Conservation Agreement and Rangewide Conservation Assessment and Strategy for the Relict Leopard Frog (*Rana onca*)

Final

Prepared by the Relict Leopard Frog Conservation Team

July 2005

ACKNOWLEDGEMENTS

The creation of this Conservation Agreement and Strategy is the result of a truly cooperative effort by a relatively large group of talented and dedicated individuals representing a diverse group of agencies and interests. As the chairman of the Relict Leopard Frog Conservation Team (RLFCT) that was assembled to develop this document I have many people I wish to thank. But before I thank the individuals, I would also like to acknowledge a number of agencies and organizations that provided support by allowing their employees to participate in this effort.

First, I would like to thank the state wildlife management agencies. The Arizona Game and Fish Department (AGFD), the Nevada Department of Wildlife (NDOW) and the Utah Division of Wildlife Resources (UDWR) all provided financial support by allowing persons under their employ to expend numerous hours helping to create this document. Similarly, it is recognized that every agency that sent representatives to help develop this plan bore part of the financial burden of its creation. Federal agencies included the U.S. Fish and Wildlife Service (USFWS), the Bureau of Land Management (BLM), the Bureau of Reclamation (BOR), the Biological Resources Division of the U.S. Geological Survey (BRD), the Environmental Protection Agency (EPA) and the National Park Service (NPS). The University of Nevada System was involved with representatives from both the University of Nevada Las Vegas (UNLV) as well as the Biological Resources Conservation Center at the University of Nevada Reno (UNR-BRRC). Local government and non-governmental organizations also helped with representation from the Southern Nevada Water Authority (SNWA), and The Nature Conservancy. Funding assistance from the Clark County Desert Conservation Program is also acknowledged and appreciated.

A few individuals deserve honorable mention even though some of their work may pre-date work on the plan itself. Dr. Randy Jennings was the first person I consulted back in 1991 about what species of frog we had living in Corral and Blue Point Springs. He brought in Dr. David Bradford (EPA), Dr. Brett Riddle (UNLV) and Jef Jaegar (UNLV) to conduct early taxonomic studies that paved the way for much of the work that followed. Zane Marshall (SNWA) was also instrumental early on (1992) in helping me put together the first interagency funding package for definitive work on the taxonomy and a preliminary assessment of the population status. Suzin Romin, initially hired as a Student Conservation Association Intern, became the primary author of a Natural Resources Preservation and Protection (NRPP) grant proposal that allowed us (NPS) to hire her, and begin more intensive management. Dr. Cecil Schwalbe (BRD-University of Arizona) helped us secure those funds.

Suzin went on to help organize the first scoping meeting (March 29, 2001) where it was decided to go forward with development of this plan through an interagency planning effort. She also was the primary editor of the document during the early stages of its development. Sean Blomquist (AGFD) deserves special thanks and recognition for taking over as editor in chief of the document after Suzin moved away. Through his efforts, the plan underwent major changes that resulted in several draft documents that grew to closely resemble the finished product. Cristina Velez (UNLV) served as editor of the document since it was first reviewed by the USFWS

Regional Office in October of 2003. She has been instrumental in making sure the comments from that review as well as a subsequent review by all of the signatory agencies in October of 2004 were incorporated into a final product.

People who attended one or more of the meetings and provided input included: Dr. Brett Riddle, Dr. Cecil Schwalbe, Zane Marshall, Suzin Romin, Mike Boyles (NPS), Kent Turner (NPS), Dr. Chester Figiel (USFWS), Bob Williams (USFWS), Jody Brown (USFWS), Jim Rorabaugh (USFWS), Amy Sprunger-Allworth (USFWS), Vicki Campbell (USFWS), Dr. Dick Tracy (UNR-BRRC), Heather Hundt (BLM), Linda Cardenas (BLM), Jerry Hickman (BLM), Dr. Ken Covay (USGS), Jim Moore (TNC), Matthew Andersen (UDWR), Kevin Wheeler (UDWR), Caireen Ulepic (BOR), Patti Clinton (BOR), Cailin Doyle (SNWA), Stephanie Harris (SNWA), Aaron Ambos (SNWA), Sean Harris (UNLV), Jeff Goldstein (UNLV), and Dr. Craig Palmer (UNLV).

Every person mentioned above was helpful and deserves thanks. However, there was a core group that deserves special thanks and recognition. These are the ones that attended most of the meetings and/or did a significant amount of writing on the document. They are: Mike Sredl (AGFD), Sean Blomquist, Jon Sjoberg (NDOW), Jim Heinrich (NDOW), Mike Burrell (NDOW), Peggy Miller (UDWR), Michael Burroughs (USFWS), Dr. David Bradford, Dr. Karin Hoff (UNR-BRRC), Jef Jaeger, Rebecca McArthur (SNWA) and Cristina Velez. These people are the primary architects and authors of this collaborative document.

Thank you, one and all, for your contributions.

Ross Haley

EXECUTIVE SUMMARY

The relict leopard frog (*Rana onca)* was first described in 1875 from a specimen collected near the Virgin River in Washington County, Utah. Subsequent reports and museum specimens provide evidence that this species had a relatively restricted range along portions of the Virgin, Muddy and Colorado Rivers, particularly in small springs that fed into those major drainages. Although there was no scientific system of population monitoring in place, anecdotal evidence suggests that populations declined for a variety of reasons. In fact, by 1950, when the last known specimens were collected from Berry Spring in southern Utah, the species was considered to be extinct (Stebbins 1951, Platz 1984, M. Jennings 1988, Platz 1988). This belief persisted for approximately forty-one years, until the species was rediscovered at 2 springs on Lake Mead National Recreation Area in Nevada on April 24, 1991 (R. Haley pers. comm.). Morphological and genetic studies conducted since 1991 have confirmed the validity of this taxon (Jennings et al. 1995, Jaeger et al. 2001).

Since their first rediscovery in 1991, additional populations have been discovered at several springs. Of the seven populations identified since 1991, six of the seven have been on lands managed by the National Park Service at Lake Mead NRA in Nevada and one was on private lands in Arizona near Littlefield, Mohave County. The Littlefield population has subsequently been extirpated, as has one of the first two populations discovered at Lake Mead NRA. The worldwide population of this species of frog is presently found in approximately 5-6 very small springs, and probably numbers fewer than 1100 total individuals based on an analysis presented in this document. Over half of this number is in one population.

On May 8, 2002, a petition to list *Rana onca* as endangered was filed by the Center for Biological Diversity and the Southern Utah Wilderness Alliance. As a separate action, the US Fish and Wildlife Service (USFWS) conducted a review of the species and determined that candidate status for this species was appropriate, with a notice of candidate status published in the Federal Register on June 13, 2002. A coalition of agencies and interest groups had already begun development of this Conservation Agreement and Strategy (CAS), before the petition was filed, with the intent of guiding and coordinating conservation efforts. This CAS has been developed to expedite implementation of conservation measures for the relict leopard frog in Clark County, Nevada; Mohave County, Arizona; and Washington County, Utah with the desired outcome of ensuring the long-term conservation of *R. onca* within its historical range.

Immediate conservation actions are needed to reduce threats to the species, increase the size and number of populations, and maintain associated riparian and wetland habitats. The actions described in this CAS are intended to stabilize existing *R. onca* populations and reduce or eliminate the potential for further species declines by reducing or eliminating known threats and expanding the number of viable, extant populations in secure habitats within its known historical range. The document is arranged to identify the five Endangered Species Act (ESA) listing factors followed by an analysis of the relevance of each factor and associated threats to the status of *R. onca.* The document then outlines actions needed to address each listing factor along with

standards for evaluating and measuring success. Implementation of the plan is intended to adequately address each listing factor and will, therefore, conserve the species.

This plan was developed with the cooperation of the USFWS, and with consideration for the recently published Policy for the Evaluation of Conservation Efforts (PECE) designed to provide guidance to the USFWS when making listing decisions. In keeping with that policy, it is intended that this agreement, when signed, will provide both the certainty that an effective conservation effort will be implemented as well as reasonable certainty that the described conservation effort will be effective. Consequently, the hope is that implementation of the conservation actions identified in this agreement and strategy will preclude the need to list the species under the provisions of the ESA. However, it is also recognized that in signing the agreement, the USFWS can make no guarantees that listing will not be necessary.

Projected costs of implementing the program listed in the implementation schedule are estimates based on input from the involved parties to the agreement. Many of those cost estimates represent anticipated staff time and other expenditures of various agencies which will be directed to the tasks described in this document from existing and future general budget appropriations, as opposed to new appropriations or other funding sources which may need to be sought out during the implementation period. Where feasible and appropriate, other potential funding sources for completion of specific conservation tasks have been identified in the implementation table.

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CONSERVATION AGREEMENT FOR THE RELICT LEOPARD FROG (*RANA ONCA*)

I. PURPOSE AND NEED

This Conservation Agreement (Agreement) has been developed to expedite implementation of conservation measures for the relict leopard frog *(Rana onca)* in Clark County, Nevada, and Mohave County, Arizona as a collaborative and cooperative effort among resource agencies, governments, landowners, and other participants. The Relict Leopard Frog Conservation Team (RLFCT) was established in March 2001, prior to the official rediscovery of the species. This team has held at least 3 meetings each year and has outlined the necessary conservation actions to ensure the long-term conservation of the relict leopard frog within its historical range. The parties to this Agreement believe that implementing the conservation actions herein defined will benefit the relict leopard frog, reduce the threat of extinction, and reduce the likelihood for its listing under the Endangered Species Act of 1973, as amended (ESA). Threats should be significantly reduced or eliminated through full implementation of the Conservation Agreement and Strategy (CAS).

Historical distribution of the relict leopard frog includes spring, stream and wetland habitats of the Virgin River and Colorado River drainages from Washington County, Utah to below Black Canyon in Nevada and Arizona. The relict leopard frog has been reduced to as few as 6 occupied sites in 2 general areas, the Overton Arm of Lake Mead, Nevada, and Black Canyon below Hoover Dam along Lake Mohave, Nevada. The decline of relict leopard frogs reflects the reduction of isolated wetland habitats in the southwestern US. Immediate conservation actions are needed to reduce threats to the species, increase the size and number of populations, and maintain associated riparian and wetland habitats. Habitat restoration is expected to benefit not only relict leopard frogs but also other plant and animal species dependent on these isolated water and wetlands sources, including humans. Those actions as described in this CAS are intended to stabilize existing relict leopard frog populations and reduce or eliminate the potential for further species declines by expanding the number of viable populations in secure habitats within its known historical range and distribution.

II. INVOLVED PARTIES

Signatory Agencies:
Arizona Game and Fish Department
Bureau of Land Management (Arizona, Nevada, and Utah state offices)
National Park Service (Pacific West Region)
Nevada Department of Wildlife
US Fish and Wildlife Service (California/Nevada Operations Office, Regions 2, and 6)

Non-signatory Parties:

Individuals

David Bradford
Karen Hoff
Jef Jaeger
Brett Riddle
Cecil Schwalbe
Richard Tracy
Kevin Zippel

Organizations and Agencies

Clark County
Detroit Zoo
Environmental Protection Agency
Mirage Casino
Nevada Division of Water Resources
Southern Nevada Water Authority
The Nature Conservancy
University of Arizona
University of Nevada, Las Vegas
University of Nevada, Reno
US Geological Survey
Utah Division of Wildlife Resources

III. AUTHORITIES

The signatory parties hereto enter into this Agreement under Federal and State laws as applicable, including but not limited to, section 6(c)(1) of the Endangered Species Act of 1973, as amended, sections 503.351 and 503.584 of Nevada Revised Statutes (NRS), and Arizona Revised Statute (ARS) 17-231.B-7. This Agreement is subject to and is intended to be consistent with all applicable Federal and State laws and interstate compacts.

Section 6(c)(1) of the ESA provides encouragement to the states and other interested parties, through Federal financial assistance and a system of incentives, to develop and maintain conservation programs that meet national and international standards. Section 6(c)(1) of the ESA is key to meeting the United States' international commitments and to better safeguard, for the benefit of all citizens, the Nation's heritage in wildlife and plants.

The Nevada Department of Wildlife's (NDOW) mission specifically identifies the protection, preservation and restoration of the state's wildlife, supported by goals and objectives in NDOW's 2004 Strategic Plan to recover State and Federal protected species and remove or reduce threats to the point that special status protection is not required. NDOW's Native Aquatic Wildlife Program plan and work programs support similar objectives. The activities described in this agreement are consistent with those goals and objectives and will be supported to the extent practicable by the Department. NRS (Nevada Revised Statute) 503.351 provides authority for the Director of NDOW to enter into cooperative agreements for the purpose of the management of native wildlife. NRS 503.584-503.589 directs NDOW to cooperate with other states and legal entities to the maximum extent practicable for the conservation, protection, restoration and propagation of species of native fish, wildlife and other fauna that are threatened with extinction. NAC (Nevada Administrative Code) 503.075 extends protected wildlife status to certain native amphibians, including *R. onca*.

An important goal of the Arizona Game and Fish Department's mission, as stated in Wildlife 2006 Nongame and Endangered Wildlife subprogram narrative, is the conservation and restoration of native biological diversity and recovery of imperiled species. Additional documents such as work plans and job descriptions for the Heritage and Section 6 Ranid Frog projects support similar objectives. The activities described in this agreement are consistent with the objectives outlined in those documents, and will be supported to the extent practicable by the Department. ARS 17-231.B.7 authorizes the Arizona Game and Fish Commission to enter into this Agreement through its administrative agency, the Arizona Game and Fish Department (AGFD).

To the extent required pursuant to ARS 12-1518, and any successor statutes, the parties agree to use arbitration, after exhausting all applicable administrative remedies, to resolve any dispute arising out of this agreement, where not in conflict with Federal Law. All parties are hereby put on notice that this agreement is subject to cancellation pursuant to ARS 38-511. Pursuant to ARS 35-214 and 35-215, and Section 41.279.04 as amended, all books, accounts, reports, files and other records relating to the contract shall be subject at all reasonable times to inspection and audit by the State for 5 years after contract completion. Such records shall be reproduced as designated by the State of Arizona. All parties are hereby put on notice that AGFD's participation in this agreement is subject to Executive Orders 99-4 and 75-11, entitled "Prohibition of discrimination in State Contracts – Non-discrimination in Employment by Government Contractors and Subcontractors". Said non-discrimination orders, by reference, are made a part of this agreement.

Bureau of Land Management (BLM) sensitive species are designated by the BLM State Directors and are protected by the policy described for candidate species as a minimum. The BLM shall carry out management, consistent with the principles of multiple use, for the conservation of candidate species and their habitats and shall ensure that actions authorized, funded, or carried out do not contribute to the need to list any of the species as threatened or endangered (BLM Manual, Section 6840.06 C).

The National Park Service was established by an act of Congress passed in 1916 generally referred to as "The Organic Act" (16 USC I). This law states that it is the mission of the National Park Service 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 Management Policies 4.1.4 encourages parks to pursue opportunities to improve natural resource management within parks and across administrative boundaries by cooperating with public agencies and interested parties. The NPS recognizes that cooperation with other resource and land mangers can accomplish ecosystem stability and other resource management objectives, when the best efforts of a single manager might fail. Therefore, parks will develop agreements with Federal, tribal, State and local governments and organizations, and private landowners, when appropriate, to coordinate plant, animal, water and other natural resource management activities in ways that maintain and protect, not compromise, park resources and values. Such cooperation may involve coordinating management activities in two or more separate areas, integrating management practices to reduce conflicts, coordinating research, sharing data and expertise, exchanging native biological resources for species management or ecosystem restoration purposes, establishing native wildlife corridors, and providing essential habitats adjacent to, or across, park boundaries (NPS 2001 Management Policies, 4.1.4). Authority to enter into such agreements is found in the National Parks Omnibus Management Act of 1998, the National Park Service Organic Act of 1916, as amended, and the Endangered Species Act of 1973, as amended.

The authorities for the involved parties to enter into this voluntary Conservation Agreement derive from the following legislation:

<u>Fish and Wildlife Service</u>

Endangered Species Act of 1973, as amended
Fish and Wildlife Act of 1956, as amended
Fish and Wildlife Coordination Act, as amended

<u>National Park Service</u>

National Park Service Organic Act of 1916, as amended
Endangered Species Act of 1973, as amended
National Parks Omnibus Management Act of 1998

<u>Bureau of Land Management</u>

Federal Land Policy Management Act
Endangered Species Act of 1973, as amended

<u>Arizona Game and Fish Department</u>

Arizona Revised Statute 17-231.B-7

Nevada Department of Wildlife

Nevada Revised Statutes 503.351 and 503.584

Information on legal protections afforded to *R. onca* by each State and Federal entity is provided in Appendix 2.

IV. Implementation of Conservation Actions

To meet the goals and objectives of this Agreement, the parties agree to undertake specific tasks, as described in this Agreement and the Strategy (Attachment A) and support activities in this agreement to the extent practicable. Where responsibility for undertaking a specific action has not yet been assigned, the parties agree to use the outcomes of reviews as proposed in this Agreement to determine and modify actions for implementation where appropriate. All cooperators agree and recognize, consistent with the goals of this Agreement, that monitoring actions and conservation measures implemented through the CAS will be conducted experimentally and be consistent with the concepts of adaptive management.

Parties to this Agreement recognize that they have specific statutory responsibilities that cannot be delegated, particularly with respect to the management and conservation of wildlife. Nothing in this Agreement or Strategy is intended to abrogate any of the parties' respective responsibilities. The Strategy is attached as Attachment A and is a part of this Agreement.

V. Administration

A. Nothing herein contained shall be construed as limiting or affecting in any way the delegated authority of the BLM.

B. This agreement provides the framework for the initiation and implementation of future agreements or modifications of existing agreements, including those involving the necessary expenditure of funds between the Participants. Such agreements will refer to this overall agreement, which shall be the primary governing document.

C. Any obligation of the BLM imposed or assumed pursuant to this agreement is subject to the availability of funds.

D. The implementation of any tasks identified under this agreement and agreed to by any signatory parties under separate, appropriate agreements is subject to required funds being available to all parties of this agreement. This agreement will not be considered as legally obligating either party to pay for services.

VI. Relict Leopard Frog Conservation Team (RLFCT)

The involved parties shall designate a representative to serve on the Relict Leopard Frog Conservation Team (RLFCT). The RLFCT shall monitor the implementation of the conservation strategy and provide a forum for exchange of information. The RLFCT shall also be responsible for specific tasks as set forth in the implementation schedule. Through unanimous mutual agreement among designated representatives of all involved parties, the RLFCT may make changes in the tasks and scheduling of task implementation, as described in the implementation schedule, if needed to better meet the goals and objectives of the Conservation Strategy. The RLFCT shall in no way make recommendations to or serve as an advisory group to a Federal agency.

Designated representatives or their alternates shall attend a minimum of two meetings of the RLFCT annually for the life of this Agreement to review progress and coordinate work priorities and schedules.

VII. Duration of Agreement

The term of this Agreement shall begin on the date the Agreement is signed by all parties. The duration of the Agreement shall be for 10 years, which the signatories have determined is the minimum time period required to implement the identified conservation actions and assess their effectiveness. The involved parties shall review the Conservation Agreement and its effectiveness at least annually to determine whether it should be revised. Within one year of completing or addressing all the tasks identified in the implementation schedule, the Conservation Agreement shall be reviewed by the involved parties and either modified, renewed, or terminated. This agreement may, at any time, be amended or terminated by mutual concurrence of all involved parties. Any party may withdraw from this Agreement by providing 60 days notice to the other parties in writing. Within 30 days of the withdrawal of any signatory party to this Agreement, the remaining parties shall meet in consultation with the US Fish and Wildlife Service to determine if the Agreement and Strategy will require amendment or modification, or if other actions may be required to insure continued effective conservation of the relict leopard frog.

VIII. Regulatory Requirements

Implementation of this agreement will require no new regulatory mechanisms (e.g., laws, regulations, ordinances). The initiating agency for any translocation action is responsible for ensuring that any compliance requirements and required documentation necessary for release of animals to the wild are completed in a timely manner that will not delay established activity timing, if at all possible. Specific compliance requirements will vary with the location and nature of translocation actions. For the augmentation of sites with existing, established populations compliance requirements may be minimal but action agencies shall insure that appropriate State and Federal permits for movement and release of live animals are in place. For the translocation and release of animals to establish populations at historically occupied or new site locations additional compliance actions may be required, including preparation of a site assessment or other project documentation, compliance checklists, and additional approval requirements from the private landowner or responsible public land management agency. Table 1 identifies the regulatory requirements that are anticipated for each major category of conservation actions included in this strategy. No new regulations are required at this time to implement the CAS.

Table 1. Anticipated regulatory/compliance requirements by conservation activity category.

Conservation action category	Project document[1]	NEPA EAC	Section 7	Landowner permission	State/NPS permitting	Other[2]
Survey/monitoring	No	C	C	C	Yes	No
Nonnative species control	C	C	C	C	C	C
Recreation management	Yes	C	No	No	No	Yes
Disease prevention	No	No	No	No	No	No
Water rights monitoring	No	No	No	No	No	No
Mitigation protocol	Yes	No	No	No	No	Yes
Captive rearing/ head starting	C	No	No	No	Yes	No
Translocation – existing populations	No	No	C	No	Yes	No
Translocation – new populations	Yes	C	C	C	Yes	Yes
Research activities	Yes	C	C	C	Yes	C
Data management	No	No	No	No	No	No

[1] preparation of a project or site-specific project proposal, site assessment, or other documentation distinct from an EA for NEPA compliance.

[2] additional compliance requirements may be imposed by individual agencies that are project specific and cannot be identified at this time.

C = Conditional, may be required depending on the specific location or characteristics of the conservation activity. NEPA and Section 7 review may be required for some activities dependent on future funding source (e.g. Section 6, SWCA).

IX. Signatures

In Witness Whereof, the parties have caused this Conservation Agreement for the relict leopard frog to be executed as of the date of the last signature below:

APPROVED:

California/Nevada Operations Office
US Fish and Wildlife Service
2800 Cottage Way, Room W-2606
Sacramento, CA 95825

for Steve Thompson — Date
Manager

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Terry Crawforth — Date
Director

Pacific West Region National Park Service 1 Jackson Center 1111 Jackson Street, Suite 700 Oakland, CA 94607	11/16/05 Jon Jarvis Date Regional Director
Bureau of Land Management Nevada State Office 1340 Financial Blvd. Reno, NV 89502	7/29/05 ~~Robert Scruggs~~ Amy Lueders Date State Director (acting)
Bureau of Land Management Arizona State Office 222 North Central Ave. Phoenix, AZ 85004	Elaine Zielinski 9-13-05 Elaine Zielinski Date State Director
Bureau of Land Management* Utah State Office 324 South State Street Salt Lake City, Utah 84145	Sally Wisely Date State Director

* At this time, Region 6 of the US Fish and Wildlife Service and the Utah State Office of the Bureau of Land Mangement have chosen not to sign this agreement. This does not preclude the participation of these agencies in any future actions to conserve the relict leopard frog.

ATTACHMENT A: RANGEWIDE CONSERVATION ASSESSMENT AND STRATEGY FOR RELICT LEOPARD FROG (*RANA ONCA*)

PURPOSE AND NEED

This Conservation Agreement and Strategy (CAS) has been developed to expedite implementation of conservation measures for the relict leopard frog in Clark County, Nevada, and Mohave County, Arizona, with the desired outcome of ensuring the long-term conservation of the relict leopard frog within its historical range. Relict leopard frogs have been reduced to as few as 6 occupied sites in 2 general areas, the Overton Arm of Lake Mead, Nevada, and Black Canyon below Hoover Dam along Lake Mohave, Nevada. Immediate conservation actions are needed to reduce threats to the species, increase the size and number of populations, and maintain associated riparian and wetland habitats. Those actions as described in this CAS are intended to stabilize existing relict leopard frog populations and reduce or eliminate the potential for further species declines by removing or reducing threats and by expanding the number of viable populations in secure habitats within its known historical range and distribution.

POLICY FOR THE EVALUATION OF CONSERVATION EFFORTS (PECE)

This document was designed to meet the requirements of a conservation agreement as specified in the USFWS policy for the evaluation of conservation efforts (68 FR 15100, 3/28/2003). These criteria are designed to ensure the certainty that the conservation effort will be implemented, and that when implemented the conservation efforts will be effective. To ensure PECE compliance, USFWS cooperators contributed extensively during the development of the plan by serving on the RLFCT. Additionally, drafts of the CAS were reviewed in 2003 by USFWS offices in regions 1 and 2, and most recently in 2004 by the USFWS Nevada State Office. A table listing PECE criteria and areas where they are addressed in the document can be found in Appendix 1.

DESCRIPTION AND ECOLOGY

A. DESCRIPTION OF SPECIES

Field Characters

Historically, three leopard frog taxa, the northern leopard frog (*R. pipiens*), the Vegas Valley leopard frog (*R. fisheri*), and the lowland leopard frog (*R. yavapaiensis*), had distributions near that of the relict leopard frog. The northern leopard frog likely occurred sympatrically with the relict leopard frog but may be distinguished from the relict leopard frog in the field, as described below. The Vegas Valley leopard frog and the lowland leopard frog are morphologically similar to the relict leopard frog, but the distribution of these frogs is mostly disjunct from that of the relict leopard frog. Those populations described as Vegas Valley leopard frogs are extinct, and the species was last documented in 1942 (Stebbins 1951). Morphological characters generally can be used to distinguish the Vegas Valley leopard frog and the northern leopard frog from the relict leopard frog (Jennings et al. 1995), although for definitive purposes, multivariate analysis of morphometrics may be required. For the lowland leopard frog, a combination of morphological characters may be used to increase the likelihood of correct field identification in relationship to the relict leopard frog, but specific identification appears to require genetic analysis (Jaeger et al. 2001).

Cope (1875) described the relict leopard frog from an adult female with the following characters "... a dermal fold on each side of the back, ... The heel extending beyond the end of the muzzle. Light brown above; below, yellow. Three rows of distinct, solid, small black spots between the dorsal folds; ... none of the spots yellow-bordered. Head unspotted; no band on lip; clouded spots on the posterior face of the femur." Since that time, a greater number of specimens and populations of this species have been identified (Jennings et al. 1995). In general, the relict leopard frog exhibits reduced spotting on the back and head compared to other species of leopard frogs. Background coloration varies from light brown or tan to dark olive-brown and charcoal. Some individuals are green, most often on the head. The inguina is pale yellow to cream colored while the rest of the venter is white or cream colored. Adults lack spots on the tympana and conspicuous supralabial stripes, especially anterior to the eyes. In comparison with other leopard frog species, the relict leopard frog is a small frog with proportionately short limbs. Adult males appear to reach sexual maturity at about 42 mm snout-urostyle length (SUL) (Bradford et al. in press). The largest females can exceed 70 mm SUL.

The following field character descriptions are adapted from Jennings et al. (1995). First, continuous dorsolateral folds generally distinguish northern leopard frogs from the 3 other species, including relict leopard frogs. Relict leopard frogs and lowland leopard frogs have folds with a short posterior segment that is broken and inset medially. In these species, the short, inset segment may be represented by a linear series of warts rather than a solid ridge. Dorsolateral folds of Vegas Valley leopard frogs are generally more truncated (terminating just posterior of the sacral hump) than those of relict leopard frogs.

Second, posterior thigh patterns are highly variable in relict leopard frogs, but generally consist of reticulations (or bands) ranging from weak and fuzzy to rather bold. These patterns are similar to those in lowland leopard frogs, but can be used to help distinguish relict leopard frogs from northern leopard frogs, which has a spotted posterior thigh. The posterior thighs of Vegas Valley leopard frogs are typically dark and unpatterned.

Third, the relict leopard frog tends to have fewer spots on the dorsum of the nose, and above and between the eyes than the other leopard frog taxa. The relict leopard frog rarely exhibits a nose spot (anterior to the eyes), a trait generally shared with lowland leopard frogs. The northern leopard frog typically has a single large nose spot, while the Vegas Valley leopard frog usually has at least 1 smaller nose spot. The relict leopard frog typically lacks spots above and between the eyes, but can also be found with 1 or 2 spots between the eyes. Northern leopard frogs and Vegas Valley leopard frogs usually have 2 or more of these spots, but the northern leopard frog occasionally has no spots above and between the eyes.

Fourth, the Vegas Valley leopard frog has a relatively short tibiofibula, almost always less than half the SUL. Although some relict leopard frogs may also exhibit short tibiofibula, most tend to have tibiofibulae that are longer than half the SUL. The tibiofibula of lowland leopard frogs and northern leopard frogs are almost always greater in length than half the SUL, and overlap in size with relict leopard frogs.

<u>Taxonomy and Systematics</u>

The relict leopard frog was described in 1875 from a single adult female likely collected within the Virgin River drainage in the vicinity of Saint George, Washington County, Utah (Cope 1875 in Tanner 1929). On the basis of numerous gross morphological similarities, this frog is considered a member of the leopard frog complex, a group consisting of numerous species in North and Central America (Hillis 1988). The taxonomy of relict leopard frogs, however, has a confused and controversial history that centered around 2 major uncertainties. One long-debated uncertainty is whether relict leopard frogs and Vegas Valley leopard frogs represent distinct species or a taxonomic synonymy (see Jennings 1988 for review). The latter taxon was described from a series of specimens collected in the nearby Las Vegas Valley, Clark County, Nevada (Stejneger 1893). The other uncertainty is whether extant populations of leopard frogs within the Virgin River drainage, in the general range of relict leopard frogs, represent disjunct populations of lowland leopard frogs, a species described in 1984 (Platz and Frost 1984). Both of these historical uncertainties raise questions about the evolutionary distinctiveness of remnant populations within the Virgin River drainage and adjacent areas.

In a phylogenetic analysis, Jaeger et al. (2001) investigated evolutionary distinctiveness of leopard frog populations within the Virgin River drainage and adjacent areas in relationship to lowland leopard frogs from the main distribution of that taxon. Results showed that leopard frogs from the Virgin River south into Black Canyon of the Colorado River were genetically very similar, and that this group of populations was genetically distinct from lowland leopard frogs. Analysis of morphological characters of leopard frogs from the Virgin River and lowland leopard frogs from the primary range of that taxon showed that these 2 groups exhibit very similar appearances but represent opposite ends of a multivariate continuum. The type specimen of the relict leopard frog was very similar to samples collected from extant populations within the Virgin River drainage. Based on these results, Jaeger et al. (2001) concluded that populations from the Virgin River and Black Canyon area are relict leopard frogs.

There are historical records of leopard frogs south of Black Canyon along the Colorado River and within the Imperial Valley in southern California. The closest known, recently extant population is from the confluence of the Bill Williams River, which is purportedly the lowland leopard frog (Clarkson and Rorabaugh 1989). The taxonomy of leopard frogs from the more southern locations on the Colorado River remains unclear, but 5 museum specimens from the Imperial Valley were included in the morphological analysis of Jaeger et al. (2001). Due to the small sample size, no definitive statements about the Imperial Valley frogs could be made, but these authors caution that the relict leopard frog genotypes may occur south of Black Canyon along the Colorado River.

The question of the systematic relationship between the relict leopard frog and the Vegas Valley leopard frog remains unresolved despite a long debate on the taxonomy. Historically, there were few actual comparisons between these taxa and the few comparisons suffered from a lack of relict leopard frog specimens. An unpublished study of morphological characters of preserved specimens compared historical samples from the Las Vegas Valley (i.e. Vegas Valley leopard frogs) to those along the Virgin River (i.e. relict leopard frogs) as well as other southwestern leopard frog taxa (Jennings et al. 1995). This study showed substantial morphological differences between leopard frogs from the Las Vegas Valley and those from the Virgin River drainage. A conclusion from that study was that the Vegas Valley leopard frog was most similar in apperance to Chiricahua leopard frog (*R. chiricahuensis*) from Arizona. A similar statement was later made by Hillis and Wilcox (2005). Although populations within the Las Vegas Valley are presumed to be extinct, the systematic relationship of these populations to relict leopard frogs is directly relevant to conservation efforts that depend on a clear understanding of the historical distribution of both species.

The molecular and morphological evidence established by Jaeger et al. (2001) is sufficient to conclude that the relict leopard frog is an evolutionarily significant unit (Moritz 1994) distinct from what appears to be a closely related taxon, the lowland leopard frog. In a phylogentic analysis of New World ranid frogs, Hillis and Wilcox (2005) suggested that the level of mtDNA genetic difference between the relict leopard frog and lowland leopard frog was more similar to that of a currently recognized subspecies pair than to observed species-level differences. Under many species concepts (Mayden 1997), however, the differences between relict leopard frogs and the lowland leopard frog are sufficient to distinguish them as separate species.

B. DISTRIBUTION

Historical Distribution

To delineate the historical range of the relict leopard frog, specimen records were solicited from 34 museums for *Rana* spp. collected from Washington Co., Utah; Clark and Lincoln Cos., Nevada; Mohave Co., Arizona; and San Bernardino Co., California (Bradford et al. 2004). In addition, herpetological literature from the region was examined to find locality records not represented by museum specimens.

Based on museum specimens, recent field surveys, and literature, the known historical distribution for the relict leopard frog is springs, streams, and wetlands within the Virgin River drainage downstream from the vicinity of Hurricane, Utah; along the Muddy River, Nevada; and along the Colorado River from the its confluence with the Virgin River downstream to Black Canyon below Lake Mead, Nevada and Arizona. All localities are within a few kilometers of these rivers, and many localities are virtually at the river. Relict leopard frogs may have also occurred at lowland localities along the Colorado River upstream from the confluence with the Virgin River. A leopard frog that is morphologically similar to the relict leopard frog and the lowland leopard frog was collected at Marble Canyon (Museum of Northern Arizona; Clarkson and Rorabaugh 1989), but specimens from this area need taxonomic resolution.

Current Distribution

The relict leopard frog is currently known to occur only in 2 general areas: near the Overton Arm of Lake Mead, Nevada, and in Black Canyon, Nevada, below Lake Mead. Historical records are reported for both areas, with specimen records dating from 1936 at the Overton Arm area and from 1955 at Black Canyon. These 2 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. Although it is possible that relict leopard frog populations may also occur in other areas, it is unlikely that many other occupied sites exist given the efforts made to date by Vitt and Ohmart (1978), Jennings et al. (1995), Bradford et al. (2003), and surveys for amphibians and fish conducted or sponsored by State and Federal agencies in Utah, Arizona, and Nevada over the past 2 decades (BIO-WEST Inc. 2001, Platz 1984, R. Fridell pers. comm., R. Haley pers. comm., Blomquist et al. 2003).

The relict leopard frog is currently known to occur at 6 localities. Populations at 2 additional localities have recently been extirpated (Littlefield, Arizona, and Corral Spring, Nevada). All sites are either historical localities (Littlefield; Blue Point, Rogers, and Corral springs) or within a few kilometers of historical localities (Gnatcatcher, Boy Scout, Salt Cedar, and Bighorn Sheep springs).

In addition, 3 individual leopard frogs have been observed on different occasions in 2000, 2001, and 2002 at the Willow Beach National Fish Hatchery at Willow Beach, Arizona, located 10 km downstream from Bighorn Sheep Spring in Black Canyon (C. Fiegel pers. comm.). One of these was collected and confirmed as the relict leopard frog based on mitochondrial DNA sequence similarity (J. Jaeger unpublished data), and another possessed a mark used in recent sampling of upstream populations.

A population of leopard frogs of undetermined identity has been found in Surprise Canyon, a tributary to the Colorado River in the lower Grand Canyon. In 1987, Barry Adams, an associate of Lawrence Stevens (ecological consultant, Flagstaff), took a photograph of a leopard frog in Surprise Canyon. The frog was not collected. In 1997, Michael Douglas (Colorado State University, Fort Collins) found a dead, badly degraded leopard frog. In 2004, surveys within Surprise Canyon documented a large population of these frogs. Analysis of mtDNA samples indicate that these frogs are most closely related to lowland leopard frogs (J. Jaeger pers. comm.).

An extant population of leopard frogs at Wahweap Creek near Big Water, Utah, and Page, Arizona is morphologically similar to the relict leopard frog and the lowland leopard frog. The taxonomy of these frogs also needs resolution, although these frogs were not similar to any known southwestern leopard frog based on mitochondrial DNA analysis (Rorabaugh et al. 2002).

C. Habitat and Environmental Setting

Habitat heterogeneity in the aquatic and terrestrial environment is unknown, but likely important to the relict leopard frog. For other leopard frog species, shallow water with emergent and perimeter vegetation provides foraging and basking habitat, and deep water, root masses, undercut banks, and debris piles provide potential hibernacula and refuge from predators (AGFD unpublished data, Jennings 1987, Jennings and Hayes 1994, Platz 1988). Historical localities were at springs, streams, and wetlands along major rivers (Bradford et al. in press). Extant populations are restricted to perennial desert springs within the Virgin and Colorado river drainages. Currently occupied habitats may reflect available rather than optimal habitat due to destruction, modification, or occupation by nonnative predators of historical habitat.

Littlefield (Reber Springs), Arizona

Reber Springs is an approximately 12-ha wetland formed by multiple springs flowing from a steep, 15-m embankment along the Virgin River. Discharge has been measured at 9 locations (Bradford et al. 2004). The springs are 200 - 350 m from the Virgin River. The wetland is characterized by thick stands of rushes (*Scirpus* spp.) overhanging the water and forming covered pools. The wetland adjoins the river, filling a wide meander opposite a point bar, but is isolated from the river by a 50 - 100 m wide band of sandy substrate, dominated by mesquite (*Prosopis* spp.) and tamarisk (*Tamarix* spp.). One spring has been converted to a stock tank, and cattails (*Typha* spp.) dominate the vegetation. Relict leopard frogs have been found from the spring discharge points throughout the wetlands, but have not been found along the river proper (Bradford et al. 2004).

