University of Nevada, Reno

The Status and Distribution of the Northern Leopard Frog (*Rana pipiens*) in Nevada

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Biology

by

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Abstract

Amphibian decline is a global problem. Declines have been attributed to harvesting, habitat loss or degradation, pollutants, ozone depletion, global climate change, anomalous weather patterns, introduced exotic species, and disease, however some decline causes remain enigmatic. In the United States, amphibian declines appear to be more severe in the western states, than those east of the Rocky Mountains. The northern leopard frog (*Rana pipiens*) was once widely distributed across the state of Nevada, however, repeated surveys of historical sites revealed that leopard frogs have severely declined in the state. Analyses have determined that suites of habitat variables where leopard frogs are present, are statistically different from habitat where leopard frogs were not found. It appears that some of the current decline hypotheses are more important for leopard frogs in Nevada than others. These causes include habitat degradation, negative interactions with introduced species, and discontinuity of habitat.

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Chapter I: The Natural History and Habitat Requirements of Leopard Frogs

Abstract

Amphibians have been declining worldwide. Declines have been attributed to harvesting, habitat loss or degradation, pollutants, ozone depletion, global climate change, anomalous weather patterns, introduced exotic species, and disease, however some decline causes remain enigmatic. In the United States, amphibian declines appear to be more severe in the western states. Leopard frog (*Rana pipiens* complex) declines have been documented in at least six western states. The biology of leopard frogs is important in understanding the basic environmental needs of these frogs. A meta-analysis of habitat determined that the minimum requirements of leopard frogs include water and at least $100 m^2$ of grass, wet meadow or marsh.

Introduction

Amphibians evolved about 350 million years ago, and have inhabited the earth since the Devonian period (Duellman and Trueb, 1986). Amphibians have adapted to life in a diversity of environments and climates and can be found on every continent (with the exception of Antarctica). Today there are more than 4500 extant species of amphibians, and many new amphibian species are being discovered each year (Stebbins and Cohen, 1995). However, recent global declines and numerous local and global extinctions are decreasing the number of amphibian species worldwide. It is also likely that species are becoming extinct before they have been discovered. Therefore, it is alarming that this prehistoric, and highly adaptable lineage is presently declining. The purpose of this chapter is to investigate documented amphibian declines, summarize the biology of leopard frogs, and define leopard frog habitat. This was accomplished by reviewing the literature on amphibian decline, *R. pipiens* biology, and using compiled information on *R. pipiens* habitat in a meta-analysis to determine the basic habitat needs for this species.

Amphibian Declines

Global amphibian declines.—Amphibian declines have been documented worldwide since the 1970s (Barinaga, 1990; Blaustein and Wake 1990; Wyman, 1990; Wake, 1991; Corn, 1994; Drost and Fellers, 1996). In recent decades, sudden and simultaneous die offs have been recorded on different continents (Barinaga, 1990). The causes of decline are likely complex, as populations of some species are declining while sympatric species remain unaffected (Drost and Fellers, 1994). Similarly, populations of the same species may be declining in some areas of their range, but thriving in other locations (Wymann, 1990; Blaustein, 1994). As a result of global declines, the Declining Amphibian Populations Task Force (DAPTF) was assembled in 1991 to assess the extent of, and investigate causes of amphibian declines, and to establish protocols for studying amphibian populations.

Causes of declines.—Amphibians are considered more susceptible to environmental change than are other vertebrates because amphibian skin is highly permeable to liquids and gasses (Duellman and Trueb, 1986; Wyman, 1990). Also, most amphibians use both terrestrial and aquatic habitats during parts of their life cycle, making them more vulnerable to changes in both kinds of environments. Consequently, amphibians could signal potentially detrimental changes in the environment long before other animals would respond to those changes. Because amphibian declines are a global phenomenon, the welfare of amphibian habitats worldwide, as well as species associated with these habitats, may be in jeopardy.

Hypotheses for global frog declines include over harvesting, habitat loss or degradation, pollutants, UV radiation exposure, global climate change, anomalous weather patterns, exotic species, diseases and parasites, and natural population fluctuations in conjunction with disturbance. There is no single cause that can explain amphibian declines. Various combinations of the above hypotheses are likely responsible. The following provide examples of each of these suspected causes.

(1) **Harvesting** in the U.S. for research and teaching reportedly consumed anywhere between 9 and 22 million frogs annually during the 1960s and 1970s (Gibbs et al., 1971; Merrell, 1971). Many frogs, including *Rana pipiens*, were also harvested for their edible legs from the late 1800s through the 1950s (Brice, 1897; Souder, 2000).

(2) **Habitat loss or degradation** is the most cited and the most likely cause of amphibian declines worldwide (Blaustein, 1994; Corn, 1994; Jennings and Hayes, 1994; Wake, 1991; Wyman, 1990; Lehtinen et al., 1999). Loss of habitat has been implicated in the near extinction of Darwin's frog (*Rhinoderma darwinii*) in Chile (Crump, 2000). In addition, the red-legged frog (*Rana aurora*), mountain yellow-legged frog (*Rana muscosa*), and the Natterjack toad (*Bufo calamita*) are among many amphibians that have suffered declines due to habitat loss and degradation (Waldman and Tocher, 1998).

(3) **Pollutants** such as pesticides, are believed to have negatively impacted leopard frogs in parts of Wyoming and Washington (Stebbins and Cohen, 1997). Agricultural run-off (e.g., fertilizers and pesticides) have been found to cause deformities in Minnesota frogs (including *Rana pipiens*) (Hayes, 1986; Souder, 2000).

(4) **Ozone depletion** causes an increase in UV radiation reaching the Earth's surface. Intense UV radiation is known to cause death and abnormalities in developing amphibian eggs (Higgens and Sheard, 1926, in Duellman and Trueb, 1986; Blaustein et al., 1994b). Exposure of anuran eggs to UV rays has been suggested as a cause of decline in high-elevation populations of the western toad (*Bufo boreas*) in Oregon (Blaustein et al., 1994b).

(5) **Global climate change** has been implicated as an underlying cause of amphibian declines. In regions that have periodic droughts, global warming may cause an increased frequency of drought. Although some amphibians are adapted to drought conditions, (e.g., spade foot toad [*Scaphiopus couchii*]; Newman, 1989), increased drought frequency may not allow these amphibians enough time to carry on their life cycles between drought periods. Similarly, changes in the timing of season can cause life cycles to be shifted, (e.g., emerging and breeding times to be earlier; Alford and Richards, 1999), which may upset the ecological balance in an area. Long-term climate change may force populations to move to new locations in order to stay within preferred climatic conditions. If populations are unable to move to suitable regions quickly enough, or if geographic boundaries prevent them from doing so (e.g., mountain ranges or large bodies of water), the population will likely perish.

(6) **Anomalous weather patterns** have been implicated in the disappearance of the golden toad (*Bufo periglenes*) and decline of the harlequin frog (*Atelopus varius*) in Costa Rica (Stebbins and Cohen, 1997; Crump, 2000). Sudden irregularities in weather can cause massive die offs if a population can not tolerate a change they have no physical or behavioral adaptations for.

(7) **Introduced exotic species** such as non-native fish and bullfrogs have been implicated in declines of the red-legged frog (*R. aurora*) and mountain yellow-legged frog (*R. muscosa*) in California and Nevada (Hayes and Jennings, 1984; Jennings and Hayes, 1994; Stebbins and Cohen, 1997). Invasive, non-native plants such as tall white top (*Lepidium latifolium*) and salt cedar or tamarisk (*Tamarix* sp.) that invade riparian habitats, can alter habitat making it unsuitable for amphibians. These plants tend to

dominate native vegetation, transforming vast areas into monocultures of the exotic species. Studies on tall white top have shown that this weed acts as a salt pump, taking sodium from the subsurface soil and depositing it on the surface. (Blank and Young, 1997; Young et al., 1997). Both plants are capable of completely changing riparian habitats in a relatively short period of time.

(8) **Disease**, for example red legged disease, caused by *Aeromonas hydrophilia*, a bacterium naturally common in amphibian habitats (Gibbs, 1973), can infect entire amphibian assemblages (K. Pope, pers. comm.). Chitrid fungus has also been suspected in amphibian die offs in the western U.S., Australia, and central and South America (K. Pope, pers. comm.; Daszak et al., 1999; Merrell, 1999). This fungus was only recently discovered (1998) and its ultimate impact on amphibians is still unknown (K. Pope, pers comm.). Chitrid fungus can be transported from water body to water body by biologists, people recreating (e.g., fishing, boating), and others who unknowingly carry the fungus on their shoes or equipment (K. Pope, pers. comm.). Birds may also transport chitrid fungus if a part of the bird remains wet from an infested water source and the bird enters another water body. Amphibians have an increased susceptibility to both red-legged disease and chitrid fungus when they are stressed or their immune systems are suppressed (K. Pope, pers. comm.; Daszak et al., 1999).

Declines of Leopard Frogs

Continental declines of leopard frogs.—Leopard frogs (*Rana pipiens* complex) are declining throughout their range. In the 1970s various reports estimate that between 9 and 22 million frogs were harvested annually in the U. S., Canada and Mexico for education and research alone, and most of these were leopard frogs (Gibbs et al., 1971; Merrell, 1971). At this time it was calculated that the frog population in the U.S. had declined by approximately 50% (Gibbs et al., 1971). Captive rearing and frog farming have since considerably decreased the amount of frogs taken from the wild for research and education, and many populations have been able to rebound, although not to their original numbers (Stebbins and Cohen, 1995). Studies begun in the 1960s indicate that population declines began in eastern North America and spread west, and that currently, declines in the western U.S. might be more severe than those east of the Rocky Mountains (Corn, 1994; Stebbins and Cohen, 1997). The general aridity of the west causes a higher human demand for what little water exists. As the human population expands we increasingly encroach on native habitats, often altering them, and rendering them unsuitable to native species. A lowering of the water table occurs when water is continually diverted for agriculture, livestock and recreation, which subsequently reduces wetland areas and impacts species that are closely associated with these wetlands. Also, natural population fluctuations could cause local extinctions if recruitment is impossible because a lack of connectivity of habitat among populations.

Examples of leopard frog declines in the western U.S.—Surveys in Colorado reveal that nine populations of leopard frogs disappeared between 1973 and 1982 and that these extirpations have been attributed to habitat degradation (Corn and Fogleman, 1984). In Targhee National Forest, Idaho, all ninety-eight sites that historically had leopard frog populations were searched in 1992, and no leopard frogs were found (Clark et al., 1993, in Stebbins and Cohen, 1997). *Rana pipiens* are now believed to be extinct in this area and causes are unknown. Similarly, the Chiricahua leopard frog (*Rana chiricahuensis*) was found in only 2 of 36 historical locations, and *R. pipiens* was found at 12 of 25 literature sites in Arizona between 1983 and 1987 (Clarkson and Rorabaugh, 1989). Habitat alteration, including water diversion, severe erosion, and clearing of land for agriculture are suspected causes of leopard frog declines here (Clarkson and Rorabaugh, 1989). As a result of severe declines, the Chiricahua leopard frog (*R. chiricahuensis*) was recently proposed for listing as a threatened species in Arizona (Humphrey and Fox, 2000; Ryan, 2000). In Washington State, all historical sites containing *R. pipiens* were surveyed in 1993, but only one leopard frog population was found (Stebbins and Cohen, 1997; Leonard et al., 1999). Bullfrogs (*Rana catesbeiana*) were found in more than half of the former *Rana pipiens* habitat in Washington (Leonard et al., 1999). In Oregon, no leopard frogs have been reported from the once populated Owyhee River drainage since the 1970s, and causes are unknown (Stebbins and Cohen, 1997). Some populations of leopard frogs in Wyoming and Minnesota that experienced declines in the 1970s, appear to have partially recovered since the applications of pesticides and commercial collection have been regulated (Gibbs et al., 1971; Moriarity, 1998).

Leopard frogs in Nevada.— Recent studies suggest that populations of leopard frogs have severely declined, been locally extirpated, or are considered extinct in Nevada (Corn, 1994; Panik and Barrett, 1994). Two endemic species of leopard frogs known from drainages near Las Vegas, in southern Nevada are the Vegas Valley leopard frog (*Rana fisheri*) and the relict leopard frog (*Rana onca*). Although *R. onca*, and *R. fisheri* were considered to be *R. pipiens* until the 1970s, these two species are only known from localized areas in Clark County. *Rana fisheri* has been considered extinct since the 1940s, whereas *R. onca*, thought to have gone extinct, was rediscovered by R. D. Jennings in 1993 (Corn, 1994). The third species of leopard frog, *R. pipiens*, was once widely distributed in the northern three-quarters of the state. Lindsdale's (1940) report on amphibians collected in Nevada between 1927-1938 stated that leopard frogs (all considered to be one species in 1940) were the "commonest and most widespread kind of frog in the state." Likewise, LaRivers (1942) stated that leopard frogs were "the most widely distributed of Nevada Ranidae," (this had to have included *R. pipiens*, *R. onca*, and *R. fisheri*). In a more recent survey (1992) of the once populated Truckee River, in Washoe County, *R. pipiens* were found in only one of 31 localities (Panik and Barrett, 1994). Anecdotal evidence suggests numerous local extirpations in western Nevada (P. F. Brussard and H. R. Panik, pers. comm.). Museum records also indicate that few leopard frogs have been collected since the 1970s. Although this later observation could reflect collection effort, it might also indicate declines. My surveys of 87 historical sites in 2000 revealed few remaining populations in areas where leopard frogs once occurred. Despite these declines, no one had undertaken a statewide study of the status of northern leopard frogs in Nevada.

The causes of many amphibian declines worldwide remain enigmatic (Barinaga, 1990; Wake, 1991; Crump et al., 1992; Blaustein et al., 1994a). It is likely that different populations respond to different threats, and some populations respond to combinations of the hypothesized causes for documented declines (Blaustein and Wake, 1990; Rabb, 1990; Wyman, 1990; Houlahan et al., 2000). Determining the causes of leopard frog declines in Nevada may offer insight into the decline causes of other populations of leopard frogs and/or other amphibian species.

The Biology of Leopard Frogs

Taxonomy and distribution.—Prior to 1968, all leopard frogs in the United States were considered to be a single species, *Rana pipiens* (Littlejohn and Oldham, 1968; Brown, 1973; Dunlap and Kruse, 1976; Hillis, 1988). Differences among populations were considered to be examples of broad geographic variation within a single species (Pace, 1974). Closer scrutiny of morphological and behavioral characteristics in several geographic areas led to the division of *R. pipiens* into four separate species by 1968, specified by call type and referred to as northern, southern, eastern and western leopard frogs (Littlejohn and Oldham, 1968; Brown, 1973; Pace, 1974). After 1973, leopard frogs went through numerous species divisions (Pace, 1974). Currently, about 25 species of leopard frogs are recognized within the *Rana pipiens* complex (Hillis, 1988) with members distributed throughout North and Central America (Stebbins, 1985). Within this complex, the northern leopard frog (*Rana pipiens*) is currently one of the most widespread anurans in the United States. The geographic range of this species is nearly trans-continental (Stebbins, 1885; Fig. 1). Leopard frogs occur in elevations from sea level to about 3300 m (Stebbins, 1985). In many western states leopard frogs exist in isolated populations, probably as a result of post-Pleistocene changes in climate, which caused extreme fluctuations in water levels and the amount of water covering the West (Fig. 2). Given the lengthy isolation of some of these populations, there are likely to be additional cryptic species. However, genetic differences, as a result of geographic isolation, may have been obscured by the commercial transport of frogs which may have provided gene flow to many populations (Hoffman, pers. comm.).

Leopard frog lineages in Nevada.—There are two extant species of leopard frogs in Nevada, the relict leopard frog (*R. onca*), and the northern leopard frog (*R. pipiens*; Fig. 3). *Rana onca* is restricted to springs surrounding Lake Mead and to marshy areas associated with the Virgin River in southern Nevada (Fig. 1). A third species, the Vegas Valley leopard frog (*R. fisheri*), from Clark County Nevada is extinct (last seen 1942; Behler and King, 1979). *Rana onca* was formerly synonymous with *R. pipiens*, and many museum specimens collected from Clark County prior to the 1970s were incorrectly identified as *R. pipiens* as well as other members of the *R. pipiens* complex (Jennings et al., 1988). However, morphological differences distinguish *R. onca* from *R. pipiens* (Hillis, 1988). *Rana onca* has shorter, less distinct dorsolateral folds, which extend three-quarters of the way down the dorsum, whereas these folds extend the entire length of the body in *R. pipiens*. The legs of *R. onca* are usually spotted rather than barred as in *R. pipiens* and the upper labial stripe is incomplete in *R. onca*. Males of *R. onca* also lack the vestigial oviducts present in *R. pipiens* (Jennings et al., 1988). Finally, the ranges of the two species do not currently overlap (Fig. 1).

Life history.—*Rana pipiens* is generally active from March through November depending on regional climate (Pace, 1974; Merrell, 1977). Leopard frogs depend on wet areas, although, they can be found far from water bodies during summer (Zenisek, 1963; Dole, 1967; Pace, 1974; Merrell, 1977; Hine et al., 1981). These frogs commonly emerge in early spring (March or April) and males immediately begin calling for mates. During this time, frogs are concentrated in or around lentic water bodies where courtship and spawning takes place (Hine et al., 1981; Merrell, 1977; Hammerson, 1999). Leopard frogs generally do not lay their eggs until the water temperature remains at least 8–13 °C for about 10 days (Merrell, 1977; Hine et al., 1981; Gilbert and Fortin, 1994). Males can be distinguished from females during the breeding season by the presence of an enlarged nuptial pads (thumb muscles), which are used for gripping the female during amplexus. Males also have enlarged vocal sacs which become evident when vocalizing (Dole, 1965a). Egg masses are attached to aquatic vegetation from 10–65 cm below the surface, usually in a shallow, warm area of the breeding pond (Zenisek, 1963; Pace, 1974; Merrell, 1977; Hine et al., 1981; Hammerson, 1999). Females produce between 600–6000 eggs per clutch (Hine et al., 1981; Gilbert and Fortin, 1994; Degenhardt et al., 1996; Hammerson, 1999), and eggs hatch approximately 14–16 days after oviposition depending upon temperatures (Hammerson, 1999). During the breeding season, females are secretive, coming to the breeding pools only to mate, whereas males are more conspicuous, calling from the surface of the water (Hine et al., 1981). This difference in behavior can give the impression of an unequal sex ratio in springtime, however studies conducted through the summer and fall show that there are similar numbers of males and females (Hine et al., 1981). After oviposition, leopard frogs leave the water and live almost exclusively in moist grassy areas surrounding the breeding pool or other nearby water sources (Dole, 1967). Males usually reach sexual maturity and begin breeding in one year, whereas females usually mature their second spring after metamorphosis (Force, 1933; Dole, 1965a; Gilbert and Fortin, 1994).

