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**HABITAT, SPATIAL POPULATION STRUCTURE, AND METHODS
FOR MONITORING BARKING FROGS (*ELEUTHERODACTYLUS
AUGUSTI*) IN SOUTHERN ARIZONA**

by

Caren S. Goldberg

**A Thesis Submitted to the Faculty of the
SCHOOL OF RENEWABLE NATURAL RESOURCES
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
WITH A MAJOR IN WILDLIFE AND FISHERIES SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA**

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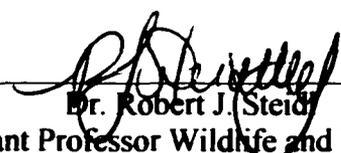
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ABSTRACT

Debate over global declines of amphibian populations has focused researchers' attention on the lack of basic life-history information about and appropriate monitoring methods for amphibian species. We studied barking frogs (*Eleutherodactylus augusti*) in a canyon in the Huachuca Mountains of southern Arizona. Annual (capture) surveys and radio-tracking revealed that most barking frogs in this canyon are strongly associated with limestone. Adults rarely moved between limestone outcrops and numbers of frogs on each outcrop were small. Subpopulations consisting of frogs on sets of neighboring outcrops probably function as a metapopulation. We evaluated four methods for monitoring population size of this species: mark-recapture, distance sampling, visual encounter surveys, and call counts. We found none of these to be able to detect changes in population size powerfully enough to alert managers to declines as they occur. We suggest resource managers focus monitoring efforts on distribution rather than population size.

CHAPTER 1 - HABITAT AND SPATIAL POPULATION STRUCTURE OF BARKING FROGS (*ELEUTHERODACTYLUS AUGUSTI*) IN SOUTHEASTERN ARIZONA

ABSTRACT

Organisms living at the edge of their species' range may rely heavily on habitat components to protect them from a physiologically challenging environment. Barking frogs (*Eleutherodactylus augusti*) are the northernmost member of the large tropical family Leptodactylidae. We investigated the ecology of this species at the northern edge of its range in a large canyon in southern Arizona. We captured 54 frogs on or in crevices in discontinuous rock outcrops; all 9 females and 39 of 45 males were on or near limestone outcrops. Seven of ten frogs radio-tracked had core home ranges (50% fixed kernel) from 94 to 100% on limestone; the other three frogs did not use limestone at all. Some frogs moved greater nightly distances when air temperatures and rainfall were high. During five years of capture efforts, no frogs were found on a different outcrop from the one where they were originally caught; no radio-tracked frogs moved between outcrops during the breeding season. We estimated that 4 to 20 barking frogs occupied each rocky outcrop; these groups probably function as subpopulations in a metapopulation. To manage for the persistence of barking frogs in southern Arizona, we must identify and protect occupied and unoccupied habitat patches and movement pathways among them.

INTRODUCTION

Understanding the relationship of species to their environment is essential to efforts to conserve biodiversity (Greene, 1994). Organisms living at the edge of their

species' range may rely heavily on habitat components to protect them from a physiologically challenging environment. To manage for the persistence of peripheral populations, we must be able to identify these essential habitat components.

Barking frogs (*Eleutherodactylus augusti*) occur farther north than any other member of the large tropical family Leptodactylidae (Lynch, 1971). The range of this species stretches from the Isthmus of Tehuantepec in southern Mexico into Arizona, New Mexico, and Texas (Zweifel, 1967). Like the Northern Territory frog (*Sphenophryne adelphe*; Zweifel, 1985) and the Great Plains narrow-mouthed toad (*Gastrophryne olivacea*; Sullivan et al., 1996), members of the mostly tropical family Microhylidae whose ranges extend into temperate areas, barking frogs breed during monsoonal rains (Adams and Comrie, 1997) in a mostly dry climate. In Arizona, barking frogs had only been seen in one canyon in the Santa Rita and one canyon in the Pajarito Mountains prior to 1993 (Slevin, 1931; Wright and Wright, 1949; Bezy et al., 1966). Barking frogs were heard calling in the Huachuca Mountains in 1978 (Cockrum et al. 1979), but frogs from that population were not observed directly until 1993 (Schwalbe et al., 1997).

Barking frogs are nocturnal and terrestrial; they are associated with caves and rocky outcrops in a variety of biotic communities (Schwalbe and Goldberg, in press). Females likely deposit their eggs in moist or rain-filled cracks and fissures (Wright and Wright, 1949) or in moist earth under rocks (Jameson, 1950). In Arizona, barking frogs spend most of the year underground, leaving overwintering sites just before the start of the summer rainy season and returning between August and October (Schwalbe et al., 1997). The calling season of this species lasts only two to four weeks, with the maximum

number of males calling within the first few nights after the start of the summer rains (Schwalbe and Goldberg, in press).

Our objectives were to quantify habitat and study the spatial population structure of barking frogs to better understand factors important to the persistence of this species in southern Arizona. To understand this species' ecology, we examined the geological formations barking frogs occupy, how geology relates to the frogs' thermal ecology, and how environmental variables correlate with nightly movements. To understand spatial population structure, we estimated the number of frogs living on each rocky outcrop in the study area, investigated territorial behavior between pairs of males occupying the same outcrop, and examined the amount of movement among outcrops.

MATERIALS AND METHODS

Study Area.- We studied barking frogs in a canyon (810 ha) in the Huachuca Mountains, Cochise County, Arizona, where frogs are distributed on rocky outcrops in Madrean Evergreen Woodland (Brown, 1994). This canyon receives an average of 52.3 cm of rainfall annually, with more than half (28.3 cm) falling in the summer monsoon season (Adams and Comrie, 1997). Undifferentiated and dolomitic limestone outcrops from the Pennsylvanian-Permian periods cover 1.1% of the canyon. This limestone is in part altered to skarn and calc-silicate hornfels and forms many caves and caverns (Doe, 1986). Other prominent geological formations in the canyon include Huachuca granite, sedimentary rock of the Morita Formation, and volcanoclastic conglomerate (Doe, 1986).

Habitat and Telemetry.- From 1996 through 2000, we used teams of volunteers to capture barking frogs on the first and/or second night after groups of frogs began calling

at the start of summer rains. We sent volunteers to areas where frogs were heard calling during first-night observations along the main drainage of the canyon. Barking frogs could often be heard from more than 600 m away; volunteers followed calls to their origin and also searched for and captured non-calling individuals. We released frogs at their point of capture on that same night (the next evening for frogs with transmitters).

We implanted transmitters (BD-2HT, BD-2GH; Holohil Systems Ltd., Ontario, Canada) in four males in 1999 and five males in 2000 (four new frogs, one repeat from 1999) within the first week of the summer rains (Goldberg et al., 2002). We implanted a transmitter (BD-2HT) in a female four weeks after the start of the summer rains in 1999 and we attached a transmitter (BD-2T) to a female one week before the summer rains in 2000 with a silicon tubing belt (Goldberg et al., 2002).

We recorded slope aspect, crevice aspect, and crevice material (limestone, other rock, other material) for every location where a frog was known to be in a crevice. We analyzed these data for each radio-tracked frog separately and used Rayleigh's test (Zar, 1996) to determine if slope and crevice aspects were evenly distributed for each frog.