Northshore Springs (also referred to as Overton Arm), Nevada

The Northshore springs originate from the Rogers Spring Fault, which extends along the southern base of the Muddy Mountains. Blue Point, Rogers, and Corral springs surface directly from the slip-strike fault; Gnatcatcher Spring flows from basin-fill deposits between the Muddy Range and Lake Mead. The source water temperatures of Blue Point, Rogers, and Corral springs are constant year round (D. Bradford unpublished data). Only Rogers Spring contacts Lake Mead. Conductivity is over 4000 µS/cm^3 at Blue Point, Rogers, and Corral springs. All are subregional 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 L/min and 2750 L/min respectively (Pohlmann et al. 1998), Blue Point and Rogers springs form the largest habitats and support the highest numbers of frogs in the Northshore complex. Blue Point Spring provides 800 m of quality habitat and over 1100 m of surface water, while Rogers Spring provides about 370 m of quality habitat and over 4400 m of surface water (S. Romin, unpublished data). Source temperature is 30°C at both springs (Pohlmann et al. 1998), but temperature varies downstream (average Rogers 29°C, average Blue Point 27°C; NPS unpublished data). The springs flow across gypsiferous soil, forming deeply incised, U-shaped channels, 25 cm wide and 60 cm deep. Channel substrate is composed of gravelly, precipitated solids.

Shallow, overflow pools are mostly-permanent features along the course margin, and provide important relict leopard frog habitat. The pools typically range from 25 - 200 cm in width (NPS unpublished data). Wider pools form in some areas, occasionally 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 relict leopard frogs as foraging, basking, and oviposition sites. Relict leopard frogs are most often found in 1 - 7 cm of water, with most choosing depths of 1 - 4 cm. At Blue Point Spring, most individuals choose locations 75 - 150 cm from the main channel and 25 - 75 cm from clumps of dense vegetation (NPS unpublished data).

Both springs have large desert fan palms (*Washingtonia filifera*) and Blue Point spring also has a few large date palms (*Phoenix dactylifera*) near the head or open water portions of the spring (Leary 1992, E. Powell pers. comm.). The head of Blue Point Spring is dominated by screwbean mesquite (*P. pubescens*). Shrub vegetation on the margins of the open water in both springs is dominated by arrowweed (*Pluchea sericea*), common reed (*Phragmites australis*), southern cattail (*T. domingensis*) and American bulrush (*S. americanus*). Both springs have a portion of the water's edge dominated by sedges (*Eleocharis* spp). The vegetation on the edge of the open water of these springs also includes southern goldenrod (*Solidago confinis*), yerba mansa (*Anemopsis californica*), Coopers rush (*Juncus cooperi*), water pimpernel (*Samolus parviflorus*), and New Mexico thistle (*Cirsium neomexicanum*). On the margins of the springs, the most common and obvious plants are screwbean and honey mesquite (*P. glandulosa*), saltgrass (*Distichlis spicata*), alkali goldenbush (*Isocoma acradenia*), seepbush (*Suaeda moquinii*) and iodinebush (*Allenrolfea occidentalis*).

Gnatcatcher and Corral Springs

Gnatcatcher and Corral springs are shorter in length (Corral Spring 30 - 240 m, Gnatcatcher Spring 75 m; NPS unpublished data, Bradford et al. 2004), have lower discharge (<1 L/min, NPS unpublished data), and are cooler (16°C at Gnatcatcher, NPS unpublished data; 27°C at Corral, Bradford et al. 2004) than Blue Point (30°C) and Rogers springs (30°C) (Pohlmann et al. 1998). The springs are much shallower than Blue Point and Rogers springs, and possess few deep pools. These smaller springs are dominated by arrowweed, common reed, and southern cattail on the margins of the open water portions of the springs (Leary 1992, E. Powell pers. comm.). At Gnatcatcher Spring there are 4 cottonwood trees (*Populus fremontii*) at the open water portion of the spring. Corral Spring has a significant portion of the spring lined by Coopers rush, alkali sacaton (*Sporobolus airoides*) and American bulrush. On the margins of the springs, the most common and obvious plants are screwbean and honey mesquite, saltgrass, alkali goldenbush, seepbush, and iodinebush.

Black Canyon Springs, Nevada

In contrast to the Northshore habitats, the Black Canyon springs occupied by relict leopard frogs flow through narrow canyons of igneous bedrock (Pohlmann et al. 1998). Boy Scout and Bighorn Sheep springs flow through steeper gradients than the Northshore springs, and are characterized by waterfalls, plunge pools, and long, shallow riffles. Discharge rates and water temperatures remain high year-round.

Boy Scout Springs (also referred to as Boy Scout Canyon Springs and Boy Scout Hot Springs)

Boy Scout Springs are created from 2 primary water sources, of temperatures 55°C and 24°C, with a combined discharge of 960 L/min (Pohlmann et al. 1998). Side seeps and small springs, most contributing <1 L/min, vary widely in temperature. The main stream includes long riffles <1 cm in depth, spilling into pools up to 60 cm deep. Boy Scout Springs flow directly into the Colorado River. The length of the stream from the springhead to the river is approximately 350 m.

Small-diameter tamarisk overhangs portions of the upper stream; open areas support seep willow (*Baccharis emoryi*) and some catclaw acacia (*Acacia greggii*) (NPS unpublished data). Cool side seeps support a variety of vegetation, including cattails, rock daisies (*Perityle emoryi*), and grasses (*Bromus rubens*, *Polypogon monspeliensis, Andropogon glomeratus*). No sedges, rushes, or reeds are present, but cattails are present in side pools. The lower portion of the stream lacks stream- and pool-side vegetation, but small, barely moist seeps at the cliff base provide moisture for small assemblages of 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 (NPS unpublished data). Pools in the main channel of Boy Scout Springs, however, can be very hot (up to 55°C) with substantially greater water velocity than at Bighorn Sheep Spring.

Bighorn Sheep Spring

Bighorn Sheep Spring has a discharge rate of 10.2 – 75.0 L/min and a temperature of 32°C (Bradford et al. 2004, Pohlmann et al. 1998). As with Boy Scout Springs, the course varies in depth, from 1 cm deep riffles to 50 cm deep pools, but does so more gradually. Small gravel bars, which relict leopard frogs use, shift regularly. Small waterfalls are common. 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 from high river level). The stream length from the springhead to the river is approximately 550 m.

Moderately dense stands of small-diameter tamarisk overhang much of the stream (NPS unpublished data). Seep willow and catclaw acacia are present. 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 and grasses, including grasses (*B. rubens*, *P. monspeliensis*), composites (*P. emoryi*, *Haplopappus gooddingii*, *Brickellia californica*, *Sonchus oleraceus*), desert tobacco, Datura (*Datura meteloides*), and borage (*Cryptantha utahensis*). A few young stands of cattails are present.

In contrast to other sites, where favorite basking sites represent a small percentage of total available space, almost all habitat components (pools, riffles, gravel bars, etc) are heavily used by relict leopard frogs as basking sites (NPS unpublished data). Seeps covered with moss (*Funaria* sp.), ferns, and other vegetation provide additional cover and feeding sites.

Salt Cedar Spring (also referred to as Salt Cedar Canyon and Salt Cedar Canyon Spring)

Salt Cedar Spring provides only shallow surface water flow. Relict leopard frogs are thought to be restricted to a narrow, 40-m length that is not entirely covered by vegetation, and temperature is not excessive (source temperature is 41°C; Bradford et al. 2004). Dense vegetation precludes an accurate survey of the remainder of the course. Vegetation primarily consists of dense stands of small-diameter tamarisk, honey mesquite, cat-claw acacia, seep willow, and a few cattails. Substrate is small-diameter gravels. Water discharge is approximately 120 L/min. The length of Salt Cedar Spring is approximately 360 m (Bradford et al. 2004).

D. NATURAL HISTORY

Life History

Breeding has been documented in September, November, and late January through March (Bradford et al. in press, NPS unpublished data). Water temperature, which differs significantly among springs, does not appear to influence breeding season. However, because eggs and tadpoles can be difficult to locate, particularly in the more complex habitat of the Northshore springs, oviposition timing and preferred location require more research.

Eggs are deposited in roughly spherical clusters 4 - 6 cm in diameter, containing up to 250 eggs (NPS unpublished data). It is not known how many clutches female relict leopard frogs produce in 1 breeding season. The clusters are attached to stems of living or dead vegetation near the bottom of shallow, low-velocity pools 5 - 7 cm deep. 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, thinning of native vegetation in pool habitat resulted in egg deposition in the thinned portion of that habitat (NPS unpublished data).

Time from egg deposition to hatching is unknown. Limited field observations at Blue Point Spring indicate hatching occurs in approximately 1 week (NPS unpublished data). Eggs collected in the field at Gosner stage <14, and maintained in the lab at room temperature, hatched in 5 - 7 days (NPS unpublished data, NDOW unpublished data). Eggs laid by a captive population of relict leopard frogs began hatching after 5 days (Malfatti 1998). Captive tadpoles metamorphose approximately 6.5 months after hatching (NPS unpublished data).

Age of females at maturity is unknown. Male relict leopard frogs may reach reproductive maturity at 42 mm SUL based on the appearance of pigmented thumb pads, within the first year following metamorphosis (Bradford et al. in press). In captivity, the relict leopard frogs collected as juveniles bred after 1 year (Malfatti 1998).

Skeletochronology using toe clips has been inconclusive in determining age distributions of adult relict leopard frogs (D. Bradford pers. comm.), but skeletochronology analysis of a population of lowland leopard frogs in central Arizona indicates that adults do not live more than 3 years (Sredl in press). Based on a mark-recapture study at a Northshore spring (Blue Point Spring) adult relict leopard frogs may be short-lived, surviving about 2 years. Population turnover in relict leopard frogs may be correspondingly high with average survivorship of 0.27 per year (Bradford et al. 2004). Comprehensive information on population structure is unknown.

Food Habits

No studies of the feeding behavior or diet of the relict leopard frog larvae or adults have been conducted, however the food preferences of adult relict leopard frogs are likely similar to other leopard frog species. Stomach analysis of other members of the leopard frog complex from the western US show a wide variety of prey items are taken, including many types of aquatic and terrestrial invertebrates (e.g. snails, spiders, and insects) and vertebrates (e.g. fish and other anurans [including conspecifics]) (Degenhardt et al. 1996, Stebbins 1951).

Larval leopard frogs are primarily herbivorous (McDiarmid and Altig 2000, Platz 1996, Sredl in press). In captivity tadpoles readily consume algae, dark leafy greens (e.g. romaine lettuce, mustard greens, turnip greens, spinach, swiss chard), duckweed, spirulina-type fish foods, peas, alfalfa-based rabbit pellets, commercial "protein gel food," and boiled egg whites (Demlong 1997, S. Romin pers. comm.).

Behavior

Extant relict leopard frog populations are restricted to narrow habitat corridors (<0.5 - 20 m; 1 - 3 m in most places), with a sharply defined boundary between riparian corridor and desert. Laterally, frogs have never been found beyond the inner saltgrass boundary of springs (NPS unpublished data). Relict leopard frogs limit movements along the course of springs. In a 3-year mark-recapture study in the isolated, 550 m long, upper reach of Blue Point Spring, the mean distance moved between captures averaged 18 m, and the longest distance recorded between recaptures was 120 m (Bradford et al. 2004). Jennings et al. (1995) noted that of 11 recaptures of marked relict leopard frogs, the longest recorded movement was 200 m.

Relict leopard frogs are active year-round, and are most often observed in shallow water along channel or pool margins, with individuals spaced 1 - 2 m apart along certain stream lengths. Some spatial and temporal separation of adult and juvenile relict leopard frogs may enhance survivorship. Seim and Sredl (1994) studied association between juvenile-adult stages and pool size in lowland leopard frogs and found that juveniles were more frequently associated with small pools and marshy areas, while adults were more frequently associated with large pools.

Relict leopard frogs are cryptically colored and will usually remain motionless to escape detection (Bradford et al. in press). During the day, they primarily rely on saltation, escaping into deep water or thick vegetation.

Relict leopard frogs call from shallow areas while concealed in vegetation. Calls are a series of soft clucks or chuckles like that of the lowland leopard frog, but historical accounts describe the call as a snore (Davidson 1996). Relict leopard frogs call singly or in response, rather than forming large choruses, call quietly, and cease calling when the habitat patch is approached. Calling has been documented January through early May, and calling occurs primarily at night. Proximity of non-calling individuals to calling individuals has not been documented. Cues that stimulate mating behavior in relict leopard frogs are unknown. Rainfall and water temperature are possible cues stimulating other southwestern leopard frogs (Degenhardt et al. 1996, Wright and Wright 1949, Zweifel 1968). The constant water temperature at warm springs occupied by known relict leopard frog populations may obscure some cues normally available to other leopard frogs in the Southwest.

Tadpoles

Hatchlings fall below the egg mass, and are often found motionless in shallow, often coverless, pool margins for approximately 1 week after hatching (NPS unpublished data). Similarly, captively hatched tadpoles are inactive for several days, gradually moving away from the disintegrating egg mass. After dispersing from the hatch site, small tadpoles share shallow pool margins.

When disturbed, tadpoles swim rapidly toward available cover including vegetation and loose mud at the Northshore Springs or large rocks and undercut ledges at Bighorn Sheep Spring (NPS unpublished data). At Blue Point Spring, large tadpoles are usually found buried in loose gypsum mud and debris, with their eyes remaining above the substrate. When disturbed, they dart a short distance (usually 30 - 45 cm) and rapidly re-bury. Based on limited observations, large tadpoles at this site may be more nocturnal than small tadpoles.

Associated Native Amphibians

Red-spotted toads (*Bufo punctatus*), Woodhouse's toads (*B. woodhousii*), Pacific tree frogs (*Pseudacris regilla*), and at least historically, Arizona toads (*B. microscaphus*) coexist with relict leopard frogs (Bradford et al. in press). Red-spotted toads are relatively uncommon in most relict leopard frog springs, but are prevalent in Corral, Boy Scout, and Bighorn Sheep springs. Red-spotted toads share shallow riffles with relict leopard frogs at Boy Scout Spring and pools at Bighorn Sheep Spring, but no direct interaction has been observed. Woodhouse's toads occur occasionally in the springs. Pacific tree frogs were collected at Rogers Spring in 1960 by J. Twente (University of Utah Specimen 2442-2442) and were observed at Rogers Spring in 1991 (Jennings et al. 1995). They have not been heard or observed recently. Pacific tree frogs have been recorded at Blue Point Spring as well (D. Bradford pers. comm.).

A list of sensitive species in the historical range of the relict leopard frog is provided in Appendix 4. These species could potentially benefit from conservation actions for the relict leopard frog.

E. POPULATION ECOLOGY

Population Estimates

Visual encounter surveys (VES) have been conducted multiple times at all sites, and mark-recapture studies have been conducted at 2 sites (Bradford et al. 2004, S. Romin pers. comm.). At the upper 555 m segment of Blue Point Spring, 96 adult frogs (≥ 42 mm SUL) were captured and marked during 13 visits over the 2-year period, 1995-1996. The estimated number of frogs averaged 36 (95% conf. limits, 27 - 45), and estimated annual survivorship averaged 0.27. Visual encounter surveys between 1991 and 2001 at this site showed considerable variation in numbers encountered (4 - 32 frogs over a 385 m reach; n = 23 visits). There was no consistent pattern of increase or decrease in numbers detected over this time period, although the data suggested an increase rather than a decrease. At Bighorn Sheep Spring in Black Canyon, which extends approximately 450 m in length, a single mark-recapture effort (60 initially marked adults) in March-April 2001 yielded an estimate of 637 adults (95% conf. limits, 381 - 1210). Visual encounter surveys on 3 - 4 visits during 1997-2001 at the sites in Black Canyon yielded average counts of 110, 5, and 13 at Bighorn Sheep Spring, Salt Cedar, and Boy Scout springs, respectively.

To obtain a rough estimate of the total number of relict leopard frog adults, mark-recapture estimates of population size, VES counts, and estimates for extent of available habitat are combined (Bradford et al. 2004). At the Northshore sites, the estimated total linear extent of aquatic habitat is 5.1 km, based on ground measurements, aerial photographs, and USGS digital orthophotoquads. Assuming a frog density similar to that observed in the upper segment of Blue Point Spring in 1995-1996 (i.e. mean of 35.9 adults/555 m), the estimated total number of frogs in the Northshore Arm area is 330 adults. This is likely an overestimate because 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. In Black Canyon, the population estimate at Bighorn Sheep Spring was 637 adults for a time when 104 frogs were counted in the VES, a factor of 6.1. Applying this factor to the average VES counts at the other 2 sites in Black Canyon (mean counts of 5 and 13), an estimate of 750 frogs is obtained for the total adult population size in Black Canyon, 85% of which are at Bighorn Sheep Spring. This yields approximately 1100 adult frogs as the rough estimate for the total population of adult relict leopard frogs, more than half of which occur at 1 site. These estimates should be interpreted with caution as numbers of relict leopard frogs in a population are expected to vary considerably within and among years (Sredl et al. 1997, Skelly et al. 1999, Sartorius and Rosen 2000).

Recent Population Extirpations

At Corral Spring, frogs were counted and marked during 16 visits between November 1991 and December 1994 (Bradford et al. 2004). The maximum number of frogs observed of all sizes was 40, but the population became extirpated by early 1995. Between 1991 and 1995, the change in habitat was conspicuous at Corral Springs. The pools that were initially largely open with scattered emergent vegetation became choked with emergent vegetation, primarily *Scirpus* spp. By early summer of 1994, most of these pools had virtually no open water. This extirpation may have been a natural process, because individuals may periodically colonize this site from Rogers Spring during wet periods after the site is scoured by flood waters, and populations may subsequently be extirpated due to shrinkage of aquatic habitat and vegetation encroachment as drier conditions prevail.

The surveys were initiated in late 1991, a year with high-precipitation storms associated with an El Nino/Southern Oscillation event that scoured vegetation at Corral Spring (R. Jennings pers. comm.). Moreover, aquatic habitats were more extensive along the creek below Rogers Spring than in subsequent years. During such wet times, frogs possibly could colonize Corral Spring from Rogers Spring by traveling 3.0 km along a drainage channel that currently contains desert wash habitat, or by traveling 1.6 km straight-line distance. Similar dispersal distances have been reported for other ranid species in the Southwest, albeit in more mesic environments (Marsh and Trenham 2001). For example, Frost and Bagnara (1977) noted movement of plains leopard frogs (*R. blairi*) for 8 km or more along a creek in the Chiricahua Mountains. Rosen and Schwalbe (1998) found up to 25 young adult and subadult Chiricahua leopard frogs (*R. chiricahuensis*) at a roadside puddle in the San Bernardino Valley, Arizona. They believed that the only possible origin of these frogs was a stock tank located 5.5 km away.

Whether the relict leopard frog persisted at Corral Spring between 1957, when several specimens were collected, and 1991 is not known. The demise of the relict leopard frog at Corral Spring may have been influenced by the construction of a fence in 1991 to exclude feral burros (*Equus asinus*) from most of the site. Prior to the fence, burros may have kept emergent vegetation from completely covering pools.

At the Littlefield site, frogs were observed during the daytime in 1992 and 1996, and 6 were counted at night in both April and July 1998. None of the frogs captured in July were those marked in April. No frogs were found during 3 nighttime surveys between March and May 2001 (Bradford et al. 2004). Bullfrogs were observed in an artificial pond at the site in 1992 and 2001, whereas relict leopard frogs were observed only within open marshy habitat near 1 spring source. As at Corral Spring, the demise of the relict leopard frog population occurred concomitantly with loss of pool habitat due to rapid encroachment of emergent vegetation. Between 1992 and 2001, vegetation cover (primarily *Scirpus* spp.) had increased dramatically such that no pools of open water remained exposed except for the artificial pond. Until some years ago, vegetation within the marsh was kept open by livestock grazing. Subsequently, with the absence of grazing, emergent vegetation grew over virtually all the former open water at the site (Bradford et al. 2004). Introduced bullfrogs have also become established in wetlands along this portion of the Virgin River (BIO-WEST, Inc. 2001).

<u>Connectivity of Extant Populations</u>

Connectivity among the extant populations has almost certainly been dramatically reduced as a result of damming the Colorado River. The formation of Lake Mead in 1935 apparently eliminated at least 1 population located between the Northshore and Black Canyon areas (Cowles and Bogert 1936), and presumably eliminated any potential for dispersal of frogs between these 2 areas. Seventy-seven years of river flow control for power management and the formation of Lake Mohave in 1951 presumably have dramatically impeded dispersal among sites in Black Canyon, which are separated from each other by 1.8 - 5.0 km via the Colorado River. Here, the river level is influenced by Lake Mohave and discharge from Hoover Dam such that the canyon floor is never exposed. Predatory game fishes are present in the river, and water is continually cold (13°C) because it emerges from below the thermocline in Lake Mead. At a local level, springs are not connected by perennial water and frog populations may be isolated from one another because of an unwillingness to cross upland desert habitat even during the wet periods (R. Jennings pers. comm.). Springs, seeps, and ephemeral drainages, however, can serve as effective corridor habitat for leopard frogs in the Southwest during wet periods of the year (Sredl et al. 1997). Downstream movement appears possible as suggested by the observations of individual frogs at Willow Beach, 10 km downstream from the nearest known population. Within the Northshore Springs area, dispersal of frogs may be possible between Blue Point and Rogers Springs. These sites are separated by a minimum of 1.6 km. Two frogs have been observed at Gnatcatcher Spring, a small spring located between Rogers and Blue Point springs (S. Romin pers. comm.). These observations indicate dispersal may occur between the two sites.

Population Genetic Structure and Diversity

What little is known about the population genetic structure and diversity within relict leopard frogs is derived from a phylogenetic analysis by Jaeger et al. (2001). In that study, mitochondrial DNA (mtDNA) was evaluated using restriction fragment length polymorphism (RFLP) analysis and by sequencing. Total genomic-wide patterns (predominately nuclear in origin) within and among populations were evaluated using randomly amplified polymorphic DNA (RAPD) markers.

Both RFLP and sequencing analyses indicate low levels of mtDNA variation within and among populations of relict leopard frogs. All relict leopard frogs evaluated in both RFLP (19 samples from 6 populations) and sequence analysis (9 samples from 7 populations) demonstrated a single mtDNA haplotype. While representative samples of lowland leopard frogs also showed low levels of genetic variation in these analyses, some variation was observed within their populations. A low level of variation was also observed within the nuclear genome as evaluated by RAPD markers in 102 relict leopard frog samples representing 6 populations. RAPD markers are methodologically variable and comparisons of the level of genetic variation should be limited to within-study comparisons. Jaeger et al. (2001) included 2 lowland leopard frog populations in the RAPD analysis with which relict leopard frog populations can be compared. Within-population similarity of the RAPD data indicates very high levels of similarity between pairs of individuals within all relict leopard frog populations, with the most variable being that of the now extinct population at Littlefield, Arizona. Within-population similarity for the relict leopard frog was 9 - 27% higher than that observed within the 2 lowland leopard frog populations.

These analyses suggest low genetic variation within the relict leopard frog as compared to that observed in lowland leopard frog populations. Low genetic variation may indicate a history of bottlenecking, or small effective population size, and high population connectivity, at least through the geographic region containing remaining populations. Jaeger et al. (2001, page 349) states, “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 the relict leopard frog.” Further genetic evaluations using higher-resolution techniques may be useful for providing a genetic basis for developing conservation strategies.

F. Putative Causes for Past Population Declines

The causes for the population declines of this species are not entirely clear, but several factors have been implicated for declines of other amphibians in the West and suggested factors for the relict leopard frog include alteration of aquatic habitat due to agriculture and water development, and the introduction of exotic predators and competitors (Jennings 1988, Jennings and Hayes 1994). The formation of Lake Mead in 1935, and Lake Mohave in 1951, inundated scores of river miles and adjacent associated scattered wetlands. Moreover, substantial areas of wetland habitat have been converted to agriculture or urban development near the Virgin and Muddy rivers in Utah, Arizona, and Nevada. Exotic species, which are often implicated as serious predators and/or competitors of native ranid frogs in the western US, have become widely distributed along the Virgin, Muddy, and Colorado rivers. Included among these are the American bullfrog (*R. catesbeiana*), many fishes, and red swamp crayfish (*Procambarus clarkii*; Jennings and Hayes 1994). Many disturbance regimes have been reduced or eliminated allowing vegetation to fill wetlands and succession to continue toward a climax stage. In many cases, the lack of disturbance exacerbates the invasion of exotic flora. Exotic plants such as tamarisk invade and out compete native vegetation, many times changing heterogeneous vegetation communities to monocultures.

G. Threats and Conservation Needs of The Relict Leopard Frog

The success of any conservation or recovery program depends on reducing or eliminating the threats to the species' existence. The following threats are arranged under headings based on the 5 listing factors used by the USFWS under Section 4(a)(1) of the ESA. For each of these factors, specific activities potentially threatening the persistence of relict leopard frog populations are described.

Wherever feasible, the involved parties in the CAS will implement actions to reduce or eliminate current threats. Following each factor, the primary needs for short-term conservation and long-term maintenance of viable populations and functional processes of the relict leopard frog have been identified based on the existing information. As a better understanding of threats to the relict leopard frog and its conservation needs are developed, the management strategy for the frog will be revised through an adaptive management process. Specific, priority conservation actions and timelines to accomplish these actions are listed in the Management Program and Implementation Schedule sections.

<u>Factor 1. Habitat Degradation</u>
Water Diversions, Spring Development, and Water Development

Water diversions and groundwater development may be a continuing threat to relict leopard frog conservation where historical populations have been extirpated or their habitats altered due to diversion of water from streams or wetlands for activities associated with livestock grazing, agriculture, urban development, and other uses. Because of legal appropriations under Arizona, California, Nevada, and Utah water laws and land use practices on public, private, and tribal lands, water diversions continue to occur and may be problematic for relict leopard frog conservation and management of occupied or historical leopard frog habitats.

Extant populations are restricted to perennial desert springs along the Virgin and Colorado river drainages. Substantial leopard frog habitat in the historical range of the relict leopard frog has been destroyed or modified by activities such as spring capping and diversions and the construction of dams and reservoirs. Modifications have not only changed the amount and quality of habitat available for relict leopard frogs, but may also have created habitat for and promoted introduction of nonnative predators (Sredl et al. 1997). In addition to local spring and stream modifications, aquifer overdrafting in areas that affect relict leopard frog habitat may be a significant threat, because these aquifers may be limited in their ability to recharge. Possible explanations of the origin of these springs and impacts that might occur from future groundwater withdrawal are considered below.

Colorado River – The Colorado River system serves as a source of water divided among 7 states for irrigation and domestic uses as well as for recreational activities, hydroelectric power, and environmental benefits. Most of the total flow into the Colorado River basin is a result of natural runoff from mountain snowmelt (USBR 2002). The Colorado River has undergone decades of alterations that have affected its ecosystems. These ecosystem alterations may have historically affected relict leopard frog populations through fragmentation of habitats and movement pathways, however, the extent of impacts are unknown. The closure of Hoover Dam potentially created a barrier in gene flow between the Northshore population and the Black Canyon population of frogs as well as inundated existing habitat for the frogs. Coldwater releases from Hoover Dam could potentially impact dispersal activities among the frogs, however, relict leopard frogs have been found downstream of Black Canyon at the Willow Beach National Fish Hatchery. These coldwater releases may have created a barrier against invasion by bullfrogs at Black Canyon which is beneficial to those populations of relict leopard frogs.

Littlefield (including Reber Springs) – The headwaters of the Virgin River originate in the Cedar and Pine Valley mountains in Utah (ADWR 1993). Annual runoff of the lower Virgin River consists of many sources including upper basin discharge, Littlefield Springs inflow, Beaver Dam Wash discharge, and surface runoff from flood events (Metcalf 1995). The origin of the Littlefield Springs has been described by Hardman and Miller (1934), Bagley et al. (1955), Trudeau, (1979) and recently by Metcalf (1995). Both Trudeau (1979) and Metcalf (1995) conclude that a portion of the flow from Littlefield Springs is from Virgin River water, however, they each identified different sources for the remainder of the spring output. Trudeau used major ion chemistry and tritium concentrations to describe the source of the Littlefield Springs as local recharge and influent river water. Metcalf used stable isotope data to rule out the local recharge component as being a potential source for the Littlefield Springs. She concluded that groundwater was more likely a factor in contributing to the recharge of the Littlefield Springs. The Southern Nevada Water Authority (SNWA) combined the tritium and stable isotope data of these 2 studies and concluded that the source of the Littlefield Springs was a mixture of Virgin River water that has seeped into the groundwater system and a deeper groundwater component (Cole and Katzer 2000).

Water discharge at Reber Springs measured 270 L/min from 9 outlets at the site and temperature was 23°C (Bradford et al. 2004). Conductivity was 3600 µS/cm. The average monthly streamflow data from 1930 - 2000 for the Virgin River, near Littlefield, Arizona, ranges from 51.4 - 201 L/min (109 - 425 ft^3/s) (USGS 2001). Historical surface water flows recorded near the town of Littlefield have ranged from 11,224,684 m^3/y in 1989 to a high of over 621,674,827 m^3/y in 1983 (ADWR 1993).

The Littlefield area was historically rural, but is becoming more urbanized. Current estimates project a human population in the Arizona Strip communities associated with the Virgin River, including Littlefield, Beaver Dam, Scenic and Arvada, of up to 25,000 by the year 2020 (Mohave County Board of Supervisors 1996). Previous development in this area has mostly been agricultural, however, historical flooding, the remoteness of the area, and the increasing lack of agricultural fields with the potential for development have all combined to limit the future for agriculture in the area (ADWR 1993). With agricultural activity in the Littlefield area restricted, future growth will most likely consist of residential, commercial, and tourism interests, including golf courses. The largest increase in water use in the Littlefield area will occur as a result of construction and irrigation of golf courses (ADWR 1993).

Northshore Springs – The most recent and thorough investigation of the Northshore Springs, including Rogers and Blue Point springs, was by Pohlmann et al. (1998) who described these springs as "subregional" and related to the Rogers Spring Fault. This fault acts as a conduit allowing upward flow from deep carbonate aquifers. "Subregional" implies that the source of water is dominated by groundwater originating outside the local topographic basin with a component of local (within the same basin) recharge. The chemical and isotopic characteristics of the Rogers and Blue Point springs water indicates that a majority of the water from the springs is carbonate aquifer water (Pohlmann et al. 1998). The origin of this carbonate aquifer water is uncertain. Higher uranium activity ratios in this water indicates longer residence times, and that the aquifer was likely charged during the wetter climate of the Pleistocene.

Because of similarities between the geologic setting west of Northshore and Moapa Valley, early researchers believed that Rogers and Blue Point springs were a terminus of the White River flow system (Pohlmann et al. 1998), a regional deep carbonate aquifer system which originates in northern Nevada and terminates at Muddy Springs. Recently, using combinations of chemical and isotopic information, various workers have proposed a wide geographic range of the possible places of origin of the carbonate aquifer water in Rogers and Blue Point springs including Virgin Valley (Prudic et al. 1993), Mormon Mountains (Pohlmann et al. 1998) and California Wash or Lower Moapa valleys (Thomas et al. 2001). Rogers and Blue Point springs are part of Lake Mead Area Flow System (Thomas et al. 2001). Waters in this flow system are a combination of

White River and Meadow Valley flow system water and local recharge from Hidden, Garnet, and California Wash, Lower Moapa Valley, and the Black Mountains area.

The average monthly streamflow data for Rogers Spring for water years 1985-2000 varies from 0.75 - 0.8 L/min and the average monthly streamflow date for Blue Point Springs for water years 1998-2000 varies from 0.23 - 0.3 L/min (USGS 2001). This variable spring discharge illustrates the local recharge component of the "subregional" classification of these springs. This means that some undefined portion of the spring discharge is from local recharge and some undefined portion is from the regional aquifer. Therefore, impacts resulting from aquifer development and/or drought are indistinguishable at present.

It is difficult to evaluate the impacts that groundwater development might have on these springs because of the uncertainty regarding both the regional component and the origin of the carbonate aquifer flow. Development in the Black Mountains area, Hidden, Garnet, and California Wash valleys had been limited until 1990 when various commercial enterprises were granted groundwater withdrawal permits (LVVWD 2001). Development in the California Wash Valley or the Lower Moapa Valley is more likely to impact Rogers and Blue Point springs because of the proximity of potential development to the springs. Groundwater development in Coyote Spring Valley and the Muddy River Springs area is currently being pursued. In an agreement between the Department of Interior, Las Vegas Valley Water District, and the SNWA, a monitoring plan has been developed to monitor impacts to the springs in the Muddy River area that might occur from groundwater pumping. This monitoring may help to anticipate future potential impacts to Rogers and Blue Point springs due to the regional component of their flow.

Black Canyon Springs – Black Canyon is located south of Hoover Dam along the Colorado River. Three springs in Black Canyon have extant populations of relict leopard frogs. Pohlmann et al. (1998) classified 3 springs to be of a local origin (2 unnamed springs and Latos Pool) and 4 additional springs (1 unnamed spring, Nevada Falls, Bighorn Sheep Spring, and Arizona Seep) to be of "subregional" origin. The local recharge component of these springs is believed to originate from the Black and Eldorado mountains and the subregional groundwater source is from Eldorado Valley. However, the subregional source is somewhat uncertain due to limited sampling of the area.

The potential for further groundwater development in Eldorado Valley is limited and, therefore, the potential to impact the springs from groundwater pumping is limited as well. Eldorado Valley is a closed basin (J. Guillory pers. comm.). This means that, except for potential temporary mining permits, the State Engineer will not issue any additional permits in that basin. Besides being a closed basin, water quality is another factor that hinders potential groundwater development of the basin. Eldorado Valley water quality is poor due to high salinities, thus making it an undesirable water source for human use, at least into the near future. As mentioned above, if Eldorado Valley is the subregional source of groundwater in Black Canyon, as it is believed to be, then the elevational gradient between the water table in Eldorado Valley and the springs in Black Canyon is such that a significant reduction in the water table would be required to impact the spring flows. If this valley is not the subregional component, then groundwater development will have no impact on the springs in Black Canyon and thus not impact the extant relict leopard frog population (J. Johnson pers. comm.).

Changes in Plant Communities

The relevance of habitat heterogeneity to frogs within the aquatic and terrestrial environment is unknown but likely important. Shallow water with emergent and perimeter vegetation provides basking and foraging habitat, and deep water provides refuge from predators and potential hibernacula (AGFD unpublished data, Jennings 1987, Jennings and Hayes 1994, Platz 1988). Leopard frogs have poor visual acuity and require both light and dark backgrounds (i.e. covered and open sky) for escape and foraging (Ingle 1973, Ingle and Hoff 1990, King and Comer 1996). Because of this need for dark and light, edge habitat and a diversity of habitat may be crucial for these 2 functions. Thick patches of vegetation are important for cover and possibly prey production. At Blue Point and Rogers springs, relict leopard frogs make extensive use of thick *Eleocharis* clumps (S. Romin pers. obs.). However, uniformly dense vegetation growth and the resultant disappearance of varied habitat structure is a significant threat to relict leopard frog populations.

Unchecked by disturbance, both native and nonnative species quickly form dense stands, eliminating open habitat and short, "understory" plant species used by relict leopard frogs. Native species of particular concern are cattails, common reedgrass, and sawgrass (*Cladium* spp.), which form tall, dense stands almost immediately upon colonizing an area, spread rapidly, and are resistant to disturbance. Cattail stalks trap large volumes of sediment in some systems (e.g. Sugarloaf Spring), further reducing pool and channel size. *Scirpus* is implicated in the reduction of habitat at Littlefield (Bradford et al. 2004). In addition to forming dense stands, aggressive nonnative species such as tamarisk and tall whitetop (perennial pepperweed *Lepidium latifolium*) can irreversibly alter plant and insect communities, soil chemistry, and disturbance regimes.

Tall whitetop has not been found in springs occupied by the relict leopard frog, but is present in the current range of the relict leopard frog and could become a threat. Tall whitetop is established and occurs sporadically in Las Vegas Wash. It is closely monitored and removed when found in the Lake Mead National Recreation Area (NRA) portion of the wash (E. Powell pers. comm.). Tall whitetop is not found elsewhere in Lake Mead NRA.

Tamarisk is prevalent along the Virgin River and the shorelines of lakes Mead and Mohave, as well as in almost every untreated spring. Tamarisk has overgrown the type locality of the relict leopard frog, changing geomorphology, soil chemistry, and available habitat. The majority of tamarisk has been removed from Northshore springs occupied by relict leopard frogs. These treatments will need to be maintained, but tamarisk is not a threat in the short term at these sites. Occupied Black Canyon springs contain substantial amounts of tamarisk. In the Black Canyon springs, tamarisk roots substantially reduce pool size by growing directly in the water and trapping sediment. Tamarisk also greatly reduces the amount of light available to forbs, which provide cover for relict leopard frogs, and each autumn fallen needles entirely cover pools. While relict leopard frogs seem to be thriving in Bighorn Sheep Spring, further encroachment and conversion to a tamarisk monoculture would likely be detrimental. Current tamarisk management maintains open areas by pulling up sprouts and trimming branches.

Livestock Grazing

Effects of livestock grazing on relict leopard frog populations may be positive and negative (Jennings 1988, Rosen and Schwalbe 1998, Sredl and Saylor 1998). While grazing has been attributed to maintaining open areas in spring systems, high levels of grazing can negatively impact amphibian habitat by removing bankside cover, increasing ambient ground and water temperatures, destroying bank structure (e.g. eliminating undercut banks), trampling egg masses, and adding high levels of organic wastes (Jennings 1988). Overgrazing may also degrade amphibian habitat by increasing runoff and sedimentation rates (Belsky and Blumenthal 1997, Jennings 1988).

Relict leopard frog populations in the Northshore area may be affected by wild burros. Burros seasonally use water sources inhabited by frogs. During those periods, they intensively graze the shallow edges of pools. This use can result in the loss of vegetative cover in riparian and emergent zones, decreased water quality, direct impacts to habitat quality at spring outflow streams and along watercourses, and accelerated drying and loss of pool habitats during spring and summer months. Direct mortality of all life stages of frogs due to trampling may also occur and is of particular concern during key periods of life history such as during breeding, oviposition and development, and emergence of metamorphs. While burro activity may sometimes benefit leopard frogs by decreasing the density of vegetation, many species of emergent vegetation colonize deeper water where they are unaffected by burro grazing, and spread toward the shallow edges, filling the pool. At a large, deep pool at Blue Point Spring previously favored by relict leopard frogs, high levels of burro activity did not stop it from becoming densely overgrown (S. Romin pers comm.).