Summer movements are generally restricted to short distances. Leopard frogs are sit-and-wait predators, and often remain in the same location for days (Dole, 1965b). During nocturnal rains they are known to travel long distances (at speeds of up to 40 m/h) perhaps to familiarize themselves with their surrounding habitat (Dole, 1965b; Merrell, 1971). In late fall, leopard frogs return to permanent water sources where they occasionally can be heard calling, although there have been no reports of autumn breeding (Pace, 1974). The onset of hibernation coincides with consistently cooler weather (Zenisek, 1963; Merrell, 1971). In general, *R. pipiens* are no longer audible or visible after the air and water temperature drop to about 10–12 °C (Zenisek, 1963; Hine et al., 1981). Winter is spent in a state of torpor in mud depressions under water. In this state, leopard frogs are capable of movement and have been observed lifting themselves and rotating, presumably to increase oxygen diffusion through the skin because silt tends to accumulate over their bodies (Emery et al., 1972). *Rana pipiens* may emerge intermittently during hibernation in periods of warm weather, but will return to winter torpor when temperatures drop below about 10 °C again (Zenisek, 1963).

Leopard frogs feed on a variety of invertebrates (Degenhardt et al., 1996) however, like most frogs, they are not selective with their food and will generally eat anything that moves and can fit in their mouths. Predators of leopard frogs include numerous snakes, tiger salamanders (*Ambystoma tigrinum*) and various species of fish and birds (Hammerson, 1999). While living terrestrially, frogs obtain their moisture by sitting on the damp soil and absorption takes place through a "seat patch" located in the groin area (Dole, 1967; Tracy, 1976).

Leopard frogs and ecosystem processes.—Leopard frog tadpoles are filter feeders and consume algae, detritus, and suspended particles. Thus, they contribute to nutrient cycling and convert particulate matter in aquatic environments (Seale, 1980). They also may reduce eutrophication of ponds and lakes by grazing on primary producers which may otherwise multiply unchecked (Seale, 1980; Stebbins and Cohen, 1997). Adult leopard frogs are primary predators of invertebrates and, therefore, may contribute to the control of insect abundance (Stebbins and Cohen, 1995). Both frogs and tadpoles are an important food source for many fishes and riparian snakes, birds, and some mammals (Stebbins and Cohen, 1995; Lannoo, 1998).

Leopard frogs as bioindicators.—The skin of amphibians in general, is a semi-permeable surface through which gasses and water are exchanged and solutes within these substrates may be absorbed. Because *Rana pipiens* use both terrestrial and aquatic habitats during their life cycle, they are susceptible to changes in both environments. Therefore, these animals are likely to experience effects of environmental perturbations before animals with less permeable skin and/or without dual habitat requirements.

Habitat Assessment of Leopard Frogs

Characterization of Rana pipiens *habitat.*—Climatic conditions and biological communities vary considerably across the broad geographic range of *R. pipiens*. Habitat in the northeastern United States can be characterized as moist deciduous forests, whereas the southwest has arid coniferous forests at higher elevations and dry sagebrush communities at lower elevations. Microhabitat similarities across regions may allow northern leopard frogs to live in a diversity of ecosystems, therefore it is important to characterize *R. pipiens* habitat at the microhabitat level. Habitat descriptions accompany many of the published studies on *R. pipiens*. These data were used to (1) characterize *R. pipiens* habitat by determining the main habitat components, and (2) determine which of Nevada's historical sites currently have the best habitat for *R. pipiens*.

Data collection.—A literature search was conducted for studies involving *R. pipiens*, which included habitat descriptions. Twenty-nine papers described habitats in areas throughout the geographic range of *R. pipiens* (Table 1). Descriptions varied in the amount of detail given. Categories of habitat variable were made. Similar vegetation types were combined into a single category (e.g., woody riparian vegetation included cottonwoods, willows, and alder). The presence of each habitat component described by the authors was tallied (Table 1). Although "marshes" could be included in "freestanding water", whereas irrigation canals and ditches could be labeled "flowing water", I kept these parameters separate because of their unique properties. Grouping these parameters did not change the overall result of the analyses.

Frequency of habitat components.—The frequency of habitat variables reported in the literature were summarized to determine the habitat features most often used by leopard frogs throughout the United States and Canada (Fig. 4). The most frequently reported habitat components at leopard frog locations were grass, water, and emergent vegetation. More specifically, leopard frogs were reported as occupying areas with freestanding water more often than flowing water and marshes. Habitat constituents such as forest/woods, springs, swamps, and circumstances such as no fish, permanent water, water > 2 m deep, low concentrations of particulates in the water, and a muddy substratum are not described in the bulk of the literature, and therefore were not concluded to be necessities components for leopard frogs.

Regional microhabitat similarities.—Although regional climates and habitats be very different throughout the geographic range of *R. pipiens*, the basic habitat components remain very similar. Some areas containing grass, water, marshes, and aquatic vegetation can be found even within the most arid of regions. Thus, it is no surprise that the geographic range of *R. pipiens* is so large. To understand how leopard frogs were able to arrive at suitable microhabitats within arid regions, we can examine the historical and geologic past.

Native and introduced frogs in Nevada.—Historical records indicate that there were both native and introduced populations of *R. pipiens* in the state in the early 1900s (R. McQuivey, pers. comm.). For example, journal entries made by explorers in the mid 1800s record the presence of leopard frogs in Nevada prior to the arrival of non-native American colonists (e.g., P. S. Ogden, Snake Country Journals, 1829; E. D. Perkins, Gold Rush Diary, 1849). Newspaper articles report that frog farming and importing frogs for commercial use was a profitable business in the late 1800s (e.g., Gold Hill News, 1871; White Pine News, 1872; Elko Independent, 1872; Carson Daily Appeal, 1878; Reno Evening Gazette, 1883). These reports indicate that both leopard frogs and bullfrogs were plentiful in Nevada in the late 1800s (e.g., Nevada State Journal [Reno], 1883; Silver State [Winnemucca], 1885; Mason Valley Tidings [Greenfield], 1893; Walker Lake Bulletin [Hawthorne], 1893; Genoa Weekly Courier, 1895). Documented introduced populations of leopard frogs were centered around major towns and cities, and these frogs likely hybridized with native leopard frogs if present. To explain how native populations of leopard frogs could have spread throughout Nevada, an understanding of the Great Basin's hydrographic history is necessary.

Post-Pleistocene changes.—The Great Basin, an interior hydrographic basin covering most of Nevada, has undergone large fluctuations in surface water levels since the Pleistocene (Grayson, 1993). For most of the Pleistocene, pluvial Lake Lahontan covered one fourth of Nevada, and sometime between 14,500 and 13,000 years B.P., water levels in Nevada reached their all time high (Benson, 1993). Since then, there have been at least three major fluctuations between high and low water levels (Grayson, 1993; Fig. 2). In times when the Great Basin received high precipitation, frogs (and other riparian-obligate species) would have greater opportunities to disperse widely within the state. In times of low precipitation, riparian species would have been confined to fewer bodies of water. During the period of western expansion in North America (early to mid 1800s), leopard frogs likely had been in Nevada for thousands of years.

On a smaller scale, even subtle changes in water levels can have large effects on organisms tied to water. As water levels drop, interstices of dry land between patches of wet habitat emerge and increase in size, potentially causing greater isolation from one water body to another. As the patches of wet habitat become increasingly isolated, organisms depending on that habitat may be unable to move from one water source to another. These hydrological events impede gene flow and recolonization and ultimately may lead to local extirpations. Water sources can become isolated from one another as a result of anthropogenic and natural causes. Isolation in conjunction with habitat degradation, climate change, anthropogenic disturbance, disease, or even natural population fluctuations can cause local extirpation and declines in virtually any species.

Conclusions.—Though regional climactic differences are large throughout the geographic range of *Rana pipiens*, basic microhabitat components in riparian areas tend to be similar. These microhabitats and the amphibian populations they support may be more isolated from each another in arid regions. Anthropogenic changes may have larger effects on amphibian species in arid areas than in moister regions because the isolation of water sources can occur quickly when connecting wet habitats are removed. The aridity of

Nevada and the constant changes (both natural and anthropogenic) occurring in this region likely contributed to the isolation and ultimately the decline of leopard frogs in this state.

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Table 1. Habitat components of *Rana pipiens* as reported in literature

Location	grass (wet meadow, field)	lakes, ponds, & small pools	rivers, streams, & creeks	marshes	aquatic/emergent vegetation	irrigation/roadside ditches	gravel/sand/rubble substratum	woody riparian vegetation	temp. breeding ponds	well-aerated waters	wet soil/substratum	forest/woods	burrows, crevices	lay eggs in shallow water	no fish	springs	permanent water	muddy substratum	swamps	brackish coastal water	water >2-m deep	clear water (low particulate)	Source
Colorado	√	√			√				√						√						√		Corn (1981); Corn and Fogleman (1984)
Colorado	√		√				√		√														Cousineau and Rogers (1991)
Colorado	√	√	√	√	√	√	√		√				√		√								Hammerson (1999)
eastern Canada	√	√		√			√	√															McAlpine and Dilworth(1989)
general	√	√	√	√	√						√			√		√	√		√	√			Pace (1974)
Iowa	√	√			√		√	√		√								√				√	Bovbjerg and Bovbjerg (1964)
Michigan	√	√		√	√		√	√			√	√	√										Dole (1965a,b)

Table 1. *(continued)*

Location	grass (wet meadow, field)	lakes, ponds, & small pools	rivers, streams, & creeks	marshes	aquatic/emergent vegetation	irrigation/roadside ditches	gravel/sand/rubble substratum	woody riparian vegetation	temp. breeding ponds	well-aerated waters	wet soil/substratum	forest/woods	burrows, crevices	lay eggs in shallow water	no fish	springs	permanent water	muddy substratum	swamps	brackish coastal water	water >2-m deep	clear water (low particulate)	Source
Michigan	√	√			√	√	√				√	√											Rittschoff (1975)
Minnesota	√	√	√	√		√			√				√						√				Breckenridge (1944)
Minnesota	√		√			√																	McKinnell and Dapkus (1973)
Minnesota	√	√	√	√		√			√					√	√								Merrell (1965, 1967, 1977)
Minnesota				√							√												Wiggins (1992)
Nevada		√	√																				Lindsdale (1940)
Nevada	√	√	√		√						√					√		√					Panik and Barrett (1994)
New England	√	√																					Whitlock et al. (1994)
New Mexico		√	√	√		√																	Degenhardt et al. (1996)

Table 1. *(continued)*

Location	grass (wet meadow, field)	lakes, ponds, & small pools	rivers, streams, & creeks	marshes	aquatic/emergent vegetation	irrigation/roadside ditches	gravel/sand/rubble substratum	woody riparian vegetation	temp. breeding ponds	well-aerated waters	wet soil/substratum	forest/woods	burrows, crevices	lay eggs in shallow water	no fish	springs	permanent water	muddy substratum	swamps	brackish coastal water	water >2-m deep	clear water (low particulate)	Source
North America	√	√	√	√	√															√			Behler and King (1979)
Ohio	√	√		√		√	√								√								Orr et al. (1998)
Ohio	√	√			√					√		√		√									Zenisek (1963)
Ontario			√				√			√			√										Cunjak (1986)
Ontario		√		√						√			√					√			√		Emery et al. (1972)
Ontario	√											√											Licht (1991)
Oregon	√	√		√																			St. John (1985)
Quebec	√		√	√	√			√															Gilbert et al. (1994)
Quebec	√	√				√		√															Leclair and Castanet (1987)

Table 1. *(continued)*

Location	grass (wet meadow, field)	lakes, ponds, & small pools	rivers, streams, & creeks	marshes	aquatic/emergent vegetation	irrigation/roadside ditches	gravel/sand/rubble substratum	woody riparian vegetation	temp. breeding ponds	well-aerated waters	wet soil/substratum	forest/woods	burrows, crevices	lay eggs in shallow water	no fish	springs	permanent water	muddy substratum	swamps	brackish coastal water	water >2-m deep	clear water (low particulate)	Source
Vermont			√							√			√										Ultsch et al. (2000)
western U.S.	√	√	√	√	√	√						√				√	√						Stebbins (1985); Stebbins and Cohen (1995)
Wisconsin & Grand Teton National Parks	√	√		√	√			√	√						√								Hine et al. (1981)
Yellowstone	√	√	√		√																		Koch and Peterson (1995)
SUM	23	22	15	15	13	9	8	6	6	5	5	5	5	4	4	3	3	3	2	2	2	1	

Figure 1. Putative geographic distribution of the northern leopard frog (*Rana pipiens*), the relict leopard frog (*Rana onca*), and the Vegas Valley leopard frog (*Rana fisheri*). Map was modified from Stebbins (1985).

Figure 2. Simulation of surface water coverage (blue) of the western Great Basin (a) 13,750 years B.P. (b) 12,500 years B. P. (c) 2,500 years B.P. (d) 1000 years B.P. Several cycles of high and low water levels have occurred since 14,000 years B.P. giving frogs ample opportunity to colonize and become established in many locations in Nevada.

Figure 3. The northern leopard frog (*Rana pipiens*). Specimen shown here was caught at Ruby Lake National Wildlife Refuge, Elko Co., Nevada (July 2000).

Figure 4. Frequency of habitat constituents at leopard frog locations reported in the literature. Grass, water, marshes and aquatic vegetation are the habitat components most often found in study areas supporting leopard frog populations.