Six of the implanted transmitters were temperature-sensing. With simple linear regression, we compared temperatures from these transmitters when frogs were underground during the day to temperatures taken above ground (1 cm) outside the burrow entrance at the same time individually for each frog. We then examined how this relationship was related to geological formation and which side of the canyon the frog occupied.

Home Range.- We located frogs carrying radiotransmitters nearly every day between 0700 and 1700h and systematically at night while transmitter batteries lasted (3.5-10.5 weeks) during both summers. During the 1999 season, we randomly assigned, without replacement, one frog to be tracked during each of four nocturnal sessions (1700-1959h, 2000-2259h, 2300-0159h, and 0200-0459h) each week. We randomly assigned, with replacement, each of the tracking sessions to a day of the week, and randomly chose the time within each session to locate the frog. In the 2000 season, in addition to diurnal tracking, we located each frog two times a week at night on randomly chosen days, with replacement, at a randomly chosen time between 1700h and 0500h.

We estimated home-range size using the minimum convex polygon method for comparison to other studies (White and Garrott, 1990) and by the fixed kernel method (Worton, 1989) using the animal movement extension (Hooge and Eichenlaub, 1997) in ArcView GIS 3.2 (ESRI; Redlands, CA).

We mapped limestone outcrops in the study area using a GeoexplorerII GPS rover unit and Pathfinder Pro XL base station and post-processed the data using Pathfinder Office software (Trimble Information Services, Sunnyvale, CA). We considered the edge of limestone to be where the bedrock was no longer exposed at the surface. We calculated the percentage of 95 and 50% (core) kernel home ranges located on limestone outcrops for each frog tracked.

Movements.- We examined the association between straight-line distances between daytime refugia, log-transformed and corrected by known nocturnal locations, and the following explanatory variables separately for each frog using multiple regression

(Ramsey and Schafer, 1997): rainfall from 0500h the previous day to 0459h on the morning of tracking, moon illumination at midnight (calculated by U. S. Naval Observatory), and average temperature and relative humidity between 1700h and 0500h (recorded every 30 min by datalogger; Onset Computer Corporation, Bourne, MA). We compared distances between daytime refugia with those corrected by nocturnal locations to determine the accuracy of this estimate of nightly movement.

Spatial Population Structure. - We marked all frogs found during surveys with passive integrated transponder (PIT) tags (Biomark; Boise, ID), inserted subcutaneously to the right of the coccyx above the ilium. We also photographed all captured frogs. We identified all frogs that were found during population surveys to see whether they were on a different outcrop than they had been in previous years.

We conducted call counts during the first four weeks of the summer rainy season at four limestone outcrops to calculate the minimum number of male frogs present on each outcrop, represented by the maximum number of frogs heard calling at one time and corroborated by capture information. We investigated sex ratio by conducting 81.5 hrs of visual encounter surveys (Crump and Scott, 1994) on three limestone outcrops after frogs stopped calling for the season and while radio-tracked frogs were still changing locations. Because we had equal probabilities of encountering a male or female frog during these surveys (assuming they spend equal amounts of time above ground), this sampling should reflect the sex ratio of the population. We extrapolated minimum population sizes from call count and sex ratio data.

In 2000, we tracked two pairs of frogs, each pair associated with the same limestone outcrop, to determine if their movements showed signs of territoriality. Both of these outcrops had a maximum number of three frogs heard calling, so this was more than half of the potential territorial male population on each outcrop (assuming territorial males would call, as in most anuran species [Duellman and Trueb, 1986]). We used dynamic interaction analysis (Millspaugh and Marzluff, 2001), to test whether the distance between frogs on any given day was different than the expected distance if they had no interaction.

RESULTS

Habitat. - Of the 54 frogs caught during this study, all but 6 (11%) were found in, on, or within 30 m of limestone outcrops. Three were found on granite and dacite tuff and three on sandstone and lithic-rich tuff. All nine females captured were on or near limestone outcrops. Frogs were found between 1600 and 1890 m elevation. Limestone covers 2% (16.2 ha) of the area within this range of elevations.

Radio-tracked frogs spent daytime hours (0500h to 1700h) in rock crevices. Those in limestone areas used only crevices made of limestone or limestone partially altered to skarn, except for three frogs (frogs 25, 28, and 49). These three frogs overwintered in the same refugium and all left the overwintering site within three days before the start of the summer rains. Both males (frogs 25 and 28) were found calling on a limestone outcrop 50 m away on the second night of the summer rains. These frogs spent 86 and 77%, respectively, of tracked days in limestone crevices. During radio-

tracking, the female (frog 49) never used a limestone crevice. Other crevices these three frogs used were made of granite and mine tailings.

Mean slope aspect of daytime refugia was southeast for three out of ten radio-tracked frogs ($P < 0.01$; Table 1.1). Mean crevice aspect was northeast to southeast for seven out of ten radio-tracked frogs ($P \leq 0.10$; Table 1.1). There was no pattern ($P > 0.10$) toward a direction for the other frogs in slope or crevice aspect.

For three frogs, changes in temperature outside the crevice were not associated with a change in frog body temperature ($P > 0.20$). For the other three frogs (20, 28, 45), higher temperatures outside were associated with higher body temperatures ($P < 0.08$, simple linear regression; Figure 1.1). One of these frogs used only limestone crevices, one used both limestone and non-limestone crevices, and one never used limestone crevices. Two had home ranges on the north side of the canyon and one lived on the south side of the canyon. All three frogs were visible in their daytime refugia on 5-10% of the days they were tracked. The other three frogs were never visible in their daytime refugia during radio-tracking.

Home Range. - We were able to track four frogs until they stopped changing location for a week or more in late August and possibly had reached their overwintering sites. The other six frogs were still changing locations when their transmitter batteries failed. Mean home-range size of ten frogs was 801.1 m^2 (95% CI 358.5 to 1790.1 m^2) when calculated by MCP, 1087 m^2 (95% CI 507.8 to 2327.2 m^2) when calculated by 95% fixed kernel, and 214.7 m^2 (95% CI 112.5 to 409.5 m^2) when calculated by 50% fixed kernel (Table 1.2). Frogs who spent any time on limestone had 51-100% of their 95%

Table 1.1. Slope and crevice aspects for daytime refugia of radio-tracked barking frogs. Z is “Rayleigh’s z ” (Zar, 1996). Slope and crevice aspects that showed a pattern toward a direction are shown with an asterisk. Angular deviation is shown as a measure of variance.