Erosion and Scouring

Erosion and scouring appear to have both negative and positive consequences for relict leopard frogs. Northshore springs flow through soft, gypsum-based soils that are prone to erosion. As water downcuts, blocks of destabilized soil fall into the stream course, blocking flows. Small course shifts, due to collapse and subsequent re-routing, at times benefit the relict leopard frog by creating larger pools and new, open habitat. Of greater concern is gypsum lens dissolution, which causes stream sections to suddenly shift underground, resulting in rapid dewatering of large areas of prime habitat.

Black Canyon springs, in narrow, high gradient drainages, are subject to occasional scouring, as evidenced by boulders up to 2 m in diameter that rest in the narrow canyons. Smaller flash flood events shifts gravel several times per year. While gravel shifts open habitat by burying vegetation, gravel also often substantially fills pools. Adult lowland leopard frogs are adept at escaping many flash floods, but larger floods may wash away entire cohorts of tadpoles (M. Sredl pers. comm.). In March 2000, vegetation debris from flooding was evident at Big Horn Sheep Spring, and the tadpole population was approximately 10% of what it had been 3 weeks prior to the flooding event (NPS, NDOW unpublished data).

Recreational Impacts

Recreational access to springs and streams is the proximate cause of a number of threats to the relict leopard frog. Recreational users deliberately introduce many nonnative species, including aquarium and sport fish, bullfrogs, turtles, snails, and alligators. At Rogers Spring, multi-colored aquarium gravel can often be found where unwanted pets have been freed. Feeding these exotic species is a favorite activity of many visitors at Rogers Spring. Recreational users may also, either through transfer of mud on their shoes or by releasing aquatic fauna, introduce or spread disease. At Rogers Spring, visitors build rock and sandbag dams to form pools, sometimes altering water flow to relict leopard frog habitat downstream. Other activities such as bathing, where soap or detergents are used, could degrade habitat quality.

Roads

Within Lake Mead NRA, a powerline access corridor runs through the Northshore area. While most public access is restricted by gates, graders conducting road maintenance at the Rogers Spring powerline crossing routinely push soil and debris into relict leopard frog habitat. The building of roads and their subsequent use and maintenance can alter the hydrology of an area by increasing runoff, erosion, and siltation (Moler 1992, Welsh and Ollivier 1998). In studies of other amphibians, vehicular traffic on roads is known to cause large numbers of direct frog mortalities (Ashley and Robinson 1996) even at very low traffic levels (Palis 1994) and can have negative impacts on population density that are correlated with traffic level (Fahrig et al. 1995). Those mortalities may affect the genetic structure of populations (Reh and Seitz 1990) and may decrease amphibian species richness in wetland areas up to 2 km from the road (Findlay and Houlahan 1997).

Air Pollution

Air pollution has been implicated as a cause of amphibian population declines in the US through acidification of water bodies from both point and non-point sources, and pesticide exposure. Anthropogenic acidification of water bodies from non-point sources, occurring downwind from large industrial and metropolitan centers, has adversely affected some pond breeding amphibians in the eastern US and Canada (Dunson et al. 1992). In the western US, however, the predominance of evidence indicates that this effect from non-point sources is not currently manifested, even in waters with low acid neutralizing capacity (Bradford et al. 1994, Vertucci and Corn 1996). In contrast, acidic deposition from a point source, a copper smelter, is thought to have been a factor in the extirpation of populations of the Tarahumara frog (*R. tarahumarae*) in southeastern Arizona (Hale et al. 1995). Recently, pesticides drifting many kilometers downwind from a major agricultural source, the Central Valley of California, have been implicated in population declines of 4 ranids in the Sierra Nevada (Davidson et al. 2001, Davidson et al. 2002, Sparling et al. 2001). Pesticides may also cause deformities and a variety of other sublethal effects in leopard frogs (Ouellet et al. 1997, Hayes et al. 2002).

Extant populations of the relict leopard frog do not occur downwind from large centers of industry, metropolitan development on the scale of the eastern US, agriculture, or smelters. Thus, it seems unlikely that air pollution is a major stressor on relict leopard frog populations. Agricultural pesticides used in the Muddy River valley and Virgin River drainage may impact relict leopard frogs in and near those areas, especially current Northshore populations that are near these agricultural areas. Within the Virgin River drainage, restrictions have been placed on the use of certain agricultural pesticides through the US EPA's Endangered Species Protection Program.

Agriculture

Groundwater pumping and surface diversion are 2 consequences of most forms of agriculture. These practices deplete the local aquifer and can lead to subsequent decreases in the amount of available aquatic habitat for amphibians. In addition, surface soil disturbance can degrade water quality (e.g. change local surface water salinity). However, certain changes in agricultural practices can actually increase biodiversity (Pimentel et al. 1992) and may be beneficial for some amphibians by creating moist foraging habitat (Hulse 1978, Rorabaugh et al. 2002). Historically, lowland leopard frogs inhabited agricultural areas in southeastern California (Jennings and Hayes 1994). Many chemicals used in agriculture and silviculture have negative effects on amphibians (Devillers and Exbrayat 1992, Herfenist et al. 1989, Sparling et al. 2000). Categories of contaminants that can affect amphibians are insecticides, herbicides, bactericides, piscicides, organics, heavy metals, and radioactive isotopes. Pesticides are not restricted to the area of application but can be transported to other, apparently pristine, areas by weather patterns (Davidson et al. 2001). Many of these contaminants can have sublethal effects on amphibians (e.g. skewed sex ratios) much below the application levels allowed in the US and can be concentrated in the food web (Devillers and Exbrayat 1992; Sparling et al. 2000; Hayes 2001; Hayes et al. 2002).

Agricultural pesticides used in the Muddy River Valley and Virgin River drainage may impact relict leopard frogs in and near those areas. Additionally, disruption of historical water regimes through construction of water impoundments and other water diversions may have impacted habitat available to relict leopard frogs. Within the Virgin River drainage, restrictions have been placed on the use of certain agricultural pesticides through the US EPA's Endangered Species Protection Program.

Fire

The effects of natural and uncontrolled human caused wildland fires, as well as human controlled burns, on native amphibian populations in the Southwest are poorly known and depend on local conditions (Abbott 1998). The few studies addressing fire and amphibians and reptiles have focused primarily on reptiles (Cunningham et al. 2000, Esque et al. 1998, Gamradt and Kats 1997, Greenberg et al. 1994, Holycross 1999, Russell et al. 1999). However, the effects of fire on fish and aquatic systems are well studied. Increased peak flows and sedimentation loads and ash flow following major precipitation events or during spring runoff after a hot burn are the primary threats to aquatic systems and species (DeBano et al. 1996). These events can move all life stages of amphibians and fish down drainage, destroy amphibian eggs, and decrease available habitat (DeBano and Neary 1996, Hendrickson and Minckley 1984, Stromberg and Ortiz-Zuazaga 1998). Smoke diffusion into water and ash flow can result in high levels of phosphorus and nitrogen (Spencer and Hauer 1991) that may be toxic to frogs. However, fire may benefit aquatic species in some systems by providing the disturbance of riparian vegetation necessary to keep succession from eliminating the aquatic system (Hobbs and Huenneke 1992).

Visitors sometimes cause fires at Blue Point and Rogers springs. Palm tree fires have occurred as a result of visitors launching fireworks into palm frond skirts. Although fires temporarily clear thick vegetation, it regrows quickly and possibly in densities greater than present prior to burning (NPS unpublished data).

Conservation needs for factor 1 is to protect and enhance occupied and nearby habitats - The relict leopard frog is highly dependent on spring and desert riparian systems. These habitats must have adequate water quantity and quality and vegetation cover at an appropriate early to intermediate successional stage. Although periodic grazing or fire may be useful to maintain appropriate vegetation communities, intense fire or grazing can result in decreased water quality and habitat suitability, increased trampling hazards and soil compaction, and accelerated seasonal drying. Excessive recreational use can alter vegetation cover characteristics and introduce nonnative species. Invasive nonnative plant species have substantially altered native riparian vegetation communities. Direct impacts to open water habitats include flow diversion and physical alteration of pools and channels, and dense vegetation growth, which limits habitat suitability and utility to all life stages of the frog. Protective or restorative efforts must be implemented at occupied and nearby sites to ensure persistence of existing frog populations, maintain connectivity between these populations, and to increase habitat availability and suitability. These goals should be accomplished through public education on the harm of nonnative species and degradation of relict leopard frog habitat, research to determine specific macro- and microhabitat needs of relict leopard frogs and how these needs can best be managed, and development of strategies to address the unavoidable degradation of relict leopard frog habitat. Conservation Actions 2 – 7, and 12 listed in the Stepdown Outline (pg. 55) apply to Factor 1.

Factor 2. Over-utilization

Collection of relict leopard frogs is currently limited to controlled, low-level sampling for scientific purposes as well as collection for use in population restoration efforts. The extent to which illegal collection occurs is unknown. It is a potential threat, especially at the Rogers and Blue Point springs sites on the Overton Arm. These sites receive a large number of visitors and are easily accessible to the public from Northshore Road. Arizona and Nevada regulate the collection of relict leopard frogs to those with a scientific collecting permit. Personal collection or possession of relict leopard frogs is prohibited under existing Nevada regulations for hobby possession of amphibians, and Arizona Commission Order 41 specifies a closed season for relict leopard frogs in that state. If relict leopard frogs exist in Utah, collection would be limited to scientific research. Existing regulations for Arizona and Nevada prohibit commercial collection or possession of relict leopard frogs for the amphibian and reptile pet trade (Appendix 2). In addition to existing State regulations, collection or take of relict leopard frogs for scientific research from extant populations on NPS-administered lands requires additional review and permitting by Lake Mead NRA. NPS regulations prohibit commercial harvest, and personal collection consistent with State statutes and regulations.

Conservation needs for factor 2 is to prevent illegal collection or use of relict leopard frogs - Arizona, Nevada, and Utah all regulate the collection or use of relict leopard frogs, and the Lacey Act provides additional protection at the Federal level by prohibiting interstate transport of animals collected in violation of State laws. These regulations prevent the collection of these frogs except to those with a scientific collecting permit. Illegal collection is a potential threat, but the extent to which it occurs is unknown. State and Federal regulations should be enforced and the public made aware of these regulations. Public outreach at easily accessible sites should be implemented to educate the public on the conservation efforts aimed at relict leopard frogs. Conservation Actions 2 and 5 listed in the Stepdown Outline apply to Factor 2.

Factor 3. Disease, Predation, Competition, and Hybridization

Parasites and Other Pathogens

Little is known of parasites of relict leopard frogs. Goldberg et al. (1998) examined parasites of lowland and Chiricahua leopard frogs and American bullfrogs collected in Arizona. They found lowland leopard frogs to be infected with 5 species of trematode (*Cephalogonimus brevicirrus*, *Glypthelmins quieta*, *Haematoloechus complexus*, *Megalodiscus temperatus*, and 1 unidentified species) and 4 species of nematode (*Falcaustra catesbeiana*, *Rhabdia ranae*, *Physaloptera* sp., and 1 unidentified species). None of the helminths identified from the 2 native species were found in the American bullfrog.

Like parasites, little is known of pathogens of relict leopard frogs. Two important pathogens have been the focus of recent research: chytrid fungus and viruses. In 1998, a chytrid fungus (*Batrachochytrium dendrobatidis*) was implicated in declines of amphibians in Australia and

Panama (Berger et al. 1998). That same year, it was first identified in Arizona (Milius 1998). Seven species of ranid frogs, Rio Grande leopard frogs (*R. berlandieri*), plains leopard frogs, American bullfrogs, Chiricahua leopard frogs, Ramsey Canyon leopard frogs (*R. subaquavocalis*), Tarahumara frogs, and lowland leopard frogs, have been affected by chytrid fungus in Arizona (Sredl et al. 2002). Salamanders, toads (including *B. punctatus*), and treefrogs have also been affected in Arizona (Davidson et al. 2000, Sredl et al. 2000, Sredl et al. 2002, Bradley et al. 2002). Chytrid fungus has been identified in 1 Columbia spotted frog (*R. luteiventris*) population and 1 boreal toad (*B. boreas boreas*) population in Utah (K. Wilson pers. comm.). Catastrophic die-offs, indicative of chytrid fungus have not been observed in these populations. Chytrid fungus has not been identified from amphibians in Nevada (J. Sjoberg pers. comm.).

Relict leopard frog tadpoles from the Bighorn Sheep Spring population were tested for chytrid fungus in 2001 by the National Wildlife Health Center and found to be chytrid negative (Green 2001). In 2003, frogs from this same location were tested for the fungus by Pisces Molecular using PCR assays, and results were again negative (R. Haley, pers. comm.). While chytrid fungus has not been confirmed as a pathogen of relict leopard frogs, there is no reason to think that the species would be immune to this pathogen. Chytrid fungus may be easily spread by transport among sites of water or wet equipment containing zoospores (Longcore et al. 1999).

Iridovirus has also been identified as a pathogen of ranids in western North America. An iridovirus, Frog Virus 3, affects the mountain yellow-legged frog (*R. muscosa*) and red-legged frog (*R. aurora*) in California, along with many other amphibians worldwide (Carey et al. in press, Green 2001). Iridovirus has also affected tiger salamanders (*Ambystoma tigrinum*) in Arizona and Canada (Jancovich et al. 1997, Carey et al. in press).

Crayfish

Native crayfish species are not known from the current or historical range of the relict leopard frog, but Louisiana red swamp crayfish has been introduced into the Colorado River drainage as bait and for sport fish forage, occurring commonly in lakes Mead and Mohave. The Louisiana red swamp crayfish inhabits Salt Cedar Spring (likely colonized from the Colorado River). Crayfish may colonize other relict leopard frog habitat if introduced, particularly in flowing water habitats such as the Rogers and Blue Point springs outflow systems. In 1970, the virile crayfish (*Orconectes virilis*) was introduced into the Sand Cove reservoirs of the upper Santa Clara River within the Virgin River drainage in Utah, possibly as forage for sportfish (Johnson 1986). Subsequently, *O. virilis* has spread into historical Virgin River habitat of the relict leopard frog.

Omnivorous crayfish directly affect native aquatic species by competing with aquatic herbivores and preying upon aquatic invertebrates and vertebrates, including leopard frogs (Creed 1994, Fernandez and Rosen 1996). Crayfish affect native aquatic species by removing vegetative matter and disrupting normal nutrient cycling in the aquatic habitat, and decreasing aquatic macroinvertebrate diversity (Fernandez and Rosen 1996, Childs 1999). Their presence would be expected to significantly impact the viability of isolated relict leopard frog populations. The Louisiana red swamp crayfish inhabits historical sites where relict leopard frogs have been extirpated and its presence may have been a factor in loss of those populations (Bradford et al. in press).

Fishes

Two of the occupied sites in the historical range of the relict leopard frog are dominated by a variety of introduced nonnative fish species, including mosquito fish (*Gambusia affinis*), tilapia (*Tilapia mossambica*), a suite of tropical aquarium fishes such as cichlids (*Cichlasoma* spp.) and mollies (*Poecilia* spp.). Areas of the Muddy and Virgin rivers within the historic range of the relict leopard frog are dominated by introduced, invasive fish species including blue tilapia (*Tilapia aurea*), red shiner (*Cyprinella lutrensis*) and mollies. Large, perennial aquatic habitats in the current range of the relict leopard frog are dominated by sportfish including members of the Centrarchidae (bass, sunfish), Ictaluridae (catfish), and Salmonidae (trout) (Deacon et al. 1964, Fuller et al. 1999, Minckley 1973, NDOW unpublished data, NPS unpublished data). Many of these nonnative fishes represent a threat to relict leopard frogs, particularly for early life stages, through competition and direct predation. Although specific threats from some of the smaller aquarium fish species are not fully understood, predation on anuran larvae by *Gambusia* spp. has been well documented (McDiarmid and Altig 2000). Dietary studies of closely related *Tilapia* species in headwater spring outflows of the adjacent Muddy River system have shown them to be aggressive, opportunistic predators on a variety of aquatic species (G. Scoppettone pers. comm.).

Trout prey on native fish and amphibians and have been linked to declines of amphibians in the western US, especially in historically fishless waters (Bradford et al. 1993, Hayes and Jennings 1986, Knapp and Matthews 2000, Pilliod and Peterson 2001, Robinson et al. 2000). These nonnative fish also compete with native fish and likely do so with amphibian larvae (Dudley and Matter 1996, Rinne 1991). While trout do occur in the Colorado River reservoirs and headwater areas of the Virgin River basin, the ecological impact of these predators on the relict leopard frog is not well documented. No areas where stocked salmonids and the relict leopard frog co-occur have been documented

Bullfrogs

Bullfrogs have been introduced throughout western North America (Conant and Collins 1991, Wright and Wright 1949). Bullfrogs are widely distributed within the current and historical range of the relict leopard frog and have been documented in occupied habitats at Littlefield, Arizona, and at historical sites where the leopard frog has been extirpated (AGFD unpublished data, Bradford et al. in press). Bullfrogs were intentionally introduced to the western US by State and Federal agencies for sport and continue to immigrate to new areas and are introduced unintentionally during sport fish introductions and by private individuals (Hayes and Jennings

1986, Sredl and Wallace 2000). Although native western ranids co-exist with bullfrogs at some sites, bullfrogs have been implicated in the decline of many ranids in the southwestern US and western North America (Adams 1999, Casper and Hendricks in press, Hayes and Jennings 1986, Moyle 1973, Rosen and Schwalbe 1995, Rosen and Schwalbe 2001). Bullfrogs compete for many of the same resources (e.g. food, breeding sites, and cover sites) and prey upon native leopard frogs. Stomach analyses of Arizona bullfrogs indicate they consume a variety of prey, including many species of aquatic and terrestrial invertebrates and vertebrates (e.g. bats, rodents, birds, snakes, turtles, frogs, and fish) (Clarkson and deVos 1986, Rosen and Schwalbe 1996). Because of their large size and aggressive behavior, bullfrogs are potential predators on all life stages of the relict leopard frog, including adults (Duellman and Trueb 1994).
Invasion of relict leopard frog habitat by bullfrogs is a significant threat. The cold water and strong current of the Colorado River coupled with the topography may keep bullfrogs from colonizing the Black Canyon sites. These sites are downstream from Hoover Dam, which forms a significant upstream barrier. They are in a steep, rocky canyon, surrounded by desert, which serves to isolate them from frog populations to the east or west, and the current, cold water and abundance of predatory fish should provide an effective barrier to colonization from downstream. Bullfrogs are present at both the northern (Overton Wildlife Management Area) and southern (Las Vegas Bay and Wash) ends of Lake Mead. These source populations of bullfrogs coupled with high recreational use of Rogers and Blue Point springs leaves these springs vulnerable to colonization by bullfrogs.

Other Frogs

The introduction of other frog species is also a potential threat to relict leopard frog populations. Hybridization with closely related, introduced species would reduce the likelihood that the unique relict leopard frog genome is passed on to subsequent generations. Although clawed frogs (*Xenopus* sp.) cannot be lawfully possessed in Arizona, Nevada, or Utah without a special permit, they are widely used in biomedical research and are available from many biomedical suppliers. The African clawed frog (*X. laevis*) is established in southern California and near Tucson, Arizona, and could become established in slow moving, perennial waters in the historical range of the relict leopard frog (Stebbins 1985). The African clawed frog is primarily insectivorous, but will consume larger prey (including other anurans) when body size allows (Measey 1998). Introduced Rio Grande leopard frogs have invaded portions of southwestern Arizona and southeastern California. This large leopard frog could prey upon or compete with the relict leopard frog, but has not yet been found on the Colorado River upstream of Laguna Dam (Rorabaugh et al. 2002). Common leopard frog species (e.g. northern leopard frog) can be obtained from pet shops in Arizona and Nevada. A variety of amphibian species can be obtained through mail order or internet suppliers despite legal protections. The unauthorized release of captive amphibians is prohibited by State and Federal laws.

Turtles

The spiny softshell turtle (*Apalone spiniferus*) occurs in littoral zones of Lake Mead within the Overton Arm and in the impoundment at Rogers Spring, adjacent to occupied leopard frog habitats. This softshell turtle is an aggressive predator on other aquatic vertebrates and predation on larval and adult relict leopard frogs would be likely if species interaction were to occur, but specific instances of predation by softshell turtles are not known.

Mollusks

New Zealand mudsnails (*Potamopyrgus antipodarum*) and zebra mussels (*Dreissena polymorpha*) have not yet been found within the historical range of the relict leopard frog, but introduction is a very plausible threat to lakes Mead and Mohave. New Zealand mudsnails are established at Lee's Ferry of the Colorado River and have been collected from as far downstream as Diamond Creek in the lower Grand Canyon (Montana State University, 2004). Both of these organisms colonize rapidly and gain extremely high densities of adults and larvae (USGS 2000, USGS 2002). Such high densities of these animals impact native aquatic invertebrates and vertebrates. Studies in Yellowstone National Park in Wyoming indicate New Zealand mudsnails reduce food availability to aquatic insects and larvae, reducing invertebrate species diversity and abundance (NPS Yellowstone; unpublished data).

Conservation needs for factor 3 is to selectively control detrimental nonnative aquatic species, and identify and control the spread of disease - Nonnative aquatic species, which currently negatively impact the relict leopard frog through competition and predation, include crayfish, nonnative fish, and bullfrogs. Direct control and elimination strategies, and where feasible, actions to reduce habitat suitability for invasive nonnative species, must be implemented at specific sites where relict leopard frogs co-occur with nonnative species. Diseases are a threat to the relict leopard frog. Diseases, such as chytrid fungus, can potentially cause massive die-offs and reduced survivorship, recruitment, and fecundity. Protocols will be adopted to prevent the introduction of chytrid and other pathogens to relict leopard frog populations and amphibians in nearby habitats, and adherence to those protocols will be required during performance of conservation activities and a mandatory condition of scientific collection and other permits issued by signatory agencies. A protocol will also be developed to address disease outbreaks in extant populations if they occur. Conservation Actions 2 - 6 listed in the Stepdown Outline apply to Factor 3.

<u>Factor 4. Inadequate Regulatory Mechanisms</u>

Arizona, Nevada, and Utah all limit the collection, study, or use of relict leopard frogs to those with a scientific collecting permit, and each state has regulations limiting or prohibiting the anthropogenic dispersal of threats, such as nonnative organisms, to the frog (Appendix 2). However, these regulations have not completely prevented illegal nonnative species introductions at some locations, such as various species of fishes at Rogers and Blue Point springs. Relict leopard frogs and their habitat are protected by Federal regulations. The relict leopard frog is a covered species under the Clark County (Nevada) Multi-Species Habitat Conservation Plan and the Lower Colorado River Multi-Species Conservation Program.

Conservation needs for factor 4 is to prevent detrimental modifications and degradation of relict leopard frog habitat - NEPA and CFR 36 provide protection for the habitat of relict leopard frogs (Appendix 2). Direct anthropogenic degradation of relict leopard frog habitat is known to occur at sites with easy public access such as Rogers and Blue Point springs. Public education (brochures and other interpretive materials) should be placed where the public is likely to encounter relict leopard frogs, and in other locations where the public has access to educational materials such as visitor centers and interpretive sites. Public outreach efforts (interpretive talks at the Lake Mead Visitor Center) should be implemented. Public closures of areas where introductions are likely to occur should be implemented if introductions of detrimental nonnatives and habitat destruction or modification continue to occur. Conservation Actions 2, 5, and 7 listed in the Stepdown Outline, as well as Appendicies 1 and 2, apply to Factor 4.

Factor 5. Other Factors

Small Population Size, Limited Habitat, and Fragmentation of Populations

Severe fragmentation and alteration of aquatic habitats in the southwestern US has likely constricted many wide ranging aquatic species into isolated pockets, and maintenance of aquatic corridors may be critical to preserving organisms in the arid Southwest (Jennings and Scott 1991). Sredl and Howland (1995) speculated that distribution of extant leopard frog populations in Arizona may reflect habitat fragmentation and extinction without recolonization, as well as habitat quality. Locality data indicate that extant relict leopard frog populations occur as small clusters (i.e. populations in the Northshore and Black Canyon areas), rather than randomly distributed populations (Bradford et al. in press). All extant populations occupy springs in the Colorado River watershed surrounding Lake Mead. The physical characteristics of the river have changed drastically. The most prominent reason for these changes is the construction of dams including the Hoover Dam in 1935. Cowles and Bogert (1936) documented relict leopard frogs at a site now under the normal water line of Lake Mead, and extant population clusters could be remnants of former metapopulations that had a large core population of frogs on or near the Colorado River. An important and ongoing reason for loss of habitat is development in or near potential riparian habitat for relict leopard frogs. For example, the Reber Springs area could be subject to residential or commercial development (J. Jaeger pers. comm.).

A likely contributing factor to leopard frog declines in the Southwest is habitat reduction and fragmentation. These disturbances disrupt metapopulation dynamics and result in small, isolated, unstable local populations (Sredl and Howland 1995). Stochastic events such as drought, flood, and fire can cause the extirpation of small populations. In addition, natural fluctuations in frog population size and recruitment can lead small populations to extirpation (Skelly et al. 1999, Sartorius and Rosen 2000). Relict leopard frog populations are currently restricted to naturally small, isolated desert spring habitats within the millions of acres that comprise the historical range of the species. The number of springs likely suitable to sustain populations of relict leopard frogs is further limited by lack of perennial surface water, deep pools, adequate cover, nonnative predators, and other habitat characteristics. Many spring courses are extremely short, and likely could not support self-sustaining populations. Damming and diverting of water have fragmented formerly contiguous aquatic habitats. This fragmentation has occurred at a variety of scales from small springs to the mainstem of the Colorado River. In many areas, fragmentation has been accentuated by nonnative predatory fishes, crayfish, and bullfrogs, leaving potential dispersal corridors between available aquatic habitats disrupted or impassable.

For leopard frogs in the Southwest, rates of reproduction, recruitment, and other population attributes are highly variable and dependent upon rainfall and other environmental influences. The life history of relict leopard frogs suggests that they, too, have highly variable population dynamics. In addition, relict leopard frogs are highly aquatic and vulnerable to desiccation, thus limited in dispersal ability. Because of the size of its current range and limited dispersal corridors, factors affecting small populations and metapopulation dynamics figure prominently into conserving the relict leopard frog.

Conservation needs for factor 5 is to develop distribution and life history information and to establish populations in new areas to alleviate small population size, limited habitat, and fragmentation of populations. Available species status and survey information of currently occupied and historical habitats is sporadic although reasonably comprehensive. Efforts since the identification of relict leopard frogs near Lake Mead in 1991 have increased knowledge of characteristics of specific occupied and historical sites, and short-term demographics. In addition, some life history information on the relict leopard frog is available from historical sources. Additional information on habitat use and needs, behavior, long-term demographics, longevity, migration and movement patterns, and the interrelationship of occupied sites is needed. This information is essential to develop a comprehensive management strategy and conservation plan, particularly accurately identifying important habitat features for protection, developing captive rearing programs, assessing potential sites for reintroduction, and reintroduction of relict leopard frogs to the wild. Additional monitoring data are necessary to develop a baseline assessment of species status and population trends. These data will provide an understanding of current conditions and allow measurement of the efficacy of implemented conservation actions. Conservation Actions 9, 11, and 12 listed in the Stepdown Outline apply to Factor 5.

MANAGEMENT PROGRAM

OVERALL GOAL

Ensure the persistence of relict leopard frog populations and groups of populations (Table 2) in a diversity of habitats and localities that reflect the Potential Management Zone (PMZ) (Figure 1) for the species for the duration of this agreement.

MANAGEMENT AND CONSERVATION OBJECTIVES

1. Remove or substantially minimize threats to extant populations and occupied habitats.
2. Enhance existing habitat and/or create new habitats where feasible.
3. Establish additional populations of relict leopard frogs in existing or created habitats.
4. Manage relict leopard frogs and their habitats to ensure persistence in diverse aquatic ecosystems, and facilitate processes that promote self-sustaining populations.
5. Monitor relict leopard frog populations.
6. Investigate the conservation biology of the relict leopard frog, and use the results of such investigations to better meet the overall conservation goal and objectives.

SUCCESS CRITERIA FOR MANAGEMENT AND CONSERVATION OBJECTIVES

1. <u>Remove or substantially minimize threats to extant populations and occupied habitats.</u>

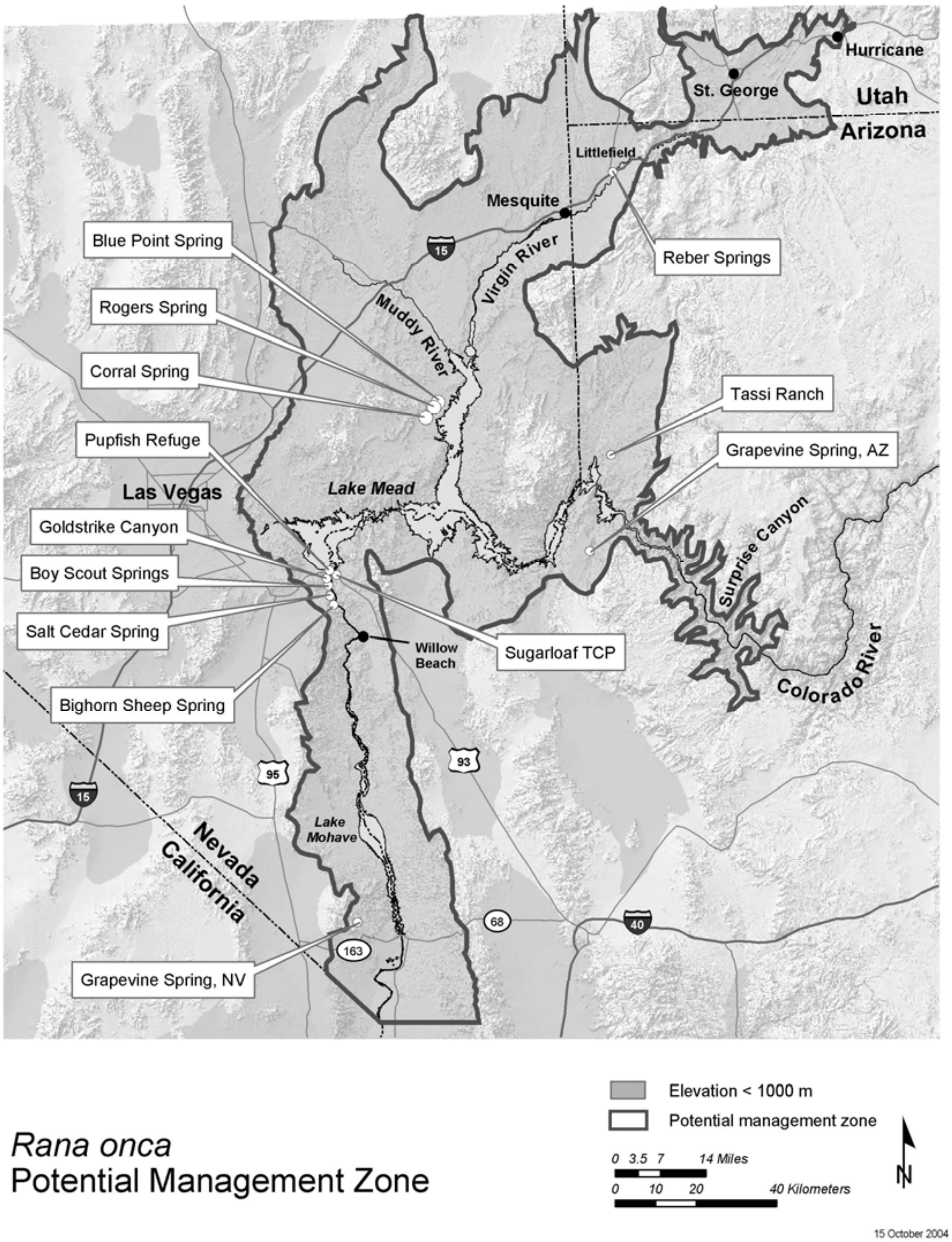
    Benefit: Ensure persistence of relict leopard frog populations and habitat across the Potential Management Zone for the duration of the agreement.

    Success Standard: Adequate water quantity and quality is maintained at all current and reestablished relict leopard frog sites. Detrimental nonnative species are eliminated or controlled, and steps are taken to minimize the likelihood of future introductions or immigration of these species. Novel diseases are not introduced to occupied spring systems. Vegetation is managed to maintain favorable habitat. State and Federal regulations pertaining to the relict leopard frog are enforced and the public made aware of these regulations.

Table 2. Current status, land ownership, potential threats, and proposed actions at *Rana onca* sites.

Current *Rana onca* Sites	Status	Land Owner	Potential threats	Action (listed from Stepdown Outline)
Bighorn Sheep Spring, NV	Natural	NPS	Disease; loss of water	6; 7
Blue Point Spring, NV	Natural	NPS	Vegetation encroachment; nonnative fish; recreation; disease; loss of water	3; 4; 5; 6; 7
Boy Scout Canyon Spring, NV	Natural	NPS	Recreation; disease; loss of water	5; 6; 7
Goldstrike Canyon Spring, NV	Experimental	NPS	Recreation; disease; loss of water	5; 6; 7
Grapevine Canyon Spring, AZ	Experimental	NPS	Recreation; disease; loss of water	5; 6; 7
Pupfish Refuge Spring, NV	Experimental	BOR	Vegetation encroachment; disease	3; 6
Red Rock Spring, NV	Experimental	BLM	Disease; loss of water	6; 7
Roger's Spring, NV	Natural	NPS	Vegetation encroachment; nonnative fish; recreation; disease; loss of water	3; 4; 5; 6; 7
Salt Cedar Spring, NV	Natural	NPS	Vegetation encroachment; nonnative crayfish; recreation; disease; loss of water	3; 4; 5; 6; 7
Sugarloaf Spring, AZ	Experimental	NPS	Disease; loss of water	6; 7

Figure 1: *Rana onca* Potential Management Zone (Described fully in Sect 1.1; pp. 55-56)



2. Enhance existing habitat and/or create new habitats where feasible.

   Benefit: Enable relict leopard frog populations to use the full potential of existing occupied habitats and expand into currently unoccupied or potential habitat.

   Success Standard: Enhancement and creation of habitat follows schedules and protocols identified in the Implementation Schedule and Stepdown Outline of Management Strategies and Conservation Actions.

3. Establish additional populations of relict leopard frogs in existing or created habitats.

   Benefit: Having more populations reduces the risk of species extinction. If individual populations are extirpated, refugia populations can serve as donor populations.

   Success Standard: Ten or more additional relict leopard frog populations that persist for the duration of the agreement are established within the historical range, of which 3 or more are outside Lake Mead NRA. Additionally, a minimum of 1 refugium population of at least 20 adult frogs is maintained at a zoo or other suitable, professional facility for the duration of the agreement.

4. Manage relict leopard frogs and their habitats to ensure persistence in diverse aquatic ecosystems, and facilitate processes that promote self-sustaining populations.

   Benefit: Ensure persistence of relict leopard frog populations and habitat across the Potential Management Zone for the duration of the agreement.

   Success Standard: Site-specific long-term population trend is stable or increasing and successful recruitment is evidenced by presence of eggs, tadpoles, or juveniles in 3 of 5 past years. The Relict Leopard Frog Conservation Team evaluates performance of focal, supplementary and refugia populations at least annually. Relict leopard frogs are found in a variety of habitats. Existing and new/enhanced habitats are monitored and managed to meet potential and ensure maintenance of self-sustaining populations.

5. Monitor relict leopard frog populations.

   Benefit: Monitoring is necessary to determine and document population viability, for evaluation and documentation of population trends, and for assessing the success or failure of management activities.

   Success Standard: Extant populations are monitored following schedules and protocols identified in the Implementation Schedule and Stepdown Outline of Management Strategies and Conservation Actions.

6. Investigate the conservation biology of the relict leopard frog, and use the results of such investigations to better meet the goal and objectives.

    Benefit: Researching baseline biological and ecological data is essential for evaluation and documentation of trend, determining appropriate management actions, and refining management strategies.

    Success Standard: Research activities identified in the Implementation Schedule and Stepdown Outline of Management Strategies and Conservation Actions including a Population Viability Analysis are carried out and research results are incorporated into adaptive management of the species.