Figure 1

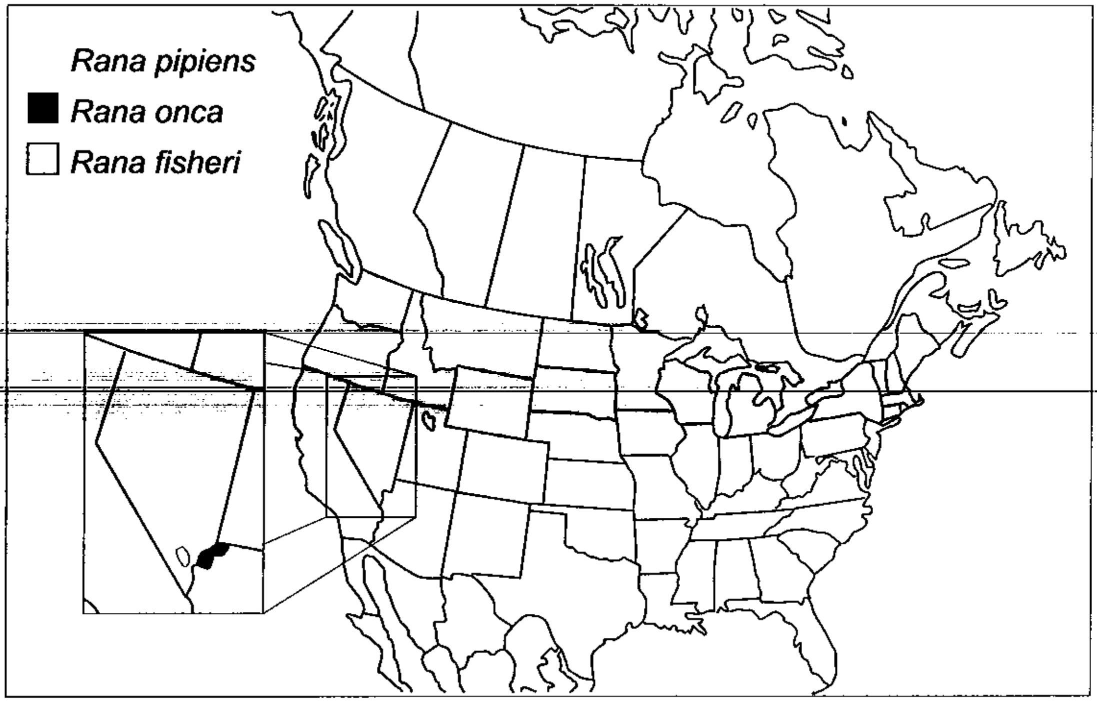


Figure 2

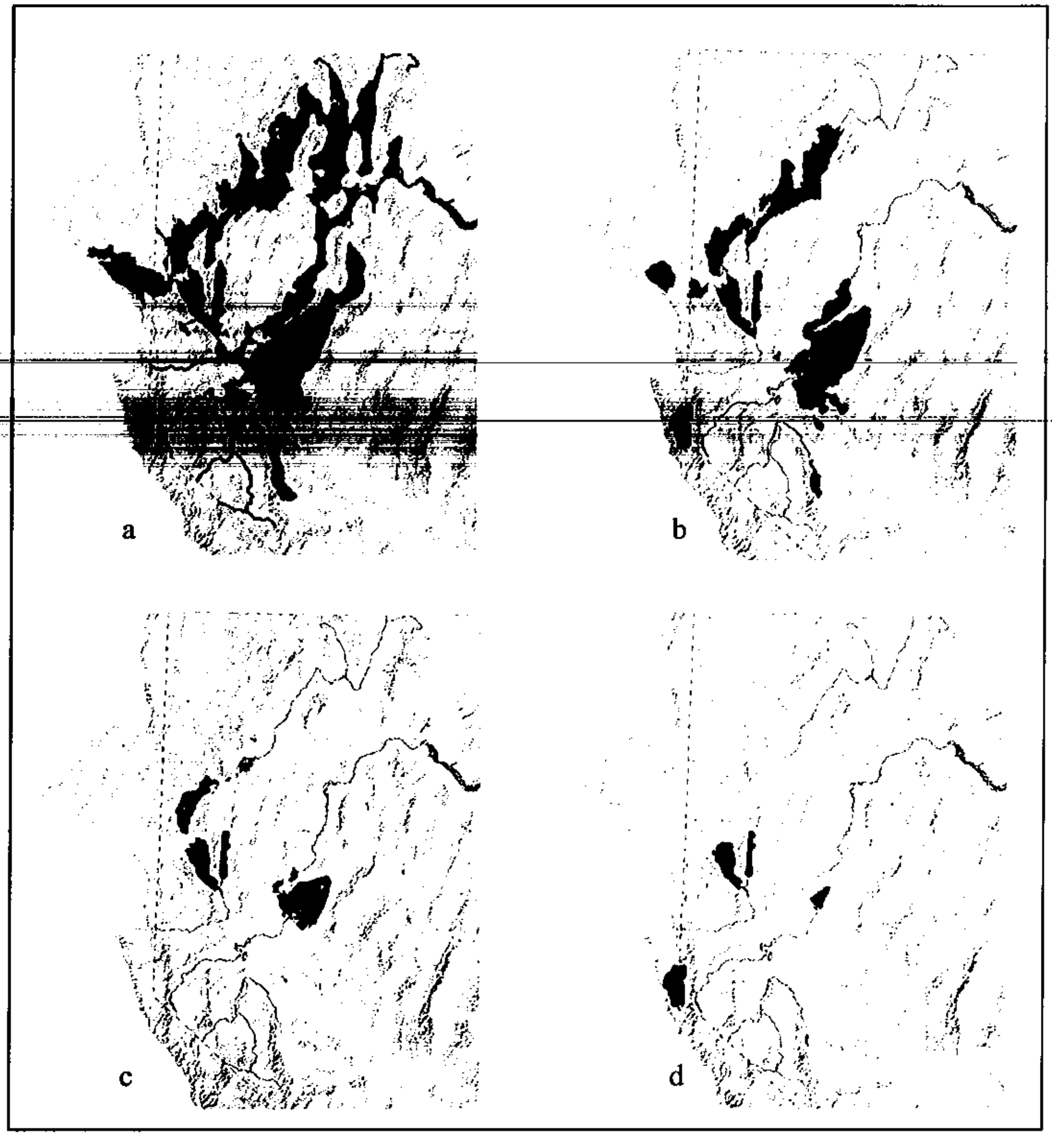


Figure 3

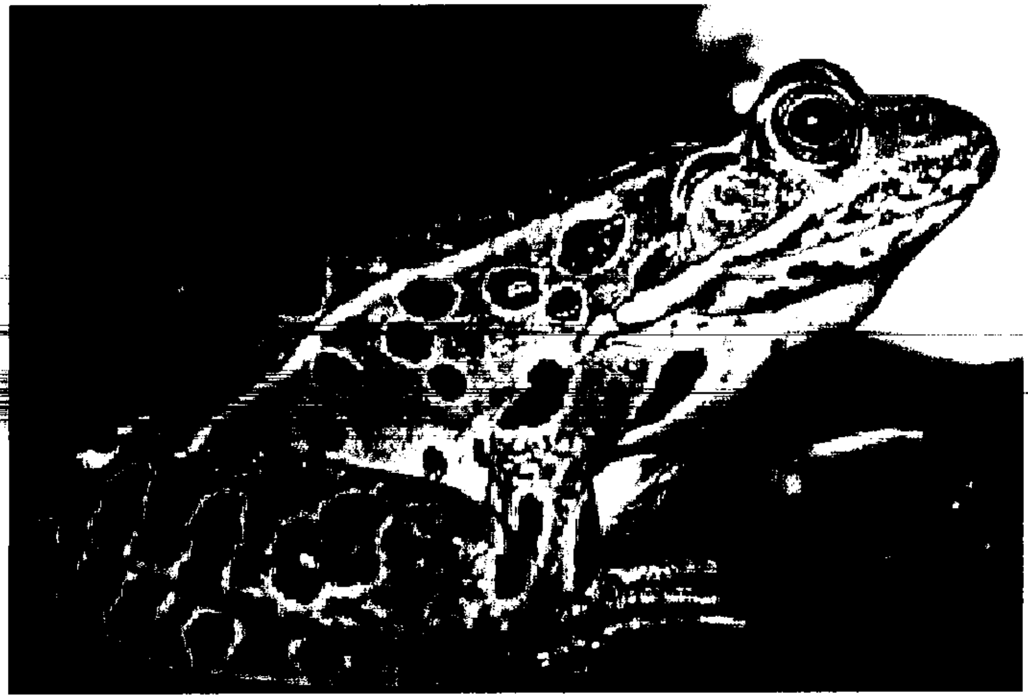
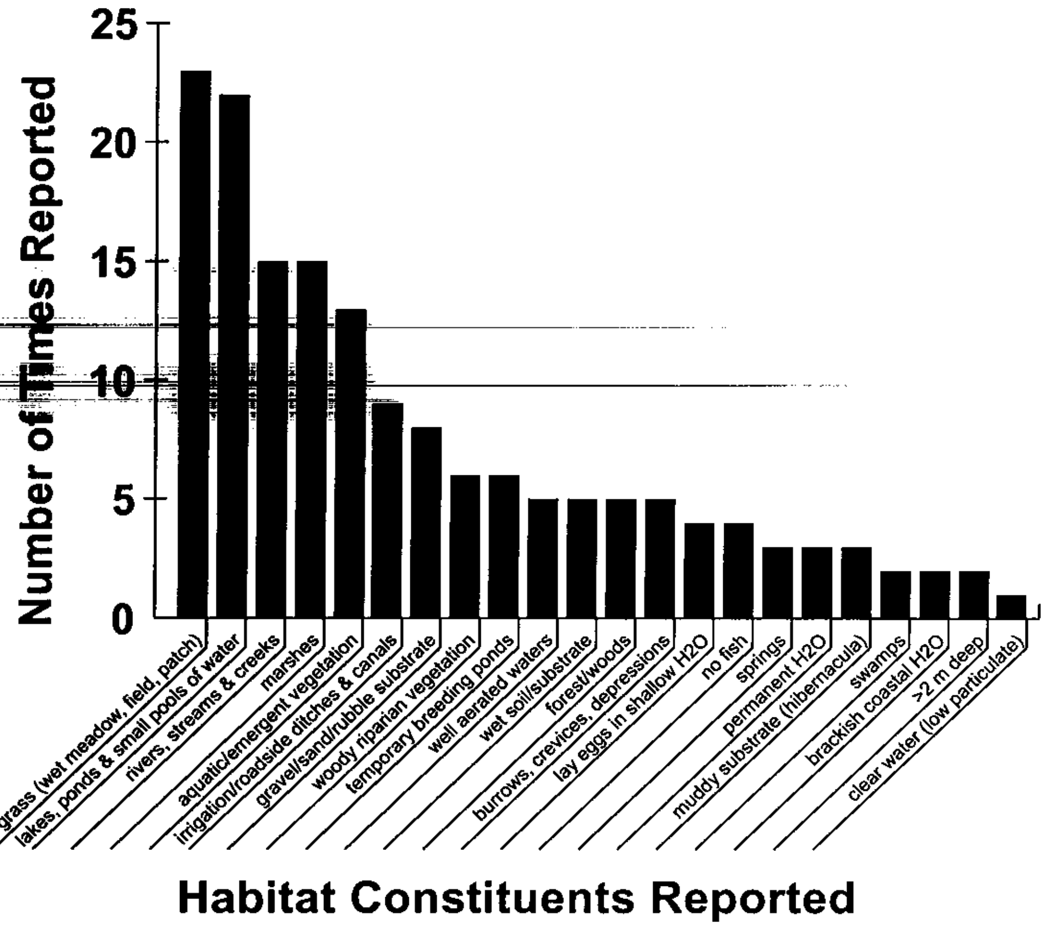


Figure 4


Number of Times Reported
25
20
15
10
5
0
grass (wet meadow, field, patch)
lakes, ponds & small pools of water
rivers, streams & creeks
marshes
aquatic/emergent vegetation
irrigation/roadside ditches & canals
gravel/sand/rubble substrate
woody riparian vegetation
temporary breeding ponds
well aerated waters
wet soil/substrate
forest/woods
burrows, crevices, depressions
lay eggs in shallow H2O
no fish
springs
permanent H2O
muddy substrate (hibernacula)
swamps
brackish coastal H2O
>2 m deep
clear water (low particulate)
Habitat Constituents Reported

Chapter II: The Status and Distribution of the Northern Leopard Frog (*Rana pipiens*) in Nevada

Abstract

Leopard frog populations are declining. Declines in the western United States appear to be more severe than declines east of the Rocky Mountains. The northern leopard frog (*Rana pipiens*) was once widely distributed across the state of Nevada, however, repeated surveys of historical sites revealed that leopard frogs have severely declined in the state. Analyses have determined that suites of habitat variables where leopard frogs are present, are statistically different from habitat where leopard frogs were not found. However, some sites without leopard frog populations appear to be similar to sites where frogs are extant for three possible reasons: (1) leopard frogs live in areas that have the same basic habitat components as sites without frogs, but only specific combinations of these components can support frogs, (2) sites without leopard frogs may be able to support leopard frog populations, but do not have the characteristics necessary for long term persistence, and (3) sites with leopard frogs are vulnerable to extirpation. Threats to leopard frogs in Nevada include habitat degradation and negative interactions with introduced species. Long-term population monitoring, and captive-breeding followed by repatriation is recommended for the persistence of leopard frogs in Nevada. Additional experimental research to gain evidence for decline causes in this state is encouraged.

Introduction

Leopard frog (*Rana pipiens* complex) populations appear to be declining on a continental scale (Jennings and Hayes, 1994; Drost and Fellers, 1994; Leonard et. al., 1999). The recent proposal for listing of the Chiricahua leopard frog (*R. chiricahuensis*) in southeastern Arizona as well as documented declines of northern leopard frogs (*R. pipiens*) in areas of Colorado, Wyoming, and Idaho have drawn attention to western United States as a region warranting immediate attention (Corn and Fogleman, 1984; Clarkson and Rorabaugh, 1989; Corn et al., 1989; Koch and Peterson, 1995; Humphrey and Fox, 2000; Ryan, 2000). Leopard frogs were the most common amphibians in Nevada when Jean Lindsdale compiled state records for amphibians and reptiles in the late 1930s. However, leopard frogs were found in only 18 of 97 historical locations during repeated surveys in 2000 and 2001. Three of these historical sites with leopard frog populations were in southern Idaho. These data suggest a severe decline. Habitat degradation and negative interactions with introduced species (e.g., tall white top and bullfrogs) are likely causes of these declines. Because leopard frogs require both terrestrial and aquatic habitats to complete their life cycle, these frogs may be useful as indicators of riparian health. This study was conducted to provide baseline information on *R. pipiens* in Nevada that is necessary for hypotheses testing and management of this species. My objectives were to determine the current status and distribution of *R. pipiens* in Nevada, compare habitat where frogs were found to where frogs were not found, and investigate likely causes of declines. This was accomplished by conducting repeated surveys of historical and random sites, recording habitat parameters and disturbances found at each site, and statistically analyzing habitat variables.

Amphibian decline.—Evidence for global amphibian declines has been collected since the 1970s (Barinaga, 1990; Blaustein and Wake 1990; Wyman, 1990; Wake, 1991; Corn, 1994; Drost and Fellers, 1996). Hypotheses proposed to explain declines include over harvesting, habitat loss or degradation, pollution, ozone depletion, global climate change, anomalous weather patterns, introduced exotic species, and disease. However, no single factor appears to explain patterns of decline, and many extinctions remain enigmatic. For example, the gastric brooding frog (*Rheobatrachus silus*) of eastern Australia, discovered in 1973, had mysteriously disappeared by 1981 (Tyler, 1989). Likewise, the golden toad (*Bufo periglenes*) of Costa Rica has not been seen since 1989 (Crump, 1992). The disappearance of both of these species was surprising because their habitats remained seemingly undisturbed. Because so many populations have unknown extinction causes, it is important to study similar species before they are on the brink of extinction to determine the process by which extinction is occurring, and deduce ways to prevent future declines.

Amphibians in western United States appear to be more vulnerable to decline and extinction than those east of the Rocky Mountains (Corn, 1994; Stebbins and Cohen, 1997). For example, leopard frogs have declined in Arizona, Colorado, Idaho, Montana, New Mexico, Oregon, Washington, and Wyoming (Gibbs et al., 1971; Hammerson, 1982; Corn and Fogleman, 1984; Hayes and Jennings, 1986; Clark et al., 1993, in

Stebbins and Cohen, 1997; Clarkson and Rorabaugh, 1989; Stebbins and Cohen, 1997; Leonard et al., 1997).

Recent studies suggest that leopard frogs have declined, been locally extirpated, or have gone extinct in Nevada (Corn, 1994; Panik and Barrett, 1993). There are two endemic species of leopard frogs in Nevada, known only from isolated drainages near Las Vegas, the Vegas Valley leopard frog (*Rana fisheri*), and the relict leopard frog (*R. onca*). *Rana fisheri* has been considered extinct since the 1940s, whereas *Rana onca* once thought to have gone extinct, was recently rediscovered by Jennings in 1993 (Corn, 1994). A third species of leopard frog, the northern leopard (*Rana pipiens*), known from across most of the northern U.S. and southern Canada (Fig. 1), was reported to be the most common and widespread amphibian in Nevada in the early part of the 20th century (Lindsdale, 1940). Until the 1970s, *R. pipiens*, *R. onca*, and *R. fisheri* were considered a single species. Recent anecdotal evidence suggests *R. pipiens* is no longer abundant in Nevada (Panik and Barrett, 1993). Despite these putative declines, a statewide study of the status and distribution of northern leopard frogs had not been undertaken in Nevada.

Study Objectives

This study was designed to determine the current status and distribution of *Rana pipiens* in Nevada and investigate likely causes of any declines. There were three objectives of this study. First, the status and statewide distribution of *R. pipiens* was determined by surveying both historical and randomly chosen wetlands for leopard frogs. Anecdotal evidence and preliminary data indicated that leopard frogs are no longer as abundant in

Nevada as they were in the 1930s. Contemporary field guides (Stebbins, 1985; Behler and King, 1998) show *R. pipiens* distributed across the entire state. However, both historical records and anecdotal evidence indicated that *R. pipiens* distribution is neither continuous nor widespread. Second, likely causes of declines were investigated through habitat assessments, and presence of disturbances. Agricultural and livestock practices, presence of exotic species, and water depletion through anthropogenic and natural changes are possible causes of leopard frog declines in Nevada. Third, differences between sites where frogs are found and where they are not found were examined to identify habitat components that predict leopard frog presence.

Methods

Historical Data

Twenty-two major U.S. museum collections were queried for records of *Rana pipiens* in Nevada (Appendix 1). Nine museums provided a total of 166 records. Locations from the literature and biologists' field notes provided 12 additional historical records, and recent observations of leopard frogs provided three more localities in Nevada. Furthermore, the number of sites with extant leopard frog populations was enhanced, by including three sites in southern Idaho, near the Nevada border, with habitat comparable to Nevada.

Identification of Historical Sites

Records with identical locality descriptions, but with specimens deposited in different museum collections, were considered a single historical site. Records determined to be

Rana onca, based on collection date and locality information, were excluded from this study. *Rana onca* was not recognized as distinct from *R. pipiens* until 1971 (Platz and Mecham, 1979), and the distributions of the two species do not appear to overlap (Stebbins, 1985; Fig. 1). Records lacking sufficient locality information (i.e., those giving only a County, or a town name, and those that I was unable to locate in the field) were excluded from analyses. Locations that had connecting habitat were considered to be the same site. These restrictions left a total of 97 distinct historical sites of *R. pipiens* for this study. These sites were located on a digitized map of Nevada and Idaho (Biological Resources Research Center, University of Nevada, Reno; www.brrc.unr.edu) using Arc/View v. 8.0, and map coordinates were recorded from the map for sites lacking this information in original records. All site localities were confirmed using a Garmin eTrex (Olathe, Kansas) GPS device in the field. Information on land, water, and trends in frog populations was obtained for several areas in Nevada by talking with biologists, park rangers, ranchers and private land owners.

Random Sites

An additional 20 wetland sites were chosen randomly from GIS coverages (not included in historical records). These sites were surveyed twice in 2001 to look for previously undocumented leopard frog populations. Site locations and descriptions, number of visits, and average number of *R. pipiens* seen are listed in Appendix 2. The random sites were generated using Excel v. 8.0 (Microsoft Corporation) and ArcView v. 8.0 (ESRI) software. In ArcView, a 12-m buffer around Nevada's lakes and streams was created. This buffer distance was chosen because terrestrial habitat at distances further than 12 m from water are likely to be dry given the general aridity of the Great Basin. The buffer coverage was merged with a map of Nevada's wetlands, riparian habitat and wet meadows, selected out of the Nevada vegetation database (Utah Cooperative Fish and Wildlife Research Unit, 1996), to create one polygon coverage of likely frog habitat within the state. Two thousand four hundred random coordinates within Nevada were generated in Excel and converted to an archived shape file within my map. The 20 wetland locations were points that intersected with the wetland coverage.

Additional Sites

Sixteen newly found records of historical locations were surveyed in 2001. These records were found after the first field season, in unpublished reports and field notes of biologists working in Nevada. The sixteen sites were surveyed once or twice depending on pre-determined criteria (see survey techniques).

Surveying and Characterization of Historical Sites

Historical Sites.—All historical sites were initially surveyed in 2000. Sites with extant frog populations in 2000 were visited once in 2001 to ensure there was no drastic population fluctuation from the previous year. Sites with suitable habitat for *Rana pipiens* but in which no frogs were found in 2000 were revisited twice in the subsequent field season (2001), during the breeding season (April–May 2001) and again in the summer (June–August 2001), to determine if individuals had escaped detection in 2000. Repeated surveys are recommended to increase the likelihood of finding current populations from historical data (Corn, 1994; Reed, 1996). Suitable habitat was defined by the presence of specific minimum habitat requirements as reported in the literature (Merrell, 1977; Hine et al., 1981) and the results of a habitat assessment conducted for this study (Chapter 1). The smallest patch of terrestrial habitat in which leopard frogs were encountered in 2000 was 100 m^2, and was therefore used as a minimum-area criterion for terrestrial habitat of leopard frogs. The four most frequently reported habitat variables at leopard frog sites were grass, freestanding water, flowing water, and marshes. Therefore, sites having freshwater and at least 100 m^2 of wet meadow, grassy field, or marsh were considered suitable habitat regardless of habitat perturbations (e.g., exotic species, agriculture, livestock, etc.). This method produced a subset of 38 of the 97 historical sites where suitable leopard frog habitat currently exists.

Sites were surveyed by trained, experienced field technicians, either alone or in pairs. Thirty-three percent of the surveys were made by one person, and the remaining 67% were surveyed in pairs. All surveys were conducted during the daylight hours and lasted from 0.6–2.8 h. Nighttime surveys are not as successful as daytime surveys in general (Dole, 1965b; Hine et. al., 1981; this study), and a single site (Ruby Marsh) surveyed both during daylight and after dark during this study revealed 19 frogs during the day and one frog at night.

The area surveyed at each historical site in 2000 depended upon the available habitat. All sites in 2001 were surveyed by walking 2 km in each direction up and downstream from documented locations and, if conditions allowed, by boating the stretch of river or pond perimeter and surveying the emergent vegetation to search for populations that may have migrated from historical locations. Two kilometers was chosen because this distance exceeds the dispersal distance of most leopard frogs (Dumas, 1964). Although a distance of 2 km may not account for decades of migration, it was biologically meaningful, especially if dispersal was recent. Drought conditions within the Great Basin in the last few years may have forced frogs to migrate up drainages.

Random and Additional Sites.—Random sites were initially surveyed in 2001. These sites were visited twice if they had water. If no water was found at the random site, the nearest water to this site was surveyed twice (N = 10). If there was no water within several kilometers of the site, the locality was visited only once (N = 3).

The locations from the 16 new records were surveyed one or two times during 2001 in accordance with methods used for 2000 (i.e., sites without frogs were resurveyed if they had water and 100 m^2 of wet meadow, field, or grass). Leopard frogs were found in eleven of the 16 new locations during the first survey and therefore were not resurveyed. Two of the remaining new locations were visited only once because one had no water at or near he location, and another site had only puddles of water, no terrestrial habitat, and heavy road-building machinery working throughout the area. The remaining three locations were visited twice.

Survey techniques.—Surveys were conducted throughout the entire active season (late March–early October) to facilitate visits to all sites at least one time during the life cycle of *R. pipiens* when they would be most conspicuous (e.g., breeding in early spring, and metamorph dispersal in late summer). During the spring (late March–May), *R. pipiens* are concentrated around breeding areas where males call for mates (Zenisek, 1963; Merrell, 1977; Hine et. al., 1981), and are therefore easier to find than during the summer when they disperse to grassy wet meadows and other damp places (Dole, 1967). *Rana pipiens* tadpoles metamorphose and disperse in late summer (July–August) and can be found in great numbers at the perimeters of water bodies at this time (Bovbjerg and Bovbjerg, 1964; Merrell, 1977). Adult *R. pipiens* congregate around water bodies again in early fall (September–October) just before hibernation (Zenisek, 1963; Pace, 1974).

