Ramsey Canyon Leopard Frog Conservation Agreement and Conservation Assessment and Strategy

Nongame Branch, Wildlife Management Division
Arizona Game and Fish Department
Phoenix, Arizona

Ramsey Canyon Leopard Frog Conservation Team

August 2007

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ACKNOWLEDGMENTS

All members, past and present, of the Ramsey Canyon Leopard Frog Conservation Team have made significant contributions to the conservation of this species. Current members are listed in Appendix 2: Principal Contacts. Kim Field, Michael Sredl, Valerie Boyarski (all of Arizona Game and Fish Department [AZGFD]), and Jim Rorabaugh (U.S. Fish and Wildlife Service – Ecological Services [USFWS-ES]) were the principal authors, compilers, and editors of this Strategy. Jim Platz (Creighton University) wrote the first Conservation Strategy, which was the basis for initial conservation activities and the 1996 Conservation Agreement. Key conservation partners have included Arizona-Sonora Desert Museum (Craig Ivanyi), AZGFD (Valerie Boyarski, Mike Demlong, Kim Field, Jeff Howland, Anne Peterson, Michael Sredl, Eric Wallace), Sarah Barchas (Barchas Ranch), Tom Sr., Tom J., and Edith Beatty (Beatty's Guest Ranch), Hank and Priscilla Brodkin (Carr Canyon), Coronado National Forest – Sierra Vista Ranger District (Tom Deecken, Glenn Frederick), Anne Craven (Sierra Vista), Angel and Mickey Rutherford (Sierra Vista), The Nature Conservancy (Bud Eldon, Brooke Gebow, Matt Killeen, Carol Lambert, Lisa Nass, Mark Pretti, Holly Richter, Tom Wood), The Phoenix Zoo (Sharon Biggs, Roger Cogan, Mike Demlong, Geoff Hall, Keri Means, Terry O'brian, Mike Seidman, Tara Sprankle, Paula Swanson, Kevin Wright), U.S. Army-Fort Huachuca (John Roberts, Sheridan Stone), U.S. Bureau of Land Management (Mark Fredlake), and USFWS-ES (Jim Rorabaugh, Maaike Shotborgh). Jim Rorabaugh, Michael Sredl, and Sheridan Stone have remained involved with the conservation of this species since the inception of the Conservation Team in December, 1993.

RAMSEY CANYON LEOPARD FROG (*Rana subaquavocalis*)
August 2007

PART 1: STATE CONSERVATION AGREEMENT

PURPOSE AND NEED

The Ramsey Canyon leopard frog, *Rana subaquavocalis*, is a recently described (1993) member of the leopard frog complex known only from several canyons on the southeastern portion of the Huachuca Mountains in Cochise County, Arizona. All populations are small and most individuals are found in man-made impoundments. The population at the type locality in Ramsey Canyon may be extirpated. Threats to the species include loss of genetic variation and demographic stochasticity that result in increased probability of extirpation in small populations, environmental stochasticity in the form of floods, drought, disease, introduction of non-native predators, and vandalism. The Ramsey Canyon leopard frog is a former candidate for Federal listing; collection of this species is prohibited by State law. This Conservation Agreement (Agreement) renews a conservation agreement that was signed in 1996. Progress in conserving the species in accordance with the 1996 Agreement and Conservation Strategy can be found in "Ramsey Canyon Leopard Frog Conservation Team. 2000. Ramsey Canyon Leopard Frog Conservation Team: Activities 1996-2000." Further information on the status, distribution, taxonomy, and threats facing this species can be found in Part 2: Conservation Assessment and Strategy.

Occupied sites and sites for potential future reestablishment are owned and managed by Fort Huachuca, The Nature Conservancy, Coronado National Forest, Bureau of Land Management, the Beatty family, Anne Craven, and the Brodkin family. A refugium population is maintained by Angel Rutherford at her home in the Sierra Vista area.

Non-Federal property owners in the general area covered by the Agreement have been informed about the potential change in species designation from the Ramsey Canyon leopard frog to the Federally listed Chiricahua leopard frog. Prior to signing this Agreement, baseline conditions of properties being enrolled were established in a manner consistent with baseline conditions determined in the Safe Harbor Agreement for the Chiricahua leopard frog in Arizona (September 2006). We anticipate a zero baseline for all properties participating in the Agreement at the time of signing and they include: Beattys, Brodkins, Craven, Rutherford, and The Nature Conservancy. For non-Federal properties currently supporting populations of frogs, a zero baseline was assigned because the frogs resulted from translocation efforts that occurred during the term of the original Agreement. Should reclassification occur, private landowners will have the opportunity to sign a certificate of inclusion to the Department's Statewide Chiricahua Leopard Frog Safe Harbor Agreement (September 2006). We anticipate the original baseline determinations to be honored in the Safe Harbor Agreement. Any landowners with baselines above zero will have the opportunity to enter into habitat conservation planning for any anticipated incidental take of frogs. Neighboring landowners will also have the opportunity to enter into the Safe Harbor Agreement as a participating neighbor and a baseline determination will be made at the time of enrollment in the Agreement. The Department and USFWS will work with landowners who have participated actively in conservation to come to the best solutions for both conservation of the frog and protection of private interests. Private landowners with captive populations (e.g., Angel Rutherford) can be issued a Federal Section 10a1A recovery permit to allow for maintaining and propagating the frogs in captivity rather than participating in a Safe Harbor Agreement.

This State Conservation Agreement has been initiated to conserve the Ramsey Canyon leopard frog by reducing threats to the species, stabilizing populations, and maintaining its ecosystem. The primary purpose of this Agreement is to conserve the Ramsey Canyon leopard frog through interim conservation measures under the Endangered Species Act of 1973, as amended.

INVOLVED PARTIES

U.S. Fish and Wildlife Service
Arizona Ecological Services State Office
2321 West Royal Palm Road, Suite 103
Phoenix, AZ 85021-4951

Arizona Game and Fish Department
Nongame Branch
2221 West Greenway Road
Phoenix, AZ 85023-4399

The Nature Conservancy
Arizona Field Office
1510 East Ft Lowell
Tucson, AZ 85705

Bureau of Land Management
San Pedro River Riparian National
Conservation Area Projects Office
1763 Paseo San Luis
Sierra Vista, AZ 85635

Coronado National Forest
Sierra Vista Ranger District
5990 South Highway 92
Hereford, AZ 85615

U.S. Army Garrison
IMSW-HUA-PWB
3040 Butler Rd., Bldg. 22526
Fort Huachuca, AZ 85613-7010

Tom Sr., Edith, and Tom Jr. Beatty
Beatty's Guest Ranch
2173 East Miller Canyon Road
Hereford, Arizona 85615

Henry and Priscilla Brodkin
3050 East Carr Canyon Road
Hereford, Arizona 85615

Angel Rutherford
4920 Corte Vista
Sierra Vista, Arizona 85635

Anne Craven
1306 East Poncho Trail
Sierra Vista, Arizona 85650

AUTHORITIES

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

<u>U.S. 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>FOREST SERVICE:</u>
Endangered Species Act of 1973, as amended.
National Forest Management Act of 1976.
Sikes Act of 1960.

<u>ARIZONA GAME AND FISH DEPARTMENT:</u>
Arizona Revised Statute 17-231.B.
Endangered Species Act of 1973, as amended.

<u>FORT HUACHUCA ARMY POST:</u>
Endangered Species Act of 1973, as amended.
Sikes Act of 1960, as amended through 1997.

<u>BUREAU OF LAND MANAGEMENT:</u>
Endangered Species Act of 1973, as amended.
Federal Land Policy Management Act of 1976, as amended.

<u>THE NATURE CONSERVANCY:</u>
Articles of Organization and Bylaws for the Arizona Chapter of The Nature Conservancy.

OTHER AGREEMENTS AND DOCUMENTS

In addition to the above-listed legislative authorities, the following interagency agreements provide a framework for cooperation and participation among involved parties in the conservation of species tending towards listing.

A. Memorandum of Understanding among the U.S. Fish and Wildlife Service, the Bureau of Land Management, the Forest Service, the National Park Service, the National Marine Fisheries Service, and the International Association of Fish and Wildlife Agencies, issued on January 25, 1994, and amended on March 20, 1994.

B. Memorandum of Understanding among the U.S. Forest Service, Department of Defense, Army Corps of Engineers, National Marine Fisheries Service, Bureau of Land Management, Bureau of Mines, Bureau of Reclamation, U.S. Fish and Wildlife Service, Minerals Management Service, National Park Service, Coast Guard, Federal Aviation Administration, Federal Highway Administration, and Environmental Protection Agency, signed September 28, 1994.

C. Integrated Natural Resources Management Plan for Fort Huachuca, Arizona. Signatory Parties include Fort Huachuca, U.S. Fish and Wildlife Service, Region 2, and Arizona Game and Fish Department, signed November, 2001.

D. Cooperative Agreement between the U.S. Fish and Wildlife Service, Arizona Ecological Services Office, Phoenix, Arizona and The Nature Conservancy, Arizona Chapter, Tucson, Arizona, signed August 10, 1993.

E. Memorandum of Agreement between the U.S. Fish and Wildlife Service and the Arizona Game and Fish Department, dated June 26, 2002.

IMPLEMENTATION OF CONSERVATION ACTIONS

All conservation actions necessary to ensure the long-term persistence of the Ramsey Canyon leopard frog are identified in Part 2: Conservation Assessment and Strategy of this document. Subject to availability of funds and compliance with all applicable regulations, the responsible parties agree to implement actions according to scheduled completion dates, as shown in the implementation schedule of the Conservation Assessment and Strategy. When and if it becomes known that there are threats to the survival of the species that are not or cannot be resolved through this or any Conservation Agreement, the U.S. Fish and Wildlife Service will reassess the species to determine whether it should be assigned candidate status, and a listing package will be prepared.

RAMSEY CANYON LEOPARD FROG CONSERVATION TEAM

A. The involved parties shall designate a representative to serve on the Ramsey Canyon Leopard Frog Conservation Team (RCLFCT). The RCLFCT shall monitor the implementation of the conservation strategy and provide a forum for exchange of information on the species. The RCLFCT shall also be responsible for specific tasks as set forth in the implementation schedule. Through mutual agreement among designated representatives of all involved parties, the RCLFCT may make changes in the tasks and scheduling of task implementation, as described in the implementation schedule of the Conservation Assessment and Strategy. The RCLFCT shall in no way make recommendations to or serve as an advisory group to a Federal Agency.

B. Designated representatives shall attend at least two meetings of the RCLFCT annually for the life of this Agreement to review progress and coordinate work priorities and schedules.

ADMINISTRATIVE CLAUSES

A. The parties to this Agreement will handle their own activities and utilize their own resources, including the expenditure of their own funds, in pursuing these objectives. Each party will carry out its separate activities in a coordinated and mutually beneficial manner.

B. Nothing in this Agreement shall be construed to require the obligation, appropriation, or expenditure of any funds by the parties to this Agreement. Specific work projects or activities that involve the transfer of funds, services, or property among the various agencies and offices of the parties to this Agreement, will require execution of separate agreements and be contingent upon the availability of appropriated funds. Such activities must be independently authorized by appropriate statutory authority. This Agreement does not provide such authority. Negotiation, execution, and administration of each such agreement must comply with all applicable statutes and regulations.

C. This Agreement is not intended to, and does not, create any right, benefit, or trust responsibility, substantive or procedural, enforceable at law or equity, by a party against the United States, its agencies, its officers, or any person.

D. All parties are hereby put on notice that this Agreement is subject to cancellation by the Governor pursuant to A.R.S. 38-511 if any person significantly involved in initiating, negotiating, securing, drafting, or creating a contract on behalf of the State or any of its departments or agencies is, at any time while the contract or any extension of the contract is in effect, an employee of any other party to the contract in any capacity or a consultant to any other part of the contract with respect to the subject matter of the contract.

E. Pursuant to A.R.S. 35-214; all books, accounts, reports, files, electronic data, and other records relating to this Agreement shall be subject at all reasonable times to inspection and audit by the State and the Federal government for five years after completion of the Agreement. Such records shall be reproduced as designated by the State of Arizona and the Federal government.

F. By signature below, the cooperator certifies that the individuals listed in this document as representatives of the cooperator are authorized to act in their respective areas for matters related to this Agreement.

G. This Agreement does not create any new right or interest in any member of the public or other non-involved party as a third-party beneficiary. The duties, obligations, and responsibilities of the parties with respect to third parties shall remain as imposed under existing law.

H. No party to this Agreement shall be liable in damages for any breach of this Agreement, any performance or failure to perform an obligation under this Agreement, or any other cause of action arising from this Agreement. The parties agree to work together in good faith to resolve any disputes, using dispute resolution procedures agreed upon by all parties.

I. Any information furnished to any Federal Party under this Agreement is subject to the Freedom of Information Act, 5 U.S.C. 552, and the Privacy Act, 5 U.S.C. 552(a).

J. This Agreement in no way restricts any of the parties or cooperators from participating in similar activities with other public or private agencies, organizations, and individuals.

DURATION OF AGREEMENT

The term of this Agreement shall begin on the date the Agreement is filed with the Arizona Secretary of State, after signature by all parties, and end when all conservation actions identified in the implementation schedule of the Conservation Assessment and Strategy or subsequent revisions thereof are completed. The involved parties shall review the effectiveness of the Conservation Assessment and Strategy and Conservation Agreement annually to determine whether they should be revised. Within a year of completing the tasks identified in the implementation schedule of the Conservation Assessment and Strategy, the Conservation Agreement and Conservation Assessment and Strategy shall be reviewed by the involved parties and either modified, renewed, or terminated. This Agreement may, at any time, be amended, extended, modified, 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. Withdrawal by one party shall not affect the ability of the remaining parties to continue this Agreement. At the end of each 5-year period, the U.S. Forest Service and the BLM (Agreement No. AZ-930-0702) will review the Agreement and determine whether to sign on for another 5 years.

LIABILITIES/WAIVERS

Each Party waives all claims against every other Party for compensation for any loss, damage, personal injury, or death occurring as a consequence of the performance of this Agreement unless gross negligence on any part of any Party is determined.

SIGNATURES

All signatories have delegated authority to enter into this Agreement

For the **U.S. DEPARTMENT OF INTERIOR, U.S. FISH AND WILDLIFE SERVICE, SOUTHWESTERN REGION**

Benjamin N. Tuggle 9/21/07

Benjamin Tuggle, Regional Director Date

Ramsey Canyon Leopard Frog Conservation Agreement and
Conservation Assessment and Strategy

For the **ARIZONA GAME AND FISH DEPARTMENT, PHOENIX, ARIZONA**

Duane S Shroufe | 8/27/07
Duane Shroufe, Director | Date

For **THE NATURE CONSERVANCY, ARIZONA CHAPTER**

Patrick Graham 9-26-07

Patrick Graham, State Director Date

For the **U.S. DEPARTMENT OF INTERIOR, BUREAU OF LAND MANAGEMENT, TUCSON FIELD OFFICE**

Patrick Madigan — 10-1-07

Patrick Madigan, Field Manager — Date

Ramsey Canyon Leopard Frog Conservation Agreement and
Conservation Assessment and Strategy

For the **U.S. DEPARTMENT OF AGRICULTURE, FOREST SERVICE, CORONADO NATIONAL FOREST**

Jeanine A. Derby

Jeanine A. Derby, Forest Supervisor

11/13/07

Date

For **U.S. ARMY GARRISON AND FORT HUACHUCA**

____

Melissa A. Sturgeon, Colonel, MI; Date
Commander, US Army Garrison Fort Huachuca

For **BEATTY'S GUEST RANCH, HEREFORD, ARIZONA**

Edith M Beatty — 03 Sept 2007
Edith M. Beatty, Co-Owner — Date

Thomas L. Beatty Sr — 03 Sept 2007
Thomas L. Beatty Sr., Co-Owner — Date

HENRY AND PRISCILLA BRODKIN, HEREFORD, ARIZONA

Henry Brodkin — 21-IX-07
Henry Brodkin — Date

Priscilla Brodkin — 9-21-07
Priscilla Brodkin — Date

ANGEL RUTHERFORD, SIERRA VISTA, ARIZONA

Angel Rutherford 09-02-07
Angel Rutherford Date

ANNE CRAVEN, SIERRA VISTA, ARIZONA

Anne Craven 9/19/07

Anne Craven Date

PART 2: CONSERVATION ASSESSMENT AND STRATEGY

PURPOSE AND NEED

The Ramsey Canyon leopard frog, *Rana subaquavocalis*, is the most recently described Arizona leopard frog (Platz 1993). At the time it was described, its known range was limited to two canyons (Ramsey and Brown canyons) on the east side of the Huachuca Mountains in Cochise County, Arizona (Platz 1993). The size of its range is the smallest of any leopard frog in the United States (Platz 1997). Following the species' description, surveyors found this frog in 3 canyons on the east side of the mountain range, in addition to a few private, residential ponds.

Due to the small number of localities and size of populations, the U.S. Fish and Wildlife Service (USFWS) considered this species a candidate for federal listing under the Endangered Species Act from 1994 - 1997, but removed it from this list after a conservation assessment and strategy was developed and a multiparty Conservation Agreement (Agreement) was signed in 1996. The Ramsey Canyon Leopard Frog Conservation Team (RCLFCT) implemented this Agreement from 1996 to the present and has been actively conserving this species during this time period. The major focus of conservation efforts has been the mitigation of threats to small populations through 1) renovation and creation of habitats, 2) augmentation or establishment of populations through translocations, and 3) removal of non-native predators and competitors. Positive preliminary results include survival and reproduction of captive-reared frogs following release to the wild. In addition, this cooperative effort has generated a great deal of favorable publicity.