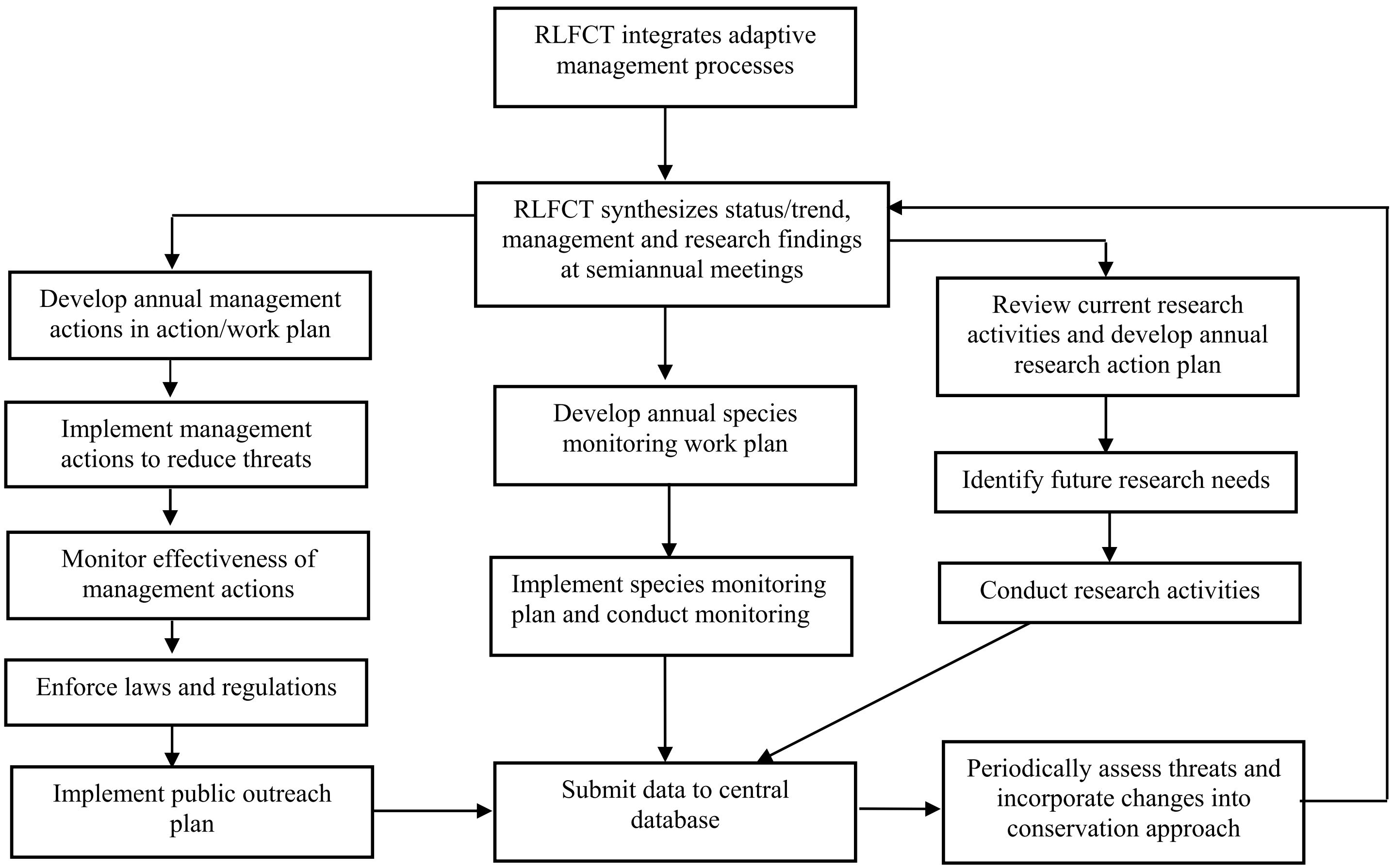
ADAPTIVE MANAGEMENT PROGRAM (AMP)

This Strategy depends upon the successful implementation of an adaptive management program (AMP). Adaptive management is designed to bring new information immediately into new management direction. All cooperators agree and recognize, consistent with the goals of this Strategy, that monitoring actions and conservation measures implemented through the CAS will be conducted experimentally consistent with the concepts of adaptive management. The effectiveness of all conservation measures and monitoring methods will be periodically reviewed and evaluated by the RLFCT. Based on such evaluation, appropriate modifications to strategies and actions will be made to ensure scientific rigor and the efficacy of conservation measures. The signatories to this agreement are committed to seeking the resources necessary to ensure successful implementation of adaptive management and its principles (Figure 2).

The essential steps of the CAS adaptive management strategy are summarized as follows:

Step 1. Implement CAS conservation actions and strategies.
Step 2. Implement annual work plans for management, monitoring and research.
Step 3. Review CAS conservation goals, objectives and strategies and adjust as necessary based on updated information.
Step 4. Prioritize locations for implementation of conservation actions and identify and prioritize research needs.
Step 5. Initiate site-specific actions to reduce or eliminate threats and complete identified research projects
Step 6. Implement monitoring plan to determine effectiveness of conservation actions.
Step 7. Analyze and evaluate monitoring and research results to determine progress towards attainment of conservation objectives.
Step 8. Return to Step 3.

Figure 2. Adaptive Management Flow Chart



STEPDOWN OUTLINE OF MANAGEMENT STRATEGIES AND CONSERVATION ACTIONS

1. <u>Administration of the strategy (Applies to all Objectives)</u>

1.1 Identify and implement a Relict Leopard Frog PMZ.

Conservation actions for the relict leopard frog will be focused on suitable aquatic/riparian habitats within the PMZ (Fig. 1). The PMZ includes existing populations and the known historical locations for relict leopard frogs derived from museum records and literature references (D. Bradford, J. Jaeger, R. Jennings, unpublished data). The purpose of the PMZ is to define a general region in which relict leopard frog habitats and populations may have once existed. Historical records for this frog are limited, and likely biased, with records generally located at sites along river courses and major travel routes, thus providing only a minimum historical distribution for this species. To delineate the borders of an area that represents a feasible approximation of the relict leopard frog's historical range, and contains sufficient potential habitat for conservation actions, the following criteria were used: (1) locations below 1000 m (the majority of historical observations for relict leopard frogs occur below 1000 m but there are some sites in Utah that occur near or just above this elevation); (2) within 1:100,000 scale hydrologic units (the best hydrologic data available were derived from Seaber et al. [1987] as modified by the Southern Nevada Water Authority to better conform to the State of Nevada Division of Water Resources designated ground water basins) that contained documented historical records or current populations (D. Bradford, J. Jaeger, and R. Jennings unpublished data); (3) extending along the Santa Clara River up to Gunlock, Utah above the 1000 m contour where a historical record exists; and (4) delineating the Nevada state line and a line extending due East of the tip of Nevada into Arizona as a functional southern limit within the hydrologic unit that extends further south.

The southern extent of relict leopard frogs along the Colorado River is not currently known. A historical leopard frog specimen exists from near the southern tip of Nevada, but no morphological evaluation to definitively identify whether the specimen is a relict leopard frog or a lowland leopard frog has been conducted because of the morphological overlap between these taxa (see Jaeger et al. 2001). Leopard frogs have been reported at the Bill Williams River near the confluence with the Colorado River further to the south, but these are purportedly lowland leopard frogs and exist in a drainage system where lowland leopard frog genotypes have been confirmed (Jaeger et al. 2001). To the east along the Colorado River, the PMZ extends along a hydrologic unit that includes Surprise Canyon where leopard frogs of unknown identity exist. Further research on the limits of the relict leopard frog along the Colorado River is needed. To the west, the hydrological unit that includes Las Vegas Valley was excluded from the PMZ. Leopard frogs once occurred in Las Vegas Valley, but the systematic status of these frog populations remains controversial and further research on their identity is needed.

The PMZ includes 3,392,658 acres (this total includes water areas such as Lakes Mead and Mojave). Only a small portion of this area represents suitable aquatic/riparian habitats for relict leopard frogs. Of the total area, more than half is covered by functioning conservation plans (see section on Other Conservation Measures Affording Protection to the Relict Leopard Frog, below). The Clark County Multiple Species Habitat Conservation Plan (MSHCP) covers 52.4% (1,779,042 acres) and Lower Colorado River Multi-Species Conservation Program (LCR MSCP) covers 5.6% (189,683 acres). All remaining natural populations of relict leopard frogs are within the areas covered by these conservation plans. Excluding major watered areas, 89.4% of the PMZ is federally controlled (2,872,215 acres). States control an additional 3.2% (102,325 acres), while private and tribal lands comprise 5.4% (172,982 acres) and 2.1% (66,824 acres), respectively. Although private lands are a minor portion of the PMZ, there are spring sites on private property. Due to the extent of publicly owned lands, participation of private landowners is not critical to the implementation of this plan; however, opportunities on private lands will be considered on a case by case basis with landowner permission.

1.2 After finalization of the CAS, amend existing and future land use plans and amendments as needed to implement the objectives, strategies, and actions identified in the CAS where possible.

1.3 Identify conservation site agreements and easements, or properties, needed within the PMZ to meet the Strategy's Goals and Objectives. Pursue these options as needed only on the basis of willing cooperator or willing seller and take appropriate measures to benefit these landowners (e.g. Candidate Conservation Agreements with Assurance, funding and technical assistance, etc.).

1.4 Establish and maintain the RLFCT. The RLFCT will include at least 1 representative from each of the signatory agencies, as well as other interested parties. Participation in this process is encouraged, with particular emphasis on review by experts on ranid conservation biology, ecology, and genetics. The responsibilities of the RLFCT are to

- 1.4.1 Identify, coordinate, and schedule conservation actions.
- 1.4.2 Develop monitoring protocols
- 1.4.3 Coordinate monitoring and research activities.
- 1.4.4 Review selected projects taken (or not taken) as part of this CAS annually.
- 1.4.5 Prepare and distribute annual progress reports; and exchange information on the biology, ecology, monitoring, and management of the relict leopard frog. The annual report will summarize, consolidate, and report on the status of the relict leopard frog and the implementation of this CAS; review new scientific information to identify additional management and research needs; consider modification of the strategies as appropriate; and

provide management recommendations. The annual report shall cover the previous calendar year, and will identify funding needs for the upcoming Federal fiscal year, and funding needs for major projects. Annual reports shall be finalized by March 1 of each year.

1.4.6 Meet a minimum of 2 times per year. A late winter meeting will serve to coordinate cooperative action plan development**,** finalize field season plans (Appendix 5) and the annual report, and an early fall meeting will serve to collect information needed for the annual report, synthesize research and field data and assess conservation progress through adaptive management review. Additional meetings may be held as needed. The National Park Service, Lake Mead National Recreation Area, will chair the RLFCT. In the event this agency is unable to fulfill this duty, the Nevada Department of Wildlife will chair the RLFCT.

1.4.7 Each signatory agency or party will designate a voting member of the RLFCT. Changes to this strategy shall require a unanimous vote by voting members. All other decisions will be made by majority vote of voting members.

1.5 Develop and implement an adaptive management process.

1.6 Seek sufficient funding to implement the commitments made in the CAS.

2. <u>Public outreach (Applies to Objectives 1 - 4)</u>

2.1 Develop brochures, interpretive signs, regulatory signs, and other materials to educate the public about the status of this species, the CAS, and regulations to protect this frog.

2.2 Place brochures and other interpretive literature at sites of high public use (e.g. Lake Mead NRA Visitors Center) and at occupied sites that are easily accessed by the public (e.g. Blue Point and Rogers springs).

2.3 Place interpretive and regulatory signs at selected occupied sites as determined by the RLFCT.

2.4 Develop educational and informational materials and programs on the frogs and their habitat and management needs for distribution through other media sources.

3. <u>Manage invasive and aggressive flora. (Applies to Objectives 1 and 2)</u>

3.1 Inspect occupied and relocation habitats for presence of nonnative, invasive, and aggressive plants.

3.2 Eradicate or control nonnative, invasive, and aggressive plants after recommendation by the RLFCT. Emphasis will be placed on nonnative species that will quickly colonize and be detrimental to relict leopard frog habitat (e.g., tall whitetop).

3.3 Evaluate the potential for tamarisk removal or control in portions of occupied springs not yet treated (e.g. Bighorn Sheep and Boy Scout).

3.4 Evaluate the status of native invasive and aggressive plants (e.g., *Scirpus spp.*) at occupied and relocation sites in terms of impacts on the quality and quantity of relict leopard frog habitat.

3.5 Manage invasive and aggressive native plants at occupied or relocation sites when necessary to maintain sufficient quality and quantity of habitat for the relict leopard frog population. Such control efforts will be recommended by the RLFCT. Findings from research will be used to govern habitat improvement projects relating to vegetation control.

3.6 Do not control invasive and aggressive plants at locations with extant relict leopard frog populations unless these plants pose a substantial threat to the frog habitat and population.

3.7 Manage or remove detrimental, invasive, and aggressive flora of concern in portions of springs identified as relocation sites prior to the introduction of relict leopard frogs when actions are likely to improve reintroduction success.

3.8 If control of invasive and aggressive plants is recommended by the RLFCT, only use herbicides in control efforts when absolutely necessary. Use only herbicides that have been approved for use in aquatic systems and that have no known negative effects on amphibians.

3.9 Monitor effectiveness of control efforts and incorporate results into future control plans through AMP review.

4. <u>Manage nonnative invasive fauna. (Applies to Objectives 1 and 2)</u>

4.1 Inspect occupied and restoration habitats for presence of detrimental, nonnative, invasive animals.

4.2 Remove or control nonnative invasive animals, with emphasis on species judged to be of significant threat to maintenance of self-sustaining populations of relict leopard frogs.

4.3 Remove or control detrimental, nonnative, invasive fauna of concern in portions of springs identified as relocation sites before introducing relict leopard frogs.

4.4 Monitor effectiveness of control efforts and incorporate results into future control plans through AMP review.

5. <u>Management of recreation (Applies to Objective 1)</u>

5.1 Manage recreational use in occupied habitats to minimize detrimental impacts to relict leopard frogs.

5.2 Evaluate proposals for new recreational development and access to prevent detrimental impacts on occupied and potential relict leopard frog habitats. Do not approve actions that would increase recreation at existing or future relict leopard frog sites.

5.3 Reduce or eliminate direct introduction of nonnative, invasive plants by humans through education, information, and enforcement efforts. These efforts should be focused on occupied sites that are easily accessed by the public (e.g. Blue Point and Roger's springs).

5.4 Reduce or eliminate direct introduction of nonnative animals, especially fishes, by humans through education, information, and enforcement efforts. These efforts should be focused on occupied sites that are easily accessed by the public (e.g. Blue Point and Roger's springs).

5.5 Monitor effectiveness of management efforts, in conjunction with conservation actions 3.9 and 4.4, where applicable, and incorporate into future management and enforcement efforts through AMP review.

5.6 Monitor effectiveness of mitigation actions and incorporate into future mitigation requirements and mitigation protocol (Appendix 3) revisions through AMP review.

6. Disease prevention (Applies to Objective 1)

6.1 Adopt and require the use of disease and pathogen protocols for all aquatic inventory and other field crews working in occupied and potential habitats (Appendix 5).

6.2 Incorporate disease and pathogen protocols into research and collection permits issued under State and Federal authorities.

6.3 Monitor for disease and malformations.

6.4 Develop a plan to address disease outbreaks, should they occur.

7. Reduce potential threats of water development, construction, and maintenance projects. (Applies to Objective 1)

7.1 Review water rights filings and respond to those that may represent active threats to occupied and potential relict leopard frog habitats.

7.2 Review and respond to proposed surface water developments for potential detrimental impacts to occupied and potential habitats.

7.3 Review, assess, and respond to other development activities which have the potential to impact groundwater or surface water resources associated with frog habitats through AMP review.

7.4 Implement a mitigation protocol that will be applied to all construction and maintenance projects in or near frog habitat (Appendix 3).

7.5 Monitor effectiveness of mitigation actions and incorporate into future mitigation requirements and mitigation protocol (Appendix 3) revisions.

8. <u>Develop and operate one or more facilities for captive or in-situ head-starting of larval relict leopard frogs. (Applies to Objectives 3 and 4)</u>

    8.1 Implement an *in-situ* headstarting program to augment existing wild populations and to provide animals for translocations.
    8.2 Follow established protocols for collection and headstarting activities as identified in Appendices 6 - 10, subject to modification through adaptive management review.
    8.3 Review headstarting efforts annually and incorporate into annual work plans through AMP review.

9. <u>Establish additional populations of relict leopard frogs in existing or created habitats. (Applies to Objective 3)</u>

    9.1 Develop target number, size, distribution of populations needed to meet the goal of the Strategy (Appendix 5).
        9.1.1 Establish 10 or more additional viable relict leopard frog populations that persist for the duration of the agreement within the historical range, of which 3 or more are outside Lake Mead NRA.
            9.1.1.1 Establish, if feasible, and under criteria in 9.3, populations at Tassi Springs and Pakoon Springs.
        9.1.2 Establish a minimum of 1 refugium population of at least 20 adult frogs at a zoo or other suitable, professional facility for the duration of the agreement.
    9.2 Survey for new sites.
    9.3 Develop site selection criteria and select sites for reintroductions (Appendix 5).
    9.4 Complete all appropriate compliance needed to introduce frogs.
    9.5 Conduct site preparation (i.e. control of nonnatives, etc.) as needed.
    9.6 Release frogs in accordance with compliance documents, health screening, transport, and release protocols (Appendix 5).
    9.7 Develop post-release monitoring protocol.
    9.8 Monitor reintroduced frogs following post-release monitoring protocol to assess success of reintroduction, and incorporate results into future reintrodution efforts through AMP review.

10. <u>Augment existing populations as needed to ensure their persistence and genetic health. (Applies to Objective 4)</u>

    10.1 Develop decision tree for determining if a population needs augmentation (Appendix 5).

10.2 Review prior augmentation efforts and determine which populations need augmentation.
10.3 Complete all appropriate compliance needed to augment populations.
10.4 Conduct site preparation and maintenance (i.e. control of nonnatives, etc.) as needed.
10.5 Release frogs in accordance with compliance documents, health screening, transport, and release protocols (Appendix 5).
10.6 Develop a genetic health management protocol for rearing facilities, reintroductions, and augmentations.

11. Monitor relict leopard frog populations. (Applies to Objective 5)

11.1 Develop monitoring program for established populations (Appendix 5).
11.2 Monitor established populations of relict leopard frogs using standard protocols (Appendix 5).
11.3 Evaluate monitoring on a regular basis, and amend the CAS as needed to apply this information through AMP review.

12. Investigate the conservation biology of the relict leopard frog, and use the results of such investigations to better meet the goal and objectives. (Applies to Objective 6)

12.1 Use research to address management questions. Design studies to help meet the goals and objectives of this strategy. As new information is developed, adaptive management will be applied and this strategy will be revised as needed to make the best use of research results.
12.1.1 Determine habitat use/needs/selection and home range or territoriality. We generally know what seems to be good frog habitat, but lack detailed data about habitat requirements and use. Studies are needed that investigate how habitat use changes with season and life stage and whether frogs establish home ranges. This information is important when choosing and developing reintroduction sites and in managing existing sites and populations.
12.1.2 Identify and describe hibernacula. Studies are needed that investigate where the frogs overwinter.
12.1.3 Describe oviposition sites.
12.1.4 Evaluate dispersal capabilities or seasonal movement.
12.1.5 Examine seasonal changes in activity.
12.1.6 Examine response to flooding.
12.1.7 Examine individual and population response to habitat manipulations. Develop and test designs incorporating vegetation and hydrological components to provide habitat for all stages of relict leopard frogs and

species on which relict leopard frogs depend, emphasizing designs which will provide an advantage to relict leopard frogs over nonnative invasive competitors.

12.1.8 Determine the best life stage for release to the wild. The success of translocating larvae or head-started juvenile frogs has not been evaluated. Survival and ultimate recruitment to the population as well as cost need to be considered.

12.1.9 Examine feeding and foraging behavior and diet.

12.1.10 Determine age and size at first reproduction and growth rates.

12.1.11 Examine interactions with nonnative predators and competitors.

12.1.12 Study population, metapopulation dynamics.

12.1.13 Conduct Population Viability Analysis (PVA) and / or Habitat Population Viability Analysis (HPVA). By doing a PVA it will be possible to estimate the number of individuals needed to maintain the population. This information could be used to modify the success criteria.

12.1.14 Improve monitoring protocols as necessary.

12.1.15 Examine frequency of disease and die-offs. Through careful monitoring it should be possible to detect die-offs early, investigate their causes, and attempt to remove the threat or salvage individuals from the population.

12.1.16 Investigate methods to treat chytrids in wild populations. Continue to communicate with chytrid researchers, and adapt procedures that reduce the potential for disease transmission or mitigate disease outbreaks when necessary.

12.1.17 Implement conservation and management actions under a science-based, hypothesis-driven framework.

12.2 Evaluate research and monitoring on a regular basis, and amend the CAS as needed through adaptive management to apply this information.

12.3 Identify additional research needs and priorities through AMP review.

12.4 Incorporate research needs into annual work plans through AMP review**.**

12.5 Amend the CAS as necessary to apply research findings through AMP review.

13. <u>Develop a process for collecting and maintaining data and information for distribution to stakeholders and decision makers. (Applies to Objective 6)</u>

13.1 Create a depository for storage of data from inventory, monitoring, and research efforts.

13.2 Maintain the depository.

13.3 Ensure data and information developed through actions of the CAS are available to and shared among involved parties.

CONSIDERATIONS FOR RESERVE DESIGN

A. BACKGROUND ON REINTRODUCTIONS AND TRANSLOCATIONS

Translocation is an important but controversial tool to conserve amphibian populations (Trenham and Marsh 2002, Seigel and Dodd 2002). Three issues are embedded in this controversy. 1) Translocations of amphibian populations have yet to demonstrate consistent success (Dodd and Seigel 1991). 2) Translocations can spread disease (Cunningham 1996). 3) Translocations can alter the genetics of wild populations (Storfer 1999). Nevertheless, translocation has a potentially important role to play in amphibian conservation, even if this role is, according to some, "experimental." To see how the CAS addresses controversial issues 2 and 3, see Sections 2, 7, and 9 in the techniques manual (Appendix 5). Section E below, "Target number, size, and distribution of populations," addresses how the success of translocations is assessed.

Key to demonstrating success of translocations is a commitment to follow-up monitoring. Without gathering detailed data, it is difficult or impossible to evaluate translocations (Scott and Carpenter 1987, Dodd and Seigel 1991). Success of translocations needs to be defined in meaningful temporal, spatial, and biological terms (Tasse 1988). For amphibians with complex life cycles (*sensu* Wilbur 1980), this would include data collected on different life stages (eggs, larvae, and adults), over a sufficiently long period of time (e.g. 1 - 2 generations of the organism, Denton et al. 1997). Some researchers have suggested a monitoring time commitment of 6 - 10 years in order to truly gain insight into the successful re-establishment of anurans (Cooke and Oldham 1995, Sredl and Healy 1999, US Fish and Wildlife Service 2000).

Using cohort-marked frogs, Sredl and Healy (1999) identified 5 temporal stages of success for Chiricahua leopard frogs (Table 3). These stages are: 1) persistence of released animals, 2) over-winter persistence of released animals, 3) long-term persistence of released animals, 4) reproduction of released animals at the site of release, and 5) recruitment in a population of released animals. Immediate or short-term success would be evaluated in the weeks following the release of animals. Ultimate success, the establishment of a self-sustaining, wild population, cannot be determined until the founding population has had the time to establish and reproduce. This suggests that monitoring needs to take place for at least 3 years, in order to evaluate whether the founders' reproductive efforts are successful.

Table 3. Stages of success to evaluate the efficacy of translocations. Techniques, duration, and frequency assume that late-stage tadpoles or juvenile frogs have been released. Duration for reproduction and recruitment stages assumes that the long-term survival stage has been attained (from Sredl and Healy [1999]).				
Stage of Success	Technique	Duration		Frequency
		Begins	Ends	
Stage 1 Short-term persistence of released individuals	VES	After release	8 weeks after release	2 times
Stage 2 Persistence of released individuals overwinter	Intensive nighttime surveys	Activity season following the first winter post-release	3 months after it is initiated	1-2 times
Stage 3 Long-term persistence	Intensive nighttime surveys	End of over-winter survival surveys	One generation post release (~3 years)	2-4 times per year
Stage 4 Reproduction	Daytime egg mass surveys	Sexual maturity	Three generations (~10 years)	1-2 times per year
Stage 5 Recruitment	Intensive nighttime surveys	Once reproduction is detected	Three generations (~10 years)	1-2 times per year

Surveys during stage 1 use Visual Encounter Survey (VES) assuming all tadpoles or juveniles encountered are from the recent release. Surveys addressing the remaining stages of success, except stage 4, would use intensive nighttime searches, involving capture and identification of cohort. Data to be collected during intensive nighttime surveys includes recording the cohort mark, snout to vent length, mass (for relative growth rates and to discern when reproductive size is reached), reproductive condition, and reproductive behavior (male vocalizations, pairs in amplexus).

Due to the potentially invasive and stressful techniques employed during the nighttime surveys, they should be conducted at least 3 but not more than 4 times a year. These surveys should occur around the potential breeding periods of the focal species (i.e. spring and summer in the case of leopard frogs). Nocturnal surveys are imperative as it is much easier to observe, approach and capture leopard frogs at night. In addition, most breeding activity takes place at night. Surveys designed to detect stage 4 should be implemented during the daytime at least 1 or 2 times per year. Either observing egg masses or tadpoles can determine success at this stage.

To measure dispersal, nearby non-release sites will also need to be monitored, and when suitable habitat exists in the nearby vicinity (#1.6 km), surveys should be conducted sporadically over the period described above to determine whether colonization occurs.

Details of parties responsible for particular monitoring particular sites can be found in the Implementation Schedule.

B. Regulatory Requirements

The initiating agency for any translocation action is responsible for ensuring that any compliance requirements and required documentation necessary for release of animals to the wild are completed in a timely manner that will not delay established activity timing, if at all possible. Specific compliance requirements will vary with the location and nature of translocation actions. For the augmentation of sites with existing, established populations compliance requirements may be minimal but action agencies shall insure that appropriate State and Federal permits for movement and release of live animals are in place. For the translocation and release of animals to establish populations at historically occupied or new site locations additional compliance actions may be required, including preparation of a site assessment or other project documentation, compliance checklists, and additional approval requirements from the private landowner or responsible public land management agency

C. Headstarting and Releases of Relict Leopard Frogs Prior to 2003

Relict leopard frogs collected as small tadpoles from Bighorn Sheep Spring in 2000 and eggs in 2001 were headstarted at the NPS headquarters in Boulder City, Nevada. These frogs were released to the Boulder City Wetlands, an artificial wetlands in downtown Boulder City in 2000 and 2001, and Sugarloaf Spring, a small spring and stream in Black Canyon in 2002 (S. Romin pers. comm.). Boulder City Wetlands were chosen to allow easy observation of frogs and success of release. It was intended to function as a refugium population of relict leopard frogs. An experimental population of 297 of the headstarted metamorphs (mean 25 mm SVL) was introduced from May - September into the wetlands. The high population count at this site was 21 adults in October 2001 (S. Romin pers. comm.). While frogs were still present at the wetlands in low numbers in January 2003, bullfrogs, crayfish and nonnative fish invaded or were illegally introduced to the wetlands. This area is no longer considered a refuge for relict leopard frogs because of these threats and lack of support for continuing the flow of water to the wetlands. Sugarloaf Spring was chosen as a release site because it was a natural site with approximately 900 m of habitat within close proximity to known populations in Black Canyon and NPS Headquarters, inaccessible to the public due to security restrictions for Hoover Dam, and was free of known threats to relict leopard frogs. This site is intended to function as another population of wild relict leopard frogs. A total of 113 frogs and tadpoles were released in September 2002.

The following observations come from Romin's efforts. These observations are similar to those working with other leopard frogs in the Southwest (Sredl et al. 2002, M. Sredl pers. comm.). Tadpoles were usually released before complete metamorphosis. Release appeared to be less stressful on slightly earlier stages compared to full metamorphs. When metamorphs with less than 2 cm of tail were raised, release had to be very delicate, with care taken to gently set the froglet in very shallow water on the edge of the pools. In contrast, earlier stages swam vigorously out of the release bucket and immediately sought cover in algae clumps and vegetation under water. Earlier stages were also easier to transport to the release site.

Tadpoles are expected to mature at different rates; at the peak of cohort metamorphosis, daily trips to the release site may be required. Monitoring of previously released individuals can occur during these trips. After all metamorphs have been released, monitoring should occur every month. In addition to counting adults, pools should be checked for eggs and tadpoles.

D. PROTOCOLS FOR CAPTIVE CARE, TRANSPORTATION, AND RELEASE OF RELICT LEOPARD FROGS

The RLFCT will base protocols for captive care, transportation, and release of relict leopard frogs on protocols developed by Sredl et al. (2002) during work with the Ramsey Canyon leopard frog (*R. subaquavocalis*) and the Chiricahua leopard frog (*R. chiricahuensis*). These protocols were modified for relict leopard frogs based on the past experiences of S. Romin and will be modified based on future data collected on the species. These protocols are compiled into a document entitled *Relict Leopard Frog Protocols and Techniques Manual* (Appendix 5). These protocols will serve as a guide for rearing facilities but should be adapted and refined at the discretion of the facility as long as survivorship and health of headstarted animals is up to the standards specified in the manual.

E. TARGET NUMBER, SIZE, AND DISTRIBUTION OF POPULATIONS

Reserve Design

Many amphibian populations vary greatly in size over time and total numbers are not necessarily indicative of population stability (Bragg 1960, Sherman and Morton 1993, Weitzel and Panik 1993, Green 1997, Meyer et al. 1998, US Fish and Wildlife Service 2000). Because of these fluctuations, the spatial distribution of the populations is important to allow natural immigration and emigration to maintain genetic and demographic health of populations (Sjögren 1991, Sjögren-Gulve 1994). Also, individual sites or a set of nearby sites should have adequate quantity and quality of habitat to support a population through such fluctuations. A matrix of sites in the PMZ will be managed to facilitate metapopulation dynamics of relict leopard frogs. Managed sites should be within dispersal distance of frogs and have adequate habitat to allow connection of sites. Ideally, individual sites or sets of sites will not be impacted by the same threats.

Four categories of sites are defined--captive, refugia, corridor, and focal--to guide conservation efforts and assess success. Conserving the frog in these 4 types of sites takes into account metapopulation concepts in the wild (corridor and focal) and addresses the need for securing frogs in controlled areas with few threats (captive and refugia). This CAS will be considered successful if at the end of this 10-year agreement, ten or more supplemental or focal populations of relict leopard frogs have been established. These populations must persist for the duration of the agreement and be within the PMZ. In addition, three or more of these populations will be outside Lake Mead NRA. Additionally, a minimum of 1 refugium population of at least 20 adult frogs is maintained at a zoo or other suitable, professional facility for the duration of the agreement.

Captive Rearing Facilities

Captive rearing facilities serve to propagate healthy, viable individuals for release into the wild. Captive rearing facilities will be managed to ensure the genetic health of offspring produced and any captive stock present in the facility. Such facilities will operate to attain the standards described in Sections 4 – 7 of the techniques manual (Appendix 5). Current captive rearing facilities include Detroit Zoo, Lake Mead NRA Facility, and Willow Beach National Fish Hatchery. Other potential sites for captive propagation include any contained facility in which healthy frogs can be reared or maintained without risk of disease or escape.

Refugia Sites

Refugia sites will be isolated from all other occupied sites with extremely low probabilities of frogs moving into or out of the sites without human control. Refugia sites will serve to ensure the genetic integrity of the relict leopard frog persists for the duration of the agreement. The sites should have adequate quantity and quality of habitat for all life stages year round. The populations at these sites will be established and periodically managed with individuals such that a natural level of genetic variability is maintained in the populations. The Boulder City Wetlands was established as a refugium for relict leopard frogs, but the effort failed.

Corridors

Corridors can range from small, natural pools within or outside of a drainage to ephemeral stock tanks to perennial waterway unsuited to frog breeding (i.e. too high flow). Corridors are sites that are unsuited to breeding by relict leopard frogs but are still used during dispersal. Corridors allow frog populations to expand to unoccupied breeding habitat and facilitate genetic exchange between breeding populations. Corridors are only successfully used by frogs during dispersal or long distance movements, and are rarely occupied. In unusually wet times, many dispersing individuals may attempt to breed at a corridor site, but recruitment to the adult population is unlikely or sporadic, given the unstable nature of the water in corridors. Given its small size, ephemeral surface water, and occasional occupation by relict leopard frogs, Gnatcatcher Spring may be a corridor for frogs at Blue Point and Rogers springs.

Focal Sites

Focal sites are a body of water or a set of nearby (<1.6 km apart) bodies of water with reliable surface water year round and year to year. These sites should have pools with a minimum depth of at least 1 m to allow breeding. In complex systems with several associated bodies of water in close proximity (for example, large pools in a stream surrounded by marsh with small isolated ponds), oviposition sites should hold water long enough to support development of eggs to metamorphosis (a minimum 6.5 months of the year; NPS unpublished data) or be connected with more permanent water. These sites should have resources available such that requirements of all life stages of the frog are met within the site. Sites should have no or few aquatic predators. Populations of relict leopard frogs at focal sites would be considered "sources" in classic metapopulation models. Focal sites can contain reintroduced or extant populations of frogs and these populations should be designed and managed to be primarily self-sustaining. The physical and biological characteristics of focal sites can be used to determine suitable sites for reintroduction. Focal sites should be established and managed during the 10-year agreement to remain intact in the future as insurance for unforeseen declines. Currently, Blue Point, Rogers, Boy Scout, Salt Cedar, and Bighorn Sheep springs could be considered focal sites.

<u>Criteria for Assessing Reintroduction Site Suitability</u>

Considerations for Site Characteristics

1. Is the site within historical range and what is the historical occupancy at the site?
2. Does the site have adequate surface water year-round?
3. Does the site have adequate habitat heterogeneity (e.g. shallow runs or pool overflows for feeding and basking, deep pools or channels for cover and escape, and sufficient / appropriate vegetation present for cover and to provide prey items)?
4. Does the site have adequate habitat available to sustain a population of frogs once established (e.g. length of run / circumference of pond)?
5. Is the site accessible to researchers/managers to permit monitoring and augmentation?
6. Is the site heavily used by the public (e.g. are anthropogenic threats likely to occur or be unmanageable at the site)?
7. Is the site isolated from other populations (for refugia sites) or can movement between nearby sites potentially occur (for supplementary sites)?
8. If there are other amphibians at the site, has disease been detected or have die-offs been observed?
9. Are predators or harmful nonnative species abundant at the site?

<u>Criteria for Assessing Donor Populations</u>

Black Canyon sites will only be populated with individuals from a Black Canyon donor site unless additional genetic data indicates that such precautions are not necessary or circumstances are deemed necessary by the RLFCT. Northshore sites will only be populated with individuals from a Northshore site. Outside these general areas, the most appropriate, available source will be used to establish populations. As of 2003, the only donor population large enough to support harvest for headstarting and release to other sites is Bighorn Sheep Spring. Bighorn Sheep Spring currently supports a comparatively large number of adults and has had by far the highest tadpole production of all the known populations.

When rearing facilities are fully functioning, the number and portion of the eggs or small tadpoles collected from the wild will be governed by the site and population characteristics such as abundance of tadpoles or eggs in a given pool, total number of eggs or tadpoles at a site, and total size of habitat at a site. As many frogs, tadpoles, and eggs as possible should be collected and headstarted when they will be lost from the wild if not collected (e.g. salvaging tadpoles or eggs from a drying pool). In all other instances, only portions of egg masses or small free-swimming tadpoles will be collected for headstarting. Egg masses or small free-swimming tadpoles will be the stages collected under normal circumstances, because they benefit more from headstarting and collection of these stages is less detrimental to the population. The details of headstarting and translocation efforts will be planned on an annual basis at meetings of the RLFCT and incorporated into the annual work plan, as described in Section 1 of the techniques manual (Appendix 5).

Augmentation Considerations

Habitat Size
1. Is there a small population at a site with ample habitat to support a large population?

Habitat Quality
2. Has habitat enhancement occurred at the site that will allow it to support more frogs?
3. Have native or nonnative predators been removed from the site?
4. Has vegetation been controlled at the site to open up breeding sites?

Other Considerations
5. Has the population suffered a loss of individuals from anthropogenic causes?
6. Has the population suffered a large loss of individuals from natural causes other than disease?
7. Has the population recently been reestablished through translocation?

