BEFORE THE SECRETARY OF INTERIOR

PETITION TO LIST THE RELICT LEOPARD FROG (*RANA ONCA*) AS AN ENDANGERED SPECIES UNDER THE ENDANGERED SPECIES ACT

CENTER FOR BIOLOGICAL DIVERSITY
SOUTHERN UTAH WILDERNESS ALLIANCE
PETITIONERS

May 8, 2002

EXECUTIVE SUMMARY

The relict leopard frog (*Rana onca*) has the dubious distinction of being one of the first North American amphibians thought to have become extinct. Although known to have inhabited at least 64 separate locations, the last historical collections of the species were in the 1950s and this frog was only recently rediscovered at 8 (of the original 64) locations in the early 1990s. This extremely endangered amphibian is now restricted to only 6 localities (a 91% reduction from the original 64 locations) in two disjunct areas within the Lake Mead National Recreation Area in Nevada.

The relict leopard frog historically occurred in springs, seeps, and wetlands within the Virgin, Muddy, and Colorado River drainages, in Utah, Nevada, and Arizona. The Vegas Valley leopard frog, which once inhabited springs in the Las Vegas, Nevada area (and is probably now extinct), may eventually prove to be synonymous with *R. onca*.

Relict leopard frogs were recently discovered in eight springs in the early 1990s near Lake Mead and along the Virgin River. The species has subsequently disappeared from two of these localities. Only about 500 to 1,000 adult frogs remain in the population and none of the extant locations are secure from anthropomorphic events, thus putting the species at an almost guaranteed risk of extinction. The relict leopard frog has likely been extirpated from Utah, Arizona, and from the Muddy River drainage in Nevada, and persists in only 9% of its known historical range.

Habitat changes due to water development, flood control projects, and agricultural and urban development impacts were responsible for eliminating much of the frog's original habitat. The damming of the Colorado River and the formation of Lake Mead Lake in 1935, and Lake Mojave in 1951 flooded the vast majority of historical relict leopard frog habitat, reduced connectivity between the remaining populations, and altered the hydrologic regime necessary to maintain optimal relict leopard frog habitats.

The remaining relict leopard frog populations suffer from low genetic variation and are very vulnerable to extinction due to population fragmentation and the small size and isolation of remaining habitat. The species is also threatened by potential water development along the Muddy and Virgin Rivers; predation and competition by introduced species such as bullfrogs (*Rana catesbeiana*), exotic fish, and crayfish (*Procambarus clarkii*); habitat alteration by invasive plants; the potential for contracting diseases that have decimated other leopard frog (*Rana pipiens* complex) species in the region; impacts from feral burros (*Equus asinus*); recreational impacts by visitors to Lake Mead; and habitat alteration due to natural flooding (e.g., erosion and scouring) and drought events.

Existing regulatory mechanisms have failed to prevent the continued elimination of relict leopard frogs from remaining sites, and a listing as endangered under the Endangered Species Act is needed to rescue this species from the brink of extinction.

NOTICE OF PETITION

The Center for Biological Diversity and the Southern Utah Wilderness Alliance ("Petitioners") formally request that the United States Fish and Wildlife Service ("USFWS") list the relict leopard frog (*Rana onca*) as an endangered species under the federal Endangered Species Act ("ESA"), 16 U.S.C. §§1531-1544. This petition is filed under 5 U.S.C. §553(e) and 50 C.F.R. part 424.14. Petitioners also request that critical habitat for the relict leopard frog be designated concurrent with its listing, pursuant to 50 C.F.R. part 414.12 and 5 U.S.C. §553.

The relict leopard frog was thought to have been extinct since the 1950s, but was rediscovered in the early 1990s. The species currently has no formal federal protection. This petition demonstrates that the relict leopard frog faces an imminent threat of extinction. The species is now confined to a tiny remnant portion of its historical range (only 6 of 64 known locations [= 9%]), and 2 of the 8 known populations during the 1990s (= 3%) have been extirpated within the last decade. There are only six remaining small springs within a few square km area that support relict leopard frogs. Each population consists of dozens to a few hundred adult frogs, and connectivity and the potential for dispersal between these populations are minimal to non-existent. The overall numbers of frogs are low enough that genetic viability of the species is a serious concern.

USFWS has jurisdiction over this petition. This petition sets in motion a specific legal process, in which the USFWS has 90 days to determine if the relict leopard frog may warrant listing under the ESA.

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The Center for Biological Diversity ("CBD") is a non-profit conservation organization dedicated to the protection of native species and their habitats in the Western Hemisphere through science, policy, and environmental law. CBD submits this petition on its own behalf and on behalf of its members and staff with an interest in protecting the relict leopard frog and its habitat.

The Southern Utah Wilderness Alliance ("SUWA") is a non-profit environmental membership organization dedicated to the sensible management of all public lands within the State of Utah, to the preservation and protection of plant and animal species, and to the preservation of Utah's remaining wild lands. SUWA has more than 15,000 members, many of whom reside in Utah and the inter-mountain west, and submits this petition on its own behalf and on behalf of its members. SUWA members use and enjoy public lands in and throughout Utah for a variety of purposes, including scientific study, recreation, hunting, aesthetic appreciation, and financial livelihood.

TABLE OF CONTENTS

I. NATURAL HISTORY AND STATUS

The relict leopard frog (*Rana onca*) is one of the first North American amphibians thought to have become extinct (*contra* Platz 1984, M. Jennings, pers. comm., 2002). The species was rediscovered at 8 springs in Nevada and Arizona in the early 1990s (R. Jennings 1993; Bradford et al. in press), but populations have since been extirpated at 2 of these springs (RLFWG 2001). The known historical distribution for *R. onca* was springs, streams, and wetlands within the Virgin River drainage in Utah, Arizona, and Nevada, downstream from the vicinity of Hurricane, Utah; along the Muddy River drainage, Nevada (Platz 1984); and along the Colorado River from its confluence with the Virgin River downstream to Black Canyon below Lake Mead, Nevada and Arizona (RLFWG 2001). The elevational range of the species was between 370 and 760 m (Stebbins 1985). Populations of leopard frogs in the Las Vegas Valley, Nevada, identified as *R. fisheri*, but which may eventually be classified as *R. onca*, were extirpated by the 1940s (Jennings 1988; Jennings and Hayes 1994a).

Currently, *R. onca* populations remain at only six sites in two general areas, both within the Lake Mead National Recreation Area in Nevada. This indicates a recent range reduction of 3% within the past decade. Relict leopard frogs inhabit three springs on the north shore of the Overton Arm of Lake Mead, Nevada (Blue Point, Rogers, and Gnatcatcher Springs), and three sites in Black Canyon, Nevada (Boy Scout, Bighorn Sheep, and Salt Cedar Tributary Springs), below Hoover Dam and Lake Mead (RLFWG 2001). A population re-discovered in the early 1990s along the Virgin River in the vicinity of Littlefield, Arizona was extirpated by 2001, as was a population at Corral Springs on the northshore of Overton Arm (RLFWG 2001). The number of adult frogs estimated to inhabit each of the remaining sites ranges from dozens to a few hundred adult frogs (AGFD 2001; NPS 2001; USFWS 2001b). An optimistic estimate of the entire adult population of the species throughout its known range is around 1,000 frogs.

This petition summarizes the natural history of the relict leopard frog, known population information and trends, and the threats to the species and its habitat. Petitioners are seeking listing of the relict leopard frog as endangered under the federal Endangered Species Act ("ESA") and request immediate action by the U.S. Fish and Wildlife Service ("USFWS") to prevent the extinction of this species.

A. NATURAL HISTORY

1. Description

Adults

The relict leopard frog is a small-sized spotted frog with an adult body length of 1 ¾ to 3 ½ inches (Stebbins 1985; Jennings 1988). The following description is based on Platz (1984) and Jennings (1988). The dorsal coloration is brown, gray, or greenish, with discrete greenish-brown spots. These spots have indefinite borders and are usually reduced or faded on the front of the body and present on the upper surfaces of the thighs. A glandular dorsolateral fold with a light stripe runs down each side of the back, becoming indistinct ½ to ¾ of the way down the dorsum. The venter (underside) is whitish, with dark mottling on the throat, and yellow or yellow-orange in the groin and on the underside of the hind limbs. Males and females are similar in appearance except males tend to be more uniform in color toward the forward part of the body and less spotted than females, have a swollen, darkened thumb base, and attain a slightly smaller size than females.

The relict leopard frog is very similar in appearance to other species of the leopard frog group (*Rana pipiens* complex), such as the northern leopard frog (*R. pipiens*), the Rio Grande leopard frog (*R. berlandieri*), and the lowland leopard frog (*R. yavapaiensis*), all of which currently occur within the range of *R. onca*. As in other leopard frogs, the hind feet of *R. onca* have well-developed webbing. However, a number of features distinguish *R. onca*, including its dorsolateral folds, generally shortened legs, incomplete supralabial stripe, spotting rather than barring on the upper surfaces of the thighs, enlarged tympana, paired vocal sacs, and lack of vestigal oviducts (Pace 1974; Platz and Mecham 1979; Platz 1984; Jennings 1988; R. Jennings et al. 1995; Jaeger et al. 2001).

The coloration and markings of the relict leopard frog have been given a wide variety of descriptions in the literature. Slevin (1928, pp. 126-127) described the coloration as:

> "brown, gray, olive, or green, with large or small discrete, dark brown spots on head, body, and limbs. These spots usually are indefinitely bordered with light blue, gray, yellow, or green, and are irregularly rounded. They may form longitudinal rows, or the spots on their light borders may be nearly absent. The dorso-lateral folds may be light or dark as the general ground color. Posterior surface of thigh may be more or less clouded, spotted or marbled with brown or gray. Lower surfaces white or yellow, sometimes clouded, marbled or reticulated with gray or brown, especially on the throat."

The distinctive color patterns of the relict leopard frog according to Wright and Wright (1949) are on the hind limbs, which are a very prominent chamois or honey yellow color, with the groin and front and rear of the hind limbs containing many reticulations of deep olive and pale olive-gray. The poetic color descriptions given by Wright and Wright (1949, p. 455 [under the name *R. fisheri*]) were:

> "*Female*. Upper parts dusky olive green, spots dark greenish olive. Sides are light grayish olive with dark olive spots. In the groin and on front of hind legs, and rear as well, are vermiculations of deep olive and pale olive-gray. Throat light grape green or light turtle green with some pale pinkish cinnamon, clouded with dark grayish olive. Breast and belly pinkish cinnamon clouded like throat. The rear of tibia and some of foot with deep colonial buff or colonial buff. Another female has throat cartridge buff, and breast and forelegs the same with no clouding. Females have more spots on the back than the males.
>
> *Male*. They range in color from cedar green to dark greenish olive. The spots range from rainette green to pois green. The greatest difference between males and females is this tendency of old

males to have spots obscured, being almost uniform like a bullfrog. The forward part of the body may be methyl green. The tympanum may be like background or may be wood brown. Honey yellow or chamois color is present on underside of hind legs."

Tadpoles

Fully developed relict leopard frog tadpoles (to 3.3 inches total length) have a dull citrine or greenish olive dorsum, a heavily mottled, elongate, pale green-yellow tail with a rounded tip, and a semitransparent venter (Wright and Wright 1949; Jennings 1988).

Name Etymology

The etymology of the relict leopard frog can be traced to the Latin "*Rana*", meaning frog, and possibly the Greek "*oncos*," meaning swelling (Jennings 1988). It is theorized that in naming the species, Cope (1875) was referring either to the swollen appearance of the first specimen he collected (Jennings 1988) or likening its spots to those of the jaguar, as in *Panthera onca* (Sredl 1992).

2. Taxonomy

Rana onca is a true frog in the family Ranidae. It was originally described by Cope *in* Yarrow (1875) from a single adult female collected in 1872 by Henry Crècy Yarrow within the Virgin River drainage, likely in the vicinity of Saint George, Washington County, Utah (Tanner 1929; Jennings 1988). Based on a number of gross morphological similarities, this frog is considered a member of the *Rana pipiens* complex (leopard frogs), a group consisting of more than 25 species in North and Central America (Hillis 1988; RLFWG 2001).

Recent morphological, molecular, and phylogenetic analysis of extant and recently extirpated leopard frog populations along the Virgin River in Arizona, and on the northshore of Overton Arm and Black Canyon areas of Lake Mead, Nevada, has confirmed these populations as *R. onca* Cope (R. Jennings et al. 1995; Jaeger et al. 2001). Leopard frog specimens collected from populations now extirpated from Las Vegas Valley, Nevada, have alternatively been described as *R. onca* or *R. fisheri*, the Vegas Valley leopard frog (Platz 1984; Jennings 1988). The question of the systematic relationship between *R. onca* and *R. fisheri* remains unresolved despite a long debate on the taxonomy. A more detailed discussion of this debate is included as Appendix 4.

This petition will consider the extant leopard frog populations within the known historical range of the species (the Virgin River drainage in Utah, Arizona, and Nevada, downstream from the vicinity of Hurricane, Utah; along the Muddy River drainage, Nevada; and along the Colorado River from its confluence with the Virgin River downstream to Black Canyon below Lake Mead, Nevada and Arizona) as *R. onca*, the relict leopard frog. The petition will discuss the historical distribution and abundance of *R. fisheri*, the extinct Vegas Valley leopard frog, with the understanding that further molecular and morphological analysis of preserved specimens of *R. fisheri* may warrant synonymy of the taxa. The implications of whether the taxa are considered to be synonymous or distinct will be discussed in section I.B.3 below on population trends.

3. Distribution

The known historical distribution for *R. onca* was springs, streams, and wetlands within the Virgin River drainage in Utah, Arizona, and Nevada, downstream from the vicinity of Hurricane, Utah; along the Muddy River drainage, Nevada (Platz 1984); and along the Colorado River from its confluence with the Virgin River downstream to Black Canyon below Lake Mead, Nevada and Arizona (RLFWG 2001). Currently, *R. onca* populations remain at only six sites in two general areas, both within the Lake Mead National Recreation Area in Nevada. A table of locations of extant and recently extirpated *R. onca* populations can be found in Appendix 2.

Rana fisheri was historically known from a number of localities at the headwaters of Las Vegas Creek and numerous artesian springs in the Las Vegas Valley (Linsdale 1940), as well as Tule Springs, Nevada (Stebbins 1951). These populations are now considered to be extinct (Jennings 1988; Jennings and Hayes 1994a). A more thorough discussion of the historical and current distribution of *R. onca* and *R. fisheri* can be found in section I.B below.

4. Habitat

Potential *R. onca* habitat includes permanent small streams, springs, and spring-fed wetlands below approximately 760 m (Jennings 1988). Relict leopard frogs did not inhabit the Colorado River proper, as the lowland leopard frog already filled this ecological niche (Jennings and Hayes 1994a). Juvenile *R. onca* have been observed in the same areas as adults and their habitat requirements are presumed to be similar (Bradford et al. in press). Historically, relict leopard frogs were presumably limited to habitats characterized by clean, clear water (both deep and shallow) and cover/forage habitat such as submerged, emergent, and perimeter vegetation (RLFWG 2001). Emergent or submergent vegetation such as bulrushes, cattails, spikerushes (*Eleocharis* sp.), or small tules (*Scirpus* sp.) is probably needed for cover and as substrate for oviposition (Jennings et al. 1994). Historical photographs from 1903 of relict leopard frog habitats in the Las Vegas area show riparian habitats with all the above characteristics (Jennings and Hayes 1994a). Present day observations suggest that adults prefer relatively open shorelines where dense vegetation does not dominate (Bradford et al. in press).

Since 1920 there have been severe habitat alterations in the range of the species as well as introductions of non-native fish, predatory invertebrates, and amphibians into these habitats. Remaining populations of leopard frogs are restricted to perennial desert springs along the Virgin and Colorado River drainages. Water sources for all six of the sites where frogs remain are geothermally influenced, with relatively constant water temperatures between 16° and 55° C (Pohlmann et al. 1998). Currently occupied habitats seem to reflect an ecological preference for minimally disturbed spring or spring-fed environments - the locations that relict leopard frogs historically occupied. Spring or spring-fed habitats may be critical for one or more life history traits (such as embryo development or larval growth) of this species (M. Jennings, pers. comm., 2002). While naturalists have collected other species of leopard frogs in the southwest in modified habitats, even in canals and roadside ditches (Jennings et al. 1994), relict leopard frogs have not been collected in such habitats in the past century. The species is a relict, not suited to extensive anthropomorphic habitat changes or introduced aquatic predators.

The three general areas recently inhabited by relict leopard frogs differ substantially. The Littlefield site is a small, marshy wetland formed by a spring near the shore of the Virgin River. Frogs there, which are now extirpated, had been found mostly near the spring source. The sites around the Overton Arm of Lake Mead are fast moving springs formed by geothermal upwelling. The stream channels are cut into gysiferous soil and are mostly overgrown with dense stands of emergent vegetation. Black Canyon habitats are geothermal springs that flow over rockier substrates, with mesquite and introduced salt cedar (*Tamarix* sp.) dominating the over-story vegetation, where present.

The following is a summary by the Relict Leopard Frog Working Group (RLFWG 2001) of the habitat characteristics at the sites of the six extant and two recently extirpated (Littlefield and Corral Spring) *R. onca* populations.

Littlefield

The Littlefield site encompasses a marshy area formed by a spring flowing from a steep embankment along the Virgin River. The site adjoins the river and is characterized by thick stands of *Eleocharis* overhanging the water and forming covered pools, filling a wide meander opposite a point bar. Frogs were found from the spring discharge point throughout the marshy area, but were not found along the river proper.

Northshore Spring Complex

The Northshore spring complex originates from the Rogers Spring Fault along the southern base of the Muddy Mountains. Blue Point, Rogers, and Corral Springs surface directly from the fault, while Gnatcatcher Spring flows from basin-fill deposits between the Muddy Range and Lake Mead. Water temperatures are constant year round and do not vary significantly from origin to end. Only Rogers Spring contacts Lake Mead. All are sub regional springs, dominated by groundwater originating outside local topographic basins and flow systems (Pohlmann et al. 1998).