Frog	slope aspect (°)	angular deviation (°)	N	Z	P	crevice aspect (°)	angular deviation (°)	N	Z	P
11	*152	59	22	5.032	0.01	*20	67	22	2.29	0.10
15	116	75	61	1.462	0.23	*86	71	61	3.18	0.04
20	29	71	41	2.082	0.13	43	74	41	1.13	0.32
25	*142	49	46	18.217	<0.01	*140	53	46	15.45	<0.01
28	180	75	57	1.209	0.30	*106	70	56	3.66	0.03
29	35	63	13	1.995	0.14	*106	62	13	2.34	0.10
45	102	75	53	0.974	0.38	*60	68	51	4.46	0.01
46	110	73	45	1.782	0.17	45	73	45	1.49	0.23
47	3	78	74	0.306	0.74	86	79	71	0.12	0.88
49	*97	41	21	11.629	<0.01	*125	58	21	4.99	0.01

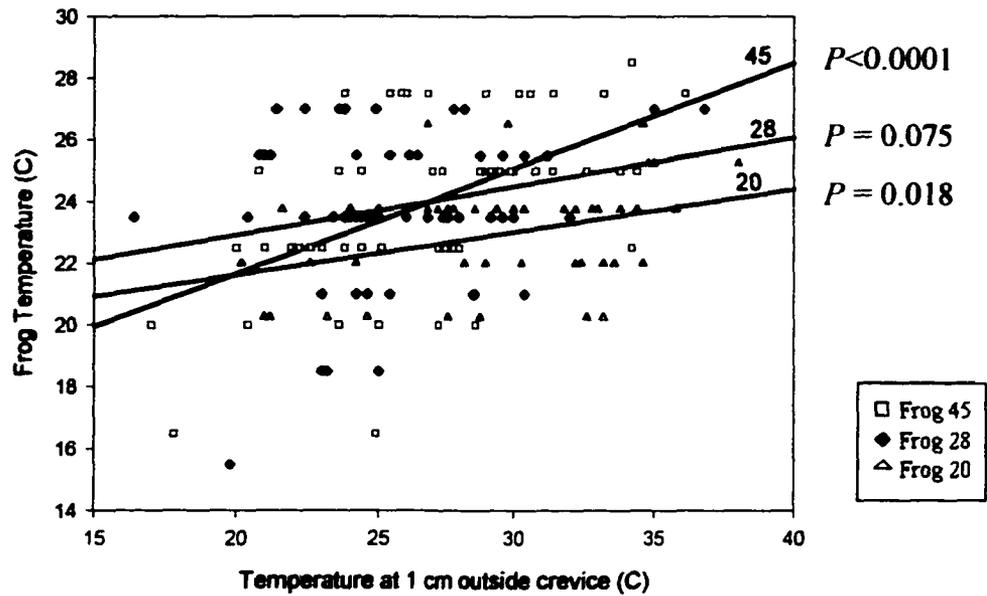


Figure 1.1. Relationship between body temperatures of barking frogs inside crevices and air temperature at 1 cm outside of the crevice at the same time during the day for 3 frogs. Numbers are frog identification numbers.

Table 1.2. Estimated home-range sizes of radio-tracked barking frogs. Female frogs indicated by (F). Percent limestone is the percent of home range that overlaps a limestone outcrop. Mean is calculated using the combined home-range size for frog 47.

Frog	N	MCP (m ²)	95% kernel		50% kernel	
			(m ²)	% limestone	(m ²)	% limestone
11 (F)	33	381.1	580.4	100	101.0	100
15	84	3178.8	2090.1	97	226.1	100
20	63	174.4	206.7	100	53.9	97
25	77	1495.8	1332.4	48	195.6	100
28	76	2815.8	4666.2	51	918.5	94
29	27	201.4	427.2	100	144.0	100
45	55	2376.1	5497.5	0	923.1	0
46	50	255.0	482.0	0	120.2	0
47 (1999)	52	1146.2	1023.4	100	164.5	100
47 (2000)	67	290.8	805.8	100	199.0	100
47 (combined)	119	1269.3	784.3	100	192.3	100
49 (F)	41	790.8	1665.2	0	300.8	0
Mean		801.1	1087.0		214.7	

fixed kernel home range on limestone and 94-100% of their 50% kernel home range on limestone.

Movements. - Three out of ten frogs moved farther when temperatures were higher ($P < 0.05$). Two frogs moved farther with more rain ($P \leq 0.05$). Two frogs moved farther with higher humidity and one frog moved farther with lower humidity ($P < 0.05$). For 55 of 80 nights that we tracked frogs, adding the nocturnal location data to our estimate of movement between daytime refugia did not affect the estimate of total distance moved. When estimates were affected, nocturnal movements were underestimated by an average of 4.6 meters (38%; 95% confidence interval 2.6-7.7 meters).

Spatial Population Structure. - We heard a maximum of 2-10 individuals calling from each of four limestone areas. These numbers equaled or exceeded the number of male frogs captured on those outcrops. No frogs were found on a different outcrop from the one where they were originally caught. One radio-tracked frog crossed a drainage to another outcrop after the calling season had ended. During visual surveys, we found three female and four male barking frogs.

We found no spatial interaction between one pair of male frogs ($t_{2160} = 1.34$, $P = 0.18$), and an attraction between the other pair of male frogs ($t_{3238} = 4.18$, $P < 0.0001$) that shared the same area. The latter pair of frogs was closer to each other by 15.6 m than expected if they had no interaction (95% C. I. 8.3 to 22.9 m).

DISCUSSION

Habitat. - Although we found some barking frogs away from limestone, the majority of frogs were on or in limestone outcrops, greatly out of proportion to the

amount of limestone available. All female frogs caught in this study were found on or near limestone outcrops. Whether or not frogs away from limestone are breeding is unknown; we found no juveniles or egg masses during this study. Limestone may offer better refugia in which to lay eggs because of its ability to absorb rainwater (Birkeland and Larson, 1989). Eggs of terrestrial frogs can dehydrate rapidly, so a moist microhabitat is essential for their survival (Duellman and Trueb 1986). In addition, limestone has a strong buffering capacity which would protect developing eggs from any acidity in the rainwater; acidity reduces the survivorship of eggs and larvae of some aquatic breeding amphibians (Freda, 1986; Griffiths et al., 1993). In Britain, the presence of limestone is associated with the distribution of newts (Denton, 1991).

Seven of 10 frogs we tracked used refugia that faced northeast to southeast; 3 out of 10 frogs used refugia with southeast slope aspects. We did not compare these patterns of use with availability, so we do not know if this is a relict of the overall structure of the frog's rocky habitat or if frogs selected these refugia. East-facing crevices and slopes could have cooler, wetter interiors than west-facing refugia because they are shielded from the afternoon sun.

Of the six frogs we radio-tracked that had temperature-sensing transmitters, three frogs had daytime body temperatures that varied with outside temperatures and three frogs had daytime body temperatures that were independent of outside temperatures. Frogs varied within these two groups in which side of the canyon and geological formation they occupied. However, all three frogs that had body temperatures that varied with outside temperatures were sometimes visible in their crevices during radio-tracking,

whereas the other three frogs were never visible in their crevices during radio-tracking. The differences between these two groups of frogs may be due to behavior with regard to how deep the frogs were in the refugia, as opposed to any physical characteristic of the substrate.

Home Range.- Barking frogs are capable of movement far beyond that indicated by home-range size. The longest distance between sequential daytime refugia for any radio-tracked frog was 116 m, greater than the maximum displacement for all but one of the other frogs tracked. Other frogs repeatedly crossed their entire home range in one night.

Frog species vary widely with respect to movement based on their habitat needs and life history, from the smaller than 20-m² home ranges of *Dendrobates pumilio* in Costa Rica (Donnelly, 1989) to long-distance migrations of 1.4 km in a night for *Hoplobatrachus occipitalis* in West Africa (Speiler and Linsenmair, 1998). The home-range sizes measured for these adult, mostly male, barking frogs encompassed the means of home ranges of groups of cliff chirping frogs [*Eleutherodactylus (Syrrhophus) marnocki*] in Texas (MCPs = 876.3 to 2296.9 m²; Jameson, 1955). Cliff chirping frogs in Texas are similar to barking frogs in that they live on isolated limestone outcrops.

