1-1-2006

Habitat selection by the relict leopard frog (Rana onca): Assessment of vegetation use at two scales

Sean Moran Harris
University of Nevada, Las Vegas

Repository Citation
Harris, Sean Moran, "Habitat selection by the relict leopard frog (Rana onca): Assessment of vegetation use at two scales" (2006). *UNLV Retrospective Theses & Dissertations*. 2034.
http://dx.doi.org/10.25669/d8b4-ijdv

HABITAT SELECTION BY THE RELICT LEOPARD FROG (*RANA ONCA*): ASSESSMENT OF VEGETATION USE AT TWO SCALES

By

Sean Moran Harris

Bachelor of Science
University of Nevada, Las Vegas
2004

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science Degree in Biological Sciences
Department of Biological Sciences
College of Sciences

Graduate College
University of Nevada, Las Vegas
August 2006

UNLV
UNIVERSITY OF NEVADA LAS VEGAS

Thesis Approval
The Graduate College
University of Nevada, Las Vegas

May 9, 2006

The Thesis prepared by

Sean Moran Harris

Entitled

Habitat Selection By The Relict Leopard Frog (Rana onca) : Assessment of Vegetation Use at Two Scales

is approved in partial fulfillment of the requirements for the degree of

Master of Science

Examination Committee Chair

Dean of the Graduate College

Examination Committee Member

Examination Committee Member

Graduate College Faculty Representative

PR/1017-53/1-00

ABSTRACT

Habitat Selection by the Relict Leopard Frog (*Rana Onca*): Assessment of Vegetation Use at Two Scales

by

Sean Moran Harris

Dr. Brett Riddle, Examination Committee Chair
Professor of Biological Sciences
University of Nevada, Las Vegas

In recent years, two populations of the rare Relict Leopard Frog (*Rana onca*) have gone extinct. These population extinctions occurred concomitantly with the encroachment of native emergent vegetation into pools in which frogs were usually observed. In order to determine if adult *Rana onca* prefer more vegetatively open habitats, a radio-telemetry study was conducted. A total of 809 radio telemetry observations were made on 34 frogs from April 2 through December 7, 2004. Binary Logistic Regression was used with both macrohabitat and microhabitat data to compare habitat characteristics between low-use and high-use segments of the spring. A more traditional multiple analysis of variance (MANOVA) approach was also used at the macrohabitat scale to compare used segments to non-used segments. Both of these analyses supported the hypothesis that adult *Rana onca* select for areas with less vegetative cover.

TABLE OF CONTENTS

LIST OF TABLES

ACKNOWLEDGEMENTS

I wish to acknowledge my advising professor, Dr. Brett Riddle, and my committee members, Dr. David Bradford, Dr. Chad Cross, and Dr. Shawn Gerstenberger for their support and guidance throughout this project. I also want to thank Dr. Jef Jaeger for his content advising during the duration of this study. Mark Sappington has also played a major role in generating GIS maps from the logistic regression models produced by these analyses. I would also like to thank all those that assisted with various aspects of the fieldwork (Scott Trujillo, Allen Calvert, Cristina Velez, Dawn Fletcher, Dawn Minnick-Trujillo, Stephanie Harris, Brian Smith, Samantha Sahni, and Tereza Jezkova). My friends and family deserve a great deal of thanks for the support and understanding they showed during this study. The United States Environmental Protection Agency (EPA), through its Office of Research and Development, as well as the National Park Service (NPS), collaborated in the research described herein.

CHAPTER 1

BACKGROUND

Conservation Biology.–Population and species extinctions are natural processes that shape the natural world. More than 99% of the species that once inhabited the earth are now extinct (Thorne-Miller, 1999). Two major extinction types are responsible for these numbers, background extinctions and mass extinctions. Background extinctions are the averaged rate of species extinctions over a defined period of time (Thorne-Miller, 1999). Rapid extinction events that involve many different phyla, habitats, and geographic regions are known as mass extinctions.

There have been five past mass extinctions during different periods of Earth's history (i.e., Ordovician-Silurian, Late Devonian, Permian-Triassic, End Triassic, Cretaceous-Tertiary). Natural catastrophes such as climate change and asteroid impacts have been implicated in these past mass extinctions. Not only were many species lost during these extinctions, but many families as well (approximately 25, 19, 54, 23, and 17% respectively) (Thorne-Miller, 1999). When an entire family goes extinct a substantially larger amount of genetic diversity is lost.

We are currently facing a similar biological crisis sometimes considered to be the sixth mass extinction. In 1993, E.O. Wilson, a Harvard biologist, estimated that approximately 30,000 species per year are going extinct. This estimation has been questioned as to its accuracy, but there is no question to the fact that species are going extinct at a relatively fast rate. These extinctions are not only alarming due to the number of extinctions, but mainly because of the causes. Unlike the natural catastrophes of the previous mass extinctions, the current mass extinction is driven primarily by humans. Some of the many human-based causes include transformation of natural landscapes through agriculture, pollution, and introduction of non-native predators and competitors. Humans are indeed an animal species, and therefore, this is the only mass extinction to have a biotic rather than a physical cause. Extinctions based on biotic causes, unlike physical causes, have the potential to be slowed through active practices such as research and education. Because each species has its own attributes and conservation issues, a vast amount of research regarding these attributes is required for both the general class of organism, and the actual species.

Amphibian Declines.–Amphibian populations as a whole have been decreasing at a dramatic rate and are the most declining class in recent history (Wilson, 1988). Amphibian populations fluctuate annually in size from many different natural interactions. Therefore, it is challenging to distinguish between typical changes in population size and a decline towards extinction (Pechmann et al., 1991). These declines have many potential human-based causes (e.g., habitat degradation, and pollution) that exploit some key adaptive characteristics of amphibians.

Amphibians do not drink water like many other animals; rather, water and air are passively moved through the skin. Chemical pollutants, in a similar way as water, can also permeate the skin of amphibians. Anuran embryos and tadpoles are very susceptible to this type of chemical toxicity (Bantle et al., 1992; Birge et al., 1979; Cooke, 1981).

Thus, aquatic pollutants have a larger affect on amphibian physiology than on animals with less permeable skin.

Amphibians are generalized as being able to live both in water and on land. Some amphibians reproduce by means of direct development. This type of reproduction eliminates the dependency on water for reproduction. The larvae are not free-swimming individuals like in typical amphibian reproduction but are encased in an egg-like structure until the young "hatch" after metamorphosing into young adults. Although direct development is an adaptation to be able to reproduce on land (e.g. Callery et al., 2001; Wake and Hanken, 1996), most amphibians do have the typical amphibious lifestyle. Typically, although there are some exceptions, fresh water is used primarily during the developmental larval stage, and land is used during the adult phase. This complex life cycle requires that both conditions be favorable in order to sustain an amphibian population. If either of these habitats are affected enough that it is no longer habitable, either for breeding (in the case of water habitats), or adult living (in the case of terrestrial habitats), the population will suffer extreme losses.

These adaptations, and their sensitivity to environmental fluctuations, make amphibians a very useful bio-indicator for measuring the effects of changes in a particular environment (Vitt et al., 1990). Bio-indicator species are usually affected first by change, indicating that other species, similar or not, will likely be affected in the future should a particular stressor remain in the environment. It has been shown many times that a negative change in an ecosystem was first denoted by a prominent decline in amphibian numbers (Stebbins and Cohen, 1995).

However, not all amphibian declines indicate a forthcoming ecosystem change. Amphibian populations are prone to wide fluctuations with some population extinctions not being uncommon. In a case of a population extinction of an amphibian with a wide range, the locality that went extinct was repopulated within a few years (Collins and Storfer, 2003). Unfortunately amphibians have been experiencing a high amount of loss in a wide geographic region within the last few decades, and species have experienced vast range reductions and fragmentation and erosing of previous metapopulation systems (e.g., Beebee et al., 1990; Bradford, 1989, Bradford, 1991; Bradford, 2004; Blaustein and Wake, 1990; Hayes and Jennings, 1986; Phillips, 1990; Tayler and Davies, 1985; Vitt et al., 1990; Wake, 1991; Wake and Morowitz, 1991). Having a smaller range as well as severed ties to other populations limits the possibility of amphibians repopulating recently extinct populations.

Rana onca.–The historical range of *Rana onca*, the Relict Leopard Frog, once stretched from Hurricane, UT to Black Canyon, NV along the Virgin and Muddy Rivers and many of their tributaries (Bradford et al., 2004). In the early 1990s *R. onca* were known to exist in three general sites comprised of seven populations. Of these three sites, two of them had three populations relatively close to one another. These two sites are the Northshore complex (i.e., Corral, Rogers, and Blue Point Springs) and the springs within Black Canyon (i.e., Bighorn Sheep, Boy Scout, and Salt Cedar Springs). Corral Spring and the solitary Reber Springs have since gone extinct leaving five populations in two general sites (Fig. 1).

In 1931 workers began construction on the Hoover Dam, which dammed the waters flowing from the Colorado, Virgin, and Muddy Rivers forming Lake Mead. Damming of these rivers led to flooding of much of *Rana onca's* historic habitat. Primary links between *R. onca* populations were severed by the formation of Lake Mead leading to the creation of isolated island populations. These isolated populations no longer had a connection to other populations besides the ones in their own complex (which are separated by desert). If these populations were now to go extinct they would likely not be recolonized as easily as before the formation of the Hoover Dam.

If the flooding of prime habitat was not bad enough, these frogs would also fall prey to the non-native predators and competitors that were introduced to the rivers and lake, such as American bullfrogs (*Rana catesbeiana*), red swamp crayfish (*Procambarus clarkii*), and various fishes (e.g., striped bass, *Morone saxatilis*; largemouth bass, *Micropterus punctulatus*; and crappie, *Pomoxis nigromaculatus*).

In lieu of recent extinctions and population declines (Bradford et. al., 2004), conservation actions need to be taken in order to keep *Rana onca* from going extinct in its native habitat. Reintroductions of individuals within the frog's historic range have recently begun (Cristina Velez pers. comm.). These reintroductions function to increase the number of populations within the historic range. Much genetic diversity is lost through reintroductions because transported individuals are often siblings collected from the same egg mass and must interbreed to establish viable populations. Reintroductions do increase the number of individual frogs and populations establishing a "safety net" in case of a natural disaster such as a flood or disease epidemic.