This assessment and strategy replaces the original assessment and strategy (Platz 1996). Commitments to implement the goals, objectives, and management actions herein will be addressed in formal conservation agreements among the Arizona Game and Fish Department (AGFD or Department), USFWS and participating agencies, zoos, and private landowners.

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 RCLFCT. Additionally, drafts of the Conservation Assessment and Strategy were reviewed in 2006 by the USFWS. A table listing PECE criteria and areas where they are addressed in the document can be found in Appendix 1.

CONSERVATION ASSESSMENT

RELATIONSHIP TO THE CHIRICAHUA LEOPARD FROG

Comparisons of mitochondrial DNA sequences from Ramsey Canyon and Chiricahua leopard frogs suggest that the two may be conspecific in southeastern Arizona (Goldberg et al. 2003, Goldberg et al. 2004). The Chiricahua leopard frog was listed as threatened under and Endangered Species Act in 2002 (U.S. Fish and Wildlife Service 2002) and a recovery plan has been finalized (June 2007). The recovery team addressed conservation of the Ramsey Canyon leopard frogs in the recovery plan, in case the two species are synonymized. This conservation assessment and strategy is consistent with the goals and recovery actions described in the Chiricahua leopard frog recovery plan and it is anticipated that the recovery team will adopt this conservation strategy as a regional plan within Recovery Unit 2 (which covers a portion of south-central Arizona and north-central Sonora). Members of the RCLFCT are serving on the Technical Subgroup and Southeastern Arizona Stakeholders Subgroup of the recovery team, which provide forums for integrating this conservation strategy into the Chiricahua leopard frog recovery plan.

Non-Federal property owners in the general area covered by the Agreement will be informed through an open house meeting, notices in the local newspaper (Sierra Vista Herald), and Department and USFWS news releases and other documents about the potential change in species designation. Prior to signing this agreement, baseline conditions of properties being enrolled will be established in a manner consistent with baseline conditions determined in the Safe Harbor Agreement for the Chiricahua leopard frog in Arizona (September 2006). We anticipate a zero baseline for all properties participating in the Agreement at the time of signing and they include: Beattys, Brodkins, Craven, Rutherford, and The Nature Conservancy. For non-Federal properties currently supporting populations of frogs, a zero baseline will be assigned because the frogs resulted from translocation efforts that occurred during the term of the original Agreement. Should reclassification of the frog occur, private landowners will have the opportunity to sign a certificate of inclusion to the Department's Statewide Chiricahua Leopard Frog Safe Harbor Agreement (September 2006). We anticipate that the original baseline determinations will be honored in the Safe Harbor Agreement. Any landowners that have baselines above zero will have the opportunity to enter into habitat conservation planning for activities that may result in incidental take of frogs. Neighboring landowners will also have the opportunity to enter into the Safe Harbor Agreement as participating neighbors and a baseline determination will be made at the time of enrollment in the Agreement. The Department and USFWS will work with landowners who have participated actively in conservation to come to the best solutions for both conservation of the frog and protection of private interests. Private landowners with captive populations (e.g., Angel Rutherford) can be issued a Federal Section 10a1A recovery permit to allow for maintaining and propagating the frogs in captivity rather than participating in a safe harbor agreement or habitat conservation plan.

DISTRIBUTION AND ABUNDANCE: HISTORICAL AND CURRENT

Because the Ramsey Canyon leopard frog was described recently, little is known of its distribution and abundance prior to 1993. Museum specimens of *Rana pipiens* do exist from Miller Canyon in the 1930s (1932 by J. Dice: UMMZ 73222, 1933 by B. Campbell: UMMZ 75742) and Ramsey Canyon in 1970 (by R.W. and T.R. VanDevender: UMMZ 134114 & 134118), of *Rana yavapaiensis* from Scotia Canyon in 1951 (UAZ 20272 & 20294), and of *Rana chiricahuensis* from Sunnyside Canyon in 1960 (UAZ 19466). *Rana chiricahuensis* was noted in Ramsey Canyon by Clarkson and Rorabaugh (1989) in the 1980s. In addition, leopard frogs that may have been Ramsey Canyon leopard frogs were historically noted at the following canyons in the Huachuca Mountains: Ash, Bear, Brown, Carr, Copper, Garden, Hunter, Montezuma, and Parker canyons (Rorabaugh personal communication, Beatty unpublished data, Belfit unpublished data, Slevin 1928, Wright and Wright 1949, Platz and Mecham 1979, Holm and Lowe 1995, Sredl 2005). The only recent observations from these canyons are of repatriated frogs in Miller, Ramsey, Brown, and Carr canyons and frogs of unknown origin in Ash Canyon. In 1994, a population of leopard frogs was found in Tinker Canyon (Platz 1996); however, the identity of this population was questioned for many years. Recent analyses show no differences in mitochondrial DNA sequences from other frogs in the Huachuca Mountains (Goldberg et al. 2003, Goldberg et al. 2004). Some speculate that the Ramsey Canyon leopard frog's historical range included the San Pedro River valley and parts of Sonora, Mexico. Museum specimens suggest the frog may occur in Chihuahua, Mexico (Platz 1997).

From 1996 to 2000, the RCLFCT established populations of Ramsey Canyon leopard frogs in ponds in the Miller and Carr canyon drainages (RCLFCT 2000). In 2002, frogs appeared at a newly constructed pond in Ash Canyon, which brought the total number of canyons with known Ramsey Canyon leopard frog localities to 6 (Ash, Miller, Carr, Ramsey, Brown, and Tinker canyons). Within these canyons, leopard frogs inhabit primarily ponds. In addition to the canyons mentioned, a couple of small ponds in residential areas support breeding populations of Ramsey Canyon leopard frogs. These small ponds have been sources of eggs for use in captive rearing, and some of the sites are considered refugia as hedges against extinction. Unfortunately, the frogs at one of the private residential ponds, and those in Tinker, Ramsey, and Ash canyons were likely extirpated by 2004. In the past, the National Amphibian Conservation Center at the Detroit Zoo and the Arizona-Sonora Desert Museum maintained a small population for security.

Platz et al. (1997) examined survey data collected between 1990 and 1995 from Ramsey Canyon and noted a decline in the population from over 90 in 1990 to 19 in 1995. Additionally, Platz (1997) summarized survey data collected intermittently between 1991 - 1996 from Ramsey Canyon and three other localities. Surveys varied from 1 to 38 individuals, and consistent reproduction was noted at only 2 localities.

Since implementation of the first Agreement, we have successfully increased overall abundance of the species. Miller Canyon supported a breeding population in 2000, 2001, 2002, 2003, and 2004 with more than 200 frogs present by 2001 (AGFD, unpublished data). Most other canyons continue to support low numbers, some without evidence of reproduction in several years.

HABITAT

No comprehensive studies examining habitat use by Ramsey Canyon leopard frogs have been conducted. Adults occupy both natural and artificial aquatic systems with slow moving water and pools in pine-oak and oak woodland and semi-desert grassland habitats between elevations of 1,501 - 1,829 m (Sredl et al. 1997, Sredl 2005).

Perennial streams that are or could be used by Ramsey Canyon leopard frogs occur throughout the Huachuca Mountains. Within these systems the favored aquatic microhabitats are pools with both riparian vegetation and open areas at the pool's perimeter for out-of-water basking. Permanent aquatic habitats are patchy and often separated by areas of intermittent flow. Other examples of small, natural aquatic systems in the area include springs, seeps, and ciénegas (wetlands). Many of these habitats have been altered or destroyed, and those that remain in a natural to semi-natural state are few and scattered (Sredl 2005).

Ramsey Canyon leopard frogs also inhabit 2 types of human-constructed water catchments. The first type is an impoundment in a drainage that collects surface water runoff (e.g., earthen basins impounded with cement or earthen dams). The second type is an excavated depression that is filled with groundwater seepage or with water that is piped in. Characteristics of these aquatic sites vary considerably from very small (< 4 m^2, 0.0004 hectares) to large, from well vegetated to barren, from deep (≥ 3 m) to very shallow, and from permanent to ephemeral. The permanence of the first type depends primarily on two factors: 1) the amount of runoff received (combination of local precipitation and size of watershed) and 2) the permeability of pond substrate. The water level of the second type is often controllable and is easier to maintain as permanent.

Few of the habitats occupied by Ramsey Canyon leopard frogs are completely natural. Sections of stream in several canyons remain in a somewhat natural state, however few frogs have been found in streams recently, especially if pools or ponds (often human made) are available nearby. In one canyon, frogs were found at a natural tinaja; however, no frogs have been seen there since summer, 2000. In Ramsey Canyon, the artificial ponds will be removed when stream restoration is complete. It is hoped that frogs will inhabit pools within the stream; however, no frogs have been seen in the canyon since 2001. Artificial aquatic systems, such as earthen cattle tanks, can be important for the continued viability of leopard frog populations in parts of Arizona (Sredl and Saylor 1998). Occupation of natural and artificial aquatic systems presents interesting opportunities and dilemmas for conservation of native leopard frogs.

The role of habitat heterogeneity within the aquatic and terrestrial environment is unknown, but is likely to be important. Shallow water with emergent and perimeter vegetation provides tadpole and adult basking habitats, while deeper water, root masses, and undercut banks provide refuge from predators and potential hibernacula (Sredl, 2005). Vegetation at sites with extant populations varies greatly. Some sites are sparsely or seasonally vegetated with native or non-native grasses such as deer grass (*Muhlenbergia rigens*), Bermuda grass (*Cynodon dactylon*), and Johnson grass (*Sorghum halepense*). Moister sites support wetland plants such as horsetail (*Equisetum* sp.), spikerush (*Eleocharis* sp.), monkey flowers (*Mimulus* spp.), watercress (*Rorippa* sp.), and cattail (*Typha* sp.).

The RCLFCT has worked to create, restore, and enhance habitat for Ramsey Canyon leopard frogs in many localities along the east side of the Huachuca Mountains. Much of this work is summarized in the RCLFCT's activities report (2000).

LIFE HISTORY AND ECOLOGY

BREEDING

Aquatic systems with slow moving water such as springs, ponds, ciénegas, earthen cattle tanks, small creeks, and slack water along rivers are considered suitable reproductive sites for the Ramsey Canyon leopard frog (Sredl 2005). The only observed reproduction, however, has occurred in artificial impoundments.

Mating begins in late March once water temperatures exceed 10 °C and continues through early October (Platz 1997, Beatty, Sredl, and Rutherford unpublished data). Males call underwater while submerged as deep as 1.4 m (Platz 1997). Although they are thought to produce calls that are inaudible in air (Platz 1993, Platz 1997, Platz et al. 1999), biologists and others have heard calling males at the type locality (C. Eldon and M. Sredl, personal observations). Whether these calls are produced while the male is above or below the water's surface is unclear. Males at other localities have been observed to call with their heads above water (K. Field and C. Eldon personal observations). Amplexus may last up to 20 hours, but oviposition is brief (Platz 1997).

Egg masses have been observed in March through October. In 1995, breeding activity at Ramsey Canyon peaked during early May, then again in mid-June, with a total of 19 egg masses produced (Platz 1997). In Miller Canyon, 28 egg masses were counted between July and October, 2000, 44 from March through September, 2001, and 129 from March to September, 2002. It is likely that females are capable of double clutching (Platz 1997). Oviposition does not appear to be correlated with rain, but instead may be correlated with changes in water temperature. Platz (1997) noted that oviposition occurred on 10 of 11 nights shortly before or slightly after a decrease in water temperature.

Females deposit eggs in spherical masses attached to submerged vegetation at a mean depth of 269 mm (n = 19, range = 110 - 710 mm; Platz 1997). Egg masses may, however, be at the surface (J. Rorabaugh, personal communication). Egg masses contain an average of 1,518 ova (n = 7, range = 1200 - 2040; Platz 1997). In the wild, eggs hatch in approximately 14 days depending on temperature (Platz 1997); in captivity they take approximately 10 days at 23 - 25 °C (M. Demlong, unpublished data).

The tadpoles look very similar to other ranid tadpoles (for description see Altig et al. 1998, Scott and Jennings 1985). The length of larval period has not been well studied, but larvae may metamorphose in the year the eggs were laid or over-winter as tadpoles (Platz and Grudzien 1993, Platz 1996, Platz et al. 1997). In captivity, larvae metamorphose as quickly as 100 days, but more commonly take 160 - 200 days (M. Demlong, unpublished data).

No comprehensive study of larval use of cover has been conducted, although Platz (1996) noted they may avoid predators by hanging motionless in algal mats. Given that many populations of

Ramsey Canyon leopard frogs are small, recent observations of high densities of carnivorous giant water bugs (Belostomatidae) and low larval survivorship (M. Sredl, personal observations) underscore the need for a greater understanding of predator avoidance and cover needs (Sredl 2005).

HOME RANGE AND TERRITORIES

Little is known of home range size and movements. Concerning establishment of territories, Platz (1996) suggests that Ramsey Canyon leopard frogs form a lek (mating priority determined by dominant status of males on communal mating grounds; Krebs and Davies 1981), but a detailed analysis of the mating system has not been done. Systematic study of within-site movement has not been conducted, although marked frogs have been observed moving hundreds of meters up and down Ramsey Canyon (Sredl, unpublished data). Movements of juvenile (Snout-Vent Length < 2 inches) leopard frogs that may be Ramsey Canyon leopard frogs have been observed in the Brown and Tinker canyon area during summer rains (Wallace, personal observations). Individuals of other species of leopard frogs in Arizona have moved distances of up to 8 km along drainages (Frost and Bagnara 1977) and likely move overland as well.

AESTIVATION AND HIBERNATION

Mechanisms that Ramsey Canyon leopard frogs use to survive the loss of surface water are unknown. Other species of leopard frogs in southwestern United States survive by burrowing into mud cracks (Howland et al. 1997). Although metamorphosed Ramsey Canyon leopard frogs are generally inactive between November and February, some individuals have been seen active during the winter months (T. Beatty, personal communication; M. Rutherford, personal communication). A detailed, wintertime study has not been done (Sredl 2005). Other species of leopard frogs often winter on the bottom of ponds, where they may bury themselves in the mud or sit under logs or rocks.

LONGEVITY AND AGE AND SIZE AT REPRODUCTIVE MATURITY

In the wild, both sexes are thought to live up to 10 years following metamorphosis (Platz et al. 1997). In one population, a higher proportion of females (15 of 20) lived 5 years or longer compared with males (11 of 22; Platz et al. 1997). In this same population, males were better represented among the 3 - 5 year age classes, while females were best represented among the 6 - 8 year age classes. Forty seven percent of all individuals examined were 6 years or older. Size was a poor indicator of age, and growth rate for males was lower than that of females (Platz et al. 1997).

Although Platz et al. (1997) speculate that Ramsey Canyon leopard frogs do not generally reach sexual maturity earlier than 6 years after metamorphosis, recently metamorphosed individuals reared in captivity and released to the wild in August, 1999, produced 31 egg masses between July and October, 2000 (Sredl, unpublished data). Similarly, metamorphs released in the fall at the Barchas Ranch ponds produced egg masses the following summer (T. Deecken, unpublished data). Captive-reared Ramsey Canyon leopard frogs also grew faster than those studied by Platz et al. (1997). Two female metamorphs and one male metamorph released in October, 1995, reached 102, 110, and 105 mm snout-urostyle length (SUL) respectively, by the end of June, 1998. None of the frogs studied by Platz (1997) reached those sizes at a similar age. His growth curves suggest sizes at a comparable age of approximately 83 mm for females and 76 mm for males. Adult females can reach at least 120 mm SUL (Platz 1997).

DIET

No comprehensive studies of the feeding behavior or diet of Ramsey Canyon leopard frog larvae or adults have been completed. Platz (1996) states that like many ranid tadpoles, larval Ramsey Canyon leopard frogs are herbivorous. Available food resources at one site examined include bacteria, diatoms, phytoplankton, filamentous green algae, water milfoil (*Myriophyllum* sp.), duckweed (*Lemna minor*), and detritus (Marti and Fisher 1998). Captive larvae ate spinach, romaine lettuce, cucumber slices, frozen trout, duckweed, spirulina-type fish foods, and rabbit pellets (Demlong 1997). The diet of Ramsey Canyon leopard frog adults has not been determined. Stomach analyses of other adult members of the leopard frog complex from the western United States show a wide variety of prey items, including many types of aquatic and terrestrial invertebrates (e.g., snails, spiders, and insects) and vertebrates (e.g., fish, other anurans [including conspecifics], and small birds; Stebbins 1951). There is one record of an adult Ramsey Canyon leopard frog eating a hummingbird (Field et al. 2003) and observations of predation on house finches (H. Brodkin, personal communication), a goldfinch, and a tarantula (A. Craven, personal communication). Captive juvenile (Snout-Vent Length < 2 inches) frogs ate crickets (Demlong 1997).

PREDATION

Detailed studies of native predators of Ramsey Canyon leopard frogs have not been conducted (Sredl 2005). Tadpoles are likely preyed upon by insects, including belostomatids, notonectids, dytiscids, and anisopterans, and vertebrates including gartersnakes (*Thamnophis* sp.), great blue herons (*Ardea herodias*), and other birds. Gartersnakes have been observed to prey on juvenile and adult Ramsey Canyon leopard frogs (K. Field, personal observation). Other likely predators of juveniles and adults include rats, coyotes, gray foxes, raccoons, ringtails, coatis, black bears, badgers, skunks, bobcats, and mountain lions (Platz 1996).