TEN YEAR IMPLEMENTATION SCHEDULE

Relict leopard frog Conservation Strategy task and implementation schedule						
Strategies and Actions	Priority	Target objective(s)	Target completion year(s)	Responsible parties	Projected cost[1] ◊ = annual cost ∞ = 10 year total cost	Potential Funding source[2]
1. Administration of the strategy						
1.1 Identify and implement a Relict Leopard Frog Potential Management Zone (PMZ).	High	1-6	Completed	RLFCT	N/A[3]	N/A
1.2 Amend existing and future land use plans and amendments as needed to implement the objectives, strategies, and actions identified in the CAS where possible.	High	1-4	As Needed[4]	BLM, NPS, USFWS	100K∞	1,2,5,6,8
1.3 Identify conservation site agreements and easements, or properties, needed within the PMZ to meet the Strategy's Goals and Objectives.	Medium	1-4	As Needed	RLFCT	IN 1.4	1,2,3,12
1.4 Establish and maintain the RLFCT (Relict Leopard Frog Conservation Team).					35K◊	
1.4.1 Identify, coordinate, and schedule conservation actions.	High	1-6	Ongoing[5]	RLFCT	IN 1.4	1,2,3,(5-13)
1.4.2 Develop monitoring protocols.	High	5-6	2005	RLFCT	20K∞	1,2,3,(5-13)
1.4.3 Coordinate monitoring and research activities.	High	5-6	Ongoing	RLFCT	IN 1.4	1,2,3,(5-13)
1.4.4 Review selected projects taken (or not taken) as part of this CAS.	High	1-6	Annually	RLFCT	IN 1.4	1,2,3,(5-13)
1.4.5 Prepare and distribute progress reports.	High	1-6	Annually	RLFCT	IN 1.4	1,2,3,(5-13)
1.4.6 Meet a minimum of 2 times.	High	1-6	Annually	RLFCT	IN 1.4	1,2,3,(5-13)
1.4.7 Each signatory agency or party will designate a voting member of the RFLCT.	High	1-6	2004	Signatories	0	5,6,8,9,10
1.5 Develop and implement an adaptive management process.	High	1-6	Ongoing	RLFCT	IN 1.4	1-12
1.6 Seek sufficient funding to implement the commitments made in the CAS.	High	1-6	Ongoing	RLFCT	IN 1.4	1-15
2. Public outreach						
2.1. Develop materials for public education.	Medium	1,4	2006	Involved Parties[6]	50K∞	1,2,3,(5-15)
2.2. Place brochures and other interpretive literature at sites of high public use.	Medium	1,4	2006-2013	Involved Parties	20K∞	1,2,3,(5-15)
2.3 Place interpretive and regulatory signs at selected occupied sites as determined by the RLFCT.	High	1,4	As Needed	Involved Parties	10K∞	1,2,3,5,6,7,8

2.4 Develop educational and informational materials and programs on the frogs and their habitat and management needs for distribution through other media sources.	Medium	1,4	2006	Involved Parties	IN 2.1	1,2,3,(5-13), 15
3. Manage invasive and aggressive flora						
3.1. Inspect occupied and relocation habitats for presence of nonnative, invasive, and aggressive plants.	High	1,2,4,5	Ongoing	NPS, BLM, USFWS	IN 9.1, 11.2	1,2,3,(5-12)
3.2 Eradicate or control nonnative, invasive, and aggressive plants.	High	1,2,4	Ongoing	NPS, BLM, USFWS	280K∞	(1-8),12
3.3. Evaluate the potential for tamarisk removal or control in portions of occupied springs not yet treated.	High	1,2,4	Ongoing	NPS, BLM, USFWS	50K∞	(1-8),12
3.4 Evaluate the status of native invasive and aggressive plants at occupied and relocation sites in terms of impacts on the quality and quantity of relict leopard frog habitat.	High	1,2,4,	Ongoing	NPS, BLM, USFWS	50K∞	(1-8),12
3.5. Manage invasive and aggressive native plants at occupied or relocation sites when necessary to maintain sufficient quality and quantity of habitat for the relict leopard frog population.	High	1,2,4,	Ongoing	NPS, BLM, USFWS	IN 3.2	(1-8),12
3.6. Do not control invasive and aggressive plants at locations with extant relict leopard frog populations unless these plants pose a substantial threat to the frog habitat and population.	High	1,2,4	Ongoing	NPS	0	5
3.7. Manage or remove detrimental, invasive and aggressive flora of concern in portions of springs identified as relocation sites, prior to introduction of relict leopard frogs, when actions are likely to improve reintroduction success.	High	2,3,4	2005-2013	NPS, BLM, USFWS	IN 3.2	(1-8),12
3.8. Only use herbicides in vegetation control efforts when absolutely necessary. Use only herbicides that have been approved for use in aquatic systems and have no known negative effect on amphibians.	High	1	Ongoing	Involved Parties	0	N/A
3.9. Monitor effectiveness of control efforts and incorporate results into future control plans through AMP review.	High	2,4,5,6	Ongoing	NPS, BLM	50K∞	(1-8),12,16
4. Manage nonnative invasive fauna						
4.1. Inspect occupied and restoration habitats for presence of detrimental, nonnative, invasive animals.	High	1,4,5	Ongoing	Involved Parties	IN 9.1, 11.2	1,2,3,(5-12)
4.2. Remove or control nonnative invasive animals, with emphasis on species judged to be of significant threat to maintenance of self-sustaining populations of relict leopard frogs.	High	1,2,4	Ongoing	Involved Parties	50K∞	1,2,3,(5-12)
4.3. Remove or control detrimental, nonnative, invasive fauna of concern in portions of springs identified as relocation sites before introducing relict leopard frog.	High	1,2,3,4	2005-2013	Involved Parties	50K∞	1,2,3,(5-12)

4.4. Monitor effectiveness of control efforts and incorporate results into future control plans through AMP review.	High	1,2,4,5,6	Ongoing	NPS, AGF, NDOW	30K∞	1-12
5. Management of recreation						
5.1. Manage recreational use in occupied habitats to minimize detrimental impacts to relict leopard frogs.	Medium	1	Ongoing	NPS	10K◊	1,2,5
5.2. Evaluate proposals for new recreational development and access to prevent detrimental impacts on occupied and potential relict leopard frog habitats.	Medium	1,4,6	Ongoing	Involved Parties	5K◊	1-12
5.3. Reduce or eliminate direct introduction of nonnative, invasive plants by humans through education, information, and enforcement efforts.	High	1,4	Ongoing	Involved Parties	IN 2.1-2.4	1-12
5.4. Reduce or eliminate direct introduction of nonnative animals, especially fishes, by humans through education, information, and enforcement efforts.	High	1,4	Ongoing	Involved Parties	IN 2.1-2.4	1-15
5.5. Monitor effectiveness of management efforts, in conjunction with conservation actions 3.9 and 4.4, where applicable, and incorporate into future management and enforcement efforts through AMP review.	Medium	1,2,4	Ongoing	NPS, BLM, USFWS	IN 3.9 & 4.4	1-6
5.6. Monitor effectiveness of mitigation requirements and mitigation protocol (App. 3) revisions through AMP review.	Medium	1,4	2008-2015	NPS, BLM, USFWS	5K◊	1-6
6. Disease prevention						
6.1. Adopt and require the use of disease and pathogen protocols for all aquatic inventory and other field crews working in occupied and potential habitats.	High	1,4,5	Completed	Involved Parties	0	N/A
6.2. Incorporate disease and pathogen protocols into research and collection permits issued under State and Federal authorities.	High	1,4,5	2004	Permitting Agencies	1K◊	5,6,9,10,11
6.3. Monitor for disease and malformations.	High	5	Ongoing	Involved Parties	50K∞	1-15
6.4. Develop a plan to address disease outbreaks, should they occur.	High	1,5,6	2005	RLFCT	5K◊	1,2,(5-13)
7. Reduce potential threats of water development, construction, and maintenance projects						
7.1. Review water rights filings and respond to those that may represent threats to occupied and potential relict leopard frog habitats.	High	1,4	Ongoing	Involved Parties	15K◊	1-13
7.2. Review and respond to proposed surface water developments with potential for detrimental impacts to occupied and potential habitats.	High	1,4	Ongoing	Involved Parties	IN 7.1	1-13
7.3. Review, assess, and respond to other development activities which have the potential to impact groundwater or surface water resources that may affect frog habitats through AMP review.	High	1,4	Ongoing	Involved Parties	IN 7.1	1-13

7.4 Implement a mitigation protocol that will be applied to all construction and maintenance projects that may affect frog habitat (App. 3).	High	1,4	2005	RLFCT	20K◊	1-13
7.5. Monitor effectiveness of mitigation actions and incorporate into future mitigation requirements and mitigation protocol revisions (App. 3).	High	1,4	2007-2015	NPS, BLM, USFWS, NDOW, AGF	5K◊	(1-12), 16
8. Develop and operate facilities for captive or in-situ head-starting of larval relict leopard frogs						
8.1. Implement an in-situ headstarting program to augment existing wild populations and to provide animals for translocations.	High	1,3,4	Ongoing	RLFCT, NPS, USFWS, DZ[7], MH[8]	200K∞	1-15
8.2. Follow established protocols for collection and headstarting activities as identified in (Appendix 5).	High	1,3,4	Ongoing	Involved Parties	5K◊	1,2,3,(5-15)
8.3. Review headstarting efforts and incorporate into annual work plans through AMP review.	High	1,3,4	Annually	RLFCT	IN 1.4	1-15
9. Establish additional populations of relict leopard frogs in existing or created habitats						
9.1. Develop target number, size, distribution of populations needed to meet the goal of the Strategy (Appendix 5).						
9.1.1. Ten or more additional viable relict leopard frog populations that persist for the duration of the agreement are established within the historical range, of which 3 or more are outside Lake Mead NRA.	High	2,3	2013	Involved Parties	400K∞	1-15
9.1.2. A minimum of 1 refugium population of at least 20 adult frogs is maintained at a zoo or other suitable, professional facility for the duration of the agreement.	High	1,3	Ongoing	RLFCT, NPS, USFWS, DZ, MH	50K◊	1,2,3,(5-15)
9.2. Survey for new sites.	High	2,3	2003-2009	Involved Parties	IN 9.1.1	1,2,3(5-13)
9.3. Develop site selection criteria and select sites for reintroductions (Appendix 5).	High	3,6	2006	RLFCT	5K◊	1,2,3(5-13)
9.4. Complete all appropriate compliance needed to introduce frogs.	High	3	Ongoing	Involved Parties	IN 9.1.1	1-13
9.5. Conduct site preparation as needed.	Medium	2,3	2006-2013	Involved Parties	IN 9.1.1	1-13
9.6. Release frogs in accordance with compliance documents, health screening, transport, and release protocols (Appendix 5).	High	3	Ongoing	Involved Parties	IN 9.1.1	1-15
9.7. Develop post-release monitoring protocol.	High	3,5,6	2005	RLFCT	10K◊	1,2,3,(5-13)
9.8. Monitor reintroduced frogs following post-release monitoring protocol to assess success of reintroduction through AMP review.	High	3,5,6	2005-2013	Involved Parties	IN 11.2	1,2,3,(5-13)
10. Augment and maintain existing populations as needed to ensure their persistence						
10.1. Develop decision tree for determining if a population needs augmentation (Appendix 5).	Medium	4,6	2004	RLFCT	IN 1.4	1,2,3,(5-13)

10.2. Review prior augmentation efforts and determine which populations need augmentation.	Medium	4,6	As Needed	RLFCT	IN 1.4	1,2,3,(5-13)
10.3. Complete all appropriate compliance needed to augment populations.	Medium	4,6	As Needed	Involved Parties	10K∞	1,2,3,(5-13)
10.4. Conduct site preparation and maintenance (i.e. control of nonnatives, etc.) as needed.	Medium	2,4,6	As Needed	Involved Parties	20K∞	1,2,3,(5-13)
10.5. Release frogs in accordance with compliance documents, health screening, transport, and release protocols.	Medium	4,6	As Needed	Involved Parties	5K◊	1,2,3,(5-13)
10.6. Develop a genetic health management protocol for rearing facilities, reintroductions, and augmentations.	High	3,4,6	2005	RLFCT	5K◊	1,2,3,(5-13)
11. Monitor relict leopard frog populations						
11.1. Develop monitoring program for established populations.	High	5,6	2005	RLFCT	IN 1.4	1,2,3,(5-13)
11.2 Monitor established populations of relict leopard frogs using standard protocols.	High	5,6	2005-2013	NPS, NDOW	50K◊	1,2,3,5,9
11.3 Evaluate monitoring on a regular basis, and amend the CAS as needed to apply this information through AMP review.	High	5,6	Annually	RLFCT	IN 1.4	1,2,3,(5-13)
12. Investigate the conservation biology of the relict leopard frog, and use the results of such investigations to better meet the goal and objectives						
12.1. Identify research needs for relict leopard frog conservation and management, and implement research activities as appropriate.					900K∞	
12.1.1. Determine habitat use/needs/selection and home range or territoriality.	High	6	Ongoing	RLFCT	IN 12.1	1,2,3(5-13)
12.1.2. Identify and describe hibernacula.	Medium	6	2013	Involved Parties	IN 12.1	1,2,3,(5-13)
12.1.3. Describe oviposition sites.	Low	6	2013	Involved Parties	IN 12.1	1,2,3,(5-13)
12.1.4. Evaluate dispersal capabilities or seasonal movement.	High	6	2013	Involved Parties	IN 12.1	1,2,3,(5-13)
12.1.5. Examine seasonal changes in activity.	Low	6	2013	Involved Parties	IN 12.1	1,2,3,(5-13)
12.1.6. Examine response to flooding.	Low	6	2013	Involved Parties	IN 12.1	1,2,3,(5-13)
12.1.7. Examine individual and population response to habitat manipulations.	Medium	2,4,6	2004-2013	Involved Parties	IN 12.1	1,2,3,(5-13)
12.1.8. Determine the best life stage for release to the wild.	Medium	3,4,6	2010	Involved Parties	IN 12.1	1,2,3,(5-13)
12.1.9. Examine feeding and foraging behavior and diet.	Low	6	2013	Involved Parties	IN 12.1	1,2,3,(5-15)
12.1.10. Determine age and size at first reproduction and growth rates.	Low	3,6	Ongoing	Involved Parties	IN 12.1	1,2,3,(5-15)
12.1.11. Examine interactions with nonnative predators and competitors.	High	1,2,3,4,6	Ongoing	Involved Parties	IN 12.1	1-15
12.1.12. Study population, metapopulation dynamics.	Medium	6	2013	Involved Parties	IN 12.1	1,2,3,(1-13)
12.1.13. Conduct Population Viability Analysis (PVA) and or Habitat Population Viability Analysis (HPVA).	Medium	1,3,4,6	2010	RLFCT	IN 12.1	1,2,3,(1-13)

12.1.14. Improve monitoring protocols.	Medium	4,5,6	2013	RLFCT	IN 12.1	1,2,3,(1-13)
12.1.15. Examine frequency of disease and die-offs.	Medium	1	2013	Involved Parties	IN 12.1	1-15
12.1.16. Investigate methods to treat chytrids in wild populations.	High	1	2013	Involved Parties	IN 12.1	1-15
12.1.17. Implement conservation and management actions under a science-based, hypothesis-driven framework.	High	1-6	Ongoing	Involved Parties	IN 12.1	1-16
12.2. Evaluate research and monitoring on a regular basis.						
12.3. Identify additional research needs and priorities through AMP review.	High	1-6	Ongoing	RLFCT	IN 1.4	1-15
12.4. Incorporate research needs into annual work plans through AMP review.	High	1-6	Ongoing	RLFCT	IN 1.4	1-13
12.5. Amend the CAS as necessary to apply research findings through AMP review.	High	1-6	As Needed	RLFCT	IN 1.4	1-13
13. Develop a process for collecting and maintaining data and information for distribution to stakeholders and decision makers.						
13.1. Create a depository for storage of data from inventory, monitoring and research efforts.	High	1-6	2004	RLFCT	10K◊	1,2,3,(1-13)
13.2. Maintain the depository.	High	1-6	2004-2013	Involved Parties	7K∞	1,2,3,(1-13)
13.3. Ensure data and information developed through actions of the CAS are available to and shared among involved parties.	High	1-6	Ongoing	RLFCT	IN 13.2	1,2,3,(1-13)

[1] Projected costs are **estimated** and are identified as either **ANNUAL (◊)** or **TOTAL FOR THE TERM OF THE AGREEMENT (∞)**. For some sub-tasks, individual costs may be rolled up as indicated within a primary task as those sub-task costs cannot be accurately estimated at this time.

[2] Key to Potential Funding sources

1=Clark County Multiple Species Management Plan funding (Section 10 ESA)
2=Southern Nevada Public Lands Management Act
3=Lower Colorado River MSCP funding (Section 10 ESA)
4=National Park Service
5=US Fish and Wildlife Service
6=Bureau of Land Management
7=Bureau of Reclamation
8=US Geological Survey
9=Nevada Department of Wildlife
10=Arizona Game and Fish Department (NOTE: AGFD total costs for strategy implementation are estimated to be approximately $10,250 per year for the term of the agreement).
11=Utah Division of Wildlife Resources
12=Southern Nevada Water Authority
13=University of Nevada
14=Detroit Zoo
15=Mirage Hotel
16=Other non-governmental organizations (NGOs)

[3]N/A = Not applicable

[4]As needed= Actions which may or may not occur because they may or may not be necessary, depending on program review and adaptive analysis.
[5]Ongoing = Actions already underway, and intended to continue during the 10 year period.
[6]Involved Parties = All agencies and interest groups participating in conservation planning and conservation actions relating to the relict leopard frog. These include, but are not limited to, the signatories to this document.
[7]DZ = Detroit Zoo
[8]MH = Mirage Hotel

Many of the activities enumerated in the Implementation Schedule are already underway. For details on accomplishments and work plans for 2003-2005 see Appendices 6-8.

PERSONAL COMMUNICATIONS

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UNPUBLISHED DATA

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[NDOW] Nevada Department of Wildlife, Reno, Nevada.
[NPS] National Park Service, Lake Mead National Recreation Area, Boulder City, Nevada
[NPS Yellowstone] National Park Service, Yellowstone National Park, WY.
[UDWR] Utah Division of Wildlife Resources, Salt Lake City, Utah.

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GLOSSARY OF TERMS

Adaptive Management: An interactive process whereby management of species populations and habitat is initiated, evaluated, and refined based on monitoring and research results.

Augmentation: Intentional release of individuals into an area occupied by that species.

Conservation: From Section 3(3) of the ESA: The terms "conserve," "conserving," and "conservation" mean to use and the use of all methods and procedures which are necessary to bring any endangered species or threatened species to the point at which the measures provided under this Act are no longer necessary. Such methods and procedures include, but are not limited to, all activities associated with scientific resources management such as research, census, law enforcement, habitat acquisition and maintenance, propagation, live trapping, and transportation.

Potential Management Zone (PMZ): Discreet geographical areas within which conservation goals and objectives are established (Sredl and Saylor 1997). Within these zones, appropriate conservation strategies are implemented and their effects monitored. PMZs combine principals of traditional wildlife management (population and “routine” habitat management) and conservation biology (small population biology, metapopulation dynamics, and reserve design).

Conservation Action: A conservation action is a management action that, when implemented, will partially or wholly achieve stated objectives for covered species or land cover types.

Contaminants: Any undesirable physical, chemical, biological, or radiological substance present in water as a result of human activities.

Emergent: In flooded or ponded areas, rooted, herbaceous vegetation with parts of the shoot both below and above water, including cattail and bulrush.

Habitat: The specific places where the environmental conditions (i.e. physical and biological conditions) are present that are required to support occupancy by individuals or populations of relict leopard frogs. Habitat may be occupied (individuals or population of the species are, or have recently been, present) or unoccupied (see also “unoccupied habitat” and “potential habitat”).

Habitat Quality: Habitat quality refers to the ability of the environment to provide conditions that support individual and population persistence. High quality habitat includes all elements needed for relict leopard frogs to complete their life cycle. Low quality habitat would include only the minimal elements that support occurrence of relict leopard frogs.

Habitat Quantity: Habitat quantity refers to the area of the environment that provides conditions that produce or could produce occupancy of a given organism.

Historical Range: Those geographic areas inhabited at the time of modern exploration and settlement, as verified by museum voucher(s) or documented in the published literature.

Introduction: Release of individuals into an area not formerly occupied by that species

Inventory: The process of conducting surveys to determine total distribution and number of frogs.

Loss of Habitat: Loss of habitat is a reduction in habitat quality or quantity that results from an adverse change in environmental conditions, such as cover, substrate, channel type, interacting species, river area, reservoir area, water quality, and groundwater depth.

Metapopulation: any assemblage of discrete local populations with migration among them, regardless of the rate of population turnover.

Native Species: A species restricted to and only known to naturally occur within a specific geographic area.

Nonnative Species: A species in a specific geographic area with no known documentation of its occurrence.

Open Water: A flooded or ponded area that does not support rooted vegetation. Deep water (>1.8 m deep) or frequent, rapid fluctuation in water depth are usually the cause for the lack of vegetation.

Oviposition (sites): The act of egg-laying and/or the location where eggs are laid.

Population: A group of individuals of the same species inhabiting a given geographic area at the same time and among which mature individuals interbreed or are likely to interbreed. Ecological interactions and genetic exchange are more likely among individuals within a population than with individuals in other populations of the same species.

Potential Habitat: Habitat that is lacking one or more of the constituent elements necessary to support a relict leopard frog population or surveys have not been conducted at the site to assess the status of the site. When a site has been surveyed and is lacking one or more of the constituent elements, such a site could support a population of relict leopard frogs if they were enhanced to establish that element.

Range: The geographic area a species is known or believed to occupy.

Reintroduction: Intentional re-release of individuals into an area not formerly occupied by that species.

Repatriation: Intentional release of individuals into an area formerly occupied by that species.

Riparian: Vegetation or other resources associated with a river or spring system that are dependent on groundwater and floodwater controlled by the river or spring. Common riparian land cover types in the historical range of relict leopard frogs are cottonwood-willow, honey mesquite, salt cedar, salt cedar-honey mesquite, salt cedar-screwbean mesquite, marsh, and arrowweed land cover types.

Saltation: Jumping motions used by anurans for locomotion.

Succession: The change in the composition and structure of a biological community over time in the absence of major disturbance (e.g. fire, flood, land clearing by humans). For example, deep open water in a backwater may gradually fill over time with organic and inorganic material and become colonized by marsh species (e.g. cattail and bulrush). The marsh may eventually be succeeded by riparian forest of willows and cottonwoods. A major flood event could scour out the backwater site, returning it to an open water condition.

SUL: Snout-urostyle length – the length of a frog measured from the anterior end the snout to the posterior end of the urostyle.

Translocation: Intentional release of individuals of a species in an attempt to introduce, reintroduce, repatriate, or augment a population.

Unoccupied Habitat: Sites that support all of the constituent elements necessary for relict leopard frogs, but where surveys have determined the species is not currently present. The lack of individuals or populations in the habitat is assumed to be the result of reduced numbers or distribution of the species such that some habitat areas are unused. It is expect that these areas would be used if species numbers or distribution were greater. See also definition of "habitat" and potential habitat."

Viable Population: A population of relict leopard frogs with the ability to survive into the foreseeable future.

APPENDIX 1: PECE CRITERIA: CERTAINTY OF IMPLEMENTATION AND EFFECTIVENESS

PECE criteria: Certainty - Implementation	Location in Document
1. The conservation effort, the party(ies) to the agreement or plan that will implement the effort, and the staffing, funding level, funding source, and other resources necessary to implement the effort are identified	Can be found on pp 9-10, 16-17, and in Implementation Schedule (IS) pp 70-78
2. The legal authority of the party(ies) to the agreement or plan to implement the formalized conservation effort, and the commitment to proceed with the conservation effort are described	Can be found on pp 10-17, and Appendix 2 (Legal Framework) pp 96-109
3. The legal procedural requirements (e.g. environmental review) necessary to implement the effort are described, and information is provided indicating that fulfillment of these requirements does not preclude commitment to the effort	Can be found on pp 15, 65, and Appendix 3 (Mitigation Protocol) pp 110-114
4. Authorizations (e.g., permits, landowner permission) necessary to implement the conservation effort are identified, and a high level of certainty is provided that the party(ies) to the agreement or plan that will implement the effort will obtain these authorizations	Can be found on pp 10-17, and Appendices 2 (Legal Framework) and 3 (Mitigation Protocol) pp 96-114
5. The type and level of voluntary participation necessary to implement the conservation effort is identified, and a high level of certainty is provided that the party(ies) to the agreement or plan that will implement the conservation effort will obtain that level of voluntary participation	Can be found on pp 56
6. Regulatory mechanisms (e.g., laws, regulations, ordinances) necessary to implement the conservation effort are in place	Can be found on pp 10-17, 65, and Appendix 2 (Legal Framework), pp 96-109
7. A high level of certainty is provided that the party(ies) to the agreement or plan that will implement the conservation effort will obtain the necessary funding	Can be found on pp 10-17, Appendix 2 (Legal Framework), pp 96-109, and in the Implementation Schdule pp 70-78. Work plans (Appendices 6-8) include MSHCP funded projects.
8. An implementation schedule (including incremental completion dates) for the conservation effort is provided requirements/sources.	Can be found in the Implementation Schedule, pp 70-78. Appendices 6 and 7 show current accomplishments of the RLFCT.
9. The conservation agreement or plan that includes the conservation effort is approved by all parties to the agreement or plan	Can be found on pp 16-17

PECE: Certainty – Effectiveness	Location in Document
1. The nature and extent of threats being addressed by the conservation effort are described, and how the conservation effort reduces the threats is described	Can be found on pp 33-62. Appendices 6 and 7 show current accomplishments of the RLFCT.
2. Explicit incremental objectives for the conservation effort and dates for achieving them are stated	Objectives can be found on pp 51, and dates for achieving those objectives can be found in the Implementation Schedule pp 70-78
3. The steps necessary to implement the conservation effort are identified in detail.	Can be found in the Stepdown Outline on pp 55-62, and in the Implementation Schedule pp 70-78
4. Quantifiable, scientifically valid parameters that will demonstrate achievement of objectives, and standards for these parameters by which progress will be measured, are identified.	Can be found on pp 51-54, and in Appendix 5 (Protocols and Techniques Manual) pp 118-150
5. Provisions for monitoring and reporting progress on implementation (based on compliance with the implementation schedule) and effectiveness (based on evaluation of quantifiable parameters) of the conservation effort are provided	Can be found on pp 53-54, in the Implementation Schedule pp 70-78, and in Sections 1 and 8 of Appendix 5 (Protocols and Techniques Manual) pp 121, 135-146
6. Principles of adaptive management are incorporated	Can be found on pp 53-54, and in Implementation Schedule pp 70-78

APPENDIX 2: LEGAL FRAMEWORK

The relict leopard frog and the habitats upon which it depends are protected by State and Federal laws, policies and regulations. Many if these do not mention the species specifically, but they provide a clear legal framework for protection, conservation and restoration of any native species and the habitat they need to survive. Some State laws in Arizona, Utah, and Nevada are directly applicable, but most of the legal authority is delegated by the legislatures to the State wildlife commissions, which have enacted more specific regulations. Similarly, some Federal laws are directly applicable, but the authority to promulgate more specific regulations has been delegated to various departments, agencies and local jurisdictions (e.g. National Park Service units). Because the legal framework is extensive, complicated, and often redundant, this Appendix provides a detailed summary of relevant State and Federal laws, rules, and regulations.

STATE LEGAL PROTECTION

Nevada

The relict leopard frog is classified as Protected by the State of Nevada (Nevada Administrative Code (NAC 503.075)). Collection or hunting relict leopard frogs in Nevada is prohibited (NAC 503.090, 503.093), except when done under the authority of a special permit. Nevada Revised Statues (NRS) protect wildlife use of springs and seeps (NRS 533.367). Legal protections afforded to the relict leopard frog in Nevada include, but are not limited to the following State laws and regulations:

Nevada Revised Statutes

NRS 501.182 – The Commission may enter into cooperative agreements with adjacent states for the management of interstate wildlife populations.
NRS 503.587 – The Commission shall use its authority 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.
NRS 533.023 – As used in this chapter, “wildlife purposes” includes the watering of wildlife and the establishment and maintenance of wetlands, fisheries and other wildlife habitats.
NRS 533.367 – 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.

Nevada Administrative Code – Chapter 503 Hunting, Fishing and trapping; Miscellaneous Protective Measures

NAC 503.075 – Amphibians: Classification. (NRS 501.105, 501.110, 501.181)

1. Amphibians are classified as game, protected, threatened, sensitive, endangered or unprotected amphibians.
2. The following amphibians are classified as game amphibians:
   Bullfrog (*Rana catesbeiana)*
3. The following amphibians are classified as protected:
   Relict leopard frog (*Rana onca*)
   Spotted frog (*Rana luteiventris*)
   Amargosa toad (*Bufo nelsoni*)
4. Unprotected amphibians are all species of amphibians which are not classified as game, protected, threatened, sensitive or endangered amphibians.

NAC 503.090 – Seasons; Protected wildlife; Unprotected wildlife.

1. There is no open season on those species of wild animal, wild bird, fish, reptile or amphibian classified as protected.
2. There is no closed season on those species of wild animals or wild birds classified as unprotected.

NAC 503.093 – License, permit or authorization to capture, kill or possess protected wildlife.

1. Except as otherwise provided in subsection 2, a person shall not capture or kill any wildlife which is classified as protected, or possess any part thereof, without first obtaining the appropriate license, permit or written authorization from the division.

NAC 503.094 Scientific permit for collection or possession of wildlife. (NRS 501.105, 501.181, 503.650)

1. The Department may issue a scientific permit pursuant to *NRS 503.650* which authorizes the taking, killing, possessing or banding of any species of wildlife, or the collecting of the nest or eggs thereof, for strictly scientific or educational purposes.
2. …
3. …
4. Not later than 30 days after the date on which the permit expires, the holder of a scientific permit shall submit to the Department a complete report which details the species of wildlife collected, the number of each species of wildlife collected at each location, the date on which each species of wildlife was collected and any other information which the Department requires.
5. Based on its evaluation of the application, the Department may make such stipulations and conditions on the use and scope of a scientific permit as the Department determines appropriate. A violation of a stipulation or condition is cause for the cancellation of the permit.

NAC 503.110 – Restrictions on importation, transportation, and possession of certain species.

1. Except as otherwise provided in this section and NAC 504.486, the importation, transportation or possession of the following species of live wildlife or hybrids thereof, including viable embryos or gametes, is prohibited:
   a) Fish: 23 species and/or genera including tilapia, walking catfish, etc.

b) Reptiles: 10 species and/or other taxa including all snapping turtles (all species in the family Chelydridae.
c) Amphibians: 2 taxa, clawed frogs (genus *Xenopus*) and Giant or Marine toads (*Bufo marinus, Bufo horribilis, and Bufo paracnemis)*

Arizona

The relict leopard frog is considered as Wildlife of Special Concern by the State of Arizona (Arizona Game and Fish Department, 1996). Commission Order 41 of the Arizona Game and Fish Department (Arizona Reptile and Amphibian regulations) prohibits collection or hunting of relict leopard frogs in Arizona, except when done under the authority of a special permit. Legal Protections afforded to the relict leopard frog in Arizona by State laws and regulations include the following:

Arizona Revised Statutes (ARS) Title 17

ARS Sec. 17-101- Definitions

A.21 "Wildlife means all wild mammals, wild birds, and the nests or eggs thereof, reptiles, amphibians, mollusks, crustaceans, and fish, including their eggs or spawn.

B. 1. Aquatic wildlife are all fish, amphibians, mollusks, crustaceans, and soft-shelled turtles.

ARS Sec. 17-306 – Importation, transportation, release or possession of live wildlife

No person shall import or transport into the state or sell, trade or release within the state or have in his possession any live wildlife except as authorized by the Commission.

Commission Order 41

D. There is no open season on *Rana tarahumarae* (Tarahumara frog), *Rana blairi* (plains leopard frog), *Rana chiricahuensis* (Chiricahua leopard frog), *Rana pipiens* (northern leopard frog), *Rana yavapaiensis* (lowland leopard frog), *Rana onca* (relict leopard frog) or *Rana subaquavocalis* (Ramsey Canyon leopard frog).

Arizona Game and Fish Commission Article 4:

Live Wildlife: Prohibited Acts
R12-4-402.A - A person shall not import or transport any live wildlife into the state, or possess, offer for sale, sell, sell as live bait, trade, give away, purchase, rent or lease, display for any purpose, propagate, stock, or release within the state any live wildlife, or export any live wildlife, or kill any captive wildlife, or operate a shooting preserve, except as authorized by this Chapter or as defined in A.R.S. Title 3, Chapter 16. A person may exhibit lawfully possessed wildlife only as authorized by this Chapter or as defined in A.R.S. Title 3, Chapter 16.

Restricted Live Wildlife
R12-4-406.A - With the exception of all live cervids, which shall not be imported, transported, or possessed except as allowed under R12-4-430, a special license or an exemption under Article 4 is required to possess restricted live wildlife or to engage in any activity prohibited by A.R.S. § 17-306 or R12-4-402.

R12-4-406.E – Amphibians listed below are “restricted live wildlife” as defined in R12-4-401.

1. The following species within the order Anura. Common names: frogs and toads.
    a) All species of the genus *Xenopus*. Common name: clawed frogs.
    b) The species *Bufo horribilis, Bufo marinus, Bufo paracnemis.* Common names: giant or marine toads.

<u>Utah</u>

Relict leopard frog is classified as a Sensitive Species in Utah (Utah Division of Wildlife Resources 1997). Although this classification does not afford any regulatory protection, it does help direct State and Federal agency actions by drawing attention to the status and conservation needs of the species. Legal protections afforded by State laws and regulations in Utah include the following:

Specific Rules and Policies

State of Utah Rule 657-53 prohibits the collection, importation, and possession of relict leopard frog without a certificate of registration. If the species was rediscovered or reintroduced into Utah, these restrictions would protect it against significant population depletion due to harvest for commercial, scientific, recreational, or educational use. The Utah Division of Wildlife Resources may issue a certificate of registration to a qualified organization or individual for scientific or educational use only. Issuance may occur only if the use of the animals will benefit the species or will significantly benefit the general public without material detriment to species. Rule 657-53 also regulates the collection, importation, transportation, and possession of amphibians and reptiles. This includes other ranid frogs the might prey upon, out compete or hybridize with the relict leopard frog.

Rule 657-3 regulates the collection, importation, transportation and possession of live species other than amphibians and reptiles such as crayfish that might prey upon or compete with relict leopard frogs. Prevention of the anthropogenic spread of crayfish into uninfested waters is regulated by R657-13, which states live crayfish may not be transported away from the waters where they were captured.

Procedures and guidelines for nonnative fish stocking in Utah have been established to prevent negative impacts to native aquatic species, including relict leopard frog. 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 sport fish recreation is to be consistent with conservation and interagency agreements.

Wildlife Resources Code of Utah

23-14-21. Transplants of big game, turkeys, or sensitive species.

(1) The division may transplant big game, turkeys, or sensitive species only in accordance with:

(a) a list of sites for the transplant of a particular species that is prepared and adopted in accordance with Subsections (2) through (5);

(b) a species management plan, such as a deer or elk management plan adopted under Section 23-16-7 or a recovery plan for a threatened or endangered species, provided that:

(i) the plan identifies sites for the transplant of the species or the lands or waters the species are expected to occupy; and

(ii) the public has had an opportunity to comment and make recommendations on the plan; or

(c) a legal agreement between the state and a tribal government that identifies potential transplants.

(2) The division shall:

(a) consult with the landowner in determining the suitability of a site for the transplant of a species;

(b) prepare a list of proposed sites for the transplant of species;

(c) provide notification of proposed sites for the transplant of species to:

(i) local government officials having jurisdiction over areas that may be affected by a transplant; and

(ii) the Resource Development Coordinating Committee created in Section 63-28a-2.

(3) After receiving comments from local government officials and the Resource Development Coordinating Committee, the division shall submit the list of proposed transplant sites, or a revised list, to regional advisory councils for regions that may be affected by the transplants of species.

(4) Each regional advisory council reviewing a list of proposed sites for the transplant of species may submit recommendations to the Wildlife Board.
(5) The Wildlife Board shall approve, modify, or reject each proposal for the transplant of a species.
(6) Each list of proposed transplant sites approved by the Wildlife Board shall have a termination date after which a transplant may not occur.

23-15-7. Taking protected aquatic wildlife or eggs unlawful except as authorized.
It is unlawful for any person to take any protected aquatic wildlife or eggs of same in any of the waters of the state, except as provided by this code or the rules and regulations of the Wildlife Board.

23-22-1. Cooperative agreements and programs authorized.
(1) The Division of Wildlife Resources may enter into cooperative agreements and programs with other state agencies, federal agencies, states, educational institutions, and individuals for the purposes of wildlife conservation.
(2) Cooperative agreements that are policy in nature must be:
(a) approved by the executive director of the Department of Natural Resources; and
(b) reviewed by the Wildlife Board.

State of Utah Division of Wildlife Resources Rule
Amphibian and Reptile Collection, Importation and Possession (R657-53)

R657-53-14. Collection of a Live or Dead Amphibian or Reptile for Personal, Scientific, or Educational Use.
(1) A person may collect a live or dead amphibian or reptile or their parts for a personal, scientific or educational use only as provided in Subsection (a) or (b).
(a) Certificates of registration are not issued for the collection of any live or dead amphibian or reptile or their parts classified as prohibited for collection, except as provided in Subsection (b) and R657-53-27.
(b) The division may issue a certificate of registration to a university, college, governmental agency, bona fide nonprofit institution, or a person involved in wildlife research to collect a live or dead amphibian or reptile classified as prohibited for collection if, in the opinion of the division, the scientific or educational use is beneficial to wildlife or significantly benefits the general public without material detriment to wildlife.
(2) A certificate of registration is required for collecting any live or dead amphibian or reptile or their parts classified as controlled for collection, except as otherwise provided by the Wildlife Board.
(3) A certificate of registration is not required for collecting a live or dead amphibian or reptile or their parts classified as noncontrolled for collection, except as provided in Subsections R657-53-18(3) and (4) and R657-53-19(5).

R657-53-15. Importation or Possession of a Live or Dead Amphibian or Reptile for Personal, Scientific, or Educational Use.

(1) A person may import or possess a live or dead amphibian or reptile or their parts for a personal, scientific or educational use only as provided in Subsections (a) or (b).

(a) Certificates of registration are not issued for the importation of any live or dead amphibian or reptile or their parts classified as prohibited for importation or for the possession of any live or dead amphibian or reptile or their parts classified as prohibited for possession, except as provided in Subsection (b) and R657-53-27.

(b) The division may issue a certificate of registration to a university, college, governmental agency, bona fide nonprofit institution, or a person involved in wildlife research to import a live or dead amphibian or reptile classified as prohibited for importation or to possess a live or dead amphibian or reptile classified as prohibited for possession if, in the opinion of the division, the scientific or educational use is beneficial to wildlife or significantly benefits the general public without material detriment to wildlife.

(2) A certificate of registration is required for importing any live or dead amphibian or reptile or their parts classified as controlled for importation or for possessing any live or dead amphibian or reptile or their parts classified as controlled for possession, except as otherwise provided by the Wildlife Board.

(3) A certificate of registration is not required for importing a live or dead amphibian or reptile or their parts classified as noncontrolled for importation or for possessing a live or dead amphibian or reptile or their parts classified as noncontrolled for possession, except as provided in Subsections R657-53-18(3) and (4) and R657-53-19(5).

(4) Notwithstanding Subsection (1) or (2), a person may import or possess any dead amphibian or reptile or its parts classified as prohibited or controlled, except as provided in Section R657-53-5, for a personal use without obtaining a certificate of registration, provided the animal was legally taken, is held in legal possession, and a valid license, permit, tag, certificate of registration, bill of sale, or invoice is available for inspection upon request.

R657-53-16. Collection, Importation or Possession of a Live Amphibian or Reptile for a Commercial Use.

(1) Pursuant to Sections 23-13-13 and 23-20-3, a person may not collect or possess a live amphibian or reptile for a commercial use or commercial venture for pecuniary gain, unless otherwise provided in this rule or a certificate of registration.

(2)(a) A person may import a live amphibian or reptile classified as non-controlled for importation or may possess a live amphibian or reptile classified as non-controlled for possession, for a commercial use or a commercial venture, except as provided in Subsection (b)

(b) A native or naturalized species of amphibian or reptile may not be sold or traded unless it originated from a captive-bred population.

(c) Complete and accurate records for native or naturalized species must be maintained and available for inspection for five years from the date of the transaction, documenting the date, and the name, address, and telephone number of the person from whom the amphibian or reptile has been obtained.

(d) Complete and accurate records must be maintained and available for inspection for five years from the date of the transfer, documenting the date, and the name, address and certificate of registration number of the person receiving the amphibian or reptile.

(3)(a) A person may not import a live amphibian or reptile classified as controlled for importation or may not possess a live amphibian or reptile classified as controlled for possession for a commercial use or commercial venture without first obtaining a certificate of registration.

(b) A certificate of registration will not be issued to sell or trade a native or naturalized species of amphibian or reptile unless it originates from a captive-bred population.