Because the remaining natural populations are only in two general sites, something with the destructive magnitude of a flood or disease, such as Chytrid fungus (Berger et. al, 2000; Daszak et. al., 1999; Rollins-Smith et. al., 2002) could lead to relatively rapid population extinctions. Recommended measures to conserve the natural populations include keeping those populations established in order to maintain as much genetic diversity as possible. In order to maintain these natural populations, subsequently maintaining biodiversity, preferred habitat characteristics of the frogs within these sites must be known.

Radio telemetry is a widely used technique that allows researchers to relocate individuals to precise locations when visual encounter surveys are not optimal. Analysis of the habitat at each relocation point enables researchers to determine if there is a statistical difference in habitat characteristics between where organisms are observed and where they are not observed (as well as if there is a difference between where they frequent and where they are seldom seen). These characteristics can be used to actively manage habitat in order to assist in population survival. Vegetation structure is the prime focus of this study. Some evidence for implicating emergent vegetation in population declines comes from a comparison of the populations. The vegetation at the two recently extinct populations (i.e., Corral Spring and Reber Springs) is visually the densest of the sites followed by Roger's Spring, then Blue Point Spring and finally the Black Canyon Springs. Population size follows this same pattern. Dense emergent vegetation has been implicated as the major cause in the two recent *Rana onca* population extinctions but with only corollary evidence. The intention of this study is to determine if *Rana onca* at Blue Point Spring are found more prominently in open vegetation rather than the dense emergent vegetation, which has been thought to be the cause of the recent extinctions. These data will then be used to manage for the preferred habitat in the Overton Arm sites.

CHAPTER 2

INTRODUCTION

Habitat disturbance has often been a factor implicated in the decline of several anuran species (e.g., Welch and MacMahon, 2005; Munger et al., 1998). Many anurans have been shown to prefer dense emergent vegetation, probably as a protective measure from predation and solar radiation (Ildos and Ancona, 1994; Munger et al., 1998; Monello and Wright, 1999; Watson et al., 2000). Reduction of dense emergent vegetation would therefore be expected to have a negative impact on population numbers. Some degree of vegetation disturbance, however, has been shown to be beneficial to the survival of some anuran species (Watson et al., 2003). For example, the Oregon spotted frog (*Rana pretiosa*) has only two reproductively healthy populations in Washington, and both of these habitats have vegetation that is disturbed by cattle grazing throughout the year (McAllister and Leonard, 1997). Low to moderate vegetation disturbance, mostly through controlled ungulate grazing, has been used as a management strategy for the conservation of Natterjack toads (*Bufo calamita*) in Britain (Denton et al., 1997).

The relict leopard frog, *Rana onca* (Cope, 1875, in Tanner, 1929; Jaeger et al. 2001) currently occupies only a few sites in two general areas – along the Virgin River drainage near the Overton Arm of Lake Mead in a complex of springs known as the Northshore complex, and from a series of springs within Black Canyon along the Colorado River below Lake Mead (Jaeger et al., 2001; Bradford et al., 2004). In the early

1990s there were two other sites with extant populations, but these have since gone extinct. These sites were Corral Spring, part of the Northshore complex, and Reber Springs which borders the flood plain of the Virgin River near Littlefield, Arizona (Jaeger et al., 2001; Bradford et al., 2004). Both of these recent population extinctions were observed to have occurred concurrently with encroachment of emergent native vegetation into more open water pools in which *R. onca* was frequently observed, and this vegetation encroachment was thought to be the major cause for these local extinctions (Bradford et al., 2004).

Vegetation disturbance at the two sites where *R. onca* was recently extirpated has changed in recent years. Historically, both of these spring areas had high levels of disturbance. Reber Springs was apparently a homestead where livestock grazed within the marsh habitat that formed on the floodplain below the springs. Although no longer present, the historical grazing, trampling, and other activities presumably restricted emergent vegetation and maintained open pools (Bradford et al., 2004). In more recent times, *R. onca* was regularly seen at this site in the small remaining open water patches during visual encounter surveys. In a similar fashion, feral burros at Corral Spring also maintained open vegetation structure, although a flash flood was noted to have reduced vegetation substantially at this site in the early 1990s (Bradford et al., 2004).

Recent vegetation disturbance at both spring systems has drastically decreased. The homestead at Reber Springs no longer exists, and the open water areas where frogs were seen have become essentially covered by dense vegetative mats of *Scirpus americana* and *Eleocharis rostellata* (Bradford et al., 2004). At Corral Spring, the National Park Service (NPS) constructed a fence around the spring in order to exclude burros from damaging the riparian vegetation, which apparently accelerated the loss of open pool habitats (Bradford et al., 2004). The other springs within the Northshore complex also have disturbance histories. The two major springs, Blue Point and Rogers, are thermal springs with obvious histories of human-caused alterations to the stream courses. Blue Point Spring once supported a tropical fish farm and both springs are occupied by tropical exotic fishes (Deacon et al. 1964). Tourists commonly visit the springheads and burros have historically grazed along the stream banks. The more recent management activities by the NPS, however, have been to limit disturbance at these sites, and the NPS has an aggressive burro removal program for this region. Currently, dense stands of emergent vegetation dominated both stream systems, and anecdotal observations in recent years indicate that the decrease in burro disturbance has begun to reduce the open areas along these springs where frogs have been observed (J. Jaeger and D. Bradford, pers. obs.).

Adult relict leopard frogs have been suggested to prefer open shorelines where dense vegetation does not dominate (Bradford et al., 2005). This perspective developed from observations made during visual encounter surveys; however, these surveys have an observational bias resulting from the unequal probability of seeing frogs in open versus dense vegetation (Crump and Scott, 1994). Whether these frogs commonly inhabit denser vegetation was not considered likely but was not truly known. Without this information, resource managers were reluctant to accept recommendations that conservation actions for *R. onca* called for greater disturbance of emergent native vegetation.

Herein, we describe a study in which radio-telemetry was used to address questions of habitat selection by adult *R. onca* along the upper portion of Blue Point Spring within Lake Mead National Recreation Area (LMNRA). Bradford et al. (2004) estimated about 36 adult *R. onca* at upper Blue Point Spring, as recently as 2001. This small population size allowed us to apply radio telemetry units to most of the adult frogs within this system (by state and federal permit up to half of the population at one time). We applied binary logistic regression to model a dependent response (i.e., present vs. absent) on a set of independent variables, as well as applying a multiple analysis of variance (MANOVA) approach. These approaches compared areas (stream segments) in which animals were observed to a random selection of segments in which no animals were observed (i.e., available habitat) and were considered traditional approaches to habitat selection studies (Johnson 1980, Thomas and Taylor 1990). A necessary assumption for these traditional approaches required that we accept the perspective that the segments where no frogs were observed constituted useable habitat. This assumption seemed reasonable since the aquatic/riparian habitat was linear and frogs have been observed at the upper and lower portions of the study area and presumably at least traverse the intervening segments. We also took a perspective common to polytomous logistic regression (Cross and Petersen, 2001) and evaluated independent variables to model a dependent response (e.g., high- vs. low-use segments; Hosmer and Lemeshow, 1989). This approach produced models that predicted the probability that a segment with given habitat characteristics will belong to a certain use-intensity category (Anderson, 1984; North and Reynolds, 1996). Assumptions that unused areas comprised useable habitat are not made with this methodology. This approach represented a narrowing of the scale at which the assessment of the habitat variables was conducted, and focused the analysis on actual areas observed to be used by the study animals.

CHAPTER 3

MATERIALS AND METHODS

Study Site.–The study was located at Blue Point Spring within LMNRA, Nevada (N 26° 23.40′, W 114° 25.59′) near the eastern edge of the Mojave Desert (Fig. 1). Blue Point Spring is thermally influenced (30°C at source) with a discharge rate of 1,040 L/min (Pohlmann et al., 1998). The stream runs approximately 2.4 km below the spring head terminating before it reaches the shore of Lake Mead (formerly the Virgin River), but cuts though gypsum soils and disappears underground at some locations (Bradford et al., 2004). This spring, along with several others, including the larger Rogers Spring, emerge from the base of the Muddy Mountains and make up the Northshore springs complex along the Overton Arm of Lake Mead (Bradford et al., 2004).

The study was focused on the "upper" portion of the spring, which was a relatively linear stream stretch 575 m in length below the springhead. The stream disappeared underground at the end of the study area, but reemerged again approximately 370 m further downstream. The stream and riparian zone were narrow (average width = 1.38 m and 2.60 m, respectively) and surrounded by lowland Mojave Desert vegetation. Based on visual observations and backed up by vegetation analyses, the aquatic and riparian areas were dominated by emergent vegetation, particularly by dense mats of tightly intertwined sedges, *Scirpus americana* and *Eleocharis rostellata*. Other dominant emergent vegetation included a cattail, *Typha domingenis*, a rush, *Juncus* sp., and several perennial herbs, including *Anemopsis californica, Samolus parviflorus*, and *Solidago* *confinis*. Some areas of the riparian zone were dominated by more woody species including *Vitis arizonica* (canyon grape vine) and *Pluchea sericea* (arroweed). Two introduced palms, *Washingtonia filifera* and *Phoenix dactilyfera*, also occurred in the spring system. From hereon, we refer to these plant species by their genus to simplify the text.

Capture, Marking, and Radio telemetry. – Frogs were captured opportunistically by hand throughout the study area over the duration of the project. In addition, frogs were also captured during systematic surveys of the entire study site conducted at night by an unbiased observer (one not directly involved with the radio telemetry). All healthy frogs ≥15 g were fitted with a beaded micro-chain waistbelt on which was adhered a radio transmitter following the protocol of Rathbun and Murphey (1996). The radio transmitters (BD-2, Holohil Systems, Ltd. Ontario, Canada) weighed up to 1.6 g when fitted, including harness and epoxy. This weight represented 7.8 % and 8.9 % of the average weight of female (n = 17) and male (n = 17) frogs that were fitted with the units, respectively. This follows the general rule that transmitter units should not exceed 10% of the total body mass of the animal (Richards et al., 1994). Carrying this weight, frogs were able to maintain themselves at the water surface, readily swim, and climb out of the water onto vegetation and stream banks. Following the first initial captures, monitored frogs were also permanently marked with a passive integrative transponder (PIT) tag (12 mm, Destron Fearing Corp., South St. Paul, MN). The PIT tags were inserted under the skin anterior to the sacral hump and pushed up behind the hump following a technique successfully used previously on *R. onca* (Bradford et al., 2004) and on other ranid frogs (e.g., Watson et al., 2003).