Sites were surveyed for frogs by walking back and forth along banks and in grassy and/or marshy areas. Dip nets were swung from side-to-side to flush out frogs that might be hidden in the vegetation (Dole, 1965a). Frogs and tadpoles were captured by hand or net. Aquatic breeding areas were visually surveyed for tadpoles and egg masses, and aquatic vegetation was probed to displace hiding tadpoles. Frogs were sexed (males have enlarged thumb pads and vocal sacs), weighed (± 0.5 g), and their body length measured (SVL ± 1 mm). Tissue samples (toe tips) were collected for genetic analysis (not part of this study). Up to five adults and tadpoles per population were taken as vouchers and were deposited in the University of Nevada, Reno Zoology Museum.

Habitat data.—Habitat components of each site were measured or estimated, and locations were photographed. Measurements and estimates made at each of several visits were averaged together to generate a single measurement for each variable for each site. Total area surveyed was measured by counting paces made through the area, or along each side of a water body converted into kilometers (1 pace [2 steps] ≈ 1.5 m). This distance was then multiplied by the width of the area covered. If more than one person surveyed an area, each person calculated their own area searched and the areas from each person were combined. Areas of large water bodies were estimated by pacing off the average width and length of the pond, lake or stream and multiplying the two distances. In areas with large expanses (≥100 m^2) of primarily one type of vegetation (e.g., grassy field, and cattail marsh), estimates were made of the area searched by the amount of paces made through that area. In general, areas (>100 m^2) were estimated instead of measured because we felt that precise measurements of large areas were unimportant biologically, and sometimes impossible to obtain. For example, areas that were extremely large (i.e., those having many hectares of marsh), or impossible to walk (i.e., those with deep mud, or with vegetation too dense to walk through), were either visually estimated on site, or estimated later using maps (map scales ranged from 1:24000–1:250,000). Dominant vegetation types were recorded and estimates of percent cover of vegetation over of the total area searched were made. Conductivity, pH, and water temperature, were measured at each site ca. 20 cm below the water surface at the waters edge. Bank height, flow, water depth, stream width and small pond areas were also measured. Cloud cover, wind velocity, and air temperature were recorded at the time of each search, and any weather changes throughout the time of the survey were noted (e.g., increased cloud cover, and precipitation). Sightings or sign of potential predators, competitors, or other animals suspected to affect frogs or their habitat were noted at each site. Habitat modifications by anthropogenic causes were also noted.

Data Analyses

Disturbance types.—Habitat disturbances were recorded at each site and compiled to determine the most prevalent type at sites with frogs and sites without frogs (Fig. 2). The 97 historical sites, but not the random sites, were included in this analysis. Random sites were excluded because their purpose was to determine if undocumented leopard frog populations existed. No undocumented populations were found, therefore it was uncertain that habitat at the random sites was comparable to habitat of historical sites. Historical sites are biologically meaningful to leopard frogs because we know that frogs inhabited these locations at some point in time. Disturbance types were tallied for each historical site and a histogram was created to illustrate frequency of occurrence for each category of disturbance. Sites were permitted to be included in more than one category if more than one disturbance type was present. A miscellaneous category was created to account for various uncommon disturbance types such as mowing, construction, trash or an undetermined cause manifested in the absence of vegetation.

Principal components analysis.—Habitat measurements from historical sites were standardized, and used in a principal components analysis (PCA). Only those variables considered to be biologically meaningful to leopard frogs were used in this analysis (Appendix 3). Many of these variables were not independent from one another, therefore, a PCA was used to create a set of new variables (factors) that were uncorrelated, for use in subsequent analyses. Principal components analysis combines the input variables linearly, such that the first combination explains the greatest amount of the variance within the data set, the second combination describes the next greatest amount without overlapping the first, and so on, until all the variance is accounted for in the form of multiple principal components. Every principal component need not be used in subsequent analyses because most of the variation within the data set will be accounted for by the first few.

There are several ways of deciding how many principal components should be used in subsequent analyses (Hair et al., 1987). It is unclear if one method is better than another, however, the most often used method is to use only those principal components with eigenvalues ≥ 1 (Gutzwiller and Barrow, 2001). The PCA was computed in StatView® v. 4.5.1 (Abacus Concepts) statistical software using 22 continuous variables and 97 sites. I used the 75% variance rule, which was the program default to create factors (Roth, 1995). The resulting seven orthogonal factor scores were used as the principal components (Hair et al., 1987). The seven principal components were then used as independent variables in a logistic regression (described below).

A correlation matrix of both the original variables and PCA factors was created in JMP® v. 4.0.4 (SAS Institute) to determine which among the original variables were correlated with each factor. All coefficients with values of ≥ 0.6 were considered correlative (showing a correlation between the original variable and the factor), which indicated the important components of each factor (Table 1). By determining what each factor represented (e.g., orthogonal factor 1 became the habitat-size factor), conclusions could be made regarding what was biologically meaningful with respect to the habitat data collected. The correlation value of ≥ 0.6 was chosen because it represented the value at which variables did not overlap, therefore permitting a correlative habitat description of each factor (e.g., habitat size was the main correlative variable for only factor 1, even though it was also represented in some portion in all of the other factors; Hair et al., 1987).

Logistic regression.—A logistic regression (JMP® v. 4.0.4; SAS Institute) was used to compare habitats between sites populated and unpopulated by leopard frogs. The seven orthogonal factors or principal components (with eigenvalues ≥ 1) from the PCA were used as independent variables and the presence/absence of frogs was used as the dependent variable for this analysis.

Multi dimensional scaling.—Ordination techniques were used to reveal patterns of similarity/dissimilarity in habitat characteristics among sites. Ordination was computed with NT-SYS-pc (Rohlf, 1995). Each variable was standardized by subtracting its minimum and dividing by its range. A Bray-Curtis distance matrix was computed based on habitat, which ordered sites according to similarities to other sites across all habitat variables. A principal coordinates analysis was conducted as a preliminary ordination, and multidimensional scaling was used to improve the representation of the distance matrix. The two resulting habitat axes were then rotated so that the first axis was aligned maximally with the presence versus absence of frogs. A variance ratio test was also conducted using the rotated principal coordinate axes from above. The variances of these axes were calculated in Excel and sites having frogs were compared statistically to sites without frogs along both axis one and two, using an F-test. A Mann Whitney-U test was used to statistically compare the means of axis one for sites with frogs and from without frogs, and the analysis was performed in StatView® v.4.5.1 (Abacus Concepts).

Dendrogram.—All historical sites were used in a hierarchical cluster analysis (using the Ward method in JMP® v. 4.0.4, SAS Institute). From the dendrogram, I made general characterizations for each group of sites and labeled them according to their known similar properties. The resulting clusters were also mapped to show where clusters fall geographically.

Results

Status and Distribution of leopard frogs in Nevada

Historic and current distribution of leopard frogs.—Data compiled on historical *R. pipiens* populations indicates that the historical distribution of these frogs is different from the distribution shown in field guides. According to historical records, leopard frogs were never documented from central Nevada (Fig. 3). This study also shows that the current distribution of leopard frogs in Nevada is much smaller than the historical distribution (Fig. 4).

Sites with leopard frogs.—Of 117 sites surveyed in 2000–2001, leopard frogs were found at 18 (15%) locations (Appendix 2; Fig. 5). No leopard frogs were found in extreme northwestern (four sites), or central (seven sites) Nevada. The majority of extant populations were found in eastern Nevada, especially in Spring and Lake Valleys. Six populations in these valleys were geographically isolated by large expanses of dry uninhabitable areas. However, because of the presence of numerous springs and connecting habitat, there is likely a network of populations throughout these valleys. Only five of 79 (6%) historical locations taken from museum records had frogs. Sites described in literature and field notes provided eight of 12 (67%) locations with frogs, and personal communications added two of three (67%) locations with frogs. The three sites visited in Idaho all had frogs. Three of four sites with leopard frogs in 2000 also had leopard frogs in 2001. These two-year sites were Ruby Marsh, Ferguson Springs, and the John's property in Wadsworth. At the site south of Minden in which only a single leopard frog was seen in 2000, no leopard frogs were seen in 2001. At three historical sites in which no leopard frogs were found in 2000 (Ambrosetti Pond Ranch, Dead Ox Wash, and Zgraggen Ranch), one or two frogs were seen at each site in 2001.

Eight additional occupied sites were found by surveying previously unsearched areas which were described in field notes, or from reports and personal communication (Hovingh, Bear, and Sada, pers. comm.). All eight sites had standing water and at least 100 m^2 of wet meadow, field, or marsh, (see Appendix 4 for more detailed site descriptions). Three locations in Idaho with *Rana pipiens* populations were visited and habitat data were obtained from these sites. Leopard frogs inspected at all sites throughout Nevada and Idaho appeared to be healthy.

Potential threats.— Fifty-three (55%) sites had fish large enough to be predators of frogs, tadpoles or eggs, and 46 (47%) had predatory birds. Forty-one (42%) sites had cattle or evidence of cattle at the time of the search. Snakes were seen at 26 (27%) sites.

Amphibians (other than leopard frogs) were seen at 26 (27%) of the historical sites, 24 (25%) of the amphibian populations were bullfrogs. Seven (7%) of the historical sites were completely dry.

Habitat analysis

Physical characteristics.—Elevations ranged from 945–2250 m. All 97 historical sites had air and water temperatures within the range acceptable for leopard frogs to be active, and all sites were located within the documented elevational range of leopard frogs (Stebbins, 1985). Sites with frogs had pHs ranging from 6.3–8.8 ($\chi = 8.1$, SD ± 0.75), and sites without frogs had pHs ranging from 6.7–10.8 ($\chi = 8.0$, SD ± 0.84). Conductivities ranged from 108–954 ($\chi = 479$, SD ± 253) at sites with frogs and from 28–6670 ($\chi = 640$, SD ± 1012) at sites without frogs. Flow ranged from 0.0–0.4 m/s ($\chi = 0.04$, SD ± 0.10) at sites with frogs and 0.0–1.0 m/s ($\chi = 0.16$, SD ± 0.23) at sites without frogs.

Types of disturbance.—All sites had at least one type of obvious disturbance. Various forms of habitat degradations were found at 72% of sites without frogs and 78% of sites with frogs. This was the largest category for both groups, which included disturbances that did not fit in tot the other categories. Percentages of each disturbance are reported at sites without frogs versus sites with frogs as follows: fragmentation (67%, 22%); erosion (57%, 44%); no water or drying up (37%, 11%); agriculture and irrigation (37%, 72%); human recreation (35%, 22%); urbanization (34%, 6%); grazing (30%, 44%); bullfrogs (25%, 22%); tall white top and tamarisk (13%; 22%); and high predator density (5%, 17%; Fig. 2). Two sites with bullfrogs and some grazing each revealed a single leopard frog (Minden and Ambrosetti Pond Ranch), and a third site with bullfrogs and some grazing supported a leopard frog population (the John's property). Two sites (Ruby Marsh and Gray's Lake) are wildlife refuges with human recreation and a high predator density, (predators included trout, bass, shore birds, and ducks), supported populations of leopard frogs. One isolated site with disturbed terrestrial habitat and evidence of human recreation supported an extremely large and dense population of leopard frogs (Ferguson Springs) within two small areas of ≈ 2500 m^2 each.

Principal components analysis.— The result of the PCA was highly significant ($P = <0.0001$). The analysis produced 11 principal components (or factors), of which seven had eigenvalues of ≥1.0. These seven factors accounted for 74% of the total variance within the original variables [factor 1 (25.5%), factor 2 (15.8%), factor 3 (9.9%), factor 4 (8.4%), factor 5 (5.2%), factor 6 (4.8%), factor 7 (4.3%)]. The correlations show that factor 1 is primarily made up of habitat size, open water size, wet ground size, and conductivity; factor 2 minimum bank height and average bank height; factor 3 cobble substratum, and rocky substratum; factor 4 elevation, and longitude; factor 5 latitude; factor 6 concrete; and factor 7 algal mats, and emergent vegetation (Table 1). Factors were evaluated according to the above correlations as follows: factor 1 corresponds primarily to potential habitat size, factor 2 to bank height, factor 3 to substratum, factor 4 to geographic location, factor 5 to latitude, factor 6 to concrete, and factor 7 to aquatic vegetation.

Logistic Regression.— Habitat at historical sites where leopard frogs are extant is statistically different from historical sites without leopard frogs ($P < 0.0001$). Four of the seven habitat factors were also significant. These included habitat size ($P = 0.0406$), bank height ($P = 0.0025$), geographic location, ($P = 0.0165$), and aquatic vegetation ($P < 0.0001$), but not substrata ($P = 0.1237$), northing ($P = 0.1856$), or concrete ($P = 0.2788$).

Multi-dimensional scaling.—The scatter plot of historical sites indicates that sites having extant populations of leopard frogs cluster, and are more similar to one another, than are sites without leopard frogs (Fig. 6). The main separation between sites with frogs and sites without frogs was found along axis one, as leopard frogs sites were statistically different from sites without frogs (U = 164; $P < 0.0001$). This plot also shows that sites with leopard frogs fall within the range of sites without frogs (i.e., sites without leopard frogs appear to be more variable than those with extant frogs). The variances of the principal coordinate axis one and axis two were statistically different ($F = 3.824$, $P_{two\text{-}tailed} = 0.003$; $F = 3.741$, $P_{two\text{-}tailed} = 0.003$) for historical sites without leopard frogs ($\sigma^2 = 0.588$; N = 79) and for sites with leopard frogs ($\sigma^2 = 0.154$; N = 18). This indicates that sites without leopard frogs are significantly more variable along both axes than those with leopard frogs.

Dendrogram.—The hierarchical cluster analysis produced 11 main clusters, which grouped sites according to their overall habitat similarities (Fig. 7). Clusters represent general site characteristics and are grouped from top to bottom by color. Cluster 1a (red) contains eight sites, three of which had leopard frogs. Sites within this group were located in western Nevada, and had small damp areas with shallow pools or ponds. Cluster 1b (red) contains five sites, one of which had leopard frogs. Sites in this cluster were located in western Nevada, had large areas of shallow water, and patchy terrestrial habitat, with the exception of site 99, which had no suitable terrestrial habitat. Cluster 2 (light blue) contains ten sites, one with leopard frogs. These sites had little or no terrestrial habitat and isolated springs or large bodies of water. Cluster 3 (green) contains four sites, three of which had leopard frogs. This site is characterized by large areas of wet grass with adjacent large bodies of water. Cluster 4a (dark blue) includes four sites, none of which had leopard frogs. This site is characterized by wet grassy areas. Cluster 4b (dark blue) has two sites, also lacking leopard frogs, and both of these sites lack terrestrial habitat. Cluster 5 (purple) has three sites, all of which had leopard frogs. Each of these sites contains small isolated springs. Cluster 6 (green) includes four sites, which had no leopard frogs. These sites had slow moving creeks with either down-cut or flattened banks, and little or no suitable terrestrial habitat. Cluster 7 (yellow) has five sites, none of which had leopard frogs. This site is characterized by pools with adjacent flowing water, and patchy wet grassy areas, with the exception of site 112, which had no grassy areas. Cluster 8 (dark blue) has six sites, none of which had leopard frogs. This cluster is characterized by no suitable terrestrial habitat and human-altered aquatic habitats. Cluster 9 (green) includes two sites, none of which had leopard frogs. These sites are distinct sections of the same creek, characterized by dry substratum and no suitable terrestrial habitat. Cluster 10 (purple) contains three sites, none of which had leopard frogs, and this cluster is characterized by large areas of human-altered aquatic habitat, and no suitable terrestrial habitat. Finally, cluster 11 (orange) includes a single site lacking leopard frogs. This was the only cemented ditch surveyed.

There were two main clusters, one that included some sites with frogs, and the other that had no sites with frogs. These two main clusters were mapped, and both frog and non-frog sites fall throughout Nevada. Therefore there is no evidence of a geographic reason for the presence/absence of leopard frogs on this level. However, ten smaller clusters within the two main clusters were also mapped. On this finer scale, the map shows that some sites cluster geographically (Fig. 8).

Discussion

Status and Distribution

Historic and current distribution of leopard frogs.—The historical distribution of leopard frogs based on records I collected, was determined to be smaller than previously reported (Stebbins, 1985; Fig. 3). However, the current distribution determined from this study, is both considerably smaller, and less continuous than the historical distribution (Fig. 4).

Sites with leopard frogs.—Leopard frogs appear to have declined in Nevada over the past 70 years. Leopard frogs reportedly used to be found over much of Nevada, but of the 114 sites in this study, *R. pipiens* were found in only 15 locations (excluding the three Idaho sites). Six sites with leopard frogs were found in the same valley. These sub-populations may function as a metapopulation because numerous neighboring springs in this valley likely allow occasional gene flow between populations, therefore enabling populations to persist through extinction and recolonization events. All other extant populations in Nevada did not appear to have the potential for a metapopulation dynamic. It is unlikely that any substantial populations of leopard frogs exist in undocumented areas because no *R. pipiens* were found at 20 random locations determined to have characteristics suitable for leopard frogs. It is also unlikely that any substantial populations of leopard frogs were missed at historical locations because multiple surveys of migration pathways were conducted. If populations are so small that they are undetectable with multiple surveys, then these populations may be on the verge of extirpation.

Comparison of Frog and Non Frog Sites

PCA and logistic regression.—This analysis provided seven uncorrelated variables for the logistic regression. Habitat from historical sites with extant frogs, as represented in these seven uncorrelated factors, was statistically different from sites where leopard frogs were not found. This result suggests that historical sites where leopard frogs were not found, no longer have the necessary combination of habitat variables to support them. Some of the sites without frogs had abundant damp grass adjacent to large areas of water, and appeared to provide suitable habitat for leopard frogs. Conversely, some of the sites with leopard frogs had habitat that did not appear to be well suited for leopard frogs (e.g., little grass, disturbed habitat).

The logistic regression indicated that the best predictors of leopard frog presence were factors one, two, four, and seven. The variables that were correlated with the significant factors included habitat size, bank height, geographic location, and percent cover of algal mats, and emergent vegetation.

There are some biological reasons why factors one, two, four, and seven should be significant predictors of where leopard frogs are found. Habitat size was expected to be a significant predictor of leopard frog occurrence. The more habitat available, the more places there are for frogs to live. Bank height, making up most of factor two, is likely associated with the presence of leopard frogs because *R. pipiens* are both terrestrial and aquatic. As such, leopard frogs require access between these two environments. As previously described, high banks would pose a barrier to normal daily and seasonal activities of leopard frogs. Emergent vegetation is used by leopard frogs to anchor their egg masses, which keeps them within the selected oviposition site where conditions are presumably most suitable for development. Algal mats may be important to *Rana pipiens* in Nevada for several reasons. Leopard frogs were observed sitting on algal mats where they could presumably absorb moisture. The largest population of leopard frogs found was confined to an area were there were more frogs than the terrestrial habitat could perceivably support. These frogs appeared to compensate for the lack of moist terrestrial habitat by using algal mats. Frogs were also seen resting on algal mats in Spring Valley (N. Millick Spring) where the grass had been grazed to a short stubble. Tadpoles in advanced stages of development are known to rest on top of algal mats presumably to breathe, because of the transformation of the respiratory system metamorphosis (Bovbjerg and Bovbjerg, 1964). I also observed this behavior in tadpoles at Grays Lake, Idaho. Algae is also food for tadpoles (Stebbins and Cohen, 1995).