Cannibalism, primarily large adults eating juvenile (Snout-Vent Length < 2 inches) frogs or large larvae, is likely but has not been studied. Fernandez (1996) speculated that lack of cover and cannibalism were the reasons for low juvenile survival in a captive colony of Chiricahua leopard frogs. Cannibalism may be avoided through different use of habitat. Seim and Sredl (1994) studied the association between juvenile and adult stages and pool size in lowland leopard frogs (*Rana yavapaiensis*) and found that juveniles were more frequently associated with small pools and marshy areas while adults were associated with large pools. Jennings (1988) noted that juvenile Chiricahua leopard frogs were more active during the day, while adults were more active at night. Oophagy (egg eating) may occur. Large Ramsey Canyon leopard frog tadpoles have been observed ingesting the gelatinous envelopes of eggs, but have not been reported to consume the ovum (Platz 1996).

Anti-predator mechanisms of tadpoles have not been studied. Platz (1996) noted that larvae may avoid predators by hanging motionless in algal mats. Metamorphosed frogs typically escape by jumping into water and seeking cover. Fright or distress calls have not been noted (Platz 1996).

REASONS FOR DECLINE AND THREATS TO SURVIVAL

HABITAT FRAGMENTATION AND DISRUPTION OF METAPOPULATION DYNAMICS

Severe fragmentation and alteration of aquatic habitats in the southwestern United States has likely constricted many wide ranging aquatic species into isolated pockets. For those species, maintenance of aquatic corridors may be critical in 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 be reflective of habitat fragmentation and extinction without recolonization, as well as habitat quality.

Locality data indicate that extant Ramsey Canyon leopard frog populations occur as small clusters, rather than randomly distributed populations (Platz 1993, 1997). All of these sites are ponds, streams, or springs in the upper San Pedro River watershed. Platz (1997) speculates that the Ramsey Canyon leopard frog occupied pools in the San Pedro River. The upper San Pedro River is considered to have been a broad marshy area consisting of a series of pools impounded by beaver dams until the late 1800s. The physical characteristics of the river have changed drastically for reasons including the extirpation of beaver by the 1880s (Rojo et al. 1999). In 1999, beaver were first reintroduced into the San Pedro River. Additional releases occurred in 2000 and 2002, and reproduction has been confirmed. With an estimated 8 colonies currently present, the river's habitat structure may be improving. We suspect that extant population clusters of Ramsey Canyon leopard frogs are remnants of former metapopulations that had a large core population of frogs on the San Pedro River. "Chiricahua" leopard frogs, which may have been Ramsey Canyon leopard frogs, occurred historically in the San Pedro River (Platz and Mecham 1979).

A likely contributing factor to leopard frog declines in the arid Southwest is habitat reduction and fragmentation resulting in disruption of metapopulation dynamics and small, isolated, unstable local populations (Sredl and Howland 1995). Damming, draining, and diverting of water have fragmented formerly contiguous aquatic habitats. In many areas, fragmentation has been accentuated by non-native predatory fishes, crayfish, and bullfrogs, leaving potential dispersal corridors between available aquatic habitats disrupted or impassable. The life history of the Ramsey Canyon leopard frog makes their populations very dynamic. Rates of reproduction, recruitment, and other population attributes are highly variable and dependant upon rainfall and other environmental influences. In addition, Ramsey Canyon leopard frogs are highly aquatic and vulnerable to desiccation. Because of the size of its current range and quality of breeding habitat and dispersal corridors, factors affecting small populations and metapopulation dynamics figure prominently into conserving the Ramsey Canyon leopard frog.

LOSS OF GENETIC VARIABILITY

Based on starch gel electrophoresis, Platz and Grudzein (2003) found limited genetic heterozygosity in frogs collected in the early and mid 1990s in Ramsey Canyon and from the Barchas Ranch in Brown Canyon. They suggested this predisposes the species to extinction in the face of novel environmental influences. It is likely that the entire population of Ramsey Canyon leopard frogs is very genetically homogeneous. Very few frogs were thought to remain in the wild in the mid-1990s. A private, residential pond (Bernstein's pond) was the only site at which egg masses were being produced regularly, and this pond was the source of animals used in translocations. The pond is small and usually occupied by several adult frogs. Stock was collected in multiple years, but the number of unique matings and relatedness of animals that produced the egg masses is unknown. The Miller Canyon ponds and a private, residential pond complex (Rutherford's ponds) now support breeding populations as a result of translocations from this source. Although translocations to Ramsey Canyon have taken place, there has been little evidence of success and no recent breeding in that canyon. Carr and Brown canyons have both received eggs and tadpoles from the breeding populations that were established in Miller Canyon and the second residential pond complex.

Important in developing a successful re-establishment program is genetic management of small populations whether they are in captivity or the wild. Genetic variability can be maintained by maximizing founder population size and equalizing founder representation (Dobson et al. 1991). Periodic augmentation of captive or wild populations may be necessary to avoid the deleterious effects of loss of variability.

NON-NATIVE ORGANISMS

Non-native aquatic organisms have been a major factor implicated in declines of native fish and amphibians throughout western North America (Jennings 1988) including Arizona (Rosen et al. 1995). Non-native species interact negatively with natives primarily through predation, but also compete for limiting resources and can significantly alter or degrade habitat. Non-native aquatic animals found in the Huachuca Mountains include crayfish (*Orconectes virilis*), sport fish (*Lepomis* spp., *Micropterus* spp., *Ictalurus* spp., and others), mosquitofish (*Gambusia affinis*), bullfrogs (*Rana catesbeiana*), and barred tiger salamanders (*Ambystoma tigrinum mavortium*) (Sredl et al. 2000b). Our opportunities to establish populations of Ramsey Canyon leopard frogs are severely limited by the presence of non-natives.

There are no crayfish native to Arizona, but two species have been introduced (*O. virilis* and *Procambarus clarki*) (Inman et al. 1998). Only the former has widespread distribution in Arizona (Inman et al. 1998) and has been found in the aquatic systems of the Huachuca Mountains (Sredl et al. 2000b). Crayfish were introduced into Arizona's aquatic systems as a prey base for sport fish (e.g., largemouth bass) and through use as bait for anglers. Crayfish can efficiently compete with many small herbivores, prey upon various invertebrates and small vertebrates, and extensively modify aquatic habitats (Fernandez and Rosen 1996). Currently, crayfish are not found at occupied Ramsey Canyon leopard frog localities. Garden Canyon on Fort Huachuca, which is one canyon north of the Ramsey Canyon leopard frog locality in Tinker Canyon, is the site nearest to a known locality of frogs that contains crayfish. Garden Canyon contains habitat that would likely be appropriate for leopard frogs, if the crayfish population could be eradicated or markedly depressed (S. Stone, K. Field, personal observation).

Bullfrogs are predators and competitors of native leopard frogs in southern Arizona (Rosen and Schwalbe 1995). Bullfrogs are native east of the Rocky Mountains, but have been introduced throughout western North America (Conant and Collins 1991). Until the early 1970s, bullfrogs were intentionally introduced to the western United States by State and Federal agencies to provide additional sporting opportunities for the public. They were also unintentionally released during sport fish introductions. In addition, private individuals who are unaware of the repercussions of their actions have moved bullfrogs around. Bullfrogs are established in many aquatic habitats in Arizona (Arizona Game and Fish Department, unpublished data). In the vicinity of the Huachuca Mountains, they are found in the slackwaters of all major drainages (e.g., San Pedro, Babocomari, and Santa Cruz rivers) and in man-made water impoundments (i.e., cattle tanks and reservoirs) (Sredl et al. 2000b). Bullfrogs have been documented at several sites in the Huachucas that might otherwise be suitable for leopard frogs. A single bullfrog was removed successfully from Tinker Pond. Bullfrogs may still be present in ponds in lower Garden Canyon despite efforts to remove them, and many bullfrogs are present in Scotia Canyon on the west side of the Huachucas.

Fish species, primarily those native to the eastern U.S., have been introduced throughout Arizona for sport, food, and ornamental purposes. These include highly predaceous members from the families Centrarchidae (bass, sunfish), Ictaluridae (catfish), and Salmonidae (trout). In addition, many species of small fish have been introduced as bait (red shiner) and for mosquito abatement (mosquitofish) (Minckley 1973). Koi and goldfish are also present in some frog areas. Even small mosquitofish have been shown to have negative impacts on ranid frog larvae (Lawler et al. 1998). Many aquatic habitats in and around the Huachuca Mountains support reproducing and/or actively stocked populations of these introduced fish species (Coleman 1990, Coleman 1991, Sredl et al. 2000b). Many of these species are highly predaceous on native amphibians. Yellow bullhead catfish were detected some time after an unauthorized introduction and successfully removed from Tinker Pond in 1999. Unauthorized introductions of goldfish or koi resulted in the successful establishment of these fish at the Carr Barn Pond and the metal storage tank at Barchas Ranch. Plans to eradicate the fish at these two sites have been developed and are being implemented. Koi and mosquito fish are present in several privately owned ponds that also have frogs; there are no plans to remove the fish. At some of these ponds the owners do move frog egg masses away from ponds containing large fish.

Tiger salamanders are present in some bodies of water on Fort Huachuca. Although Sonoran tiger salamanders (*Ambystoma tigrinum stebbinsi*) are native to the areas at the crest and east slope of the Huachuca Mountains and in the adjacent San Rafael Valley, non-native barred tiger salamanders (*Ambystoma tigrinum mavortium*) are also present and widespread in the Upper San Pedro basin. There is evidence for the hybridization of these two salamanders on Fort Huachuca (Storfer et al. 2002). Salamanders and leopard frogs seem to coexist in sites throughout Arizona (AGFD, unpublished data); however, predation of tadpoles by salamander larvae is likely.

DISEASES AND PARASITES

Several diseases and pathogens have been noted in Arizona amphibians. Demlong (1997) described a "bloating malady" and malformed tails in captive tadpoles. An unknown fungus commonly infects egg masses, but whether this infection is the primary or secondary cause of mortality is unknown (Platz 1996). Gram-negative bacteria, including species implicated in the disease red leg, are often detected on dead frogs. It is unclear whether these bacteria are actually causing disease or invading the diseased or dead organisms opportunistically.

In 1998, a chytrid fungus (*Batrachochytrium dendrobatidis*) was implicated in declines of amphibians in Australia and Panama (Berger et al. 1998). Later that same year, it was first identified in Arizona (Milius 1998), although analyses of museum specimens show that the fungus was in Arizona as early as 1972 (Cashins and Davidson 2003). Data support the hypothesis that chytrid fungus is a newly emerged pathogen (Morehouse et al. 2003). Bradley et al. (2002) found lowland leopard frogs, Chiricahua leopard frogs, and canyon tree frogs collected from populations in Arizona that experienced cool season die-offs during 1992 - 1999 to be infected with chytrid fungus. Presently, 1 salamander (*Ambystoma tigrinum stebbinsi*), 7 species of ranid frogs (*Rana berlandieri*, *R. blairi*, *R. catesbeiana*, *R. chiricahuensis*, *R. subaquavocalis*, *R. tarahumarae*, *R. yavapaiensis*), 2 treefrogs (*Hyla arenicolor*, *Pseudacris triseriata*), and 1 toad (*Bufo punctatus*) have been affected by this fungus in Arizona (Davidson et al. 2000, Sredl et al. 2003). Specimens positive for chytrid fungus have been collected from the Carr, Ramsey, Brown, and Tinker canyon drainages (Sredl et al. 2002, AGFD, unpublished data, Table 1). Dead Ramsey Canyon leopard frogs have been found in lower Carr Canyon and Ramsey Canyon. Ramsey Canyon leopard frogs have disappeared after metamorphosis from lower Brown Canyon and have also disappeared from Tinker Pond. Chytrid fungus likely plays a large role in our lack of success in translocations to several localities.

Goldberg et al. (1998) examined parasites of two closely related native ranids (Chiricahua and lowland leopard frogs) and one non-native ranid (bullfrogs) collected in Arizona. None of the helminths identified from the two native species were found in American bullfrogs.

ROADS, TRAILS, AND FIREBREAKS

Roads and trails provide access to lands for recreation, ranching, mining, and other activities. Most vehicle travel is likely by canyon residents in passenger vehicles on paved or graded dirt roads. On Coronado National Forest (Forest), recreational driving of both passenger and off-highway vehicles is common. Military training and outdoor recreation are the primary uses of pathways on Fort Huachuca. Military exercises using vehicles generally take place away from steeper slopes and wetlands areas. Muddy areas and pond basins are attractions sought out by some OHV users.

A network of firebreaks has been constructed on ridgelines, canyons, and slopes on Fort Huachuca to reduce risk of catastrophic fire. Construction and maintenance of roads, trails, and firebreaks can negatively affect leopard frog habitats by increasing runoff, erosion, and siltation. However, roads and firebreaks can reduce the incidence or extent of wildfire and protect against ash flow and sedimentation of frog habitats.

Table 1. Detection of chytrid fungus in specimens collected at Ramsey Canyon leopard frog localities. Sites are organized generally from north to south along the east side of the Huachuca Mountains. All specimens were *Rana subaquavocalis* (RASU) unless otherwise noted. ? = all specimens undiagnosable, NA = information not available, BUWO = *Bufo woodhousii*, SCMU = *Scaphiopus multiplicata*. Samples marked with* were scrapings of skin taken from live animals and analyzed using PCR, while all other samples were specimens examined histologically.

Site	Sample Date	Number Chytrid Positive/Sample Size	Collected live or dead	Life Stage
Rutherford Ponds	09/20/99	0/5	Live	Adult
	mm/dd/00	0/2	NA	Adult
	08/12/03	0/1	Dead	Adult
Tinker Pond	07/16/94	0/1	NA	Adult
	07/20/97	2/4	NA	Larvae
	10/17/97	1/4	NA	Larvae
	01/06/00	1/1	Dead	Adult
	05/02/00	1/1	Live	Larvae
Bernstein Pond	mm/dd/01	?/1	NA	NA
	03/30/02	?/1	Dead	Adult
	01/09/03	0/3	Live	Larvae
	12/10/03	1/1*	Live	Adult
Barchas Ranch ponds	04/04/03	1/1	Live	Larvae
	10/29/03	16/16*	Live	Adult
	04/27/04	1/1*	Live	Juvenile
Ramsey Canyon	03/28/96	0/2	Dead	Juvenile
	09/08/97	?/1	Dead	Adult
	mm/dd/00	1/1	Dead	Adult
	01/08/01	1/1	Dead	Adult
	07/28/01	1/1	Dead	Juvenile
	06/06/02	0/1	Dead	Adult (BUWO)
Carr Barn Pond	01/29/02	?/3	Dead	Adult
	04/05/02	1/1	Live	Larvae
	07/18/02	0/1	Live	Adult (SCMU)
	01/09/03	?/4	Dead	Adult
Carr Canyon	01/09/03	0/2	Live	Larvae
Miller Canyon	mm/dd/00	0/2	NA	Adult, Juvenile
	mm/dd/00	0/1	NA	Larvae
	mm/dd/02	0/2	Dead	Larvae
	07/18/02	0/9	Live	Adult (3), Larvae (6)
	07/25/02	0/1	Live	Larvae
	01/08/03	0/1	Dead	Adult
Stump Canyon	04/06/00	0/1	Live	Adult

FIRE

Wildland fires, whether controlled or uncontrolled, have effects on frog populations that are poorly known and are dependant on local conditions (Abbott 1998). Direct effects include mortality from heat or suffocation. Indirect effects include the incineration of riparian and terrestrial habitats, siltation of aquatic habitats following precipitation (DeBano et al. 1996), and high levels of phosphorus and nitrogen in water (Spencer and Hauer 1991) that may be toxic to frogs. Removal of habitat components, which provide foraging areas and refugia from desiccation and predation, is expected to decrease the chances of frogs surviving following fire (Abbott 1998). Siltation, shifts in water chemistry, and changes in insect prey base following a burn may also have indirect effects on amphibians (Rinne and Neary 1996, Abbott 1998).

Sometimes water is removed from cattle tanks, reservoirs, and large rivers with heavy equipment (pump trucks and helicopters with buckets) to fight a fire and replaced with water from another source. This practice could inadvertently translocate non-native aquatic species and pathogens (see non-native species and disease sections, this document). Recent fires on the eastside of the Huachuca Mountains (e.g., Oversite Fire) have been fought using well water (S. Gunzel, personal communication). Currently, sources of water are carefully chosen because of commitments to conservation of the Sonoran tiger salamander (S. Gunzel, personal communication).

The frequency and intensity of fires in southwestern forests have been altered from pre-settlement regimes (Dahms and Geils 1997). In montane pine forests, such as those that occur in the Huachuca Mountains, surface fires burned at least once per decade prior to 1900. Fire suppression and intensive livestock grazing brought these frequent fires to a halt (Swetnam and Baisan 1996) and encouraged the buildup of woody debris. Currently, infrequent and intense crown fires threaten the forest in the Huachuca Mountains. Recent sources of ignition include lightning strikes and the campfires of illegal immigrants. The intense fires expose soils to erosion, which can dramatically change the downstream drainages (DeBano and Neary 1996). In 1977, a crown fire in Miller Canyon resulted in erosion and scouring of the canyon, after which leopard frogs were no longer seen in the canyon (T. Beatty personal communication). In the nearby Chiricahua Mountains, a debris flow following the 1994 Rattlesnake fire filled Rucker Lake and many pools within the Rucker Canyon. Many canyons in the Huachucas, from which there are historical records of leopard frogs, no longer contain natural pools. Since 2000, there have been fires in Oversite Canyon (above Miller Canyon) and Ash Canyon, neither of which seems to have affected localities currently known to contain frogs.

HUMAN POPULATION GROWTH AND GROUNDWATER DEPLETION

Urban development is on the rise along the east side of the Huachucas. The population of Sierra Vista is expected to grow at an annual rate of 1.5 to 3.0 percent through 2005 (Sierra Vista 2000). Most of this growth is expected to occur in the unincorporated area surrounding Sierra Vista (Wade 1998).