Radio tracking was conducted approximately every other day from April 7 through December 2, 2004. During each of these visits, monitored frogs were located during the daylight (usually late afternoon) and again at night (starting at least one hour after sunset). To relocate monitored frogs along the narrow and densely vegetated stream, we relied on both directional 'H' and pigtail antennas and then manipulated the gain and power setting of the radio receiver (Model WTI-1000, Wildlife Track Inc., Livermore, CA) until a location was pinpointed.

Habitat Characterization.–Habitat variables were measured at two scales. The first was an assessment of the entire stream performed at the beginning of the study (these data are referred to hereafter as 'macrohabitat' data). A second set of habitat measurements were taken within days of each observation and measured conditions within 25 cm of each frog location (hereafter referred to as 'microhabitat' data). The microhabitat data were a more precise measurement of habitat conditions selected by the frog, and thus represented a subset of the macrohabitat data.

Macrohabitat Data.–Data for macrohabitat assessments were collected by line transects run across the riparian width (from edge to edge, perpendicular to the stream), and collected every meter down the length of the stream channel where possible (hereafter, we refer to these generally meter long stretches of stream and riparian vegetation as 'segments'). Stream width was measured directly. Riparian width was measured as the broadest distance between emergent vegetation on each bank (see list of plant species above), with the exclusion of *Anemopsis* and *Juncus*, and woody species, which often occurred many meters from the water's edge in areas not readily used by *R. onca*. A total of 17 habitat variables were measured (Table 1). Vegetation cover ≤25 cm and > 25 cm tall by dominant species, substrate type (i.e., stream, wetted substrate, dry ground), and stream depth (when in stream) was determined at 30 points evenly distributed along the transect line. A percentage of dominant vegetation type (i.e., percentage of the segment that is *Eleocharis, Scirpus, Typha*, and other vegetation) across each segment, both above and below 25 cm, was calculated by dividing the number of hits of that vegetation type by the 30 points. Observations indicated that taller vegetation (> 25 cm) impeded frog movements, whereas shorter vegetation (≤25 cm) was easily traversed. Thus, in this study, vegetation > 25 cm was viewed as vegetation that impeded frog movement.

Because the total riparian width varied (range 0.19 – 8.75 m), the distance between points along transects varied among segments. Similarly, the percentages of points representing each substrate type varied among segments in response to variation in stream width (including wetted substrate with standing water on surface) and riparian (moist and dry ground) width. In certain types of dense vegetation, collecting data associated with the physical aspects of the stream channel was practically impossible or substantially time consuming. In these vegetatively homogeneous areas, transects were run at least at the beginning and ending meter of the dense vegetation. These segments were then given the habitat characteristics of the nearest measured segment. Within the study area, the stream forked and then remerged in two locations. In these cases, both stream stretches were measured. Thus, a total of 591 segments were measured along the 575 m length of the study area.

Microhabitat Data.–A 0.25 m^2 grid (plot) was centered on each frog location site in a method similar to that described by Watson et al. (2003). The square grid was intersected by 25 evenly distributed points. At each point on the grid, substrate type (i.e., aquatic = main channel or wetted substrate; terrestrial = riparian or upland) and dominant vegetation ≤and > 25 cm high (using the same vegetation categories describe above; Table 1) were recorded. If over water, depth was measured at each point. Thus, a total of 15 habitat variables were measured for microhabitat assessment. Distance to nearest water (when on bank) and distance to nearest bank (when in water) were also measured from a center point. To avoid eliciting a flight response in frogs at night when frogs were often active, locations were marked at a distance (usually 1 m from the frog location) and a compass angle to the observation was recorded from this reference mark. This allowed habitat measurements to be taken during the next daytime tracking event. The daytime locations were measured at the time of observation.

Day and Night Observations.–The radio tracking data consisted of observations on many individuals, not just a single animal, thus autocorrelation was not considered a problem (Otis and White, 1999). Furthermore, because our analytical approach compared stream segments (i.e., high- versus low-use, use versus non-use), consecutive observations within a stream segment were generally not considered to greatly bias these analyses (see below). General observations indicated that at the macrohabitat scale, differences between day and night locations were not substantial; thus, in macrohabitat analyses daytime and nighttime observations were combined instead of treated as separate data sets. This procedure was statistically assessed by including time (day and night) as an independent variable in a preliminary logistic regression analysis, which was not found to be significant for model determination (data not shown). For microhabitat assessments, observations between day and night were considered biologically and statistically relevant and data were grouped accordingly.

High- vs. Low-use Categories.–At the macrohabitat scale, telemetry observations of frogs were assigned to the stream segment in which the frog was observed. To compare habitat variables among segments in which frogs were actually observed, the use intensity of each segment was determined by pooling telemetry observations among all frogs and counting the number of observations within a segment. Classification of use-intensity levels was based on patterns observed in histograms of the number (or frequency) of observations among segments (Cross and Petersen, 2001). Examination of the histograms (Fig. 2) implied a natural break between ≤3 (low-use) and ≥4 (high-use) telemetry observations per segment. Assessments of the sensitivity of results to modification of these categories were also conducted. Assessments of three categories (i.e., polytomous logistic regression of low-, medium-, and high-use) were first performed, but these preliminary analyses (not shown) indicated that these data were naturally dichotomous. Further sensitivity analyses were conducted in which the dichotomous categories were varied (breaks as low as between 2–3 and as high as between 7–8).

At the microhabitat scale, data were grouped according to time (day or night). The 0.25 m^2 plots were then grouped by the segment in which they were recorded and then these segments were categorized for use-intensity based on visual interpretation of histograms (Fig. 2). As might be expected from the grouping methodology, these histograms showed similar dichotomous breaks between 3 and 4 plots (or 'observations') per segment noted in the macrohabitat data. Sensitivity analyses were also performed on the microhabitat data by adjusting the breakage point between categories in order to determine if the outcome changed based on possible misinterpretations of what is considered to be a 'natural break' in the histogram.

Use- vs. Non-Use Categories.–At the macrohabitat scale, stream segments with frog telemetry observations ('use') were compared against an equal number of randomly selected segments in which no telemetry observations were documented ('non-use'). This approach allowed for an assessment of the entire study area as opposed to only those segments in which frogs were observed during radio telemetry. For these comparisons, a random selection of the non-use segments comparable in number to the use segments were selected in SPSS (SPSS Inc., Chicago, Illinois); since there were 173 use segments (see below), an equal number of the 418 non-use segments were randomly selected in order to optimize the statistical power of this analysis.

Binary Logistic Regression Procedure.–Binary logistic regression (LR) was used as the major approach to assess habitat selection at both macrohabitat and microhabitat scales. The nature of habitat variables like those used in this study, however, often leads to highly skewed distributions (Cross and Peterson, 2001). Most of the variables measured were a percentage of an area covered by vegetation. These variables tended to have either very high (near 100%) or very low (near 0%) coverage with few intermediate values. Raw data were used in preliminary logistic regression analyses, which indicated that too much intra-variable variation (resulting from using percentages) limited the ability to accurately compare segments based on variable similarities. In order to decrease the effects of bimodality, percentage values were assigned to categories based on 10% increments prior to final analyses. This process groups the intermittent data together thus decreasing the drastic changes between them and the segments with 0% and 100 % coverage.

To determine the required independence of variables prior to use in LR, correlation analyses were administered in a pairwise fashion to all variables using SPSS software. Under the high- versus low-use categories, the macrohabitat data were grouped in three ways, by both use-categories (low and high-use) and over all 591 stream segments combined. Variables that had strong Spearman rank correlations (> 0.70 correlations with significance ≤0.05; Hosmer and Lemeshow, 2000) across all three assessments were considered significantly correlated. Only one of the correlated variables was then used in LR under the assumption that the two variables reflected the same habitat characteristic. Of the 17 habitat variables in the macrohabitat data set, only two pairs of vegetation cover variables were determined to be significantly correlated (Table 1). In a comparable analysis of the macrohabitat data included in the use versus non-use categories, these same variables were determined to be correlated.

To determine independence of variables among the microhabitat data, day and night data sets were grouped by use-intensity (i.e., high- and low-use). Variables that were significantly correlated for both high- and low-use data sets (under the same requirements stipulated above) were then assessed for appropriateness to the model. Of the 15 microhabitat variables, 2 pairs were correlated across the night data set and 1 of these same variable pairs was correlated across the day data (Table 1). Thus, 13 variables were included in the nighttime microhabitat LR while 14 variables were included in the daytime microhabitat LR.

SAS software (SAS Institute Inc., North Carolina) was use to perform all LR analyses. A stepwise procedure with an entry criterion of 0.3 and an exit criterion of 0.15 (Hosmer and Lemeshow, 1989; and Mickey and Greenland, 1989) was used to select the variables that best differentiated use-intensity categories. Model variables were considered significant at a $P < 0.1$ level of significance (Hosmer and Lemeshow, 1989). Tukey post-hoc tests (Neter et al., 1990) were used in two-way comparisons to assess trends. As implemented in SAS, quasi-jackknife validation/reclassification percentages were generated to determine the robustness of the models.

Nonparametric MANOVA Procedure.–At the macrohabitat scale, the assessment of habitat variables among use and non-use segments was conducted using a Multiple Analysis of Variance (MANOVA) approach, as a more traditional habitat assessment methodology. Standard MANOVA requires normality of data, but vegetation data rarely meet this assumption (Reinert, 1984; Noon, 1986). Komolgorov-Smirnov tests performed on the major vegetation variables rejected normality (all P values < 0.05), and the distributions of these data generally appeared to be bimodal which could not be normalized. The data were analyzed as a combined data set disregarding the time of the observation and with all 17 habitat variables included in the analysis. Data were ranked prior to analysis and a nonparametric MANOVA procedure (Mann-Whitney test) was performed with a Bonferoni correction (Holm, 1979) in order to determine differences between use and non-use segments. As in the LR analyses, Tukey post hoc tests were used to assess trends. A Kruskal-Wallis analysis was also performed followed by Nemenyi post hoc analyses, but this approach provided similar results to the Mann-Whitney approach and is not presented herein.

CHAPTER 4

RESULTS

Radio Tracking.–A total of 34 frogs (17 males and 17 females) were tracked during the study for a total of 809 observations (413 day and 396 night observations). The number of observations per frog varied from 1-134 (Table 2). This variability was a result of several factors but mostly because of radio losses. Frogs that lost their radios were generally captured the following night and refitted with a radio. The lowest numbers of individual observations resulted from the earliest radio telemetry efforts when frogs had not yet been PIT tagged and we could not be certain of individual identity upon all recaptures. Frogs were generally relocated around the same area with some movement at times. The average total frog movement for the duration of the monitoring is approximately 33 meters (Table 2). Only one frog died while carrying a radio and another was suspected of being eaten by a coyote. Because of the number of radio losses, this number of detected deaths cannot be used to provide a meaningful estimate of mortality.