Several factors, which were expected to be significant, were not good predictors of leopard frog habitat. For example, because leopard frogs hibernate under water (Bovbjerg and Bovbjerg, 1964; Emery, 1972; Pace, 1974), the availability of deep water was expected to be important to *Rana pipiens*. Leopard frogs should be found in places where they could hibernate in water that was deep enough so as not to freeze solid, or experience anoxic conditions over winter. However, results suggest that water depth is not a strong predictor of the presence of leopard frogs. Substrate was also expected to be important to leopard frogs because this component may play an important role for cover and hibernacula for leopard frogs. Not only do leopard frogs hibernate in the mud at the bottoms of lakes and streams (Bovbjerg and Bovbjerg, 1964; Emery, 1972), but leopard frogs also jump into water and seek cover in mud or silt as a means of predator avoidance. Leopard frogs select oviposition sites in areas of little or no water flow (Pace, 1974). Flowing water could dislodge eggs and wash them to areas where conditions may be suboptimal for development, and therefore was expected to be significant, however this could not be confirmed for the study.

The limitation of PCA with respect to my study is that if there are many variables measured within a site that have a value of zero, or with no data (blank cells), the data will tend to violate the assumption of normality. Our data had sites containing variables with values of zero or no value (blank cells), therefore not all of the variables were normally distributed. Because of this limitation, additional analyses were performed to confirm results. Sites with data containing many zeros or blank cells were those lacking water, or with little vegetation. For example, if a site had no water, then variables such as flow, conductivity, water depth, riparian width, and bank height could not be measured. Sites with little vegetation received values of zero for each missing vegetation type, and sites with one type of substratum received zeros for the other categories of substrata.

Multi-dimensional scaling.—The ordination shows that sites with leopard frogs cluster. The axes represent habitat similarity indices. In general, axes created from multi-dimensional scaling cannot be specifically defined. Sites with leopard frogs are significantly different from sites without frogs in their mean value of axis one, and their variance in axes one and two. The higher variance seen among sites without leopard frogs could indicate that these sites have been altered more than sites without leopard frogs. However, the scatter plot also indicates considerable overlap in sites without frogs and sites to where frogs are present. If the necessary habitat characteristics are present at sites historically inhabited by frogs, and had remained intact since the record was reported, factors other than habitat alteration must have played a role in extirpation of that population. Possible explanations include the proximity of occupied patches and the availability of connecting habitat between populations.

Sites with the "necessary habitat characteristics" were not necessarily unaltered. Some sites with obvious habitat disturbances also had leopard frogs. In these cases, either sites were not so disturbed as to eliminate all of the leopard frogs, or sites may have additional components (unmeasured in this study) that are important for leopard frog persistence despite habitat perturbations. For example, Spring Valley, Rupes bog hole, and Lake Valley had all been heavily grazed, yet all had leopard frogs. These sites were also connected to a network of springs throughout the valleys, which may facilitate recruitment from less disturbed areas. White Horse Pass, the site with the largest and densest population of leopard frogs, was not only disturbed but also completely isolated from other water sources making immigration and emigration impossible. Although there was pavement on two sides of one of the ponds and a small area of grass around either pond, this site is possibly infrequently used by humans because it is ≈ 48 km from any town, and not visible from the nearest road. Isolation from other suitable habitat, and isolation from human contact may be why this population has grown so large. Interestingly, all individuals seen at this location were brown morphs, suggesting that the population has experienced a genetic bottleneck. Another possible explanation is that there was a small founder population, as the isolation of this area from other water sources may indicate that this population was introduced by humans.

The John's property in Wadsworth had some grazed areas but it also had areas of tall bull rush (*Scirpus* sp.) which are relatively unaltered by cattle. The ponds here are adjacent to the Truckee River, which at one time likely served as a corridor for leopard frogs. Invasive, and non-native tall white top (*Lepidium latifolium*) is now the dominant and frequently the only vegetative cover along the banks of the Truckee River. Also, dense populations of bullfrogs inhabit inlets and oxbow ponds along the river. Juvenile bullfrogs also inhabit the ponds on the John's property however, the bullfrog population here is not as dense as populations found closer to the river. Leopard frogs at the John's property may be able to sustain their population in the presence of bullfrogs because the bullfrogs here are small and less abundant.

In conclusion, the similarities between sites with leopard frogs and those where we did not find leopard frogs indicate that (1) leopard frogs live in areas that have the same basic habitat components as sites without frogs, but only specific combinations of these components can support frogs, (2) sites without leopard frogs may be able to support leopard frog populations, but do not have the characteristics necessary for long term persistence, and (3) sites with leopard frogs are vulnerable to extirpation. However, other environmental variables not measured in this study also may explain why *R. pipiens* are able to persist in some locations despite obvious habitat disturbance.

Dendrogram.—Hierarchically clustering sites indicated which sites were most similar (Fig. 5). The basis for some of these groupings was obvious, but others were difficult to identify. Many sites without frogs were clustered with sites that had frogs. This is additional evidence that the habitat characteristics alone do not predict the presence of leopard frogs. Furthermore, the ten mapped clusters indicate that there are geographic reasons for some clusters (Fig. 8).

Limitations of Using Historical Data

There is some controversy as to how to use historical records to determine if populations have declined. For example, many historical records only represent where the individuals occurred in the sample at the time the record was taken. Furthermore, the inability to detect a species in an area where it has historically occurred does not mean that the species is locally extinct (Corn, 1994; Orr et al., 1998). Making such assumptions can erroneously indicate, or overestimate the severity of a decline. However, several steps were taken in this study to alleviate bias. For example, random sites were surveyed to determine if undocumented populations had escaped detection. Migratory pathways were surveyed to account for populations that may have migrated from the original location. Random sites, and historical sites having the minimum habitat requirements for leopard frogs were surveyed several times.

Habitat Perturbations

Types of disturbance.—Nearly all 117 historical and random sites had some type of habitat alteration. Of 97 historical sites, various disturbances were the largest category. These included mowing/haying, landscaping, trash, and construction. The other categories were more specific, and therefore more informative. Several categories of disturbance were present at more sites without frogs than sites with frogs. These disturbances were associated with the presence of a large human population, and included fragmentation, erosion, no water or drying, and urbanization. Fragmentation causes habitat to be disconnected, and consequently, patchy. Patchy habitat may become increasingly more isolated from other habitat, which would cause genetic isolation as well. Nearly half (45%) of all sites surveyed had been eroded, most of these severely. Banks that were down-cut ≥1 m were common among these sites. Because leopard frogs live both in water and on land throughout the year (Chapter 1), they need be able to move back and forth between these two environments. Down-cut banks would tend to hinder this movement and may prevent frogs from inhabiting an area with this type of disturbance. Evidence of recent drying was apparent at more sites without frogs than sites with frogs. At 32 sites, water levels had visibly dropped as indicated by rings from previous water levels. Nevada has many ephemeral creeks and pools and the water levels in these typically fluctuate, however, perennial creeks and lakes surveyed also showed evidence of drying. Lowering surface water may also lower water tables, which can change bank vegetation and cause inlets, oxbow lakes, and shallow pools to dry up, thus decreasing frog habitat. Also, when wet areas that may have connected water bodies disappear, leopard frogs may experience difficulty immigrating or emigrating. Urbanization was evident at one site where a single leopard frog was found. This site was along a main highway but also abutted agricultural land. No other leopard frogs were found in association with urbanization.

Several disturbances were more prevalent at sites with frogs than at sites without frogs. Therefore hypotheses for decline in Nevada should not focus on these types of disturbances. These disturbances included agriculture and irrigation, grazing, tall white top and tamarisk, and a high predator density. Agriculture and irrigation are disturbances that may allow areas to have enough water and grass for leopard frog persistence. Livestock grazing was evident at 44% of the sites with frogs. Areas without vegetative cover generally do not retain ground moisture, and would therefore prevent frogs from absorbing water terrestrially. However, grazing may open up breeding areas for frogs. Therefore, grazing may be a disturbance that leopard frogs can tolerate. Tall white top and tamarisk cannot be interpreted accurately from this analysis because the degree of invasion of these non-native plants was not measured. For example, a mono-culture of tall white top would completely alter the terrestrial habitat, whereas if the white top was in early succession, and only a few plants were seen, grass and other native vegetation could still be dominating the area. A high predator density was present at 17% of the sites with frogs. Two of the three sites were large wildlife refuges where many native riparian and shore birds resided or migrated through. The presence of many predators is generally an indicator of a healthy ecosystem, and predators in these areas had a large choice of prey.

Non-native Biotic Perturbations

Of the biotic disturbances recorded, bullfrogs (*Rana catesbeiana*) were the most prevalent type in this study and were found at 22% of the total locations, (28% of the locations that had water). A single leopard frog was found in each of three sites that also had bullfrogs. Leopard frogs were also found co-existing with juvenile bullfrogs at a fourth site, however no adult bullfrogs were ever seen at that location. Non-native bullfrogs were first introduced to Nevada in the late 1800s (McQuivey, pers. comm.). Bullfrog populations grow rapidly, and individuals can reach large body sizes at adulthood (up to 20 cm; Stebbins, 1985). Bullfrogs will prey on other frogs (Porter, 1967; Moyle, 1973; Hayes and Jennings, 1986; McAlpine and Dillworth, 1989). Large dense populations of these non-natives, encountered throughout the surveys in 2000 and 2001, could therefore impact leopard frog populations in the state. Introduced bullfrogs as well as exotic fish have been shown to affect other populations of southwestern ranids negatively (Jennings and Hayes, 1986). Non-native salmonids are known to prey on ranid species, and therefore may pose a threat to leopard frog populations in Nevada, however, I made no attempt to systematically survey for non-native fish in this study. Additional studies of predators are recommended to determine their impact on leopard frog populations in Nevada.

Conclusions

Leopard frogs have severely declined in Nevada since the 1930s. Repeated surveys revealed only 18 of 97 historical sites with extant populations of leopard frogs. It is unlikely that a single variable explains these declines, and the causes of local extirpations. However, aggregates of habitat characteristics can be used to predict where frogs can be found. Some sites without leopard frog populations appear to be similar to sites where frogs are extant for three possible reasons: (1) leopard frogs live in areas that have the same basic habitat components as sites without frogs, but only specific combinations of these components can support frogs, (2) sites without leopard frogs may be able to support leopard frog populations, but do not have the characteristics necessary for long term persistence, and (3) sites with leopard frogs are vulnerable to extirpation.

Water levels in the Great Basin have been fluctuating Pleistocene, however in a general drying trend is also apparent (Grayson, 1993). Although this trend has occurred over thousands of years, it continues slowly to change water regimes in Nevada. Because of the aridity of Nevada and recent rapid human population growth, the demand and competition for water for humans, livestock, and wildlife has become great. Among the disturbances threatening leopard frog populations in Nevada, habitat degradation, and interactions with introduced species are likely to have played the largest role in declines of leopard frog in Nevada. Direct and indirect influences of habitat degradation and introduced plants and animals were frequent in the surveys. Agricultural practices, human recreation, and the growth of towns have altered or destroyed former habitat for *Rana pipiens*. Several historical locations that once supported leopard frog populations are now cattle ranches, street corners, parking lots, and city parks. The use of water from the Truckee River for irrigation caused Winnemucca Lake to dry up by 1939 (Grayson, 1993). It is likely that similar occurrences happened throughout Nevada as the human population has expanded. Habitat alteration in areas used as corridors between frog populations also would contribute to the decline of this species. Once leopard frog populations have become increasingly isolated, immigration becomes increasingly unlikely, and should a local extirpation occur, it could be permanent. Introduced species such as tall white top and bullfrogs now dominate many areas where native species once thrived. Tall white top and bullfrogs have invaded even those areas that are not heavily altered by humans. Attempts to eradicate either of these exotics would be extremely difficult given their wide distribution, and the lack of available methods to exterminate them without harming the habitat or native species.

To preserve and enhance the remaining leopard frog populations in Nevada, we should initiate monitoring of the extant populations and begin a captive rearing program. Habitat reparation and leopard frog reintroductions should follow. Where there is a lack of suitable habitat connecting populations, then riparian corridors must be developed. Because the populations of leopard frogs were most widely spread in an area with interconnected springs (Spring Valley), a metapopulation dynamic may be important for populations to be self-sustaining in arid environments. There are multiple components to each of the causes of extirpation (e.g., habitat degradation includes grazing and agriculture, human recreation, road construction and urbanization, erosion, and fragmentation). Interactions among these presumed causes are likely different at each site. Therefore, management of leopard frogs in Nevada should be implemented on a site-by-site basis. Management techniques should be based on experimental data (not provided by this study) and consequences of management practices should be considered before they are implemented. For example, intermittent grazing may keep vegetation from overgrowing while retaining enough cover and terrestrial moisture to support leopard frogs. However, cattle can also cause banks to be down-cut or trampled, and water to be over abundant in nitrates and silt. Therefore, further experimental research and hypothesis testing is needed.

This study has revealed a number of potential causes for leopard frog declines in Nevada. Several of these causes have been suggested in other locations or with other ranid species (Hammerson, 1982; Corn and Fogleman, 1984; Jennings and Hayes, 1994), but some remain enigmatic. Exploring the potential causes of declines in Nevada may be useful for understanding declines in other arid regions as well.

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Appendix 1. Museums queried for *Rana pipiens* records in Nevada.

Museum	# records
1.) University of Michigan Museum of Zoology	42
2.) University of Nevada, Reno	42
3.) Museum of Vertebrate Zoology	33
4.) University of Nevada, Las Vegas	24
5.) California Academy of Sciences & San Diego	12
6.) Personal Communication	5
7.) California Academy of Sciences	4
8.) University of Kansas	3
9.) Los Angeles County Museum of Natural History	1
10.) Academy of Natural Science, Philadelphia	0
11.) American Museum of Natural History, NY	0
12.) Brigham Young University	0
13.) Carnegie Museum of Natural History	0
14.) Conner Museum Washington State University	0
15.) Idaho State Museum	0
16.) National Museum of Natural History, Smithsonian Institution	0
17.) San Diego Natural History Museum	0
18.) Texas A&M, Austin	0
19.) University of Arizona, Tucson	0
20.) University of California, Davis	0
21.) University of Colorado Museum	0
22.) University of New Mexico, Albuquerque	0

Appendix 2. Site localities surveyed, site type (P = personal communication, L = literature, M = museum record, R = randomly chosen location, N = field notes), number of visits, and average number of pipiens seen.

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Bannock	Inkom	Portneuf River: 8 mi S of Inkom	401105	4730896	1440	P	1	1
Bonneville	Wyan	Grays Lake NWR: ag. ditch at E entrance, 3 mi N of refuge hqts.	468140	4767697	2029	P	1	8
Caribou	Inkom	Toponce Creek: beaver ponds on side of Innman Creek Road	409445	4744642	1933	P	1	9
Churchill	Fallon	Carson Lake: NE corner of Carson Sink, in canals and adjacent wet meadow	357194	4357581	1200	L	2	0
	Fallon	Lahontan State Park: Marshy area E of dam	323567	4370148	1240	M	3	0
	Fallon	4 mi. west of Fallon, intersection of rte 50 and Scheckler Cut Off	339028	4371877	1216	M	1	0
	Fallon	West of Fallon, south side of Lahontan Dam, marshy area 200 m from dam	322464	4369987	1238	M	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Churchill	Fallon	7 mi. north-northeast of Fallon, Indian Lakes Road	354311	4381192	1186	M	1	0
	Fallon	2 mi. south of Fallon, St. Clair Road off hwy 95	346372	4367575	1208	M	1	0
	Fallon	West end of town, west side of bridge over Carson River on south side of rte. 50	343811	4371398	1216	M	1	0
	Fallon	West of Fallon, just below Lahontan Dam along Carson River	322598	4370156	1232	M	1	0
	Fallon	Stillwater NWR: Channel and marsh at SW end of Stillwater Point Reservoir	368179	4372877	1205	P	2	0
	Fallon	Stillwater WMA: 0.8 mi from Leter Rd at lat 39 36'34" (followed bearing due W to small dirt road then followed road to spring)	352988	4386080	1190	R	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Churchill	Fallon	Stillwater WMA: N on Indian Lakes Rd; take L fork at ~mi 10, stay straight until road ends at abandoned corral, walk NNW ~800m	358356	4395279		R	1	0
	Fallon	Stillwater WMA: Indian Lakes Rd, 1. 15 mi N of fork at Leter Rd (lake area and wet meadow adjacent to lake)	354864	4381516	1190	R	2	0
	Fernley	approx. 1 mi. southeast of Fernley, water diversion ditch; Truckee River Canal	308077	4383567	1275	M	1	0
	Lovelock	Humboldt WMA: Took dirt road along Humboldt Lake to end of road, area was dry. Searched small marshy area just S of bridge over ag channel	363025	4432715	1190	R	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Churchill	Toulon	Lower Humboldt Drain: 10 mi S of Toulon	356087	4419758		R	1	0
Douglas	Gardnerville	off 395, west side of hwy. east side of east fork Carson River, aprox. 5 mi. southeast of Gardnerville, right after mobile home park	266744	4306494	1500	M	1	0
	Carson City	Carson River: 10 mi S of Carson City on Rte 395, Ambrosetti Pond Ranch	258629	4324971	1431	P	2	1
	Minden	Centerville: S of Minden, marshy area between agriculture fields on corners of Rtes 88 and 756	258768	4310490	1440	M	2	1
Elko	Carlin	Upper Maggie Creek: 10 mi N and 15 N of Carlin off Rte 766 mi N of Carlin on Rte 766	565501	4519586	1534	M	3	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Elko	Carlin	Upper Susie Creek, 2.5 mi. from prison/rte 80/Carlin	578483	4512285	1542	M	1	0
	Carlin	Lower Susie Creek, 1.5 mi. north of Carlin/rte 80/prison	578181	4510831	1503	M	1	0
	Carlin	Upper Maggie Creek - 10 mi. north of Carlin/ rte 80,on rte 766	565501	4519586	1534	M	1	0
	Deeth	Zgraggen Ranch: SW off Rte 230 approximately 1 mi after exiting to Deeth, wet meadow and Humboldt River	643196	4545379	1620	M	3	1
	Deeth	Deeth: dry pond 1.5 km NE of Deeth	634963	4548541	1620	N	1	0
	Henry Siding	Salmon Falls Creek: W of Rte 93,1 km N of Vineyard Ranch	685291	4623935	1615	N	2	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Elko	Jackpot	Trout Creek Ranch: Various sections along southern Goose Creek, 5 km W of Utah state line and ponds on Trout Creek Ranch	739558	4641565	1580	M	3	0
	Jackpot	near Trout Creek Ranch: Goose Creek, 2 mi. from Utah boarder, just south of bridge that crosses creek; Also, further down Goose Cr. to ranch headquarters	741322	4644291	1569	M	3	0
	Jackpot	Shoshone Creek: 2 mi E of Jackpot, creek crosses under Progressive Road at Y3 Ranch	394437	4647868	1570	M	2	0
	Jackpot	Rancho Grande: Goose Creek, 2.8 km upstream from ranch house	730565	4645766	1664	N	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Elko	Jarbidge	Corral Creek: Jarbidge Wilderness	626284	4636654	2359	R	2	0
	Lamoille	Lamoille Creek: At Maggie Creek Ranch near Lamoille and in Lamoille Canyon Recreation Area beaver ponds	628809	4505871	1191	M	3	0
	Mountain City	Mountain City: Off Rte 225 over "The Bridge to Nowhere," along W side of Owyhee River	585857	4632181	1714	M	3	0
	Ruby Lake	Ruby Lake NWR: W side of Ruby Lake 3 mi N of Elko County line (200 m SW of park outhouse)	629479	4448127	1840	M	2	15
	Wells	Weeks Creek: Off Clover Valley Road (Rte 221),S of Wells	661671	4534234	2150	R	2	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Elko	Wells	Pequop Spring: 19 mi E of Wells, 11 mi from Moor exit 360	700134	4563265	1910	R	2	0
	Wells	Tabor Creek: Tabor Creek Recreation Management Area, N of Wells	659401	4587263	1877	R	2	0
	Wendover	Ferguson Spring: Aprox 30 mi S of Wendover off Alt 93 towards Whitehorse Pass, take hwy maintenance station road into wild life viewing area. Small pond about 1/4 mi up road behind chain link fence and Small pond further up the road	738850	4479264	1633	L	3	100
Eureka	Crescent Valley	Crescent Valley: Alkali flats between Dewey Dann Ranch and Dean Ranch, down Danna Road	538938	4464221	1454	R	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Humboldt	Denio	Onion Valley: Alder Creek surveyed both directions from where road crosses creek	352816	4619204	1640	R	2	0
	Gerlach	W side of Black Rock Desert, reservoir on county road 217 (Soldier Meadows Road) ≈ 34 mi. north of Gerlach	318799	4565608	1309	M	1	0
	Paradise Valley	up rte 290 past town of Paradise Vy., just after pavement ends on W side of road	454567	4598226	1446	M	1	0
	Quinn River	Quinn River Road off rte 140 crosses over Quinn River Crossing	382440	4602783	1524	M	1	0
Lander	Austin	Austin: 4 mi N on Grass Valley Road off Rte 50,7 mi E of Austin; third creek bed up	501572	4374869	2000	R	2	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Lander	Austin	Belmont: Turn off Rte 50 of Austin onto Rte 376 then L onto FR 100; near Toquima Cave Campground	517291	4338599	2321	R	2	0
	Battle Mountain	Buffalo Valley: Take Rte 305 16 mi SW of Battle Mountain then follow signs to Buffalo Valley/Copper Canyon and then to Mary Hudson Ranch 2, past the ranch, turn L at the T intersection then L again into a corral	467351	4476389	1415	R	2	0
	Battle Mountain	Willow Creek Reservoir/Willow Creek: Take Rte 30516 mi SW of Battle Mountain, follow signs to Buffalo Valley/ Copper Canyon and then to Willow Creek	486172	4490640	1740	R	2	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Lander	Belmont	Antone Creek: 5 mi N of Belmont in Toquima Range,Toiyabe Natl Forest; in Antone Canyon, off Round Meadow Canyon	507800	4279831	2400	R	2	0
Lincoln	Alamo	Lower Pahranagat Lake: E side, midway	669953	4121610	982	M	3	0
	Alamo	down S end of Upper Pahranagat Lake: SW side of lake, channel below dam, and marsh below dam	666593	4128244	1010	M	3	0
	Alamo	Maynard Lake	675245	4117608	945	M	2	0
	Alamo	town of Alamo, corner of Broadway And First St.	662223	4136615	1070	M	1	0
	Alamo	Lone Tree Spring: S boundary of Pahranagat NWR, just off Rte 93, S of the highway	674349	4117977	967	P	3	5

