.

Classification of Case and Control Sites

The AGFD survey database included 549 sites in central and southern Arizona where adult native ranid frogs had been detected historically at least once, and AGFD biologists had more current data on site characteristics, location, and presence or absence of frogs (i.e., data from surveys during or after 1993, when the overall survey intensity increased). We classified the status (present or absent) of native ranids for each of the sites for every year that had historically documented observations or AGFD site visits. This spanned the time period from 1891–2003; nevertheless, for most sites, detailed environmental data were not collected until the 1990s. Sites were classified in accordance with detectability data from validation studies of AGFD visual encounter surveys, data from repeated negative surveys (when frogs were not found), and knowledge of ranid frog activity patterns in the arid Southwest. Field studies show that the detectability of Arizona frogs is high, even when population size is small, especially if the visual encounter survey is repeated (Howland et al. 1997; Sredl et al. 1997*a*). Simulations based on field data have also confirmed the usefulness of presence–absence surveys (Pollock 2006). Therefore, if biologists did not detect any of the target ranid species in any survey in a given calendar year, we classified the native ranid status of that site as "absent" for that year. If biologists detected target species during at least one survey in a given calendar year, then we classified the native ranid status of the site as "present" for that year. If native ranids could not be distinguished from non-native ranids or if native ranids were not detected when survey conditions were not favorable for finding frogs, then we classified the site as "unknown" for that year and the survey was not used for classification of ranid status. Any surveys occurring after the introduction of native ranids (for conservation purposes) at a given site were also classified as unknown.

We defined case sites as those with 2 or more consecutive survey years (i.e., a year in which a site was surveyed) of native ranid absence. Potential control sites had either all survey years classified as present or had single, embedded absent classifications preceded and followed by present classifications.

We were unable to classify all eligible sites as cases or controls. Some sites ($n = 105$) did not meet initial screening criteria because either environmental characteristics could not be assessed during post-1993 surveys or all post-1993 surveys were classified as unknown (no distinction between native and non-native ranids [$n = 54$]; local drought with dried ponds [$n = 32$]; cool temperatures [$n = 1$], introduction of native ranids [$n = 18$]). We excluded sites where the last observation was a single absent year ($n = 112$) because we could not distinguish new cases from controls with single years in which frogs were undetected. Sites where the first observations yielded 2 or more consecutive absent years ($n = 8$) were also excluded because we could not ascertain conditions or time of disappearance.

Case sites were included in the study at the time of documented frog disappearance (i.e., when a site was first classified as a case). An equal number of control sites were randomly selected from all control sites classified as present during the same year. Thus, a control site could convert to a case site, but once a site became a case, it was analyzed as such in this study. The final data set for analysis consisted of one or more cases from each year and an equal number of controls with the same distribution of sampling over time (a method known as time-based density sampling, which controls for time-related confounding and heterogeneity) (Wacholder et al. 1992; Rothman & Greenland 1998).

Risk Factors

We evaluated risk factors that were consistent with Arizona amphibian decline hypotheses. Complete habitat destruction (e.g., paving over a site) is an unequivocal cause of local extirpation (Pimm et al. 1995), so we focused on other factors for populations where habitat remained. Data on moderate habitat alteration, effects of proximal urbanization, and pollution were sparse and incomplete in this data set and therefore were not evaluated. The final list of evaluated variables included presence or absence of non-native species, water type (lentic or lotic), water pH, status of nearby sites, aspect, soil characteristics, and elevation. Additional information on selected variables follows.

Presence or absence of non-native species was evaluated separately for bullfrogs (*R. catesbeiana*), fish, and crayfish, at each study site based on observations made during site-specific AGFD surveys. Two hydrological characteristics were evaluated. We calculated median water pH for each site from serial pH readings taken with a calibrated pH meter and recorded during site surveys. Sites were then classified as relatively more alkaline (median pH value above 8.35, the median pH of all sites) or relatively more acidic (median pH value below 8.35). We classified water type for each site as lentic (still water) or lotic (streams containing natural pools that flowed at least some time during the year).