Although systematic surveys of the study site were often conducted to capture frogs, most captures and locations tended to occur within certain segments. Monitored frogs were relocated in only 173 of the 591 segments measured along the stream length, and the used segments were generally clustered in certain areas throughout the length of the stream (Fig. 3).

High-use vs. Low-use Categories.–In the macrohabitat assessments comparing segments in which frogs were actually observed (i.e., high- versus low-use segments), the LR used to evaluate categories considered to represent a natural break in the data (i.e., low-use $\leq$ 3, high-use $\geq$4 observations) resulted in 110 low-use and 63 high-use segments (Fig. 2). In this LR, 5 of the 15 independent habitat variables included in the analysis were determined to be significant (Table 2). From the trend analyses of model results, high-use segments had broader riparian widths and lower amounts of stream cover regardless of plant height (i.e., both $\leq$and > 25 cm). Other vegetation (i.e., a category that defined plants other than the dominant *Eleocharis*, *Scirpus*, and *Typha*) regardless of plant height (both < and > 25 cm) were also identified in the model with high-use segments having more of this vegetation type than low-use segments. The model validation/reclassification percentage was 69 % (Table 3), which indicates a fairly good model. The differences in mean cover and standard deviation of the macrohabitat variables are shown in Table 4.

In the sensitivity analysis of categories used to define high- versus low-use, raising the break point by single increments up to between 6 and 7 observations resulted in a series of models (data not shown) in which riparian width and stream cover > 25cm were the common significant variables. The reclassification rate of these models, however, increased dramatically as the break-point rose, which was an artifact of having fewer and fewer segments within the high-use category which limited chances for misclassification. Lowering the break to between 2 and 3 observations per segment resulted in 3 significant variables (stream cover > 25 cm, riparian cover $\leq$25 cm, and other vegetation ≤25 cm) although the model was less robust than that for the natural break (i.e., quasi-jackknife validation/reclassification rate = 61%).

In the LR for the daytime microhabitat data, only two of the habitat variables were determined to be significant (Table 5A). Although the validation/reclassification rate of this model was 66%, it was not considered robust because this rate was highly skewed with the low-use segments correctly reclassified only 17% of the time. In the sensitivity analyses (data not shown), raising and lowering use-categories by a single step from the natural break point did not affect the model outcome, nor did it substantially improve reclassification rate of the low-use category. The LR model for the nighttime microhabitat data was considered robust with 70% validation/reclassification. In this model three variables were determined to be highly significant (Table 5B). From the trend analyses, microhabitats within high-use segments contained lower amounts of stream cover > 25 cm (which was correlated to total cover > 25 cm), and lower amounts of total cover ≤25 cm (in this analysis, this variable was not correlated with stream cover ≤25 cm). Of the vegetation within 0.25 m^2 patches of frog locations *Eleocharis* ≤25 cm was identified in the model and the trend assessment indicated that high-use segments contained more of this vegetation type than low-use segments. In the sensitivity analyses (data not shown), raising the use-categories by a single step from the natural break did not affect the model outcome, but did lower the validation/reclassification rate to 64%. Lowering the use-category by one-step from the break point (i.e., to between 2 and 3 observations per segment) caused the loss of significance for short *Eleocharis* and the gain of significance for a higher percent of shallow water, but also resulted in highly skewed validation/reclassification in which few (11%) of the low-use segments were correctly classified.

Use vs. Non-use Categories.–When evaluating the segments where frogs were observed against those segments without observations (i.e., use versus non-use categories), five variables were determined to be significant (Table 6). From the trend assessment, adult frogs were found in segments in which the stream had a higher percentage of shallow water, and where there was less stream cover > 25 cm (correlated with total cover > 25 cm) and less riparian cover > 25 cm; although the latter variable was only weakly supported in the model ($P = 0.083$). Of the vegetation species occurring within the use- and non-use segments, use-segments had more *Eleocharis* > 25 cm, and more *Typha* ≤25 cm; although this latter result was only weekly supported ($P = 0.088$). The validation/reclassification for the model was 66%.

When these data were evaluated using a non-parametric MANOVA approach, 9 of the 17 variables included in the analysis were determined to be significant in distinguishing between the use and non-use segments (Table 7). Based on the Tukey post-hoc trend assessments, adult frogs were found in segments with broader stream widths and in segments in which the stream had a higher percentage of shallow water. Frogs were more often in segments that had less stream cover and less total cover regardless of plant height, and of the vegetation species occurring within the use- and non-use segments, frogs were positively associated with other vegetation ≤25 cm and with *Eleocharis* regardless of plant height; although the association with *Eleocharis* > 25 cm was not as strongly supported as the other significant variables ($P = 0.038$).

CHAPTER 5

DISCUSSION

Low- vs. High-use Categories.– Segments commonly used by adult frogs (i.e., high-use) had lower levels of vegetation cover over stream regardless of vegetation height than occasionally used segments (i.e., low-use). The significance of one of the stream cover variables (stream cover ≤ 25 cm) identified in the primary LR model (that was based on the natural break in data), however, was only weakly supported ($P = 0.057$), and this variable tended to drop out of models in the sensitivity analyses when use-categories were modified. Average values of both stream cover and total cover > 25 cm showed substantial differences (up to 20%) between high-and low-use segments (Table 4) suggesting a biologically relevant difference in habitat quality. In these analyses, stream cover was correlated with total cover for both height classes. Total cover, however, comprised both riparian cover and stream cover, and since riparian cover was not identified in most models as being significant, the important variable appeared to be the amount of tall vegetation (> 25 cm high) over the stream. In general, the average percentages of riparian cover > 25 cm were much higher than that for both stream and total cover (Table 7).

The microhabitat data provided a similar but more refined perspective on the selection of vegetation cover by adult frogs. These analyses focused on determining the possible selection of microhabitat patches (within 25 cm of actual frog observations) within segments that might not have been captured at the macrohabitat scale. The daytime microhabitat model, however, was not robust, indicating no strong selection for microhabitat variables within segments during daytime hours. Microhabitat patterns were stronger at night and model results were consistent with the perspective that adult frogs tended to select more vegetatively open habitat, particularly that with less tall vegetation cover over the stream. At night frogs used microhabitat patches within high-use segments that contained less tall stream cover (> 25 cm high; correlated in these analyses with total cover > 25 cm high) and less total cover $\leq$25 cm high (for this height class total cover and stream cover were not correlated in the analyses).

Of the vegetation species occurring within the high- and low-use segments, frogs were positively associated with other vegetation that was not one of the three main plants (i.e., *Eleocharis*, *Scirpus*, and *Typha*) regardless of plant height. The significance of other vegetation > 25 cm, however, was not strongly supported in the primary model and was lost from models in the sensitivity analyses when use-intensity categories were modified. In the nighttime microhabitat model, adult frogs were positively associated with short ($\leq$25 cm high) *Eleocharis*. These results were consistent with the perspective that adult frogs avoided the dense, tall mats of *Scirpus* and *Eleocharis* that covered large sections of the stream and riparian (Bradford et al. 2004), and that these frogs do not commonly use stream segments where *Typha* grows in dense stands. Other vegetation types occur more often in areas not dominated by these three main plants. The association of frogs with shorter *Eleocharis* at the microhabitat scale may be explained by the fact that burros graze and trample on the edges of stream banks often forming narrow bands of vegetatively open patches in which shorter *Eleocharis* dominates. This vegetation was easily traversed by adult frogs and appears to be a favored habitat.

Use vs. Non-use Categories.– At the scale of the entire study area, adult frogs also showed a preference for segments with lesser amounts of tall (> 25 cm high) vegetation cover over the stream. In the LR analyses of these data (Table 5), stream cover was correlated with total cover, but in this analysis riparian cover > 25 cm was also identified, although weakly. These results indicate an overall selection for segments with lesser amounts of tall vegetation cover. Comparisons of the average values for these variables also suggest a biologically meaningful difference in habitat quality between use- and non-use segments, particularly for stream and total cover > 25 cm high (average difference in cover of about 13-15%; Table 7). The significance of *Eleocharis* > 25 cm tall in this model is a bit of an enigma, but frogs appeared to prefer taller vegetation to be *Eleocharis* as opposed to other vegetation types. This may result from the selection of recently disturbed areas where *Eleocharis* often grows in quickly reaching levels above 25 cm, or this could result from a general lack of areas within the study site without high amounts of tall vegetation cover (see Table 7) and frogs selecting segments with higher amounts of *Eleocharis* rather than plants like *Typha* and *Scirpus* which grow more densely and may be harder to traverse. There was also weak support for the selection of *Typha* ≤25 cm, which in some areas was also eaten by burros.

The primary LR model based on use-categories also indicated a significant selection for segments with greater percentages of shallow water habitat and in general there was about 10% more shallow water in stream segments used by frogs than those segments where frogs were not observed. Several stretches of the stream within the study areas were cut deep into the porous soils with undercut banks resulting in only narrow areas of surface water and riparian vegetation. Frogs have rarely been seen in most of these areas.

The results from the non-parametric MANOVA of these data supported the general results found in the LR analyses. Additional variables identified as significant included stream width, *Eleocharis* ≤25 cm, and other vegetation ≤25 cm, but the latter two habitat variables had previously been identified as significant in other analyses. The inclusion of these additional variables in this analysis was not surprising given that nonparametric procedures in comparison to parametric procedures lack discriminatory power and the inclusion of variables that are close to the probability criterion is more likely (Sokal and Rohlf, 1995).

Selection for Vegetatively Open Habitats.– The important conclusion from this research is that at both spatial scales evaluated adult *R. onca* tended to select more vegetatively open areas where tall vegetation cover was substantially less, and particularly where there was less cover over the stream. Within the study area, this tall vegetation was predominately formed by mats of *Eleocharis* and *Scirpus*, or consisted of very dense stands of *Typha*. This does not mean that all amounts of vegetation cover are thought to be detrimental to these animals. These frogs obviously use vegetation for cover and camouflage to protect themselves from desiccation and predation, especially during daylight hours which may be the reason that the LR analysis of the daytime microhabitat variables lacked power. The vegetation types that appeared to be favored consisted of the more varied species, as well as *Eleocharis*, that tend to be structurally shorter and not common within the dense stands of *Eleocharis*, *Scirpus*, and *Typha*. The nighttime selection for more vegetatively open habitats provides the perspective that selection for optimal foraging habitats may be the major factor. Indeed, our sampling avoided most of the winter months when frogs may have sheltered in dense vegetation, although the thermal waters of this spring system may have made such winter behavior unnecessary.