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Lincoln	Ash Springs	Ash Springs Hot Springs: 7 mi N of Alamo, across from Texaco Station; public recreation area	659827	4147785	1130	M	3	0
	Caliente	in Caliente along Meadow Valley Wash,. corner of N Spring St. and Minnie St.	719713	4166326	1336	M	1	0
	Caliente	Meadow Valley Wash; 4.7 mi. south of Caliente after rte. 317 turn-off, pull -off on side of road and walked west to river	714935	4159332	1288	M	1	0
	Crystal Springs	Crystal Springs: Follow path from historical information sign	656064	4155339	1200	M	3	0
	Hiko	Key-Pittman Wildlife Mgmt Area: between Hiko and Crystal Springs, 3 mi N of Crystal Springs	656913	4159157	1180	M	3	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Lincoln	Hiko	north east Pahranagat Valley, approx. 1mi. NE of Hiko on Cannon Ranch	643561	4207635	1190	M	1	0
	Panaca	Panaca: Pond/spring 1.3 mi N of Panaca	730549	4187637	1450	M	3	0
	Ursine	Spring Valley State Park: marshy area at N end of Eagle Valley Reservoir	746869	4212473	1770	M	3	0
	Ursine	25 mi. north of Ursine, above Big Jack Ranch, headwaters of Camp valley Wash	738442	4240838	2064	M	1	0
	Ursine	just north of Ursine, Eagle Valley	745020	4208509	1649	M	1	0
	Ursine	Delmue Ranch: Aprox 18 mi N of Eagle Valley Lake	745306	4240685	2110	R	2	0
Lyon	Dayton	along Carson River in Dayton & just north of Dayton, south side of river	276722	4346305	1330	M	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Lyon	Smith	5 mi. east of Smith, rest stop along 208 (access to Walker River), Wilson Canyon Recreational area	306680	4297802	1450	M	1	0
	Wabuska	6 mi. west of Wabuska off Julien Ln., Walker River	318529	4335457	1310	M	1	0
	Wellington/ Smith	north of Wellington, rte 823 to Artist View Way, then down 338 and Smith Gauge Road	294439	4294366	1448	M	1	0
	Yerrington	Mason Valley WMA: Miller's Marsh, adjacent to Fort Churchill Pond	315460	4331723	1319	R	2	0
	Yerrington	0-8 mi. south of Yerrington along rte. 208	312194	4311402	1470	M	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Mineral	Hawthorne	Fletcher Spring: off Rte 359 S of Hawthorne, ≈9 mi W of Lucky Boy summit off Lucky Boy Pass Road	334257	4247313	1870	M	3	0
Nye	Sunnyside	Wayne Kirch WMA: 70 mi S of Ely off Rte 318; Hot Creek from spring down to parking area, and Hot Creek, W from end of Adams-McGill Reservoir, and grassy area below dam road	661683	4249361	1590	M	3	0
	Sunnyside	Kirch Wildlife Area, where Sunnyvale-Sharpe Road and White River used to cross	667322	4254133	1465	M	1	0
	Tonapah	Eden Creek; 0.25 mi east of Eden Creek Mine, Eden Creek Ranch	553222	4202180	1980	M	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Pershing	Lovelock	3 mi. northeast of Lovelock off Irish American Road along Humboldt River	293477	4454384	1219	M	1	0
Washoe	Derby	exit 36 Derby Dam off I-80 east, Truckee R.	290868	4384656	1293	M	1	0
	Franktown	Washoe County Park: On Susan Lee Circle, off William Brent Road, off Rte 429	255417	4350154	1546	R	2	0
	Nixon	Truckee River: 2 mi E of mouth of Truckee R. at Pyramid Lk. In shallow pools and marshy areas on N side of Truckee R.	277669	4474312	1158	M	2	0
	Nixon	Truckee River: Near mouth at Pyramid Lk., 3-5 mi W of Nixon	277752	4477088	1158	M	2	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Washoe	Reno	Hunter Creek: 2.5 mi hike up Hunter Canyon from Mayberry St. Above waterfall: spring/beaver ponds / grassy wet meadow area next to creek	249061	4372292	1890	M	3	0
	Reno	9.5 mi. east of Reno, north side of Truckee River	273676	4379065	1524	M	1	0
	Reno	east of 395 between Hilton hotel and 4th street, south of rte. 80 - trucking company found at coordinates	260809	4379415	1219	M	1	0
	Reno	Rancho San Raphael Park behind arboretum	257352	4381101	1372	M	1	0
	Reno	North Virginia on east side of road across from Reno sports complex	257221	4382416	1372	M	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Washoe	Reno	corner of Arlington and Plumb, #1885 Arlington-Plumb Plaza	257084	4375865	1219	M	1	0
	Reno	north UNR campus between Evans and McCarran	258382	4381302	1372	M	1	0
	Reno	off of Holcomb on corner of Lakeside and Kinney	258357	4370550	1490	M	1	0
	Reno	off Skyline Blvd., dog run along stream u-turns to Manzanita Dr.	255934	4362011	1219	M	1	0
	Spanish Springs	Spanish Springs Valley	266304	4386691	1402	M	1	0
	Sparks	0.25 mi. east of Sparks	263646	4397328	1219	M	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Washoe	Sparks	junction of Steamboat Creek and Truckee R.	267704	4378981	1219	M	1	0
	Verdi	Crystal Peak Park: E side of Truckee River in wet meadow/field	242350	4377925	1490	M	3	0
	Wadsworth	Nixon: Paiute Indian Reservation, The John's property; E side of Truckee R. in irrigation pond	305464	4393139	1220	M	4	3
	Wadsworth	Wadsworth: Dead Ox Wash, Aprox 7 mi N of Wadsworth on Pyramid Hwy (Rte 447), 1 mi N of fish hatchery	301383	4401428	1200	M	2	1
	Wadsworth	East side of Truckee River past gravel pit. property slated for gravel pit	305077	4395395	1210	M	1	0
	Wadsworth	Truckee River, .25 mi. east of Wadsworth	304039	4389169	1234	M	1	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
Washoe	Wadsworth	Big Bend section of Paiute Indian Reservation, behind trailers	303454	4387908	1238	M	1	0
	Washoe	S end of Washoe Lake: SE shore and marshy area to S	258990	4346113	1540	M	3	0
	Washoe	north end of Washoe Lake, east of rte 395	258682	4356870	1540	M	1	0
White Pine	Ely	N Spring Valley: 30.5 mi N on Rte 893, W side of road; also, 30 mi N on Rte 893, t hen 0.5 mi E	714046	4371426	1739	L	1	3
	Ely	N Spring Valley: 19.5 mi N on Rte 893 near the 'nudist colony', E side of road beside fence	717279	4354319	1709	L	1	4
	Ely	S Spring Valley: aprox 1 mi up Highline Road, off Rte 894	723576	4313366	1773	L	1	3

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
White Pine	Ely	S Spring Valley: Shoshone Ranch, off Rte 894	723790	4304411	1771	L	1	5
	Ely	Lake Valley: Geyser Ranch, 25.7 mi S of Majors Junction on Rte 93, 'bass pond', 1.3 mi S of ranch; also, Wamboldt Spring, next to C & A Cattle Co. Ranch off Rte 93, 27.8 mi S of Majors Junction	706117	4280825	1817	L	1	15
	Ely	White River Valley: Ruppes Bog Hole, 6 mi S of Lund then 1 mi W. Also, Spring S of Ruppes Bog Hole, 6 mi S of Lund then 1 mi W	669039	4291583	1664	L	1	1

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
White Pine	Ely	N Spring Valley: N Millick Spring, 11 mi N on Eight Mile Ranch Road, hen 0.5 mi NNE; also, 11 mi N on Eight Mile Ranch Road, then 0.5 mi NE; also, 12 mi N on Eight Mile Ranch Road, then 1 mi W (1 mi N of the Millick Springs)	725682	4354008	1706	L	1	37
	Ely	Ely: Hercules Gap, about 5 mi N of Ely on Bothwick Rd	681552	4357829	1910	M	3	0
	Ely	CCC Ranch: 5.5 mi S of Ely; Marsh just S of CCC Ranch house and W side of Rte 93, across hwy from CCC ranch; Commins Lk.	689131	4337563	1990	M	3	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
White Pine	Ely	CCC Ranch, Steptoe Creek: 5.5 mi S of, Ely on Rte 93 ≈1.1 mi N of CCC ranch house and human-made pond 0.5 mi N of ranch house	689276	4339779	1970	M	3	0
	Ely	56 mi. southeast of Ely and 26 mi. north of Pony Springs on rte. 93 .25 mi. north of Lincoln/White Pine County line, just N of Geyser Ranch on west side of 93	705583	4285153	1849	M	1	0
	Ely	35 mi. north of Ely, Warm Springs Siding, east side of RRX	689237	4391545	1819	M	1	0
	McGill	Duck Creek: 16 mi up Rte 486, off Rte 93 N of Ely	697124	4357271	2250	M	3	0

Appendix 2. (continued)

County	Town	Location	Easting	Northing	Elevation (m)	Site Type	No. Visits	Avg. no. *R. pipiens*
White Pine	McGill	Steptoe Slough: 2 mi W of McGill in marshy pond, off Bassett Lake Rd just past 2nd cattle guard	685985	4367873	1840	M	3	0
	McGill	old sewage pond (1971), now owned by Kennecut Mining Co. @ far west end of center of town	690829	4365151	1950	M	1	0
	McGill	Bassett Lake: Marshy areas S of dam and E of reservoir	684549	4371479	1850	R	2	0

Appendix 3. Variables used in analyses, transformations performed, and rankings used.

Easting, Northing, and Elevation .... (no transformation)

Average flow .... (square root transformation)

Conductivity .... (log transformation)

Average pH .... (no transformation)

Maximum water depth .... (square root transformation)

Average water depth .... (square root transformation)

Minimum bank height .... (square root transformation)

Average bank height .... (square root transformation)

Average riparian width .... (square root transformation)

Potential habitat size rank .... (1-5, 1 = smallest)

Open water size rank .... (1-5, 1 = smallest)

Wet ground size rank .... (1-5, 1 = smallest)

Percent cover of algal mats .... (1 + log transformation)

Percent cover of emergent vegetation .... (1 + log transformation)

Average soil moisture .... (1-4, 1 = submerged, 5 = dusty dry)

Percent cover of silt/mud/clay/sand substratum ...... (no transformation)

Percent cover of gravel substratum......................... (no transformation)

Percent cover of cobble substratum .... (no transformation)

Percent cover of rocky substratum.......................... (no transformation)

Percent cover of concrete substratum .... (no transformation)

Appendix 4. Descriptions of sites occupied by *Rana pipiens* in Nevada.

Ambrosetti Pond Ranch

Approximately 16 km south of Carson City, just south of the Carson River is Ambrosetti Pond Ranch (4324971 N; 258629 E; 1431 m). An inlet from the Carson River feeds a large shallow pond on the ranch. The pond is encircled by bull rush, some grass, and a human made stone embankment. Hundreds of bullfrogs (*Rana catesbeiana*) were seen or heard in the pond. A single leopard frog was found in the water, below the only grassy bank at the edge of the pond on 15 July 2001. The bank was located on the western side of the pond and was about 30–cm high. The pond extends onto an adjacent property, which was not searched. The leopard frog was toe clipped, taken as a voucher, and deposited in the UNR museum.

Dead Ox Wash

Along the east side of the Truckee River, halfway between Wadsworth and Nixon, about 2 km north of the Paiute Indian fish hatchery is a spring outflow that forms a wet meadow and several marshy pools surrounded by bull rushes (4401428 N; 301383 E; 1200 m). One juvenile leopard frog was found in the wet grass adjacent to a pool on 16 April 2001. Tall white top has filled in the area between the river and the spring-fed wet meadow and marsh. There are plans to burn the area and pump the spring to create a water tank and grazing area for cattle (D. Grabowski, pers. comm.). This frog was not toe clipped or taken, in accordance with the Paiute Tribe regulations.

Ferguson Springs at White Horse Pass

Ferguson Springs at White Horse Pass in northeastern Nevada (40.42987 N; 114.18398 E; 1850 m), is adjacent to Alternate Route 93, ≈ 48 km south of Wendover. The spring is partially contained in a steel water tank, but overflows into a 200 m^2 pond, which is surrounded on two sides by an infrequently used paved road. The water then flows under the road and through grass and cottonwood trees downhill and east for ≈ 200 m where it seeps into another 200–m^2 pond encircled by grass and cattails (*Typha* sp.). Hundreds of leopard frogs in all life stages (except egg masses) were found in and around both ponds and in the wet grass on 14 August 2000, 3 July 2001, and 23 July 2001. Twenty-two frogs were sexed, weighed, measured and toe-clipped. Seven adults and seven tadpoles were taken as vouchers for the UNR museum.

The John's Property

Just north of Wadsworth, Washoe Co. (39.66444 N; 119.27861 E; 1220 m), along the eastern bank of the Truckee River adult leopard frogs were observed several times in 2000 and 2001. Bullfrogs were also present in this area. The area holds an agricultural pond (600 m^2) and two smaller shallow ponds (≈ 200 m^2 each) adjacent to the river. The main pond is fringed with grass and some cattails, whereas the smaller ponds are encircled mainly by bull rushes with some grassy areas. The ponds are all fed via an irrigation ditch that flows from a gravel pit (about 2 km southeast of the property) and empty into the Truckee River. Water containment in these ponds is controlled by the property manager. Leopard frogs were found in greater numbers in the ponds surrounded by bull rush and in the damp grass than in the pond closest to the river that was encircled by a fringe of grass. One burnsi morph (lacking dorsal spots) leopard frog was sited and photographed on the John's property. The burnsi morph has been recorded previously in Minnesota, South Dakota, western Wisconsin, northwestern Iowa, and occasional sitings have been reported from Colorado and two New England states (Hoppe and McKinnell, 1991; D. M. Hoppe, pers. comm.). No frogs were toe clipped or taken from this property as requested by the Paiute Indian Tribe.

Lone Tree Spring

There are two locations locally known as Lone Tree Spring in Pahranagat Valley (both were visited to make sure we surveyed the correct one), and frogs were found at one of these locations. The southern most spring in Pahranagat Valley is located at 4117977 N; 674349 E; 967 m. The spring is in a hollow that is enclosed by Route 93 and a steep hillside. Densely vegetated bull rush and cattails to 2–m tall, cover most of the water making it difficult to survey. Only very small (≤ 10 m^2) patches of open water were observed. An area of low vegetation, mostly yerba mansa (*Anemopsis californica*), densely covers parts of the hollow that were not wet during our surveys. This spring is directly across the road from Maynard Lake which had a historical population of leopard frogs in 1938, and the two areas were probably connected before the highway was built in the 1930s. Maynard Lake is now virtually dry and the area is dominated by yerba mansa and sagebrush. Small pools of water can be found here occasionally throughout the year. At Lone Tree Spring, at total of five *Rana pipiens* were seen, one was taken as a voucher for the UNR museum.

Minden

A single leopard frog was found on 15 September 2000 near a small marshy pond ≈ 4.8 km south of Minden, (38.91019 N; 119.78212 E; 1440 m). The pond was approximately 1200 m^2 and is surrounded mostly by cattails with some grass. At the northern border of the marsh is Route 756, to the east is Route 88, and running diagonally between the two roads to the south and west of the marsh was a fenced field that had been heavily grazed. Several juvenile bullfrogs were observed in the pond. The lone leopard frog was found in a patch of grass, just outside the fence at the southwestern end of the pond. Unfortunately, it escaped before we were able to sex, weigh, measure, and toe-clip the individual. No leopard frogs were found at this location in 2001.