Blue Point and Rogers Springs

With discharges of 1040 liters/minute ("L/min") and 2750 L/min respectively, Blue Point and

Rogers Springs form the largest habitats and support the highest numbers of frogs in the Northshore complex. The springs flow through gypsiferous soils, forming deeply incised channels, 60 cm deep and 25 cm wide. Channel substrate is composed of gravelly, precipitated solids.

Shallow overflow pools are mostly-permanent features along the course margin, and provide important *R. onca* habitat. The pools are typically narrow, ranging from 25 to 200 cm in width, along both sides of the channel. Wider pools form in some areas, sometimes developing into marshy areas. Pool depth ranges up to 30 cm, but typically does not exceed 5 cm. Pool substrate is gypsum mud combined with organic matter.

Pools are used by *R. onca* as foraging, basking, and egg deposition sites. Frogs are most often found in 1 to 7 cm of water, with most choosing depths of 1 to 4 cm. At Blue Point Spring, most individuals choose locations 75 to 150 cm from the main channel and 25 to 75 cm from clumps of dense vegetation.

Gnatcatcher and Corral Springs

Gnatcatcher and Corral Springs are significantly smaller and cooler than Blue Point and Rogers Springs and are much shallower, but possess a few deep pools. Discharge is <1 L/min. Water temperature is 16° C and does not vary significantly along the course lengths.

<u>Black Canyon Springs</u>

In contrast to the Northshore habitats, the Black Canyon springs occupied by relict leopard frogs flow through narrow canyons of igneous bedrock. Boy Scout and Bighorn Sheep Springs in particular possess steeper gradients than the Northshore springs, and are characterized by waterfalls, plunge pools, and long, shallow riffles. Non-flood discharge rates and water temperatures remain constant.

Boy Scout Spring

Boy Scout Spring is derived from two primary water sources, of temperatures 55° C and 24° C, with a combined discharge of 960 L/min. Side seeps and small springs, most contributing <1 L/min, also vary widely in temperature. Features of the main stream include long riffles of <1 cm in depth, spilling into pools up to 60 cm deep.

Small-diameter tamarisk (*Tamarix ramosissima*) overhangs portions of the upper stream; open areas support Emory's baccharis (*Baccharis emoryi*) and some catclaw acacia (*Acacia greggii*). Cool side seeps support a variety of vegetation, including cattails (*Typha domingensis*), rock daisies (*Perityle emoryi*), and grasses (*Bromus rubens, Polypogon monspeliensis, Andropogon glomeratus*). The lower portion of the stream mostly lacks stream- and pool-side vegetation, but small, barely moist seeps at the cliff base provide moisture for small assemblages of maidenhair ferns (*Adiantum capillus-veneris*), rock daisy, desert tobacco (*Nicotiana trigonophylla*), and rock nettle (*Eucnide urens*).

These small communities of vegetation may provide important cover for relict leopard frogs, which are found at night in the shallow riffles of the lower drainage. In the upper portion of the stream, frogs are sometimes found in the riffles, and more reliably in cooler side pools formed by seeps and filled with emergent vegetation. Adults or tadpoles have not been observed using plunge pools in any portion of the main stream, in contrast to Bighorn Sheep Spring, where pools are an important habitat component. Pools in the main channel of Boy Scout Spring, however, are very hot with substantially greater water velocity.

Bighorn Sheep Spring

Bighorn Sheep Spring has a discharge rate of 10.2 L/min and a temperature of 16º C. As with Boy Scout Spring, the course varies in depth, but does so more gradually. Small gravel bars appear regularly. Large boulders from past flood events tend to block the channel, forming small waterfalls. Although a small gravel cove on the river marks the Bighorn Sheep drainage, the spring disappears underground before reaching the Colorado River (approximately 100 m above the high water mark of the river).

Fairly dense stands of small-diameter tamarisk overhang much of the stream. Riffles tend to be disguised by vegetation growing in the shallow centers, or invaded by thread-like tamarisk roots. Areas open overhead support a variety of perennial and seasonal forbs, including grasses (*Bromus rubens* and *Polypogon monspeliensis*), daisies (*Perityle emoryi*, *Haplopappus gooddingii*, *Brickellia californica*), sow weed (*Sonchus oleraceus*), desert tobacco (*Nicotiana trigonophylla*), Datura (*Datura meteloides*), and borage (*Cryptantha utahensis*). A few young stands of cattails (*Typha*, likely *T. domingensis*) are present.

Relict leopard frogs heavily utilize almost all habitat components (pools, riffles, gravel bars, etc.) at Bighorn Sheep Spring, in contrast to other sites, where favorite basking sites represent a smaller percentage of total available space. Seeps covered with moss (*Funaria sp.*), maidenhair ferns (*Adiantum capillus-veneris*), and other vegetation provide additional cover and feeding sites.

Salt Cedar Spring

Salt Cedar spring currently supports only very shallow surface water flow, likely caused by high evapotranspiration of the tamarisk choking the drainage. Relict leopard frogs are restricted to a very narrow tributary. Substrate is small-diameter gravels.

5. Behavior

Relict leopard frogs are apparently active year-round, although they must have hibernated at certain historical locations (above 600 m) where it would most certainly freeze during the winter months. There is no evidence of torpor or hibernation during cold weather, although adult frogs are reported to be more difficult to find during the coldest months, even in geothermally influenced springs (Bradford et al. 2001).

Although individuals can be found basking or sitting at any hour regardless of season, activity appears to generally shift seasonally. During summer months frogs are primarily nocturnal, shifting to an increasingly diurnal schedule during the winter (RLFWG 2001). The species is found only in permanent wet sites and there is no evidence of diminished activity or aestivation during summer or dry periods (Bradford et al. 2001).

a. Movement

The remaining leopard frog populations are restricted to narrow habitat corridors, with a sharply defined boundary between riparian corridor and desert. During a 3-year mark-recapture study in the isolated, 550 m upper reach of Blue Point Spring, the mean distance moved by frogs between captures averaged 18 m, and the longest distance recorded between recaptures was 120 m (D. Bradford, unpublished data, as cited in RLFWG 2001). R. Jennings et al. (1995) noted that of 11 recaptures of marked relict leopard frogs, the furthest recorded movement was 200 m. Studies have shown no evidence of seasonal migration or hibernation (Bradford et al. 2001). Adult *R. onca* are known to reside in and near breeding habitat and breeding migrations are unknown (Bradford et al. 2001). Because all known spring habitats are not connected with one another, either by an aquatic connection or a vegetative connection, these frog populations are effectively isolated from one another because of their unwillingness to cross desert habitats during the relatively few wet periods of the year (M. Jennings, pers. comm., 2002).

b. Reproduction and Growth

Relict leopard frogs breed in late January through April, with peak oviposition occurring in February and March. However, oviposition has also been documented in November (Bradford et al. 2001). Water temperature, which differs significantly among springs, does not appear to influence breeding season. Favored breeding habitat seems to be quiet, shallow pools outside the channel or slow moving side areas of streams (Bradford et al. 2001). Eggs discovered thus far are deposited in roughly spherical clusters 4 to 6 cm in diameter, containing up to 250 eggs. The number of clutches per female is unknown. The egg clusters are attached within a few centimeters of the water surface to stems of living or dead vegetation (Bradford et al. 2001; RLFWG 2001). Although failure to find eggs in dense cover may be due to sampling difficulty, sites with little to moderate cover seem to be preferred. In February 2000, random sections were experimentally thinned in a favored relict leopard frog pool, which was becoming densely overgrown with native vegetation. Before clearing, a careful search revealed no eggs in the pool, although several calling relict leopard frogs were present. Within a week of vegetation removal, eggs were deposited in the pool in the thinned sections (NPS, unpublished data, as cited in RLFWG 2001).

Time from egg deposition to hatching is unknown. Limited field observations at Blue Point Spring indicate hatching occurs in approximately one week (RLFWG 2001). Limited observations suggest that several months are required for relict leopard frogs to reach metamorphosis (Bradford et al. 2001). Tadpoles maintained in the laboratory at room temperature, with natural photoperiods and abundant food and space, metamorphose approximately 6.5 months after hatching. Lab tadpoles demonstrate the ability to substantially hasten or delay metamorphosis depending on tadpole density and water quality. Lab tadpoles metamorphosing earlier are active and evasive, but are noticeably smaller (NPS, unpublished data, as cited in RLFWG 2001).

Fully developed tadpoles can reach 85 mm in length (Wright and Wright 1949), although a maximum of 70 mm is more typical (S. Romin, NPS, pers. comm., 2002). Hatchlings fall below the egg mass, and hatchlings and small tadpoles are usually found in motionless groups in shallow, often coverless, pool margins for approximately 1 week after hatching. After dispersing from the hatch site, small tadpoles sometimes share shallow edges of pools, but pooling/flocking does not appear to occur. Small, medium, and large tadpoles are active diurnally, resting and browsing on substrate (RLFWG 2001).

Based on the timing of the appearance of pigmented thumb pads, male relict leopard frogs appear to reach reproductive maturity within the first year, at 42 mm snout-to-vent length (D. Bradford, unpublished data, as cited in RLFWG 2001). The age of females at maturity is unknown. The oldest individual frogs found in a four-year mark/recapture study were 2 years old (n=14). Population turnover was relatively high, with the survivorship of adults averaging only 27% per year (D. Bradford, unpublished data, as cited in RLFWG 2001).

Population Structure

Although there is no comprehensive information on the population structure of relict leopard frogs, R. Jennings et al. (1995) made some observations of population composition at the springs on the northshore of Overton Arm.[1] Bradford and Jennings (1997) noted that growth rate and population turnover appear to be relatively high, speculating this may be a consequence of geothermal influence and the associated long period of activity by terrestrial arthropods which are the food base. Bradford and Jennings (1997) estimated an annual adult survivorship of only 27%.

c. Feeding

The relict leopard frog's diet is unknown, but presumably it is similar to other *Rana* species. Most ranid adults eat small invertebrates such as insects, spiders, and crustaceans, and rarely will consume small vertebrates (AGFD 1997). Ranid larvae eat algae, plant tissue, organic debris, and probably small invertebrates (AGFD 1997). In captivity, *R. onca* tadpoles choose a predominately vegetarian diet (RLFWG 2001).

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1 At the apparently stable population at Blue Point Spring, there were no observations of breeding activity (such as calling males, eggs, or tadpoles), and the population consisted of almost exclusively large adult animals, suggesting that oviposition and tadpole development were occurring elsewhere. Eggs or calling males were noted at Rogers Spring during 1992 to 1994. The frog population at Corral Spring both before and during its decline consisted primarily of adult frogs, although eggs, early staged tadpoles, or calling males were observed there in 1991, 1992, and 1993. Prior to early 1993, the sex ratio of adults was approximately 1:1, whereas males tended to predominate after October 1993. (Jennings et al. 1995).

d. **Basking/Sitting**

Relict leopard frogs are most often observed sitting motionless in shallow water along channel edges, with individuals spaced 1 to 2 m apart along certain stream lengths. Favorite sites are almost always occupied, often with two or three individuals clustered within 10 to 30 cm of each other. Favored sites are very specific, as frogs are often found sitting the next morning exactly under the flagging of precisely marked locations from the previous night's surveys (RLFWG 2001).

e. **Escape**

Adult relict leopard frogs leap into deep water or thick vegetation when disturbed. During the day, individuals generally leap immediately, before being seen. At night, frogs usually sit motionless unless threatened. Once individuals leap, they are almost impossible to relocate. However, if observers remain quietly nearby, most will reappear within 10 to 15 minutes, indicating that even those fleeing into swift currents of primary spring channels do not move far if not further threatened (RLFWG 2001).

Tadpoles randomly flee when disturbed, rather than exhibiting flocking behavior. Disturbed tadpoles were observed to seek cover among vegetation and loose mud at Northshore springs and under large rocks and undercut ledges at Bighorn Sheep Spring (RLFWG 2001). At Blue Point Spring, large tadpoles are almost always found buried into loose gypsum mud and debris, with their eyes remaining above the substrate. When disturbed, they dart a short distance (30 to 45 cm) and rapidly re-bury (RLFWG 2001).

f. **Calling**

Relict leopard frogs call from shallow areas while concealed in vegetation. Calls are a series of soft clucks. Relict leopard frogs call singly or in response, rather than forming large choruses. Calling has been documented only during winter and early spring. Although more effort to locate calling individuals has occurred during these months, calling noted while performing unrelated work also occurs most often in January through March. Proximity of non-calling individuals to calling individuals has not been documented (RLFWG 2001). A sonogram of this frog's call has not been made to date.

During January, February, and March 1997, calling surveys took place at Corral, Blue Point, and Rogers Springs. Calling surveys were not pursued as a survey method after concurrent investigations at known egg deposition sites at Blue Point Spring revealed the surveys are not a viable survey method for *R. onca*. Relict leopard frogs call too quietly to be heard from more than approximately 15 m, and cease calling when the habitat patch is approached (RLFWG 2001).

g. **Response to Rain**

The effect of wind and rain on relict leopard frog behavior has been studied (RLFWG 2001).

Searches for frogs at known locations occurred during and after high winds and rain to determine the effect, if any, on activity patterns. The number of frogs observed did not correlate with wind speed (wind in excess of 40 mph was not uncommon), perhaps because velocity was significantly reduced within the vegetation at ground level. Rain, however, did influence *R. onca* activity. Relict leopard frogs were never active during or immediately following rain, despite significantly higher humidity and the temporary availability of larger pools. Precipitation, even during summer, results in cooler pool temperatures, which may be unattractive to *R. onca* (RLFWG 2001).

B. STATUS

1. Historical Distribution and Abundance

Rana onca historically occurred at the edge of the ranges of *R. chiricahuensis* (the Chiricahua leopard frog), *R. pipiens* (the northern leopard frog), and *R. yavapaiensis* (the lowland leopard frog), and survived as a relict population in the marginal habitats of desert springs and creeks. To delineate the historical range of *R. onca*, Bradford et al. (unpublished data, as cited in RLFWG 2001) solicited specimen records from 34 museums for *Rana* spp. from Washington County, Utah; Clark and Lincoln Counties, Nevada; Mohave County, Arizona; and San Bernardino County, California (as followed in Jennings and Hayes 1994a, 1994b). Herpetological literature from the region and recent collections were also examined.

Based on these museum specimens, recent collections, and literature, the known historical distribution for *R. onca* is considered to have been springs, streams, and wetlands within the Virgin River drainage downstream from the vicinity of Hurricane, Utah; along the Muddy River drainage, Nevada; and along the Colorado River from its confluence with the Virgin River downstream to the Black Canyon area below Lake Mead, Nevada and Arizona (RLFWG 2001). Since 1920 there have been severe habitat alterations as well as introductions of non-native fish and amphibians into these habitats, and *R. onca* was considered to have been extinct throughout its range from the 1950s (Platz 1984; Jennings 1988) until it was re-discovered in 1991.

All known localities are within a few kilometers of these rivers – probably because spring habitats are most abundant here. This apparent restriction in proximity to the main rivers, however, may be partially an artifact of historical collecting activities (RLFWG 2001). Jennings and Hayes (1994) did not note occurrences along the Colorado River proper, but Cowles and Bogert (1936) reported collecting relict leopard frogs from the mouth of Boulder Wash, Arizona at the Colorado River as the waters filling Lake Mead were flooding the area. Prior to the formation of Lake Mead, spring habitats along portions of the Colorado River may have been more favorable (Bradford et al. 2001) to the species. Speculatively, dispersing juveniles or subadults of *R. onca* may have also occurred at lowland localities along the Colorado River upstream from the confluence with the Virgin River (RLFWG 2001).

There have been efforts to locate *R. onca* in southeastern California. C. Fleming collected a leopard frog from an unspecified location in California on July 16, 1925 that he thought might have been *R.*

onca (FMNH 2001). However, in looking for evidence of *R. onca* in California, Jennings and Hayes (1994a, 1994b) extensively reviewed available museum specimens, field notes of naturalists, and interviewed knowledgeable individuals. The chance that they overlooked an important source of information is probably remote. Bradford et al. (unpublished data, as cited in RLFWG 2001) solicited museum specimen records from San Bernardino County, California and searched herpetological literature without finding any historical records from California. All known records of leopard frogs from southeastern California have turned out to be either native *R. yavapaiensis* or recently introduced *R. berlandieri* (Jennings and Hayes 1994a).

Rana fisheri was historically found in Nevada in creeks, springs, and seeps in the vicinity of Las Vegas Valley, Clark County, at elevations between 370 and 760 m (Platz 1984; Stebbins 1985). *Rana fisheri* was presumably extirpated throughout its historical range by 1942 (Stebbins 1951; Jennings and Hayes 1994a).

A map of the historical distribution of *R. onca* and *R. fisheri* can be found in Appendix 1.

a. Nevada

Rana onca was historically found in Nevada around the Overton Arm of what is now Lake Mead; along the Muddy River and Meadow Valley Wash, northwest of the Overton Arm; and within Black Canyon along the Colorado River, below Hoover Dam (Bradford, et al. 2001; USFWS 2001b). Specimen records are known from 1935 along the Virgin River in Clark County (Los Angeles County Museum specimen, as cited in R. Jennings et al. 1995); from 1955 at Black Canyon (RLFWG 2001); from 1957 at Corral Springs (Bradford and Jennings 1997); and from 1968 at Rogers Spring (Carnegie Museum specimen, as cited in R. Jennings et al. 1995).