CHAPTER 6

RECOMMENDATIONS

Presumably, *Rana onca* once occupied major habitats along the Virgin, Muddy and Colorado Rivers. These habitats have been anthropogenically altered by water developments and agriculture conversion, as well as occupied by exotic predators and competitors such as the American bullfrog and red swamp crayfish (Bradford et al. 2004). What remains appears to represent fringe habitats. The remaining habitats within the Northshore springs complex are far from pristine and have been greatly altered by human actions. Although these are some of the last sites occupied by *R. onca* (Bradford et al. 2004), these springs and streams may be far from ideal because of the prevalence of dense vegetation, and there may be very little optimal habitat for adult frogs along these streams. The major outcomes from this research may represent selection for the best foraging conditions within a system of generally poor habitat conditions. Indeed, when all segments of the stream within the study areas were classified or reclassified into high-use and low-use categories based on the equation from our primary LR analysis comparing macrohabitat data among segments in which frogs were actually observed (Fig. 4), few of the segments in which frogs were not seen were classified as high-use, indicating that most of the habitat at this site was not optimal for the significant variables.

The attributed loss of the Corral and Reber Springs populations of *R. onca* to vegetation encroachment implied that emergent native vegetation was a threat to local populations of this frog (Bradford et al. 2004). The results of this study confirm that *R. onca* avoid dense tall cover of native emergent vegetation and that these frogs do prefer more vegetatively open habitats. These results are consistent with the perspective that the recent extinction of *R. onca* at some sites was caused by encroachment of native emergent vegetation into the more open habitats. The springs within the Northshore complex are densely covered in emergent plants, and the small areas along the banks of these streams where vegetation was reduced by burros grazing and trampling appear to be in decline as the NPS reduces the burro population within the region. This change in the disturbance of emergent vegetation should be viewed as a real and present threat to the populations of *R. onca* within these spring systems.

For these reasons, it is recommended that the NPS begin active management, within a research context, to reduce vegetation cover at Northshore spring sites. In general, we recommend that in these areas, tall (> 25 cm), dense mats of vegetation that impedes frog movements be removed or reduced. The effect should be to setback succession of emergent vegetation and to encourage shorter, more open vegetation structure. At upper Blue Point Spring these actions should encourage 'other' native vegetation (e.g., *Solidago*, *Samolus*) and short *Eleocharis*. Although not a direct result of our analyses, other plants that form tall dense stands, such as grapevine, arrowweed, and sawgrass should be discouraged.

Broader riparian widths and wider shallow water habitats (< 13 cm) were identified as significant habitat variables within the study areas. In areas of upper Blue Point Spring the stream channel has narrowed and undercut banks, and in some areas the stream is practically underground with little surface water or riparian area. As the stream continues to cut deeper, there is a potential that the water table could drop in areas enough to dry up substantial stretches of riparian vegetation (exactly the current situation at the lower end of the study area where the stream goes underground). The porous soils and potential for impacting large segments of downstream habitat suggests a cautious approach to recommendations to mitigate this process. We suggest a hydrological study to determine the speed and potential impact of this process and to suggest potential mitigation actions that could protect and improve *R. onca* habitat.

Figure 1. Map of the known extant *Rana onca* populations in the 1990s. The black circle indicates Blue Point Spring, while the yellow (light) circles are the recently extinct populations, Reber Springs near Littlefield, AZ, and Corral Spring within the Overton Arm of Lake Mead National Recreation Area. This map is modified from Bradford et. al., 2004.

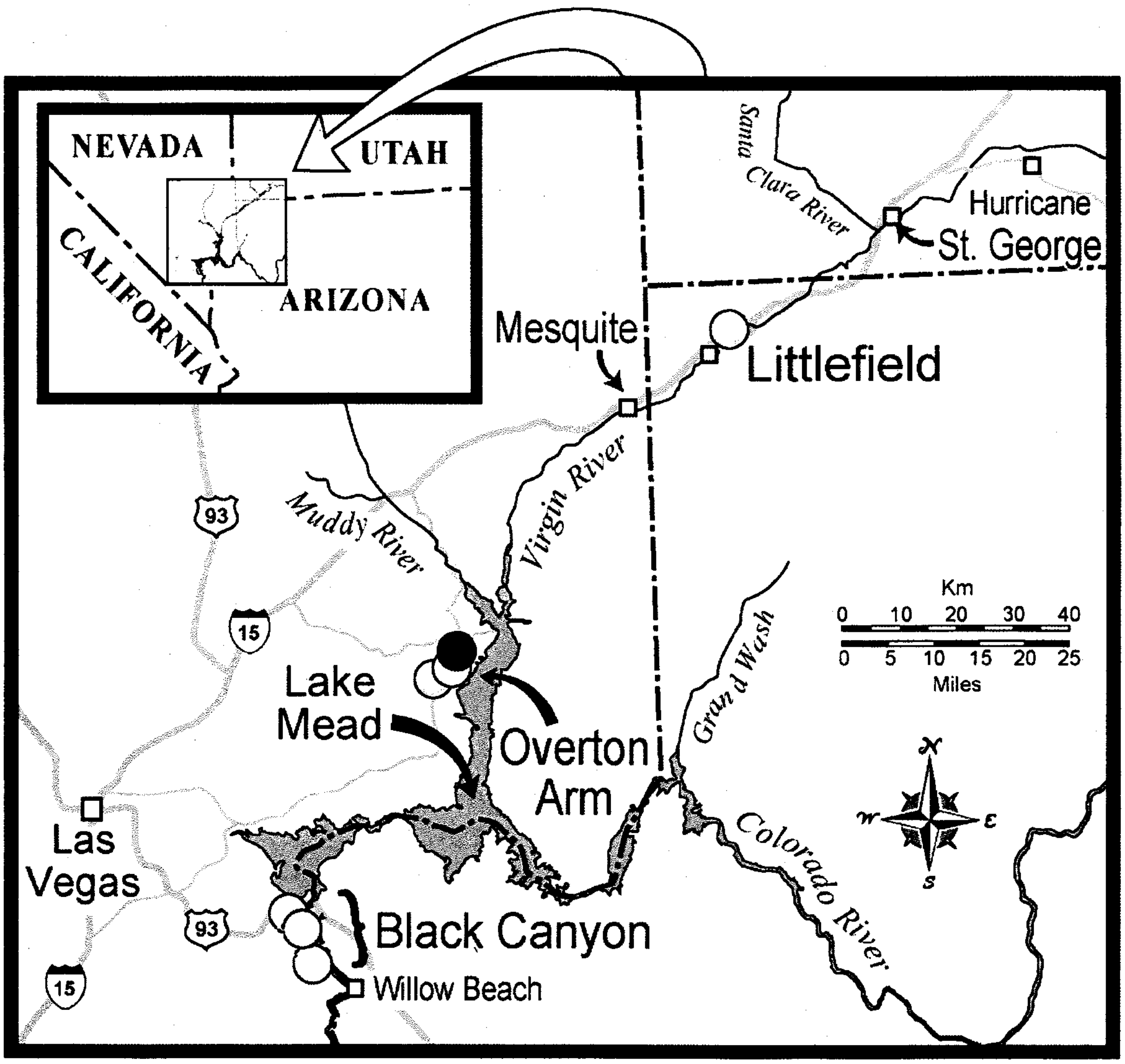


Figure 2. Number of radio-telemetry observations of *Rana onca* per stream segment based on (A) macrohabitat data (daytime and nighttime combined; n=173 segments), (B) daytime microhabitat data, and (C) nighttime microhabitat data. The natural break in the data used to identify use-intensity categories for binary logistic analyses were determined visually by dramatic changes in the amount of segments with a specific number of observations, and are indicated by arrows (low-use = 1-3 observations, high-use ≥4 observations).