(c) It is unlawful to transfer a live amphibian or reptile classified as controlled for possession to a person who does not have a certificate of registration to possess the amphibian or reptile, except as follows:

(i) the amphibian or reptile is captive-bred;

(ii) the transferee is not domiciled in Utah;

(iii) the transferee is exporting the amphibian or reptile out of Utah; and

(iv) the transferee follows the transport provisions in Section R657-53-20.

(d) Complete and accurate records must be maintained by the buyer and the seller for five years from the date of the transaction or transfer, documenting the date, and the name, address, and telephone number of the person from whom the amphibian or reptile has been obtained and the person receiving the amphibian or reptile.

(e) The records indicated in Subsection (d) must be made available for inspection upon request of the division.

(4)(a) A certificate of registration will not be issued for importing a live amphibian or reptile, classified as prohibited for importation, or for possessing a live amphibian or reptile, classified as prohibited for possession, for a commercial use or commercial venture, except as provided in Subsection (b) or R657-53-27.

(b) The division may issue a certificate of registration to a zoo, circus, amusement park, aviary, or film company to import or possess a live amphibian or reptile classified as prohibited for importation or possession if, in the opinion of the division, the importation or possession for a commercial use is beneficial to wildlife or significantly benefits the general public without material detriment to wildlife.

(c) The division's authority to issue a certificate of registration to a zoo, circus, amusement park, or aviary under this Subsection is restricted to those facilities that keep the prohibited amphibian or reptile in a park, building, cage, enclosure or other structure for the primary purpose of public exhibition or viewing.

(5) It is unlawful to sell or trade any turtle, including tortoises, less than 4" in carapace length.

R657-53-18. Classification and Specific Rules for Amphibians.

(1) Amphibians are classified as follows:

(a) American bullfrog, Ranidae Family (*Rana catesbeiana*) is prohibited for collection, importation and possession, except as provided in Subsection (5);
(b) Clawed frog, Pipidae Family (*Xenopus*) (All species) is prohibited for collection, importation and possession;
(c) Columbia spotted frog, Ranidae Family (*Rana luteiventris*) is prohibited for collection, importation and possession;
(d) Green frog, Ranidae Family (*Rana clamitans*) is prohibited for collection, importation and possession, except as provided in Subsection (5);
(e) Lowland leopard frog, Ranidae Family (*Rana yavapaiensis*) is prohibited for collection, importation and possession;
(f) Northern leopard frog, Ranidae Family (*Rana pipiens*) is controlled for collection, importation and possession;
(g) Pacific treefrog, Hylidae Family (*Pseudacris regilla* or *Hyla regilla*) is controlled for collection, importation and possession;
(h) Relict leopard frog, Ranidae Family (*Rana onca*) is prohibited for collection, importation and possession;
(i) Tiger salamander, Ambystomatidae Family (*Ambystoma tigrinum*) is controlled for importation, and noncontrolled for collection and possession as provided in Subsection (4);
(j) Arizona toad, Bufonidae Family (*Bufo microscaphus*) is controlled for collection, importation and possession;
(k) Cane (marine) toad, Bufonidae Family (*Bufo marinus*) is prohibited for collection, importation and possession; and
(l) Western toad, Bufonidae Family (*Bufo boreas*) is prohibited for collection, importation and possession.

(2) All species and subspecies of amphibians not listed in Subsection (1) are classified as noncontrolled for collection, importation and possession, except as provided in Subsection (3).
(3) A person must obtain a certificate of registration to collect four or more amphibians of each species classified as noncontrolled within a calendar year, except as provided in Subsection (4) and (5).
(4) A person may collect or possess for personal use up to 50 Tiger salamanders (*Ambystoma tigrinum*) without a certificate of registration.
(5) A person may collect or possess any number of American bullfrogs (*Rana catesbeiana*) or Green frogs (*Rana clamitans*) without a certificate of registration provided they are either killed or released immediately upon removing them from the water. A person may not transport a live bullfrog or green frog from the water from which it was collected without first obtaining a certificate of registration.

Collection, Importation, Transportation, and Possession of Zoological Animals (R657-3)

R657-3-22. Classification and Specific Rules for Invertebrates.
(1) Crustaceans are classified as follows:

(a) Asiatic (Mitten) Crab, Grapsidae Family (*Eriocheir*, All species) are prohibited for collection, importation and possession;
(b) Brine shrimp, Mysidae Family (All species) are classified as controlled for collection, and noncontrolled for importation and possession;
(c) Red-claw crayfish, Astacidae Family (*Cherax quadricarinatus*) is prohibited for collection, and controlled for importation and possession;
(d) Crayfish, Astacidae, Cambaridae and Parastacidae Families (All species except *Cherax quadricarinatus*) are prohibited for collection, importation and possession;

Taking Fish and Crayfish (R657-13)

R657-13-12 (4) Use of live crayfish for bait is legal only on the water where the crayfish is captured. It is unlawful to transport live crayfish away from the water where captured.

FEDERAL REGULATORY PROTECTION

The Federal Land Policy Management Act of 1976 (43 U.S.C. 1701 et seq.) and the statutory requirements contained in 16 USC Ia-7(b) direct agencies to prepare management plans to guide management decisions.

The National Environmental Policy Act of 1969 (NEPA) (42 U.S.C.4321-4370a) requires Federal agencies and other entities using Federal funds 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 National Park Service, the Bureau of Land Management, and other Federal agencies that affect the relict leopard frog are subject to the NEPA process.

The Lacey Act (16 U.S.C. 3371 et seq.), as amended in 1982, provides some protection for the relict leopard frog. This legislation prohibits the import, export, sale, receipt, acquisition, purchase, and engagement in interstate or foreign commerce of any species taken, possessed, or sold in violation of any law, treaty, or regulation of the US, any Tribal law, or any law or regulation of any State.

Additional legal protections are afforded to the relict leopard frog by the National Park Service through various Federal laws and regulations pertaining to the national park system as a whole as well as laws and regulations specific to the Lake Mead National Recreation Area. Some of those regulations are outlined below:

The National Park Service was established by an act of Congress passed in 1916 generally referred to as "The Organic Act" (16 USC I). This law states that it is the mission of the National Park Service 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.

As the National Park System grew, it became more diverse. The original "parks" and "monuments" were followed by military parks, seashores, recreation areas, etc. creating a confusing array of over 20 different designations for various units of the National Park System. In 1970, Congress responded by amending the Organic Act to state that it was the intent of Congress to establish a nationwide system of parks, and that each unit was to be administered not only under the terms of its own authorizing law, but also under a unified standard. They would all fall under the same systemwide laws, "including but not limited to the" Organic Act. This position was reaffirmed in 1978 with yet another amendment to the Organic Act, commonly referred to as the Redwood Act.

Lake Mead National Recreation Area was established as a unit of the National Park System by the 88th Congress through Public Law 88-639 passed on October 8, 1964. This law includes provisions for certain activities such as hunting and fishing specific to the Recreation Area, but other guidance for management of the area derives from systemwide policies, procedures and regulations. Title 36 of the Code of Federal Regulations (CFR) Parks Forests and Public Property contains regulations specific to the National Park System. Some of the regulations that provide protection for *R. onca* populations found on NPS lands at Lake Mead NRA include, but are not limited to the following:

CFR Title 36 Chapter 1 – National Park Service, Department of the Interior

Part 2 – Resource Protection, Public Use and Recreation

§ 2.1 Preservation of natural, cultural and archeological resources

a) Except as otherwise provided in this chapter, the following is prohibited:
   1) Possessing, destroying, injuring, defacing, removing, digging, or disturbing from its natural state:
      i) Living or dead wildlife or fish, or the parts or products thereof, such as antlers or nests.
      ii) Plants or the parts or products thereof.
   2) Introducing wildlife, fish or plants, including their reproductive bodies, into a park area ecosystem.

§2.2 Wildlife Protection

a) The following are Prohibited:
   1) The taking of wildlife, except by authorized hunting and trapping activities…
   2) The feeding, touching, teasing, frightening or intentional disturbing of wildlife nesting, breeding or other activities.

3) Possessing unlawfully taken wildlife or portions thereof.

d) The following are prohibited:

1) Fishing in fresh waters in any manner other than by hook and line, with the rod or line being closely attended.
2) Possessing or using as bait for fishing in fresh waters, live or dead minnows or other bait fish, amphibians, nonpreserved fish eggs or fish roe, except in designated waters. Waters which may be so designated shall be limited to those where nonnative species are already established, scientific data indicate that the introduction of additional numbers or types of nonnative species would not impact populations of native species adversely, and park management plans do not call for elimination of nonnative species.
3) Chumming or placing preserved or fresh fish eggs, fish roe, food, fish parts, chemicals, or other foreign substances in fresh waters for the purpose of feeding or attracting fish in order that they may be taken.
4) Commercial fishing, except where specifically authorized by Federal statutory law.
5) Fishing by the use of drugs, poisons, explosives, or electricity.
6) Digging for bait, except in privately owned lands.

f) Authorized persons may check fishing licenses and permits; inspect creels, tackle and fishing gear for compliance with equipment restrictions; for compliance with species, size and other taking restrictions.

g) The regulations contained in this section apply, regardless of land ownership, on all lands and waters within a park area that are under the legislative jurisdiction of the United States.

§2.4 Weapons, traps and nets.

a) (1) Except as otherwise provided in this section and Parts 7 (special regulations) and 13 (Alaska regulations), the following are prohibited:

(i) Possessing a weapon, trap or net
(ii) Carrying a weapon, trap or net
(iii) Using a weapon trap or net

(2) Weapons, traps or nets may be carried, possessed or used:

(i) At designated times and locations in park areas where:

(A) The taking of wildlife is authorized by law in accordance with §2.2
(B) The taking of fish is authorized by law in accordance with §2.3

INTERNATIONAL PROTECTION

The relict leopard frog is not protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora, which regulates international trade.

OTHER CONSERVATION MEASURES AFFORDING PROTECTION TO THE RELICT LEOPARD FROG

Virgin Spinedace Conservation Agreement and Strategy

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 (*Micropterus salmoides*), 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.

Lower Colorado River Multi-Species Conservation Program (LCR MSCP)

The LCR MSCP is being developed by Arizona, California, Nevada, and the Department of the Interior. The LCR MSCP is intended to reduce conflicts between protecting listed species and conducting economic development activities by integrating land and water use activities with conservation goals (SAIC/Jones and Stokes 2003). The plan provides long-term mitigation to offset any incidental take of listed and sensitive species that may occur as a result of the actions and programs of Federal, State, tribal, and other resource users within the LCR MSCP planning area, while contributing to recovery and conservation of those species. The LCR MSCP specifically addresses *R. onca* in section 5.4.23, and sets forth the following goal: "MSCP program activities will assist and contribute to existing relict leopard frog research and conservation programs where appropriate. In particular, the MSCP will contribute $10,000 per year for 10 years to support implementation of planned, but unfunded, conservation measures for the relict leopard frog."

Clark County Multi-Species Habitat Conservation Plan (MSHCP)

The MSHCP provides for conservation of 78 plant and animal species, including *R. onca*, and their habitats throughout Clark County. The permit issued by the USFWS under the authority of Section 10(a)(1(B) of the ESA to Clark County authorizes take of listed species on no more than 145,000 acres of non-Federal land over a 30-year period. Disturbance fees collected from developers fund conservation actions for the covered species on Federally-managed land to offset impacts from development on non-Federal land in Clark County. Conservation actions include public information and education, research, inventory and monitoring, protective measures, and habitat restoration and enhancement. Specifically, the permit requires Clark County to participate with the Federal land management agencies in the development of conservation management plans for certain areas or covered species, including desert riparian habitats, such as the Muddy and Virgin rivers, Meadow Valley Wash, and low elevation springs, which contain amphibians and aquatic snails.

Recovery Plan for the Rare Aquatic Species of the Muddy River Ecosystem

The recovery plan for the Muddy River ecosystem was written after the ESA listing of the Moapa dace and covers 7 other sensitive species in 9.5 km of stream habitat in 5 thermal headwater spring systems and the main stem of the upper Muddy River (USFWS 1995). This plan does not specifically list the relict leopard frog, but conservation measures aimed at the covered species (e.g. removal of nonnative fish) should benefit relict leopard frogs.

APPENDIX 3: MITIGATION PROTOCOL

A. MITIGATION

Mitigation measures should, to the extent practicable, minimize effects of proposed projects on the relict leopard frog and its habitat. In order of preference, mitigation should avoid, minimize, rectify, reduce, and/or compensate for the impacts of a project. The objective of mitigation should be no net loss of frog habitat quantity and quality, and maintenance or enhancement of movement corridors among populations and future reestablishment sites. The following mitigation measures shall be incorporated into all projects where applicable. The measures are to be modified to conform to the nature of the project.

LIVESTOCK, BURROS

Manage cattle/burro use to diversify the habitat; provide a refuge for egg masses, tadpoles, and frogs from trampling and cattle/burro-caused water quality problems, and to prevent loss of open-water habitats. Potential measures include but are not limited to partial fencing and fencing during breeding season. Options for measures such as permanent fencing with periodic vegetation treatments may be needed at some sites on a case-by-case basis.

SURFACE-DISTURBING CONSTRUCTION PROJECTS

1. To the extent possible, surface-disturbing projects shall be located outside of occupied relict leopard frog habitat, reestablishment sites, and the immediate watersheds of such habitats. If a project must be located in habitats or in the immediate watershed of habitat, effort shall be made to locate the project in a previously disturbed area, in an area where habitat quality is poor, or where impacts to the frog and habitat will be minimized. A survey of the project site shall be conducted prior to construction in order to assist in locating the project. Prior to project initiation, an individual from the RLFCT and appropriate lead agency shall be designated as the field contact representative. The field contact representative shall have the authority to ensure compliance with protective measures for the relict leopard frog and will be the primary agency contact dealing with these measures. The field contact representative shall have the authority and responsibility to halt activities that are in violation of mitigation measures.

2. All project work areas shall be clearly flagged or similarly marked at the outer boundaries to define the limit of work activities. All construction and restoration workers shall restrict their activities and vehicles to areas that have been flagged to eliminate adverse impacts to the relict leopard frog and its habitat. All workers shall be instructed that their activities are restricted to flagged and cleared areas.

3. Within relict leopard frog habitats, the area of disturbance of vegetation, soils, and water shall be the minimum required for the project. If possible, specify a maximum disturbance allowable based on the specifics of the project. Project activities shall be located out of wetted sites to the extent practicable. Locate equipment staging areas, borrow sites, and material stockpiles well away from occupied habitat. Clearing of vegetation and grading shall be minimized. Wherever possible, rather than clearing vegetation and grading, equipment and vehicles shall use existing surfaces or previously disturbed areas. Shrubs that cannot be avoided should be crushed rather than graded out of the way, if possible. Where grading is necessary, surface soils shall be stockpiled and replaced following construction to facilitate habitat restoration. Soils shall be stockpiled outside of riparian and wetland areas, and shall not be placed upstream or upslope of such sites.

4. Existing roads shall be used for travel and equipment storage whenever possible.

5. Where feasible and desirable, in the judgment of the field contact representative, newly created access routes in the action area shall be restricted by constructing barricades, erecting fences with locked gates at road intersections, and/or by posting signs. In these cases the project proponent shall maintain, including monitoring, all control structures and facilities for the life of the project and until habitat restoration is completed.

6. Measures shall be designed and implemented to ensure hazardous materials, including, but not limited to, pesticides, fuels, oil, and other chemicals are stored well away and not upstream of frog habitats. Use of such materials shall not occur in frog habitats and only in such a way that these materials do not enter frog habitats. If use of such materials is necessary, only use those that have been approved for use in aquatic systems and that have known effects on amphibians where possible. Measures shall be taken to avoid or minimize runoff into and sedimentation of frog habitats.

7. A biological monitor, approved by the action agency, shall be present in each area of active surface disturbance occurring in frog habitat, or in the immediate watershed of such habitats. Monitors shall remain onsite throughout the work day from initial clearing through habitat restoration. The monitor(s) shall perform the following functions:
   a) Develop and implement a worker education program. Wallet-cards summarizing this information may be provided to all construction and maintenance personnel. The education program shall include the following aspects at a minimum:
      - biology and status of the relict leopard frog,
      - protection measures designed to reduce impacts to the species and its habitat,
      - function of flagging designating authorized work areas, and
      - reporting procedures to be used if a frog is encountered on project sites.
   b) Ensure that all project-related activities are in compliance with these measures. The biological monitor shall have the authority and responsibility to halt activities that are in violation of agreed upon mitigation measures.

c) Monitor frog habitats in the action area periodically to ensure effects are minimized. In addition, all hazardous sites (e.g. open pipeline trenches, holes, or other deep excavations) shall be inspected for the presence of frogs prior to backfilling.

8. Work with the project supervisor to take steps, as necessary, to avoid disturbance to relict leopard frogs and their habitat. For example, if stream crossings by trucks or other heavy equipment are required, have monitors check for egg masses, frogs, and tadpoles. If avoiding disturbance to a frog, egg mass, or tadpole is not possible, or if a frog is found trapped in an excavation, the affected animals shall be captured and relocated, or held for release at a suitable facility following cessation of project activities as designated by the field contact representative. Affected animals should not be held in captivity for more than 1 year, and should not be relocated more than 1.6 km away from the point of capture unless otherwise designated by the field contact representative.

9. Take measures as needed to minimize the risk of disease transmission associated with construction projects. If vehicles/equipment use will occur in more than one frog habitat, ensure that all equipment is clean and dry or disinfected before it moves to another habitat (if the presence/absence of the disease is well known in the area, these rules could be varied).

RECREATIONAL DEVELOPMENTS, ACTIVITIES

1. Avoid promoting public use (i.e. fishing, trails, campsites, OHVs, etc.) of occupied relict leopard frog habitat and reestablishment sites.

2. Sign and enforce public use regulations as needed to limit use. Provide the public with interpretive signage and brochures to explain the need for use restrictions.

3. Close routes/trails as needed.

4. Close areas around occupied habitats to OHV use and fishing.

MONITORING AND RESEARCH ACTIVITIES

Adhere to disease prevention protocols.

PROJECTS WITH LONG-TERM EFFECTS

Sites of permanent or long-term (greater than one year) effects, where continuing activities are planned that pose a hazard to frogs, may be enclosed with barrier fencing to prevent frogs from wandering onto the project site where they may be in harm's way. Barrier fencing should consist of flashing or other solid barrier material at least 0.3 m high and buried sufficiently to ensure gaps do not form under the barrier. Hardware cloth with a 0.6-cm mesh may also be used if the top is folded over and out, away from the project site, to prevent frogs from climbing over the barrier.

ADDITIONAL MEASURES FOR ROAD CONSTRUCTION OR UPGRADES

Construction of new roads within 0.5 km of occupied frog habitats or habitats selected for reestablishment shall include a frog barrier fence on each side of the road that is exposed to occupied frog habitat. In cases where such barriers could isolate populations, culverts shall be installed to facilitate movement of frogs under the road. Roads farther away than 0.5 km from occupied frog habitats may also need to be equipped with barrier fencing and culverts if the road would act as a substantial barrier to movement of frogs among populations, or to colonization of suitable habitats. Engineer and maintain roadways to minimize erosion/watershed degradation in the vicinity of suitable habitat. Also design them (or fence them) so as not to promote OHV use, camping near habitats, and other recreational activities that may adversely affect the frogs or their habitats. Barrier fences and culverts are measures to be considered in addition to those described above under "Surface-Disturbing Construction Projects".

RESTORATION FOR SURFACE-DISTURBING ACTIVITIES

A project-specific habitat restoration plan shall be developed by the project proponent under approval by the lead agency. The plan shall consider and include as appropriate the following methods: expansion or enhancement of affected wetlands, seeding or planting of plant species native to the project area, control of nonnative plants or animals (without pesticides), erosion control, or other measures, as appropriate. Generally, the restoration objective shall be to return the disturbed area to pre-project conditions, or at a minimum, to result in no net loss of frog habitat quality or quantity. The project proponent shall conduct periodic monitoring of the restored area. Restoration shall include eliminating any hazards to frogs created by construction, such as hazardous materials, areas of erosion, and holes or trenches in which frogs might become entrapped. Disturbance of existing perennial shrubs during restoration shall be minimized, even if such shrubs have been crushed by construction activities.

GROUNDWATER PUMPING, IMPOUNDMENTS, AND SURFACE WATER DIVERSIONS

To the extent possible, groundwater pumping, impoundments, and surface water diversions shall not be authorized by signatory agencies where they would adversely affect occupied relict leopard frog sites or reestablishment sites, unless such activities are unavoidable. If unavoidable, the action agency shall take every reasonable measure to ensure effects are mitigated to the maximum extent practicable. Mitigation measures will need to be tailored to each project, but may include:

1) Relocating the project to a site where effects are minimized
2) Minimizing the amount or duration of water pumped, diverted, or impounded
3) Providing replacement water to frog habitats to offset impacts
4) Temporarily relocating frogs if disturbance to hydrology is temporary
5) Replanting riparian and wetland vegetation if temporary impacts desiccate these plants

APPENDIX 4: SENSITIVE SPECIES THAT CO-OCCUR WITH *RANA ONCA*

Note: This list includes species that may be found in the *Rana onca* Management Zone and are: (1) listed as endangered or threatened, or candidates for listing under the Endangered Species Act, (2) listed as sensitive by the states of Nevada, Utah, or Arizona, and (4) USFWS bird species of concern.

<u>Plants</u>
Cliff jamesia (*Jamesia americana* var. *zionis*)
Grand Canyon evening primrose (*Camissonia specuicola* ssp. *hesperia*)
Holmgren milkvetch (*Astragalus holmgreniorum*)
Las Vegas bearpoppy (*Arctomecon california*)
Sticky buckwheat (*Eriogonum viscidulum*)
Threecorner milkvetch (*Astragalus geyeri* var. *triquetrus*)
Virgin River thistle (*Cirsium virginense*)
Zion daisy (*Erigeron sionis*)
Slender evening primrose (*Camissonia exilis*)
Rosy twotoned beardtongue (*Penstemon bicolor* ssp. *roseus*)

<u>Invertebrates</u>
Amargosa naucorid (*Peolcoris shoshone shoshone*)
California floater (*Anodonta californiensis*)
Desert springsnail (*Pyrgulopsis deserta*)
Grand Wash springsnail (*Pyrgulopsis bacchus*)
Grated tryonia (*Tryonia clathrata*)
MacNeil sooty wing skipper (*Pholisora gracielae*)
Moapa pebblesnail (*Fluminicola avernalis*)
Moapa warm spring riffle beetle (*Stenelmis moapa*)
Tiger beetle (*Cicindela oregona*)
Utah hydroporous diving beetle (*Hygrotus utahensis*)
Utah minute moss beetle (*Limnebius crassalus*)
Utah water scavenger beetle (*Chaetarthria utahensis*)
White desert snail (*Eremarionata immaculate*)

<u>Fish</u>
Bonytail (*Gila elegans*)
Desert sucker (*Catostomus clarki*)
Flannelmouth sucker (*Catostomus latipinnis*)
Humpback chub (*Gila cypha*)
Meadow Valley Wash desert sucker (*Catostomus clarki* ssp.)
Moapa dace (*Moapa coriacea*)
Moapa speckled dace (*Rhinicthyes osculus moapae*)
Moapa White River springfish (*Crenichthys baileyi moapae*)

Razorback sucker (*Xyrauchen texanus*)
Roundtail chub (*Gila robusta robusta*)
Virgin River chub (*Gila seminuda*)
Virgin spinedace (*Lepidomeda mollispinis*)
Woundfin (*Plagopterus argentissimus*)

Amphibians
Southwestern (= Arizona) toad (*Bufo microscaphus*)
Pacific treefrog (*Pseudacris regilla*)
Northern leopard frog (*Rana pipiens*)

Reptiles
Banded gila monster (*Heloderma suspectum cinctum*)
California kingsnake (*Lampropeltis getulus californiae*)
Utah banded gecko (*Coleonyx variegatus utahensis*)
Southwestern black-headed snake (*Tantilla hobartsmithi*)
Utah blind snake (*Leptotyphlops humilis humilis*)
Plateau striped whiptail (C*nemidophorus velox*)

Birds
American peregrine falcon (*Falco peregrinus anatum*)
Bald eagle (*Haliaeetus leucocephalus*)
Bell's vireo (*Vireo bellii*)
Belted kingfisher (*Ceryle alcyon*)
Black tern (*Chlidonias niger*)
Blue grosbeak (*Guiraca caerulea*)
Common yellowthroat (*Geothlypis trichas*)
Crissal thrasher (*Toxostoma crissale*)
Lewis' woodpecker (*Melanerpes lewis*)
Loggerhead shrike (*Lanius ludovicianus*)
Lucy's warbler (*Vermivora luciae*)
Osprey (*Pandion haijaetus*)
Phainopepla (*Phainopepla nitens*)
Southwestern willow flycatcher (*Empidonax trailii extimus*)
Summer tanager (*Piranga rubra*)
Vermilion flycatcher (*Pyrocephalus rubinus*)
Western burrowing owl (*Athene cunicularia*)
White-faced ibis (*Plegadis chihi*)
Yellow-billed cuckoo (*Coccyzus americanus*)
Yuma clapper rail (*Rallus longirostris yumanensis*)

Mammals
Allen's big-eared bat (*Indionycteris* [=*Plecotus*] *phyllotis*)
Big free-tailed bat (*Nyctinomops macrotis*)
Cactus mouse (*Peromyscus eremicus*)
California leaf-nosed bat (*Macrotus californicus*)
Cave myotis (*Myotis velifer*)
Desert kangaroo rat (*Dipodomys deserti*)
Desert shrew (*Notiosorex crawfordi*)
Fringed myotis (*Myotis thysanodes*)
Greater western mastiff bat (*Eumops perotis californicus*)
Long-eared myotis (*Myotis evotis*)
Long-legged myotis (*Myotis volans*)
Merriam's kangaroo rat (*Dipodomys merriami*)
Occult little brown bat (*Myotis lucifugus occultus*)
Pale Townsend's big-eared bat (*Corynorhinus townsendii pallescens*)
Pocketed free-tailed bat (*Nyctinomops femorosaccus*)
Ringtail (*Bassariscus astutus*)
Small-footed myotis (*Myotis ciliolabrum*)
Spotted bat (*Euderma maculatum*)
Virgin River montane vole (*Microtus montanus*)
Western (desert) red bat (*Lasiurus blossevillii*)
Western small-footed myotis (*Myotis ciliolabrum*)
Western yellow bat (*Lasiurus xanthinus*)
Yuma myotis (*Myotis yamanensis*)

APPENDIX 5: RELICT LEOPARD FROG PROTOCOLS AND TECHNIQUES MANUAL

A collection of protocols and techniques designed to accompany the Conservation Agreement and Rangewide Conservation Assessment and Strategy for the Relict Leopard Frog (*Rana onca*)

Prepared by the Relict Leopard Frog Conservation Team

October 2004

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INTRODUCTION

This document was created by the Relict Leopard Frog Conservation Team (RLFCT) to provide guidance to agencies and individuals working with Relict Leopard Frogs (*Rana onca).* Most, if not all, of the sections in this manual were at one time included as appendices to various drafts of the Conservation Agreement and Rangewide Assessment and Strategy for Relict Leopard Frog (CAS). However, due to the need for a working document that could be modified and updated through the principles of adaptive management, it was decided to remove these appendices from the CAS and bind them together into a techniques manual. It is intended that this manual will be reviewed and updated at least annually by the RLFCT to insure that best management practices for this species are readily available, and to insure that decisions are based on the most current data and agreed upon by the appropriate regulatory and scientific authorities.

SECTION 1: ANNUAL WORK PLANS AND REPORTS

As described in Section 1.4 of the Stepdown Outline in the Relict Leopard Frog CAS, an annual work plan will be developed by the RLFCT to guide management actions and an annual reporting process will be developed in order to track accomplishments, tie activities to the management program outlined in the CAS, and provide feedback necessary for an adaptive management process to function.

Annual Work Plan

The CAS calls for a minimum of two meetings per year of the RLFCT, one in the fall and one in the late winter. Annual work plans will be topics of discussion at both of these meetings. The fall meeting will primarily focus on reviewing the previous field season's accomplishments and organizing the reporting process, and the late winter meeting will use the annual report to create an appropriate work plan for the upcoming field season. In general, work plans will be simple lists of activities the group expects to accomplish during each calendar year.

During the creation of the list, or very soon thereafter, leads will be assigned to each project or activity on the list. Leads will be voluntary, but every attempt will be made to assign leads based on expertise, legal authority, and any other factors that the group feels are appropriate to make sure the tasks delineated in the plan get accomplished. The lead for each activity is then responsible to track that activity over the course of the year, so that he or she can report back to the RLFCT.

Annual Report

Individuals leading projects or major activities and other RLFCT members will provide input for the annual report to an Annual Report Coordinator appointed by the RLFCT, who will compile and edit these reports into a single annual report. The annual reports will be organized by projects and activities that were identified in the annual work plan and cite the appropriate strategies and actions in the Implementation Schedule (IS). Activities not identified in an annual work plan will be organized as they are in the IS, citing the appropriate strategies and actions from the IS. Project and activity leads are strongly encouraged to submit their contributions (digital submissions are requested) to the Annual Report Coordinator throughout the year, but no later than the end of September.

Sources of information that are appropriate to submit include, but are not limited to, emails, grant proposals, meeting notes, photos, field notes, reports, data, and journal articles. In addition to reporting progress implementing the strategies and actions of the CAS, the annual report will include all meeting notes from the reporting year.

When the Annual Report Coordinator receives input from conservation team members, she/he will compile the report, and distribute it for review by the RLFCT. Final edits will be made after the fall meeting of the RLFCT, at which time the report will be distributed to all signatory agencies and interested individuals. The NPS will maintain the administrative record.

SECTION 2: LOCATIONS SURVEYED FOR *RANA ONCA*

Updated July 2004

Numerous surveys have been conducted in habitats within the Potential Management Zone for *R. onca*. These surveys include those by a variety of researchers directed at various riparian dwelling taxa such as plants, fish, bats, and amphibians. The following list documents only those surveys directed specifically at detecting amphibians including *R. onca*. The surveys or reports documenting these surveys are arranged chronologically.

Vitt, L.J. and R.D. Ohmart. 1978. Herpetofauna of the Lower Colorado River: Davis Dam to the Mexican border. Western Foundation of Zoology 2(2): 35-72.

Vitt and Ohmart surveyed for amphibians and reptiles in the 5 miles on either side of the lower Colorado River. They surveyed the Colorado River between Davis Dam and the Mexican border. They did not find leopard frogs.

Platz, J.E. 1984. Status report for *Rana onca*. Unpublished report submitted to Office of Endangered Species, US Fish and Wildlife Service, Albuquerque, New Mexico. 27 pp.

Platz surveyed specifically for *R. onca* and other leopard frogs to determine taxonomy of ranid frogs at suitable aquatic sites (springs, streams, lakes, man-made ponds, and ditches with permanent water) in and around Las Vegas, Nevada and northeast along the Virgin River to the vicinity of St. George, Utah. Platz found no leopard frogs at Blue Point, Corral, and Rogers springs and determined, based on morphology, that frogs at Littlefield were lowland leopard frogs (*R. yavapaiensis*).

National Park Service – Lake Mead National Recreation Area, unpublished surveys 1991 (R.Haley).

Haley searched Blue Point, Corral, and Rogers springs for amphibians. Three new populations of *R. onca* were discovered.

Jennings, R.D., B.R. Riddle, and D.F. Bradford. 1995. Rediscovery of *Rana onca*, the relict leopard frog, in southern Nevada with comments on the systematic relationships of some southwestern leopard frogs (*Rana pipiens* complex) and the status of populations along the Virgin River. Report prepared for Arizona Game and Fish Dept., US Bureau of Land Management, Las Vegas Valley Water District, US National Park Service, and Southwest Parks and Monuments Association. 71 pp. (also includes Bradford et al. in prep., in review until published).

Field surveys were conducted at historical and other sites containing potential habitat for leopard frogs (e.g. permanent water with pools > 30 cm deep and > 1.6 km apart). The authors surveyed a total of 64 localities within the historical range of *R. onca*, 12 of which were historical localities for *R. onca*. Some historical localities were not searched because either habitat was 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.

Areas surveyed were as follows: within 22 km of St. George, Utah; Virgin River between approximately 6 km SW of Riverside, Nevada, and 5 km NE 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 SW 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).

Three populations were discovered in Nevada Black Canyon below Hoover Dam, and one population (previously identified as *R. yavapaiensis* by Platz) was found near Littlefield Arizona.

National Park Service – Lake Mead National Recreation Area, unpublished surveys 1996-2002 (S. Romin).

Romin searched all springs and seeps in the Overton Arm of Lake Mead, Gold Butte, Temple Bar, and Grapevine areas for leopard frogs. All surveys were within Lake Mead NRA boundaries. One occupied spring was discovered (Gnatcatcher Spring).

Utah Division of Wildlife Resources, unpublished surveys, 1998-2002 (K. Wheeler).

Surveys of springs, streams, rivers, and stock tanks in southern Utah for leopard frogs and other amphibians. A total of 149 surveys were completed on the Virgin River and tributaries (10 rivers/streams). Surveys were performed from either the confluence with a bigger stream or the state line and done every mile upstream. Each survey was 500 m long. No new populations of *R. onca* were discovered.

Stevens Ecological Consulting, unpublished surveys, 2000-2001 (L. Stevens).

Stevens searched over 150 sites in the lower Grand Canyon and Arizona Strip for leopard frogs. One documented record (photo-voucher) of a leopard frog at Surprise Canyon (Colorado River mile 248) from 1987 was found during this study.

Rorabaugh, J.C., M.J. Sredl, V. Miera, and C.A. Drost. 2002. Continued invasion by an introduced frog (*Rana berlandieri*): southwestern Arizona, southeastern California, and Rio Colorado, Mexico. Southwestern Naturalist 47(1): 12-20.

Rorabaugh et al. searched potential habitat along the Colorado River below Davis Dam for leopard frogs and other amphibians. No *R. onca* populations were found.

Rorabaugh, J.C., J.M. Howland, and R.D. Babb. In press. Distribution and habitat use of the Pacific treefrog (*Pseudacris regilla*) on the lower Colorado River and in Arizona. Southwestern Naturalist.

Rorabaugh et al. searched potential habitat along the Colorado River below Davis Dam for Pacific treefrogs and other amphibians. No *R. onca* populations were found.

Bradford, D., A.C. Neale, M.S. Nash, D.W. Sada, and J.R. Jaeger. In press. Habitat patch occupancy by the toads *Bufo punctatus* in a naturally fragmented desert landscape Ecology.

Bradford et al. conducted amphibian surveys south of the Black Canyon area at springs in the Eldorado Mountains near Lake Mohave, Nevada. These revealed little permanent water and no leopard frogs.

Blomquist, S.M., D.A. Cox, and M.J. Sredl. 2003. Inventory and habitat assessment of the relict leopard frog (Rana onca) in Arizona. Nongame and Endangered Wildlife Program Technical Report 219. Arizona Game and Fish Department, Phoenix, Arizona.

Blomquist and Cox searched springs and stock tanks with permanent water in and around the Virgin Mountains, Virgin River corridor, Grand Wash, lower Grand Canyon, and Lake Mead for leopard frogs. All surveys were conducted in Arizona. No leopard frog populations were detected.

SECTION 3: RANID FROG EGG MASS COLLECTION AND TRANSPORTATION PROTOCOL

Updated July 2004

1) If possible, record the water and air temperature at the site, location of the egg mass in the pond or creek, and current and recent weather events. Forward this information with the egg mass to a member of the RLFCT.

2) Egg masses should be freshly laid (< 5 days) or show little sign of development.

3) Use a new, 1 gallon, self-closing (zip lock) plastic bag to transport the egg mass. Rinse the bag thoroughly before use and write the name of the collection site on the bag. Place only 1 egg mass per bag.

4) To transfer the egg mass into the bag, submerge the bag and fill with clear water. Next, carefully cut away any vegetation or sticks attached to the egg mass, without dividing the egg mass. In your cupped hand(s), gently move the egg mass into the submerged, opened, plastic bag. Be careful not to transfer aquatic invertebrates, mud, leaves, and other organic debris into the bag.

   If only a portion is being collected, use 2 plastic spoons and your fingers to separate the egg mass. Place 1 hand underneath the egg mass, to prevent the eggs from touching the substrate or breaking apart. Take caution not to remove the portion of the egg mass attached to the supporting vegetation or debris.

5) Once the egg mass is in the bag, bring it to the surface and seal the bag. Allow approximately ½ - 1" of air space. Once sealed, placed the filled bag into a second bag in case of leakage.

6) You may want to collect an additional 2 – 5 gallons of water from the site in clean plastic bags or plastic buckets, for captive rearing needs.

7) Transport the egg mass in the plastic bag, within a styrofoam or hard plastic cooler. The bag should be supported within the cooler to prevent leakage through the seam and excess sloshing during transport. Towels, newspaper, or air filled bags work well in supporting the egg mass bag in the cooler. Ice or freezer packs may be added to the cooler to maintain a suitable temperature (60-75 degrees F.), provided the frozen material does not directly contact the egg mass bag.

8) Coordinate with the captive rearing facility prior to departure to alert them to your estimated time of arrival and minimize transit time.

SECTION 4: RANID FROG CAPTIVE CARE PROTOCOL ADAPTED FROM DEMLONG (1997).

Updated July 2004

I. Containment

A. Holding containers

1. All should be constructed of easily disinfected materials like plastic, glass, or fiberglass.
2. No metal containers, galvanized or not.
3. Aquaria and plastic kiddy pools work well.
4. Containers of cement based products are 1 alternative, provided they are well aged and no longer leaching alkaline.
5. PVC or plastic pond liners are also acceptable, provided they are labeled as "fish safe" by the manufacturer.

B. Lids

1. All containers should have screened or solid lids to prevent larvae or metamorphs from jumping out or escaping.
2. An alternative is to use taller containers and keep the water level low.
3. When not being serviced, cover the holding containers with a solid cloth or other material to minimize stress on the animals.
4. Disturbance can be minimized by setting up the holding containers in low (human) activity areas.

C. Hiding devices

1. Artificial floating plants provide larvae with resting and hiding places.
2. Live plants or algae may be used if obtained from the same location as the animals, or if the plants are thoroughly rinsed and stored in tap water for 30 days.
3. Another alternative is plastic window screen mesh they to serve as rafts.