Rana fisheri was historically collected from Las Vegas Creek and numerous artesian springs in the Las Vegas Valley, Clark County (Linsdale 1940) as well as from Tule Springs, 25.7 km north of Las Vegas (Stebbins 1951). The densest populations of *R. fisheri* were at three large springs that were at the western edge of what is currently Las Vegas, at the headwaters of Las Vegas Creek (Wright and Wright 1949).

After E. W. Nelson collected the paratype of *R. fisheri* from Las Vegas Ranch near Las Vegas on March 9, 1891 (Platz 1984), there were numerous collections and sightings of leopard frogs in the Las Vegas area until the 1940s, when *R. fisheri* was thought to have gone extinct as a result of habitat alterations (Stebbins 1951; Jennings and Hayes 1994a).[2] Water was originally diverted from the

2 Other collections in the vicinity of Las Vegas include 2 frogs taken by Nelson as well as 2 others collected by Bailey from Vegas Valley on April 13, 1891, which are in the U. S. National Museum collection (Platz 1984); numerous specimens (20 frogs taken on May 1 and 79 frogs on August 10-13) collected by Slevin from a small stream 1 mile northwest of Las Vegas in 1913 (Van Denburgh and Slevin 1921; CAS 2001); single frogs taken by Camp on March 23, 1923, and by Compton on May 5, 1934 (MVZ 2001); 7 frogs taken by Linsdale on May 11, 1934, as well as 7 more he collected in two trips on April 19 and 24, 1936 (MVZ 2001); and 2 specimens in the Los Angeles County Museum collection from 1941 and 11 specimens in the University of Michigan Museum of Zoology collection from Vanderhorst collected during January 1942 (Jennings et al. 1995). Records at Tule Springs include 6 frogs

headwaters springs and Las Vegas Creek for agricultural use. After 1900, urbanization of the valley led to capping of the springs and overdraft of groundwater (Jones and Cahlan 1975). Bullfrogs were introduced into the Las Vegas area around 1920 and had become common by the mid-1930s (Cowles and Bogert 1936). The last recorded specimens of *R. fisheri* were ten frogs collected at Tule Springs on January 13, 1942 by A. Vanderhorst, which are in the University of Michigan Museum of Comparative Zoology collection (Platz 1984).

Rana fisheri was apparently historically abundant at the headwaters of Las Vegas Creek (Wright and Wright 1949). Collections of large numbers of specimens, such as 99 frogs taken from one location near Las Vegas on two days in 1913 (Van Denburgh and Slevin 1921; CAS 2001) and 14 specimens taken in one day from another location in 1938 (CAS 2001; MVZ 2001), indicate the species was relatively abundant and easily found at one time. *Rana fisheri* was presumably extirpated throughout its historical range by 1942 (Stebbins 1951; Jennings 1988).

b. Arizona

The Arizona Game and Fish Department reported that no historical records of *R. onca* exist from Arizona (AGFD 1997). However, Cowles and Bogert (1936) reported collecting a single relict leopard frog from Littlefield and five frogs from the Muddy River in the spring of 1935. The Los Angeles County Museum has a specimen collected in 1970 from the Virgin River in Mojave County (R. Jennings et al. 1995). It is likely that the species occurred in Arizona historically, since some leopard frog specimens could have been misidentified as *R. yavapaiensis* (AGFD 1997), and a small *R. onca* population was discovered along the Virgin River drainage near Littlefield, Arizona in the early 1990s (R. Jennings et al. 1995; Bradford and Jennings 1997; Bradford et al. in prep.). There is no known information on historical abundance of the species in Arizona.

c. Utah

Rana onca was historically found in Utah from the vicinity of Hurricane, Washington County, downstream through the Virgin River valley, at elevations between 370 and 760 m (Sandmeier and Van der Meijden 2001). Most known historical localities are from the St. George area, and range from just east of Hurricane to west and south of Bloomington (R. Jennings 1993). Since Cope (1875) first identified *R. onca* from a specimen Yarrow collected somewhere in the Virgin River Valley in Utah in 1872, there were a number of collections made until 1950, when the last known Utah relict leopard frogs were taken from Berry Springs (just east of the Virgin River about 700 yards downstream from the confluence with Quail Creek).[3] Berry Springs was drained in 1973 to create a catfish (*Ictalurus* sp.) pond, and a survey of this

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collected by the Wrights on August 20, 1925 (Wright and Wright 1949), and 14 specimens taken on July 15, 1938 by Calhoun, Miller, Hubbs, and Rodgers (CAS 2001; MVZ 2001).

3 *Rana onca* specimens collected from Utah include 8 specimens collected in 1921 from the Virgin River in the California Academy of Sciences collection (Jennings et al. 1995); 2 frogs taken by Tanner (1929) in June 1928 from a small stream south of St. George; 5 specimens collected in 1928 from the Virgin River (these may include Tanner's frogs) in the Brigham Young University

spring and surrounding sites by Platz (1984) did not find any more individuals. *Rana onca* populations along the Virgin River drainage were thought to have gone extinct sometime after 1950 (Platz 1984; Jennings 1988). The only indication of the historical abundance of the species in Utah is the fact that 14 frogs could still be collected from a single location at Berry Springs on a single day in 1950, at a time when the species was presumably near extirpation in Utah (Platz 1984).

2. Current Distribution and Abundance

a. Current Distribution

The relict leopard frog was thought to be extinct since the 1950s, although there were unpublished reports of leopard frogs (thought to be *R. fisheri*) inhabiting Rogers and Blue Point Springs from 1970 and 1974 (NPS 2001a). It is unknown whether *R. onca* persisted at Corral Springs between 1957, when a specimen was collected, and 1991, when the species was re-discovered (Bradford and Jennings 1997). The species was confirmed in 1991 at three historical locations, including Blue Point and Rogers Springs (R. Jennings et al. 1995; Bradford and Jennings 1997).

Surveys of potential habitat within the historical range of the species were conducted at a total of 64 localities, 12 of which were historical localities for *R. onca* (R. Jennings et al. 1995; Bradford and Jennings 1997; Bradford et al. in prep.).[4] Some other historical localities were not searched because either suitable habitat is no longer present, or the site could not be reliably located. The Las Vegas Valley was excluded from surveys because nearly all aquatic habitat for leopard frogs has been eliminated or greatly modified, and no sightings of leopard frogs have been made at potential sites since the 1940s. As part of another study (Bradford et al., in review) amphibian surveys south of the Black Canyon area at springs in the Eldorado Mountains near Lake Mojave, Nevada revealed little permanent water and no leopard frogs. (RLFWG 2001).

Leopard frogs have been found recently at only eight sites (see Fig. 1 in Jaeger et al. 2001), two of

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collection (Jennings et al. 1995); 3 frogs collected in 1940 from the Virgin River in the University of Michigan Museum of Zoology collection (Jennings et al. 1995); 3 frogs collected in 1941 from the Virgin River in the Los Angeles County Museum collection (Jennings et al. 1995); a single frog collected by Schwartz on August 22, 1949, 6 miles east of St. George, which is now in the University of Michigan Museum of Vertebrate Zoology collection (Platz 1984); a single frog taken on March 22, 1950 from Berry Springs by H. M. Goldschmidt (FMNH 2001); 14 frogs collected by V. Tanner from Berry Springs on April 20, 1950, which are now in the Brigham Young University Museum of Natural History collection (Platz 1984); and 3 frogs collected in 1970 from Washington County now in the Los Angeles County Museum collection (Jennings et al. 1995).

4 Field surveys were conducted at historical and other sites containing potential habitat for leopard frogs, such as permanent water with pools > 30 cm deep and > 1.6 km apart (Jennings et al. 1995; Bradford et al, in prep.). Areas surveyed were: within 22 km of St. George, Utah; the Virgin River between approximately 6 km southwest of Riverside, Nevada, and 5 km northeast of Littlefield, Arizona; Muddy River and Meadow Valley Wash, Nevada, below approximately 750 m elevation; springs to the east and west of the Overton Arm of Lake Mead, Nevada (i.e., within the Virgin River Valley prior to the creation of Lake Mead in 1935); springs in Nevada and Arizona that drain to the Colorado River between Lake Mead and 6 km southwest of Willow Beach, Arizona (i.e., Black Canyon area); and the Grand Wash area of northwestern Arizona and adjacent Nevada (even though this area lacked historical records for native ranids).

which subsequently have been extirpated (Littlefield, Arizona and Corral Spring, Nevada). All of these eight localities were either historical localities (Littlefield; Overton Arm sites - Blue Point, Rogers, and Corral Springs) or within a few kilometers of historical localities (Gnatcatcher, Black Canyon sites - Boy Scout Canyon, Salt Cedar Tributary, and Bighorn Sheep Springs). Genetic analyses indicate a common mitochondrial haplotype for the Littlefield, Lake Mead, and Black Canyon populations, and all the newly discovered populations have been classified as *R. onca* (R. Jennings et al. in prep; Jaeger et al. 2001).

Individual leopard frogs were subsequently observed on different occasions in 2000 and 2001 in the fish hatchery at Willow Beach, Arizona, located 10 km downstream from Bighorn Sheep Spring in Black Canyon (C. Fiegel, pers. comm., as cited in RLFWG 2001). One of these was collected and confirmed as *R. onca* based on mitochondrial DNA sequence similarity (J. Jaeger, unpublished data, as cited in RLFWG 2001), but the fish hatchery is a highly unlikely locality for a viable population.

Rana onca is currently known to occur only in two general areas within the Lake Mead National Recreation Area: near the Overton Arm area of Lake Mead, Nevada, and Black Canyon, Nevada, 4 km downstream from Hoover Dam. Both areas represent historical localities, with specimen records dating from 1936 at the Overton Arm area and from 1955 at Black Canyon. These two areas, encompassing maximum linear extents of only 3.6 and 5.1 km, respectively, comprise a small fraction (= <1%) of the original distribution of the species. Although it is possible that *R. onca* populations may also occur in other localized areas, it is unlikely that many other occupied sites exist given the efforts made to date by R. Jennings et al. (1995), Bradford et al. (in prep.), and surveys for amphibians and fish conducted or sponsored by state and federal agencies in Utah, Arizona, and Nevada over the past 2 decades (Platz 1984; BIO-WEST Inc. 2001; R. Fridell, R. Haley, and M. Sredl, pers. comm., as cited in RLFWG 2001).

A table and maps of locations of the extant and recently extirpated *R. onca* populations can be found in Appendices 2 and 3.

Nevada

Rana onca was rediscovered in November 1991 at Corral Spring. R. Jennings subsequently conducted more extensive surveys along the Virgin River in Nevada and at eight springs on the east and west shores of the Overton Arm of Lake Mead (R. Jennings et al. 1995). The species was found at three springs; Blue Point, Rogers, and Corral Springs about 3.2 km west of the Overton Arm of Lake Mead, Clark County, Nevada (R. Jennings et al. 1995; Bradford and Jennings 1997). The population at Corral Spring has since gone extinct (D. Bradford, pers. comm., as cited in USFWS 2001b; NPS 2001). There have been individual frog sightings at Gnatcatcher Spring, midway between Blue Point and Rogers (NPS 2001; USFWS 2001a). The species was also rediscovered in the mid-1990s at three sites (Boy Scout Canyon, Salt Cedar Tributary, and Bighorn Sheep Spring) in Black Canyon, 4 km downstream from Hoover Dam (R. Jennings et al. 1995; NPS 2001; AGFD 2001).

R. Jennings et al. (1995) also surveyed six sites in the Pahranagat Valley, Lincoln County, as well as a number of sites in Meadow Valley Wash, Lincoln and Clark counties, Nevada, but found no relict leopard frogs in these areas. R. Jennings et al. (1995) noted that some microhabitats may exist along Meadow Valley Wash and at Panaca Spring, Nevada, that could potentially support the species, and that these areas should be surveyed.

In 2000, small tadpoles were taken from the Bighorn Sheep population and raised to metamorphosis. An experimental population of 297 of these metamorphes (mean = 25 mm SVL, 55 mm snout-to-tail length) was introduced from May through September into the Boulder City Wetlands Park (USFWS 2001, 2001a). The Wetlands Park is an artificially created creek and wetlands designed to treat sewage effluent from Boulder City, although currently raw lake water is being used rather than treated effluent, as intended in the design (S. Romin, NPS, pers. comm., 2002). The site has potential conflicting wildlife management needs and goals (the ponds are important razorback sucker grow-out sites) and the City of Boulder City wants to eliminate the stream or substantially reduce it to keep the channel clear of vegetation (USFWS 2001a). Other problems include periodic herbicide spraying in the wetlands to keep cattails down, and the fact that bullfrogs have invaded the site (NPS 2001; USFWS 2001a) and are apparently flourishing (S. Romin, NPS, pers. comm., 2002). The high population count at this site was 21 adults in October 2001 (S. Romin, NPS, pers. comm., 2002), although the long-term viability of this population is unknown.

Arizona

The only population of relict leopard frogs recently found in Arizona was at a spring-fed wetland adjacent to the Virgin River near Littlefield in the extreme northwest corner of the state. This was a small population that apparently was extirpated by 2001 (USFWS 2001b). *Rana onca* could potentially still occur in small numbers in scattered localities along the Virgin River drainage of Arizona, as well as in the drainages of small tributaries to the Colorado River from Grand Wash to Davis Dam. However, six previous surveys (Sredl 1997) conducted within the potential Arizona range found frogs only at Littlefield (AGFD 1997). R. Jennings et al. (1995) surveyed 8 sites in Grand Wash and 7 spots along the Arizona section of the Virgin River and failed to find relict leopard frogs.

There have been individual leopard frogs found near the confluence of Surprise Canyon and the Colorado River in the Grand Canyon, at the upper end of Lake Mead, and also at the fish hatchery at Willow Beach, Arizona, located 10 km downstream from Bighorn Sheep Spring in Black Canyon (RLFWG 2001; USFWS 2001a). Neither of these findings represent new viable populations. *Rana onca* is apparently completely or very nearly extirpated from Arizona.

Utah

The relict leopard frog is likely extirpated from Utah, since it has not been observed there since 1950. Surveys by Jennings (1993) of over 20 sites in Utah in the historical range of the species, and by the Utah Department of Natural Resources in the Virgin River drainage in southern Utah failed to locate *R.*

onca. The species is unlikely to occur in any other localized areas along the Virgin River because the Utah Division of Wildlife has conducted thorough surveys in suitable habitat, and state and federal agency personnel are on the lookout for this species during surveys of suitable aquatic habitat (Bradford and Jennings 1997). (RLFWG 2001).

b. Current Abundance

Nevada

<u>Northshore of Overton Arm</u>

Blue Point and Rogers Springs

The *R. onca* populations at Blue Point (which includes "Slim Creek") and Rogers Springs, which were rediscovered in 1991, are the most stable populations of the species. As of 1997, there had been 113 surveys conducted of Rogers Spring, with 75 observations of adult frogs, and with no more than 8 adults observed on any one survey (NPS 2001). During surveys from December 1996 to June 1997 at Rogers, the average frog observations/visit was 4.3 frogs; there was never more than one adult frog observed at the upper portion of Rogers Spring and the high at the lower portion of Rogers Spring was 7 frogs (NPS 2001).

These same surveys found a high of 24 frogs at the upper portion of Blue Point Spring and a high of 35 frogs at the lower portion of Blue Point Spring. Adult population estimates at Blue Point Spring in 1997 ranged from 100 to 150 frogs, based on observed habitat preferences, to 300 frogs, based on total available habitat (NPS 2001).

Surveys from July 1999 to June 2000 found an average of 10.8 frogs/visit at Blue Point upper spring and 11.9 frogs/visit at Blue Point lower spring (NPS 2001); surveys in March and April 2001 found 32 frogs at Blue Point upper spring, considered similar numbers to previous years (D. Bradford, pers. comm., as cited in USFWS 2001b). The adult population estimate for the entire Blue Point-Rogers Springs complex was about 300 frogs (AGFD 2001; NPS 2001).

Corral Spring

The *R. onca* population at Corral Spring was first rediscovered in 1991 (R. Jennings et al. 1995). Ten frogs were observed here in April and 15 more were seen in August of 1991 (NPS 2001a). The maximum numbers of frogs observed here of all sizes was 40 in November 1991 and 33 in June 1992 (R. Jennings et al. 1995; RLFWG 2001). Frog numbers declined dramatically during 16 visits between 1991 and 1994. By November 1994, only 2 adult frogs could be located and the population eventually went extinct by early 1995 (R. Jennings et al. 1995; D. Bradford, unpublished data, as cited in RLFWG 2001). Between 1995 and 1997, there were 51 visual attempts and 48 calling attempts (99 total) to locate the species, and no *R. onca* could be found at the Corral Spring site (NPS 2001). Surveys from July 1999 to June 2000 and in March and April 2001 also failed to locate the species here (NPS 2001; D. Bradford, pers. comm., as cited in USFWS 2001b). The species is apparently extirpated from Corral Spring.

Gnatcatcher Spring

The species was first located at Gnatcatcher Spring in April 1997. Nine surveys during 1997 each located a single individual adult frog at Gnatcatcher Spring (NPS 2001). Surveys from July 1999 to June 2000 failed to locate any frogs (NPS 2001), although a single juvenile (implying 3 frogs) was found on October 24, 2000. The very small size of this site makes this population very vulnerable and the species may be close to extirpation here.

Black Canyon

Boy Scout Canyon

The species was first located at Boy Scout Canyon Hot Springs in July 1997 (USFWS 2001a). During surveys from July 1999 to June 2000, an average of three frogs were seen per visit at the Boy Scout Canyon site (NPS 2001). The population estimate as of January 1999 was a few tens of adults (NPS 2001). Surveys during March and April 2001 located 18 frogs, considered to be similar numbers to previous years (D. Bradford, pers. comm., as cited in USFWS 2001b). The AGFD guessed the adult population was less than 100 in March of 2001 (AGFD 2001), but this is an optimistic estimate.