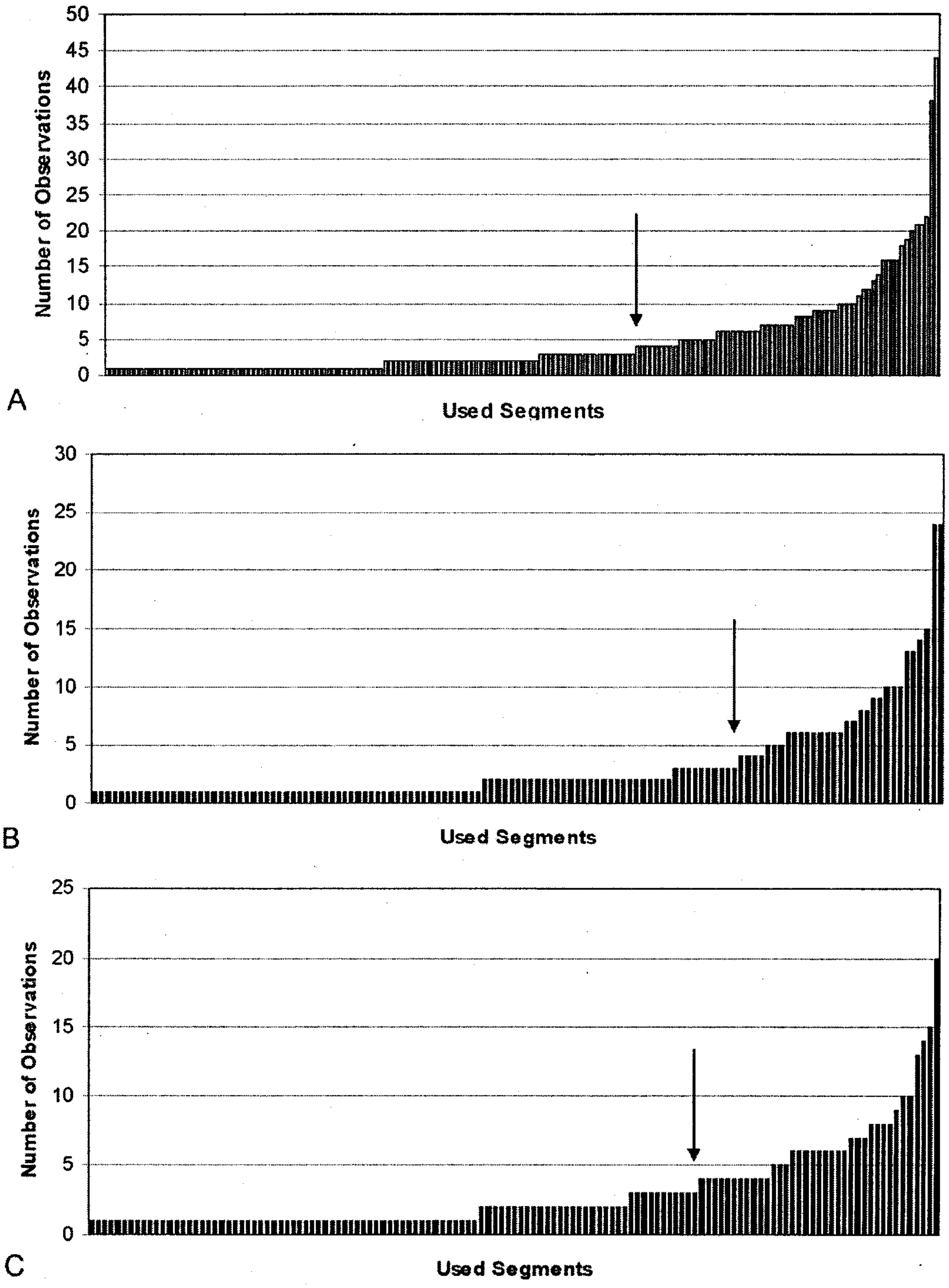


Figure 3. Arial photo of upper Blue Point Spring, Lake Mead National Recreation Area, showing frog observations based on radio telemetry. Monitored frogs were relocated in only 173 of the 591 segments (approximately 1 meter long) measured along the stream length. Low-use segments (≤3 observations per segment) are indicated in yellow (light), and high-use segments (≥4 observations per segment) are in red (dark). The spring source is located 10 m upstream (left) from the gauging station.

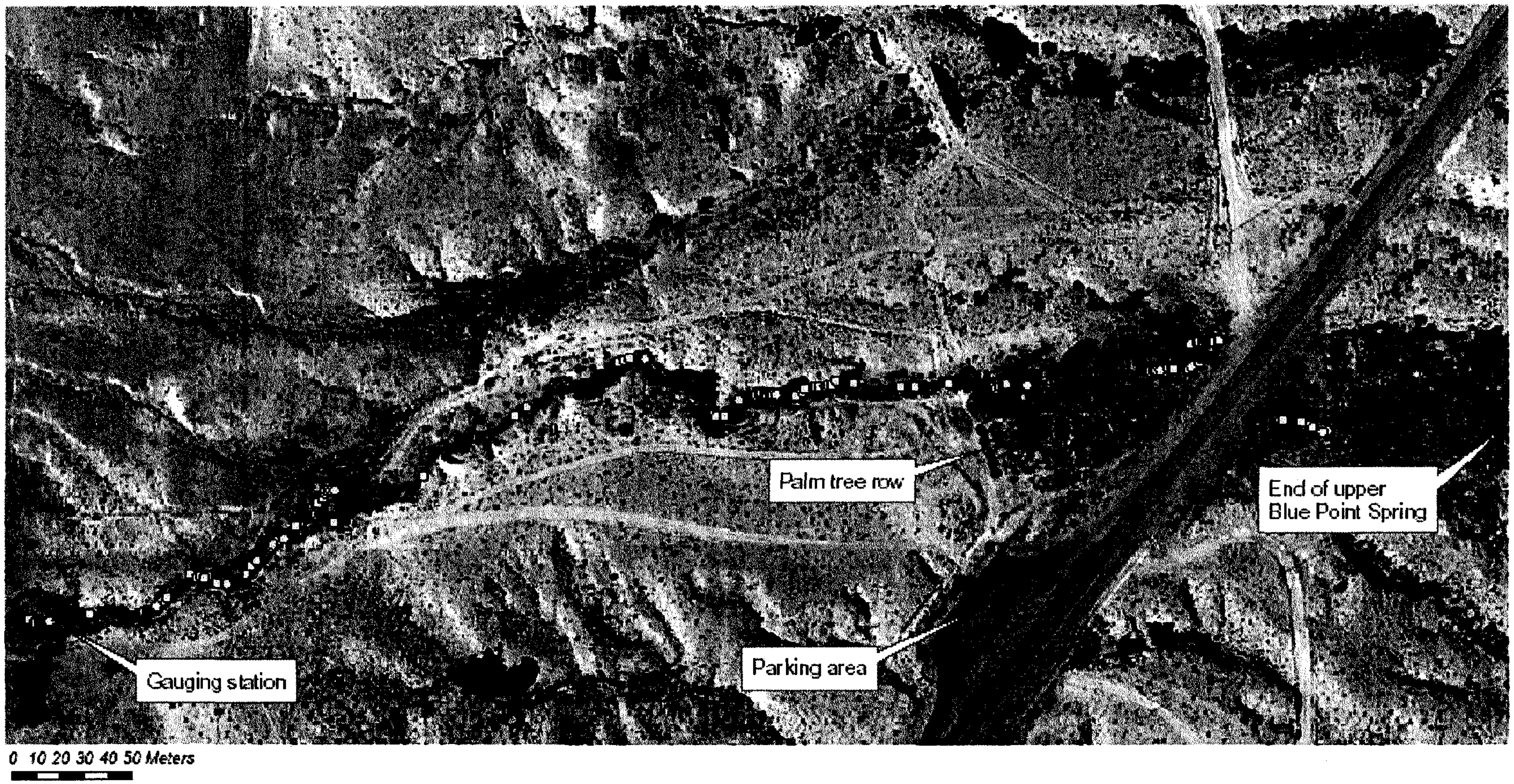


Table 1. Macrohabitat and microhabitat variables measured for relict leopard frogs (*Rana onca*) at Upper Blue Point Spring, Lake Mead National Recreation Area, Nevada. For macrohabitat variables, measurements were taken along transect lines run perpendicular to the stream at approximately one-meter intervals. For microhabitat variables, measurements were taken within 0.25 m^2 plots at exact frog locations. Stream Width and Riparian Width were not measured at the microhabitat scale.

Habitat Variable	Variable Description
Stream Width	Width (cm) of the wetted perimeter of the stream
Riparian Width	Distance (cm) between furthest riparian species (see text) on either side of the stream (excluding *Anemopsis* and *Juncus*)
Percent Shallow	Percent of water that is ≤12 cm deep (about the depth where an adult frog could keep its head above water while touching bottom)
Total Cover ≤25 cm*	Percent vegetation cover ≤ 25 cm high from one edge of riparian zone (see Riparian Width) to the other (including stream)
Total Cover > 25 cm**	Percent vegetation cover > 25 cm high from one edge of riparian zone (see Riparian Width) to the other (including stream)
Stream Cover ≤ 25 cm*	Percent vegetation cover ≤ 25 cm high over the stream
Stream Cover > 25 cm*	Percent vegetation cover > 25 cm high over the stream
Riparian Cover ≤25 cm	Percent vegetation cover ≤25 cm high over the riparian banks (i.e., riparian zone exclusive of stream)
Riparian Cover > 25 cm	Percent vegetation cover > 25 cm high over the riparian banks (i.e., riparian zone exclusive of stream)
Eleocharis ≤25 cm	Percent of total vegetation cover ≤25 cm high that is *Eleocharis*
Eleocharis > 25 cm	Percent of total vegetation cover > 25 cm high that is *Eleocharis*
Scirpus ≤25 cm	Percent of total vegetation cover ≤25 cm high that is *Scirpus*
Scirpus > 25 cm	Percent of total vegetation cover > 25 cm high that is *Scirpus*
Typha ≤25 cm**	Percent of total vegetation cover ≤25 cm high that is *Typha*
Typha > 25 cm**	Percent of total vegetation cover > 25 cm high that is *Typha*
Other Vegetation ≤ 25 cm	Percent of total vegetation cover ≤25 cm high that is not one of the three major plants (i.e., *Eleocharis*, *Scirpus,* or *Typha*)
Other Vegetation > 25 cm	Percent of total vegetation cover > 25 cm high that is not one of the three major plants (i.e., *Eleocharis*, *Scirpus,* or *Typha*)

* Total Cover regardless of height was correlated (P > 0.7) with Stream Cover in the macrohabitat data, and Total Cover > 25 cm was correlated (P > 0.7) with Stream Cover > 25 cm for the microhabitat data regardless of time of observation (i.e., day or night data).
** Typha ≤25 cm was correlated (P > 0.7) with Typha > 25 cm for the nighttime macrohabitat data only.

Table 2. Relocation and movement data from frogs radio-tracked at Upper Blue Point Spring from 07 April 2004 – 02 December 2004. The first five frogs were generally unable to be relocated because of early methodological problems involving radio loss. Individuals with no observations were unable to be relocated, and in most cases, the radio units were found not attached to the frog.

Frog Number	Number Of Observations	Number Of Segments Frogs Were Observed	Most Upstream Segment Observed	Most Downstream Segment Observed	Maximum Distance Traveled
1	0	0			0
2	0	0			0
3	1	1	120	120	0
4	0	0			0
5	0	0			0
6	134	21	441W	464E	23
7	28	10	040	065	25
8	5	3	510	512	2
9	4	3	374	376	2
10	2	2	302	304	2
11	2	2	115	120	5
12	69	18	371	397	26
13	87	19	364	386	22
14	77	23	001	122W	121
15	10	6	243	263	20
16	10	7	032	070	38
17	16	10	458W	507	49
18	86	20	110	161	51
19	33	9	030	043	13
20	19	9	290	312	22
21	0	0			0
22	31	13	320	375	55
23	27	9	442W	462E	20
24	12	8	038	070	32
25	19	10	036	105	69
26	14	11	101	134E	33
27	0	0			0
28	40	8	112	125W	13
29	0	0			0
30	34	13	297	324	27
31	7	5	099	114	15
32	11	6	118	205	87
33	14	9	068	136E	68
34	17	11	287	337	50

Table 3. Binary Logistic Regression analysis of macrohabitat variables associated with frog locations based on segment use-intensity classification. The low-use category is 1-3 observations per segment and the high-use category is ≥4 observations per segment. The quasi-jackknife validation/reclassification correctly classified 69% of the data. For the trend assessment, downward arrows indicate that use-intensity had a lower amount of the given habitat variable while an upward arrow indicates that use-intensity had a higher amount of the given habitat variable.