D. Lighting

1. Some lighting can be provided with natural sunlight or by using artificial fluorescent light fixtures with full-spectrum bulbs.
2. Ideally the fixture must be fairly close, within 12", to be effective.

E. Inserts

1. Holding containers can be fitted with mesh bottom inserts that contain the larvae when removed from the water. This insert is then placed into a clean container of the same size.

II. Stage specific considerations

A. Housing-Embryos

1. Gently aerate water in embryo holding tank with an air stone and aquarium pump or an aquarium power head.
2. Embryo masses should be suspended off the bottom of the holding container. Plastic window screen mesh or rinsed cheese cloth material are useful for building a "hammock" underneath the embryos to suspend them in the water.

3. Remove dead embryos or eggs covered with fungus from the mass if possible with minimal disturbance.
4. Stocking density: 1 embryo mass per container (10 gallon aquarium).

B. Housing-Larvae
1. Undergravel filters, filter sponges, and external filters help keep the holding containers cleaner but are not substitutions for water change.
2. Stocking density:
Sizes can be mixed; there is no evidence that large tadpoles harm small individuals. Stocking capacity declines as tadpoles grow larger, so it is important to monitor water quality closely and check for signs of overcrowding.
a. for maximum growth 25-50 per 10 gallon aquarium,
b. 100-350 per kiddy pool (39" diameter by 7" tall) or
c. 100-300 per 200 gallon container.

C. Housing-Metamorphs
1. Provide cover/hiding places and dry haul out areas.
2. Provide basking light with 75-150 watt full spectrum light.
3. To help keep the animals from drowning and reduce stress during metamorphism place the metamorphs in a separate tank when they have 4 legs and a tail.
4. Also separate the frogs by size to keep cannibalism to a minimum.
5. Stocking density:
a. 10 metamorphs per 10 gallon aquarium or
b. 40 per kiddy pool (39" diameter by 7" tall).

III. Diet
A. Type of food for larvae:
1. Larvae feed well on spinach. Fresh spinach bunches that are frozen overnight or boiled are superior to fresh or canned.
2. They will also feed on fresh spinach, but it must be weighted down to the bottom of the tank.
3. It is also helpful to weigh down the frozen greens.
4. Blanched romaine lettuce, mustard greens, turnip greens, cucumber slices, duckweek (*Lemma* sp.), spirulina type fish foods (good for younger larvae), peas and alfalfa-based rabbit pellets are also taken.
5. Bok Choy and Kale are not recommended, it doesn't break down enough for them to eat when frozen.
6. For protein, bloodworms and egg whites (hard-boiled) work well and do not carry the parasites found in aquaculture reared fish.
7. Algae wafers mold quickly, so use sparingly.
8. Another item to supplement their diet is calcium.
9. Rocks covered with algae or floating filamentous algae are a great source of natural food.

10. Preparing food:
    a. Fresh greens and vegetables must be thoroughly rinsed before being fed to remove soil, and residual pesticides or herbicides.
    b. When algae is being used it should be cultivated in uncontaminated water to avoid the risk of introducing parasites and disease.
    c. Food can be provided to the larvae free choice, or fed once, twice, or a few times a day. Food should not sit longer than 24 hours in the tanks.

B. Types of food for metamorphs and juveniles:
1. They feed well on commercially reared crickets, mealworm larvae, adult beetles, and flightless houseflies.
2. In outdoor open air facilities a black light can be hung near the edge of the pond to attract wild night flying insects. The light should be hung low enough to the ground so the frogs can easily catch the flying insects, but high enough to attract insects from a distance.

IV. Water Quality and Changing Schedule

A. Changing Schedule
1. All holding containers should ideally be cleaned daily by siphoning off a minimum of 20% and a maximum of 50% of the water in the larvae holding containers, and then replacing it with one of the water types under water quality.
2. The frequency of water changes will depend on the stocking density of larvae and presence/absence of a filtration system.
3. Water for the metamorphs can be changed once a week to minimize stress, with dead crickets being skimmed daily.

B. Water Quality
1. If tap water is used for water changes it should be allowed to sit 24 or more hours in an open container to allow the chlorine to dissipate.
2. Aeration helps remove the chlorine quicker.
3. Stream or pond water from which the animals originated or distilled water, are also acceptable.
4. De-chlor and similar products can be used to quickly remove chlorine and chloramines. Easy to use and inexpensive chlorine test kits are available from any aquarium store. Biological aquarium supplements are useful in tanks containing undergravel filtration systems.
5. If only 20%-30% water change is done and tap water is used it is not required to add dechlorinating agent.
6. Replacement water should be the same temperature as the water in the holding container to minimize stress.
7. External charcoal and reusable fine and course filters can be used for water filtration.
8. UV light for sterilization is desirable.

C. Air Quality

1. Larvae holding tanks and pools should be aerated by an aquarium air pump and 1 or more air stones.
2. Tanks should be sufficiently aerated so that the larvae are not gasping for air at the top of the tank or looking distressed.

SECTION 5: RELICT LEOPARD FROG CAPTIVE RELEASE PROTOCOL

Updated July 2004

Qualifications For A Release Program:

1. No mortalities in the release group during the previous 30 days (Release groups may be defined as groups of frogs or larvae confined to an individual container, such as a fish tank, at a rearing facility). No "cause of death unknown" or diagnosis of contagious disease as cause of death for 30 days prior to release. All mortalities should be examined by a pathologist skilled in diagnosing amphibian diseases. If sections of skin are submitted to the pathologist (instead of the whole animal), the sections should include at least 2 pieces of skin from the ventral pelvic region and/or ventral hind limb and/or feet or toes.

2. No unthriftiness or diagnosed illness in the release group during the previous 30 days. No obvious physical abnormalities – missing limbs, deformities of long bones, vertebral scoliosis or kyphosis, corneal lesions, skin lesions – detected. Diagnosis of certain diseases, such as mycobacteriosis, in a single individual may render the entire group unfit for release.

3. No medical treatments of the release group during the previous 30 days.

4. All animals designated for release should be in permanent quarantine so that there is no overlap with care of exotic animals or native animals that have been exposed to exotic animals. Caretakers should "shower in" if they care for other herps either as part of their job or as pets. If a wild population has a known incidence of a given infectious agent (e.g. Lucke's herpesvirus), it may be safe to assume that released animals with that agent represent an acceptable risk.

5. All enclosures should be worked with separate tools and equipment to reduce cross-transmission. Disposable gloves should be worn and new ones used for each enclosure. Any enclosures with unthrifty animals should be worked last.

6. Water quality logs should be maintained. Adjustment to release site water conditions should occur 30 days prior to release.

Pre-Release Screening Protocol:

Depending on the size and life stage of the specimens to be released as well as the number of specimens destined to be released, a random sample of animals may need to be assessed rather than an individual assessment of all animals within a group.

1. Obtain weight.

2. Perform physical exam; note body position, alertness, and evaluate musculoskeletal system for obvious bony abnormalities.

3. Obtain toe clip for identification; toe clip may be saved for chytrid histopathology, DNA banking or frozen for future pathogen recovery attempts. Consider PIT tags as permanent identification.

Immediate Pre-Release Activities:

4. 10 days and 2 days before release:

   Soak in an antifungal solution (if this has never been used on this species before, try the treatment on a few individuals well ahead of time to determine tolerance). Use one of the following two treatments.

   Itraconazole: itraconazole diluted to 0.01% concentration in 0.6% saline (Sporanox, Janssen Pharmaceutica, Titusville, NJ) for up to 1 hr. (Rx: Add 5 ml of a 1% itraconazole solution to 445 ml of 0.6% saline [6 g NaCl added to 1 liter of water]. Soak frogs for 1 hour.)

   Miconazole: miconazole diluted to 0.01% concentration in 0.6% saline as alternative (Conofite lotion, Schering-Plough Animal Health Corp., Union, NJ) for up to 1 hr. This solution is generally not tolerated as well as itraconazole since it contains alcohol.

5. At time of packing for transport:

   Dip in benzalkonium chloride (2.0 mg/L) for at least 15 seconds. Rinse with fresh water before packing animal. If this has never been done before, try the treatment on a few individuals well ahead of time to determine tolerance. (Rx: Add 0.04 ml of a 50% benzalkonium chloride solution to 10 L of water. Soak frogs for 15 to 20 seconds.)

   Do a visual assessment of animals and approve or reject packing for transport.

6. At release site:

   Do a final visual assessment of animals and approve or reject release.

   Aquatic animals: Equilibrate water temperature and chemistries of transport container with release site water

   Terrestrial animals: Equilibrate container temperature with release site temperature

SECTION 6: RANID FROG TRANSPORTATION PROTOCOL ADAPTED FROM DEMLONG 1997.

Updated July 2004

I. Transportation

A**.** General Container Information

1. Use only plastic containers, no metal or glass.
2. Containers should be water tight when tipped upside down.
3. Do not use bags more than once. Use only new, rinsed bags.
4. Carry 1 or 2 extra containers filled with water in case of an emergency (i.e. leak).

B. Type of Containers per animal size

1. Larvae at any stage, ship well in 11" x 10.5" (1 gallon self closing bags (e.g. Ziplocs®) or in aquarium grade plastic bags sealed with a rubber band. Aquarium grade bags can be inflated and sealed with rubber bands to prevent collapsing. Double bagging should be considered for trips longer than 4 hours or when driving on rough roads.
2. Larvae may also be transported in hard plastic buckets or containers that have tight fitting lids.
3. GladWare® is highly recommended for transportation of metamorphs, juveniles, and adults. They keep them from being crushed and they are reusable.

C. Preparing Containers

1. Thoroughly rinse all shipping containers with water. Do not use any type of detergent or soap to clean the containers.
2. The GladWare® also needs holes drilled in the top. A standard hole punch works well, approximately 16 holes.
3. If desired, mark each bag with identification of eventual destination and the number of animals in the container.

D. Stocking densities

1. Per gallon bag for short shipments.
   a. Eggs: 1 mass per bag, minimize disturbance and division of mass
   b. Larvae under ½": 25 per bag
   c. Larvae 1" - 1 ½": 15 per bag
   d. Larvae over 1 ½": 10 per bag
   e. Recently metamorphosed frogs: 5 per container or bag
2. Avoid overcrowding

E. Water

1. Water put in the bags must be chlorine and chloramine free. Dechlorinating chemicals can be used to immediately remove chlorine.
2. Stream or pond water from which the animals originated can be used. Avoid capturing aquatic invertebrates or organic debris.

3. Other alternatives are bottled drinking water, distilled water, or tap water left uncovered for 24 or more hours. Avoid capturing decomposing food or feces.
4. For larvae, fill bags by approximately 75% or greater volume water to avoid excessive sloshing.
5. For metamorphs, juveniles, or adults place 20 ml of water with a leaf of romaine or iceberg lettuce for hiding. If transporting from the wild, use algae or leaves instead.

F. Shipping

1. Blow out bags with a breath or an oxygen cylinder to prevent collapse during shipping. Allow a little space within the bag to allow for expansion with elevation changes.
2. Foam or plastic insulated ice chests work well for protecting bags from temperature extremes and accidental damage. Foam boxes that fit within a cardboard box are commercially available from tropical fish dealers.
3. Use towels, newspapers or bags blown full of air to fill in empty spaces between bags in the shipping container.
4. Battery operated air pumps are useful in aerating buckets of animals during transport.

G. Temperature

1. Optimal shipping temperature is a compromise between the captive and anticipated release temperature.
2. To keep animals cool in warm weather, place a 1-3 inch layer of cubed ice inside plastic bags on the bottom of an insulated ice chest. Cover the ice with a layer of plastic, then a few layers of towels, newspaper, or cardboard to insulate the animals from the direct cold. It is suggested to place a piece of foam between ice and animals, so if ice melts the animals will float instead of settling in the water.
3. A thermometer with a remote sensor inside the container can assist in monitoring the temperature while shipping.
4. Alternatively, animals could be moved in open containers if kept inside air-conditioned vehicles capable of maintaining the appropriate desired temperature.
5. When tadpoles arrive at the rearing facility, it is important to equalize the temperature of the shipping container and that of the tank into which the animals will be released. This is easily achieved by floating the plastic bag or container in the tank for 15-20 minutes. An aquarium thermometer can be used to ensure that the two containers are within one or two degrees of each other before transferring the animals.

SECTION 7: LIST OF POTENTIAL RELEASE SITES

Updated July 2004

The following is a list of potential release sites agreed upon by the Relict Leopard Frog Conservation Team for immediate introduction of frogs as soon as necessary agency clearances and NEPA compliance can be completed. Additional sites will be evaluated and added to the list as conditions warrant, including the ability to produce adequate quantities of frogs for a larger scale reintroduction program.

1) Nevada Hot Spring (in Goldstrike Canyon) (Nevada, Hoover Dam, UTM Zone 11, 703565E, 3986117N)
2) Corral Spring (Nevada, Echo Bay, UTM Zone 11, 727948E, 4027849N)
3) Tassi Spring (Arizona, Gyp Hills, UTM Zone 12, 234320E, 4016210N)
4) Grapevine Spring (Arizona, Meadview North, UTM Zone 11, 768020E, 3992790N)
5) Pumphouse Outflow (UTM Zone 11, 694820E, 3993679N)
6) Hoover Dam Pupfish Refugium (UTM Zone 11, 703380E, 3987627N)
7) Sugarloaf Spring (Arizona, UTM Zone 11, 703643E, 3986218N)
8) Lone Palm Spring (Arizona, UTM Zone 11, 703787E, 3985687N)

SECTION 8: MONITORING PROTOCOL, VISUAL ENCOUNTER SURVEY PROTOCOL, DATA SHEET, AND INSTRUCTIONS

Updated July 2004

MONITORING PROTOCOL

All sites occupied by relict leopard frogs will be monitored. A minimum of 2 surveys per year will be conducted using visual encounter survey (VES) at each site. One survey will be conducted during the breeding season (January – March, September, and November) and 1 survey will be conducted outside of the breeding season. The purpose of these surveys will be to detect threats to the population (e.g. drought, invasion of nonnative predators), assess habitat condition, and monitor presence of relict leopard frog breeding activity (i.e. production of egg masses), and recruitment (i.e. presence of metamorphs). Day surveys will be used to detect presence of egg masses, assess habitat condition, and detect threats. If necessary, night surveys will be used to detect adult frogs and breeding activity. The number of surveys will be conditional upon detection of threats or unforeseen changes in the population. If no frogs are detected at a formerly occupied site or threats are detected, more surveys will be conducted. These surveys are not intended to give statistically valid estimates of trends in abundance (e.g. Gerrodette 1987, 1993; Gibbs 1996).

VISUAL ENCOUNTER SURVEY PROTOCOL

This standard VES protocol was developed by AGFD and is to be used for surveys attempting to specifically detect relict leopard frogs. This protocol was adopted from Heyer et al. (1994) and modified based on statewide surveys in Arizona from 1991-2002. The protocol is designed to be simple and repeatable with minimal training of personnel. However, all personnel should be trained and have survey technique checked periodically by a more experienced individual. The VES protocol described here will generate presence/absence data if used independently and generate information from which inferences about abundance and trends can be made if used in a statistically valid monitoring program.

<u>Equipment needed:</u>

The observer should always have the following when conducting a VES:

- a dip net
- a Global Positioning System unit set to read in the North American Datum 1983 and the appropriate Universal Transverse Mercator (UTM) Zone
- a clipboard with the Riparian Herp Survey Form and Instructions
- a pen with waterproof ink
- a time piece set to local time with a stop watch

- a pH meter
- 2 thermometers
- a conductivity meter
- a sling psychrometer or hygrometer
- binoculars
- the appropriate US Geologic Survey quadrangles
- bleach or Quat128 for disinfecting all gear before and after surveying each site

Other suggested items are the following:

- a counter or clicker for keeping a tally of frogs observed
- a field notebook
- a headlamp or spotlight for night surveys
- rubber boots, hip waders, or chest waders depending on the habitat
- guides to identification of aquatic insects, fish, amphibian larvae, and adult amphibians
- a "Field Guide to Western Amphibians and Reptiles" by Stebbins (1985 or 2003)
- a camera with slide film
- the appropriate land ownership maps
- database reports of historical surveys done in the area
- wind meter
- measuring tape
- "dead box" (whirl pack or ziplock bags, MS 222, and formalin for collecting specimens)
- pocket magnifier (to help identify tadpoles, look at mouthparts, etc.)
- tape player (for call backs)
- compass

Survey Protocol:

Lentic systems:

Upon approaching a survey site, stop approximately 20 m from the bank and search the site with binoculars. Search for frogs floating in water away from the bank as well as scanning the bank as best as possible. Proceed to walk around the entire perimeter of site if the site is a small lentic system. If the site is a large lentic system and the entire perimeter cannot be surveyed, record the start and stop points as UTM coordinates. While walking along banks, use a dip net to sweep vegetation to flush frogs that do not respond to the observer's approach. After the initial perimeter survey, search mud cracks, divots, under rocks and downed branches, and any other places where frogs might find cover. If the lentic system allows, walk though the site in a zigzag fashion to further flush frogs that may be sitting on the bottom of the water. Dip net to determine the presence of amphibian larvae, fish, and aquatic insects. Record all visual observations and audible "plops" of frogs escaping into water. Be careful not to count frogs more than once.

Lotic systems:

Upon arriving at the starting point of a lotic system, record the UTM coordinates of the starting point. Proceed upstream (preferably) or downstream searching the banks, surrounding vegetation, and water along a minimum of 400 m of a lotic system. Search under rocks, downed branches, undercut banks, and any other places where frogs might find cover as well as possible. Where the lotic system allows, walk though the site in a zigzag fashion to further flush frogs that may be sitting on the bottom of the water. Dip net to determine the presence of amphibian larvae, fish, and aquatic insects. Record all visual observations and audible "plops" of frogs escaping into water. Be careful not to count frogs more than once. Record UTM coordinates of the end point.

Data collection:

Data should be collected according to the Complete Riparian Herp Survey Form Instructions. Collect the following data at the specified locations, but note any major changes that occurred during the survey on the data form. Record the site name, UTM points, elevation, USGS quad, date, observers, and time the survey starts at the starting point of the survey. Record time the survey stops, time spent actively searching for herps, effort, any voucher specimens taken, water class, water type, search methods, water pH, relative humidity, air and water temperature, habitat characteristics (water clarity, vegetation types present, primary substrate, site width and/or length), weather conditions (wind, cloud cover, precipitation), land use, sign of potential vertebrate and invertebrate predators, as well as comments at the end point of the survey. Record any herps observations when observed.

New Site: Y N

RIPARIAN HERP SURVEY FORM

AZ Game and Fish Department May 2002

Locality Data

*SITE:	SITE AT:

*NUM: __ __ __ --- __ __ __ __	*UTM ZONE: 11 12	*EASTING	*NORTHING	*ELEVATION ft

*QUAD:	*MIN: 7.5 15	*YEAR: __ __ __ __	*COUNTY: __ __---__ __ __ __

DIRECTIONS:

Site and Visit Conditions

*DATE: m m d d y y y y	*START TIME	*STOP TIME	*SEARCH TIME min	*OBSERVERS:

*EFFORT: meters	Total Perimeter	Partial Perimeter	Left Bank	Right Bank	Both Banks	* VOUCHERS: Specimen Photo :__ __ Habitat Photo :__ __ Specimen(s) : Y N Specimen #s:

*H_2O CLASS: Lentic Lotic

*H_2O TYPE: Canal Plant outflow Riverine Wetland Stock tank Lake Reservoir Small metal/concrete tanks or drinkers

* SEARCH METHODS: Dip net Seine Trap Hand exploration Snorkel Boat Call playback Other

TDS: µs pH: RH: %

*T_{AIR}: °C *T_{WATER}: °C

WATER CLARITY: Extremely clear Somewhat clear Moderately clear Somewhat heavily turbid Extremely heavily turbid

*LENTIC LENGTH: m *LENTIC WIDTH: m *LOTIC WIDTH: 0-2m 3-5m 6-10m 11-20m 21-50m 51-100m >100m

*RIPARIAN WIDTH: 0-2 m 3-5 m 6-10 m 11-20 m 21-50 m >50 m

*PRIMARY SUBSTRATE (mark 1-3) : Mud/Silt Sand Gravel Cobble Boulder Bedrock

*WIND: < 1 mph 1-3 mph 4-7 mph 8-12 mph 13-18 mph 19-24 mph >24 mph

*CLOUD COVER: 0-20% 21-40% 41-60% 61-80% 81-100%

*PRECIPITATION: None Intermitent Steady & Light Steady & Heavy Snow/Sleet

*LAND USE: Agric Devel Graze Log Mine Rec

VEGETATION	%	PROMINENT SPECIES
FLOATING		
SUBMERGED		
EMERGENT		
PERIMETER		
CANOPY		

*PREDATORS: (include scat and tracks)
Leeches Boatmen/Backswimmers Dragonflies
Belostomatids Beetles Warm water fish
Cold water fish Tiger salamanders Bullfrogs
Mud turtles Garter snakes Wading birds
Hawks (black or zone-tailed) Mammals Crayfish

*OTHER ORGANISMS:

OTHER ORG. NOTES:

SITE / SURVEY NOTES:

Herpetofauna Observations

*SPECIES	CERTAINTY	LIFE STAGE	#	NOTES
	Uncertain Certain	Egg Larvae Juvenile Adult		
	Uncertain Certain	Egg Larvae Juvenile Adult		
	Uncertain Certain	Egg Larvae Juvenile Adult		
	Uncertain Certain	Egg Larvae Juvenile Adult		
	Uncertain Certain	Egg Larvae Juvenile Adult		
	Uncertain Certain	Egg Larvae Juvenile Adult		
	Uncertain Certain	Egg Larvae Juvenile Adult		
	Uncertain Certain	Egg Larvae Juvenile Adult		

- All fields are to be filled out for historical sites and sites with relict leopard frogs.
- Fields with an asterisk (*) are to be filled out for every survey, regardless of results.
- If the site has never been surveyed for relict leopard frogs, circle Y (yes) at the top of the form. Otherwise circle N (no) and check the site's upon returning to the office for consistency.
- Upon return to the office, check each Survey Form for completeness, conciseness, and clarity.

Locality Data:

*****SITE: A "site" is any aquatic system (or piece of an aquatic system) that is > 1 mile from any other survey locality, or if less than 1 mile apart, represents a **distinct** change in aquatic habitat types (e.g. riverine vs. lake or cienega). Features with unique names are considered unique sites regardless of how far apart they are. Record the site name as it is marked on the US Geologic Survey (USGS) quadrangle (hereafter quadrangle or quad). If the site is unnamed on the quad, refer to the corresponding land management map (e.g. US Forest Service map, Surface Management Responsibility map). If the site doesn't have a name, write "unnamed" preceding the feature; similarly, if the site is not marked on any map, write "unmarked" preceding the feature (e.g. Unnamed Wash, Unmarked Tank).

SITE AT: This field should always be filled out for unnamed and unmarked sites and for large/long aquatic systems. For other localities, use this field *as needed* to enhance a site name (i.e. to verbally pin-point a site in space). Use such features as the nearest road crossing (e.g. East Verde River at **Highway 87**) stream confluence (e.g. East Verde River at **Webber Creek**) or topographic feature (e.g. East Verde River **N of Piety Hill**) in the description.

*****NUM: Write the site number. A site number is a unique number that, once assigned to a site, will always be used in conjunction with that site. The site number starts with a 3-letter code that describes the land manager. These 3 letters are followed by a hyphen and then a 4-digit number (e.g. TON-0001, COC-0153). Sites are numbered in ascending, consecutive order within each management unit. Management unit codes are as follows:

Arizona Game and Fish -- AGF
Bureau of Land Management -- BLM
Land Grants -- LGR
Military -- MIL
National Forests -- APA, COC, COR, KAI, PRE, TON
National Parks/Monuments -- NPS
National Wildlife Refuges -- NWR
Private Lands -- PVT
State Lands -- ARZ
Tribal Lands (note: the final "R" = Reservation):

Ak Chin -- AKR	Fort McDowell -- MCR
Fort Apache -- APR	Fort Mojave -- MOR
Cocopah -- CCR	Navajo -- NAR
Chemehuevi -- CHR	San Carlos -- SCR
Colorado River -- CRR	Salt River -- SRR
Camp Verde -- CVR	San Xavier -- SXR
Gila Bend -- GBR	Tonto-Apache -- TAR
Gila River -- GRR	Tohono O'odham -- TOR

Havasupai -- HAR	Yavapai-Prescott -- YPR
Hopi -- HOR	Fort Yuma -- YUR
Hualapai -- HUR	Zuni -- ZUR
Kaibab -- KAR	

*UTM ZONE: Circle "11" or "12" to note whether the **starting point** of the survey is in UTM grid zone 11 (west of 114 degrees longitude) or 12 (east of 114 degrees longitude). Most of Arizona except for the extreme western portion of the state is Zone 12.

***EASTING:** Record the **starting point** of the survey as a 6-digit number. An example of a UTM x-coordinate is 295440E. Use a Global Positioning System (GPS) unit to measure the UTM coordinate. The UTM coordinate should be measured in North American Datum 1983. Check that the GPS unit is set to the appropriate Zone (most of Arizona is Zone 12, most of Nevada is Zone 11). Alternatively, read the UTM coordinate from the quad. The first 3 numbers will be found on the top or bottom edge of the quad. These numbers are in 100,000-meter increments. The fourth number describes a point with ∀ 100-meters accuracy. The fifth number describes a point with ∀ 10-meters accuracy. The last number will be a zero. Use a coordinate scale to determine the fourth and fifth numbers.

*NORTHING: Record the **starting point** of the survey as a 7-digit number. An example of a UTM y-coordinate is 4318410N. Use a Global Positioning System (GPS) unit to measure the UTM coordinate. The UTM coordinate should be measured in North American Datum 1983. Check that the GPS unit is set to the appropriate Zone (most of Arizona is Zone 12, most of Nevada is Zone 11). Alternatively, read the UTM coordinate from the quad. The first 4 numbers will be found along the left or right edge of the quad. These numbers are in 1,000,000-meter increments that tell you how far north of the equator you are. The fifth number describes a point with ∀ 100-meter accuracy. The sixth number describes a point with ∀ 10-meter accuracy. The last number will be a zero. Use a coordinate scale to determine the fifth and sixth numbers.

***ELEVATION:** Record the elevation at which the **starting point** of the survey occurs. Read the elevation off of the survey quad. Be sure to indicate the elevation in feet (ft). The contour interval and unit (meters or feet) is written in the center of the bottom margin of the quadrangle. To convert meters to feet multiply by 3.281.

***QUAD:** Record the quadrangle name as it appears on the quadrangle except in the situations outlined below. The name of the quadrangle appears in the upper and lower right hand corners of the quadrangle. If more than 1 quad is used in the survey, record the name of the quad in which the survey starts and note the name(s) of the other quad(s) in the DIRECTIONS.

- Do not use periods.
- Do not use apostrophes.
- Change the word "Mountain" to "Mtn" if it appears anywhere in the quad name other than the first word.
- Composite polar coordinates (e.g. Southeast, Northwest) should be abbreviated (e.g. SE, NW) if they appear anywhere in the quad name other than the first word
- Never abbreviate the 4 cardinal directions

*****MIN: Circle "7.5" or "15" to note whether the quadrangle series is 7.5 or 15 minutes. The series of the quadrangle can be found in the upper right hand corner of the quadrangle.

*****YEAR: Record the year of the quadrangle as it is printed in the lower right corner of the quadrangle. If more than 1 year appears on the map, record the year of the most recent revision.

*****COUNTY: Record the state abbreviation (e.g. AZ) followed by a hyphen and then the first 4 letters of the county (e.g. AZ-MARI, AZ-YAVA). The county name can be found in the upper right corner of the quadrangle if the quad covers an area within a single county. For quads that cover areas in two or more counties, the names of the counties will appear somewhere in the topographic region of the quad. National Forest maps and the Arizona Highway road map, and the Arizona Atlas & Gazetteer are also useful in identifying counties. Please use the following abbreviations for county: APAC, COCH, COCO, GILA, GRAH, GREE, LAPA, MARI, MOHA, NAVA, PIMA, PINA, SANT, YAVA, YUMA

DIRECTIONS: Write the directions to the site. **Keep them short and pertinent** (e.g. on FS 105 –4.3 MI N of FS 105/FS 393 jct.). Directions are especially important when there are no roads or when existing roads are not marked on your maps. Use the directions N, NE, E, SE, S, SW, W, and NW instead of "turn right" or "veer left". This field can also contain any information or comments you want to convey to other field personnel. For example: "Dry 05/1994"; "Contact landowner for permission to access (602)555-9683"; "Also survey adjacent tank and draw"; etc.

Site and Visit Conditions:

*****DATE: Record the date of the survey as 8 numbers giving the month first, followed by the day then the year (e.g. 10-27-1993, 06-02-1994).

*****START TIME: Record the time the surveyor begins searching for herps using a 24-hour clock.

*****STOP TIME: Record the time the surveyor stops searching for herps using a 24-hour clock.

*SEARCH TIME: Record the time spent actively searching for herps in minutes. The time recorded should include only time spent actively searching for herps and should not include time taken to write field notes, complete data sheets, read data sheet instructions, or other activities that may be performed while at the site.

*****OBSERVERS: List the names of all people present during the survey. Record the names as: first initial, second initial, and full last name (e.g. M.J. Sredl, K.J. Field, and S.M. Blomquist).

*****EFFORT: There are 5 categories of effort:

TP = Total Perimeter
PP = Partial Perimeter
LB = Left Bank
RB = Right Bank
BB = Both Banks

Circle all category(s) that apply. For all categories other than TP, record the distance surveyed in meters. The minimum acceptable survey distance for linear systems and large lentic systems (> 20 acres) is 400m (0.25 mile). Use category BB for any lotic system in which it is possible for you to access both banks (i.e. to meander from shore to shore). Use categories LB and RB for large, deep, and/or swiftly flowing lotic systems in which you are unable to meander shore to shore. LB and RB should always be filled out together even if you didn't survey, or were unable to access, one of the shores (e.g. LB = 0000m, RB = 0350m; RB = 0050m, LB = 0200m). Left and right banks are in reference to a person looking upstream. To calculate meters walked use a map wheel, range finder, or pace off the distance. If using a map wheel to determine the distance in kilometers (or miles), be sure to use the scale on the map wheel that corresponds to the scale of your map or quad. Multiply your result by 1000 to get meters. Round the final result to the nearest 25-meter value. Alternatively, multiply the value generated from the map wheel in miles by 5,280 feet/mile. Multiply this new value by 0.3048 meters/foot. Remember, during the course of any survey, the surveyor should dip net, comb through bushes and grasses, turn over rocks, and scan the water and shore for herpetofauna.

*****VOUCHERS: Note how many photo vouchers of specimens were taken at a site. Write the number as 2 digits (e.g. 00 or 13). Photo vouchers of specimens should be close-ups (i.e. macro shots) of diagnostic characters (e.g. thigh pattern and dorsolateral folds of leopard frogs, scale row of lateral stripes in garter snakes, dorsal and cranial views of Arizona toads). Note how many habitat photographs were taken at a site. Write the number as 2 digits (e.g. 00 or 02). Habitat photos should be taken at any site in which target riparian herps were found, at any historical locality regardless of results, and at any survey site that has habitat even if no target riparian herps were found. Keep a detailed log of all photos taken with the camera. Circle "Y" (yes) or "N" (no) as an indication of whether voucher specimens were collected at a site. If "Y" is circled, the collection tag number(s) should be written in the Specimen #s field.

*****H_2OCLASS: Circle 1 category that best describes the hydrological class of the water system you have surveyed.

Lentic = still water
Lotic = flowing water

*****H_2OTYPE: Circle 1 category that best describes the type of water you have surveyed. The categories are based upon lotic/lentic characteristics as well as the size/magnitude of the water body:

Canal = manmade (metal, concrete or earthen) diversion of riverine water
Plant outflow = sewage and electric plants; any chemical or mechanical processing of water; storm drainages
Riverine = natural flow, from raging rivers to streams to seeps
Wetland = an inland body of water that is primarily emergent vegetation (e.g. cienega)
Stock tank = an earthen-dammed or dredged basin that catches run-off for livestock or wildlife
Lake = an inland body of water that is primarily open water
Reservoir = a dammed riverine system that is primarily used for recreation and/or human water supply

Small metal/concrete tanks and drinkers = manmade water holding structures

*SEARCH METHODS: Circle all methods used to search for herps. If needed, include a description of other techniques used to search in the SITE / SURVEY NOTES with a footnote reference. Remember, during the course of any survey, the surveyor should dip net, comb through bushes and grasses, turn over rocks, and scan the water and shore for herpetofauna.

TDS: Use a dissolved solids meter to measure. The water sample should be taken 1 centimeter below waters' surface and 1 meter from shore. For bodies of water less than 2 meters wide, take the sample from the center. Record value as µS (micro-Seimens). Be sure to: 1) take the cap off the meter before using, 2) keep the level of the water sample below the mark on the meter, 3) turn the meter on before measuring the conductivity of the sample, and 4) turn the meter off when finished sampling. Meters should be calibrated monthly.

pH: Measure pH using a pH meter. The water sample should be taken from water column 1 meter from shore. For bodies of water less than 2 meters wide, take the sample from the center. Be sure to: 1) take the cap off the meter before using, 2) keep the level of the water sample below the mark on the meter, 3) turn the meter on before measuring the pH of the sample, and 4) turn the meter off when finished sampling. Meters should be calibrated monthly.

RH: With a sling psychrometer or hygrometer, measure relative humidity 1.5 meters above ground and 1.5 meters from water. Record as percent.

*T_{AIR}: Measure air temperature to the nearest 10^{th} of a degree (degrees Celsius) 1.5 meters above ground and 1.5 meters from the water. Be sure thermometer is shaded and completely dry.

*T_{WATER}: Measure water temperature to the nearest degree (degrees Celsius) 1 centimeter below water's surface and 1 meter from shore. For bodies of water less than 2 meters wide, measure temperature at the center. Be sure to shade the thermometer.

WATER CLARITY: Circle 1 phrase that best describes the survey area.

*LENTIC LENGTH: For lentic systems, record the length (i.e. longest axis) of the system in meters. Measure the entire system (not just the portion surveyed), and use the standing water at the time of the survey as your boundaries. Do not measure the normal waterline or highwater mark. For large systems, estimate the length using a map. Do not rely on a visual estimate for large systems.

*LENTIC WIDTH: For lentic systems, record the width (i.e. shortest axis) of the system in meters. The width should be the maximum distance perpendicular to the length axis. As with the length, the width should reference the entire lentic system, not just the portion surveyed, and should be determined based upon the standing water present at the time of the survey, not the usual waterline or high water mark. Use a map as a guide for larger systems.

*LOTIC WIDTH: For lotic systems, select 1 range that best describes the width of water at the time of the survey. Do not measure the normal waterline or the high water mark.

*RIPARIAN WIDTH: Circle the category that includes the maximum width of the riparian area in meters. Riparian width should be measured from the boundary of riparian vegetation and upland vegetation. For a lentic system, include the area of riparian vegetation along the shore of the body of water and any vegetated waters. For a small lotic system in which both banks can be surveyed simultaneously, include the zone of riparian vegetation on both banks of the body of water surveyed and any vegetated waters. For large or swiftly flowing lotic systems, include only bank that was surveyed or the maximum width of riparian vegetation on both banks. Riparian width is measured for the area surveyed.

*PRIMARY SUBSTRATE: Circle from 1 - 3 categories as appropriate. All substrate types may be present, but choose only those that best describe the area potentially inhabited by target species.

Mud/Silt = 0.001-0.1 mm
Sand = 0.1-2 mm
Gravel = 2-32 mm
Cobble = 32-256 mm
Boulder >256 mm
Bedrock = exposed sheet of rock

*WIND: Circle 1 category as appropriate. Wind should be measured 1.5 meters above the ground and 1.5 meters from the water. If using a wind meter, be sure to: 1) hold meter near the top so that you are not blocking any holes, 2) face into the direction of the wind while reading the meter, and 3) use the left scale for wind strengths < 10 mph, and use the right scale (by putting your index finger over the red knob on top of the meter) for wind strengths ≥10 mph. Wind categories are those used in the Beaufort scale:

≤1 mph = smoke rises vertically
1-3 mph = wind direction shown by smoke drift
4-7 mph = wind felt on face, leaves rustle
8-12 mph = leaves and small twigs in constant motion, wind extends light flag
13-18 mph = raises dust and loose paper, small branches are moved
19-24 mph = small trees begin to sway, crested wavelets form on inland waters
>24 mph = greater effect than above

*CLOUD COVER: Circle 1 category as appropriate. Categories are based on percent cover.

*PRECIPITATION: Circle 1 category as appropriate.

*****LAND USE: Circle all categories that best indicate the land use at a survey site. For noteworthy land uses that are not immediately at the survey site but which may potentially impact the study site (e.g. large agricultural fields within 1 mile of survey site, active mining operation 0.5 mile upstream of survey area), fill out the land use field as described here, and also make written comments about the land use in the SITE / SURVEY NOTES. The land-use categories are:

Agric = agriculture (include agriculture fields, diversion canals, etc.)
Dev = human development (include road construction, dam site, housing development, etc.)

Graze = cattle grazing (include manure, hoofprints, invading species and grass length as evidence of grazing use); note elk/deer grazing in OTHER ORGANISMS and OTHER ORG. NOTES, but only if heavy
Log = logging
Mine = mining (include 50+ year tailings/shafts, currently active mines, small claims, and large developments)
Rec = recreation (include developed and primitive campsites trails, litter, etc.)

VEGETATION % & PROMINENT SPECIES: Record the percent of the area potentially inhabited by target species that is covered by floating vegetation (e.g. broad-leafed macrophytes and dense algal mats), submerged vegetation, emergent vegetation (e.g. cattails, sedges, rushes), perimeter vegetation (i.e. up to 1 m from waters edge), and canopy vegetation. Use increments of 5% (i.e. 1% effectively = 0). Record the genus name or common name (only if positively identified) of the 1-4 most prominent species that best describe the surveyed area.