Salt Cedar Tributary

During surveys from July 1999 to June 2000, no frogs were located at the Salt Cedar Tributary site (NPS 2001). The population estimate as of January 1999 was a few tens of adults (NPS 2001). Four frogs were seen in Salt Cedar Tributary during surveys in March and April of 2001, considered to be similar numbers to previous years (D. Bradford, pers. comm., as cited in USFWS 2001b). The AGFD again guessed the adult population was less than 100 in March of 2001 (AGFD 2001), but this is a very optimistic estimate, as frogs are only found in a 40 m length (S. Romin, pers. com, 2002).

Bighorn Sheep Spring

The species was first located at Bighorn Sheep Spring in July 1997 (USFWS 2001a). During surveys from July 1999 to June 2000, an average of 30 frogs were seen per visit at Bighorn Sheep Spring (NPS 2001). The population estimate as of January 1999 was several hundred adults (NPS 2001). During surveys in March and April of 2001, 104 frogs were located on the first visit, and 199 were seen on the second visit (D. Bradford, pers. comm., as cited in USFWS 2001b). Bradford gave a population estimate of 637 early spring adults, greater numbers than in previous years. The AGFD observed 5 adults here in February 2001 and guessed the adult population was several hundred in March 2001 (AGFD 2001).

Arizona

Littlefield

Relict leopard frogs were re-discovered at a wetland near Littlefield in the 1990s (AGFD 1997). Frogs were observed during the daytime in 1992 and 1996, and 6 adults were counted at night in both April and July 1998 (RLFWG 2001). None of the frogs captured in July were those marked in April. The population estimate at this site as of January 1999 was a few tens of adults (NPS 2001). However, no relict leopard frogs could be found during three surveys of the site from March through May of 2001 (D. Bradford, pers. comm., as cited in USFWS 2001b). Three bullfrogs were found there in March, and virtually all the original *R. onca* habitat was overgrown with vegetation (USFWS 2001b). The species appears to be extirpated from the Littlefield site. The AGFD gave a population estimate in March of 2001 of less than 50 adults (AGFD 2001), but the species was likely already extirpated from the site by this time.

Willow Beach Fish Hatchery

Two individual leopard frogs, one of which was subsequently identified as *R. onca*, were observed on different occasions in 2000 and 2001 at the fish hatchery at Willow Beach, Arizona (C. Fiegel, pers. comm., as cited in RLFWG 2001). The site is located 10 km downstream from Bighorn Sheep Spring in Black Canyon. The frogs were found within the hatchery, and there is no suitable relict leopard frog habitat around the hatchery. The river is very cold and swift here with little shoreline vegetation, making unassisted dispersal of these frogs unlikely. It has been theorized that these frogs were likely taken from one of the springs by a visitor to the NRA, then let go near the hatchery area (S. Romin, pers. comm., 2002).

Surprise Canyon

A single dead, decomposed specimen of a leopard frog, appearing to possibly be *R. onca*, was found in June 1997 near the confluence of Surprise Canyon and the Colorado River in the Grand Canyon, at the upper end of Lake Mead (USFWS 2001a).

Utah

The relict leopard frog is likely extirpated from Utah.

Overall Abundance

Overall abundance estimates were made for the relict leopard frog populations along the Virgin

River (at Overton Arm and Littlefield), before additional populations were located in Black Canyon. Based on mark-recapture data for 96 adults from 1995 to 1996 at Blue Point Spring, and assuming similar densities at other localities (i.e. mean of 35.9 adult frogs/555 m of stream habitat, or 1 frog/15.5 m of habitat), the total population was estimated at less than three hundred adults (Bradford and Jennings 1997; Bradford 1999; AGFD 2001).[5] Bradford et al. (in prep.) refined this estimate using a combination of the mark-recapture estimates of population size and subsequent visual encounter surveys, producing an estimate of 330 adult frogs in the Overton Arm area.[6] At the Overton Arm sites, the estimated total linear extent of aquatic habitat is 5.1 km, based on ground measurements, aerial photographs, and USGS digital orthophotoquads (RLFWG 2001; Bradford et al., in prep.).

These figures likely overestimate abundance because they do not account for spatial variation of frog densities, and the density of frogs encountered in most of the aquatic habitat in this area is conspicuously lower than the density seen at the upper Blue Point Spring area (RLFWG 2001). Also, the Corral Spring, Littlefield, and possibly the Gnatcatcher Spring populations on the Overton Arm have subsequently been extirpated (RLFWG 2001), so that some of the sites included in the 5.1 km of estimated habitat are now known to have no frogs at all.

The discovery of additional populations in Black Canyon was initially guessed to have doubled the known population. A rough estimate of the total population in Black Canyon was ventured based on visual encounter surveys (VES) conducted at Bighorn Sheep Spring, again, the densest relict leopard frog population in the Black Canyon area. The population estimate at Bighorn Sheep Spring was 637 adults, based on a multiplying factor of 6.1, at a time when 104 adult frogs were counted in the VES (RLFWG 2001).[7] Applying this multiplying factor to the average VES counts at the other two sites in Black Canyon (mean counts of 5 and 13), the estimate for the total adult population size in Black Canyon was 750 frogs, 85% of which were presumed to be at Bighorn Sheep Spring (RLFWG 2001).[8] Again, this estimate is based on extrapolation of densities at the largest population in Black Canyon, and may be an overestimate.

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5 Visual encounter surveys were conducted multiple times at all known relict leopard frog sites, and mark-recapture efforts were done at two sites where populations remained (Bradford et al., in prep.; S. Romin, pers. comm., as cited in RLFWG 2001). At the upper 555 m segment of Blue Point Spring in the Overton Arm area, 96 adult frogs (<42 mm snout-urostyle length) were captured and marked during 13 visits over a 2-year period from 1995 to 1996. The estimated number of frogs averaged 36 (95% confidence limits, 27 - 45), and estimated annual survivorship averaged 27%.

6 Visual encounter surveys between 1991 and 2001 at Blue Point Spring showed considerable variation in numbers encountered (4 to 32 frogs over a 385 m reach; n=23 visits). There was no consistent pattern of increase or decrease in numbers over time period, although the data suggest an increase rather than a decrease.

7 Bighorn Sheep Spring extends approximately 450 m in length. A single mark-recapture effort (60 initially marked adults) in March and April 2001 yielded an estimate of 637 adults (95% conf. limits, 381 - 1210) (RLFWG 2001).

8 Visual encounter surveys on three to four visits from 1997 to 2001 at the sites in Black Canyon yielded average counts of 110, 5, and 13 frogs at Bighorn Sheep Spring, Salt Cedar Tributary Spring, and Boy Scout Canyon Spring, respectively (RLFWG 2001).

This yields an estimated maximum total *R. onca* population of 1080 adult frogs, more than half of which occur at one site.[9] This is a disturbingly low number of individual frogs, and many of the remaining sites harbor populations estimated to number only in the tens, an extremely tenuous situation, as shown by the extirpations at Littlefield and Corral Spring. The methodology used to obtain this estimate applies multiplier factors (1 frog/15.5 m of habitat at Overton sites, and 6.1 times the numbers observed during VES at Black Canyon sites) based on frog densities at the best populations, and applies them to the linear extent of aquatic habitat (not all of which is necessarily occupied) in the Overton Arm area, and to the mean numbers of frogs encountered during visual encounter surveys in the Black Canyon area. This population estimate, as based on total habitat, rather than preferred habitat, is probably high. Even at the highest estimates, however, the numbers are critically low, especially when fragmentation is considered.

3. Population Trends

Survey efforts by Bradford and Jennings (1997) rediscovered *R. onca* at 4 of 51 potential habitat sites (Rogers, Blue Point, and Corral Springs on Overton Arm, and Littlefield, Arizona) surveyed from 1991 to 1993. This survey included 12 historical localities and 39 other sites with potential habitat within the historical range of the species (Bradford and Jennings 1997). The species was subsequently extirpated from Littlefield and Corral Spring, meaning *R. onca* has been lost from 10 of the 12 historical localities surveyed by Jennings and Bradford (1997). All of the historical localities for the species in Meadow Valley Wash, Nevada, and the St. George, Utah area have been lost.

Rana onca populations have subsequently been located at Gnatcatcher Spring, between Rogers and Blue Point Springs on Overton Arm, and at three sites in Black Canyon. It is unknown whether the Gnatcatcher population, which in any case is very near extirpation, represents a historical locality, or whether the site has recently been colonized by frogs from the adjacent populations. Leaving out Gnatcatcher, the Black Canyon sites potentially represent one historical area or three historical localities, as specimens were collected from the Black Canyon area in 1955 (RLFWG 2001). Thus the species is known to have been lost from 10 of 13 or 10 of 15 historical localities. Given that the known historical localities likely represent a small sample of all the places the species probably once occurred, the relict leopard frog currently occupies only a tiny fraction of its likely historical range.

There were a number of historical localities for populations of *R. fisheri* in the Las Vegas Valley, all of which are now extirpated. Should *R. fisheri* prove to be synonymous with *R. onca*, the historical range of the species would have also included the Las Vegas Valley and the Tule Springs, Nevada area, and the reduction in historical localities will have been significantly greater (e.g. lost from 58 of 64 known total locations or 91%). Even if *R. fisheri* proves to be a distinct species (the Vegas Valley leopard frog), *R. onca* has still been extirpated from almost all of its historical range, has suffered a tremendous loss of habitat and populations, and survives in a tiny remnant of its former habitat.

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9 2001 population estimates at individual sites, detailed in Appendix 2, yield a total adult population range of <700 to <1200 (AGFD 2001; NPS 2001; USFWS 2001b).

Based on predominately monthly Visual Encounter Surveys (VES) for numbers of juvenile and adult frogs, the populations at Rogers and Blue Point Springs (the only populations with enough data to hazard a guess at short-term population trends) are thought to be stable or slightly increasing since 1992 (Bradford and Jennings 1997; USFWS 2001; NPS 2001). The Bighorn Sheep Spring population in Black Canyon is the largest *R. onca* population, with an estimated 85% of the total population (RLFWG 2001). Not enough data has been collected to analyze trends for the Black Canyon populations, but the Boy Scout Canyon and Salt Cedar Tributary populations are thought to be quite small, with far fewer adult frogs seen at either site during VES than were observed at Corral Spring before the population disappeared.

<u>Discussion of Recent Population Extirpations</u>

Corral Spring

At Corral Spring, although up to 40 frogs had been observed at a time during surveys from 1991 to 1994, the species was completely extirpated by early 1995. This decline apparently resulted from loss of adults, rather than lack of reproduction or recruitment, as tadpoles were evident during the period of decline in 1993 (RLFWG 2001). Adult numbers declined dramatically in late 1993, and the body size of female frogs declined immediately prior to this (Bradford and Jennings 1997).

Between 1991 and 1995, habitat change was conspicuous at Corral Spring. The pools that were initially largely open with scattered emergent vegetation became choked with emergent vegetation, primarily tules (*Scirpus* sp.). By early summer of 1994, most of these pools had virtually no open water. Surface water also diminished greatly during this time, although it is unknown whether the cause was increased evapotranspiration from the dense vegetation, natural subsurface diversions, or a combination (S. Romin, pers. comm., 2002). These changes suggest that adult frogs either emigrated or died as a result of habitat change. Surveys were initiated and frogs discovered at the site in 1991, after a flood-scouring event that may have allowed frogs to colonize the site from Rogers Spring (Bradford and Jennings 1997).

Littlefield

At the Littlefield site, although up to 12 frogs were observed in 1998, the species was apparently extirpated from the site by 2001. As at Corral Spring, the demise of the *R. onca* population occurred concomitantly with a loss of pool habitat due to rapid encroachment of emergent vegetation (thought to result from lack of scouring flood flows and cessation of livestock grazing) and with the establishment of bullfrogs in the area (RLFWG 2001).

These rapid extirpations do not bode well for the small relict leopard frog populations at Boy Scout Canyon and Salt Cedar Tributary, or at Gnatcatcher Spring.

II. CRITERIA FOR ENDANGERED SPECIES ACT LISTING

A. THE RELICT LEOPARD FROG IS A "SPECIES" UNDER THE ESA

The Endangered Species Act ("ESA" or "Act") provides for the listing of all species warranting the protections afforded by the Act. The term "species" is defined broadly under the Act to include "any subspecies of fish or wildlife or plants and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature." 16 U.S.C. § 1532 (16). Petitioners believe there is sufficient evidence, as discussed in the above section on taxonomy and in Appendix 4, that the relict leopard frog is a valid species, and conforms to the definition of a "species" under the Act. The relict leopard frog qualifies for an endangered listing to afford it the protections of the Act.

B. THE RELICT LEOPARD FROG IS ENDANGERED UNDER THE ESA

The Fish and Wildlife Service is required to determine, based solely on the best scientific and commercial data available, whether a species is endangered or threatened because of any of the following factors: (1) the present or threatened destruction, modification, or curtailment of its habitat or range; (2) overutilization for commercial, recreational, scientific, or educational purposes; (3) disease or predation; (4) the inadequacy of existing regulatory mechanisms; or (5) other natural or manmade factors affecting its continued existence. 16 U.S.C. §§ 1533(a)(1) and 1533(b).

Petitioners believe that all of these factors except for (2) play a role in threatening the relict leopard frog with imminent extinction.

1. PRESENT OR THREATENED DESTRUCTION, MODIFICATION, OR CURTAILMENT OF ITS HABITAT OR RANGE

As noted above, *R. onca* has been extirpated from all historical localities in Utah and Arizona, primarily due to habitat loss and alteration. The species currently persists in only two general areas: near the Overton Arm area of Lake Mead, Nevada, and Black Canyon, Nevada, below Lake Mead. These two areas, encompassing maximum linear extents of only 3.6 and 5.1 km, respectively, comprise a small fraction of the original distribution of the species. *Rana fisheri* has been extirpated from its entire historical range in Nevada. Taken in summary, there are 64 known locations for the relict leopard frog and only 6 populations are left. This represents a 91% locality reduction. The 6 known populations of *R. onca* are confined to small springs within a small area and are therefore susceptible to extirpation by localized human-induced or natural impacts. Frog populations at 2 of 8 recently known sites have been extirpated in the last decade, apparently by natural habitat alteration.

Water development for agriculture and urban development and the resultant alteration of the hydrologic regime of the river and wetlands within the range of the frog are primarily responsible for the historical decline of the species (ACFG 1996, 1998). Future water development is a serious threat to remaining *R. onca* habitat. Other factors which have modified habitat or curtailed the range of the species include invasive plants, native plant succession (which is related to alteration of the hydrologic regime), introduction of non-native predators, natural erosion, cattle grazing and feral burro impacts, and recreational and right-of-way impacts. These factors continue to threaten the existence of the species, and are discussed below.

a. Water Development

Water development within the range of the relict leopard frog has flooded historical frog locations, impeded dispersal and reduced or eliminated connectivity between remaining populations, and dramatically changed the hydrologic regimes of the Colorado, Virgin, and Muddy Rivers. The formation of Lake Mead by Hoover Dam in 1935, and Lake Mojave by Davis Dam in 1951, inundated scores of river miles and adjacent associated scattered wetlands. Several relict leopard frog populations located between the Overton Arm and Black Canyon areas were apparently eliminated by the filling of Lake Mead (Cowles and Bogert 1936).

Connectivity and potential for dispersal between the Overton Arm and Black Canyon areas has almost certainly been dramatically reduced if not outright eliminated as a result of the damming of the Colorado River. The control of river flow for power management since 1935, and the formation of Lake Mojave presumably has also dramatically impeded dispersal among frog sites in Black Canyon, which are separated from each other by 1.8 to 5.0 km via the Colorado River. Here, the river level is influenced by Lake Mojave such that the canyon floor is never exposed, predatory game fishes are constantly present in the river, and the water is continually cool because it emerges from the bottom of Lake Mead.

The recent demise of the *Rana onca* population at Littlefield may have resulted from anthropogenic processes. Along the Virgin River, the hydrological regime has been disrupted by upstream impoundments. Prior to the establishment of reservoirs in the Virgin River watershed, the emergent vegetation at the Littlefield site would have been scoured periodically by flooding of the Virgin River. With the absence of flood action, emergent vegetation grew over virtually all the former open water at the site, resulting in a loss of pool habitat. The cessation of livestock grazing and exclosure of feral burros at the site may have contributed to this habitat change. Sredl (1997) correlated a significant increase in a *R. yavapaiensis* population at Tule Creek, Yavapai County, Arizona, with a major scouring flood in 1993. The flood removed sediment and increased open water habitats preferred by lowland leopard frogs at a site that had been choked by vegetation.

Periodic flood scouring events may be required to eliminate emergent vegetation from spring sites and create the open water habitats relict leopard frogs appear to prefer. The discovery of an *R. onca* population at Corral Spring correlated with high-precipitation storms associated with an El Nino/Southern Oscillation event that scoured vegetation there in 1991. During such wet times, frogs possibly could colonize Corral Spring from Rogers Spring by traveling a 1.6 km straight-line distance. Such dispersal distances have been reported for other *Rana* species, albeit in more mesic environments (Marsh and Trenham, 2001). It is not known whether leopard frogs persisted at Corral Spring between 1957, when several specimens were collected, and 1991. Frog populations may subsequently be extirpated from sites due to shrinkage of aquatic habitat and vegetation encroachment as drier conditions prevail (Bradford and Jennings 1997; RLFWG 2001). Under this scenario, the long-term viability of relict leopard frogs would depend upon periodic scouring floods, connectivity and potential for dispersal between sites, and metapopulations of frogs able to re-colonize scoured sites.

Since it requires a year-round water supply, the relict leopard frog is highly susceptible to lowering of the water table through diversions and ground water pumping (AGFD 1996, 1998). The primary threats to remaining *R. onca* habitat are massive destruction and modification of habitat along the Muddy and Virgin Rivers. Water from these rivers is used for agriculture and a power plant is proposed near St. George, Utah. Sections of the Virgin River have been also been channelized, reducing available *R. onca* habitat (Sredl 1982).

