United States Department of the Interior

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In Reply To:
R2/ES-TE

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Memorandum

To: Field Supervisor, Arizona Ecological Services Field Office, Phoenix, AZ

From: Assistant Regional Director - Ecological Services, Region 2

Subject: Request for Intra-service Consultation: Reintroduction of Tarahumara Frog into South Central Arizona

This responds to your April 15, 2003, letter and Biological Assessment (BA) requesting formal consultation on the reintroduction of the Tarahumara frog into two canyons on the Coronado National Forest, Pima, and Santa Cruz counties, Arizona. Your request for formal consultation was received on April 21, 2003. This document transmits the Service's biological opinion of the proposed reintroduction, and its effects on the threatened Mexican spotted owl, Chiricahua leopard frog, Sonora chub, and critical habitat designated for the chub in accordance with section 7 of the Endangered Species Act (Act) of 1973, as amended (16 U.S.C. 1531 et seq.).

This biological opinion is based on information provided in the April 15, 2003, BA, the Draft Proposal to Re-establish Tarahumara Frogs into Southcentral Arizona, and other information contained in our files. A complete administrative record of this consultation is on file in this office.

Consultation History

A draft proposal to re-establish Tarahumara frogs into south central Arizona was submitted to the U.S. Fish and Wildlife Service (Service) in December 2000 (FWS Agreement No. 1448-20181-99-J827). Formal consultation on this project was requested on April 15, 2003.

Biological Opinion

It is the Service's biological opinion that the reintroduction of the Tarahumara frog in Arizona is not likely to jeopardize the continued existence of any of the species considered, or destroy or adversely modify any designated critical habitat.

Description of Proposed Action

The re-establishment proposal (Field *et al.* 2000) is included as Appendix 1. To summarize, the proposal calls for a cooperative effort among the participants of the Tarahumara Frog Conservation Team (TFCT) to: 1) obtain stock of Tarahumara frogs from Sonora, Mexico, 2) re-establish populations of the frog from imported stock into Big Casa Blanca Canyon, Santa Rita Mountains, and Sycamore Canyon, Pajarito Mountains, 3) monitor the releases and adapt management as needed to ensure the re-establishment is successful, and 4) continue coordination through the TFCT to ensure all issues and concerns are addressed. The TFCT, which was first convened in 1991, consists of representatives from Arizona Game and Fish Department (AGFD), Service's Arizona Ecological Services Field Office, Coronado National Forest, Arizona-Sonora Desert Museum (ASDM), U.S. Geological Survey - Biological Resources Division, University of Arizona, and other interested parties. Meetings have been scheduled to coordinate the re-establishment project with livestock grazing permittees on the Coronado National Forest that hold grazing leases for the areas proposed for release. If approved by the AGFD, the TFCT would guide implementation of the re-establishment program.

Biology and Status of the Tarahumara Frog

The Tarahumara frog (*Rana tarahumarae*), is a medium-sized (adults range from 2.6 to 4.5 inches in snout-urostyle length), drab green-brown frog with small brown to black spots on the body and dark crossbars on the legs. The hind feet are extensively webbed. This species lacks a distinct dorsolateral fold, characteristic of related leopard frogs. Larvae are greenish-yellow with small dark spots over the dorsum and larger spots on the tail. Larvae grow as large as 3.8 inches prior to metamorphosis. Both sexes, and adults and juveniles, have a call consisting of snores of 0.5-1.5 second in duration, as well as occasional "eeps," "phoots," and other sounds.

Rorabaugh and Hale (in press) reviewed the biology, status, and reasons for decline of the Tarahumara frog. Much of the following discussion is taken from that manuscript. Tarahumara frogs were known from 63 localities in the montane canyons of southern Arizona, south to northern Sinaloa and southwestern Chihuahua, Mexico, (Campbell 1931, Zweifel 1968, Hale *et al.* 1977, 1995, Hale 2001). The range of Tarahumara frogs is centered in the northern Sierra Madre Occidental of Mexico (McCranie and Wilson 1987); however, the eastern and southern distributional limits are not clear. Tarahumara frogs may not occur south of the Sierra Surutato in Sinaloa.

In the United States Tarahumara frogs were known historically from six locales, including three locales from the Santa Rita Mountains and three locales from the Atascosa-Pajarito-Tumacacori Mountains complex, which are located north and west, respectively, of Nogales in Santa Cruz County, Arizona, (Campbell 1931, Zweifel 1968; Hale *et al.* 1977, 1995). Tarahumara frogs have been extirpated from all localities in Arizona. The last observation of Tarahumara frogs in Arizona, and thus in the U.S., was in May 1983 in Big Casa Blanca Canyon in the Santa Rita Mountains. Surveys from May 1998-May 2000 in Sonora yielded Tarahumara frogs at 6 of 11 historical localities and 3 new localities (Hale *et al.* 1998, Hale 2001).