*****PREDATORS: Circle all predators seen or otherwise detected at a survey site. Most predator categories lump together similar organisms and/or organisms with similar effects on riparian herps. Record herp predators in the Herpetofauna Observations table. For **crayfish**, include claws and carapaces as evidence of presence. For **dragonflies**, do not include damselflies. For **beetles**, include any large aquatic beetles observed, such as hydrophilids and dytiscids. **Warm water fish** include bass, carp, catfish, perch, sunfish, and walleye. **Cold water fish** include trout and pike. **Large wading birds** include American bittern, black-crowned night heron, egrets, great blue heron, and green-backed night heron. **Mammals** include only medium-sized mammals such as skunk, ring-tail, and raccoon.

*****OTHER ORGANISMS: This field is to be used for observations of species other than riparian herpetofauna. Riparian herps are to be recorded in the "Herpetofauna Observations" table. List all non-riparian herps by 4-letter genus/species code (see Herpetofauna List -Derived from Stebbins (1985). List Federal or State sensitive species of other organismal groups or any other species whose occurrence merits noting by common name. No words other than the species name(s) should be listed (e.g. UXOR, SCC, great horned owl, elk). Use the OTHER ORG. NOTES field as needed to expand upon why you listed a species.

OTHER ORG. NOTES: Use this field to write out noteworthy observations about any or all of the species listed in OTHER ORGANISMS (e.g. UXOR observed mating, great horned owl roost site observed, area heavily impacted by elk grazing).

SITE / SURVEY NOTES: Use this field to describe the most outstanding features of a survey or site. **Don't be redundant** with fields already completed. Write short, specific comments that emphasize habitat quality and why you think you did or did not find herps. Be sure to comment on any land use in, around, or in proximity of the survey area that may potentially impact the study site (e.g. large mining operation 0.5 mile upstream of survey site, agricultural spraying 1 mile from survey site). You can also use this field to describe any noteworthy similarities or dissimilarities between the area searched and the total area (e.g. wash devoid of vegetation except in area of survey, survey covered the north end of the lake which was the only area with emergent vegetation).

Herpetofauna Observations:

*SPECIES: Record all riparian herp species (target or non-target) detected during a survey in this column. Record non-riparian herpetofauna in the OTHER ORGANISMS and OTHER ORG. NOTES. If no species are observed, record “NONE.” Use the unique 4-letter Genus-species code (see "Herpetofauna List - Derived from Stebbins (1985)") for all riparian herp species. When an organism cannot be identified to species (e.g. "I saw a ranid-like frog", or "I saw an anuran egg mass"), use the 4-letter code corresponding to the taxonomic classification for which you are confident in your identification. For the examples above, the ranid-like frog would be assigned the code "RANA", and the egg mass would be coded as "ANUR". If you are confident you saw a leopard frog but are not certain which species you saw, use the code "RAPC." Do not use historical information to bias your decision on species identification. Record your most confident observation and justify it in the NOTES or COMMENTS.

CERTAINTY: Circle 1 word to indicate your level of certainty about your identification of each species. Certainty of identification should be based on species-specific diagnostic characters (e.g. thigh pattern and dorsolateral folds in leopard frogs, scale row of lateral stripes in garter snakes, lack of dorsal stripe and cranial crests in Arizona toads). For information on diagnostic characters of species, see Stebbins (1985), "Characteristics of Arizona Leopard Frogs", and "Garter Snakes of Coconino National Forest."

LIFE STAGE: Circle the life stage of each species observed. Use separate rows for different life stages of the same species. A juvenile leopard frog is usually < 55 mm SVL, while an adult is > 55 mm SVL or exhibits obvious sign of breeding condition (e.g. swollen thumbpads, stretched vocal sacs)

OBSERVED: Enter the number of individuals of each species and life stage you encountered. Do not estimate total numbers within the survey area, but record only the number that you saw. For egg masses, estimate the number of eggs, note the overall size of mass, condition, and stage of embryos in the NOTES or COMMENTS sections (see Gosner 1960).

NOTES: Record any relevant notes specific to the species or life stage observed. Types of observations to include are as follows: 1) what criteria were used to identify a species; 2) if species identification is uncertain, what was observed including both physical features and behaviors would be of use (e.g. “dorsal spots obs.,” “ranid like plop,” “no bullfrog peep”); 3) record the collection number (e.g. AGFD field tag #) of any voucher specimens taken; 4) record any photo vouchers taken; and 5) note the presence of disease or deformities.

COMMENTS: Use this field to elaborate upon species observations. Be sure to reference your comments with the species observation to which it relates by using numbers or letters (i.e. a footnote).

SECTION 9: DISEASE PREVENTION PROTOCOL

Updated July 2004

The Relict Leopard Frog Conservation Team (RLFCT) adopts the disease prevention protocol for working in wetlands from the Lake Mead National Recreation Area (NRA). All resource and land management agencies are encouraged to follow this or a similar protocol to prevent or reduce the spread of amphibian and other aquatic borne diseases. This protocol for working in wetland habitats is adapted from the DAPTF's (Declining Amphibian Populations Task Force) Fieldwork Code of Practice, which provides guidelines for use by anyone conducting fieldwork in amphibian or other aquatic habitats. Chytrid fungus and other highly contagious and deadly diseases are being reported worldwide, and may be a significant cause of amphibian population declines. Within the historical range of the relict leopard frog, viability of the known, small and scattered populations of this species is of particular concern. Pathogens and parasites can easily be transferred between habitats on equipment and footwear of fieldworkers, spreading organisms to new locations containing species that have little or no resistance to the agents. It is vitally important for anyone involved in amphibian research and other types of wetland studies, including those on fish, bats, invertebrates and plants, to take steps to prevent the introduction of disease agents and parasites. For further DAPTF information, see http://www.open.ac.uk/daptf/index.htm.

<u>Requirements for Working in Wetland and Aquatic Systems</u>

1. Dedicated equipment will be used by staff, crews, and permitees frequently working in springs occupied by relict leopard frogs. This includes footwear. Dedicated equipment will be cleaned and stored separately.
2. Equipment which cannot be duplicated or can be easily cleaned must be disinfected between visits to springs. Equipment will be rinsed and all debris removed. Surfaces, which should appear clean, will be scrubbed with a 10% bleach, 1.6% Quat-128, or 70% ethanol solution and rinsed with tap water. Footwear belonging to occasional users must be completely cleaned before and between visiting spring sites, with special attention paid to grips, cleats, and laces. Felt-bottomed wader boots are very difficult to clean completely and should be avoided whenever possible. To further reduce the risk of disease transfer, all equipment will be completely dried before re-use. Bat and bird netting which has remained out of the water does not have to be wetted. Poles and stakes need to be completely cleaned as above. Trowels used to collect plants need to be dedicated or completely disinfected between springs.
3. In remote locations, clean all equipment as described above upon return to the lab or base camp. If disinfecting in the field is necessary, sanitize all items before arriving at the next location. Do not use solutions in the immediate vicinity of the springs or in other habitats. Used cleaning materials (including liquids) must be disposed of safely and if necessary taken back to the lab for proper disposal.

4. Staff, crews, and permitees will be provided with a chytrid information sheet, and advised of known chytrid locations throughout the region. Boots and equipment used in known chytrid locations should not be used in Lake Mead NRA.
5. When animals are collected, separation of specimens from different sites will be ensured and great care taken to avoid indirect contact between them (e.g. via handling, reuse of containers) or with other captive animals. Isolation from unsterilized plants or soils that have been taken from other sites is also essential.
6. Amphibians that are headstarted for release into refugia will be grown using clean lab methods and disinfected prior to release (See Section 4).

UNPUBLISHED DATA

[NPS] National Park Service, Lake Mead National Recreation Area, Boulder City, NV.

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APPENDIX 6: 2003 ANNUAL WORK PLAN AND REPORTS

2003 ANNUAL WORK PLAN

1. Bring Willow Beach Hatchery on-line as a functioning frog rearing facility. (Chester Figiel; Action 8 in Implementation Schedule)
2. Complete the Conservation Agreement and Strategy (CAS). (RLFCT; Action 1 in Implementation Schedule)
3. Begin radio telemetry study as described in Jef Jaeger and Brett Riddle's Clark County MSHCP proposal. (Jef Jaeger; Action 12 in Implementation Schedule)
4. Conduct a survey of historical habitat in Arizona, and begin evaluating sites for translocations. (Cristina Velez; Action 9 in Implementation Schedule)
5. Augment Sugarloaf Spring translocation site. (Cristina Velez; Action 10 in Implementation Schedule)
6. Monitor Sugarloaf Spring population. (Cristina Velez; Action 11 in Implementation Schedule)
7. Monitor all existing populations. (Cristina Velez; Action 11 in Implementation Schedule)
8. Initiate metabolic study at UNLV as described in Karin Hoff and Stan Hillyard's Clark County MSHCP proposal. (Karin Hoff and Jeff Goldstein; Action 12 in Implementation Schedule)
9. Improve National Park Service (NPS) frog rearing facility. (Cristina Velez; Action 8 in Implementation Schedule)
10. Establish a monitoring protocol. (Mike Sredl and RLFCT; Actions 1 and 11 in Implementation Schedule)
11. Finalize the translocation protocol. (Mike Sredl and RLFCT; Actions 1 and 9 in Implementation Schedule)
12. Develop site-selection criteria for translocations. (Mike Sredl and RLFCT; Action 9 in Implementation Schedule)
13. Initiate Pakoon Springs evaluation. (Cristina Velez; Action 9 in Implementation Schedule)
14. Nevada site evaluation. (Cristina Velez and Jeff Goldstein; Action 9 in Implementation Schedule)

2003 ANNUAL REPORTS

1. Bring Willow Beach Hatchery on-line as a functioning frog rearing facility. (Chester Figiel; Action 8 in Implementation Schedule)

Rana onca tadpoles were brought onto the Willow Beach NFH in November and December 2002 to determine whether the hatchery could: 1) accommodate tadpoles (i.e., space and water), 2) prevent disease cross-contamination with fish, and 3) rear tadpoles to metamorphosis. At that time, the hatchery asked for approval from the Region 2 USFWS to bring a new species onto station. This request was put forth to the Washington office and on April 30, 2004, the hatchery received this approval. The hatchery successfully accommodated tadpoles and prevented disease cross-contamination between amphibians and fish, and was successful in rearing tadpoles to metamorphosis.

2. Complete the Conservation Agreement and Strategy (CAS). (RLFCT; Action 1 in Implementation Schedule)

The CAS went through several drafts in 2003, but was still incomplete at the end of the year.

3. Begin radio telemetry study as described in Jef Jaeger and Brett Riddle's Clark County MSHCP proposal. (Jef Jaeger; Action 12 in Implementation Schedule)

The grant money for this project did not become available until late in the year. Our work on this project during 2003 was limited to formalizing agreements, acquiring permits, and formalizing data collection designs.

4. Conduct a survey of historical habitat in Arizona, and begin evaluating sites for translocations. (Cristina Velez; Action 9 in Implementation Schedule)

One site in Arizona, Grapevine Spring, was evaluated and approved for translocations.

5. Augment Sugarloaf Spring translocation site. (Cristina Velez; Action 10 in Implementation Schedule)

164 froglets were released into Sugarloaf Spring in 2003.

6. Monitor Sugarloaf Spring population. (Cristina Velez; Action 11 in Implementation Schedule)

No night visual encounter surveys were conducted at Sugarloaf Spring in 2003.

7. Monitor all existing populations. (Cristina Velez; Action 11 in Implementation Schedule)

The National Park Service hired a fulltime wildlife biologist to monitor frog populations at the end of 2003. Only one night visual encounter survey was conducted in the fall of 2003, at Blue Point Spring. 22 adult relict leopard frogs were observed in the upper portion of the spring.

8. Initiate metabolic study at UNLV as described in Karin Hoff and Stan Hillyard's Clark County MSHCP proposal. (Karin Hoff and Jeff Goldstein; Action 12 in Implementation Schedule)

Funding did not start until Feb. 2004. See 2004 report (Appendix 7).

9. Improve National Park Service (NPS) frog rearing facility. (Cristina Velez; Action 8 in Implementation Schedule)

The frog facility was improved by adding an external filtering system to all tadpole aquariums, and by adding artificial vegetation and more dry land to the frog tanks.

10. Establish a monitoring protocol. (Mike Sredl and RLFCT; Actions 1 and 11 in Implementation Schedule)

The monitoring protocol is found in Appendix 5 of the CAS: Relict Leopard Protocols and Techniques Manual.

11. Finalize the translocation protocol. (Mike Sredl and RLFCT; Actions 1 and 9 in Implementation Schedule)

The translocation protocol is found in Appendix 5 of the CAS: Relict Leopard Protocols and Techniques Manual.

12. Develop site-selection criteria for translocations. (Mike Sredl and RLFCT; Action 9 in Implementation Schedule)

The site-selection criteria for translocations are found in Appendix 5 of the CAS: Relict Leopard Protocols and Techniques Manual.

13. Initiate Pakoon Springs evaluation. (Cristina Velez; Action 9 in Implementation Schedule)

Pakoon Spring has many bullfrogs and is currently not a suitable area to release *Rana onca*.

14. Nevada site evaluation. (Cristina Velez and Jeff Goldstein; Action 9 in Implementation Schedule)

Hiko Springs, Nevada (BLM land) and Gnatcatcher Springs, Nevada were surveyed as potential frog relocation sites, but neither one seemed suitable for frogs. Funding for UNLV work did not start until Feb. 2004. See 2004 report (Appendix 7).

Other items not included in the 2003 work plan:

The following talk was given (Action 2 in Implementation Schedule):

Jaeger J.R., Bradford D.F., Jennings R.J., Riddle B.R. Conservation of the relict leopard frog (*Rana onca*): our limited understanding of the distribution, size, structure, and dynamics of

extant and recently extinct populations. Presentation given at the BIOS Symposium, October 18, 2003. Department of Biological Sciences, University of Nevada, Las Vegas.

APPENDIX 7: 2004 ANNUAL WORK PLAN AND REPORTS

2004 ANNUAL WORK PLAN

1. Improve the frog rearing capabilities at the Willow Beach Hatchery. (Chester Figiel; Action 8 in Implementation Schedule)
2. Initiate planning for artificial habitat at Willow Beach (Warm water well and outflow). (Chester Figiel; Action 8 in Implementation Schedule)
3. Complete the Conservation Agreement and Strategy (CAS). (RLFCT; Action 1 in Implementation Schedule)
4. Begin field activities on the radio telemetry study as described in Jef Jaeger and Brett Riddle's Clark County MSHCP proposal. (Jef Jaeger; Action 12 in Implementation Schedule)
5. Continue to conduct site evaluations in both Nevada and Arizona for translocations. (Cristina Velez and Jeff Goldstein; Action 9 in Implementation Schedule)
6. Augment Sugarloaf Spring Translocation Site. (Cristina Velez; Action 10 in Implementation Schedule)
7. Monitor Sugarloaf Spring population. (Cristina Velez; Action 11 in Implementation Schedule)
8. Monitor all existing populations. (Cristina Velez; Action 11 in Implementation Schedule)
9. Initiate metabolic study at UNLV as described in Karin Hoff and Stan Hillyard's Clark County MSHCP proposal. (Jeff Goldstein and Karin Hoff; Action 12 in Implementation Schedule)
10. Review and revise the monitoring protocol. (Mike Sredl; Actions 1 and 11 in Implementation Schedule)
11. Review and revise site-selection criteria for translocations. (Mike Sredl; Actions 1 and 9 in Implementation Schedule)
12. Develop field identification protocol for tadpoles. (Cristina Velez, Karin Hoff, and Mike Sredl; Action 12 in Implementation Schedule)
13. Enhance habitat at the Pupfish Refuge Spring, and augment the population. (Cristina Velez and Mike Burrell; Actions 3 and 10 in Implementation Schedule)
14. Complete the development of microsatellite markers for population genetics. (Jef Jaeger; Action 12 in Implementation Schedule)
15. Identify at least two new sites that are completely ready to receive transplanted frogs, including all compliance documentation, by 2005. (Cristina Velez; Action 9 in Implementation Schedule)
16. Work with the appropriate partners toward developing a refuge on (or near) the Muddy River. (Jon Sjoberg; Action 9 in Implementation Schedule)
17. Introduce tadpoles to Grapevine Spring Arizona and Goldstrike Spring (both on Lake Mead NRA), and begin monitoring the success of those transplants. (Cristina Velez; Actions 9 and 11 in Implementation Schedule)

2004 ANNUAL REPORTS

1. Improve the frog rearing capabilities at the Willow Beach Hatchery. (Chester Figiel; Action 8 in Implementation Schedule)

Frog rearing capabilities were improved at Willow Beach NFH in two ways: 1) A 20’ x 2 1/2’ raceway was used to increase the number of tadpoles reared to metamorphosis and 2) an 8’ x 2 1/2’ tank was used to house 19 adults from the previous year.

2. Initiate planning for artificial habitat at Willow Beach (Warm water well and outflow). (Chester Figiel; Action 8 in Implementation Schedule)

The hatchery grounds were explored for potential sites for the creation of a wetland area. We wanted this area to be 1) similar to sites where *Rana onca* currently are found, i.e., similar pools and runs, 2) be protected from the public and other hatchery operations, and 3) have a good water source. A 100 m section on the hatchery appears to be acceptable for creation of this artificial habitat. Water would have to be pumped or piped to this site and pools would have to be developed.

3. Complete the Conservation Agreement and Strategy (CAS). (RLFCT; Action 1 in Implementation Schedule)

The CAS was sent out for agency review, but because of edits and comments a copy was not finalized in 2004.

4. Begin field activities on the radio telemetry study as described in Jef Jaeger and Brett Riddle’s Clark County MSHCP proposal. (Jef Jaeger; Action 12 in Implementation Schedule)

During 2004, we completed field activities necessary to develop a model of habitat selection by relict leopard frogs at upper Blue Point Spring. As part of our methodological evaluation for this project, a preliminary evaluation of radio-belting procedures on adult *Rana onca* was completed in early 2004 prior to field efforts. We conducted field tracking of adult frogs from April 7 through December 2, 2004. A total of 93 daytime and 90 nighttime tracking events were conducted for a total of 413 daytime and 396 nighttime individual frog observations. Measurements of habitat characteristics (e.g., various measures of vegetation cover, stream width and depth, etc.) were completed during the spring. These habitat data were collected by line transects run across the riparian width (perpendicular to the stream) approximately every meter down the length of the stream channel. In addition, microhabitat features within 0.25 m of each frog observation were also measured. Analyses of these data are planned for 2005 using Polytomous Logistic Regression and multiple analyses of variance. Quarterly reports on this project are available on the web from the Clark County Multiple Species Habitat Conversation Plan Database.

5. Continue to conduct site evaluations in both Nevada and Arizona for translocations. (Cristina Velez and Jeff Goldstein; Action 9 in Implementation Schedule)

Nevada done as per contract with UNLV. Twenty-one spring sites on Gold Butte (with water rights owned by Clark County, NV) were examined in mid-summer of 2004 to determine the extent of water availability and the presence of riparian vegetation. Only two of these sites had both surface water and riparian vegetation. Both would require significant restoration work before frogs could be translocated to those sites. Examination of one additional Gold Butte spring site was recommended by the RLFCT. That site will be examined in early 2005.

Tassi Spring, Arizona was evaluated and approved for translocations, and compliance was initiated.

6. Augment Sugarloaf Spring Translocation Site. (Cristina Velez; Action 10 in Implementation Schedule)

161 froglets were released into Sugarloaf Springs during 2004.

7. Monitor Sugarloaf Spring population. (Cristina Velez; Action 11 in Implementation Schedule)

Sugarloaf Spring was visited eight times in 2004, twice (spring and fall) for night visual encounter surveys (VES). During the spring VES, 39 adult, 2 juvenile, and 22 *Rana onca* tadpoles were seen. By late July much of the spring had dried up; fortunately, we still observed 32 adults and 3 tadpoles during the fall VES.

8. Monitor all existing populations. (Cristina Velez; Action 11 in Implementation Schedule)

All natural and experimental populations of relict leopard frogs were monitored in 2004:
Natural:
<u>Bighorn Sheep Spring</u>- Found 32 egg masses in January. During the spring VES, we counted 188 *Rana onca* (RAON) adults, 10 juveniles, over 300 tadpoles, and another 22 egg masses. During the fall VES, we counted 354 RAON adults, 19 juveniles, and 69 tadpoles.
<u>Blue Point Spring</u>- Found 18 RAON adults and 4 tadpoles during spring VES, and 32 adults and 3 juveniles during fall VES.
<u>Boy Scout Spring</u>- During spring night VES, 21 adult RAON observed.
<u>Roger's Spring</u>- During spring VES we observed 5 very skinny RAON adults. During the fall VES we only observed 1 adult RAON.
<u>Salt Cedar Spring</u>- In the spring VES, we observed 4 RAON adults, 3 juveniles, and 32 tadpoles.

Experimental:
<u>Goldstrike Canyon</u>- During fall night VES we observed 15 adult RAON.

Grapevine Spring, AZ- During a day VES in the fall we observed 6 small RAON, but did not see any during the fall night VES.
Pupfish Refuge Spring- During night VES in the fall, we observed 18 adult RAON.

9. Initiate metabolic study at UNLV as described in Karin Hoff and Stan Hillyard's Clark County MSHCP proposal. (Jeff Goldstein and Karin Hoff; Action 12 in Implementation Schedule)

Due to delay of funding, experiments did not start until tadpoles were almost 2 months old. Growth and development data were collected for tadpoles at 5 acclimation temperatures. Tadpoles at 20, 25 and 30 degrees C developed normally. Tadpoles started losing mass at 35 degrees, so that acclimation temperature was discontinued. Tadpoles at 15 degrees increased in size, but did not progress developmentally, so after 5 months the temperature was increased at a rate consistent with the onset of spring. The 15 degree tadpoles then developed normally. Size at metamorphosis was greatest in the tadpoles held at the lowest temperatures, and was also proportional to the length of larval life. Twenty-five degrees produced large tadpoles in a short time and had by far the smallest variance in time to metamorphosis of any temperature group. Of the temperatures examined, 25 degrees appears to be optimal for captive rearing. The rearing results further suggest that thermally influenced springs with temperatures above 30 degrees C should not be considered as translocation sites, while ambient temperature waters that can be very cold in the winter should not be excluded from consideration.
Preliminary metabolic and performance experiments showed some responses to change in temperature, but because of the late start there was insufficient data collection to determine a clear acclimation effect. These experiments will be finished in 2005 when acclimation can begin very early in larval life.

10. Review and revise the monitoring protocol. (Mike Sredl; Actions 1 and 11 in Implementation Schedule)

The monitoring protocol in Appendix 5 (Relict Leopard Protocols and Techniques Manual) of the CAS was revised in 2004.

11. Review and revise site-selection criteria for translocations. (Mike Sredl; Actions 1 and 9 in Implementation Schedule)

The site-selection criteria for translocations in Appendix 5 (Relict Leopard Protocols and Techniques Manual) of the CAS were revised in 2004.

12. Develop field identification protocol for tadpoles. (Cristina Velez, Karin Hoff, and Mike Sredl; Action 12 in Implementation Schedule)

Not done, needs further description.

13. Enhance habitat at the Pupfish Refuge Spring, and augment the population. (Cristina Velez and Mike Burrell; Actions 3 and 10 in Implementation Schedule)

The Pupfish Refuge Spring is located on United States Bureau of Reclamation (USBR) property near the base of Hoover Dam. Because of its proximity to Hoover Dam and associated security concerns, access is restricted by the USBR. The spring and high gradient outflow are on the Nevada side of the Colorado River. The outflow is less than 300m in length and discharges directly into the river. The approximate UTM is 11S 0703317 3987207 (taken from a map, not on site). Estimated flow is less than .5 cfs.

The outflow stream was thickly overgrown with vegetation. The thick vegetation resulted in minimal areas of open riparian habitat. The exotic *Tammarix gallica (*tamarisk) is the primary plant chocking the outflow. In an effort to improve the habitat for the benefit of *Rana onca* (relict leopard frog), sections of the outflow stream were cleared of vegetative overgrowth. In 2004 approximately 30 meters of stream were opened using hand tools and a chainsaw (Figure 3). In an effort to provide pool habitat, several small rocks and/or sandbag dams were created in the cleared sections. Labor was provided by the Nevada Department of Wildlife (NDOW) and the National Park Service (NPS). The work required 4 man-days of effort.

To facilitate large scale removal of vegetation and debris in early 2005, additional coordination with the USBR was required in 2004. Clearance was obtained to allow the NPS exotic plant management team access in early 2005.

391 froglets were released into the Pupfish Refuge Spring between October 2003- August 2004.

14. Complete the development of microsatellite markers for population genetics. (Jef Jaeger; Action 12 in Implementation Schedule)

Our assessments of population genetic structure within remnant populations of *R. onca* will require development of quickly evolving molecular markers to produce meaningful results. As part of our strategy, we developed microsatellite markers within *R. onca*. Four microsatellite-enriched libraries were prepared from *R. onca* DNA with each of the libraries containing between 10,000 and 15,000 recombinant cells. We attempted enrichment for four different microsatellite motifs, and based on a sampling of clones, greater than 78% of clones within each library contained repeat motifs. A total of 99 clones were successfully sequenced from these libraries (about an equal number from each library). In total, we identified 79 microsatellite-containing clones from sequencing runs. Using primer designing software, we designed PCR primers for 47 microsatellite-containing loci. The next step in the process will be to screen these primers for their usefulness and variability in populations of *R. onca*. Funding for analysis of populations using these markers was sought in a proposal to the Clark County Multiple Species Habitat Conservation Plan (see below).

15. Identify at least two new sites that are completely ready to receive transplanted frogs, including all compliance documentation, by 2005. (Cristina Velez; Action 9 in Implementation Schedule)

Tassi Spring and Red Rock Spring have been identified as potential new release sites, and compliance documentation was initiated.

16. Work with the appropriate partners toward developing a refuge on (or near) the Muddy River. (Jon Sjoberg; Action 9 in Implementation Schedule)

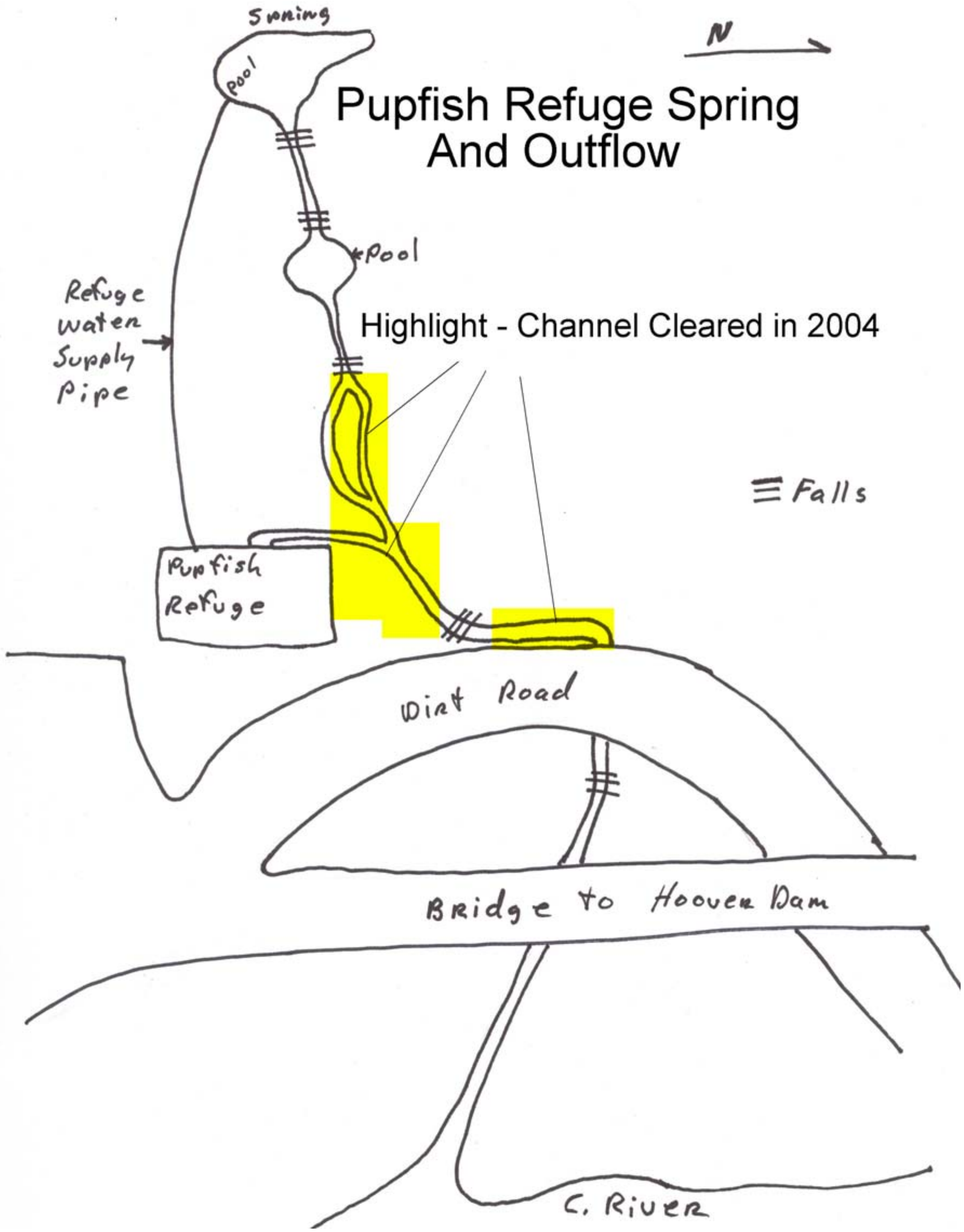
Field coordination and site evaluation for potential relict leopard frog refugium sites in the upper Muddy River area were pursued in 2004 with The Nature Conservancy (TNC), BLM, and USFWS – Refuges. The Perkins Ranch property on the Muddy River near the Warm Springs Road crossing, recently acquired by TNC from private ownership, contains a pond which appears to have appropriate size, depth and habitat characteristics for use as a refugium site. However, initial evidence suggests that the site or adjacent habitats contain bullfrogs, which could be problematic for relict leopard frogs due to predation and competition. NDOW, TNC and other partners are developing a strategy to reduce bullfrog presence and increase suitability for refugium use, which would require renovating the pond itself and providing barriers to bullfrog access. TNC is currently developing a Preliminary Management Plan for acquired properties in the upper Muddy River area, including the Perkins Ranch. That plan will address relict leopard frog as a target species for management planning and restoration.

The Moapa NWR and additional private lands in the upper Muddy River drainage may contain suitable habitats to support relict leopard frogs or refugium sites. Evaluations of these additional sites were not completed in 2004 but should be pursued in 2005 as time is available. The FWS refuge contains a number of thermal spring brook and spring pool outflow systems which may be capable of supporting frog populations but the relict leopard frog is not included as a priority species for refuge management and restoration planning at this time.

17. Introduce tadpoles to Grapevine Spring Arizona and Goldstrike Spring (both on Lake Mead NRA), and begin monitoring the success of those transplants. (Cristina Velez; Actions 9 and 11 in Implementation Schedule)

In 2004, 905 tadpoles were released to Grapevine Spring, Arizona, and 879 tadpoles were released to Goldstrike Canyon. See #8 above for monitoring results.

Figure 3. Diagram of Pupfish Refuge Spring and outflow. Highlighted areas were cleared of vegetation in 2004. (not to scale)



Other items not included in the 2004 work plan:

In association with the assessment of population genetic structure, we have focused on evaluation of sequence variation within mitochondrial DNA. We have sequenced 1082 bp of the ND2 gene which contained a small amount of variation between individuals from the two remaining *R. onca* population centers (i.e., Black Canyon and the North Shore springs). We sequenced population-level samples from all sites with extant natural populations (n=5) and from the recently extinct population at Littlefield. Population-level samples from three regional northern populations of *R. yavapaiensis* were also sequenced for comparative purposes. This data set was used to assess the identity of the newly found leopard frog population at Surprise Canyon. These data were presented at the Declining Amphibian Task Force, California and Nevada Working Group, January 15-16, 2004.

The following manuscript was published:

Bradford D.F., J.R. Jaeger, R.D. Jennings. 2004. Population status and distribution of a decimated amphibian, the relict leopard frog (*Rana onca*). Southwestern Naturalist 49(2):218-228.

The following talks were given (Action 2 in the Implementation Schedule):

Harris S.M., Jaeger J.R., Bradford D.F., Riddle B.R. Evaluating vegetation encroachment on relict leopard frogs: a precursor to habitat management. Presentation given at the BIOS Symposium, October 23, 2004, Department of Biological Sciences, University of Nevada, Las Vegas.

Jaeger J.R., Bradford D.F., Jennings R.J., Riddle B.R. Status of the relict leopard frog (*Rana onca*): our limited understanding of the distribution, size, and dynamics of extant and recently extinct populations. Presentation given at the Declining Amphibian Task Force, California and Nevada Working Group, January 15-16, 2004. University of Nevada, Reno.

The following proposals were developed and submitted to the Clark County Multiple Species Habitat Conservation Plan (Action 12 in the Implementation Schedule):

Delineation of Distribution, Evaluation of Relatedness, and Assessment of Connectivity for Leopard Frog Populations (*Rana* spp.) within the Management Zone of the Relict Leopard Frog (*Rana onca*). Authored by Jaeger J.R. and Riddle B.R. Submitted (in August) to the Clark County Multiple Species Habitat Conservation Plan ($147,836 requested).

Evaluation of Experimental Habitat Manipulations on Relict Leopard Frog Populations. Authored by Jaeger J.R. and Riddle B.R. Submitted to the Clark County Multiple Species Habitat Conservation Plan ($87,267 requested).

APPENDIX 8: 2005 ANNUAL WORK PLAN

2005 ANNUAL WORK PLAN

1. Improve the frog rearing capabilities at the Willow Beach Hatchery. (Chester Figiel; Action 8 in the Implementation Schedule)
2. Initiate planning for artificial habitat at Willow Beach (warm water well and outflow) as described in the MSHCP proposal, *Establishment of a Rana onca population in a Created Aquatic Habitat* (2005-USFWS-595-P). (Chester Figiel; Action 8 in the Implementation Schedule)
3. Complete the Conservation Agreement and Strategy (CAS). (RLFCT; Action 1 in the Implementation Schedule)
4. Complete radio telemetry study as described in the Clark County MSHCP project, *Evaluation of the Impact of Vegetation Encroachment on Relict Leopard Frog Populations* (2003-NPS-232-P-2004-01) by Jef Jaeger and Brett Riddle. (Jef Jaeger; Action 12 in the Implementation Schedule)
5. Develop habitat model and make recommendations for habitat manipulations, if appropriate, at Blue Point Spring. (Jef Jaeger; Action 12 in the Implementation Schedule)
6. Continue to conduct site evaluations at Tassi Spring, Red Bluff Spring, Red Rock Spring, and a pond at Kingman's community college. (Cristina Velez, Jeff Goldstein, Gerry Hickman, and Mike Sredl; Action 9 in the Implementation Schedule)
7. Augment Sugarloaf Spring, Pupfish Refuge Spring, Goldstike Canyon Spring and Grapevine Spring, AZ translocation sites. (Cristina Velez; Action 10 in the Implementation Schedule)
8. Conduct site maintenance at Sugarloaf Spring and Boyscout Canyon spring after heavy January rains. (Cristina Velez; Action 10 in the Implementation Schedule)
9. Monitor all translocated populations: Sugarloaf Spring, Pupfish Refuge Spring, Goldstike Canyon Spring and Grapevine Spring, AZ. (Cristina Velez; Action 11 in the Implementation Schedule)
10. Monitor all naturally existing populations: Bighorn Sheep Spring, Blue Point Spring, Roger's Spring, Boyscout Canyon Spring, and Salt Cedar Spring. (Cristina Velez; Action 11 in the Implementation Schedule)
11. Finish metabolic study at UNLV as described in Karin Hoff and Stan Hillyard's Clark County MSHCP proposal, *Temperature Acclimation and Oxygen Consumption of Rana onca larvae* (2003-NPS-230-P-2004-16). (Jeff Goldstein; Action 12 in the Implementation Schedule)
12. Review and revise the monitoring protocol. (Mike Sredl; Action 1 and 11 in the Implementation Schedule)
13. Enhance habitat at the Pupfish Refuge Spring and Salt Cedar Spring. (Cristina Velez and Mike Burrell; Action 3 in the Implementation Schedule)

14. Work on preparing Tassi Spring and Red Rock Spring to receive transplanted frogs by 2006. (Cristina Velez; Action 9 in the Implementation Schedule)
15. Work with the appropriate partners toward developing a refuge on (or near) the Muddy River. (Jon Sjoberg; Action 9 in the Implementation Schedule)
16. Develop GIS map of natural, transplanted and potential sites. (Cristina Velez; Action 13 in the Implementation Schedule)
17. Work with the Nevada Division of State Parks to develop an agreement and move forward toward the establishment of an Ash Grove Spring Refugium. (Jon Sjoberg; Action 9 in the Implementation Schedule)
18. Begin implementation of the Clark County MSHCP proposal *Relict Leopard Frog Monitoring and Management* (2005-NPS-476-P). (Ross Haley and Cristina Velez; Actions 9, 10, and 11 in the Implementation Schedule)
19. Begin implementation of the Clark County MSHCP proposal *Establishment of a Rana onca population in a Created Aquatic Habitat* (2005-USFWS-595-P). (Chester Figiel; Action 8 in the Implementation Schedule)
20. Begin implementation of the Clark County MSHCP proposal *Delineation of Distribution, Evaluation of Relatedness, and Assessment of Connectivity for Leopard Frog Populations (Rana spp.) within the Management Zone of the Relict Leopard Frog (Rana onca)* (2005-UNLV-575-P). (Jef Jaeger; Action 12 in the Implementation Schedule)
21. Begin implementation of the Clark County MSHCP proposal *Evaluation of Experimental Habitat Manipulations on Relict Leopard Frog Populations* (2005-UNLV-597-P). (Jef Jaeger; Actions 3 and 12 in the Implementation Schedule)