Variable	Wald Chi-Square	*P*-value	Trend
Riparian Width	8.2796	0.004	Low (↓) : High (↑)
Stream Cover ≤25 cm*	3.6235	0.057	Low (↑) : High (↓)
Stream Cover > 25cm*	6.4059	0.011	Low (↑) : High (↓)
Other Vegetation ≤25 cm	5.9710	0.015	Low (↓) : High (↑)
Other Vegetation > 25 cm	3.3454	0.067	Low (↓) : High (↑)

* Total Cover was correlated (P > 0.7) with Stream Cover regardless of height; thus, Total Cover variables were excluded from the analysis because they essentially measured the same characteristic.

Table 4. Mean and standard deviation (within parentheses) of the macrohabitat data, for major habitat variables based on segment classifications at upper Blue Point Spring. Riparian width is presented in cm, all other values are percentages based on the number of hits per 30 points along linear transects across the stream segments.

Variable	Use (SD)	Non-use (SD)	High-use (SD)	Low-use (SD)
Stream Width	159.4 (119.2)	137.9 (125.0)	180.2 (143.0)	147.6 (120.6)
Riparian Width	287.1 (141.5)	270.1 (171.1)	322.8 (167.2)	266.6 (120.6)
Percent Shallow	0.617 (0.241)	0.513 (0.288)	0.657 (0.214)	0.594 (0.253)
Total Cover ≤25 cm	0.876 (0.138)	0.914 (0.146)	0.828 (0.157)	0.904 (0.118)
Total Cover > 25 cm	0.614 (0.323)	0.744 (0.272)	0.489 (0.306)	0.685 (0.311)
Stream Cover ≤ 25 cm	0.856 (0.160)	0.898 (0.187)	0.806 (0.168)	0.885 (0.148)
Stream Cover > 25 cm	0.605 (0.331)	0.759 (0.283)	0.492 (0.319)	0.669 (0.321)
Riparian Cover ≤25 cm	0.961 (0.159)	0.976 (0.098)	0.940 (0.194)	0.973 (0.134)
Riparian Cover > 25 cm	0.727 (0.378)	0.772 (0.339)	0.630 (0.411)	0.782 (0.348)
Eleocharis ≤25 cm	0.408 (0.360)	0.247 (0.343)	0.508 (0.325)	0.351 (0.368)
Eleocharis > 25 cm	0.159 (0.239)	0.083 (0.161)	0.195 (0.278)	0.138 (0.212)
Scirpus ≤25 cm	0.117 (0.204)	0.117 (0.236)	0.136 (0.190)	0.106 (0.212)
Scirpus > 25 cm	0.272 (0.308)	0.279 (0.350)	0.320 (0.316)	0.245 (0.302)
Typha ≤25 cm	0.018 (0.068)	0.010 (0.055)	0.019 (0.075)	0.017 (0.064)
Typha > 25 cm	0.052 (0.170)	0.040 (0.161)	0.061 (0.182)	0.047 (0.163)
Other Vegetation ≤25 cm	0.121 (0.173)	0.124 (0.239)	0.161 (0.164)	0.098 (0.174)
Other Vegetation > 25 cm	0.291 (0.336)	0.294 (0.351)	0.281 (0.318)	0.297 (0.346)

Table 5. Binary Logistic Regression analysis of microhabitat variables in the immediate vicinity (within 25 cm) of frog relocations based on use-intensity classification. Two models are presented based on (A) daytime observations and (B) nighttime observations. In both models, the low-use category is 1-3 observations per segment, and high-use category is ≥4 observations per segment. The quasi-jackknife validation/reclassification correctly classified 66% of the data for the daytime model and 70% for the nighttime model, but the daytime model is not considered robust because few low-use segments were correctly reclassified giving a highly biased result.

A. Daytime

Variable	Wald Chi-Square	P-value	Trend
Eleocharis ≤25 cm	4.8727	= 0.027	Low (↓) : High (↑)
Other Vegetation > 25 cm	15.7904	< 0.001	Low (↓) : High (↑)

B. Nighttime

Variable	Wald Chi-Square	P-value	Trend
Stream Cover > 25 cm*	14.2048	= 0.001	Low (↑) : High (↓)
Total Cover ≤25cm	11.1839	< 0.001	Low (↑) : High (↓)
Eleocharis ≤25 cm	13.7887	< 0.001	Low (↓) : High (↑)

* Stream Cover > 25 cm was correlated with Total Cover > 25, and the latter variable was excluded from the analysis.

Table 6. Binary Logistic Regression analysis of macrohabitat variables associated with frog use and non-use of segments. Use was determined by radio telemetry observations regardless of the number of observations per segment. The quasi-jackknife validation/reclassification correctly classified 66% of the data.

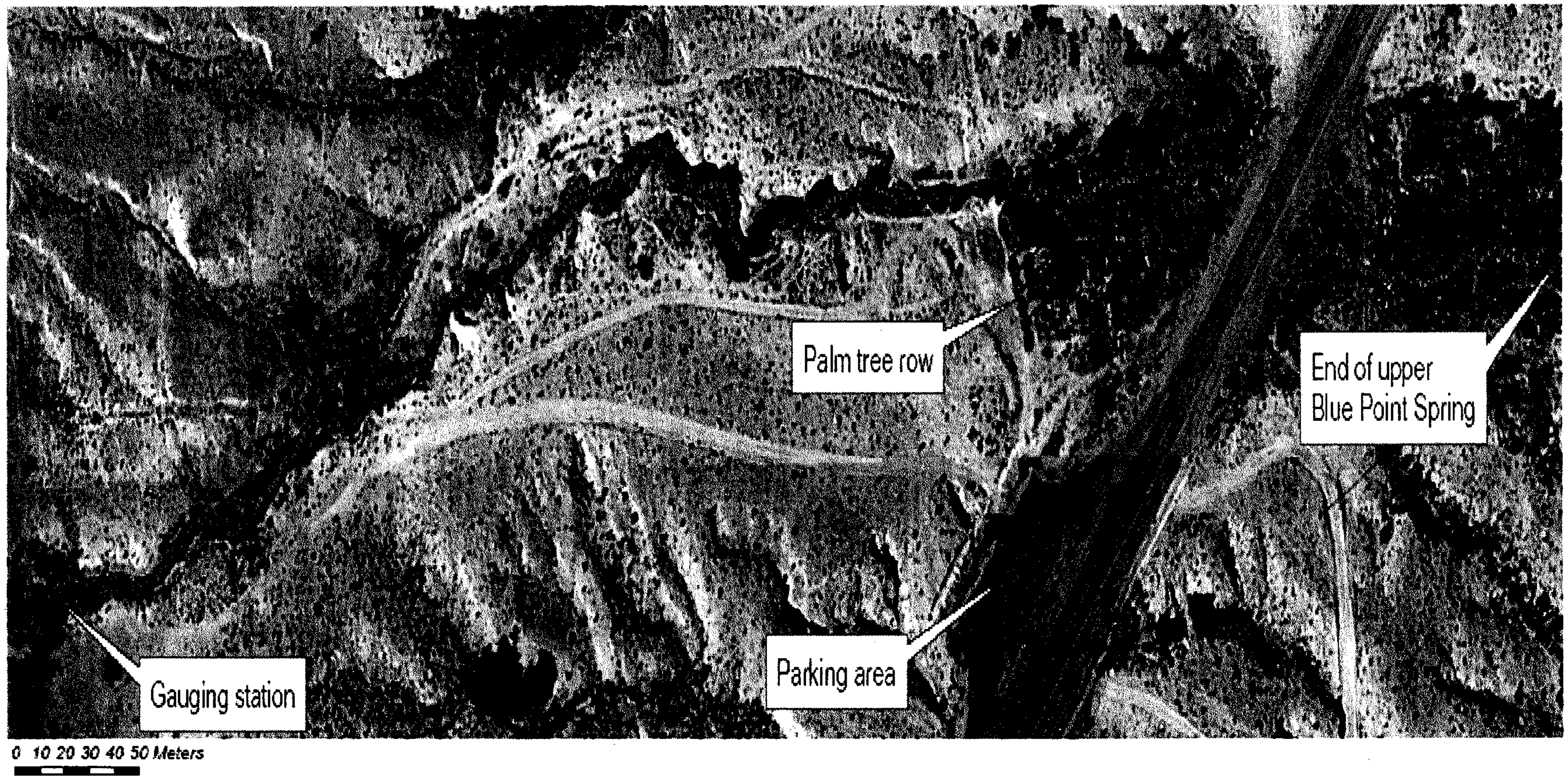
Variable	Wald Chi-Square	P-value	Trend
Percent Shallow	6.8432	= 0.009	Non-Used (↓) : Use (↑)
Stream Cover > 25 cm*	12.0185	= 0.001	Non-Used (↑) : Use (↓)
Riparian Cover > 25cm	3.0112	= 0.083	Non-Used (↑) : Use (↓)
Eleocharis > 25 cm	8.4598	= 0.004	Non-Used (↓) : Use (↑)
Typha ≤25 cm	2.9155	= 0.088	Non-Used (↓) : Use (↑)

* Stream Cover was correlated with Total Cover at both vegetation heights, the Total Cover variables were excluded from analysis. .

Table 7. Nonparametric (MANOVA) comparisons of variable ranks between use and non-use segments. The Mann-Whitney F-statistic and *P*-value are indicated, with Tukey post-hoc (Trend column). Post-hoc results are significant at $P < 0.05$.

Variable	Test Statistic	*P*-value	Trend
Stream Width	2.699	= 0.013	Non-Used (↓) : Use (↑)
Percent Shallow	13.242	< 0.001	Non-Used (↓) : Use (↑)
Stream Cover ≤25cm	5.026	< 0.001	Non-Used (↑) : Use (↓)
Stream Cover > 25 cm	21.591	< 0.001	Non-Used (↑) : Use (↓)
Total Cover ≤25 cm	6.126	< 0.001	Non-Used (↑) : Use (↓)
Total Cover > 25cm	16.404	< 0.001	Non-Used (↑) : Use (↓)
Eleocharis ≤25 cm	18.249	< 0.001	Non-Used (↓) : Use (↑)
Eleocharis > 25 cm	12.103	= 0.038	Non-Used (↓) : Use (↑)
Other Vegetation ≤25 cm	0.016	< 0.001	Non-Used (↓) : Use (↑)

Figure 4. Arial photo of upper Blue Point Spring, Lake Mead National Recreation Area, showing probable low- and high-use segments (blue or light and red or dark dots respectively) for all 591 measured segments based on the equation from our primary LR analysis comparing macrohabitat data among segments in which frogs were actually observed. Few of the non-use segments were classified as having a high-use probability, indicating that most of the habitat at this site was not optimal for the significant variables. The spring source is located 10 m from the gauging station (left side of photograph).