The insatiable human demand for water in the arid region inhabited by *R. onca* poses a serious threat to the species. The Rogers and Blue Point Springs populations are presently threatened by water withdrawals upstream near Moapa on the Muddy River, which feeds the Overton Arm. The Moapa dace, a federally listed fish species, has already been endangered partly due to these water withdrawals. Flows have been steadily decreasing in the Muddy River for the past 30 years, probably because of existing ground-water withdrawals and surface-water diversions (NPS 1995). The Las Vegas Valley Water District and Nevada Power Company filed for ground water rights in Moapa Valley in 1989. The Moapa Valley Water District has been attempting to increase their water withdrawal from a well that taps into the aquifer feeding Muddy Springs, which is the main source for the Muddy River (NPS 1995).

The National Park Service filed 146 water rights protests in 1989 over appropriation and diversion applications from the Las Vegas Valley Water District for approximately 200,000 acre feet of water in areas north of Rogers and Blue Point Springs. These diversions would impair or impact springs in the Lake Mead National Recreation Area, including Blue Point, Rogers and Corral Springs (NPS 2001, 2001a). Hearings began on these protests in 2001. The waters issuing from Rogers and Blue Point Springs may originate in the Mormon Mountains, Beaver Dam Wash, and the Muddy Mountains in the Virgin River Basin (Prudic et al. 1993; Pohlman et al. 1998; NPS 2001). These springs are discharge areas for regional groundwater flow systems and could have their outflow reduced by Las Vegas Valley Water District and other water diversions up gradient from the Lake Mead NRA (NPS 2001).

b. Agricultural and Urban Development

The extinction of relict leopard frog populations throughout the species' range has occurred concurrently with the elimination or alteration of aquatic habitat due to marsh draining and water development for agriculture and urban development (Jennings 1988; Jennings and Hayes 1994). For example, frog populations at Berry Springs, Utah, and in the Vegas Valley, Nevada were eliminated when springs were capped or altered for human use (Jones and Cahlan 1975; Platz 1984). Urbanization of the Las Vegas valley led to overdraft of groundwater (Jones and Cahlan 1975), which contributed to the demise of *R. fisheri*. Much of the former wetland habitat near the Virgin and Muddy Rivers in Utah, Arizona, and Nevada has been converted to agriculture or urban development. Explosive human population growth and development (including subdivisions, a golf course, and commercial development) began in the 1980s in the Virgin River flood plain near Beaver Dam Wash, in the vicinity of the Littlefield *R. onca* population (Deacon and Deacon 1998).

The recent extirpations of small *R. onca* populations at Littlefield Arizona, and Corral Spring, Nevada demonstrate that small fragmented populations may not be viable over the long term. Reestablishing relict leopard frogs at a number of formerly occupied suitable habitats with connectivity between occupied locations will be absolutely necessary to prevent extinction and recover the species. Much of this potential frog habitat is on private land in Utah and Arizona, and is threatened by agricultural and urban development. For example, potential relict leopard frog habitat in southern Utah is threatened by proposed projects such as the St. George Bypass (Interstate 15), the St. George Airport, and the Sand Hollow Recreation Area (L. Thomas, SUWA, pers. comm., 2002).

The counties within the current and historical range of the relict leopard frog are undergoing explosive human population growth. The population of Washington County, Utah increased 500% (from 18,000 to 90,000) from 1974 to 2000 and is projected to increase another 378 to 583% (340,000 to 525,000) by 2050 (BE 1998; SLT 1999). Clark County, Nevada has grown 308% (463,000 to 1,429,000) from 1980 to 2000, and currently has an 8% annual growth rate (CC 2000). Las Vegas, Nevada in particular is the fastest-growing metropolitan area (USCB 1999) in the U. S. - the population of the area, now at 1.3 million, is slated to double every ten years. Lincoln County, Nevada has a 1.9% annual growth rate (LVRJ 1998). Mohave County, Arizona has grown by 248% (56,000 to 139,000) from 1980 to 1998 (MC 1998).

c. Cattle Grazing and Feral Burro Impacts

The relict leopard frog appears to require open water habitats that historically were maintained by flood scouring and native grazers. Although it has been posited that managed grazing may benefit relict leopard frog habitat, livestock and feral burros can also cause significant habitat degradation.

Habitat alteration by livestock grazing (due to trampling, water quality impacts, and impacts to riparian vegetation) is an important factor in the decline of *Rana yavapaiensis* in Arizona (Jennings and Hayes 1994a) and of ranid frogs in California (Jennings 1988a; USFWS 2000a). Livestock grazing is known to decrease the suitability of riparian and aquatic habitat in general (Behnke and Raleigh 1978; Buckhouse et al. 1981; Kauffman et al. 1983; Kauffman and Krueger 1984; Bryant 1985; Marlow and Pogacnik 1985; Siekert et al. 1985) and negatively impacts habitat for herpetofauna (Jones 1979, 1988; Szaro et al. 1985; Jennings 1988a; Jennings and Hayes 1994b; USFWS 2000a). Exclusion of cattle grazing in Contra Costa County, California, resulted in reestablishment of suitable habitat and expansion of red-legged frog (*Rana aurora draytonii*) populations (Dunne 1995).

Cattle can draw down water levels when drinking from small water bodies, leaving amphibian egg masses desiccated or subject to disease such as fungal infections (USFWS 2000a). Cattle can also crush and disturb egg masses, larvae, and metamorphosing frogs (USFWS 2000a). Loss of streamside vegetation due to cattle grazing can reduce habitat for insects and small mammals (USFWS 2000a), which are important dietary components for aquatic species (Cordone and Kelley 1961), including the relict leopard frog. Livestock grazing can also cause nutrient loading problems due to urination and defecation in areas where cattle are concentrated near the water (Doran et al. 1981).

However, in some situations, carefully managed grazing programs may benefit relict leopard frogs by providing open water habitats in the absence of flood scouring. Bighorn sheep may have historically kept pools open, without causing the negative impacts to riparian and wetland amphibian habitat associated with cattle, which tend to congregate in wetlands areas and cause significant damage (Jennings 1996; Belsky et al. 1999). Although evidence of bighorn activity was not striking at Corral Spring in 1993, prior to the decline of *R. onca* at the site, bighorns cleared two pools there of vegetation through trampling during the summers of 1994 and 1995 (Bradford and Jennings 1997).

At the Littlefield site, the discontinuation of livestock grazing and subsequent overgrowth of emergent vegetation is thought to have contributed to habitat changes that led to the extirpation of the leopard frog population there (RLFWG 2001). However, livestock grazing was discontinued in the area of Blue Point and Rogers Springs in 1989 (NPS 2001a) and these springs contain one of the most stable relict leopard frog populations.

The introduction of feral burros has been another factor in the reduction in *R. onca* numbers, as burros also overgraze and trample leopard frog habitat. Burros at Blue Point Spring had trampled and eaten vegetation along the shoreline as well as urinated and defecated in the water in 1996 (M. Malfatti, pers. comm. in NPS 2001). A 1999 survey of the wild horse and burro population in the Muddy Mountains Habitat Management Area, which includes the Northshore springs, estimated that approximately 86 burros and 17 horses utilized the area (NPS 2001a). Bradford and Jennings (1997) postulated the demise of *R. onca* at Corral Spring may also have been influenced by the construction of a fence in 1991 to exclude feral burros from most of the site, which allowed emergent vegetation to choke out the open water habitat. This raises the question of how frogs here survived before burros were introduced, or whether they had recently colonized the site after a flood-scouring event in 1991.

2. OVERUTILIZATION FOR COMMERCIAL, RECREATIONAL, SCIENTIFIC, OR EDUCATIONAL PURPOSES

Remaining *R. onca* populations are few enough and small enough in numbers that any collecting of adult frogs could potentially be a threat for local extirpation at individual sites. Collections from existing populations in the Lake Mead NRA for experimental captive breeding and attempted introduction at the artificial wetlands in Boulder City (USFWS 2001, 2001a; NPS 2001a) are not likely to pose a major threat to the numbers or viability of any of the extant populations, as mostly eggs or tadpoles up to 20 mm (an age class that generally has only a 1% chance of survival) were taken (S. Romin, pers. comm., 2002).

3. DISEASE AND PREDATION

a. Disease

Little is known about pathogens of relict leopard frogs. A red-leg bacterial infection caused by *Aeromanas hydrophila* killed a major portion of a population of lowland leopard frogs, *R. yavapaensis*, at Big Spring, Graham County, Arizona, in 1992 (Sredl 1997). The *A. hydrophila* bacteria is ubiquitous in freshwater systems, and the infection is likely brought on by stress (Sredl 1997). Two important pathogens that have been the focus of recent research are the chytrid fungus and viruses. (RLFWG 2001).

In 1998, a chytrid fungus (*Batrachochytrium dendrobatidis*) was identified in numerous Arizona amphibians. This fungus presently affects seven species of ranid frogs in Arizona [Rio Grande leopard frogs, plains leopard frogs (*R. blairi*), Chiricahua leopard frogs, Ramsey Canyon leopard frogs (*R. subaquavocalis*), lowland leopard frogs, Tarahumara frogs (*R. tarahumarae*), and bullfrogs], as well as four other amphibians [canyon treefrogs (*Hyla arenicolor*), striped chorus frogs (*Pseudacris triseriata*), Sonoran tiger salamanders (*Ambystoma tigrinum stebbinsi*), and red-spotted toads (*Bufo punctatus*)] (Sredl et al., 2000; Collins, unpublished data, and Sredl, unpublished data, as cited in RLFWG 2001; Bradley et al. 2002). Chytrid fungus damages the mouthparts of tadpoles, then damages keratin in the skin of metamorphosed frogs, eventually killing them. Chytrid fungi are ubiquitous in soil, but the aquatic chytrid infecting frogs is relatively new to science (Berger et al. 1998).

Fatal chytrid fungus infections in *R. yavapaiensis* and *R. chiricahuensis* populations in Arizona correlated with major die-offs and population decreases (Bradley et al. 2002). The chytrid fungus has also caused die-offs of mountain yellow-legged frog (*Rana muscosa*) and Yosemite toad (*Bufo canorus*) populations in the Sierra Nevada of California (Carey et al. 1999; Vredenberg et al. in press; V. Vredenberg pers. comm., R. Knapp, pers. comm., as cited in CBD and PRC 2000), has been found in declining populations of Wyoming toads (Taylor et al. 1999), and decimated frog populations in Australia and Central America (Laurence et al. 1996; Lips 1998; Berger et al. 1998). Since five other species of leopard frogs in Arizona have been affected, there is no reason to think that relict leopard frogs would be immune to fatal outbreaks of this pathogen. Although there have been recent observations of suspicious mouthpart abnormalities in relict leopard frogs in the Bighorn Sheep Spring population, tests from the USGS National Wildlife Health Center showed no indication of chytrid infection (S. Romin, pers. comm., 2002).

It has been hypothesized that introduced fish may act as a vector for new diseases to infect amphibians. Numerous introduced aquarium fish apparently coexist with relict leopard frogs at Blue Point and Rogers Springs (see discussion in section II.B.3.b). It has been demonstrated that a virus is capable of being transmitted from fish to amphibians under natural conditions. An iridovirus (viruses with DNA as the genetic material, that occur in insects, fish, and amphibians, and may cause death, skin lesions, or no symptoms) has been linked to a salamander die-off in 1995 in southern Arizona (McLean 1998). A study by Mao et al. (1999) isolated identical iridoviruses from wild sympatric fish (threespine stickleback, *Gasterostelus aculeatus*) and amphibians (the red-legged frog, *Rana aurora*), evidence that this pathway is probable. An iridovirus, SRV, has apparently affected tiger salamanders (*Ambystoma tigrinum*) in Arizona, and a second iridovirus, Frog Virus 3, has affected other members of the true frog family, common frogs (*Rana temporaria*) and green frogs (*Rana esculenta*) in Europe (RLFWG 2001).

Like pathogens, little is known about parasites of relict leopard frogs. Goldberg et al. (1998) examined parasites of lowland and Chiricahua leopard frogs and bullfrogs collected in Arizona. They found lowland leopard frogs to be infected with five species of trematode (*Cephalogonimus brevicirrus*, *Glypthelmins quieta*, *Haematoloechus complexus*, *Megalodiscus temperatus*, and one unidentified species) and four species of nematode (*Falcaustra catesbeiana*, *Rhabdia ranae*, *Physaloptera* sp., and one unidentified species). None of the helminths identified from the two native species were found in the bullfrog (RLFWG 2001).

Because of the small and isolated nature of many of the remaining relict leopard frog populations, disease could rapidly extirpate the species from individual sites and potentially cause the extinction of the species. Of potential concern is the possibility that recreational visitors (and their pets) to the occupied springs could unwittingly transfer pathogens between sites. It is also imperative that frog researchers (who fairly intensively handle a significant portion of the remaining *R. onca* population at all known remaining sites as well as other ranid species in Arizona) take special precaution not to act as vectors for transmission of these diseases. Any local extirpations caused by disease would further isolate the remaining populations and probably reduce the time to extinction for the entire species.

b. Predation/Competition

Natural predation upon *R. onca* is not known to be a threat to existing populations. Relict leopard frogs are likely eaten by western terrestrial garter snakes (*Thamnophis elegans*) (Bradford, et al. 2001). Natural predators of other adult leopard frog species include raccoons (*Procyon lotor*), ringtails (*Bassariscus astutus*), and various kinds of snakes [Colubridae] (Sredl 1992). Herons, garter snakes, fish, and large aquatic invertebrates (especially giant water bugs [Belostomatidae]) are known to feed on eggs and tadpoles of other leopard frog species (Sredl 1992).

Rana onca coexists with red-spotted toads (*Bufo punctatus*), Woodhouse's toads (*B. woodhousii*), Pacific tree frogs (*Hyla regilla*), and at least historically, Arizona toads (*B. microscaphus*). Red-spotted toads are relatively uncommon in most *R. onca* springs, but are prevalent in Corral and Boy Scout Springs. In Boy Scout Spring, red-spotted toads share shallow riffles with *R. onca*, but no direct interaction has been observed. Woodhouse's toads occur occasionally in the springs, but are much more common along the lakeshores and backwaters. Pacific tree frogs were reported at Rogers springs in the 1990s, but have not been heard or observed there since. (RLFWG 2001). Rio Grande leopard frogs, which have been introduced into spring habitats in Arizona (Sredl 1997) and are rapidly spreading along the lower Colorado River Valley (Jennings and Hayes 1994a), may compete with relict leopard frogs for habitat and food. Although Rio Grande leopard frogs are not presently known to occupy *R. onca* habitat, it is likely just a matter of time before the species spreads into *R. onca* springs (M. Jennings, pers. comm., 2002).

There are many introduced exotic species that predate upon and/or compete with native ranid frogs in the western U.S. that have become widely distributed along the Virgin, Muddy, and Colorado Rivers. These include the bullfrog; predatory fishes such as bass (*Micropterus* spp.), sunfish (*Lepomis* spp.), and catfish; red swamp crayfish (*Procambarus clarkii*); and western spiny soft-shell turtles (*Trionyx spiniferus emeryi*) [Jennings and Hayes 1994a; RLFWG 2001]. These introduced species are suspected to have contributed to population declines of relict leopard frogs, as well as northern leopard frogs, spotted frogs, and other amphibians (Corn, 1994; Jennings and Hayes 1994a, 1994b; AGFD 1996, 1998). These species may all exert a strong, negative influence on relict leopard frog populations through competition or predation on egg masses, tadpoles, and post-metamorphic individuals.

The presence of introduced bullfrogs is known to preclude the persistence of native leopard frogs in the western U. S. through competition and predation (Hayes and Jennings 1986). Lawler et al. (1999) found that less than 5% of California red-legged frog (*Rana aurora draytonii*) tadpoles survived to metamorphosis when raised with bullfrog tadpoles. Introduced bullfrogs have become established in wetlands and springs in Arizona, Nevada, and Utah, and have been found where *R. onca* and *R. fisheri* have been extirpated (BIO-WEST, Inc. 2001). No bullfrogs were found in 1992 at the Littlefield, Arizona habitat occupied by *R. onca* along the Virgin River, although they occupied adjoining habitat (Bradford and Jennings 1997). By 2001, bullfrogs inhabited the springs at Littlefield that had previously supported leopard frogs (BIO-WEST, Inc. 2001; USFWS 2001b). Bullfrogs were introduced into wetland habitats in the Las Vegas area around 1920, prior to the decline and eventual extirpation of *R. fisheri* (Jennings and Hayes 1994a). Bullfrogs had not been found at the three sites on Overton Arm (Blue Point, Rogers, and Corral Springs) that still supported leopard frogs in 1997 (Bradford and Jennings 1997). In September 2001, bullfrogs also invaded the Boulder City Wetlands Park in Nevada, where an experimental population of relict leopard frogs is being introduced (USFWS 2001a).

A Nevada Fish and Game Department ("NFGD") report in 1953 noted that Rogers Spring was devoid of fish; in 1954, NGFD used the spring as a temporary holding pond for threadfin shad (*Dorosoma petenense*) prior to introduction in Lake Mead (NPS 2001a). Blue Point Spring was operated as a fish hatchery from 1956 to 1960, holding 10-15 species of exotic fish (NPS 2001a). A 1963 poisoning program at Rogers Spring identified 9 species of exotic fish and a 1971 report identified 12 introduced fish species there (NPS 2001a).

The Nevada Department of Wildlife treated Rogers and Blue Point Springs with rotenone in 1984 and planted largemouth bass (*Micropterus salmoides*) in Rogers Spring on three occasions in 1991 in attempts to reduce the numbers of predaceous fish (NPS 2001a). Larval amphibians may be particularly susceptible to rotenone treatment (Fontenot et al. 1994; McCoid and Bettoli 1996). The bass themselves may predate upon ranid frogs (Jennings and Hayes 1994b). Five exotic fish removals using seines were conducted at these springs in 1996 and 1997 and another effort was made in March 2001. Seven species of exotic fish were identified (NPS 2001a). Pilot projects during 1998 demonstrated that dip-netting and minnow traps may not be feasible solutions for removing exotic fish from these springs (NPS 2001).