Movements.- Nocturnal tracking revealed that distances between daily locations underestimated nocturnal movement at least a third of the time. Because of this underestimation, we may not have been able to detect the association between movement and environmental variables for some of the frogs. For animals that forage away from refugia and then return, distance between daily locations may have no correlation with

actual nightly movement (Laundré et al., 1987). In addition, the relationship of movement to actual moon illumination may have been obscured by clouds, as our measurement of moon illumination came from the proportion of the moon lit on a particular night, not on how much of that light was reaching the ground. The fact that increased temperature and rainfall were associated with larger movements for some frogs may indicate that dryness and cold are physiologically challenging for barking frogs in temperate areas, in addition to the evidence that they are only active above ground during the warm wet season.

Spatial Population Structure.- The groups of barking frogs that occupy the isolated habitat patches in the study area are so small that it seems impossible for them to persist as populations in the face of demographic and environmental stochasticity (Gilpin and Soulé, 1986). Even if demography and the environment are both remarkably stable, a population of 10 individuals is predicted to go extinct in only 99 generations due to accumulation of deleterious mutations in the genome without input from other populations (Holsinger et al., 1999). The largest estimated group size for barking frogs in this canyon is 20 individuals (assuming a sex ratio of 1:1) so even this group probably could not persist without occasional immigration. Because they do manage to persist, these groups most likely function as subpopulations in a metapopulation, with limited exchange of individuals with neighboring subpopulations and some subpopulations blinking on and off over time (Akçakaya et al., 1997).

There was no sign of territoriality between two pairs of male frogs. Because most barking frogs are confined to limestone outcrops, territorial behavior could have

explained the small numbers of frogs occupying each outcrop. Other factors that could be keeping group size so small include low reproductive rate and a high level of juvenile dispersal or mortality. The movement of juveniles away from their natal habitat patch (juvenile dispersal) is the primary way that genetic material moves between subpopulations in metapopulations of natterjack toads (*Bufo calamita*; Sinsch, 1997). This seems likely to be the case for barking frogs as well. Almost nothing is known about the movements of juvenile terrestrial breeding amphibians (Pough et al., 1998). Juvenile dispersal rates for two aquatic breeding amphibians were at least 18% for wood frogs (*Rana sylvatica*; Berven and Grudzien, 1990) and at least 27% for Fowler's toads (*Bufo fowleri*; Breden, 1987).

Management Implications.- There are four scenarios under which stable metapopulations become extinct (Hanski, 1991): the rate of establishment of new populations becomes lower than the rate of local extinction of habitat patches, population size falls below a threshold point by stochasticity, immigration is reduced, or there is regional disruption. In order to manage for metapopulation persistence, therefore, we need to further understand the movements (and barriers to movement) among habitat patches, be able to identify both occupied and unoccupied patches, and limit disturbance to as few patches as possible.

If we want to manage for barking frogs in southern Arizona, we must understand the relationship of this species to geological formations. The main threat to patches of barking frog habitat in southern Arizona may be copper mining. Copper deposits in the southwestern United States are typically layered with limestone formations (Jerome,

1968). There are many large open-pit copper mines in the mountains of southern Arizona, ore bodies from several of which are closely associated with extensive limestone formations (Mule Mountains, Bryant and Metz, 1968; Sierrita Mountains, Kinnison, 1968; Silverbell Mountains, Richard and Courtright, 1968; White Mountains, Moolick and Darek, 1968). Open-pit mining removes habitat for almost every species and would certainly destroy any barking frog population living within the mined area.

To manage for the persistence of this species in southern Arizona, we need to survey for and protect additional populations. Surveys should be focused between 1500 and 2150 m elevation in the mountains of southern Arizona. To preserve populations, both occupied and unoccupied habitat patches and movement pathways between them must be protected. We are currently locating genetic markers to measure gene flow among habitat patches and identify movement corridors.

CHAPTER 2- METHODS FOR MONITORING BARKING FROG (*ELEUTHERODACTYLUS AUGUSTI*) STATUS IN SOUTHERN ARIZONA

ABSTRACT

Determining how to monitor the status of amphibian populations is a challenging task for both scientists and resource managers. Western barking frogs (*Eleutherodactylus augusti cactorum*) are secretive and have small populations in their narrow U. S. range. We attempted to use four methods for estimating population size of barking frogs in southern Arizona: mark-recapture, distance sampling, visual encounter surveys, and call counts. A minimum of five years of data were necessary for calculating population size using an open population mark-recapture model and subsequent power to detect changes in population size was low. We found distance sampling to be unusable due to the effect of the observer's presence on calling frogs. Visual encounter surveys were problematic due to differences between habitat structure of sites and observer biases. Call counts gave minimum numbers of male frogs occupying outcrops, but numbers of calling frogs varied greatly with temporal and environmental variables. For cryptic species such as barking frogs, estimating population size is a time-consuming endeavor with low power to detect trends. We propose that a more powerful and feasible method of monitoring secretive species may be to focus on the distribution of populations over time; in the case of barking frogs this would mean monitoring the presence of calling males from habitat patches in the area of concern.

INTRODUCTION

Whether amphibian populations are declining globally was a matter of debate for many years (Blaustein et al., 1994; Pechmann and Wilbur, 1994; Travis 1994).

Distinguishing natural population fluctuation from long-term population trajectories can be difficult (Pechmann et al., 1991) and estimates of frog population size are among the most variable of all vertebrate taxa due to both population fluctuation and sampling error (Gibbs et al., 1998). Long-term data (Pechmann et al., 1991; Blaustein et al., 1994) and standardized efficient surveying techniques (Heyer et al., 1994) are essential for monitoring the status of amphibian populations.

We evaluated methods for estimating population size of western barking frogs (*Eleutherodactylus augusti cactorum*) to develop a monitoring strategy. Ideally, monitoring programs would be able to both assess the distribution of populations and their likelihood of persistence. Often, monitoring programs focus on estimating the number of individuals in populations (Thompson et al., 1998), although these methods have a high level of uncertainty and require intensive efforts to be focused on a selected area, especially for rare or secretive species (Link et al., 1994; Becker et al., 1997; Green, 1997; Freilich et al., 2000). We attempted to determine if there were any useful ways to monitor population size for barking frogs.

The western barking frog reaches the northernmost limit of its range in the mountains of southeastern Arizona. Barking frogs in Arizona are associated with isolated limestone or rhyolite outcrops (Bezy et al., 1966), where they live in rock crevices most of the year. Their above-ground season starts with the onset of the summer rains, when

males call in groups on rainy nights during the first two weeks of the rainy season (Schwalbe and Goldberg, in press). Barking frogs are cryptic and nocturnal, making visual detection difficult. In Arizona they probably end all surface activities for the year in late summer or early fall (Schwalbe et al., 1997).

We investigated four methods for estimating population size for barking frogs in a small park in southern Arizona: mark-recapture, distance sampling, visual encounter surveys, and call counts. We assessed each method for feasibility, accuracy, and power to detect long-term trends.

MATERIALS AND METHODS

Study Area. - In 1996, we began marking barking frogs in a small park (1922 ha) in the Huachuca Mountains with passive integrated transponder (PIT) tags. The study area consists of one large canyon, with barking frogs distributed throughout on isolated rocky outcrops at 1600-1890 m elevation. The majority of barking frogs were found on or near limestone rock outcrops; we also found a few frogs on granite, sandstone, and dacite and lithic-rich tuff.