We evaluated the potential for a rescue effect (Brown & Kodric-Brown, 1977) from nearby extant ranid populations. The entire AGFD database was searched to determine whether native ranid frogs were recorded as present at any nearby site in the year before, during or after the case or control site entered the study. We used a geographic information system (GIS; ArcMap-ArcView 9.0, ESRI, Redmond, California) to delineate circular areas 2, 4, 6, and 8 km in diameter around each study site. These time frames and distances were chosen based on frog population biology; studies documenting ranid frog dispersal estimate distances that range from 2 to 8 km (Frost & Bagnara 1977; Rosen et al. 1996; Funk et al. 2005; Sredl & Jennings 2005). The most predictive of the 4 buffer sizes (4 km) was selected for inclusion in the multifactorial model based on the magnitude and associated variance estimates of the odds ratios (OR) in the univariate and multifactorial analyses.

We similarly evaluated the association between case or control status and disappearances at nearby sites within a window of 2 years before or 2 years after each site entered the study. This time window was selected because the temporal spread of a disease or non-native species responsible for a disappearance at a nearby site could occur over a longer period than indicated by the date the disappearance was recorded. Again, we used GIS to delineate circular areas of 2, 4, 6, and 8 km in diameter around all study sites for the year they were selected. We identified sites with at least one disappearance within the each circular area separately for all 4 distances. The most predictive distance (6 km) was selected for inclusion in the multifactorial model based on the magnitude and associated variance estimates of the OR in both the univariate and multifactorial analyses.

We determined 2 soil characteristics by overlaying site locations on digital soil maps from the 1994 U.S. Department of Agriculture, Natural Resources Conservation Service State Soil Geographic (STATSGO) database. For each site, we found the soil profiles of the underlying soil polygon. We calculated available water capacity (AWC), the amount of water a soil can store for use by plants, by summing weighted averages of AWC for each soil layer in each profile (weighted by layer depth). The AWC-per-profile calculations were normalized to the percent composition of the polygon (U.S. Department of Agriculture 1995). Based on standard AWC classification tables (U.S. Department of Agriculture 1993), we divided AWC into "very low" soil AWC (0–7.5 cm/cm^2 of soil) and higher AWC values (>7.5 cm/cm^2 of soil). Similarly, we determined the percentage of the topsoil layer that was composed of organic matter (hereafter, soil organic matter) as the average organic content for the topsoil layer, normalized by the percent composition of the polygon (U.S. Department of Agriculture 1995). Soil organic matter was evaluated as a continuous predictor of disappearance and ranged from 0% to 2.9% with a mean, median, and standard deviation of 1.3, 1.2, and 0.5%, respectively.

Elevation was determined from 7.5-min U.S. Geological Survey (USGS) topographical maps and 7.5-min Digital Elevation Model (DEM) files. Elevation among case and control sites was normally distributed and ranged from 171 m to 2524 m, with a mean of 1522 m (SD 499). We overlaid maps with point locations for each site on 7.5-min DEM maps with the GIS to determine aspect values for each location. We calculated the difference between each site's aspect and 180° (south), the aspect receiving the most sun exposure throughout the year. Differences from a south-facing aspect for all sites ranged from 0° to 180°, with a mean of 105° (SD 55). We similarly calculated the difference between each site's aspect and 315° (northwest), the aspect receiving predominant northwesterly winds throughout most of the year (Douglas et al. 1993; Adams & Comrie 1997). Differences from a northwest aspect for all sites ranged from 0° to 180°, with a mean of 57° (SD 51).

Statistical Methods

To evaluate associations between risk factors and disappearance, we calculated univariate ORs and Fisher's exact tests (categorical variables) or Wald statistics (continuous variables). Associations with $p \leq 0.25$ were further examined in multifactorial analyses. We screened for plausible confounders and effect modifiers with stratified contingency table analyses (Hosmer & Lemeshow 2000).

We used multifactorial, unconditional logistic regression with the best subsets method of model selection (Hosmer & Lemeshow 2000; King 2003) to identify and separate concurrent effects of multiple risk factors. We evaluated estimated coefficients, their respective standard errors, Wald statistics, and changes in other coefficients as variables were added and removed from the model. We compared nested models with likelihood ratio tests.

We evaluated potential confounding (i.e., when a covariate was associated with both the factor of interest and the outcome, thereby masking or inflating the apparent effect of the factor of interest) by examining the change in magnitude of the coefficient for the risk factor from models fit with and without potential confounding variables. We evaluated interaction by comparing main-effects models with those containing both main effects and their first-order interaction terms (Rothman & Greenland 1998; Hosmer & Lemeshow 2000). We evaluated influential observations by examining the change in the Pearson chi-square statistic, deviance, and parameter estimates when a particular covariate pattern was deleted (Hosmer & Lemeshow 2000; Peng & So 2002). We examined the residuals for spatial autocorrelation (González-Megías et al. 2005) with Moran's I (ArcMap-ArcView 9.0, Spatial Statistics Toolbox, ESRI, Redmond, California).