Among potential impacts of this population growth on resident amphibian populations is the increasing demand for water. Water consumption in the upper San Pedro River Valley has steadily increased for many years. This demand has led to the creation of a cone of depression in regional groundwater level, which may affect base flows of the San Pedro River (Corell 1996, Rojo et al. 1999). These changes in regional groundwater level will likely have major impacts to amphibian populations by changing flows in perennial springs, creeks, and rivers and by changing vegetative communities (Scudday 1977, Stromberg et al. 1996). Currently, the City of Tombstone diverts up to 1000 acre-feet of water each year from Carr Canyon and upper Miller Canyon (Arizona Department of Water Resources 1991), and this diversion limits the amount of natural stream habitat present for frogs in these canyons. Water is also diverted from the stream in Ash and Brown canyons (S. Gunzel, personal communication).

Regional population growth coupled with urban development continues to encroach on amphibian habitats in foothill grassland habitats. With this growth, the frequency of intentional and unintentional introductions of non-natives is likely to increase. Well-intentioned, but misguided, individuals are known to intentionally release unwanted pets in aquatic systems in the Huachuca Mountains. Backyard pond enthusiasts could unintentionally create a conflict between native and non-native species, because these ponds are usually stocked with non-native plants (e.g., lilies), fish (primarily goldfish and their relatives), and bullfrogs. At least one Sierra Vista vendor of backyard pond supplies sells bullfrog tadpoles (A. Craven, personal communication).

COLLECTION AND VANDALISM

Over-collection for commercial purposes is known to be a contributing factor in the decline of other species of ranid frogs (Jennings and Hayes 1985, Corn and Fogelman 1984). Collection of Ramsey Canyon leopard frogs is illegal except with a special permit (see Existing Regulatory Protection below). In 1991-1992, Platz (1995) noted the disappearance of large tadpoles from one site in Brown Canyon and suggested that their disappearance may have resulted partly from an act of vandalism. Also, in 1995, many large adult frogs reportedly were illegally collected from the Barchas Ranch ponds following publicity about the rare status of the frog (USFS notes from May 25, 1995 Ramsey Canyon leopard frog conservation team meeting). Illegal collection and vandalism are potential threats to small populations of rare or highly prized reptiles and amphibians (Hoover 1998).

MINING ACTIVITIES

Historically, mining occurred throughout the Huachuca Mountains (Taylor 1991). Adits, shafts, tunnels, and other evidence of mining can be found at or near locations of many extant populations and potential habitat of the Ramsey Canyon leopard frog. Ramsey Canyon leopard frogs have been found in a flooded adit. Few mines are currently active and most do not directly affect the habitats occupied by the species. Mining activities were much more widespread historically and may have constituted a greater threat to this species in the past. The effect of mining on these populations is unclear, but could include changes in water quality and flow rates. Mining of gold, copper, iron, or other materials may threaten the habitat of the Ramsey Canyon leopard frog.

OVERGRAZING

The effects of grazing on amphibian habitats can be divided into local and watershed effects. These effects may be positive or negative (Jennings 1988, Rosen and Schwalbe 1998, Sredl and Saylor 1998).

In the late 1800s and early 1900s, construction of earthen cattle tanks in upland drainages became a common range management practice (U.S. General Accounting Office 1991), and it continues to this day. These tanks are primarily built to provide water for livestock. A secondary benefit is the water and aquatic habitat they provide to many species of wildlife, including amphibians.

Locally, prudent management of livestock may even benefit leopard frogs. Removal of vegetation by livestock keeps the water column open. Cattle may benefit frog populations inhabiting earthen tanks by compacting the soil and stabilizing an earthen berm. In addition, organic waste from cattle attracts insects and provides detritus for tadpoles to feed on. Leopard frogs do use cattle hoof prints in moist mud as refugia (M. Sredl, personal observation).

Overgrazing negatively impacts 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 waste. Overgrazing in upland habitats may degrade amphibian habitat by increasing runoff and sedimentation rates (Jennings 1988, Belsky and Blumenthal 1997).

Cattle grazing is extremely limited in the current range of the Ramsey Canyon leopard frog. On the Forest, grazing is limited to Miller Canyon by a small number of cattle in the winter months. Other allotments, except for one shared with Coronado National Memorial, are currently vacant (S. Gunzel personal communication). There is no cattle grazing on Fort Huachuca or The Nature Conservancy lands.

AGRICULTURAL ACTIVITIES

Irrigated agriculture in the upper San Pedro River Valley has been decreasing over the last 30 years (Rojo et al. 1998). Nonetheless, groundwater pumping and surface diversion for agriculture add to the demands for water in the valley. These activities lead to a decrease in the local aquifer and subsequent changes in the aquatic habitat for amphibians. Many chemicals used in agriculture and silviculture have negative effects on amphibian populations (Herfenist et al. 1989); however, certain changes in agricultural practices can actually increase biodiversity (see Pimentel et al. 1992). Leopard frogs currently (*R. blairi*, *R. berlandieri*) and historically (*R. yavapaiensis*, *R. chiricahuensis*) have been noted in agricultural areas (Frost and Bagnara 1977, Clarkson and Rorabaugh 1989, Rorabaugh et al. 2002). Ramsey Canyon leopard frogs may have benefited from agricultural developments in the past; however these habitats in the San Pedro River Valley are presently overrun with non-native competitors and predators.

EXISTING REGULATORY PROTECTION

The U.S. Fish and Wildlife Service considered this species a candidate for federal listing from 1994 - 1997, but removed it from the list after a conservation assessment and strategy were developed and a multiparty Conservation Agreement was signed and implemented in 1996. Signers of the Agreement include: Department of Defense – Fort Huachuca (DOD), Arizona Game and Fish Department (AGFD), U.S. Fish and Wildlife Service (USFWS), The Nature

Conservancy (TNC), U.S. Forest Service (USFS), Sarah Barchas, and Bureau of Land Management (BLM). Also participating in activities under this agreement were the Phoenix Zoo (PZ), the Arizona-Sonora Desert Museum (ASDM), and several residential pond owners. The RCLFCT has been responsible for management and oversight of activities under the terms of the agreement.

A variety of existing international conventions and Federal and State regulations provide limited protection to the Ramsey Canyon leopard frog and its habitat. Arizona Game and Fish Commission Order 41 (Arizona Reptile and Amphibian Regulations) prohibits collection or hunting of Ramsey Canyon leopard frogs in Arizona, except when done under the authority of a special permit. The frog is on the sensitive species list for Region 3 of the Forest Service, which requires an analysis of all proposed projects to determine effects and needed modifications, or to develop mitigation.

The Lacey Act (16 U.S.C. 3371 et seq.), as amended in 1982, provides some protection for the Ramsey Canyon 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 United States, any Tribal law, or any law or regulation of any State.

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

The Federal Land Policy Management Act of 1976 (43 U.S.C. 1701 et seq.) and the National Forest Management Act of 1976 (16 U.S.C. 1600 et seq.) direct Federal agencies to prepare management plans to guide management decisions. In addition, the Forest Service is required to "maintain viable populations of existing native and desired non-native species" in their planning areas (36 CFR 219.19).

The National Environmental Policy Act of 1969 (NEPA) (42 U.S.C.4321-4370a) requires Federal agencies to consider the environmental impacts of their actions. The NEPA process requires these agencies to describe a proposed action, consider alternatives, identify and disclose potential environmental impacts of each alternative, and involve the public in the decision-making process. Most actions taken by the Forest Service, the Bureau of Land Management, and other Federal agencies that affect the Ramsey Canyon leopard frog are subject to the NEPA process.

CONSERVATION STRATEGY

GOAL

Our goal is to ensure the long-term persistence of Ramsey Canyon leopard frog populations in a diversity of habitats and localities in the Huachuca Mountains and the upper San Pedro River Valley.

We will strive to have populations in habitats as natural as possible. We will emphasize localities that are natural to semi-natural streams, pools, and cienegas or that are in close proximity to such settings. Privately owned ponds within canyons, which are linked to appropriate natural to semi-natural systems, may be appropriate for use in our conservation efforts. Habitats that are highly maintained or require intensive, frequent management are not desirable. Ponds in the backyards of homes within residential areas of Sierra Vista have minimal value in the conservation of this species, except where currently established as refugia and sources of animals for translocation (e.g., Rutherford ponds). Additional ponds of this type will not be sought.

OBJECTIVES

Implementation and administration of this Conservation Strategy will focus efforts on removing threats to Ramsey Canyon leopard frogs and their habitats and on improving the metapopulation structure. Specifically, we will 1) remove threats to and secure occupied and unoccupied focal habitats, create new habitats where feasible, and manage habitats for the foreseeable future; 2) establish, re-establish, and augment wild populations through translocations to suitable habitats when feasible; 3) monitor extant and translocated frog populations to direct adaptive management practices; 4) maintain or maximize genetic diversity; and 5) conduct research that investigates the biology of and threats to Ramsey Canyon leopard frogs. Conservation Agreements with cooperators will help to insure that these 5 specific actions are carried through in locations most appropriate for achieving success.

SUCCESS CRITERIA

Evidence of successful breeding and recruitment over a long period of time at multiple sites within the Ramsey Canyon leopard frog's range will be a better measure of the trajectory or persistence of the population than will the total number of individuals in the population. Anuran population numbers can fluctuate drastically (e.g., Tinker Pond) and total numbers are not necessarily indicative of population stability (Green 1997, U.S. Fish and Wildlife Service 2000). The spatial distribution of the populations is important as well. There should be some natural immigration and emigration, yet localities should be distinct enough such that factors influential to population declines are not affecting all sites at the same time. It may be beneficial to have some sites that are isolated to buffer against problems such as spread of disease.

Three types of sites, focal, supplementary, and refugia, will be defined to guide our attempts to secure the Ramsey Canyon leopard frog population. Tables 2 and 3 list sites with the potential to be developed into or designated as focal, supplementary, or refugia sites. Conserving the frog in these three types of sites takes into account metapopulation concepts and addresses the need for securing some populations in controlled areas with few threats.

Focal sites will be defined as aquatic sites that will, on average, have reliable water year round and year to year in 5 of 6 years, resources available such that requirements of all life stages of the frog are met within the site (frogs of various life stages appear to occupy the site throughout the year), depth of at least 1 m, and a surface area of at least 9 m^2. Focal sites within a canyon should be at least 1600 m apart with no more than 2 sites within a single canyon to be counted towards the objective.

Supplementary sites will be defined as aquatic sites that: may or may not have surface water year round; have resources available such that frogs may occupy the site at least part of the year; are not isolated (no evidence of being a sink, frogs are able to move to other areas with water if site begins to dry); and have no minimum size. No more than 4 sites within a single canyon's drainage may be counted towards the objective of 10 total sites.

Refugium sites will be isolated from all other sites with extremely low probabilities of frogs moving into or out of the sites without human control. The sites should have water and adequate habitat for all life stages year round. The populations at these sites will be established and periodically managed at the individual level such that genetic variability is maintained. Refugium populations that are primarily self-sustaining should remain intact for as long as possible to maintain insurance for unforeseen declines.

We will consider our conservation efforts successful if in 5 years we have: populations at 5 focal sites (requires at least 3 separate canyons), 3 of which are natural to semi-natural settings, that have demonstrated successful breeding and recruitment in 2 of the last 3 years; frog presence observed at 10 supplementary sites (in at least 3 separate canyons); and 2 refugium populations that are capable of reproduction, which produce egg masses in 3 of 5 years.

Recruitment is best confirmed through intensive mark-recapture study designs. The production and survival of breeding adults is important in assuring population persistence (Green 1997), so detection of recruited adults needs to be considered. After observations of developing egg masses and larvae and the presence of unmarked juveniles, we will allow for unmarked adults to indicate recruitment within a site. Only adults will be counted as individuals recruited to the population. All frogs that we release into sites will be marked. Frogs existing at sites prior to augmentations will also be marked to the best of our ability. Although unmarked individuals that appear at sites may be immigrants, we hope that through careful monitoring we can document egg masses hatching, the presence of tadpoles, and eventually the appearance of metamorphs and adults. The necessity of a long-term monitoring plan becomes evident when considering how to define success in re-establishment. See Monitoring (part 4) in narrative outline.

Table 2. Possible designations of focal, supplementary, and refugium sites. Map will be provided to signatories and RCLFCT members (see attachment).

Focal sites	Supplementary sites	Refugium sites
Frog (House) Pond, Miller Canyon	other ponds and stream, Miller Canyon	Rutherford Ponds
McCoy Spring Pond, Miller Canyon	Tinaja and stream, Brown Canyon	Phoenix Zoo
Barchas House Pond, Brown Canyon	Mano Pond, Brown Canyon	Detroit Zoo
Barchas Wild Duck Pond, Brown Canyon	Mine adit (between Ramsey and Carr)	Scottsdale Community College
Upper Stream, Garden Canyon	Craven Pond	Arizona–Sonora Desert Museum
Meadow Ponds, Ramsey Canyon	Carr Barn Pond	
Trout Pond, Ramsey Canyon	Brodkin Pond, Carr Canyon	
Ramsey Creek, Ramsey Canyon	Tinker Pond, Tinker Canyon	

Table 3. Potential sites to investigate.
Veal property ponds
Herrin property ponds
San Pedro RNCA
Scotia Canyon
Birdland Ranch / Algerita Canyon (Scott/Heath property)
Upper Carr Canyon
Pat Scott Canyon
Ash Canyon springs
Clark Springs, Miller Canyon
Upper Sunnyside Canyon
Bear Creek
Upper Miller Canyon
Tony Battiste property, Miller Canyon
Ash Canyon Bed and Breakfast
Rail Oaks Ranch, Carr Canyon

OUTLINE OF CONSERVATION STRATEGY TASKS

1. Administration of the Conservation Strategy

1.1. Formalize the Ramsey Canyon Leopard Frog Conservation Team

The diverse backgrounds and expertise of RCLFCT members make the team's input an essential asset to specific activities within this Agreement. Members should include at least one representative of each signatory agency or party as well as people with expertise relevant to conservation of the frog. RCLFCT members should be distinct from interested parties; however all are welcome to participate in general planning meetings. The RCLFCT should continue to be represented on the Chiricahua leopard frog recovery team.

1.2. Plan activities and implement conservation actions

Activities will be planned and priorities set by the RCLFCT. Each signatory agency or party will designate a voting member. Significant changes to the strategy require a unanimous vote. All other changes will be accepted by a majority vote.

1.2.1. RCLFCT to meet twice annually

The RCLFCT should meet a minimum of two times annually. In a late winter meeting (February) of each year, the RCLFCT would coordinate and finalize plans for the upcoming field season. A second meeting, held in early fall (October-November), would allow for review or synthesis of information collected during the previous field season and formulation of preliminary plans for the upcoming field season. Additional meetings should be held as needed, and small working groups may be formed to focus on particular tasks.

1.2.2. Coordinate activities

RCLFCT coordinators will be chosen by the RCLFCT for one-year minimum appointments. Should the Agreement be constructed as an umbrella agreement, an individual from the entity holding the umbrella permit should consider being the coordinator. Coordinators will organize specific activities and conservation actions year round. These activities will be planned during RCLFCT meetings.

1.2.3. Prepare annual work plans

Work plans will be prepared by RCLFCT members. Draft plans of future years' activities will be prepared for review, so that the activities may be worked into future budget cycles.

1.2.4. Explore funding sources and develop proposals

Organizations or individuals will seek funding to insure that conservation actions will be implemented. Team members will develop proposals for monitoring and research priorities as information needs dictate.

1.3. Develop educational and outreach activities

Through education and outreach we should be able to reduce some threats and gain cooperation in establishing populations. Private landowners may gain interest in becoming signatories of the Agreement.

1.3.1. General public outreach

This may include educational signs, outreach through pet and garden pond industries, presentations to local clubs or groups (i.e., Koi Club, schools), and meetings with landowners.

1.3.2. Media coverage of releases and conservation activities

Press releases will be prepared for releases of frogs, and local media will be kept informed about noteworthy activities. By publicizing events and successes of this cooperative effort, conservation of the RCLF as well as other species may be viewed more positively.

1.4. Compile reports

Formal reports will help to archive the team's activities and are a requirement by the USFWS for parties entering into Candidate Conservation Agreements.

1.4.1. Annual Progress Reports

A draft report will be developed by the RCLFCT in December, which incorporates summaries of activities prepared by each cooperator. The report will be distributed to the RCLFCT for review in January, and finalized and submitted to USFWS in February. The annual report will address the following: 1) report the previous Federal fiscal year's work, 2) include a work plan for the current fiscal year, 3) include a detailed budget request for the upcoming fiscal year, and 4) summarize out year budget requests for 2 years beyond. The annual report will satisfy the requirement by USFWS to submit annual reports related to the actual Agreement. Unique reporting requirements will be described in the Agreement(s).

1.4.2. 5-Yr. Progress Report

Members of the RCLFCT will draft a 5-yr progress report using information from the annual progress reports. This report will address whether the goal and objectives of the Conservation Strategy have been met. The RCLFCT will review the report before it is finalized.

2. Secure, enhance, and create habitat

2.1. Secure Habitat

Efforts will be focused on key areas of importance in maintaining the metapopulation structure and in areas where efforts will be effective. Securing habitat includes gaining consent to partake in activities to conserve frogs at sites and taking preventative measures to reduce threats.

2.1.1. Develop agreements with landowners

Conservation easements, conservation agreements with assurances, safe harbor agreements, or other agreements that would help secure habitat for frogs should be pursued with willing landowners.