We focused on three limestone sites in the park for exploring techniques for monitoring population size (Figure 2.1). In annual intensive capture efforts over five years, no barking frog was found to have moved between rock outcrops. We radiotracked nine frogs for one summer each and one frog for two summers. Only one frog ever moved between outcrops; for purposes of population estimation, therefore, we consider the two outcrops (2a and 2b) that the radiotracked frog moved between to contain one population. Because no frogs were found to have moved into or out of

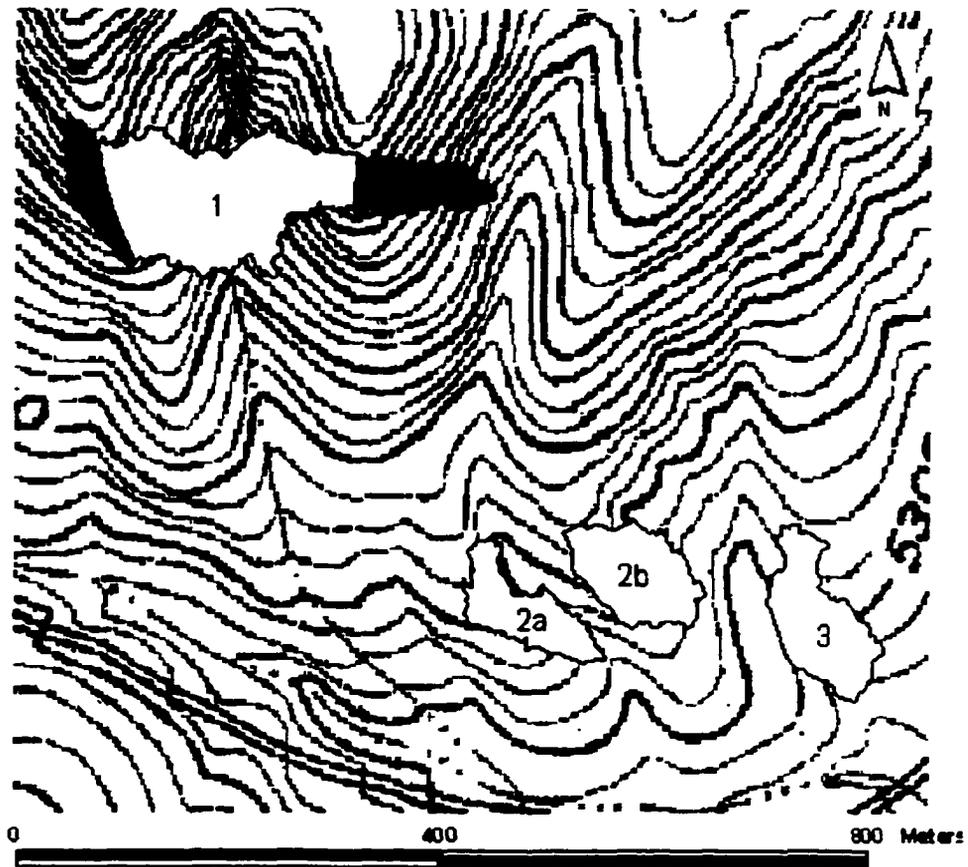


Figure 2.1. Limestone sites where we investigated methods for monitoring population size of barking frogs. Shaded parts of site 1 are limestone areas outside of the drainage and not included in the analysis.

sites 1 and 3, we consider those to contain a single population each. The dominant vegetation in the area is Madrean evergreen woodland (Brown, 1994). Elevation at these sites ranges from 1600-1770 m.

Mark-recapture.- Each year from 1996 through 2000, we used groups of 7-30 volunteers to capture frogs in the first few nights of the summer rains. We used the Cormack-Jolly-Seber (CJS) model for open populations in Program Mark (White, 1999) to estimate the original number of individuals at site 3 and both the CJS and the Pradel (1996) model to estimate rate of change in population size at site 3 with five years of data. Due to the small data set, we were unable to model the data with differential probability of capture by sex using either the CJS or the Pradel model. Data for sites 1 and 2 were insufficient to reach numerical convergence using the CJS model.

We used the modified Lincoln-Petersen model (Chapman, 1951) to estimate the number of individuals in all three sites. We analyzed data both from capture occasions on two consecutive nights in 1999 and from all captures in consecutive years (1999 and 2000) for sites 1 and 3; low sample size for site 2 in 1999 precluded using two nights in 1999 as capture events, so we used only the two years as capture occasions to estimate population size for this site. We used the 95% binomial confidence limit chart in Krebs (1999) to calculate confidence intervals for the Lincoln-Petersen estimates.

Distance Sampling.- On the first night of summer rains in 2000, we surveyed for calling (male) barking frogs using the variable circular plot method of distance sampling (Reynolds et al., 1980; Buckland et al., 1993). We assigned the locations of listening points using ArcView GIS 3.2 (ESRI; Redlands, CA) Animal Movement extension

(Hooge and Eichenlaub, 1997) at sites 2 and 3 at random locations at least 25 m from the edge of the outcrop and 50 m from each other. We set the detection limit at 25 m. After arriving at the point, we waited quietly for one min (Reynolds et al., 1980) and then began a 10-min listening period. We noted all barking frogs we could hear calling during this listening period and then visually searched the area at the point. Next, we attempted to locate calling individuals by walking in the direction of the calls. Using our voices or a tape player, we attempted to elicit response calls from each individual until it could be located. In most cases, the frog did not respond and we estimated its location from our original observations.

Visual Encounter Surveys. - We conducted visual encounter surveys (VES) for 45.5 hrs at site 3 and for 36 hrs at site 2 during the two and a half months after the first summer rain. We calculated time spent searching per individual found as a measure of relative abundance (Morrison et al., 1995). We mapped each limestone outcrop using a GeoexplorerII GPS rover unit and a Pathfinder Pro XL as a base station, post-processed the data using Pathfinder Office software (Trimble Information Services; Sunnyvale, CA), and calculated the area using ArcView GIS 3.2 (ESRI; Redlands, CA). We compared the relative abundance of frogs on each outcrop with the density of frogs estimated using mark-recapture and the area of each outcrop to see if the development of an index for population estimation was feasible.

Success of VES depends strongly on the ability of the observer (Crump and Scott, 1994). We explored this variable by establishing a VES-sampling area containing 20 adult and 7 juvenile model barking frogs on a limestone outcrop in barking frog habitat.

Twenty-eight observers spent 45 min each searching for these models at night when there was no moonlight. In the afternoon before each set of trials, we placed each model at a designated, randomly chosen site. Each model had a 50% probability of being in a crevice, a 25% probability of being under vegetation, and a 25% probability of being in the open. Once we assigned a model to a location, that model had the same location for all trials. We wrote a number on the ventral side of each model and the observer recorded this number when they found the model. Observers filled out datasheets after the trial, recording their level of experience searching for small animals, experience working at night or in the dark, experience conducting visual surveys, experience hiking in rough terrain, and eye color. We ranked experience level on an ordinal scale ranging from no experience to more than five years experience. We used multiple regression (Ramsey and Schafer, 1997) to determine which factors had explanatory power for the number of models the observer found.