The final model was selected for biological plausibility, strength of associations, concordance between predicted and observed outcomes, and the Hosmer–Lemeshow goodness-of-fit χ^2 statistic. Odds ratios and 95% confidence intervals were calculated from the final model. For elevation the OR was reported per 100-m increase and was calculated with the equations $e^{100(\beta)}$ and $e^{100(\beta)\pm 1.96(SE)(100)(\beta)}$ to determine the OR estimate and associated 95% confidence intervals, respectively. Univariate and multifactorial analyses were conducted with the statistical software program SAS (version 9.2, SAS Institute, Cary, North Carolina).

Results

Of the 549 sites in the AGFD database with historical ranid documentation and current site characteristic data, 324 were classified as either a case site or a potential control site. Thirty-six percent (117/324) of all classified sites became cases between 1986 and 2001. New cases occurred in new localities every year except 1988. We were unable to include 12 cases because we could not find enough contemporaneous control sites, leaving 105 case sites and an equal number of controls for further statistical evaluation. These case and control sites were located throughout central and southern Arizona and generally reflected the same geographic distribution as all 549 ranid localities surveyed in the AGFD database (Fig. 1).

Fourteen of the 18 variables examined with univariate analyses met screening criteria ($p < 0.25$) (Table 1) and were further considered in the multifactorial modeling process. The final model included all 210 sites and consisted of 4 variables (Table 2). Odds for disappearance of frogs were significantly higher for sites at higher elevations (OR = 1.9 for every 100-m increase in elevation, $p < 0.01$), sites with introduced crayfish (OR = 2.6, $p = 0.04$), and sites with a nearby disappearance within 6 km (OR = 4.3, $p < 0.01$). In contrast the odds of

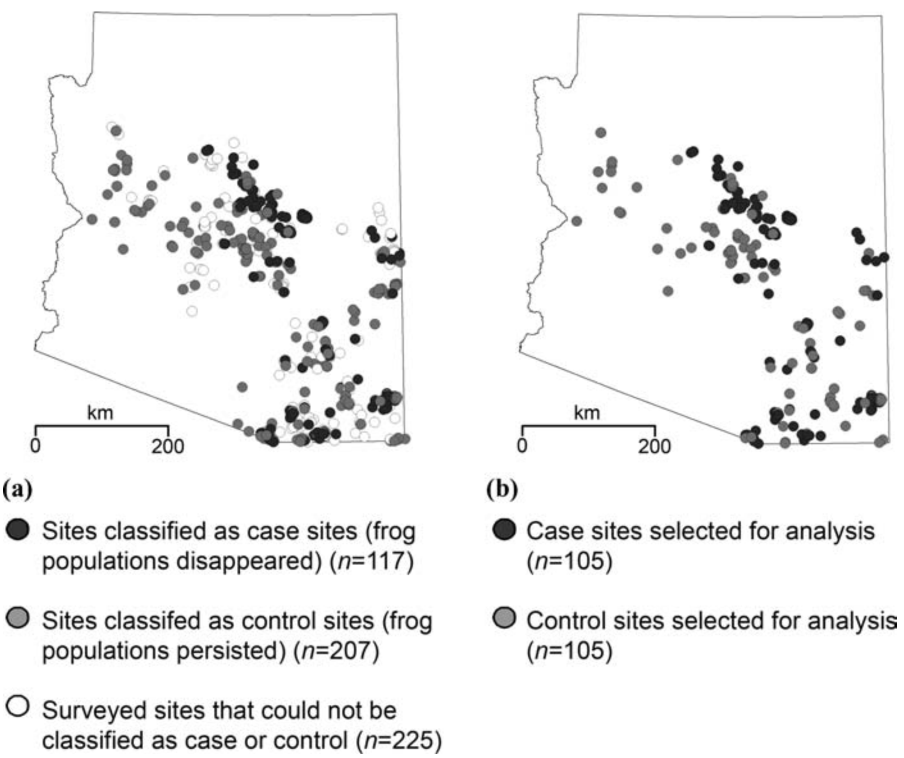

Figure 1. (a) All sites in the Arizona Game and Fish Department's survey database that were visited during or after 1993 (n = 549) and their classification status. (b) Case and control sites selected for univariate and multifactorial analysis (n = 210).