A large number of tropical aquarium fish such as mosquitofish (*Gambusia affinis*), mollies (*Poecilia* spp.), and cichlids (Cichlidae) exist in Blue Point and Rogers Springs, potentially threatening *R. onca* eggs and tadpoles (ORWG 2001; M. Malfatti, pers. comm. in NPS 2001; NPS 2001). Cichlids in particular are voracious and may consume eggs or tadpoles (Romin 1997). The fact that *R. onca* populations persist at these springs has been considered evidence that they can coexist with small introduced tropical fish (Courtenay and Deacon 1983; Bradford et al. 2001).

Louisiana red-swamp crayfish were observed in the Salt Cedar Tributary site in 1998 and 2001 (USFWS 2001b). Introduced crayfish have been documented to prey upon California newt (*Taricha torosa*) eggs and larvae, in spite of toxins that the species has developed in many parts of its range, and may be a significant factor in the loss of newts from several streams in southern California (Gamradt and Kats 1996). Introduced crayfish may be a threat to *R. onca* eggs and larvae. Introduced turtles, including the red-eared slider (*Trachemys scripta elegans*) and the western spiny soft-shell turtle, have been caught at Rogers Spring (R. Jennings et al. 1995; Bradford and Jennings 1997). Malfatti (1998) believed that soft-shell turtles were eating eggs, larvae, and adult leopard frogs at this site, but he lacked any firm data to support this statement. Live turtle trapping was attempted in at Rogers Spring in fall 1997 and spring 1998 (NPS 2001). Visitors to Rogers Spring feed, and probably introduce additional turtles into the impoundment (RLFWG 2001).

The presence of these introduced predators and their continued introduction into springs occupied by the species (see the discussion of Recreational and Right of Way Impacts in section II.B.5.f below) pose a major threat to *R. onca* and other native invertebrates and small vertebrates of the area (M. Jennings, pers. comm., 2002).

4. INADEQUACY OF EXISTING REGULATORY MECHANISMS

The perilous status of the relict leopard frog reflects the overall failure or inability of existing federal and state regulatory mechanisms to protect relict leopard frog habitat and provide for the conservation of the species. Current estimates, as discussed in this petition, find *R. onca* extirpated from 58 of 64 (= 91%) known locations. The recent extirpations of populations at Littlefield and Corral Spring are de facto evidence of the inadequacy of existing regulatory mechanisms to date for protecting remaining populations of *R. onca*.

<u>Federal Regulatory Mechanisms</u>

The relict leopard frog currently has no formal federal protection. Because this species was presumed extinct, it was listed by the U. S. Fish and Wildlife Service ("USFWS") as Federal Category

3/3A, meaning it used to be considered for listing but found to be inappropriate for listing at that time - 3A meant it was indeed extinct (AGFD 1995).

The USFWS is working with a consortium of federal and state agencies, water districts, and universities on a Rangewide Conservation Assessment and Strategy for the relict leopard frog. This consortium, known as the Relict Leopard Frog Working Group ("RLFWG"), consists of the USFWS, National Park Service, U. S. Bureau of Reclamation, U. S. Bureau of Land Management, U. S. Environmental Protection Agency, U. S. Geological Survey, Arizona Game & Fish Department, Nevada Department of Wildlife, Utah Department of Wildlife Resources, Las Vegas Valley Water District, University of Nevada Las Vegas, University of Nevada Reno, Southern Utah University, and Nevada Natural Heritage Program. However, this document is still in the draft stages, and specific recommendations of the Rangewide Conservation Assessment and Strategy to benefit *R. onca* populations and habitat are far from being finalized, funded, or implemented. Thus it cannot be relied upon to protect or recover the species, especially given the loss of 2 of the 8 remaining populations during the last decade.

Existing federal regulatory mechanisms that have the potential to provide some form of protection for the relict leopard frog include occurrence on federally protected land, consideration under the National Environmental Policy Act ("NEPA"), coverage under Habitat Conservation Plans ("HCPs"), or co-occurrence with other listed species.

Federally Protected Land

All of the known remaining viable populations of relict leopard frogs occur within the Lake Mead National Recreation Area, managed by the National Park Service ("NPS").

The congressionally-mandated mission of the NPS is to "conserve the scenery and the natural and historic objects and the wildlife therein and to provide for the enjoyment of the same in such a manner and by such means as will leave them unimpaired for the enjoyment of future generations" in the areas under their jurisdiction (NPS 2002).

Some of the regulations that provide protection for *R. onca* populations found on NPS lands at Lake Mead NRA include the Code of Federal Regulations Title 36, Chapter 1, §§ 2.1 and 2.2. These prohibit "possessing, destroying, injuring, defacing, removing, digging, or disturbing" wildlife; introducing wildlife, fish, or plants into a park area ecosystem; taking or disturbing wildlife; and possessing or using amphibians as bait. The only one of these activities that has been identified as a threat to the relict leopard frog is introduction of non-native predators by park visitors into springs supporting this frog. The existence of these regulations has not prevented this activity (RLFWG 2001). See the discussion of recreational impacts in section II.B.5.e below.

As part of its participation in the Clark County Multi Species Habitat Conservation Plan ("MSHCP"), the NPS is developing a management plan for the relict leopard frog. The MSHCP identifies the NPS as having responsibility for a number of species-specific conservation measures for the relict leopard frog (RECON 1998).

Before discussing the proposed protections in this management plan, it is important to note that the Fish and Wildlife Service ("USFWS") may not consider promised or future management actions when considering whether or not to list a species. The USFWS must instead consider only the current management and status of the species. States, federal agencies, and private interests can easily promise to protect and recover species in order to avoid or delay a listing they consider potentially controversial, but there is no way of knowing whether promised actions will be implemented or result in recovery.

To protect species from ongoing destruction, modification, or curtailment of habitat or range, listing under the ESA is required while management actions are being tested and implemented. If promised management actions turn out to result in substantial recovery, then at that point they can be incorporated into a recovery plan for the species. Clearly the relict leopard frog is in danger of extinction and thus requires ESA protection, regardless of untested and promised management actions.

Proposed action items in the NPS management plan include:

- installation of signs at springs on a case-by-case basis, explaining the need for their protection and reiterating state laws that prohibit camping within 30 m (100 ft) of water sources;

- inventory and monitoring of populations of relict leopard frogs and other amphibians, as time allows (this measure is currently being implemented, but it is unclear how long the requisite funding will last);

- evaluation of the potential for reintroduction of relict leopard frog populations into managed areas outside the NRA, such as Las Vegas Wash Wetlands and Park, in Henderson, Nevada; Boulder City Wetlands Park; and Big Springs Refugium, in downtown Las Vegas (relict leopard frogs have already been introduced into Boulder City Wetlands Park, but there are concerns about de-watering, herbicide use, and bullfrogs that have invaded the site, and the long-term success of this reintroduction is unknown); and

- development and implementation of a NPS management plan to ensure long-term protection and conservation of relict leopard frog populations. The plan should address measures to monitor the remaining populations, grazing management, conservation agreements, conservation easements with private landowners, deterrence of poaching through regular ranger patrols, assessment of the need for refugia, and control of exotic fish and bullfrog populations.

A relict leopard frog plan has not yet been completed by the NPS. There is as of yet no published timeline or dedicated funding for all of these action items. Development of a NPS management plan, however ambitious, will likely not be enough to ensure long-term protection and conservation of the relict leopard frog. These measures may only cover *R. onca* habitats on NPS land, not all the potential habitat needed to protect and recover the species.

Current NPS management has not prevented extirpations of relict frog populations on NPS lands. One of the few known populations of the species was recently extirpated at Corral Spring, and the population at Gnatcatcher Spring is apparently close to extirpation, both within the Lake Mead National Recreation Area. There are ongoing anthropomorphic threats to the remaining populations of relict leopard frogs on NPS lands (see the discussion of recreational impacts in the Lake Mead NRA in section II.B.5.e below) and also from outside NPS land, such water diversions up gradient from occupied springs.

National Environmental Policy Act

The National Environmental Policy Act of 1969 (NEPA) (42 U.S.C.4321-4370a) requires Federal agencies to consider the environmental impacts of their actions. The NEPA process requires these agencies to describe a proposed action, consider alternatives, identify and disclose potential environmental impacts of each alternative, and involve the public in the decision-making process. Most actions taken by the federal agencies such as the National Park Service, U. S. Forest Service, and the Bureau of Land Management that could affect the relict leopard frog are subject to the NEPA process. NEPA does not, however, prohibit these agencies from choosing alternatives that will negatively affect individual frogs, populations of relict leopard frogs, or potential relict leopard frog habitat. De facto evidence of NEPA's inability to protect the relict leopard frog is that the species has declined precipitously in spite of the existence of NEPA.

Habitat Conservation Plans

The Habitat Conservation Plan ("HCP") provisions of the ESA were intended to provide a net benefit to threatened and endangered species, in return for providing landowners with regulatory certainty and permits to impact or otherwise "take" listed species and their habitats (Kareiva et al. 1999). In theory, HCPs can help protect and restore habitat, including habitat for non-listed species covered under the plan. Unfortunately, most HCPs fail to live up to this promise, and simply function as exemptions from the species and habitat protection policies of the ESA (Hood 1997; Kareiva et al. 1999). Arguably, a few HCPs make the best of difficult situations on private lands, and may even help species' recovery to some extent. There is considerable controversy over whether HCPs adequately function to protect and recover listed species that are covered under HCP agreements (Hood 1997; Kareiva et al. 1999).

A nationwide study of HCPs by the National Center for Ecological Analysis & Synthesis and the American Institute of Biological Sciences (Kareiva et al. 1999) found that most HCPs contributed to habitat losses for the targeted species, failed to meet recovery goals, and suffered from poor planning and plan evaluation. According to Kareiva et al. (1999): nearly 30% of HCPs "take" 100% of the focal species' populations or habitat in the permit area; about 50% of HCPs allowed 50% or more of the species' populations or habitat in the plan area to be "taken"; 43% of the time, HCPs failed to provide sufficient mitigation measures; 23% of the time, species and their habitats were "taken" before mitigation measures were implemented and found effective (most HCPs failed to reduce allowed "take" levels or use other more conservative approaches in the face of inadequate information or uncertainties); 33% of HCPs failed to secure up-front funding to ensure that mitigation actually occurs; and 81% of HCPs studied had irreversible impacts.

Not surprisingly, HCPs that failed to adequately conserve species also tended to lack rigorous impact assessments and planning. The Kareiva et al. (1999) study found that: 75% of the time, impacts to species were not adequately studied by HCPs; 42% to 49% of the time, HCPs failed to quantify how much of a species' habitat and population, respectively, would be "taken"; most HCPs used low quality data to evaluate their mitigation measures; and 25% of the time, sufficient information did not exist to determine how HCPs would affect the species' viability.

The relict leopard frog is a covered species under only one HCP, the Clark County Multi Species Habitat Conservation Plan ("MSHCP") in southern Nevada (RECON 1998). A Lower Colorado River HCP is being prepared by the three lower Colorado River basin states (California, Nevada, and Arizona), in concert with the U. S. Bureau of Reclamation and other federal agencies, but the relict leopard frog is not a covered species in this HCP. This HCP is still in the planning stages and only will cover the 100-year flood plain along the Colorado River, from Lees Ferry below the Glen Canyon Dam in northern Arizona to the southerly international boundary with Mexico. Thus it will not cover most of the known occupied *R. onca* habitat, which is outside of this floodplain. It also is questionable whether the HCP will be effective in preventing the extinction of native species and assuring the overall ecological health of the region (Hood 1997).

The Clark County MSHCP is significant because all of the remaining known populations of relict leopard frogs are in Clark County. Permittees and cooperators in the MSHCP are the National Park Service ("NPS"), University of Nevada at Reno, Nevada Division of Wildlife, and U. S. Fish and Wildlife Service. The MSHCP is a 30-year plan completed in 1994, covering 2,125 km^2 (525,000 acres). Unfortunately, the Clark County MSHCP mostly focuses on habitat for the desert tortoise (*Gopherus agassizii*), which is not a riparian or wetlands-dependent species, and thus will not provide much protection for *R. onca* habitat. Also, the MSCHP allows more than half of the covered area to be developed. 688 km^2 (170,000 acres) of the plan area have already been developed and the plan allows for development of an additional 461 km^2 (114,000 acres) - development that will likely require attendant water development projects in the area, which will potentially impact *R. onca* habitat.

There is no guarantee that the Clark County MSHCP will not suffer from the inadequacies discussed by Kareiva et al. (1999) when it is implemented. Also, the fact that the plan expires after thirty years does not guarantee that potential or occupied relict leopard frog habitat will be protected in perpetuity.

Proposed conservation actions in the MSHCP for the relict leopard frog include general and ecosystem level actions for desert riparian habitat, including environmental education programs; riparian habitat and spring protection; habitat restoration and enhancement; livestock, wild horse, and burro management; and potential reestablishment of extirpated populations. Although some *R. onca* have been bred in captivity, it is not yet known whether the species can be successfully reintroduced and reestablished at former habitats.

Funding priorities for the Clark County MSHCP include the following programs for the relict leopard frog: continued identification of occupied sites and initiation of monitoring; protection and enhancement of both occupied and potential habitat at springs; and genetic studies.

The MSHCP identifies the NPS as having responsibility for a number of species-specific conservation measures for the relict leopard frog, as mentioned above. These measures may only cover *R. onca* populations and habitat on NPS land, not all potential *R. onca* habitat that may be necessary for recovery of the species.

Co-existence With Other Federally Listed Species

The Virgin Spinedace Conservation Agreement and Strategy (Lentsch et al. 1995) provides procedures for controlling stocking, introduction, and spread of nonnative aquatic species specifically in the Virgin River basin. Stocking of salmonids is to be restricted to areas where salmonid populations already exist or areas where they will not conflict with native species of special concern. Stocking of other nonnative species, including channel catfish (*Ictalurus punctatus*), largemouth bass, and bluegill sunfish (*Lepomis macrochirus*) is prohibited without a certificate of registration. Certificates of registration are issued only for stocking of standing water impoundments, including reservoirs and isolated ponds. Stocking of these nonnative species is not permitted where conflicts with native species of special concern could occur. Fish species classified as prohibited under State of Utah Rule 657-3 may not be stocked into the Virgin River basin.

Unfortunately, the relict leopard frog has already been extirpated from the Virgin River basin. Non-native predatory fish, such as bass, sunfish, and catfish, have already become widely distributed along the Virgin, Muddy, and Colorado Rivers (Jennings and Hayes 1994; RLFWG 2001). The existence of these procedures will not necessarily prevent further introductions of non-native fish, as NPS policies prohibiting introduction of non-native species into relict leopard frog habitat in Lake Mead NRA has not prevented the practice by recreational visitors. See the discussion of recreational and impacts in section II.B.5.e below.

<u>State Regulatory Mechanisms</u>

Nevada

The Nevada Natural Heritage Program ranks the relict leopard frog as S1, "critically imperiled due to extreme rarity, imminent threats, and/or biological factors." *Rana onca* is a Nevada State Protected Species under Nevada Administrative Code §503 (AGFD 1996; RLFWG 2001). While this code makes it illegal to kill or possess individual frogs, collecting has not been a threat to the relict leopard frog. The code offers no habitat protection, and does not address the factors threatening the continued existence of the species nor aid in its recovery in any way.

Nevada Revised Statutes that could potentially benefit the species include NRS 501.182 and NRS 503.587, which authorize the Nevada Game and Fish Commission to "enter into cooperative agreements with adjacent states for the management of interstate wildlife populations" and to "manage land to carry out a program for conserving, protecting, restoring and propagating selected species of native fish, wildlife and other vertebrates and their habitats which are threatened with extinction and destruction." Petitioners are not aware of any such agreements for the relict leopard frog.

NRS 533.367 requires that before a person may obtain a right to the use of water from a spring or water that has seeped to the surface of the ground, he must ensure that wildlife which customarily uses the water will have access to it. The state engineer may waive this requirement for a domestic use of water. This statute did not prevent springs supporting *R. fisheri* from being capped or diverted.

Despite the existence of these statutes, the relict leopard frog has been recently extirpated from one location (Corral Spring), and is probably close to extirpation from a second location (Gnatcatcher Spring), out of only seven remaining sites (= at least 14%) in Nevada.

Arizona

The relict leopard frog is considered as Wildlife of Special Concern by the state of Arizona (AGFD 1996). Such designation may call attention to the species and prompt more information to be collected about the loss of its habitat in Environmental Impact Reports and other documents, but offers no substantive species or habitat protection and has not halted the habitat loss or other factors causing the decline of the species. Arizona Game and Fish Commission Order 41 prohibits collection or hunting of relict leopard frogs in Arizona, except when done under the authority of a special permit. Again, collecting has not been a threat to the relict leopard frog, the code offers no habitat protection, and it does not address the factors threatening the continued existence of the species nor aid in its recovery.

In 1997, AGFD developed a ranid frog conservation and management program in an effort to successfully orchestrate the stabilization and recovery of Arizona's native ranid frogs (Sredl 1997). Management actions suggested by AGFD nongame branch personnel included: (1) determining taxonomic relationships with other leopard frogs, particularly the lowland leopard frog; (2) determining the current distribution and status of the species by conducting surveys throughout the potential range of the species (e. g. drainages feeding into Lake Mead, especially the Virgin River, and springs along the Colorado River mainstem below Hoover Dam); and (3) conserving and managing known populations in spring systems below Hoover Dam. There is now a more complete understanding of the first two objectives, taxonomic clarification, and determination of population distribution and status.