Call Counts. - From listening stations, we counted number of frogs we heard calling at two-hr intervals from 1800-0500h in 1999 (sites 2a and 3). The maximum number of frogs calling each night was reached between 1800 and 2330h; in 2000 we did counts hourly between these hours at sites 1 and 3. We opportunistically recorded observations of numbers of calling frogs from site 2b. We did not use recorded or imitated calls to elicit response calls. In 1999, we randomly chose the beginning time of the sampling session to be odd or even (i.e., 1800h, 2000h, etc. or 1900h, 2100h, etc.) and alternated observers by night. In 2000, the two stations were sampled simultaneously by different observers. We conducted these counts from the first night of the summer when

barking frogs called until the first night with rain over three mm when frogs did not call. On the first night of calling in both years, both observers counted at the same site simultaneously and compared observations until they reached agreement on how many frogs were calling.

The observer arrived at the listening station and waited quietly for one minute before beginning to collect calling data so that frogs might start calling again after possible disturbance from the observer's movement (Reynolds et al., 1980). The observer then recorded how many frogs called from that area within a ten-minute period. The observer recorded cloud cover (<25%, 25-75%, or >75%), wind speed (Beaufort scale; List 1949) and rain (Tru-Check® direct-reading raingauge [Edwards Manufacturing Company, Albert Lea, Minnesota]; or datalogging raingauge [Onset Computer Corporation, Bourne, MA]) for each listening session. Temperature and relative humidity were recorded by datalogger (Onset Computer Corporation, Bourne, MA). We did not conduct any activities on the outcrops 30 minutes before each call count.

We investigated variability in numbers of males calling on sites 1 and 3 by quantifying the square root of this count in relation to environmental and temporal variables (cloud cover, wind speed, rain during the previous two hours, rain since 0500h that day, night of the summer rainy season, temperature, and humidity) separately for each site using multiple regression. We assessed differences among observers by adding this variable to a model of these data built using stepwise regression (Ramsey and Schafer, 1997). We used JMP® software to perform these analyses (SAS Institute Inc. JMP version 4; SAS Institute Inc., Cary, NC, 1989-2000).

RESULTS

Mark-recapture.- We did not detect differences between years in either survival ($P = 0.66$) or probability of capture ($P = 0.13$) at site 3, nor did we detect a change in population size between years ($\lambda = 0.95$ [95% C.I. 0.82 - 1.10] from CJS model, 0.99 [95% C.I. 0.84 - 1.17] from Pradel model; Table 2.1). Survival rate was estimated to be 0.93 (95% C. I. 0.57 - 0.99). The CJS model fit the data acceptably ($P = 0.34$ from goodness-of-fit testing).

Distance Sampling.- In four of six listening sessions of distance sampling, frogs that had been calling consistently during other listening sessions stopped calling when the observer was within approximately 14 to 22 m of their location. Of 11 males heard calling in the survey area during the distance sampling listening sessions, only five resumed calling and were detected within the predetermined radius (25 m) of the observation point. We did not attempt to estimate population size using this method.

Visual Encounter Surveys.- At site 3, where the density of frogs was estimated to be one per 736 m² (95% C. I. one per 356 - one per 859 m²), we took 7.6 hours to find each frog visually. At site 2, where the density of frogs was estimated to be one per 3507 m² (95% C. I. one per 723 - one per 4525 m²), we took 5.1 hours to find each frog visually. All frogs found at site 2 were found at site 2b.

Of 27 barking frog models inside the search area, 28 searchers found between 2 and 11 model frogs. The mean number of models found was 5.9 (95% C. I. 4.7 - 7.1 models). Five or more years of experience hiking in rough terrain was associated with finding more model frogs ($P = 0.04$). Level of experience searching for small animals,

Table 2.1. Population estimates of barking frogs from sites 1, 2, and 3. CJS = Cormack-Jolly-Seber model, L-P = Lincoln-Petersen model. No estimate of variance is available for site 3 in 1999 because all frogs captured on the first night were also captured on the second night. Estimates of population size for site 1 are for males only because no females were captured there.

Site	Model	Number of frogs estimated	95% C.I.
1 (1999 only)	L-P	14	6.8 – 333.3
1 (both years)	L-P	12	8.7 – 22.9
2 (both years)	L-P	4	3.1 – 19.4
3 (1996-2000)	CJS	11.9	10.2 – 24.6
3 (1999 only)	L-P	6	NA
3 (both years)	L-P	12	8.5 – 53.3

experience working at night or in the dark, experience conducting visual surveys, and eye color did not explain variability in the results ($P>0.45$).

Call Counts. - In 1999, frogs called as a group (>1) on only 8 nights, while in 2000 frogs called as a group from site 3 on 16 nights (Figure 2.2). The maximum number of frogs heard calling from site 1 was ten, from site 2, five, and from site 3, five in 1999 and nine in 2000. We did not observe any frogs calling outside of the listening session that were not counted within the session.

Greater numbers of frogs called in association with days closer to the beginning of the summer rains, at higher humidities, lower temperatures, less wind, and higher hourly rainfall ($P<0.05$). After accounting for these variables, cloud cover and the amount of rain during the day were not associated with larger numbers of frogs calling ($P>0.20$). There is no evidence of inter-observer bias in the count of frogs ($P>0.20$).

DISCUSSION

Mark-recapture. - Only male barking frogs call, so capture probabilities for this species during the calling season were inherently biased when we attempted to estimate total population size. Because the calculation of capture probabilities was based mostly on males, which are more likely to be captured than females, this estimate was artificially high and estimates of population size produced using mark-recapture models with these data probably underestimate numbers of frogs. We could not differentially calculate capture probabilities for the sexes for any site because of small sample sizes. Although we did not detect this violation of the assumption that every marked animal present in the population has the same probability of capture using the CJS model, we had low power to

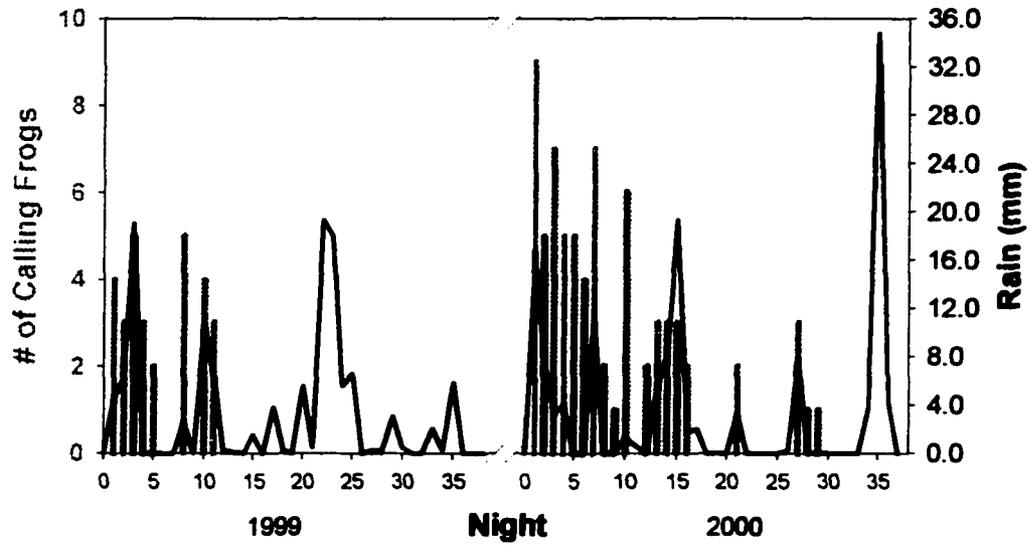


Figure 2.2. Numbers of calling barking frogs by night of the summer rainy season, site 3, 1999 and 2000. The first night of rain each year is designated as night 1. Gray bars are number of frogs heard calling, black line is the amount of rainfall between 0500h the morning before calling until 0459h on the night of calls.

detect this because of small sample size. Differences in capture effort among years may have added variability to this estimate.