2.1.2. Monitor for threats

Monitoring of sites to detect threats such as non-natives, disease, drying, malfunction of water delivery systems, problems with construction (pond liners, cracked cement, etc.), vandalism, incompatible human uses, erosion, siltation, and other things that threaten the persistence of frogs will occur.

Federal and private landowners have the primary responsibility of monitoring sites under their management, unless arrangements have been made for monitoring to be done by another party. Private landowners should alert the RCLFCT when threats are detected.

2.1.3. Develop plans to remove or mitigate threats

When a threat can be addressed swiftly by using measures previously approved by the RCLFCT, further consultation with the RCLFCT is unnecessary. The RCLFCT should be consulted when complex or unforeseen situations arise that require expert opinion or cooperative efforts.

As soon as non-native organisms are detected at a site, eradication should be attempted. At sites that have contained non-natives for some time, plans should be developed to prevent the spread of the non-natives. In addition, plans that can be implemented when conditions are favorable should be developed. Drought conditions provide an opportunity to attempt eradication while sites have little water and habitat suitable for the non-native organisms is reduced.

Chytrid fungus is a threat that is already present in four drainages containing frogs. Individuals visiting frog habitat should follow the disinfection protocol (Appendix 9). It is critical to prevent movement of frogs, plants, mud, and water into sites where chytrid has not been detected. If appropriate and feasible, plans to restrict access to chytrid positive sites should be developed. Because frogs can be treated for chytridiomycosis, plans that include attempts to clear a site of chytrid fungus should be considered once appropriate research provides suggestions.

2.1.4. Implement plans to remove or mitigate threats

Prompt implementation of plans is required.

2.2. Enhance focal, supplementary, and refugium habitats

Habitats that could better support frogs if enhanced will receive priority. Most effort should be applied towards focal sites, with supplementary and refugium sites following after focal sites have become productive. Examples of enhancements include increasing the size of a wetland, improving vegetative cover, and improving water quality.

2.3. Create new habitats in strategic locations

New habitats will be created in accordance with attaining the criteria for success. The purpose and type of site (focal, supplementary, refugium) needed and location will be carefully reviewed by the RCLFCT prior to directing resources towards this action.

3. Identify, establish, and enhance populations

3.1. Reconnaissance surveys

We will continue to gather information from knowledgeable individuals about key places to investigate, and survey sites with unknown potential.

3.1.1. Detect undocumented, natural populations of frogs

All new populations of frogs will be reported to the RCLFCT.

3.1.2. Identify potential re-establishment sites

Information should be gathered about each potential site so that sites can be evaluated and prioritized in 3.2.2. Potential sites should be ranked using Appendix 10, Translocation Site Evaluation guidelines, and presented to the RCLFCT for review.

3.2. Enhance population or metapopulation dynamics

Populations can be enhanced through translocations of animals to sites.

3.2.1. Develop and implement a genetic management plan to maintain genetic diversity.

The genetic variability of frogs needs to be considered when planning for translocations. Platz and Grudzien (1993, 2003) found levels of heterozygosity to be low when compared to published estimates for other anurans. Limited genetic variability can have negative consequences if organisms are under strong selection pressure to adapt to changing conditions.

3.2.2. Evaluate potential release sites

The suitability and feasibility of sites will be evaluated using the Translocation Site Evaluation guidelines (Appendix 10).

3.2.3. Choose site(s) and complete environmental compliance review and documentation

Environmental compliance documentation has been completed for sites on the east side of the Huachuca Mountains and is effective through 2010.

3.2.4. Collect eggs, larvae, or frogs from donor sites to be used for translocation

Individuals or organizations with breeding populations of RCLFs on their property will inform the Coordinator or designated individuals when egg masses are observed (see Appendices 4-7)

3.2.5. Head-start eggs and larvae

This step is not required, but will result in numerous juveniles or late stage larvae for release (see Appendix 3. ARIZONA LEOPARD FROG CAPTIVE CARE PROTOCOL for techniques used by the Phoenix Zoo).

3.2.6. Supplement extant populations when necessary and translocate individuals to unoccupied habitat

Translocations may be done directly from one site to another or may use stock that was head-started in captivity. Appropriate precautions will be taken to avoid the transfer of disease and non-native organisms (see Appendix 11. TRANSLOCATION GUIDELINES and Appendix 8. CAPTIVE RELEASE PROTOCOL).

3.2.7. Maintain refugium populations

Populations should be maintained beyond the term of this agreement to ensure the availability of animals into the future.

4. Monitor extant populations and occupied or suitable habitats

Monitoring is a crucial step in any conservation effort. Only through a carefully designed monitoring plan will we be able to determine whether our conservation actions are successful and formulate future plans based on the successes and failures. We need to refine our monitoring techniques, so that we are better able to determine the status of extant populations and the outcome of our re-establishment attempts. Through monitoring, we may also gain insight into causes of population declines, or into key environmental and habitat components that seem associated with population persistence. Monitoring will occur throughout the term of any associated Agreement (and is required by the USFWS as a part of such Agreements).

4.1. Evaluate the status of extant populations

Techniques used to monitor extant Ramsey Canyon leopard frog populations will include day and night visual encounter surveys (VES) and periodic intensive surveys during which individuals will be captured. Capture will be necessary in order to read toe clips or PIT tags for survival estimates after more than one cohort has been released. Handling time will be as brief as possible. The results of previous surveys of Ramsey Canyon leopard frogs underscore the importance of performing nighttime VES to document the presence and abundance of all life stages.

4.2. Evaluate habitat

We will monitor the habitat of extant populations and unoccupied but suitable habitats using established techniques. Parameters that may prove particularly insightful include water quality, habitat structure, presence of competitors or predators, presence of disease, and variables such as temperature and precipitation.

4.3. Evaluate effectiveness of conservation actions

Follow-up monitoring is required to determine the effectiveness of conservation actions. General population monitoring will be done by visual encounter survey (VES) following the General Visual Encounter Survey Method (Appendix 12). (A sample data sheet and instructions are included as Appendices 13 and 14).

Translocation requires commitment to a specialized follow-up monitoring plan. Some researchers have suggested a monitoring time commitment of 6 to 10 years in order to truly gain insight into the successful re-establishment of anurans (Cooke and Oldham 1995, Sredl and Healy 1999, U.S. Fish and Wildlife Service 2000, Semlitsch 2002). Success of a translocation should be evaluated on multiple temporal scales. Immediate or short-term success would be evaluated in the weeks following the release of animals.

Complete success, the establishment of a self-sustaining population, cannot be determined until the founding population successfully reproduces and should not be considered until the resultant second-generation adults breed as well (Semlitsch 2002). This suggests that monitoring needs to take place for at least 3 years, to evaluate whether the founders' reproductive efforts are successful. Five temporal stages of success, which may be used as guidelines, were identified for Chiricahua leopard frogs (Table 4).

Shortly after releases, population and environmental monitoring should be frequent in order to evaluate the short-term success of translocation and to observe any dispersal or catastrophic events.

Areas surrounding the release sites should be surveyed, so that movement into nearby habitats can be documented. These surveys should occur at least every 3 months, with particular attention to areas where the frogs are likely to expand. Connected habitats should be surveyed for the duration of the project.

Table 4. 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 Survival of release	VES	After release	8 weeks after release	2 times
Stage 2 Survival over-winter	Intensive nighttime surveys	Activity season following the first winter post-release	3 months after it is initiated	1-2 times
Stage 3 Long-term survival	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

5. <u>Research</u>

As part of the implementation of this conservation strategy, we plan to address some questions in need of research. Studies will be designed to help us better meet the goals and objectives of this strategy. Without these studies, it is difficult to practice adaptive management, choose re-establishment sites that are likely to be successful, and monitor the frogs appropriately. Through research and effective monitoring we should be able to determine: which life stage is best to release, in terms of survival rate and cost; why some sites are more productive than others; and answers to natural history questions**.** As new information is gained, adaptive management will be applied and this strategy will be revised as needed to make the best use of research results.

5.1. <u>Examine taxonomic relationships</u>

Resolving taxonomic questions is critical. We will investigate whether or not Ramsey Canyon leopard frogs and Chiricahua leopard frogs are distinct species. One researcher suggests there are differences (J. Platz, unpublished data), while others have found no differences (N. Benedict, unpublished data). Work investigating this question is underway, and our most recent data suggest that all of the frogs in question are the same species (Goldberg et al. 2003, Goldberg et al., 2004).

5.2. Determine habitat use and home range or territoriality

We generally know what seems to be good frog habitat, but we lack detailed data about habitat requirements and how habitat is used. Studies are needed that investigate where the frogs over-winter, how habitat use changes with season and life stage, how much the frogs move, and whether they establish home ranges. This information is important when choosing and developing sites in attempts to meet our success criteria.

5.3. Describe oviposition sites

5.4. Evaluate dispersal capabilities or seasonal movement

5.5. Examine seasonal changes in activity

5.6. Examine response to flooding

5.7. Examine individual and population response to habitat manipulations, including fire

5.8. Determine the best life stage for release to the wild

The success of translocating larvae or head–starting (rearing from egg mass to late stage tadpole or young frog in captivity) juvenile frogs has not been evaluated. Survival and ultimate recruitment to the population as well as cost need to be considered.

5.9. Examine feeding and foraging behavior and diet

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

5.11. Examine interactions with non-native predators and competitors

Determine how presence of non-native predators and competitors affects the frogs.

5.12. Study population and metapopulation dynamics

5.13. Conduct PVA/PHVA

By doing a PVA we will be able to estimate the number of individuals needed to maintain the population or better define factors that limit population viability. This information could be used to modify the success criteria or redirect management activities to better meet the goal of this strategy.

5.14. Develop more effective means to monitor populations

5.15. Examine cause and frequency of disease and die-offs

Because four sites (Ramsey Canyon, Tinker Pond, Carr Barn Pond, Barchas Ranch House Pond) have had die-offs and produced frogs infected with chytrid fungus, it is critical that we gain knowledge about the frequency of this and other diseases in populations. Careful monitoring will alert us to die-offs early, so that we can investigate their causes and attempt to remove the threat or salvage individuals from the population. Our investigations will make use of the latest techniques, which may include PCR for the detection of chytrid.

5.16. Investigate methods to treat chytrids in wild populations

We will continue to communicate with outside researchers about their chytrid studies. We will be available to field test new procedures when appropriate.

5.17. Investigate possible causes of previous extirpations of frogs from specific localities

Frogs have been extirpated from several localities in the Huachuca Mountains in the recent past. Studies should be conducted to illuminate possible causes of the extirpations and to determine if those factors are still present at the localities.

6. Adaptive Management

The new information that we collect during monitoring and as the result of research will be incorporated back into the planning process to improve our ability to meet the goal of this strategy. Evaluation of our efforts will be ongoing, and we will have the ability to adapt our tasks and this strategy if needed. Temporary variances from this strategy are permissible to react rapidly as emergencies dictate. Formal changes in wording of the strategy will be proposed by signatories or RCLFCT members and approved by the RCLFCT as described in 1.2.

6.1. Assess new information

This task will be ongoing as information is gathered.

6.2. Incorporate new information into activity plans

New information will help to guide priorities and will be used in planning (see 1.2 and all subtasks). The strategy is flexible and is designed to allow for adapting to changing situations, priorities, and techniques. In emergency situations, new information may be acted on rapidly. If deviations from the strategy are temporary, no written modifications to the strategy are necessary.

6.3. Modify strategy to reflect major changes in direction

The strategy may require modifications in wording should significant changes occur in threats to the frog or in the best ways to address those threats. Any proposed modifications should have data to support the changes. Signatories and RCLFCT members may propose changes. The RCLFCT must approve (see 1.2) deviations from the signed strategy that require changes in wording.

Table 5. Implementation Schedule.
This implementation schedule outlines the tasks that need to be completed to manage Ramsey Canyon leopard frogs effectively for 5 years.

Task Number	Task Description	Task Priority	Task Completion (years)	Responsible Parties	Total Cost Estimate (in $1,000 units)	Funding Sources
1	**Administration of the Conservation Strategy**					
1.1.	Formalize the Ramsey Canyon leopard frog Conservation Team	High	1	RCLFCT	1	Involved Parties
1.2.	Plan activities and implement conservation actions					
1.2.1.	RCLFCT to meet twice annually	High	5	RCLFCT	20	Involved Parties
1.2.2.	Coordinate activities	High	5	RCLFCT	20	Involved Parties
1.2.3.	Prepare annual work plans	High	5	RCLFCT	5	Involved Parties
1.2.4.	Explore funding sources and develop proposals	High	5	RCLFCT	2	Involved Parties
1.3	Develop educational and outreach activities					
1.3.1.	General public outreach	Medium	5	Involved Parties	15	AGFD, FWS
1.3.2.	Media coverage of releases and conservation activities	Low	5	AGFD, FWS	1	AGFD, FWS
1.4.	Compile reports					
1.4.1.	Annual Progress Reports	High	5	RCLFCT	15	Involved Parties
1.4.2.	5 Yr. Progress Report	High	1	RCLFCT	10	Involved Parties
2.	**Secure, Enhance, and Create Habitat**					
2.1.	Secure Habitat					
2.1.1	Develop agreements with landowners	High	5	RCLFCT, FWS	12	Involved Parties
2.1.2.	Monitor for threats	High	5	Involved Parties	5	Involved Parties
2.1.3.	Develop plans to remove or mitigate threats	High	5	RCLFCT, Signatories	3	Involved Parties
2.1.4.	Implement plans to remove or mitigate threats	High	5	RCLFCT, Signatories	15	Involved Parties
2.2	Enhance focal, supplementary, and refugium habitats	High	5	RCLFCT, Signatories	75	FWS, Involved Parties
2.3	Create new habitats in strategic locations	Medium	3	RCLFCT, Signatories	24	Involved Parties

Task Number	Task Description	Task Priority	Task Completion (years)	Responsible Parties	Total Cost Estimate (in $1,000 units)	Funding Sources
3.	**Identify, Establish, and Enhance Populations**					
3.1.	Reconnaissance surveys					
3.1.1.	Detect undocumented, natural populations of frogs	Medium	5	AGFD, FWS, CNF, DOD, TNC	0.5	Involved Parties
3.1.2.	Identify potential re-establishment sites	Medium	5	AGFD, FWS, CNF, DOD, TNC	15	Involved Parties
3.2.	Enhance population or metapopulation dynamics					
3.2.1.	Develop and implement a genetic management plan to maintain genetic diversity.	Medium	5	RCLFCT	20	TBD
3.2.2.	Evaluate potential release sites	Medium	5	AGFD, FWS,	13	Involved Parties
3.2.3.	Choose site(s) and complete environmental compliance review and documentation	Medium	5	AGFD, CNF, DOD, BLM,	15	Involved Parties
3.2.4.	Collect eggs, larvae, or frogs from donor site to be used for translocation	Medium	5	AGFD, CNF, DOD	5	Involved Parties
3.2.5.	Head-start eggs and larvae	Medium	5	PZ, SCC, ASDM	TBD	TBD
3.2.6.	Supplement extant populations when necessary and translocate individuals to unoccupied habitat	Medium	5	AGFD, CNF, TNC, FWS, DOD	5	Involved Parties
3.2.7	Maintain refugium populations	High	5	Involved Parties	TBD	FWS, AGFD, Involved Parties
4.	**Monitor extant populations and occupied or suitable habitats**					
4.1.	Evaluate the status of extant populations	High	5	RCLFCT	9	TBD
4.2.	Evaluate habitat	High	5	RCLFCT	15	Involved Parties
4.3.	Evaluate effectiveness of conservation actions	High	5	RCLFCT	20	Involved Parties
5.	**Research** [1.]					
5.1.	Examine taxonomic relationships	High	1	AGFD	20	FWS, AGFD
5.2.	Determine habitat use and home range or territoriality	Medium	2	AGFD	TBD	TBD
5.3.	Describe oviposition sites	Medium	2	AGFD, TNC	TBD	TBD
5.4.	Evaluate dispersal capabilities or seasonal movement	Medium	2	AGFD	TBD	TBD

Task Number	Task Description	Task Priority	Task Completion (years)	Responsible Parties	Total Cost Estimate (in $1,000 units)	Funding Sources
5.5.	Examine seasonal changes in activity	Low	2	AGFD, TNC	TBD	TBD
5.6.	Examine response to flooding	Low	1	AGFD, TNC	TBD	TBD
5.7.	Examine individual and population response to habitat manipulations, including fire	Low	1	AGFD, TNC, CNF, DOD	TBD	TBD
5.8.	Determine the best life stage for release to the wild	Medium	3	AGFD	TBD	TBD
5.9.	Examine feeding and foraging behavior and diet	Low	1	Involved Parties	TBD	TBD
5.10.	Determine age and size at first reproduction and growth rates	Low	3	Involved Parties	TBD	Involved Parties
5.11.	Examine interactions with non-native predators and competitors	Low	1	DOD, AGFD	TBD	Involved Parties
5.12.	Study population, metapopulation dynamics	Medium	5	AGFD	TBD	TBD
5.13.	Conduct PVA/PHVA	Low	1	RCLFCT	TBD	TBD
5.14.	Develop more effective means to monitor populations	Medium	1	RCLFCT	TBD	Involved Parties
5.15.	Examine cause and frequency of disease and die-offs	High	5	AGFD	TBD	Involved Parties
5.16.	Investigate methods to treat chytrids in wild populations	High	2	AGFD, FWS, DOD, PZ	TBD	Private Grant, Involved Parties
5.17	Investigate possible causes of previous extirpations of frogs from specific localities	High	4	AGFD, CNF, TNC, FWS	TBD	Involved Parties
6.	**Adaptive Management**					
6.1.	Assess new information	High	5	RCLFCT, Signatories	2	Involved Parties
6.2.	Incorporate new information into activity plans	High	5	RCLFCT	2	Involved Parties
6.3.	Modify strategy to reflect major changes in direction	High	5	RCLFCT	2	Involved Parties
Total Cost: $356,500 over 5 years Estimated Annual AGFD cost: $16,500 annually for 5 years Estimated Annual cost covered by other participants: $54,800 annually for 5 years						

[1]Research will be conducted when funding is available.