Table 1. Risk factors for disappearance of Arizona ranid frogs based on univariate logistic regression analysis.[a]

Risk factor	*Case sites*		*Control sites*		*Odds ratio*	*95% CI[b]*	p
	yes	*no*	*yes*	*no*			
Crayfish present	32	73	12	93	3.4	1.6–7.1	0.001[c,d]
Non-native fish present	45	60	32	73	1.7	1.0–3.0	0.085[d]
Bullfrogs present	15	90	8	97	2.0	0.8–5.0	0.18[d]
Lentic water system	57	48	41	64	1.9	1.1–3.2	0.038[c,d]
More alkaline pH	44	55	44	41	0.8	0.4–1.3	0.38
Extant population nearby at 8 km	59	46	77	28	0.5	0.5–0.8	0.014[c,d]
Extant population nearby at 6 km	52	53	68	37	0.5	0.5–0.9	0.036[c,d]
Extant population nearby at 4 km	40	65	62	43	0.4	0.4–0.7	0.004[c,d]
Extant population nearby at 2 km	18	87	46	59	0.3	0.1–0.5	<0.0001[c,d]
Disappearance nearby at 8 km	55	50	24	81	3.7	2.1–6.7	<0.0001[c,d]
Disappearance nearby at 6 km	51	54	17	88	4.9	2.6–9.3	<0.0001[c,d]
Disappearance nearby at 4 km	44	61	8	97	8.7	3.9–20.0	<0.0001[c,d]
Disappearance nearby at 2 km	14	91	1	104	16.0	2.0–124.0	<0.0001[c,d]
Low soil available water capacity (<7.5 cm/cm^2 of soil)	84	20	65	40	0.4	0.2–0.7	0.0035[c,d]
	mean, median, range		mean, median, range				
Soil organic matter (%)	1.3, 1.2, 0.6–2.9		1.3, 1.2, 0–2.3		1.0	0.6–1.8	1.0
Southern aspect (difference from 180°)	104, 113, 0–180		106, 119, 0–180		1.0	1.0–1.0	0.74
Northwest aspect (difference from 315°)	55, 35, 0–180		60, 49, 0–180		1.0	1.0–1.0	0.48
Elevation (meters)	1741, 1792, 507–2524		1302, 1274, 171–2499		2.9[e]	2.9–2.9[e]	<0.0001[c,d]

[a] *Case sites are localities where frog populations disappeared; control sites are localities where frog populations persisted.*
[b] *Exact confidence intervals.*
[c] *Significantly* ($p < 0.05$) *associated with disappearances.*
[d] *Met screening on criteria* $p < 0.25$ *for multifactorial analyses.*
[e] *Statistic calculated to estimate the odds of exposure associated with every 1-m increase in elevation with a beta coefficient of 0.00212. Odds ratio and associated 95% CI are reported to reflect odds associated with every 500-m increase in elevation, based on the formulas* $e^{500(\beta)}$ *and* $e^{500(\beta)\pm 1.96(SE)(500)(\beta)}$*, respectively, where SE = 0.000362.*

disappearance were 6.7 times less (OR = 0.15, $p < 0.01$) for sites with source populations within 4 km than those without a nearby extant population. No significant interactions were identified, and other variable combinations did not improve the fit of the model or indicate additional sources of confounding. There was no significant spatial autocorrelation in the model residuals (Moran's $I = 0.02$, Z score $= 0.17$). The concordance between predicted and observed case status was 85%, and there was no significant lack of fit (Hosmer–Lemeshow goodness-of-fit test, $p = 0.86$).

Discussion

Studies of naturally occurring amphibian declines have been limited by scarcity of repeatedly measured, quantitative data for multiple populations over time (Pechmann et al. 1991; Blaustein 1994). The AGFD database provides many years of longitudinal information for a wide variety of frog locations. Nevertheless, it does not represent a complete inventory or a random sample of native Arizona ranid populations. Survey effort also varied over time. These sampling issues are general characteristics

Table 2. Multifactorial model[a] of factors associated with native ranid frog disappearance.