Ruby Lake

Ruby Lake is in northeastern Nevada (40.24823 N; 115.47110 E; 1820 m). The lake and marsh are approximately 24 km long by 4 km wide. This national wildlife refuge, managed by the U.S. Fish and Wildlife Service, has many riparian birds, is stocked annually with rainbow (*Oncorhynchus mykiss*), brown (*Salmo trutta*), and eastern brook trout (*Salvelinus fontinalis*), and stocked occasionally with Lahontan cutthroat trout (*O. clarki henshawi*; E. Pauch, pers. comm.). Largemouth bass (*Micropterus salmoides*)also live and reproduce in the lake but are not stocked. Three areas of the marsh at Ruby Lake were searched. These included "the Fingers," located on the western side of the marsh approximately 5 km north of the refuge headquarters (4456468 N; 630085 E; 1830 m; 25 leopard frogs were seen here); the fishing area, also on the west side but approximately 3 km south of the headquarters (4448127 N; 629479 E; 1840 m; five leopard frogs were seen here); an area of cattail marsh on the east side of Ruby Lake at the end of North Dike Road (4452230N; 631431 E; 1819 m; no leopard frogs were seen here); and Narcissus boat launch (4511384 N; 624882 E; 1825 m; one adult *Rana pipiens* was seen here). Other locations around the marsh are likely to have leopard frogs as well, however my intention was simply to find them in the general area, not to determine every location within marsh complex. A total of nine *R. pipiens* were identified between the hours of 0800 and 1600 on 25 June 2000, and 25 between the hours of 1400 and 1630 on 24 May 2001. Only adult leopard frogs were seen at Ruby Lake in 2000, however mostly juvenile frogs were found in 2001. All frogs were found in grassy areas within 40 m of water. Eighteen frogs were sexed, weighed, measured and toe-clipped. Five *R. pipiens* were taken as vouchers and deposited in the UNR museum.

Ruppes Bog Hole

This is a series of several springs located south of Lund, ≈ 4291583 N; 669039 E; 1664 m. One leopard frog was found in the wet grass but escaped capture. Although this location is close to Spring and Lake Valleys, a mountain range separates the two areas. At least three local ranchers have grazing rights to the land surrounding the springs. There is minimal open water, but the springs form a corridor of wet ground, which is vegetated with sedges and grass. This wet corridor was trodden by cattle and grazed to the ground when I visited the site. Beyond the area searched, the valley floor was not explored, but looked dry. However, other springs may exist in the valley beyond where I surveyed.

Spring and Lake Valleys

Spring and Lake Valleys are the eastern-most valleys in Nevada, and are east and southeast of Ely, respectively. Although six populations of leopard frogs were defined in this region, it is likely that other leopard frog populations exist and intermingle within each valley. These locations are different from all other areas surveyed because of the network of springs and creeks that are found throughout both valleys. During times when the eater level is high, it is conceivable that all populations found in each valley could be connected. Populations were defined in these valleys on the basis of large intervening areas of dry ground. Much of the land in these valleys is privately owned, and some of the public land (managed by the Bureau of Land Management) is used by local ranchers for grazing livestock. Alfalfa is grown mainly on the private land, which is watered periodically. Moist ground is also naturally available in many areas, and pronghorn, mule deer, and sandhill crane were observed using this habitat. A total of 26 leopard frogs were found within these valleys, 24 were toe clipped, and nine were taken as vouchers.

Zgraggen Ranch

Dusty Zgraggen's ranch is located on the south side of the Humboldt River in Deeth (4545379 N; 643196 E; 1620 m). Two recent metamorphic leopard frogs were taken at the edge of the river 22 July 2001, adjacent to a hay field that had recently been mowed and bundled. Earlier visits were made 26 June 2000 and 11 June 2001 when the hay field was being irrigated, and was completely submerged. The ranch owner reported seeing leopard frogs in the wet hay field in past years but not in 2000 or 2001. The Humboldt River was extremely low in 2001, which may have decreased the amount of breeding sites and dispersal corridors available to leopard frogs. It is therefore curious that I did not find frogs here in 26 June 2000, or during my first visit 11 June 2001 when the hay field was wet.

Table 1. Correlation matrix of original sites and PCA factors. Values that are ≥ 0.6 (in **boldface** type) indicate those variables that make up a significant proportion of the corresponding factor.

Original variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Flow of water	0.3781	0.2067	0.5965	-0.0205	0.0330	0.0816	-0.3343
soil moisture	-0.0893	0.5149	-0.0023	-0.2632	-0.0078	0.0355	-0.3304
concrete substrate	-0.1356	-0.0275	-0.0849	-0.2291	0.0014	**-0.8453**	-0.0792
minimum bank height	-0.1027	**0.8854**	0.1752	0.0139	0.0547	0.0011	-0.0276
elevation	0.0264	-0.0580	-0.0470	**0.8205**	0.2430	0.1102	-0.0274
cobble substrate	0.1138	0.1564	0.7383	0.0980	0.3063	-0.1303	-0.0151
gravel substrate	-0.0441	0.0927	0.0930	0.1919	0.1080	0.0200	-0.0002
rocky substrate	0.0004	0.0211	**0.8404**	-0.1992	-0.0712	0.1710	0.0037
northing	0.1082	0.0764	0.1202	0.1042	**0.9022**	0.0172	0.0205
water size	**0.7762**	0.1448	0.2473	-0.1146	0.1887	0.0846	0.0429
habitat size	**0.8021**	-0.0793	0.1601	0.0950	0.0392	0.1669	0.0508

Table 1. (continued)

Original variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
average bank height	0.1808	**0.8710**	0.0759	-0.0191	0.0595	0.1715	0.0780
average water depth	0.4749	0.3301	0.1165	-0.4170	0.0664	0.5371	0.1047
pH	0.5843	0.4084	-0.1183	-0.2123	-0.1594	0.1368	0.1081
maximum water depth	0.5507	0.3302	0.1872	-0.2939	0.1025	0.4848	0.1920
easting	0.1189	0.0076	-0.1108	**0.6500**	-0.4236	-0.0156	0.2387
wet ground size	**0.6436**	-0.2174	-0.0126	0.2872	0.1459	0.0828	0.3964
silt substrate	0.3549	0.2614	-0.3831	-0.1396	-0.0650	0.5423	0.3985
average riparian width	0.5755	-0.1797	-0.0334	0.2484	-0.0773	0.1103	0.3993
conductivity of water	**0.6042**	0.1198	-0.1789	0.0990	-0.1736	-0.0189	0.5518
emergent vegetation	0.3755	-0.1378	0.0114	0.0477	0.0175	0.1582	**0.7809**
percent algal mats	0.0414	0.1082	-0.0900	-0.0358	0.0145	0.0794	**0.9018**

Figure 1. Putative geographic distribution of the northern leopard frog (*Rana pipiens*), the relict leopard frog (*Rana onca*), and the Vegas Valley leopard frog (*Rana fisheri*). Map was modified from Stebbins (1985).

Figure 2. Types of disturbance found at all historical sites surveyed (2000 and 2001). Site may be included under more than one category. Miscellaneous habitat degradation includes trash, construction, mowing and/or an undetermined cause manifested in the absence of vegetation.

Figure 3. Historical distribution of *R. pipiens* in Nevada based on historical records collected.

Figure 4. Current distribution of *R. pipiens* in Nevada based on this study.

Figure 5. Historical and random site locations for leopard frogs in Nevada that were searched in summer, 2000 and 2001. Red indicates that frogs were found, and green indicates that frogs were not found.

Figure 6. Scatter plot of rotated principal coordinate axes showing that sites with leopard frogs are similar to each other whereas sites without leopard frogs are more varied.

Figure 7. Hierarchical clustering of 58 of 97 historical sites. Dendrogram shows which sites are similar to each other and describes general characteristics of the sites within each cluster. Sites with a star next to them indicate that leopard frogs were present at that site.

Figure 8. Geographic interpretation of sites that clustered according to their habitat components. Some clusters appear to cluster geographically as well as clustering by habitat.

Figure 1

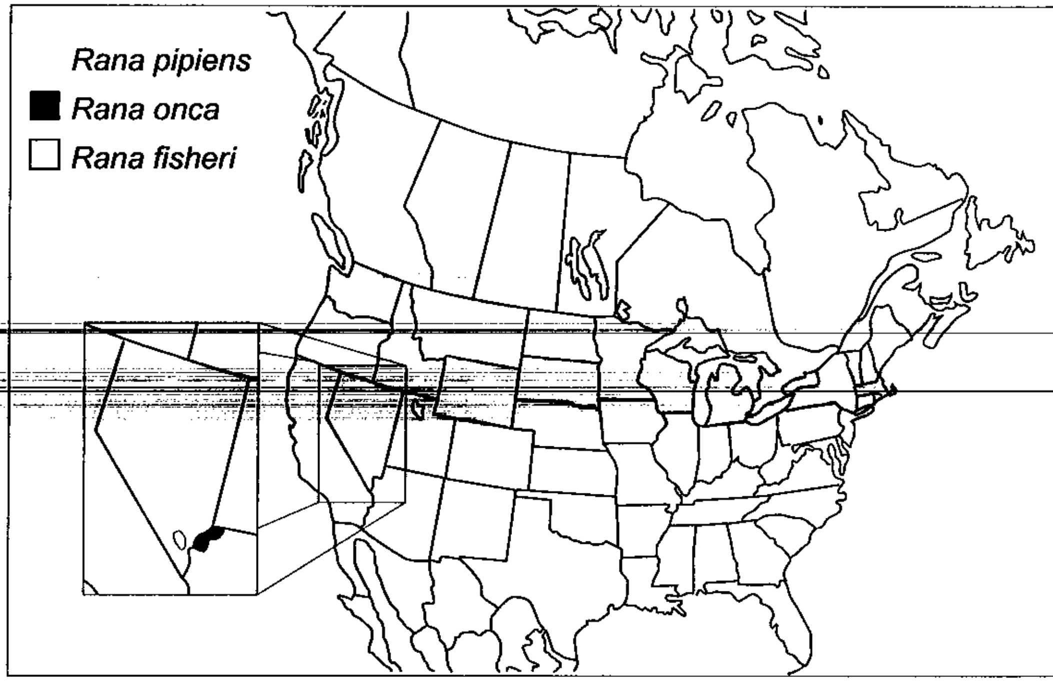
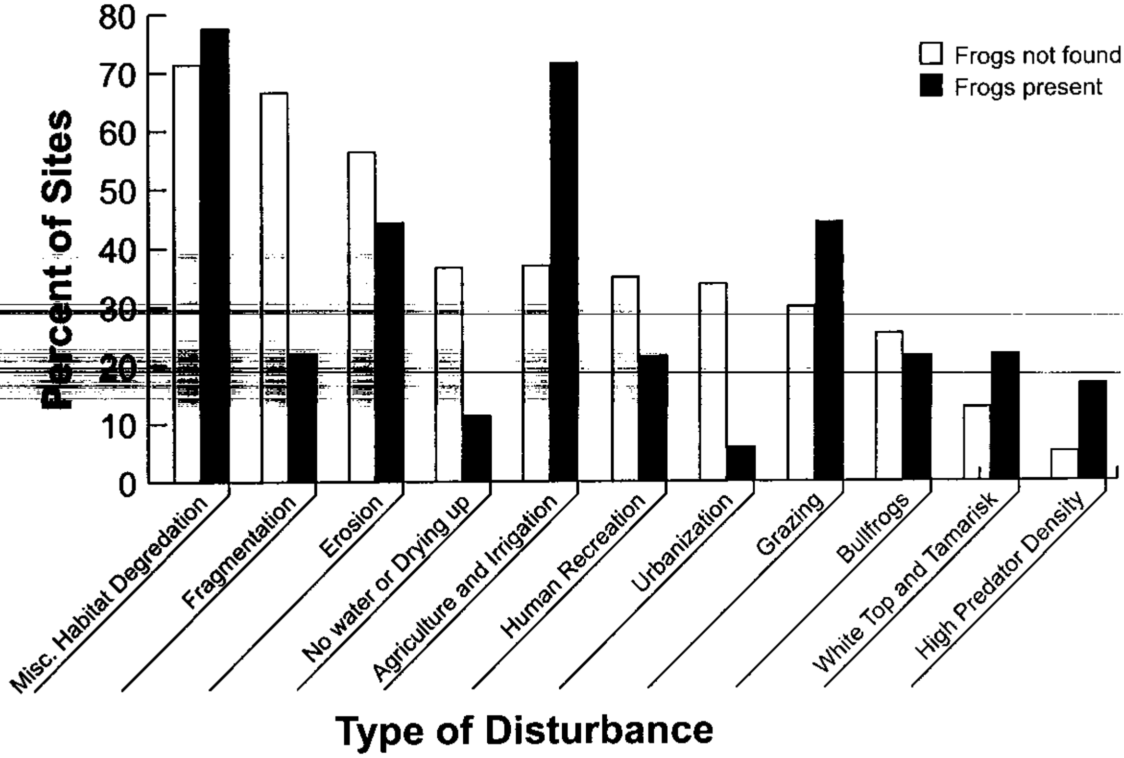


Figure 2



Frogs not found
Frogs present
Percent of Sites
80
70
60
50
40
30
20
10
0
Misc. Habitat Degredation
Fragmentation
Erosion
No water or Drying up
Agriculture and Irrigation
Human Recreation
Urbanization
Grazing
Bullfrogs
White Top and Tamarisk
High Predator Density
Type of Disturbance