Lincoln-Petersen estimates should be unbiased if the number of recaptures at a site were at least seven (Robson and Regier, 1964); only one estimate in this study meets this criterion. Chapman (1951) states that using this model with less than 10 recaptures may fail to give an estimate of the correct order of magnitude. When we used data from two nights in a row to calculate population size, this model produced no estimate or a very large estimate of variance, even though assumptions of closure were more likely to be met when the data were divided this way. When different years were used as capture occasions, the estimates show no difference in population size between sites, whereas call counts and numbers of individuals captured indicated that the number of frogs at one site may be twice the number at another.

The population estimated using the CJS model would have to be declining by 16 to 18% for 5 years before the decline was detected. If the 12 frogs estimated to occupy this site had a rate of population decline of 18% per year, by the end of the five years of this study there would be only six frogs left and we may or may not have detected a change in population size using this method. Mark-recapture is a difficult technique to use with barking frogs because capture efforts must be conducted with multiple people in a short window of time. Even where this effort is feasible, low capture numbers mean we have very little power to detect changes in population size (Taylor and Gerrodette, 1993).

Distance Sampling.- Two major assumptions of distance sampling are that the likelihood of finding an animal increases with decreasing distance to the observer and

that all animals at the observation point are observed (Buckland et al., 1993). In this study, frogs closer to the observer were less likely to be detected, making distance sampling an inappropriate method for estimating population size for this species at this scale. If we choose not to limit the distance at which a frog can be detected, estimating the locations of this highly ventriloquistic frog would be difficult to impossible.

Visual Encounter Surveys. - We spent more time searching per frog found where the density of frogs was higher. One explanation for this is that the more densely populated site is steeper and more heavily vegetated, making visual detection of frogs difficult. If frogs were easier to find at the lower-density site because of differences in habitat structure, then visual encounter surveys may still be useful for monitoring individual habitat patches. However, the time required is prohibitive and the variability introduced by observer behavior greatly reduces the power of this method to detect trends.

Most experience levels had no explanatory power for how many barking frog models observers found. Freilich and LaRue (1998) also found this to be true for visual surveys for desert tortoise sign. All searchers in this study who found nine or more frog models had greater than five years experience hiking on rough terrain; however, other searchers with this level of hiking experience found few models. This is unlike observer differences in aerial surveys, where the experience of the observer greatly affects the accuracy of their work (Caughley et al., 1976; Frei et al., 1979). Although the models in this study did not move or make noise, we feel that the variation demonstrated in these

trials is representative of that of observers looking for real frogs, which often do not move when approached.

Call Counts. - Call counts have been considered most useful as a monitoring method for species that call over a prolonged period of weeks or months and possibly not usable for monitoring species which call for only a short time each year (Zimmerman, 1994). For any species, counts taken at different times will vary due to temporal and environmental factors (Green, 1997; Shirose et al., 1997; Bridges and Dorcas, 2000). The between-year variability in numbers of barking frogs calling from the same site when capture data indicated no change in population size demonstrates the low power of call counts to detect changes in population size even when a full data-set is available. Call counts for barking frogs are best conducted on cool rainy nights with low wind as close as possible to the beginning of the summer rainy season. Because numbers of calling frogs drop off dramatically over time, the most informative count would be taken within the first two nights after the start of the summer rains and can only be considered a minimum number of male frogs occupying an outcrop.

We found no differences in call count data collected by different observers. Barking frogs have a very simple call, and the density of barking frogs is low. Therefore, it is fairly easy to determine how many frogs are calling in an area despite the ventriloquistic aspect of this species' call (Bezy et al., 1966). This differs from results of multi-species call counts conducted in Ontario, Canada, where novice observers tended to underestimate numbers of calling frogs (Shirose et al., 1997).

Management Implications.- We found that distance sampling, visual encounter surveys, and call counts were not useful ways of estimating population size for barking frogs. Mark-recapture efforts produced a useful estimate after five years of intensive monitoring in a single site, but the power to detect changes in population size was so low that the estimate is nearly useless for monitoring purposes.

The amount of variability associated with estimating population size for some animals (including amphibians and insects) is so high that detecting trends may require an extraordinary commitment of resources if it is possible at all (Gibbs et al., 1998). In the current amphibian extinction crisis, populations are disappearing at a much higher rate than they are being established (Green, 1997). Monitoring the distribution of species across the landscape is an important predictor for understanding the risk of extinction (Green, 1997; Channell and Lomolino, 2000) and may be a more powerful and feasible way of monitoring the status of species than attempting to monitor population size (Swann, 1999).

If we wish to monitor the status of barking frogs in the park by focusing on their distribution, we could record on which rock outcrops they are present every year by listening for their calls. This method potentially involves a low amount of error, associated with falsely attributing absence to an area where frogs are present. By working at the scale of presence/absence, we would be relieved of the variation that causes an unacceptable level of error in methods attempting to estimate population size for such small and secretive groups of individuals. If we wish to know the status of barking frogs in a region or as a species, we must focus on monitoring their distribution

region- or range-wide. Although long-term datasets on individual populations are indispensable to understanding population processes, monitoring the status of species on a range-wide scale is essential to understanding the current extinction crisis.

APPENDIX A – BARKING FROG CAPTURES AND MEASUREMENTS

Table A.1. Barking frog capture dates, sexes, and measurements. SVL = snout-vent length.

Frog Number	Sex	Captured		SVL (mm)	Mass (g)	Tympanum Width (mm)	Head Width (mm)	Tibia Length (mm)	Foot Length (mm)
		1999	2000						
1	M			80	34				
2	M			76	25				
3	M	x	x	70	29	5.0	31	32	31
4	M	x	x	77	29	5.5	30	29	31
5	M	x	x	78	37	5.5	31	37	30
6	M		x	76	33	5.0		36	30
7	F	x		81	41	4.0	30		
8	M	x		72	34	5.5	32		
9	F			85	56				
10	M			80	36				
11	F	x		81	45	5.5	39		
12	M	x	x	76	33	6.0	29	33	28
13	J			21	0.8				
14	M			75	27				
15	M	x	x	75	37	5.0	34	34	30
16	M	x		74	34	4.0	31		
17	M	x	x	76	31	5.0	30	35	31
18	M			74	28				
19	M	x		74	35	4.0	33		
20	M	x	x	76	39	5.0	33	32	31
21	M			79	39				
22	M			75	34				