REFERENCES

Anderson, J.A. 1984. Regression and ordered categorical variables. J.R. Stat. Soc. B 46:1-30

Bantle, J.A., J.N. Dumont, R.A. Finch, and G. Linder. 1992. Atlas of Abnormalities: A guide for the performance of FETAX. Oklahoma State University Press, Stillwater, Oklahoma.

Beebee, T.J.C., R.J. Flower, A.C. Stevenson, S.T. Patrick, P.G. Appleby, C. Fletcher, C. Marsh, J. Natkanski, B Rippey, and R.W. Battarbee. 1990. Decline of the Natturjack toad *Bufo calamita* in Britain: Paleoecological, documentary and experimental evidence for breeding site acidification. *Biological Conservation* 53: 1-20.

Berger L, Speare R, Hyatt AD. 2000. Chytrid fungi and amphibian declines: Overview, implications and future directions. In: Campbell A, editor. Declines and disapparances of Australian frogs. Environmental Australia, Canberra, Australia: Environmental Australia: 21-31.

Birge, W.J., Black, J.A., Westerman, A.G. 1979. Evaluation of aquatic pollutants using fish and amphibian eggs as bioassay organisms. National Academy of Sciences, Washington, D.C.

Blaustein, A.R., and D.B. Wake. 1990. Declining amphibian populations: a global phenomenon? *TREE* 5: 203-204.

Bradford, D.F. 1989. Allotopic distribution of native frogs and introduced fishes in high Sierra Nevada lakes of California: Implication of the negative effect of fish introductions. Copeia 1989: 775-778.

Bradford, D.F. 1991. Mass mortality and extinction in a high elevation population of Rana muscosa. Journal of Herpetology 25(2): 174-177.

Bradford, D.F., J.R. Jaeger, and R.D. Jennings. 2004. Population Status and Distribution of a Decimated Amphibian, The Relict Leopard Frog (*Rana onca*). The Southwestern Naturalist 49(2): 218-228.

Bradford, D.F., R.D. Jennings, and J.R. Jaeger. 2005. *Rana onca* Cope, 1875, Relict leopard frog. In: Status and Conservation of U.S. Amphibians. Lannoo MJ (editor). University of California Press, Berkeley, CA

Callery, E.M., Fang, H., and Elinson, R.P. 2001. Frogs without polliwogs: evolution of anuran direct development. BioEssays 23: 233-41.

Collins, J.P., and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. *Diversity and Distibutions* 9: 89-98.

Cooke, A.K. 1981. Tadpoles as indicators of harmful levels of pollution in the field. Environmental Pollution 25: 123-133.

Cope, E.D. 1875. *Rana onca*, sp. Nov., p. 528-529. In: Dr. H.C. Yarrow, report upon the collections of batrachians and reptiles made in portions of Nevada, Utah, California, Colorado, New Mexico, and Arizona, during the years 1871, 1872, 1873, 1874. Report upon the U. S. Geographical Explorations and Surveys west of the One Hundredth Meridian 5:509-589.

Cross, C.L., and C.E. Petersen. 2001. Modeling snake microhabitat from radiotelemetry studies using polytomous logistic regression. Journal of Herpetology 35(4): 590-597.

Crump. M.L. and N.J., Scott Jr. 1994. Visual encounter surveys. In: Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, M.S. Foster (Eds.). 1994. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution Press, Washington.

Daszak, P., L. Berger, A.A. Cunningham, A.D. Hyatt, D.E. Green, and R. Speare. 1999. Emerging Infectious Diseases and Amphibian Population Declines. Emerging Infectious Diseases 5: 735-748.

Deacon, J.E., C. Hubbs, and B.J. Zahuranec. 1964. Some effects of introduced fishes on the native fish fauna of southern Nevada. Copeia 1964(2):384-388.

Denton, J.S., S.P. Hitchings, T.J.C. Beebee, and A. Gent. 1997. A recovery program for the natterjack toad (*bufo calamita*) in Britain. Conservation Biology 11: 1329-1338.

Hayes, M.P., and M.R. Jennings. 1986. Decline of ranid frogs in western North America: Are bullfrogs (*Rana catesbeiana*) responsible? *Journal of Herpetology* 20: 490-509.

Hosmer, D.W., and S. Lemeshow. 1989. Applied logistic regression. John Wiley and Sons, New York.

Hosmer, D.W., and S. Lemeshow. 2000. Applied logistic regression. John Wiley and Sons, New York.

Ildos, A.S., and N. Ancona. 1994. Analysis of amphibian habitat preferences in a farmland area (Po plain, northern Italy). Amphibia-Reptilia 15: 307-316.

Jaeger, J.R., B.R. Riddle, R.D. Jennings, and D.F. Bradford. 2001. Rediscovering *Rana onca*: evidence for phylogenetically distinct leopard frogs from the border region of Nevada, Utah, and Arizona. Copeia 2001:339-354.

Johnson, D.H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology 61: 65-71

McAllister, K.R., and W.P. Leonard. 1997. Washington state status report for the Oregon spotted frog. Unpublished report. Washington Department of Fish and Wildlife, Olympia.

Mickey, J., and S. Greenland. 1989. A study of the impact of confounder selection criteria on effect estimation. American Journal of Epidemiology. 129:125-137

Monello, R.J., and R.G. Wright. 1999. Amphibian habitat preferences among artificial ponds in the Palouse region of northern Idaho. Journal of Herpetology 33: 298-303.

Munger, J.C., M. Gerber, K. Madrid, M. Carroll, W. Petersen, and L. Heberger. 1998. U.S. national wetland inventory classifications as predictors of the occurrence of Columbia spotted frogs (*Rana luteiventris*) and Pacific treefrogs (*Hyla regilla*). Conservation Biology 12: 320-330.

Noon, B.R. 1986. Summary: biometric approaches to modeling–the researcher's viewpoint. In: C.L. Cross, and Petersen, C.E. 2001. Modeling snake microhabitat from radiotelemetry studies using polytomous logistic regression. Journal of Herpetology 35(4): 590-597.

North, M.P., and J.J. Reynolds. 1996. Microhabitat analysis using radiotelemetry locations and polytomous logistic regression. Journal of Wildlife Management 60:639-653.

Otis, D.L., and G.C. White. 1999. Autocorrelation of location estimates and the analysis of radiotracking data. Journal of Wildlife Management 63: 1039-1044.

Pechmann, J.H., D.E. Scott, R.D. Semlitsch, J.P. Caldwell, L.T. Vitt, and J.W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. Science 253: 892-895.

Phillips, K. 1990. Where have all the frogs and toads gone? *BioScience* 40: 422-424.

Pohlmann, K. R., D.J. Campagna, J.B. Chapman, and S. Earman. 1998. Investigation of the origin of springs in the Lake Mead National Recreation Area. Publication Number 41161, Desert Research Institute, Las Vegas, Nevada.

Rathbun, G.B., and T.G. Murphey. 1996. Evaluation of a radio-belt for ranid frogs. Herpetological Review 27(4):187-189.

Richards, S. J., U. Sinsch, and R. A. Alford. 1994. Radio Tracking. *In* E. Muths, 2003. A Radio Transmitter Belt for Small Ranid Frogs. Herpetological Review 34: 345-348.

Reinert, H.K. 1984. Habitat separation between sympatric snake populations. Ecology 65:478-486.

Rollins-Smith, L.A., C. Carey, J. Longcore, J.K. Doersam, A. Boutte, J.E. Bruzgal, and J.M. Conlon. 2002. Activity of antimicrobial skin peptides from ranid frogs against *Batrachochytrium dendrobatidis*, the chytrid fungus associated with global amphibian declines. Developmental and Comparative Immunology 26: 471-479.

Stebbins, R.C. and N.W. Cohen. 1995. A natural history of amphibians. Princeton University Press, Princeton.

Tayler, M.J., and Davies, M. 1985. The gastric brooding frog Rheobatrachus silus. The Biology of Australasian Frogs and Reptiles. 469-470.

Thomas, D.L., and E.J. Taylor. 1990. Study designs and tests for comparing resource use and availability. Journal of Wildlife Management 54: 322-330.

Thorne-Miller, B. 1999. The Living Ocean: Understanding and Protecting Marine Biodiversity. Island Press, Washington, DC.

Vitt, L.J., J.P. Caldwell, H.M. Wilbur, and D.C. Smith. 1990. Amphibians as harbingers of decay. *BioScience* 40: 418.

Wake, D.B. 1991. Declining amphibian population. *Science* 253: 860.

Wake, D.B., and H.J. Morowitz. 1991. Declining amphibian populations – A global phenomenon? Findings and recommendations. Report to Board on Biology, National Research Council, on workshop in Irvine, California 19-10 February 1990; reprinted 1991. *Alytes* 9: 33-42.

Wake, D. B. and J. Hanken. 1996. Direct development in the lungless salamanders: What are the consequences for developmental biology, evolution and phylogenesis? Internat. J. Devel. Biol. 40: 859-869.

Watson, J.W., K.R. McAllister, and D.J. Pierce. 2003 Home Ranges, Movements, and Habitat Selection of Oregon Spotted Frogs (*Rana pretiosa*). Journal of Herpetology 37(2): 292-300.

Watson, J.W., K.R. McAllister, D.J. Pierce, and A. Alvarado. 2000. Ecology of a remnant population of Oregon spotted frogs (*Rana pretiosa*) in Thurston County, Washington. Washington Department of Fish and Wildlife, Olympia.

Welch, N. E. and J. A. MacMahon. 2005. Identifying Habitat Variables Important to the Rare Columbia Spotted Frog in Utah (U.S.A.): an Information-Theoretic Approach. Conservation Biology 19(2): 473-481.

Wilson, E.O. 1988. Biodiversity. National Academy Press, Washington, D.C.

VITA

Graduate College
University of Nevada, Las Vegas

Sean Moran Harris

Home Address:
1466 Lorilyn Ave. #1
Las Vegas, Nevada, 89119

Degrees:
Bachelor of Science, 2004
University of Nevada, Las Vegas

Thesis Title: Evaluation of the Impact of Vegetation Encroachment on Relict Leopard Frog (*Rana onca*) Populations

Thesis Examination Committee:
- Chairperson, Dr. Brett Riddle, Ph.D.
- Committee Member, Dr. David Bradford, Ph.D.
- Committee Member, Dr. Shawn Gerstenberger, Ph.D.
- Graduate Faculty Representative, Dr. Chad Cross, Ph.D.