Figure 3

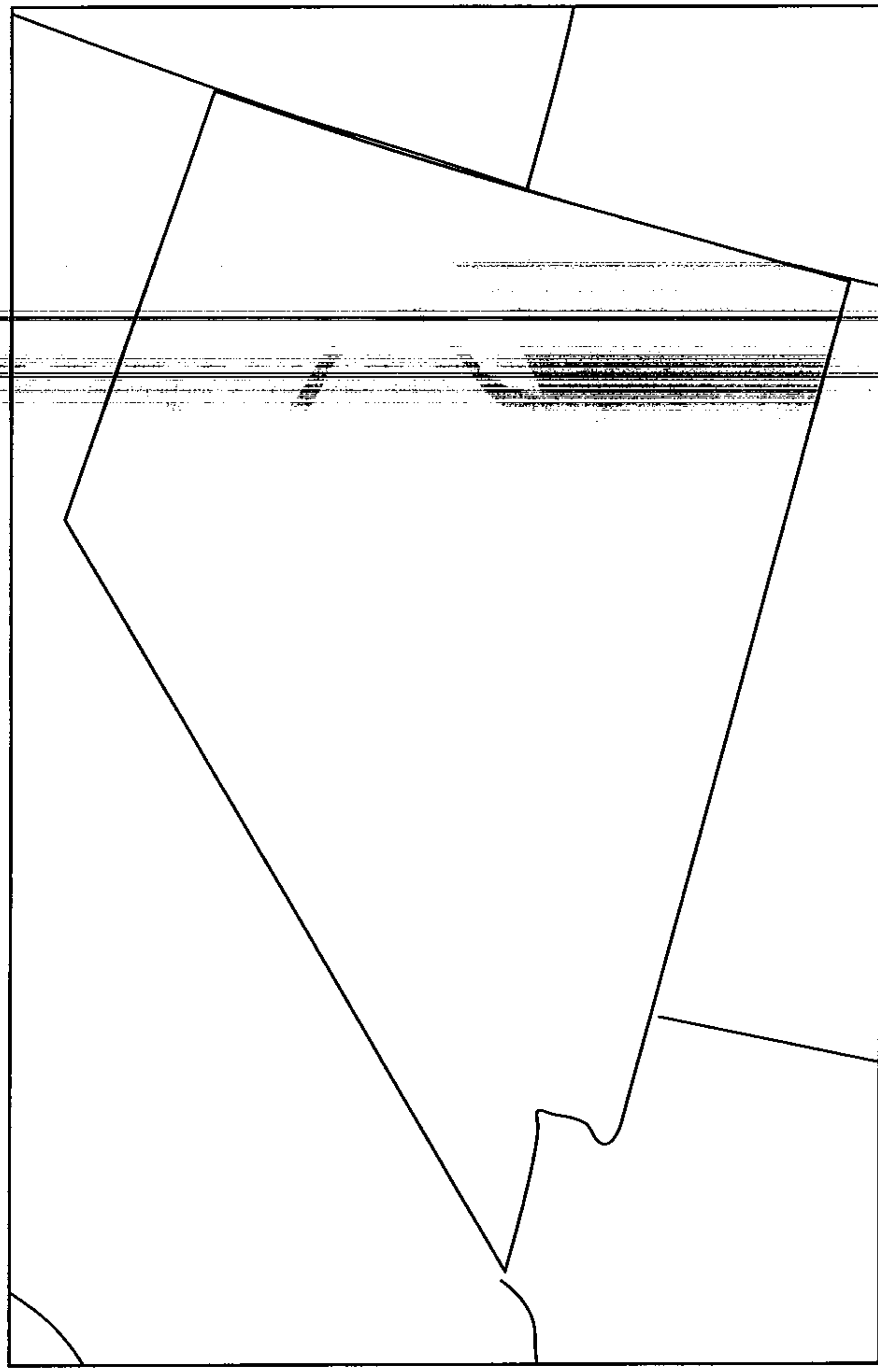


Figure 4

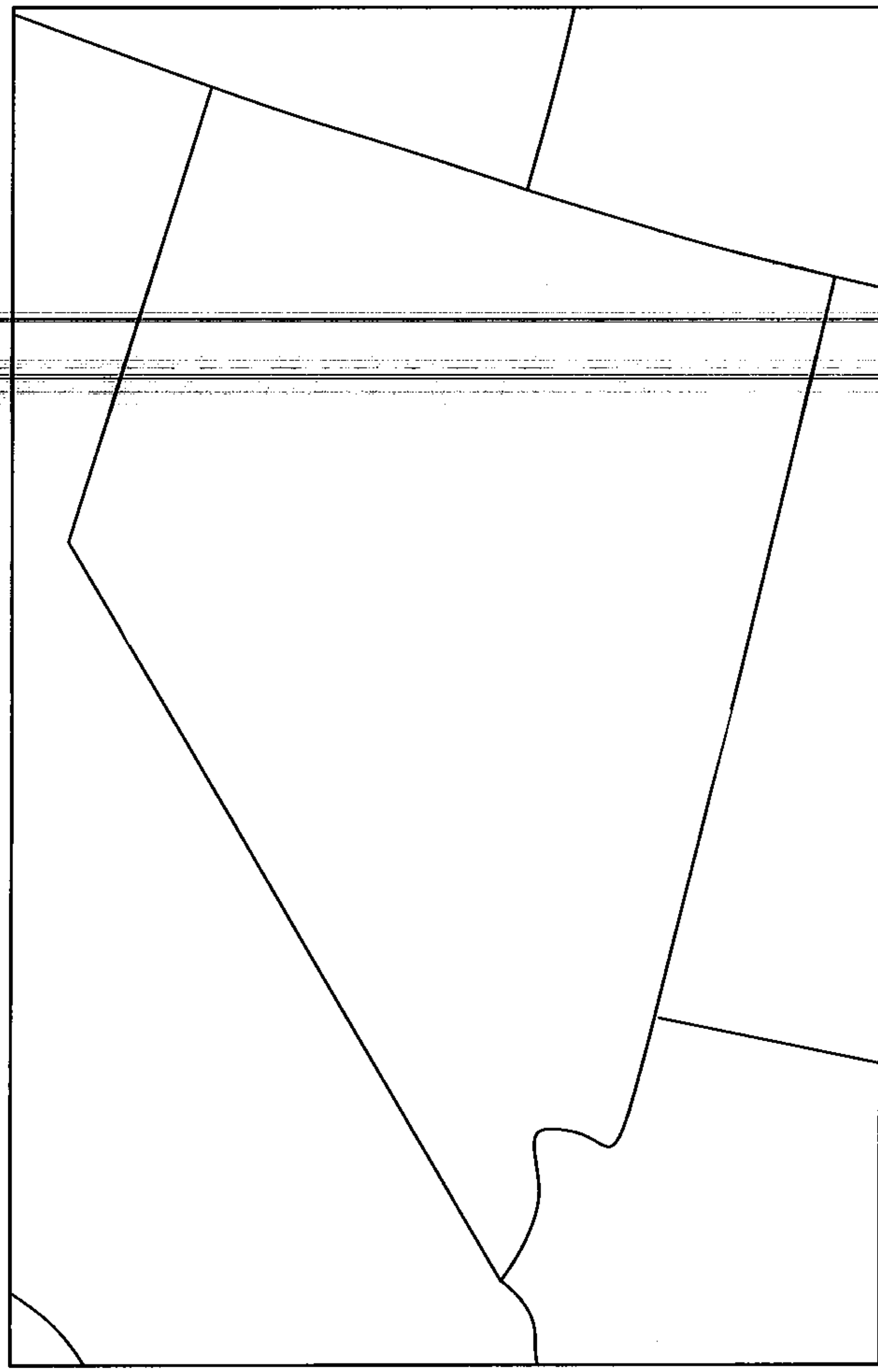


Figure 5

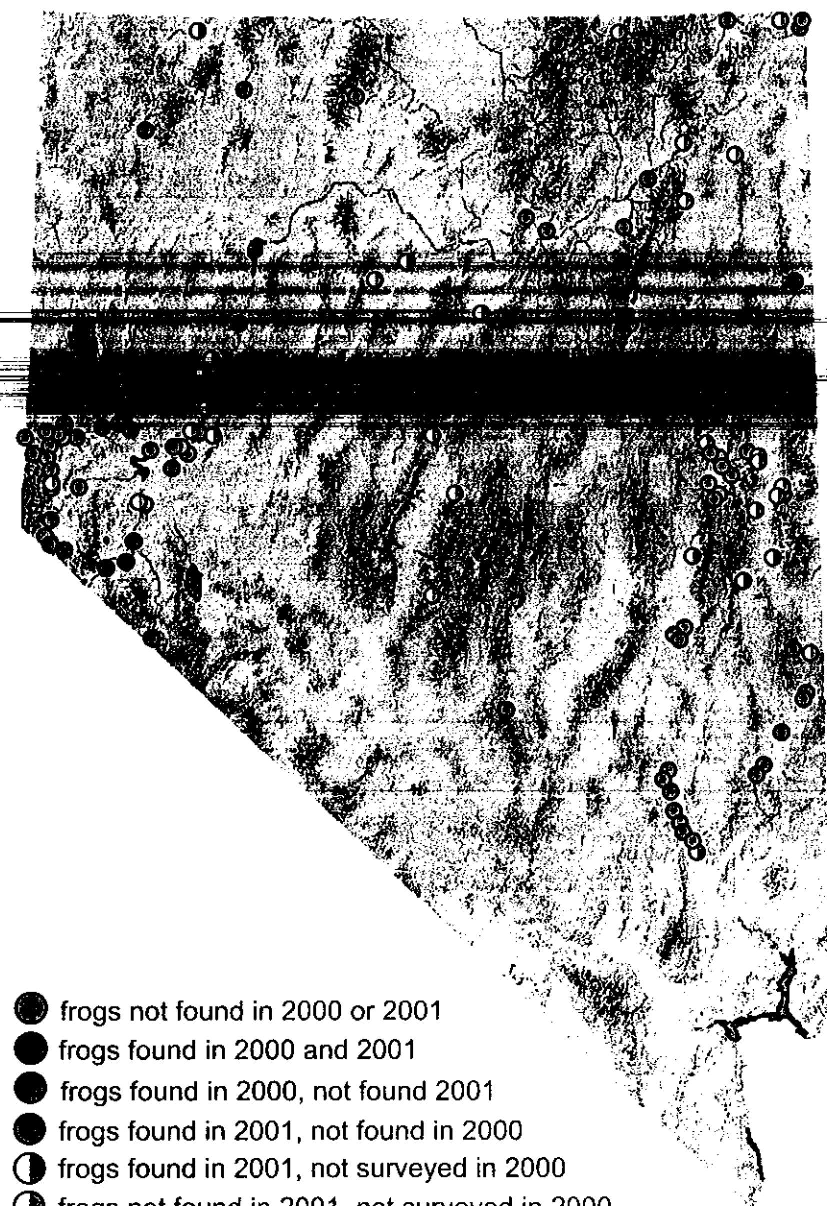
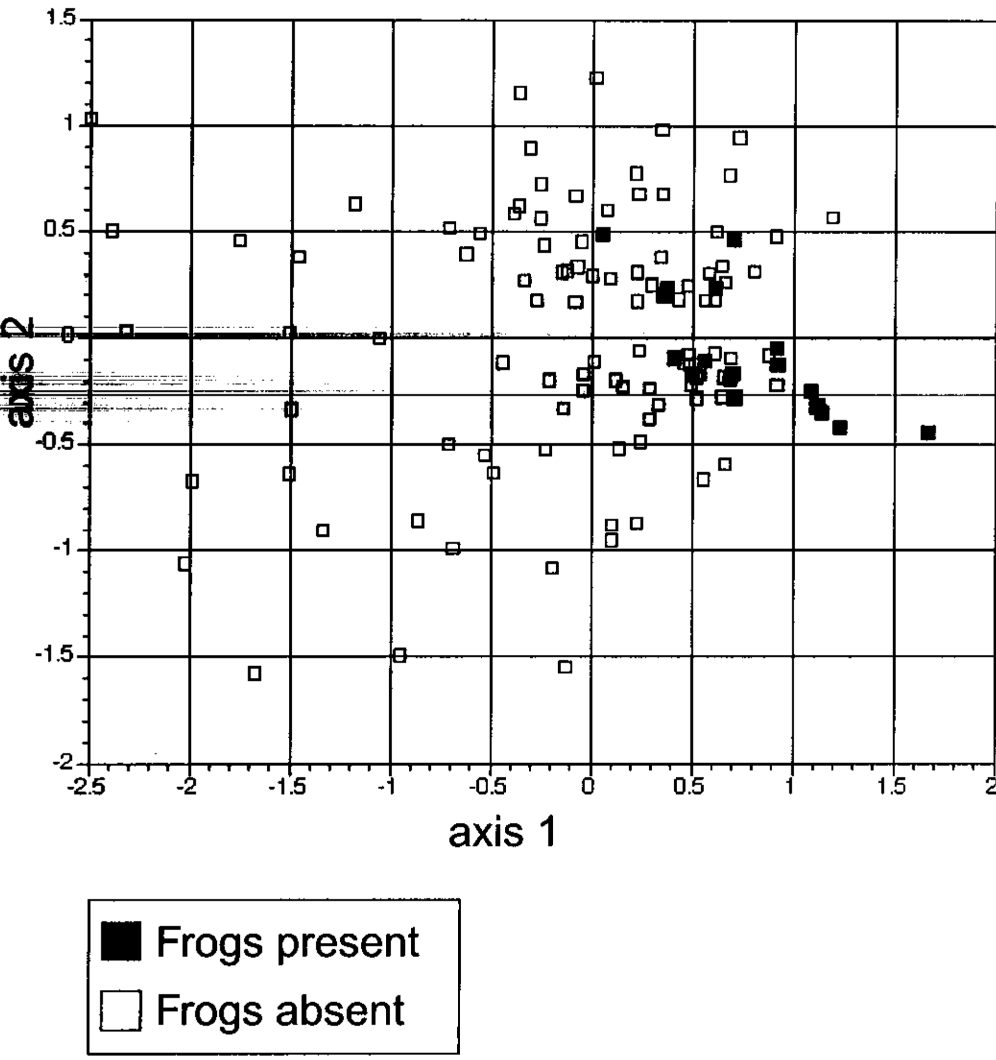

frogs not found in 2000 or 2001
frogs found in 2000 and 2001
frogs found in 2000, not found 2001
frogs found in 2001, not found in 2000
frogs found in 2001, not surveyed in 2000
frogs not found in 2001, not surveyed in 2000

Figure 6



1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
axis 2
axis 1
Frogs present
Frogs absent

Figure 7

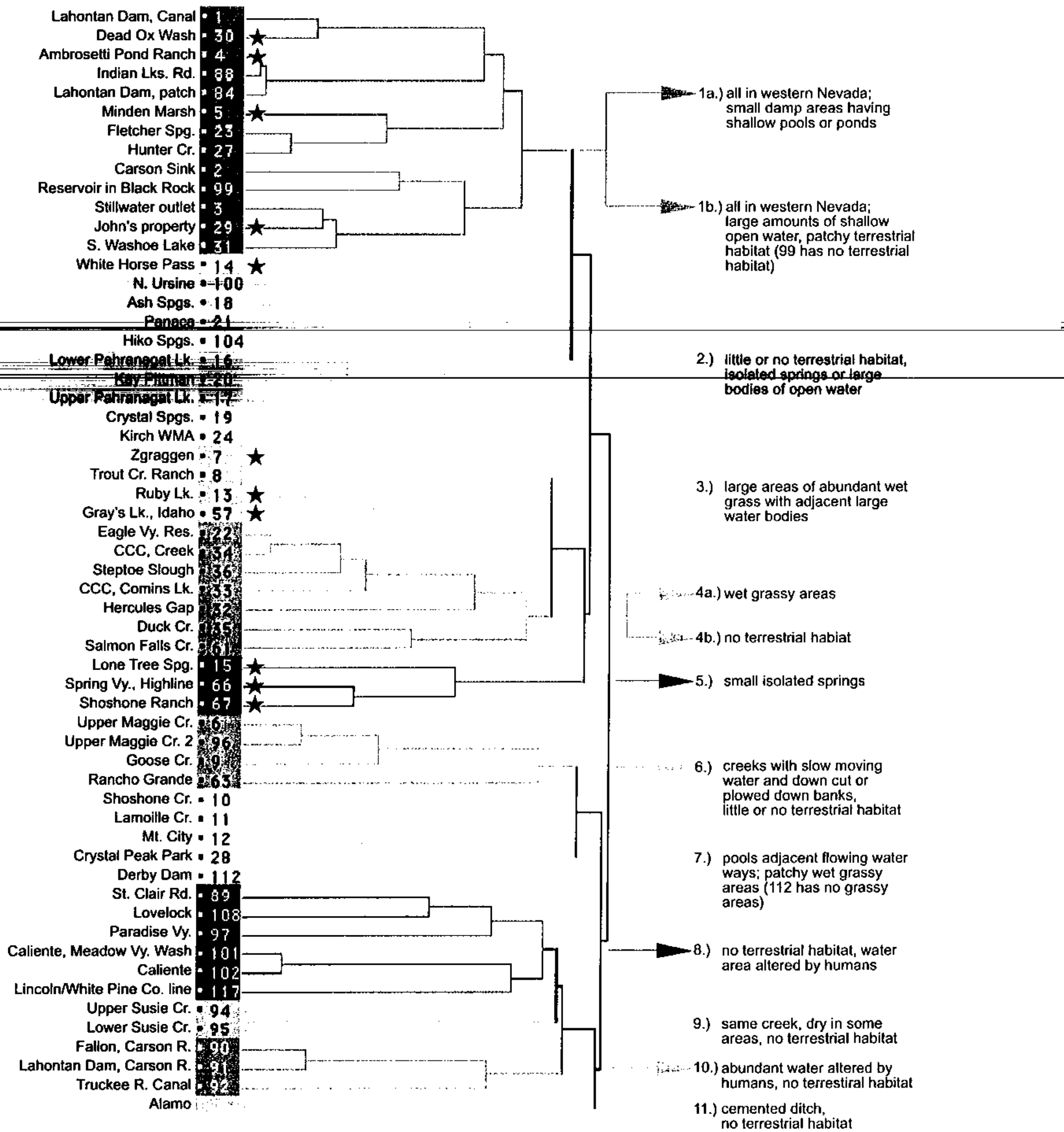
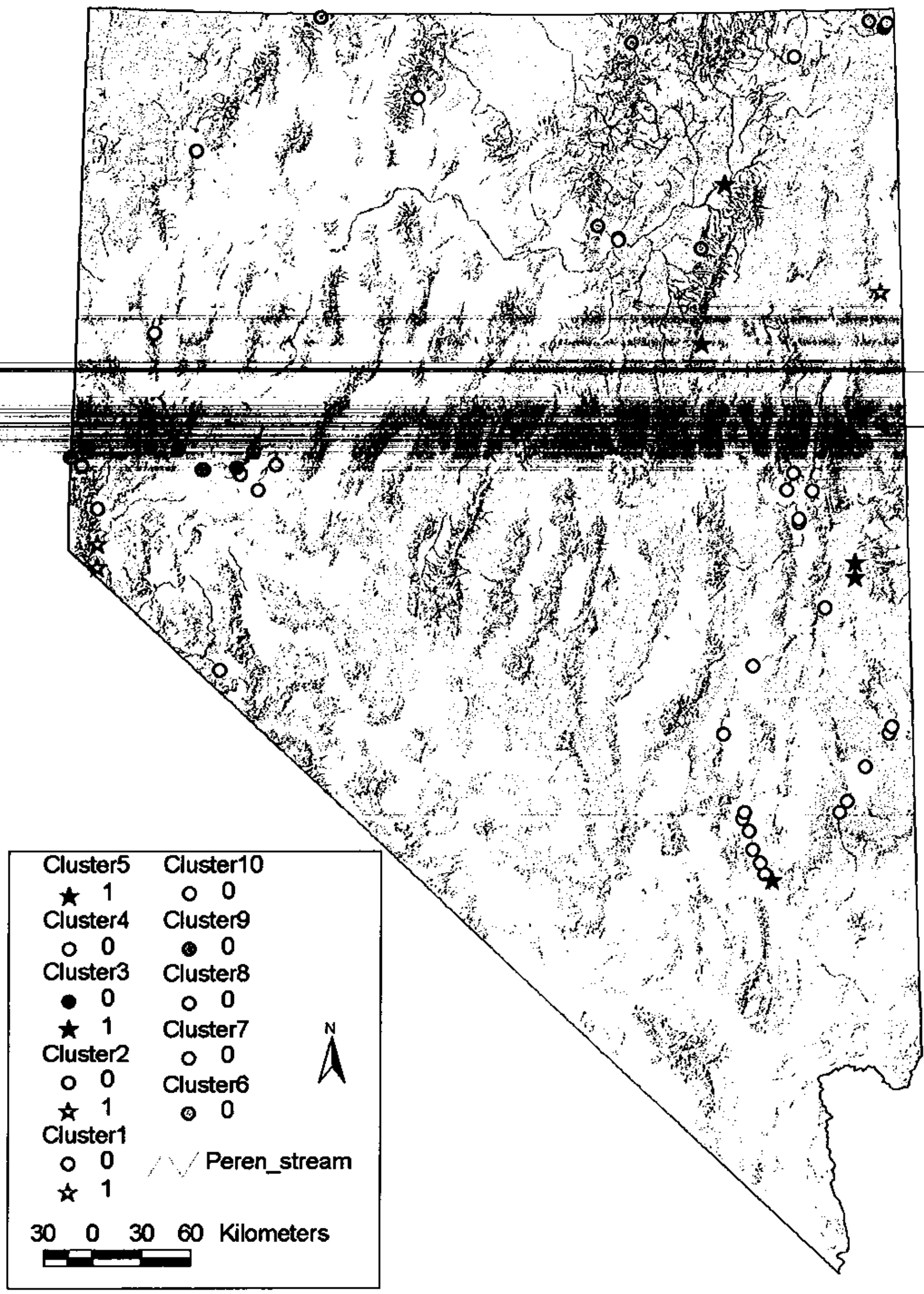

Lahontan Dam, Canal 1
Dead Ox Wash 30
Ambrosetti Pond Ranch 4
Indian Lks. Rd. 88
Lahontan Dam, patch 84
Minden Marsh 5
Fletcher Spg. 23
Hunter Cr. 27
Carson Sink 2
Reservoir in Black Rock 99
Stillwater outlet 3
John's property 29
S. Washoe Lake 31
White Horse Pass 14
N. Ursine 100
Ash Spgs. 18
Panaca 21
Hiko Spgs. 104
Lower Pahranagat Lk. 16
Key Pittman 20
Upper Pahranagat Lk. 17
Crystal Spgs. 19
Kirch WMA 24
Zgraggen 7
Trout Cr. Ranch 8
Ruby Lk. 13
Gray's Lk., Idaho 57
Eagle Vy. Res. 22
CCC, Creek 34
Steptoe Slough 36
CCC, Comins Lk. 33
Hercules Gap 32
Duck Cr. 35
Salmon Falls Cr. 61
Lone Tree Spg. 15
Spring Vy., Highline 66
Shoshone Ranch 67
Upper Maggie Cr. 6
Upper Maggie Cr. 2 96
Goose Cr. 9
Rancho Grande 63
Shoshone Cr. 10
Lamoille Cr. 11
Mt. City 12
Crystal Peak Park 28
Derby Dam 112
St. Clair Rd. 89
Lovelock 108
Paradise Vy. 97
Caliente, Meadow Vy. Wash 101
Caliente 102
Lincoln/White Pine Co. line 117
Upper Susie Cr. 94
Lower Susie Cr. 95
Fallon, Carson R. 90
Lahontan Dam, Carson R. 91
Truckee R. Canal 92
Alamo
1a.) all in western Nevada; small damp areas having shallow pools or ponds
1b.) all in western Nevada; large amounts of shallow open water, patchy terrestrial habitat (99 has no terrestrial habitat)
2.) little or no terrestrial habitat, isolated springs or large bodies of open water
3.) large areas of abundant wet grass with adjacent large water bodies
4a.) wet grassy areas
4b.) no terrestrial habiat
5.) small isolated springs
6.) creeks with slow moving water and down cut or plowed down banks, little or no terrestrial habitat
7.) pools adjacent flowing water ways; patchy wet grassy areas (112 has no grassy areas)
8.) no terrestrial habitat, water area altered by humans
9.) same creek, dry in some areas, no terrestrial habitat
10.) abundant water altered by humans, no terrestiral habitat
11.) cemented ditch, no terrestrial habitat

Figure 8


Cluster5
1
Cluster10
0
Cluster4
0
Cluster9
0
Cluster3
0
1
Cluster8
0
Cluster7
0
Cluster2
0
1
Cluster6
0
Cluster1
0
1
Peren_stream
N
30 0 30 60 Kilometers