23	M			74	23				
24	F			51	10				
25	M	x	x	70	25	5.0	30	31	29
26	M	x		67	33	5.0	29		
28	M	x	x	70	27	5.0	32	35	28
29	M	x	x	79	29	5.0	31	37	30
30	M	x	x	71	28	5.0	27	34	28
31	M	x		73	43	5.0	35		
32	M	x		73	42	5.0	32		
33	M	x		75	47	4.0	32		
34	M	x		76	32	4.5	32		
36	M	x	x	74	29	7.0	29	32	28
37	M	x	x	76	28	6.0	30	33	27
38	M	x	x	77	33	5.0	31	35	31
39	M	x		69	26	4.0	29		
40	M	x	x	77	32	5.0	31	35	30
41	M	x	x	70	29	5.0	29	34	28
42	M	x	x	72	30	5.0	31	35	30
43	M	x	x	76	19	4.0	24	32	29
44	M	x		70	24	4.5	30		
45	M	x	x	70	34	5.0	30	35	30
46	M	x		80	38	4.5	34		
47	M	x	x	74	29	5.0	32	34	27
48	M	x		63	21	3.5	27		
49	F		x	75	32	6.0			
50	F		x						
51	M		x	69	24	4.0		31	30

52	F	x	80		6.0			
53	F	x	79	44	5.5	35	37	30
55	M	x	66	23	4.0		34	27
56	M	x	65	22	6.0		31	26
57	M	x	60	16	4.5		29	24
58	M	x	56	16	5.0		28	25
59	M	x	80	44	5.0		36	29
60	M	x	69	24	5.0		34	28
61	M	x	66	25	5.0		31	26
62	F	x	79	40	5.0	33	33	32

APPENDIX B- PREY ITEMS FOUND IN BARKING FROG SCAT

Table B.1. Prey items found in barking frog scat

Frog	Date	Prey Item	
6 (M)	13 Jul 96*	centipede 13 field crickets 1-2 scorpions	Chilopoda <i>Acheta assimilis</i> Scorpionida
13 (juv)	20 Aug 96*	13 field crickets 2 leafhoppers oribatid mite	<i>Acheta assimilis</i> Cicadellidae Oribatidae
20 (M)	28 Jun 99	4 short-horned grasshoppers spider	probably Acrididae Araneae
29 (M)	28 Jun 99	adult ant lion	<i>Hesperoleon niger</i>
46 (M)	5 Jul 99	longhorned katydid	Tettiganiidae
15 (M)	23 Jun 00	2 field crickets	<i>Acheta assimilis</i>

*Scat from 1996 collected by Barbara Alberti, National Park Service.

All prey items identified by Carl Olson, Department of Entomology, University of Arizona.

APPENDIX C- SEXUAL DIMORPHISM AND MORPHOMETRICS OF BARKING FROGS

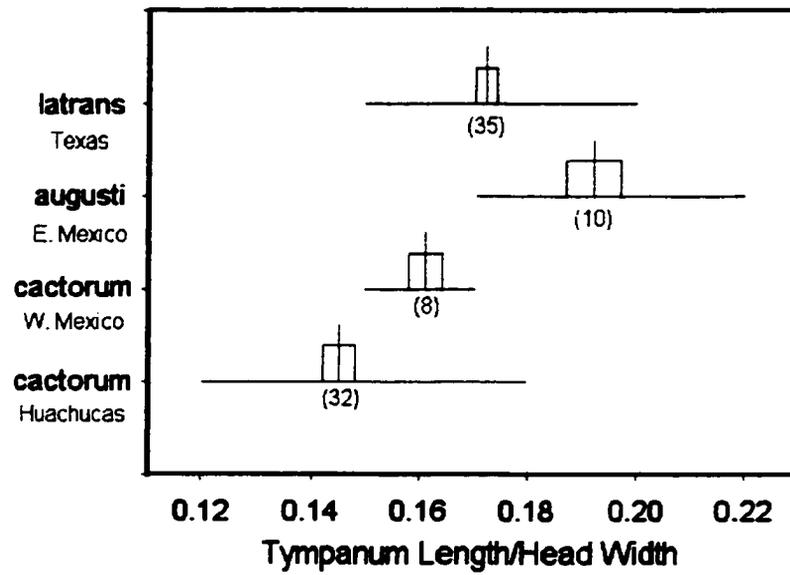
There is no reliable way to differentiate between the sexes of barking frogs in Texas and New Mexico (J. Malone, pers. comm.). In Coronado National Memorial (CNM), female barking frogs (defined as those carrying eggs) had white throats and pink tympana, whereas males (defined as those frogs calling) had dark throats and tympana during the calling season. Later in the summer, male throat color became mottled gray and remained that way through the winter into late spring. We found that snout-vent length (SVL) of female barking frogs (N = 7, mean = 80.0 mm, 95% C.I. 77.2 to 82.8 mm) was larger than that of males (N = 49, mean = 72.9 mm, 95% C.I. 71.4 to 74.4 mm; $t_{54} = 3.53$, two-sided $P = 0.0009$) in our study area, as they are in Texas (Zweifel, 1956).

Maximum body sizes for both male and female barking frogs in our study area exceeded the sizes of the largest preserved specimens of this subspecies (*E. a. cactorum*) measured by Zweifel (1956) from throughout Mexico by 13.6 and 8 mm, respectively. Barking frogs in this area had wider heads by an average of 5.0 mm (95% C.I. 2.9 to 7.0 mm) than those measured by Zweifel (1956; $t_{44} = 4.88$, $P < 0.001$), but this may be due to their larger body size. Only the maximum sizes of specimens were published (Zweifel 1956), so we were not able to compare mean body sizes between these groups. Zweifel (1956) found that females had longer hind feet compared to their body length than males in this subspecies, but we found no difference between the sexes for this measurement ($t_{29} = -0.20$, $P = 0.84$).

The relative size of the tympanum has been used to differentiate between subspecies of barking frog (Zweifel 1956). For females at CNM, the ratio of tympanum width to head length was not different from that found by Zweifel (1956) for this subspecies, mostly from specimens collected in Mexico ($t_{17} = 0.34$, two-sided $P = 0.74$). For males at CNM, this ratio is smaller than that found for Mexican specimens of this subspecies by Zweifel (1956; $t_{38} = 2.57$, two-sided $P = 0.014$; Figure C.1). This difference is estimated to be 0.016 (95% C.I. 0.0034 to 0.0286). The ratios for this study area were also smaller than those measured by Zweifel (1956) for males of *E. a. augusti* ($t_{40} = 7.76$, $P < 0.0001$) and for females ($t_{13} = 2.69$, $P = 0.019$) and males ($t_{65} = 8.84$, $P < 0.0001$) of *E. a. latrans*. These differences are estimated to be 0.047 (95% C.I. 0.035 to 0.059), 0.038 (95% C.I. 0.008 to 0.068), and 0.027 (95% C.I. 0.021 to 0.033), respectively. Unlike Zweifel (1956), we did not find a difference between sexes in relative tympanum size ($t_{34} = 0.061$, $P = 0.95$).

Our data support the distinction between subspecies based on relative tympanum size, as these measurements in our study were closest to that for the *E. a. cactorum* in Zweifel's study and smaller than those of the other subspecies. Some of the differences discussed here may be due to problems associated with the unpredictable distortion of characters on preserved specimens (Lee 1982).

Figure C.1. Relative tympanum width for male barking frogs from different areas and subspecies. Horizontal lines are range of data, vertical lines are mean, boxes are ± 1 standard error. Numbers in parentheses are sample sizes. Data are from Zweifel (1956) except for data from the Huachucas.



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