Variable	*Beta coefficient*	*SE*	*Odds ratio*	*95% CI*	p
Intercept	−3.09	0.62			
Elevation	0.00196	0.000405	2.66[b]	2.66–2.67[b]	<0.01
Extant population nearby at 4 km	−1.89	0.42	0.15	0.07–0.35	<0.01
Disappearance nearby at 6 km	1.46	0.41	4.32	1.93–9.64	<0.01
Crayfish present	0.96	0.46	2.61	1.06–6.44	0.04

[a] *Model reflects risk factors among 105 cases (localities where frog populations disappeared) and 105 controls (localities where frog populations persisted).*
[b] *Statistic calculated to estimate the odds of exposure with every 1-m increase in elevation with a beta coefficient of 0.00196. Odds ratio and associated 95% CI are reported to reflect odds associated with every 500-m increase in elevation, calculated with the formulas* $e^{500(\beta)}$ *and* $e^{500(\beta)\pm 1.96(SE)(500)(\beta)}$*, respectively, where SE = 0.000405.*

of observational data collected for natural populations (Rothman & Greenland 1998). Theory and methods to make appropriate conclusions about population health from observational data have been extensively developed in the field of epidemiology, primarily for humans (Rothman & Greenland 1998; Nelson et al. 2001) but also for animals (Dohoo et al. 2003; Thrusfield 2007). We used these epidemiological methods to identify factors associated with local frog disappearances observed in these incomplete, but extensive AGFD surveys.

Disappearances at higher elevations revealed that the pattern of decline in the arid climate of Arizona is similar to amphibian disappearances occurring worldwide and in places with very different climate regimes (Stuart et al. 2004). Global warming and other climate changes may challenge ranids and/or favor pathogens at higher elevations (Pounds et al. 2006), and chytridiomycosis-related mortalities and declines, caused by the infectious agent *Batrachochytrium dendrobatidis*, have often been associated with higher elevations (e.g., Berger et al. 1998; Bosch et al. 2001; Lips et al. 2006). Although *B. dendrobatidis* has been documented in Arizona ranid die-offs and in museum specimens dating back to the 1970s (Bradley et al. 2002; M.J.S., unpublished data), no information on specific pathogens was available for analysis for most of our sites. The high-elevation sites we examined in the analyses were likely to have higher AWC values and lentic water. Both of these factors were significant in the univariate analyses, but did not improve the multifactorial logistic regression model when elevation was included. Although elevation better explained the pattern of disappearances, these co-occurring factors may offer insight into mechanisms of elevation-related declines.

Nearby disappearances and nearby extant frog populations were both independently associated with local disappearances, exerting opposite effects. The patterns we found are consistent with the spread of disease or invasive species as well as augmentation or recolonization of populations by native ranids within a metapopulation framework. Increased dispersal ability between close patches of habitat (Lidicker & Caldwell 1982) would facilitate direct spread of pathogens or invasive species. Close spacing of habitat patches significantly increases infection prevalence in other species (Grosholz 1993). Our results suggest that the probability of a local disappearance may increase as distance from a nearby disappearance decreases. Conversely, presence of a nearby extant population may be critical as a source for site repopulation. The high rates of juvenile dispersal over distances of at least 5 km for ranid frogs in Colombia (Funk et al. 2005) support this connectivity between sites. Metapopulation models also predict breeding ponds can blink in and out of existence, with colonization rates related to spatial arrangement of habitat patches (Marsh & Trenham 2001). In experimental pond-recolonization studies sites closer to the initial source of amphibians maintained larger populations (Halley et al. 1996). In AGFD surveys frogs were later found to be present again at 8 of the 117 case sites. This does not estimate the proportion or rate of recolonization because case sites were not a representative sample of all sites without frogs and more recent declines had less time for frog returns to be observed. Nevertheless, this observation does further support the potential for a rescue effect. Our results suggest conservation efforts may need to focus on groups of populations that can infect or recolonize each other, rather than on individual sites.