AGFD--Arizona Game and Fish Department
ASDM--Arizona-Sonora Desert Museum
BLM--Bureau of Land Management
CNF--Coronado National Forest
DOD--Department of Defense, Fort Huachuca
FWS--U.S. Fish and Wildlife Service
Involved Parties--All parties participating in activity
PZ--Phoenix Zoo
TBD--To be determined
SCC--Scottsdale Community College
RCLFCT--Ramsey Canyon Leopard Frog Conservation Team
TNC--The Nature Conservancy

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Anne Craven (formerly Bernstein), private pond owner, Sierra Vista, Arizona
Tom Deecken, Coronado National Forest, Sierra Vista Ranger District, Arizona
Charles (Bud) Eldon, TNC volunteer, Ramsey Canyon, Arizona
Kim Field, former AGFD employee, current USFWS-Nevada employee
Stephen Gunzel, District Ranger, Coronado National Forest, Sierra Vista Ranger District
Jim Platz, Creighton University, Nebraska
Jim Rorabaugh, USFWS Phoenix, Arizona
Angel Rutherford, private pond owner, Sierra Vista, Arizona
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Eric Wallace, former AGFD employee, current University of Arizona – Tucson student

Appendix 1. PECE CRITERIA: CERTAINTY OF IMPLEMENTATION AND EFFECTIVENESS

PECE: 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 p. 4, and in the Implementation Schedule (IS) pp. 48-50.
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 p. 3.
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 p. 4.
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. 1-3, and 19-20.
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 p. 4.
6. Regulatory mechanisms (e.g., laws, regulations, ordinances) necessary to implement the conservation effort are in place.	Can be found on pp. 3.
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 in the Implementation Schedule pp. 48-50.
8. An implementation schedule (including incremental completion dates) for the conservation effort is provided with requirements/sources.	Can be found in the Implementation Schedule, pp. 48-50.
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. 2 and 7-17.

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. 1-2, 19, 26-35.
2. Explicit incremental objectives for the conservation effort and dates for achieving them are stated.	Can be found on pp. 39-50.
3. The steps necessary to implement the conservation effort are identified in detail.	Can be found on pp. 39-50.
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. 39-50.
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. 39-50.
6. Principles of adaptive management are incorporated.	Can be found on pp. 47-50.

Appendix 2. PRINCIPAL CONTACTS

Principal contacts and members of the Ramsey Canyon Leopard Frog Conservation Team are:

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Appendix 3. ARIZONA LEOPARD FROG CAPTIVE CARE PROTOCOL. Author: K. Wright

I. Minimization of disease transfer
    a. Make sure that all enclosure materials that may have housed other amphibians have been disinfected to prevent spread of chytrid fungus. Various methods have been shown effective: 1) rinsing with 1% sodium hypochlorite (household bleach); 2) 20-second exposure to 70% ethanol or 1 mg/ml benzalkonium chloride; 3) desiccation and exposure to 50-60°C heat for 30 minutes; and, 4) soak in either 0.012% Path-X™ or 0.008% quaternary ammonium compound 128 (both contain DDAC, didecyl dimethyl ammonium chloride as active ingredient), rinse, and allow to dry. Johnson, ML, L Berger, L Philips, and R, Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 57:255-260.

II. Preparation time
    a. Enclosures should be functioning at least 14 days ahead of amphibian arrival to ensure that the systems are maintaining stable water quality parameters and to allow initial colonization of filter media with organisms crucial to each stage of the nitrogen cycle (i.e., capable of converting ammonia to nitrite and nitrite to nitrate).

III. Enclosures
    a. The largest enclosure possible should be used to provide maximum water capacity. A large water volume with proper filtration maintains more stable water quality parameters than a smaller water volume similarly equipped.
    b. All should be constructed of easily disinfected materials like plastic, glass, or fiberglass.
        i. Containers of cement-based products are one alternative, provided they are well aged and no longer leaching alkaline. Unsealed concrete can be problematic to disinfect between groups of animals. Rough concrete surfaces have been linked to mycobacterial infections in aquatic frogs, an incurable fatal infection.
        ii. No metal containers, galvanized or not. These may leach metal ions that are known toxicants to amphibians.
        iii. Aquaria, plastic kiddy pools, plastic cattle troughs, and aquaculture tubs work well. The specific enclosure depends on the husbandry plan to be implemented. In most cases, the largest enclosure as possible should be chosen so that the filtration system is as complete as possible (i.e., mechanical, chemical, biological, UV irradiation) to achieve and maintain stable water quality within appropriate parameters.
        iv. PVC or plastic pond liners are also acceptable, provided they are labeled as “fish safe” by the manufacturer.
        v. Water depth should be at least 5 inches for swimming larvae and no more than 5 inches for metamorphosing larvae and recently metamorphosed frogs.
    c. Lids.

        i. All containers should have screened or solid lids to prevent larvae, metamorphs, or adults from jumping out or escaping. The screens should be plastic rather than metal to avoid oxidized metal falling into the enclosure.
            1. An alternative is to use taller containers and keep the water level low.

d. Cage furniture.
    i. Hiding spots, basking spots, and aquatic perches are essential for frogs to feel comfortable in their enclosures. Visual barriers are important to reduce stress between frogs within the same enclosure and to reduce stress caused by activity outside the enclosure.
    ii. Disturbance should be minimized by setting up the holding containers in low (human) activity areas.
    iii. Artificial floating plants provide larvae with resting and hiding places.
    iv. 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. More stringent disinfection measures may be appropriate depending on the level of quarantine desired for the population of frogs. Copper sulfate, levamisole, and chlorhexidine baths may be used to eliminate protozoa, helminthes, and other pathogens that may find refuge in the plants. Chytrid fungus may survive on aquatic plants but may be eliminated by soaking the plants in water maintained at 37.5°C (99°F) for at least 18 hours.
    v. Plastic window screen mesh can be used as rafts and feeding platforms. Tadpoles often prefer resting above the bottom of the water column.
    vi. PVC pipe and fixtures can be used as underwater refuges.
    vii. The underwater perches should be stratified so that an animal can seek refuge at a comfortable depth of water. Some of the perches should be placed beneath overhead basking lights.

e. Lighting.
    i. Where practical, access to natural sunlight at levels approximately equal to the wild habitat is beneficial.
    ii. Artificial lighting can be provided using fluorescent lights.
    iii. Ultraviolet B may be provided using specific fluorescent bulbs. The need for this is uncertain at present.
    iv. Multiple basking sites should be provided on the land and on underwater perches using incandescent lights or ceramic bulb heaters.
    v. Light should be provided in a patchwork mosaic so an animal can choose between light and dark spaces.

f. Temperature.
    i. Water temperature should be maintained between 68°F and 80°F.
        1. Basking lights may be suspended over underwater rocks to provide thermal variation that offers larvae the chance to thermoregulate.
    ii. Larval growth rates are directly correlated with environmental temperatures. Within the biologically appropriate temperature ranges, higher temperatures typically yield faster growth rates.
    iii. Temperatures above 72°F are recommended to reduce the risk of the fungal disease saprolegniasis.

g. Inserts for rapid movement of animals.
    i. Holding containers can be fitted with mesh bottom inserts that contain the larvae or adults when the inserts are removed from the water. This insert is then placed into a clean container of the same size. This is not practical for complex systems but is often useful for small enclosures maintained on a sponge filter.

IV. Stage specific considerations
  a. Housing-Embryos.
    i. In general, the enclosure should be large enough so that the pump produces minimal current to agitate the egg mass or recently hatched larvae.
    ii. Gently aerate water in the embryo holding tank with a sponge filter and aquarium pump or an aquarium power head. If a sponge filter is not available, an airstone may be used. A sponge filter is preferred as it provides biological filtration if it has been properly aged. An airstone does not provide any filtration.
    iii. Egg masses and recently hatched larvae 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 eggs to suspend them in the water.
    iv. Remove dead hatchlings or eggs covered with fungus from the mass if possible with minimal disturbance. Ammonia levels can quickly rise to toxic level from decomposing eggs or hatchlings even with biological filtration.
    v. Stocking density: 1 egg mass (up to 1000 eggs) per 10 gallons of filtered aerated water.
  b. Housing-Larvae.
    i. Filtration.
      1. Mechanical, chemical, and biological filtration is essential to maintain water quality. UV sterilizers may also be beneficial.
      2. External canister filters are best for maintaining high volumes of water and moderate to high stocking densities. Undergravel filters and filter sponges are best for low water volumes and low stocking densities.
      3. Even with filtration, water changes are important to reduce build-up of organic waste products. Approximately 10% of the water volume should be changed weekly.
      4. Systems that include algal growth and living plants are encouraged as it provides additional buffering of water quality parameters. Additionally, algae is excellent food for larvae.
    ii. Stocking density.
      1. Sizes can be mixed; with Chiricahua leopard frogs 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.
      2. For maximum growth.
        a. 25-30 larvae per 10 gallons of filtered aerated water.
  c. Housing-Metamorphosing Larvae.

i. Water depth should be decreased to no more than 5 inches for larvae showing hindlimbs only.
ii. Edges of the enclosures should have areas where the metamorphosing larvae may crawl out of the water along a gradual incline similar to that of natural stream or pond banks (haul-out areas) and underwater perches. Some larvae may drown if haul-outs have not been provided.
iii. Cover should be provided on dry land and underwater.
iv. Some haul-out areas should be beneath a basking light.
    1. Wattage of light should be adjusted to provide a hotspot of 85-90°F.
v. Larvae that have developed 4 legs but retain a tail should be maintained in a separate tank from the 2-legged larvae. The water level can be decreased to 3 inches or less to reduce risk of drowning.
vi. Newly metamorphosed frogs should be separated by size to keep cannibalism to a minimum. Although larvae are not cannibalistic, juvenile and adults frogs are.
vii. Stocking density:
    1. No more than 10 metamorphs or recently metamorphosed frogs per 10 gallons of filtered aerated water.

V. Diet
  a. Many of the problems with metamorphosis are due to inadequate nutrition as a tadpole. Mistakes during tadpole development may result in dying tadpoles, stunted metamorphs, or recently metamorphosed frogs that have irregularities.
  b. Leopard frog tadpoles typically graze off the bottom of the water column or on the surface of objects. Food should be placed on the bottom of the enclosure to ensure the tadpoles find it easily. Some food items are buoyant, such as thawed frozen spinach, and may need to be weighted with stones so they don't float.
  c. Types of food for larvae:
    i. Live algae and aquatic plants are excellent food sources for tadpoles.
      1. Where possible, enclosures should be heavily planted so that tadpoles can graze on live food plants.
        a. Duckweed (*Lemma*) is easy to raise and a good food source. It may need to be harvested and crushed to sink to the bottom of the enclosure where it is easily found by tadpoles.
        b. Other aquatic plants are useful food sources (e.g., *Elodea*).
      2. If it is not practical to maintain algae and live plants in the rearing enclosures, algae cultures can be started in other enclosures and used as a food source.
        a. Firm plastic sheets, pieces of tile, or nonporous stone may be placed into an algae-rich environment and seeded with algae. Once a layer of algae is growing, the "plot" of algae can be removed and placed in with the tadpoles for grazing.
        b. If multiple plots are maintained, fresh algae is available for harvesting continuously.
    ii. Larvae feed well on dark green leafy produce.
      1. Dark green leafy produce should not exceed 50% of the total diet offered.

a. Spinach.
    i. Use either frozen thawed spinach or fresh spinach that has been frozen overnight. Freezing breaks down the cell walls of the spinach and makes it more digestible by the tadpoles.
    ii. Spinach contains oxalates that can interfere with tadpole development if consumed to excess. Spinach should comprise no more than 15% of the diet offered.
    iii. Spinach is not an essential part of the diet, merely an option.

b. Romaine lettuce.
    i. Should be frozen overnight to break the cell walls and increase its digestibility.
    ii. Romaine lettuce should comprise no more than 15% of the diet offered.

c. Mustard greens.
    i. Should be frozen overnight to break the cell walls and increase its digestibility.
    ii. Mustard greens should comprise no more than 15% of the diet offered.

d. Turnip greens.
    i. Should be frozen overnight to break the cell walls and increase its digestibility.
    ii. Turnip greens should comprise no more than 15% of the diet offered.

2. Other produce may be offered not to exceed 15% of the total diet offered.
    a. Cucumber slices.
        i. Should be frozen overnight to break the cell walls and increase its digestibility.
        ii. Cucumber should comprise no more than 15% of the diet offered.
    b. Green peas.
        i. Should be frozen overnight to break the cell walls and increase its digestibility.
        ii. Peas comprise no more than 15% of the diet offered.
3. Bok Choy and Kale are not recommended as their cell walls seem more resistant to bursting during the freezing process. They have low digestibility for tadpoles.

iii. Processed fish foods and protein sources.
    1. Spirulina-based fish foods and algae wafers designed for herbivorous cichlids work well.
        a. They may comprise up to 50% of the diet.
        b. Sinking wafers or pellets are preferred to floating wafers or pellets.

2. High protein fish foods should comprise at least 25% of the offered diet.
    a. Dehydrated bloodworms, tubifex worms, and earthworms are excellent sources of protein.
    b. Sinking foods are preferred to floating foods.
3. Frozen bloodworms, daphnia (water fleas), and rotifers are excellent protein sources and should comprise at least 5% of the offered diet.
4. Cooked egg white can be used as a protein source. It should not exceed 5% of the offered diet.
5. Alfalfa-based rabbit pellets may be used as a temporary diet if no other foods are available.

iv. A complete tadpole diet will vary from species to species and depends on water quality in part. However, a good starting diet consists of 5 oz of frozen thawed dark green leafy produce, 2 oz of frozen thawed peas, 5 oz of spirulina algae wafers, 2 oz high protein fish food, 2 oz frozen bloodworms.
1. All of these materials can be mixed together and frozen into small cubes for later use.
2. One 400 mg tablet of human-grade calcium carbonate and one multivitamin tablet should be ground into a powder and mixed into every pound of food.
3. One pound of the diet can be mixed together with hot water and one packet of unflavored gelatin to form more durable cubes that sink to the bottom of the water table.
    a. This may be kept in the refrigerator (45°F) for up to 5 days.
    b. If longer storage is desired, freeze the cubes. This reduces the potency of some of the water-soluble vitamins.
4. Food should be offered *ad libitum*. This means that fresh food is constantly available for feeding throughout the entire day and night.
5. Uneaten decomposing food should be removed daily.

v. Calcium is a critical supplement for the diet.
1. Calcium carbonate blocks or calcium carbonate pills (designed for human consumption) should be scattered on the bottom of the enclosures even if the food has been supplemented with calcium.
2. Calcium hardness of the water needs to be high for most species of native Arizona Leopard Frogs. Calcium supplements used to increase the hardness of water for freshwater tropical cichlids may be used. Ranges of 350-450 ppm are appropriate.
3. Vitamin D3 supplements, such as those used for supplementing the water source of feeder chickens, pigs, or calves, may need to be added to the water in some instances if the diet was poor.

d. Types of food for recently metamorphosed frogs and juveniles.

i. They feed well on domestic crickets, mealworm larvae, mealworm adult beetles, flightless houseflies, silkworm larvae, earthworms, small fish, and small roaches.
1. Food must be offered alive.

2. Since frogs often hunt more intensively at night, food items should be introduced to the enclosure at dusk, either just before or just after the lights over the enclosure have been turned off.
3. Insects should be dusted with calcium carbonate prior to feeding to increase the calcium content ingested by the frog.
4. Insects should be dusted with a multivitamin powder once a week prior to feeding.

ii. In 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.

1. Do not use this technique if there is a risk that pesticides are sprayed in nearby areas.

VI. Air Quality

a. If the air smells bad to you for any reason, it may contain chemicals that are harmful to amphibians.

i. Do not smoke around amphibian enclosures.

ii. Avoid the use of strong smelling chemicals in the airspace around an enclosure.

iii. Make sure that the ventilation leading to the amphibian enclosure does not interface with any air that contains dangerous chemicals.

b. Water in enclosures should have sufficient aeration so that the larvae are not gasping for air at the top of the tank or looking distressed.

VII. Water Quality and Changing Schedule

a. The importance of appropriate water for raising young amphibians cannot be overstated. Larval development and metamorphosis are incredibly complex and demanding life stages for amphibians. In addition to diet, some dissolved substances in the water provide nutrients for growth of larvae. Conversely, some dissolved substances are toxic and create metabolic demands that can interfere with normal growth and metamorphosis.

b. Water samples from natural breeding sites should be analyzed for various parameters and efforts made to reproduce those parameters in the captive setting.

i. Unfortunately, many times there is little or no data about the water quality *in situ*. The guidelines in Table 1 are applicable settings for most Leopard frog species native to Arizona.