The third objective was postulated to include habitat enhancement and maintenance, removal of nonnative predators and competitors, and establishment of new breeding populations (perhaps in association with aquatic habitats established for native fishes that are managed to preclude invasion by nonnative species such as bullfrogs, Rio Grande leopard frogs, crayfish, and others). These measures have not yet been implemented, and there is no published timeline or dedicated funding for all of these action items. There are no other known AGFD agency-mandated recovery goals for the relict leopard frog.

Despite the existence of state regulations and the proposed ranid frog conservation and management program, the relict leopard frog has apparently been recently extirpated from Arizona.

Utah

The relict leopard frog is classified as a Sensitive Species in Utah (UDWR 1997). Although this classification may direct state and federal agency actions by drawing attention to the status and conservation needs of the species, it does not afford any regulatory protection.

State of Utah Rule 657-3 prohibits the collection, importation, and possession of relict leopard frogs without a certificate of registration (for scientific or educational use only, if the use will benefit the species or will significantly benefit the general public without material detriment to species). If the species were to be rediscovered or reintroduced into Utah, these restrictions would protect it against population depletion due to harvest for commercial, scientific, recreational, or educational use. However, these activities are not known to be a threat to the relict leopard frog, and the rule does not address habitat protection or the factors known to threaten the species.

Procedures and guidelines for nonnative fish stocking in Utah have been established to prevent negative impacts to native aquatic species. Under State of Utah Policy W2ADM-1, fish stocking and transfer is to be conducted in a manner that does not adversely affect the long-term viability of native aquatic species or their habitat. Stocking for sportfish recreation is to be consistent with conservation and interagency agreements. The existence of these procedures will not necessarily prevent introductions of non-native fish, as NPS policies prohibiting introduction of non-native species into relict leopard frog habitat in Lake Mead NRA has not prevented the practice by recreational visitors. See the discussion of Recreational and Right of Way Impacts in section II.B.5.f below.

Despite the existence of these rules and procedures, the relict leopard frog has likely been extirpated from Utah since the 1950s.

<u>Regional and Local Government Plans</u>

Petitioners are not aware of any regional or local government plans that effectively provide for conservation of existing relict leopard frog populations or ensure protection of potential relict leopard frog habitat suitable for reintroduction efforts.

5. OTHER NATURAL OR ANTHROPOGENIC FACTORS

a. Population Fragmentation

A number of factors make the extant populations of relict leopard frogs extremely vulnerable to extinction due to natural or anthropogenically caused fluctuations in population numbers or habitat conditions (Bradford and Jennings 1997; Bradford et al. 2001). The amount of habitat in which the species persists is very small. For example, at the Salt Cedar Tributary site, frogs are found only in a 40 m length (S. Romin, pers. comm., 2002) and the habitat at Littlefield, where *R. onca* is extirpated, was only a few hectares (Bradford and Jennings 1997). All known populations occur within a few square kilometers. The total population size of the species is small, estimated at less than 1100 adults (RLFWG 2001), but could easily be half that. Small populations are more susceptible to extinction due to chance events than larger ones (Wilson and Bossert 1971; Hartl 1988). Amphibian populations in general and small ranid populations in Arizona, specifically, are known to be highly vulnerable to local extirpation due to habitat fragmentation (USFWS 2000; AGFD 2001). Population turnover in the extant populations appears to be rapid, with an annual survivor rate for adults of only 27% (Bradford and Jennings 1997).

The recent population extirpations at the Littlefield and Corral Spring sites do not bode well for the species as a whole. Bradford and Jennings (1997) theorized that the extirpation of frogs at Corral Spring might have been a natural process. Historically, individuals may have been able to periodically colonize this site from Rogers Spring during wet periods, after flood waters have scoured the site, and habitat conditions are more suitable (i.e. not choked with vegetation). Populations may have subsequently been extirpated due to shrinkage of aquatic habitat and vegetation encroachment as drier conditions prevail. This sort of re-colonization is more likely if there is a large source meta-population and under more pristine habitat conditions. Given the small population size, limited habitat availability, and human alteration of the hydrologic regime and habitat in relict leopard frog habitat, the ability of frogs to disperse between extant populations is essential to the persistence and recovery of the species.

Population fragmentation is one of the major threats to the survival of the relict leopard frog (AGFD 1996). Connectivity and potential for dispersal among the extant populations been dramatically reduced as a result of damming the Colorado River. Since dispersal among most remaining populations was precluded or greatly reduced by the formation of Lake Mead in 1935, natural re-colonization after local extirpation events is very unlikely. Some downstream movement of frogs in Black Canyon appears possible as suggested by the observations of individual frogs at Willow Beach, 10 km downstream from the nearest known population. Within the Overton Arm area, dispersal of frogs might be possible between Blue Point and Rogers Springs. These sites are separated by a minimum of 1.6 km. Moreover, two frogs have been observed at a small spring located between Rogers and Blue Point Springs (S. Romin, pers. comm., as cited in RLFWG 2001). (RLFWG 2001). However, the great dependence of *R. onca* on permanent water may effectively subdivide frogs at individual springs into small distinct populations rather than one large population (Pratt and Jennings 1992). There is no known overland movement of frogs (Bradford et al. 2001) and the greatest movement recorded between recaptures of marked frogs was 120 m (D. Bradford, unpublished data as cited in RLFWG 2001), less than one-tenth the distance between Blue Point and

Rogers.

b. Low Genetic Variation

With an estimated adult population of no more than 1,100 relict leopard frogs, more than half of which occur at one site, there is concern that the species is near the critical threshold for genetic viability. What little is known about the population genetic structure and diversity within *R. onca* is derived from a phylogenetic analysis by Jaeger et al. (2001). In that study, mitochondrial DNA (mtDNA) was evaluated using restriction site variation (RFLP) analysis and by sequencing. The study also evaluated total genomic-wide patterns (predominately nuclear in origin) within and among populations, using randomly amplified polymorphic DNA (RAPD) markers. Both RFLP and sequencing analyses indicate low levels of mtDNA variation within and among populations of *R. onca*.[10] All 19 *R. onca* in the RFLP analysis shared the same RFLP haplotype, and in the sequencing analysis, all 9 *R. onca* (representing samples from all 7 populations extant in the 1990s) demonstrated a single mtDNA haplotype. The now extinct *R. onca* population at Littlefield showed the most genetic variation. Jaeger et al. (2001) also demonstrated low levels of variation within the nuclear genome as evaluated by RAPD markers in 102 *R. onca* samples representing 6 populations.[11] These analyses suggest low genetic variation within *R. onca* as compared to that observed in *R. yavapaiensis* populations from the northern portion of its range.[12]

c. Invasive plants

Invasive plants can modify *R. onca* habitat by displacing native vegetation and forming dense monocultures, substantially altering native riparian communities. Tamarisk invasion is of particular concern,

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10 In the RFLP analysis, 19 leopard frogs representing samples from 6 of the 7 populations that were extant during the 1990s were evaluated for about 2150 basepairs (bp) of mtDNA consisting predominately of the NADH subunit 2 gene and a portion of the cytochrome oxidase subunit 1 gene using 11 restriction enzymes. All 19 *R. onca* shared the same RFLP haplotype. In comparison, from the same analysis, 3 haplotypes were found in 6 *R. yavapaiensis* from 2 populations within the northern portion of this species distribution. In control region sequencing, about 959 bp were analyzed for 9 *R. onca* representing samples from all 7 populations extant in the 1990s. All *R. onca* evaluated demonstrated a single mtDNA haplotype, and while representative samples of *R. yavapaiensis* sequenced for the same region also showed low levels of genetic variation, some variation was observed within populations.

11 RAPD markers are notoriously methodologically variable and comparisons of the level of genetic variation should be limited to within-study comparisons. Jaeger et al. (2001) included 2 *R. yavapaiensis* populations in the RAPD analysis with which *R. onca* populations can be compared. In their Table 4, within-population similarity of the RAPD data indicates very high levels of similarity between pairs of individuals within all *R. onca* populations, with the most variable being that of the now extinct population at Littlefield, Arizona. Within population similarity for *R. onca* was about 9 to 27% higher than that observed within the 2 *R. yavapaiensis* populations.

12 This signal of low genetic variation potentially indicates a history of low, or bottlenecked, effective population sizes and high population connectivity, at least through the geographic region containing remaining populations. Jaeger et al. (2001) state, " given the high level of similarity in all evaluated genetic markers, little information can be derived from our study regarding current gene flow and population structure... beyond recognition of the distributional limits of *R. onca*." These authors suggest further genetic evaluations using higher-resolution techniques may be useful for providing a genetic basis for developing conservation strategies.

since tamarisk has a high rate of evapo-transpiration, and sucks many times the amount of water of the willow-cottonwood association it replaces, lowering the water table (Robinson 1965; Weeks et al. 1987). It also carpets riparian corridors with salty needles, reduces the insect supply, and generally reduces biodiversity by forming monocultures. Tamarisk is a regional problem, and is prevalent along the Virgin River (including at the Littlefield site) and the shorelines of Lakes Mead and Mojave, as well as in almost every untreated spring. The majority of tamarisk has been removed from Northshore springs occupied by relict leopard frogs. The National Park Service does not consider tamarisk to be a short-term threat at these sites, but control treatments will need to be maintained. Black Canyon springs occupied by relict leopard frogs contain substantial amounts of tamarisk. (RLFWG 2001).

Although dried palm (*Washingtona* sp.) frond skirts can provide important wildlife habitat, palms also displace native vegetation. Mature palms are present at Blue Point and Rogers Springs, and a large number of recruits sprout each season in Blue Point, Rogers, and Gnatcatcher Springs. Mature palms also occur within the Black Canyon drainage; recruits have been found in Bighorn Sheep Spring. (RLFWG 2001).

Tall whitetop (*Lepidium latifolium*) is quickly becoming an invasive threat in the general range of the relict leopard frog. It has not yet been found in springs occupied by *R. onca*, but has been found in Las Vegas Wash, which empties into Lake Mead (RLFWG 2001).

Not all nonnative plants are detrimental. In the Black Canyon springs, nonnative, weedy species such as *Bromus rubens*, *Polypogon monspeliensis,* and *Sonchus oleraceus* colonize disturbed gravel bars in moderate densities, providing cover and foraging areas for *R. onca* (RLFWG 2001).

d. Native Plant Succession

Unchecked, native plant succession produces dense vegetation marginally suitable as relict leopard frog habitat, since the species requires open habitat and deep pools. Species such as *Eleocharis* and *Scirpus* generally require 3-5 years to become overgrown. Native species of particular concern are *Typha*, *Phragmites*, and *Cladium*, which form tall, dense stands almost immediately upon colonizing an area, spread rapidly, and are resistant to disturbance. (RLFWG 2001).

Choking of relict leopard frog habitats by unchecked emergent vegetation may be related to the establishment of reservoirs in the Virgin and Colorado River watersheds, and changes in grazing and trampling action by native (bighorn sheep) and non-native (feral burros, horses, and cattle) animals. Historically, floods would have periodically scoured emergent vegetation along these river systems. Sredl (1997) correlated a significant increase in a *R. yavapaiensis* population at Tule Creek, Yavapai County, Arizona, with a major scouring flood in 1993. The flood removed sediment and increased open water habitats preferred by leopard frogs at a site that had been choked by vegetation. *Rana onca* populations at the Littlefield and Corral Spring sites were extirpated after emergent vegetation grew over virtually all the former open water at the sites, resulting in a loss of pool habitat.

e. Recreational and Right of Way Impacts

Recreational access to springs occupied by the relict leopard frog results in the continuous introduction of nonnative aquatic species. Within Lake Mead National Recreation Area ("NRA"), the Northshore springs are easily accessed, as Blue Point and Rogers Springs have parking areas and picnic facilities. At Rogers Spring, multi-colored aquarium gravel can often be found where unwanted pets have been freed. Fish may also be commonly released into Blue Point Spring. Lake Mead NRA entrance station personnel at the Las Vegas Boulevard entrance report visitors sharing their intent to release pet fish, not realizing regulations prohibit such activities. Planned construction of entrance stations near Overton may help reduce the amount of fish released in the Northshore area. Visitors to Rogers Spring feed, and probably also introduce nonnative soft-shell turtles into the impoundment. (RLFWG 2001).

Human visitors to the NRA are naturally attracted to the warm springs in which extant populations of relict leopard frogs exist. Visitors regularly build rock dams across Boy Scout and Rogers Springs. The remainder of the springs occupied by *R. onca* are too cool or inaccessible to attract bathers, or presently lack attractive pools. At Boy Scout Spring, damming occurs in the very hot primary channel and thus has minimal effect on relict leopard frogs, which mostly use the cooler, vegetated, side pools. At Rogers Spring, damming is generally restricted to the uppermost pool and concrete spillway. There the rock dams alter flow to a *R. onca* site immediately downstream of the spillway. Unauthorized damming near the source of Rogers Spring in 1999 substantially rerouted and changed the water flows. These dams are regularly dismantled, and the rocks removed from the site to discourage re-building. Although law enforcement and interpretive staff assist in reporting or dismantling new dams, as a well as educating visitors, dams continue to appear. NPS personnel also have to regularly remove dislodged algae mats (which are not a natural phenomenon, and adversely affect frog habitat below the outflow) and garbage at Rogers Spring. (NPS 2001; RLFWG 2001).

Within Lake Mead NRA, road maintenance is a concern. At the Rogers Spring power line crossing, owned by Nevada Power, grader operators routinely create a wider road each year, often pushing soil and debris into *R. onca* habitat (RLFWG 2001).

f. Natural Erosion And Scouring

Springs on the north shore of the Overton Arm flow though soft, gypsum-based soils prone to water erosion. As the springs down cut, blocks of destabilized soil fall into the course. Soil blocks typically crumble and absorb course water, resulting in shallow spots. This is particularly a problem at Gnatcatcher Spring where water volume is not sufficient enough to carry away collapsed soil. Of greater concern is the dissolution of gypsum lenses, resulting in streambed collapse. Small course shifts, due to collapse and subsequent re-routing, benefit *R. onca* by creating larger pools and new, open habitat. However, collapses also lead to the conversion to underground sections and rapid dewatering of large areas of prime habitat. (RLFWG 2001).

The natural process of dissolution of gypsum soils reduces aquatic habitat. Sinkholes are common downstream of Blue Point and Rogers Springs, and the streams go underground in some places, sometimes for hundreds of meters. A dewatering of frog habitat that occurred recently due to subsurface dissolution may represent a permanent reduction in aquatic habitat (Bradford and Jennings 1997). Early in 1996 a large section of prime *R. onca* habitat at Blue Point Spring dried up completely due to dissolution of gypsum soils and streambed shift (Romin 1997; NPS 2001).

Black Canyon springs, in narrow, high gradient drainages, are subject to occasional scouring. Large, smooth boulders of up to 2 meters in diameter rest in the narrow canyons. Smaller flash flooding often deposits gravel downstream, substantially filling pools. Gravel shifts often in Big Horn Sheep Spring. While adult relict leopard frogs are adept at escaping flash floods (M. Sredl, as cited in RLFWG 2001), tadpoles may be washed away. In March 2000, vegetative debris from flooding was evident, and the tadpole population at Bighorn Sheep Spring was approximately 10% of what it had been 3 weeks prior (NPS & NDOW, unpubl. data, as cited in RLFWG 2001).

III. CONCLUSION

The relict leopard frog is clearly an extremely endangered amphibian. The species has been lost from 91% of its known distributional sites over the past 100 years, with 2 of the 8 known sites lost within the past decade. At most, 1100 adult frogs remain in a fraction of their former range. Population fragmentation, lack of connectivity between populations, and low genetic variation threaten several of the remaining small populations with immediate extirpation. The remaining sites are vulnerable to a host of impacts from potential water development, predation and competition by introduced species, habitat alteration by invasive plants and natural erosion, disease, and recreational visitors to inhabited springs.

IV. CRITICAL HABITAT

Petitioners request the designation of critical habitat for the relict leopard frog concurrent with its listing. The relict leopard frog already has already vanished from 91% of the areas in its historical range. Currently unoccupied habitats are absolutely essential to the survival and recovery of the species. Critical habitat should encompass all springs, streams, wetlands, and seeps within the historical range of the species, as well as a protective buffer around those features. Additionally, the entire drainage upstream from each spring should be protected as critical habitat, to ensure the hydrological integrity of springs with habitat value for the relict leopard frog.