Introduced species (i.e., fish, crayfish, cane toads, bullfrogs) can act as predators, competitors, or disease reservoirs and have been associated with amphibian declines worldwide (Collins & Storfer 2003; Kats & Ferrer 2003; Knapp et al. 2007). The presence of introduced crayfish negatively affected native ranids (OR $= 0.15$, $p = 0.04$), regardless of elevation or the presence–absence status of nearby populations. The spread of crayfish is a recognized threat to Arizona's aquatic systems, and native leopard frogs have completely disappeared from some habitats following crayfish introduction (Fernandez & Rosen 1996; AGFD, unpublished data). These invertebrates can act as direct predators and competitors of frogs and reduce aquatic vegetation, habitat heterogeneity, and protective cover (Fernandez & Rosen 1996; Gamradt & Kats 1996). Their role as disease reservoirs is unknown. Crayfish are not native to Arizona, but 2 species, the northern crayfish (*Orconectes virilis*) and the red swamp crayfish (*Procambarus clarkii*), were introduced in the 1970s to aquatic systems through stocking and were used as both fish food and recreational bait (Fernandez & Rosen 1996; Gamradt & Kats 1996; Kats & Ferrer 2003). Current management practices include managing waters to prevent crayfish spread, trapping and removing crayfish, and passing state legislation that limits the possession and transportation of live crayfish (Arizona Revised Statue 17-309A1, R12-4-316). Nevertheless, additional methods of crayfish eradication are urgently needed to prevent further spread into ranid habitat.

The negative impact of introduced fishes and bullfrogs on amphibian populations is well documented (e.g., Kats & Ferrer 2003; Knapp et al. 2007), but their role in large-scale disappearances is less clear (Clarkson & Rorabaugh 1989; Mahoney 1996; Stuart et al. 2004). Introduced fishes were not significantly associated with frog disappearances in our study, but information distinguishing fish species was not available for many sites. Presence of fish may also serve as an indicator of water permanency in Arizona, which could have a beneficial effect on ranid frog survival in this desert climate.

The final data set included only a small number of sites with bullfrogs ($n = 23$ sites). Other surveyed sites where bullfrogs were present were not included in our study because they lacked historical records to determine whether native ranid species were ever present. These sites may represent historical, native ranid localities now inhabited by introduced bullfrogs. These observations emphasize the need for further research on the effects of bullfrog introductions.

Case–control studies are used widely in human and domestic-animal epidemiology and offer many advantages for investigating disease. Nevertheless, we found challenges in applying these methods to an existing amphibian population database. Cases were characterized by at least 2 successive survey years that had favorable conditions, but no native ranids were found. Control sites could have a single year when frogs were undetectable. Although this classification scheme was designed to separate "normal" fluctuations from more serious declines, disappearances where at least one frog had migrated in within 2 years would be missed. This would, in general, misclassify some cases as controls and bias the associations toward the null hypothesis. Nevertheless, it could also emphasize factors that caused short-term disappearances to persist, for example, increasing the significance of a recolonization source.

Our case definition was conservative because it only included sites where presence of native ranids had been documented before a decline. Many additional sites with apparently suitable habitat were not included in this study because frogs were never found during any AGFD site visit ($n = 1076$). It is unknown whether these sites represent localities that were never inhabited or localities where disappearances occurred prior to recent surveys. Some case sites may have suffered the loss of several native ranid species, making our case definition even more conservative. Clarkson and Rorabaugh (1989) document 94% of historical *R. chiricahuensis* localities as being uninhabited by the mid-1980s. We used density sampling to assure that cases and controls were contemporaneous, but quantitative data from early disappearance (occurring in the late 1980s and the early 1990s) may have identified risk factors different than those that led to disappearances in the latter part of the study period.

Conclusions

We found the application of epidemiologic methods useful for identifying environmental and spatial variables associated with population declines of ranid frogs in Arizona. Our findings increase understanding of frog disappearances occurring in natural populations and will guide future wildlife conservation management decisions. As AGFD continues to actively monitor suitable frog habitat throughout the state, implicated threats described herein can be prioritized to aid in reestablishing and protecting native ranids.

Reintroducing populations to locations of historical distribution at lower elevations may offset the strong negative correlations between disappearances and elevation and may benefit the overall population. Conservation plans should be developed with knowledge of the status of nearby populations that may either benefit or threaten the local population of interest. Dispersal facilitation between patches of habitat may promote beneficial repatriation of sites, although a cautious approach should be taken because neighboring sites may harbor diseases or non-native species that could negatively affect native ranids. Wildlife managers may need to establish populations in localities distant from sites with known hazards (such as introduced crayfish), to avoid the potentially harmful threats these could pose to neighboring sites.

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