1. Values should be adjusted if the species is known to inhabit hard alkaline water (e.g., limestone seep) or soft acidic water (e.g., sphagnum bogs, pine forests) or it is likely that larval growth and metamorphosis may be abnormal.
   a. For example, Ramsey Canyon leopard frogs, *Rana subaquavocalis*, developed nutritional secondary hyperparathyroidism when maintained in water that had lower calcium hardness than the levels detected in natural breeding sites.
2. Simple dipstick water quality tests are readily available (Hach Company, PO Box 389, Loveland, CO 80539, phone (800) 227-4224).

c. Changing Schedule.

i. Ideally, all holding containers should be cleaned daily by siphoning off a minimum of 10% 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.
ii. The frequency of water changes will depend on the stocking density of larvae and presence/absence of a filtration system.
iii. Water for the recently metamorphosed frogs can be changed once a week to minimize stress as long as dead prey items are being skimmed daily.

d. Water Issues.
    i. If tap water is used for water, the faucet should be opened and run for a few minutes prior to collecting water. This allows the residual water in the pipes that may have a high copper content to be flushed out of the system.
        1. Tap water should be allowed to sit 24 or more hours in an open container to allow the chlorine to dissipate.
        2. Aeration helps to remove the chlorine more quickly.
        3. Check with your local water provider. If chloramines are used to disinfect the water, you may need to use dechlorinating chemicals instead of aeration. It may take 3-5 days for chloramines to be eliminated from the water by aeration.
        4. Carbon filters can be placed in-line to eliminate the need for aeration to remove chlorine or chloramines.
            a. These filters need to be changed regularly.
            b. Water should be checked with chlorine test kits (e.g., Hach Company dry strips) to make sure the filters are functioning properly.
    ii. Stream or pond water from which the animals originated is acceptable.
        1. The water temperature may be raised to 95°F or higher to eliminate chytrid fungus.
        2. During a water change, replacement water should be the same temperature as the water in the holding container to minimize stress.

Appendix 3, Table 1. Water quality parameters suitable for Arizona Leopard Frogs.

Parameter	**Range** **Frequency of Sampling**	**Comments**
Temperature	68-74°F Daily	Temperature may be maintained outside these ranges depending on the growth rate desired. Gastrointestinal gas and slow development are signs of inappropriately low temperatures.
pH	7.8-9.0 Daily	Requires at least a 10% water change if outside this range. If the pH is outside this range for more than 3 days in a row, the filter media may need to be changed. Lower pH to 6.5-7 for acidic species by adding peat moss. Higher pH may be achieved using calcium supplements or water quality supplements for alkaline-dwelling cichlid fish.
Ammonia	not to exceed 0.2 ppm Daily	If outside this range, change water immediately. Make sure uneaten food and organic debris are being removed frequently. The volume of water to be changed depends on level of ammonia. May need to add Amquel™ or other ammonia-neutralizer designed for tropical fish. Filter may need to be changed and new activated carbon added. Even a minor rise in ammonia can cause immediate death or immunosuppression and subsequent outbreaks of infectious disease. Ideally, ammonia levels should never exceed 0.1 ppm
Nitrite	not to exceed 0.1 ppm Every 2 or 3 days	Requires at least a 10% water change if above this limit. Make sure uneaten food and debris are removed. Filter may need to be changed.
Nitrate	not to exceed 10 ppm Every 2 or 3 days	Requires at least a 10% water change if above this limit. Make sure uneaten food and debris are removed. Filter may need to be changed.
Total Hardness	50-350 ppm Every 7 days	Specimens may show white plaques if hardness is too high. Change water and refill with distilled or deionized water if hardness is too high. Add calcium blocks or cuttle bone if hardness is too low. Water supplements designed for hardwater (alkaline) cichlid fish may be used to increase hardness instead.
Calcium Hardness	Not to exceed	See comments for total hardness.

Parameter	**Range** **Frequency of Sampling**	**Comments**
	total hardness (350 ppm) Every 7 days	
Alkalinity	50-200 ppm Every 7 days	See comments for total hardness.
Free Chlorine	Not to exceed 0 ppm after water change	Chlorine-free water should be used to prepare an enclosure. Any detected chlorine indicates that the carbon filter on the water supply line needs to be changed. Dechlorinating agents such as sodium thiosulfate may be added if it is impractical to change water.
Total Chlorine	Not to exceed 0 ppm after water change	See comments for free chlorine.
Copper	Not to exceed 0 ppm after water change	Water supply lines should be flushed for several minutes to avoid adding copper to an enclosure. If copper is detected, a 50-100% water change is recommended. Copper can be toxic or immunosuppressive depending on the concentration.
Iron	Not to exceed 2 ppm after water change	See comments for copper.

Appendix 4. GENERAL GUIDELINES FOR TRANSPORTATION OF LEOPARD FROG LIFE STAGES
Author: K. Wright, Phoenix Zoo

I. Transportation
   a. General Container Information.
      i. Use only plastic containers, no metal or glass.
      ii. Containers should be water tight when tipped upside down.
      iii. Do not use bags more than once. Use only new, rinsed bags.
      iv. Carry 1 or 2 extra containers filled with water in case of an emergency (i.e., leak).
   b. Type of Containers per animal size.
      i. 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.
      ii. Larvae may also be transported in hard plastic buckets or containers that have tight fitting lids.
      iii. GladWare® plastic containers are highly recommended for transportation of metamorphs, juveniles, and adults. These containers keep the frogs from being crushed and are reusable.
   c. Preparing Containers.
      i. Thoroughly rinse all shipping containers with water. Do not use any type of detergent or soap to clean the containers.
      ii. The GladWare® also needs holes drilled in the top. A standard hole punch works well, approximately 16 holes. Drill from the inside out so that no sharp edges protrude into the animal holding space.
      iii. If desired, mark each bag with identification of eventual destination and the number of animals in the container.
   d. Stocking densities.
      i. Per gallon bag for short shipments.
         1. Eggs: 1 mass per bag, minimize disturbance and division of mass.
         2. Larvae under ½”: 25 per bag.
         3. Larvae 1” - 1 ½”: 15 per bag.
         4. Larvae over 1 ½”: 10 per bag.
         5. Recently metamorphosed frogs: 5 per container or bag.
      ii. Avoid overcrowding.
   e. Water.
      i. Water put in the bags must be chlorine and chloramine free. Dechlorinating chemicals can be used to immediately remove chlorine.
      ii. Stream or pond water from which the animals originated can be used. Avoid capturing aquatic invertebrates or organic debris.
      iii. Other alternatives are bottled drinking water or tap water left uncovered for 24 or more hours.
      iv. For larvae, fill bags to at least 75% capacity to avoid excessive sloshing.

v. 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.

vi. 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.

f. Temperature.

i. Optimal shipping temperature is a compromise between the captive and anticipated release temperature.

ii. 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 the ice and animals, so if the ice melts the animals will float instead of settling in the water.

iii. A thermometer with a remote sensor inside the container can assist in monitoring the temperature while shipping.

iv. Alternatively, animals could be moved in open containers if kept inside air-conditioned vehicles capable of maintaining the appropriate desired temperature.

v. 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.

Appendix 5. LEOPARD FROG EGG MASS COLLECTION AND TRANSPORTATION PROTOCOL
Author: K. Wright, Phoenix Zoo

- Ensure that all field equipment (boots, nets, truck tires, etc.) that may have been used at other locations has been disinfected to prevent spread of chytrid fungus. Various methods have been shown effective: 1) rinsing with 1% sodium hypochlorite (household bleach); 2) 20-second exposure to 70% ethanol or 1 mg/ml benzalkonium chloride; 3) desiccation and exposure to 50-60°C heat for 30 minutes; and, 4) soak in either 0.012% Path-X™ or 0.008% quaternary ammonium compound 128 (both contain DDAC, didecyl dimethyl ammonium chloride as active ingredient), rinse, and allow to dry. Johnson, ML, L Berger, L Philips, and R, Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 57:255-260.

- 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. Additional water quality data may be collected at this time as resources and circumstances permit (pH, dissolved oxygen, ammonia, nitrite, nitrate, total hardness, calcium hardness, alkalinity, chlorine, copper, iron). Forward this information with the egg mass and send a copy to V. Boyarski.

- Egg masses should be freshly laid (< 5 days) or show little sign of development. Reduced hatched rate and mortality of tadpoles increases greatly once the embryonic tadpoles are developed to the point they are able to wriggle within their eggs.

- Use a new, 1 gallon, self-closing plastic bag to transport the egg mass. Rinse the bag with the source water thoroughly before use and write the name of the collection site on the bag. Place only 1 egg mass per bag (approximately 500 eggs or fewer). If the egg mass is larger, divide into smaller portions of approximately 300-500 eggs each.
    - 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.
    - 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.
    - If the situation permits, collect an additional 2 – 5 gallons of water from the site in clean plastic bags or plastic buckets. This source water may be important for the initial acclimation of egg masses in the captive environment.

- Transport the egg mass in the plastic bag within a styrofoam or hard plastic cooler to provide a stable appropriate thermal environment. 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.

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

Appendix 6. LEOPARD FROG TADPOLE COLLECTION AND TRANSPORTATION PROTOCOL
Author: K.Wright, Phoenix Zoo

- Ensure that all field equipment (boots, nets, truck tires, etc.) that may have been used at other locations have been disinfected to prevent spread of chytrid fungus. Various methods have been shown effective: 1) rinsing with 1% sodium hypochlorite (household bleach); 2) 20-second exposure to 70% ethanol or 1 mg/ml benzalkonium chloride; 3) desiccation and exposure to 50-60°C heat for 30 minutes; and, either 0.012% Path-X™ or 0.008% quaternary ammonium compound 128 (both contain DDAC, didecyl dimethyl ammonium chloride as active ingredient). Johnson, ML, L Berger, L Philips, and R, Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 57:255-260.

- If possible, record the water and air temperature at the site, location of the tadpoles in the pond or creek, and current and recent weather events. Additional water quality data may be collected at this time as resources and circumstances permit (pH, dissolved oxygen, ammonia, nitrite, nitrate, total hardness, calcium hardness, alkalinity, chlorine, copper, iron). Forward this information with the tadpoles and send a copy to V. Boyarski.

- Tadpoles to be collected should be moving independently and have already absorbed their yolk. Recently hatched tadpoles that rest and only move when stimulated have higher mortality during transportation and acclimation to captivity than older more active tadpoles.

- Use a new, 1 gallon, self-closing plastic bag to transport the tadpoles. Rinse the bag with the source water thoroughly before use and write the name of the collection site on the bag.
  - Use a soft nylon net to collect the tadpoles to minimize damage to their skin. A clear plastic bag may be used instead of a net in some circumstances and causes even less damage to the tadpoles.
    - If the tadpoles are small (< 1 inch Snout-Tail Length), place no more than 25 tadpoles per bag.
    - If the tadpoles are large (> 1 inch Snout-Tail Length), place no more than 15 tadpoles per bag.
  - Fill the 1 gallon transport bag with clear water.
    - If you are using a nylon net to collect tadpoles: once you have netted the tadpoles, quickly lift them out of the water and place the entire net below the waterline in the transport bag. Gently swish the net back and forth to release the tadpoles into the transport bag.
    - If you are using a plastic bag to collect tadpoles: let the bag drift open underwater as you swoop it toward the tadpoles. Lift the bag above the water surface and seal the bag with just a slight gap so that excess water can be squeezed out. Transfer the tadpoles and the small amount of water into the 1 gallon transport bag.

        - Avoid placing aquatic invertebrates, mud, leaves, and other organic debris into the transport bag.
    - Close the transport bag and allow approximately 1” of air space. Once sealed, placed the filled bag inside a second bag in case of leakage.
    - If the situation permits, collect an additional 2 – 5 gallons of water from the site in clean plastic bags or plastic buckets. This source water may be important for initial acclimation of tadpoles in the captive environment.

- Transport the tadpoles in the plastic bag within a styrofoam or hard plastic cooler to provide a stable appropriate thermal environment. 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 tadpole 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 tadpole bag.

- Coordinate with the captive rearing facility prior to departure to alert them to your estimated time of arrival and minimize transit time.
    - If the water quality of the source water and the captive rearing enclosure are radically different with respect to pH and hardness, effort should be made to adjust the enclosure water prior to introduction of tadpoles. In any case, the tadpoles should be floated in their bags in the water of enclosure to allow for them to reach equilibrium with the enclosure water temperature before release. If the pH and hardness cannot be adjusted, add small amounts of enclosure water to the bags to gradually acclimate the tadpoles. You may want to add the extra source water to the enclosure to try and ameliorate the effects of the different water quality parameters.

Appendix 7. JUVENILE AND ADULT LEOPARD FROG COLLECTION AND TRANSPORTATION PROTOCOL Author: K. Wright, Phoenix Zoo

- Make sure that all field equipment (boots, nets, truck tires, etc.) that may have been used at other locations have been disinfected to prevent spread of chytrid fungus. Various methods have been shown effective: 1) rinsing with 1% sodium hypochlorite (household bleach); 2) 20-second exposure to 70% ethanol or 1 mg/ml benzalkonium chloride; 3) desiccation and exposure to 50-60°C heat for 30 minutes; and, 4) soak in either 0.012% Path-X™ or 0.008% quaternary ammonium compound 128 (both contain DDAC, didecyl dimethyl ammonium chloride as active ingredient), rinse, and allow to dry. Johnson, ML, L Berger, L Philips, and R, Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 57:255-260.

- If possible, record the water and air temperature at the site, location of the frog in the pond or creek, and current and recent weather events. Additional water quality data may be collected at this time as resources and circumstances permit (pH, dissolved oxygen, ammonia, nitrite, nitrate, total hardness, calcium hardness, alkalinity, chlorine, copper, iron). Forward this information with the tadpoles and send a copy to V. Boyarski.

- Frogs may be transported in a new, 1 gallon, self-closing plastic bag. No more than 5 juveniles (Snout-Vent Length < 2 inches) should be placed per bag. Larger frogs (Snout-Vent Length ≥ 2 inches) should be transported with no more than 1 frog per bag. Hard plastic containers may be used depending on the circumstances (e.g., disposable Gladware™, Rubbermaid© containers, 5 gallon buckets with lids, etc.). Hard containers should have ventilation holes on the lid—make sure the ventilation holes have no rough edges projecting inward that could harm the frogs. The containers should be rinsed with the source water thoroughly before use and approximately ½" to 1"deep water added. Write the name of the collection site on the container.
    - Many frogs benefit from soft plant material added to the water. This can be aquatic vegetation, sphagnum moss, or shredded deciduous leaves. This material provides underwater perch sites that help to calm down some frogs. It also provides some padding if the container is jostled during transport. Be wary of putting too much material in the container as this can trap and drown frogs.
    - If the situation permits, collect an additional 2 – 5 gallons of water from the site in clean plastic bags or plastic buckets. This source water may be important for initial acclimation of smaller frogs in the captive environment.

- Transport frogs in a plastic bag by placing them within a styrofoam or hard plastic cooler to provide a stable thermal environment. 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 frog 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 tadpole bag.
    - If the containers are too large to be managed this way, care should be taken to limit the speed of temperature change during transport. Newspaper or insulation

can be duct-taped around the container and ice-packs slipped between the bucket and the insulation material.

- Coordinate with the captive rearing facility prior to departure to alert them to your estimated time of arrival and minimize transit time.
  - For frogs with a known history of mortality following transport, initial efforts at the captive rearing facility may include some anti-shock measures. This may involve supplemental oxygen bubbled through the water, addition of no more than 4.5 g of salt or sea salt to liter of water in the enclosure (which should be completely rinsed and refilled with freshwater 48 hr after arrival to removal all supplemental salts), and addition of artificial slime products used for stressed tropical fish. Initial treatment with itraconazole baths (1% solution dissolved to 0.01% strength in a 0.6% salt solution as a 5 minute soak) is warranted if the frogs come from an area known to be contaminated with chytridiomycosis or if there have been recent mortalities in the population.
  - If the water quality of the source water and the captive rearing enclosure is radically different with respect to pH and hardness, effort should be made to adjust the enclosure water prior to introduction of frogs. In any case, the frogs should be floated in their bags in the water of enclosure to allow for them to reach equilibrium with the enclosure water temperature before release. If the pH and hardness cannot be adjusted, add small amounts of enclosure water to the bags to gradually acclimate the frogs. You may want to add the extra source water to the enclosure to try and ameliorate the effects of the different water quality parameters.

Appendix 8. CAPTIVE RELEASE PROTOCOL FOR LARVAE, JUVENILE, AND ADULT LEOPARD FROGS NATIVE TO ARIZONA From: K. Wright, Phoenix Zoo.

- <u>Qualifications For A Release Program</u>
    - 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.
            - Each release group should be screened by PCR tests to identify chytrid fungus at least 30 days prior to release.
                - Only chytrid-negative groups should be released.
    - No irregularities 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.
    - No medical treatments of the release group during the previous 30 days.
    - All animals designated for release should be in permanent quarantine to prevent exposure to novel pathogens.
        - Open enclosures which allow access of free-ranging insects and other food animals are still considered quarantine as long as there is a low risk of other amphibians entering the facility.
        - Staff caring for animals known to harbor diseases communicable to leopard frogs (including but not limited to other amphibians) should have no contact with quarantined leopard frogs. If this is not practical:
            - Caregivers should work the leopard frogs first before they have cared for other animals.
            - Caregivers that have contacted other animals either as part of their job or as pets should "shower in" and change clothes before entering the leopard frog facility.
        - 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 acceptably low risk.
    - 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 containing animals with irregularities should be worked last.
    - Water quality logs should be maintained. Adjustment to release site water conditions should occur 30 days prior to release.

    - Animals that have recently been exposed to ammonia or nitrite spikes within 30 days of may be under substantial stress. Potential impact should be discussed with all parties involved before a release is approved.