V. SIGNATURE PAGE

Submitted this 8th day of May, 2002

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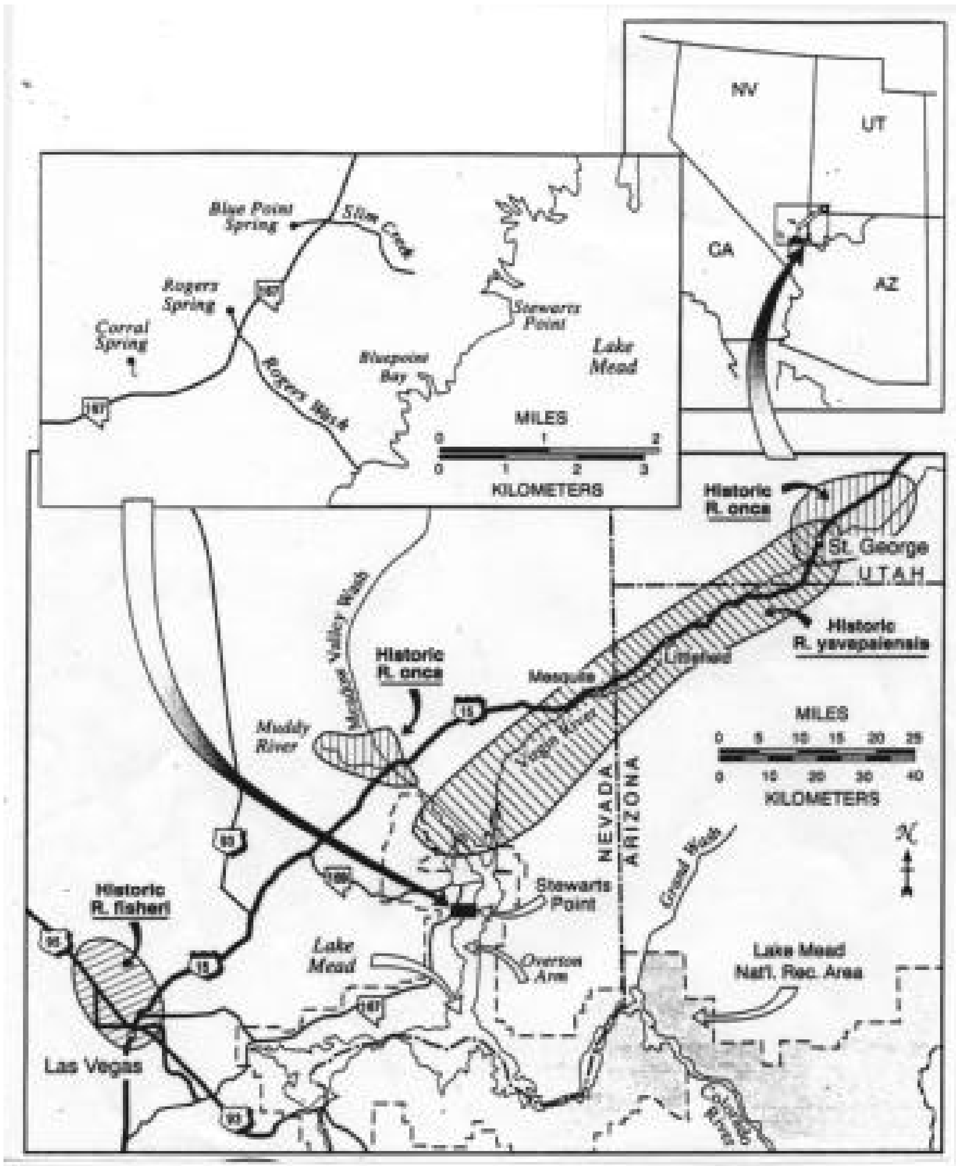
APPENDIX 1

Historical Distribution Map of *R. onca* and *R. fisheri*



Map of historical distribution of *R. onca* and *R. fisheri* from Bradford and Jennings (1997)

[Does not include Black Canyon, NV area, where *R. onca* was historically found (Bradford, et al. 2001; USFWS 2001b) and was rediscovered in 1997 (USFWS 2001a). Leopard frogs at Littlefield, AZ, identified as historical *R. yavapaiensis* range, have been confirmed as *R. onca* (Jaeger at al. 2001)]

APPENDIX 2

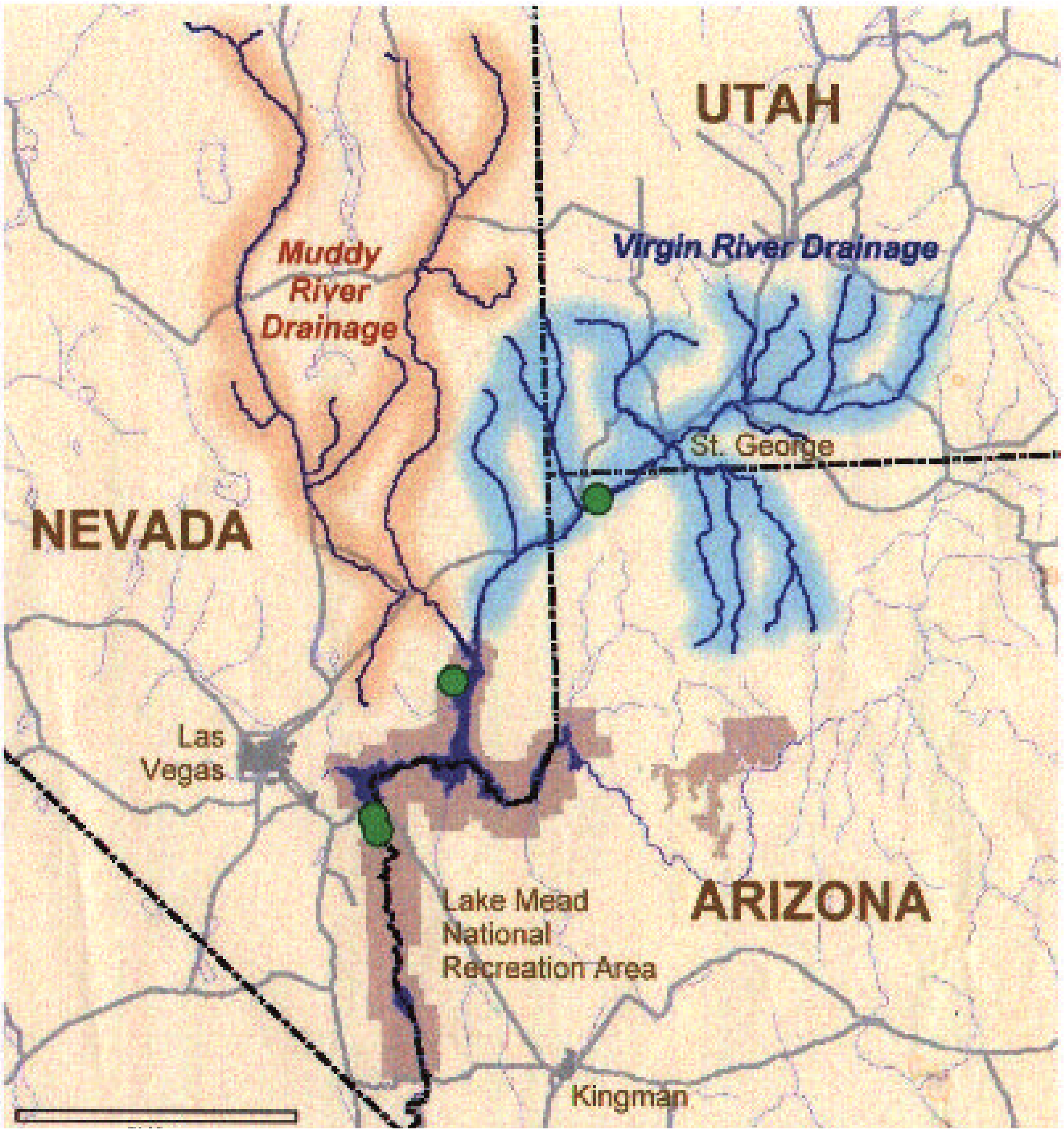
Locations of Extant and Recently Extirpated *R. onca* Populations

Location	**Historical Locality?**	**Year Relocated**	**Current Status**	**2001 Adult Pop. Estimate**[a]
<u>Overton Arm of Lake Mead, Nevada</u>				
Blue Point Spring	Yes	1991	Extant	~300 total for
Rogers Spring	Yes	1991	Extant	both springs
Corral Spring	Yes	1991	Extirpated	0
Gnatcatcher Spring	w/in a few km	1997	Extant	few tens
<u>Black Canyon, Nevada</u>				
Boy Scout Spring	w/in a few km	1997	Extant	few tens to <100
Bighorn Sheep Spring	w/in a few km	1997	Extant	few tens to <100
Salt Cedar Spring	w/in a few km	1997	Extant	several hundred to 637
<u>Virgin River, Arizona</u>				
Littlefield	Yes	1991?	Extirpated	0

a Sources: AGFD 2001; NPS 2001; USFWS 2001b.

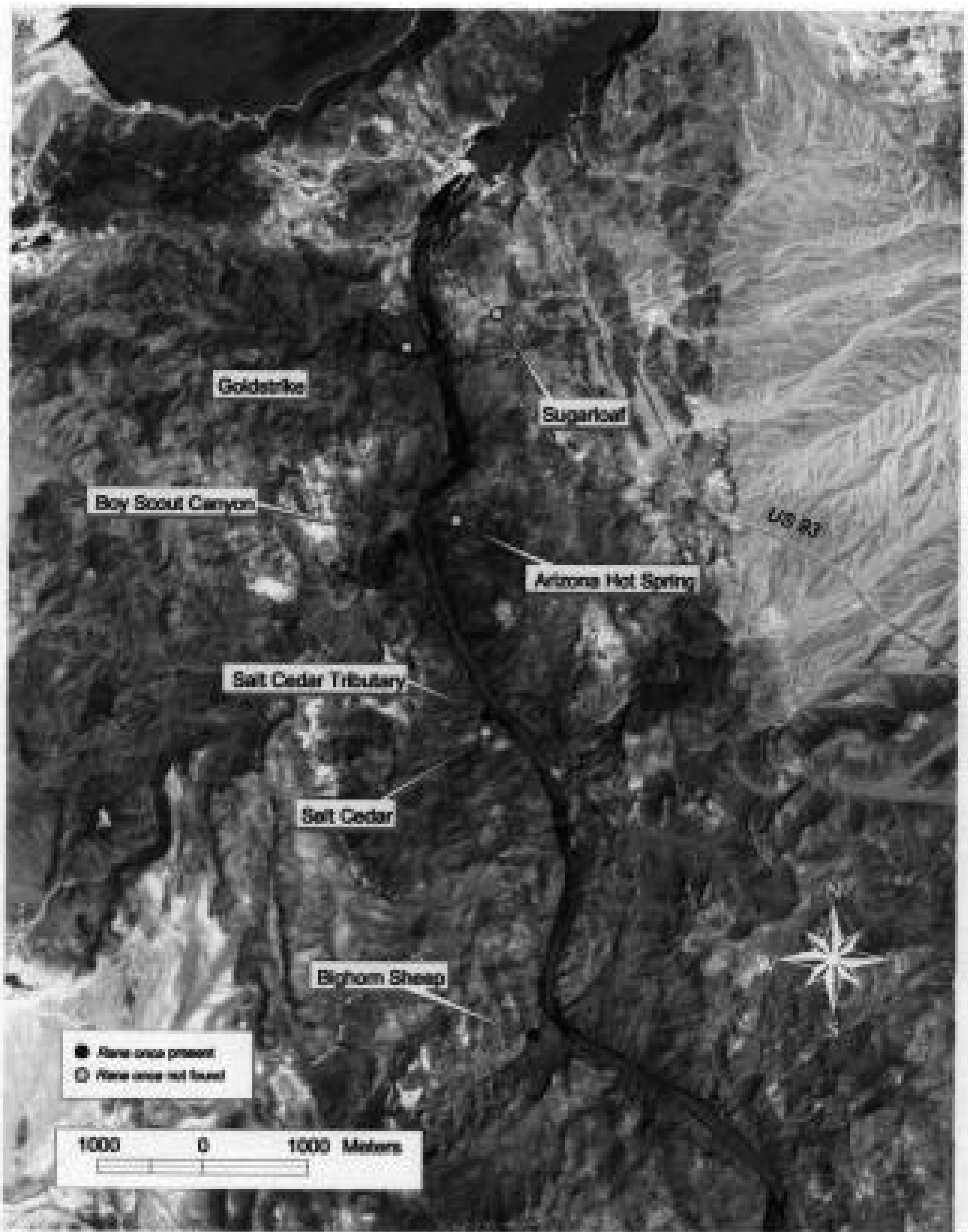
APPENDIX 3

Maps of Extant and Recently Extirpated *R. onca* Populations



Locations of recently known *R. onca* populations shown in green (from USFWS 2001b).

Rana onca distribution status in the Northshore Springs Complex as of March 2001. Base Map: Color IR Satellite Image (SPOT, 1994).



Rana onca distribution status in Black Canyon as of March 2001.
Base Map: Color IR Satellite Image (Spot, 1995).

APPENDIX 4

Taxonomic Relationship Between *R. onca* and *R. fisheri*

The taxonomy of *R. onca* has a confusing history. Cope (1872) described the relict leopard frog, *R. onca*, from an adult female likely collected in Washington County, Utah (see Tanner 1929). Stejneger (1893) described *R. fisheri* from specimens collected in the nearby Las Vegas Valley, Clark County, Nevada. The question of the systematic relationship between *R. onca* and *R. fisheri* remains unresolved despite a long debate on the taxonomy.

Should extinct *R. fisheri* populations prove to be synonymous with *R. onca*, this would not change the taxonomic nomenclature because the name *R. onca* would take precedence. Although Vegas Valley leopard frog populations are extinct, the question of their systematic relationship to *R. onca* is directly relevant to conservation efforts because many conservation actions may depend on or benefit from a clear understanding of the historical distribution of the species.

Numerous early researchers considered *R. fisheri* to be synonymous with *R. onca*. However, there were apparently few actual early comparisons between these taxa and the few comparisons suffered from a perceived lack of *R. onca* specimens (Slevin 1928; Pace 1974). Dickerson (1906) synonymized the two species, but gave no basis for that conclusion, and Boulenger (1919) followed Dickerson. Van Denburgh and Slevin (1921) synonymized the species after examining 99 specimens of *R. fisheri* found in the California Academy of Sciences. Slevin (1928), who examined the same 99 specimens and the type specimen of *R. onca*, maintained that the species were the same. Tanner (1931) also considered them synonymous, but it is unclear whether he examined any *R. fisheri* specimens.

Linsdale (1940) disagreed with synonymy after examining a series of *R. onca* specimens from Utah, including additional recently obtained specimens, and *R. fisheri* from Nevada. Wright and Wright (1949) noted the unresolved taxonomic status of the two taxa. Stebbins (1951, 1959) considered the taxa distinctive, and suggested that *R. fisheri* might be a subspecies of *R. pipiens*.

Pace (1974) examined types of *R. onca* and *R. fisheri*, and supported synonymy based on similarities in general appearance. Platz and Mecham (1979), Platz (1984), and Platz and Frost (1984) supported synonymy after a multivariate analysis of 8 morphometric characters obtained from the type specimen of *R. onca* and a series of *R. fisheri* specimens (along with three other species of southwestern leopard frogs). Jennings (1988) also considered the two taxa synonymous.

Leopard frogs collected as *R. fisheri* in the Las Vegas area prior to 1910 show the morphological characteristics of *R. onca*, including short dorsolateral folds, while specimens collected there after this period (especially during the 1930s) show characteristics of *R. yavapaiensis*, the lowland leopard frog (Platz 1984; Jennings 1988). Adding to the confusion were publications in the literature such as Dickerson (1906) showing a photograph labeled "*R. onca* collected from Las Vegas" that actually depicted a *R.*

yavapaiensis (Jennings 1988; Platz 1988). *R. yavapaiensis* may have been introduced to the area, as locals were known to have transplanted other frog species around Las Vegas (Cowles and Bogert 1936; Linsdale 1940; Stebbins 1951), or may have been native to some Las Vegas springs habitats but not collected by early naturalists.

A study analyzing morphological characteristics of preserved specimens (including the length of the tibiofibula, condition of dorsolateral folds, number of spots on the nose and above the eyes, and the thigh pattern) compared historical samples from the Las Vegas Valley (i.e., *R. fisheri*) to those along the Virgin River (i.e., *R. onca*) as well as other southwestern leopard frog taxa (R. Jennings et al. 1995). R. Jennings et al. (1995) showed substantial morphological differences between leopard frogs from the Las Vegas Valley and all of the other leopard frog taxa examined, including *R. onca* collected from the Virgin River drainage. Jennings et al (1995) concluded that *R. fisheri* is not synonymous with *R. onca* and should be considered to be a valid, distinct species, the Vegas Valley leopard frog.

When leopard frogs were re-discovered in the 1990s at Littlefield, Arizona, within the Virgin River drainage and in the general range of *R. onca,* it was incorrectly posited that they represented disjunct populations of *R. yavapaiensis*. *R. yavapaiensis* has a relatively continuous distribution from Sonora, Mexico into southern and central Arizona and southwestern New Mexico, with additional populations (now thought to be extinct) around the Imperial Valley of southern California (Platz and Frost 1984; Platz 1988; Jennings and Hayes 1994). The northern leopard frog, *Rana pipiens*, also occurs within the upper reaches of the Virgin River.

A recent molecular and phylogenetic analysis by Jaeger et al. (2001) demonstrated that the Littlefield frogs (now extirpated) were *R. onca*. The phylogenetic analysis by Jaeger et al. (2001) focused on the evolutionary distinctiveness of frog populations within the Virgin River drainage and adjacent areas (i.e., *R. onca*) in relationship to *R. yavapaiensis* from the main distribution of that taxon. They did not consider the question of the identity of leopard frogs historically collected within the Las Vegas Valley (i.e., *R. fisheri*), predominately because these populations are now extinct. Jaeger et al. (2001) evaluated several molecular markers of samples from extant, or recently extirpated, populations of both *R. onca* and *R. yavapaiensis*, and also evaluated morphological characters from recent and historically preserved specimens from representative regional populations.

The molecular analysis by Jaeger et al. (2001) showed that leopard frogs from the Virgin River south into the adjacent Black Canyon of the Colorado River are genetically very similar, and that this group of populations is genetically distinct from *R. yavapaiensis*. Morphologically, the type specimen of *R. onca* was very similar to samples collected from extant populations within the Virgin River drainage, which indicates that the current populations represent the organism originally described as *R. onca* Cope. Morphological analysis showed that the Virgin River leopard frogs, *R. onca*, and *R. yavapaiensis* from the main distribution of that taxa demonstrated differentiation of multivariate variation, but also showed that these two groups exhibit similar appearances that represent opposite ends of a multivariate continuum. The molecular and morphological evidence established by Jaeger et al. (2001) is sufficient to conclude that *R.*

onca is an evolutionarily significant unit (Moritz 1994) distinct from what appears to be a closely related taxon, *R. yavapaiensis*, and the differences between these taxa are sufficient to distinguish them as separate species. (RLFWG 2001).