- <u>Pre-Release Screening Protocol (Up to 30 Days Prior to Release)</u>
  - Depending on the size, life stage, and number of the specimens to be released, a random sample of animals may need to be assessed rather than an individual assessment of all animals within a group.
  - Data to obtain and evaluate.
    - Weight.
    - Physical exam noting.
      - Body position.
      - Alertness.
      - Nose-to-toes visual examination noting.
        - Any abnormalities.
        - Any musculoskeletal conditions, including obvious bony abnormalities (e.g., long bone curvature or asymmetry, spinal curvature, mandibular bowing, etc.).
    - Skin scrape sample for chytrid PCR testing.
      - Only animals testing negative within 30 days of release should be released.
      - Positive animals should be treated.
    - If post-release monitoring is scheduled, mark animal with permanent or temporary technique consistent with goals of monitoring program.
      - Toe clip.
        - Toe clip may be saved for chytrid histopathology, DNA banking or frozen for future pathogen recovery attempts.
      - PIT tags.
        - Intracoelomic placement may not be permanent.
        - Subcutaneous placement may need surgical glue closure of injection site to prevent tag loss.
      - Injectable elastomeres
        - *Insert protocol from Kevin Zippel.*

- <u>Pre-Release Activities</u> (10 Days and 2 Days Before Release)
  - Chytrid fungus prophylaxis (method to prevent spread of disease).
    - Soak in an antifungal solution 10 days and 2 days prior to release (or packing for transport for release).
      - 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: diluted to 0.01% concentration in 0.6% saline (Sporanox, Janssen Pharmaceutica, Titusville, NJ) for up to 1 hr.

    - 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.

- <u>Immediate Pre-Release Activities (At Time of Packing for Transport)</u>
  - Do a visual assessment of animals and approve or reject packing for transport.
  - Antibacterial prophylaxis.
    - If this has never been done before, try the treatment on a few individuals well ahead of time to determine tolerance.
    - Dip in benzalkonium chloride (2.0 mg/l) for at least 15 seconds.
    - Rinse with fresh water before packing animal.
    - Repeat visual assessment and approve or reject for packing for transport.

- <u>Activities At Release Site</u>
  - Do a final visual assessment of animals and approve or reject release.
  - Aquatic life stages.
    - Equilibrate water temperature and chemistries of transport container with release site water.
      - Float containers in release site water for at least 30 minutes.
      - Do a 50% water change with release site water and wait for 10 minutes.
      - Release all animals that appear to behave normally.
  - Terrestrial life stages.
    - Equilibrate container temperature with release site temperature.
      - Sit containers in shaded location for at least 30 minutes.
    - Release all animals that appear to behave normally.

Appendix 9. GUIDELINES FOR PREVENTION OF PATHOGEN TRANSFER IN AQUATIC SYSTEMS

The RLFCT will take precautionary measures to prevent the spread of diseases in aquatic systems. All agencies and private individuals are encouraged to follow these guidelines when working in aquatic systems regardless of their involvement in RCLFCT activities. These guidelines are adapted from the Declining Amphibian Populations Task Force's (DAPTF) Fieldwork Code of Practice (also shown below), which provides guidelines for anyone conducting fieldwork in amphibian or other aquatic habitats. Pathogens and parasites can be transferred between habitats on equipment and footwear of fieldworkers. It is important for anyone involved in amphibian research and other types of aquatic studies 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.

GUIDELINES FOR PREVENTION OF PATHOGEN TRANSFER IN AQUATIC SYSTEMS

1. When possible, use dedicated equipment at sites. This includes footwear. Clean and store dedicated equipment separately.

2. Equipment must be disinfected between visits to sites. Remove mud and debris from all equipment. Give special attention to grips, cleats, and laces on footwear. Felt-bottomed wader boots are very difficult to clean completely and are not recommended. Scrub all surfaces with 10% bleach or 1.6% Quat-128 (Waxie Enterprises Inc.) solution, and let sit for 5 minutes before rinsing with tap water or water at the next aquatic site visited. To further reduce the risk of disease transfer, all equipment should be dried completely before reuse whenever possible.

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 water. Used cleaning materials (including liquids) must be disposed of safely and if necessary taken back to the lab for proper disposal.

4. Animals collected from different sites must be kept separately. Take care to avoid indirect contact between animals from different sites and those already in captivity (e.g., via handling, reuse of containers, exchange of water). Isolation from unsterilized plants or soils that have been taken from other sites is essential.

5. Amphibians that are head-started for release into refugia will be grown using clean methods and disinfected prior to release.

6. Do not transfer aquatic vegetation to sites without disinfection.

7. The RCLFCT will announce which localities are known to be positive for chytrid. All equipment used in known chytrid locations must either be dedicated or disinfected as above with complete drying mandatory before reuse.

THE DAPTF FIELDWORK CODE OF PRACTICE

1. Remove mud, snails, algae, and other debris from nets, traps, boots, vehicle lyres, and all other surfaces. Rinse cleaned items with sterilized (eg. boiled or treated) water before leaving each study site.

2. Boots, nets, traps, etc., should then be scrubbed with 70% ethanol solution and rinsed clean with sterilized water between study sites. Avoid cleaning equipment in the immediate vicinity of a pond or wetland.

3. In remote locations, clean all equipment as described above (or with a bleach solution) upon return to the lab or "base camp". Elsewhere, when washing-machine facilities are available, remove nets from poles and wash with bleach on a "delicates" cycle, contained in a protective mesh laundry bag.

4. When working at sites with known or suspected disease problems, or when sampling populations of rare or isolated species, wear disposable gloves and change them between handling each animal. Dedicate sets of nets, boots, traps and other equipment to each site being visited. Clean and store them separately at the end of each field day.

5. When amphibians are collected, ensure the separation of animals from different sites and take great care to avoid indirect contact between them (e.g., via handling, reuse of containers) or with other captive animals. Isolation from unsterilized plants or soils which have been taken from other sites is also essential. Always use disinfected/disposable husbandry equipment.

6. Examine collected amphibians for the presence of diseases and parasites soon after capture. Prior to their release or the release of any progeny, amphibians should be quarantined for a period and thoroughly screened for the presence of any potential disease agents.

7. Used cleaning materials (liquids etc.) should be disposed of safely and if necessary taken back to the lab for proper disposal. Used disposable gloves should be retained for safe disposal in sealed bags.

The DAPTF Fieldwork Code of Practice has been produced by the DAPTF with valuable assistance from Begofia Arano, Andrew Cunningham, Tom Langton, Jamie Reaser, and Stan Sessions.

For further information on this Code or on the DAPTF, contact:

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Appendix 10. TRANSLOCATION SITE EVALUATION.

The potential sites for release will initially be chosen in reference to likely historical distribution. Sites of release should be pools that are a minimum of one meter deep. Pools should have overhanging banks or soft soil and vegetation available within one meter. There should be multiple areas for basking along the banks that receive full sun. Ideally, the release pools and those in close proximity should not contain crayfish, bullfrogs, or non-native fish. Also refer to focal, supplementary, and refugium site definitions.

The following factors will be considered when evaluating site suitability: 1) availability of potential habitat for all leopard frog life stages; 2) habitat characteristics such as water permanence and quality (heavy metals and other contaminants and temperature), dispersal corridors, foraging areas, and quality of aquatic and terrestrial vegetation; 3) possible challenges to success such as land use or presence and abundance of non-native species; 4) necessary habitat renovations; 5) land ownership; 6) site accessibility; 7) history of frog inhabitance and/or die-offs; 8) history of diseases detected at the site; and 9) locations of nearby extant populations of Ramsey Canyon leopard frogs.

Appendix 11. TRANSLOCATION GUIDELINES.

COLLECT EGGS, LARVAE, OR ADULTS

To establish or augment wild or captive populations, we will collect frogs, larvae, or eggs from suitable sources to be released directly to the wild or to be head-started in captivity prior to release. Whenever possible, the number of individuals collected will be sufficiently large and from geographic locations such that genetic variability will be maintained. Prior to collection, possible source populations will be surveyed to investigate their ability to withstand removal of some frogs, larvae, or eggs.

The collection of eggs or tadpoles, rather than adults, will minimize impacts to the source population and when head-started in captivity or at in-situ facilities will produce large numbers of metamorphs or late stage tadpoles for release. Removal of 10% of the eggs from several egg masses should have few negative impacts (U.S. Fish and Wildlife Service 2000). A detailed protocol for the transport of eggs is provided as Appendix 5 and for the transport of tadpoles and frogs as Appendices 6-7.

Any stock that is to be translocated directly will come from sites where no disease has been detected, as long as such sites are available. Prior to release, stock will be treated in anti-fungal solutions using methods known to rid animals of chytrid infection.

REAR EGGS AND LARVAE TO LATE STAGES OR METAMORPHOSIS

Captive rearing will take place at the Phoenix Zoo, Scottsdale Community College, Arizona-Sonora Desert Museum, or similar facilities. The Phoenix Zoo and Arizona-Sonora Desert Museum successfully head-started Ramsey Canyon leopard frogs for translocations during the 1996-2001 Conservation Agreement period. In addition, frogs or tadpoles may be reared in small facilities operated by private individuals or *in situ* in temporary facilities within the Ramsey Canyon leopard frog's area of distribution (Meadow Ponds, Ramsey Canyon; metal tank at Barchas Ranch). The husbandry protocol for head-starting frogs should be similar to that developed by the Phoenix Zoo (Appendix 3).

EVALUATE SUITABILITY OF STOCK FOR RELEASE

Diseased animals will not knowingly be released. Phoenix Zoo has developed a pre-release health screening (Appendix 8). A sample of the frogs intended for release will be screened for diseases. The screening will have minimal impact on the number of animals released to the wild. We will use the latest techniques in disease detection, prevention, and treatment.

MARK AND MEASURE INDIVIDUALS TO BE RELEASED

Prior to release into the wild, all frogs will be given at least a cohort mark to identify the date and place of release by removing portions of toes (Martof 1953) or by injection of elastomers into the webbing (Nauwelaerts et. al 2000). Toe clipping will take place at the captive rearing facility prior to release. Alternatively, frogs may be individually marked with unique numbers by toe clipping, elastomer implants, or insertion of a passive integrated transponder (PIT) tag (Camper and Dixon 1988). These marks will allow for estimations of survival rates and tracking of movements.

Although there are methods for marking larvae, many methods are unreliable and costly (Muths et al. 2000). Injected polymers may be used (see Anholt et al. 1998), although many tadpoles are likely to be released unmarked. Animals to be released will be transported to the release site in coolers packed inside vehicles. Once at the release areas, the animals will be acclimated by floating their containers in the water at the site and through the addition of small amounts of site water into their containers. The techniques used to transport Ramsey Canyon leopard frogs to the release areas should be similar to techniques developed by Phoenix Zoo (Appendix 4).

Individuals should be measured (snout-vent length for frogs, total length for tadpoles) prior to release. This will allow for estimation of growth rates in captivity and the wild. In addition, size at time of release may affect ultimate success of translocation.

RECORD NUMBERS AND STAGES OF INDIVIDUALS RELEASED

The number of animals released at each site depends upon the capacity of the habitat to support all life stages of Ramsey Canyon leopard frogs and the number of individuals available for translocation. In order to increase the chances of success, some researchers suggest that releases of amphibians should take place in at least two consecutive years (Beebee 1996). The RCLFCT recommends that releases take place in the late spring, when water temperatures have reached at least 15 °C, through early fall. Releases outside of this suggested time period may be appropriate under certain circumstances and will be evaluated accordingly. While in captivity, the individuals to be released will be acclimated to temperatures at the release areas.

Because mortality could be quite high in the wild and frogs have a secretive nature, many more individuals will be released than will likely be observed in follow up surveys. Platz (1996) suggests the release of 100 metamorphs for each 200 feet of exposed shoreline. In small, ornamental ponds the release of as few as 5 metamorphs or tadpoles has resulted in the establishment of small populations (AGFD, unpublished data).

Appendix 12. GENERAL VISUAL ENCOUNTER SURVEY METHOD

(Arizona Game and Fish Department, May 2002)

This standard visual encounter survey (VES) method is to be used for Chiricahua leopard frog surveys. This method was adopted from Heyer *et al*. (1994) and modified based on statewide ranid surveys in Arizona. The method is designed to be simple and repeatable with minimal training of personnel. However, all personnel should be trained and survey techniques should be checked periodically by a more experienced individual. The VES method 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. Before designing a monitoring program, it is recommended that the user consult Gibbs' (1996) program MONITOR or Gerodette's (1987, 1993) program TRENDS to test the statistical power of the proposed monitoring program.

Equipment needed:

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 1927 (NAD27Conus) and the appropriate Universal Transverse Mercator (UTM) Zone
- a clipboard with the Chiricahua leopard frog Survey Form and instructions
- a pen with waterproof ink
- a time piece with a stop watch
- a pH meter
- 2 thermometers
- a conductivity meter
- a sling psychrometer or hygrometer
- binoculars
- the appropriate United States 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 camera with slide film
- the appropriate land ownership maps
- database reports of historic 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 and identifying calls)
- taped recordings of anuran calls (e.g., Davidson 1996)
- compass

Survey Method:

All “suitable” habitats within an action area (area to be affected by a project) should be surveyed.

Suitable Habitat is defined as follows: The frog is a habitat generalist that is found in cienegas, pools, beaver ponds, livestock tanks, lakes, reservoirs, streams, and rivers at elevations of 1,000 to 2,710 meters (m). They are occasionally found in livestock drinkers, irrigation sloughs and acequias, wells, abandoned swimming pools, back yard ponds, and mine adits. On the Coronado National Forest, the species occurs from 1,000 to 2,013 m. On the other Forests in Arizona, Chiricahua leopard frogs occur between 1,080 and 2,525 m. The frog uses permanent or nearly permanent pools and ponds for breeding. Most sites that support populations of this frog will hold water year long in most years. Time from hatching to metamorphosis is shorter in warm waters than cold water, thus water permanency is probably more important at higher elevation and in the northern portion of the species’ range. The species is rarely found in aquatic sites inhabited by non-native fish, bullfrogs, or crayfish. However, in complex systems or large aquatic sites, Chiricahua leopard frog may occur with low densities of non-native predators.

Surveys in suitable lentic and lotic systems should be conducted as follows:

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. Walk around the entire perimeter of the site. If the entire perimeter is not 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 starting point (or the most downstream point of the site) as UTM coordinates. Proceed upstream 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 best 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.

Data collection:

Data should be collected according to the Chiricahua Leopard Frog Survey Form Instructions (Attachment 3). 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 herp observations.

Appendix 14. ADAPTED FROM RIPARIAN HERP SURVEY FORM INSTRUCTIONS (Arizona Game and Fish Department—March 2003)

- Fields with an asterisk (*) are to be filled out for every survey, regardless of results.
- Check the site's Locality Data upon returning to the office for consistency (i.e., the site name filled out is consistent with the site name used in previous surveys).
- Upon return to the office, check each Survey Form for completeness, conciseness, and clarity prior to submitting for entry.

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 United States 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., U.S. Forest Service map, BLM 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 Fork Gila River at **Diamond Creek**) or topographic feature (e.g., San Francisco River, W of Glenwood) in the description.

NEW SITE: This field is used for central database management purposes only and is not to be filled out by survey personnel.

NUM: This field is used for central database management purposes only and is not to be filled out by survey personnel. 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).

*UTM ZONE: Circle "11", "12" or "13" 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. Most of New Mexico, except for the extreme western portion is in Zone 13.

*****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 1927 (NAD27Conus for Garmin units). Check that the GPS unit is reading the appropriate Zone (most of AZ is Zone 12, most of NM is Zone 13). 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 1927 (NAD27). Check that the GPS unit is reading the appropriate Zone (most of AZ is Zone 12, most of NM is Zone 13). 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.

*ELEV: Record the elevation at which the **starting point** of the survey occurs. Read the elevation off of the survey quad or GPS unit. Be sure to indicate the measurement units (ft or m). 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. To convert feet to meters multiply by 0.3048. If using a GPS unit, ensure you have adequate satellite coverage for an accurate elevation reading (at least 4 satellites).

*QUAD: Record the quadrangle name as it appears on the quadrangle. The name of the quadrangle appears in the upper and lower right hand corners of the quadrangle. If more than one 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.

*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 one year appears on the map, record the year of the most recent revision.

*COUNTY: Record the state abbreviation (e.g., AZ, NM) followed by a hyphen and then the first 4 letters of the county (e.g., AZ-MARI, AZ-YAVA, NM-CATR, NM-SIER). 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, road maps, and gazetteers are also useful in identifying counties.

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: "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 eight 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, period, second initial, period, space, and full last name (e.g., M.J. Sredl, C.W. Painter).

***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 measuring tape. 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 gartersnakes, 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 suitable 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. In New Mexico, all specimens collected should be given to the New Mexico Dept. of Game and Fish, Endangered Species Program for identification and deposition in the Museum of SW Biology at Univ. of New Mexico. In Arizona, give specimens to the Arizona Game and Fish Dept., Nongame Branch in Phoenix for identification and deposition in the Arizona State University Museum.

***H_2O CLASS:** Circle 1 category that best describes the hydrological class of the water system you have surveyed.

Lentic = still water (e.g., pond)
Lotic = flowing water (e.g., stream)

*****H_2O TYPE: 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 water's 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.

REL. HUM.: 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 preferred, circle C or F) 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 preferred, circle C or F) 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 one 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 to 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.

*DRY SITE: Circle Y (yes), if the site has no standing or flowing water on the surface. Circle N (no) water is present.

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 amphibians and reptiles that are predators on other herpetofauna 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 following the list derived from Stebbins (1985) or common name. List federal or state sensitive species of other organismal groups or any other species whose occurrence merits noting by common name. 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., side-blotched lizard 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 (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 historic 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 gartersnakes, lack of dorsal stripe and cranial crests in Arizona toads). For information on diagnostic characters of species, see the references listed in the Survey Protocol or other appropriate diagnostic keys.

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, record the number of egg masses, note the overall size of mass, condition, and stage of embryos in the NOTES or COMMENTS sections.

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) note the presence of disease or deformities.

Attachment. Possible designations of focal, supplementary, and refugium sites (see Table 2).