Breeding habitat is located within oak and pine-oak woodland and the Pacific coast tropical area (foothill thornscrub and tropical deciduous forest; Hale and May 1983, McCranie and Wilson 1987). Breeding occurs primarily towards the end of the dry season (April-May), when permanent water is often restricted to springs and "plunge pools" (deep [> 3 feet] pools in bedrock or among boulders) with deep underwater and streamside retreats. Plunge pools in canyons with low mean flows (< 0.2 cubic feet per second) and relatively steep gradients (> 200 feet per 0.6 mile of stream) provide the best breeding sites (Hale and May 1983; Hale 2001). Permanent water is necessary for metamorphosis. At Pena Blanca Spring, Arizona, and Arroyo El Salto, northeastern Sonora, Tarahumara frogs inhabited artificial impoundments (Hale and May 1983, Hale 2001). The presence of hibernacula where frogs can remain moist and protected from predators and freezing temperatures is an important habitat feature (Hale and May 1983), particularly in the northern portion of the species' range or at higher elevation sites. Hibernacula may include moist refugia among rocks and boulders along streams and at plunge pools.

Prey items of Tarahumara frogs are diverse, and include juvenile Sonoran mud turtles (*Kinosternon sonoriense*); Sonora chubs (*Gila ditaenia*); snakes, including black headed snakes (*Tantilla atriceps*); beetles (including Tenebrionidae, Scarabaeidae, and Buprestidae); moths (Leptodoptera); water bugs (Belostomatidae); scorpions (Scorpionida); centipedes (Chilopoda); grasshoppers (Agrididae); mantids (Mantidae); wasps (Hymenoptera); spiders (Lycosidae); crickets (Gryllidae); caddisflies (Tricoptera); and katydids (Tettigoniidae; Zweifel 1955, Hale and May 1983). Both diurnal and nocturnal feeding is indicated based on the activity patterns of prey species. As with most ranid tadpoles, larvae are likely omnivorous with a strong tendency towards algivory. Tadpoles reared in captivity ate spinach, sliced vegetables, fish food, algae, and boiled egg whites (Rorabaugh and Hale, in press).

Hale and Jarchow (1988) list the following possible causal mechanisms in the extirpation of Tarahumara frog populations: 1) winter cold; 2) flooding or severe drought; 3) competition; 4) predation; 5) disease; and 6) heavy metal poisoning. Tarahumara frogs may be excluded from habitats that support large populations of nonnative predators, such as American bullfrogs and fishes (i.e., green sunfish [*Lepomis cyanellus*], largemouth bass [*Micropterus salmoides*]; Hale and May 1983). Hale (2001) suggested predation by a large chub species (*Gila* sp.) may have eliminated Tarahumara frogs from two sites in Sonora.

Metals occur naturally in streamside deposits and may be mobilized by acid precipitation events. Cadmium toxicity is a possible cause of observed Tarahumara frog die-offs in Arroyo La Carabina, Arroyo Pinos Altos, and Arroyo La Colonia in northeastern Sonora, and Big Casa Blanca and Sycamore canyons in Arizona (Hale and Jarchow 1988, Hale *et al.*. 1998). Cadmium is highly toxic due to its propensity to substitute for zinc and/or copper in enzymes (Coombs 1979). Absorption through the skin or ingestion of zinc by frogs may act to reduce cadmium toxicity. Cumulative sedimentation from physical erosion and deposition in drainages likely result in elevated concentrations of cadmium in downstream reaches. Thus, stream headwaters and springs may be important refuges for frogs when toxic conditions exist in downstream reaches (Hale *et al.* 1998).

Die-offs of ranid frogs (Tarahumara frogs, lowland leopard frogs, and Chiricahua leopard frogs) in Sycamore Canyon, Arizona, are similar to die-offs of Chiricahua leopard frogs reported by Scott (1993) in New Mexico. Scott attributed the New Mexico events to "postmetamorphic death syndrome." The die-offs are also consistent with chytridiomycosis, a fungal disease infecting Tarahumara frogs collected during a die-off in Sycamore Canyon in 1974 (T.R. Jones and P.J. Fernandez, personal communication). Chytridiomycosis was confirmed in frogs from Sycamore Canyon, and in the northeastern and east central Sonora at Arroyo La Carabina (1981, 1982), Arroyo el Tigre (1999), Arroyo La Colonia (1982), Arroyo el Trigo (1982), and Arroya el Aguaje (1999). Tarahumara frogs are extirpated from Sycamore Canyon, Arroyo La Carabina, and Arroyo La Colonia; however, they have persisted at Arroyo el Trigo despite the presence of chytridiomycosis. Hale (2001) also presents evidence of population persistence despite chytridiomycosis at Arroyo El Aguaje, Arroyo La Colonia, and Arroyo El Cobre.

Tarahumara frogs are considered an endangered species by the AGFD (1988), and are included on a draft state list of species of concern (Arizona Game and Fish Department 1996). Tarahumara frogs currently have no status under the Federal Endangered Species Act, CITES, or under Mexican law. However, under the Endangered Species Act, the frog was a category 2 candidate from 1982-1985, a category 1 candidate from 1985-1994, and then was reclassified again as category 2 from 1994-1996. A team of state, Federal, and other partners are working to re-establish this species back into suitable habitats in Arizona. A re-establishment plan, awaiting approval by the Arizona Game and Fish Commission (AGFC), proposes to re-establish Tarahumara frogs into Sycamore and Big Casa Blanca Canyons (Field *et al.* 2000; Rorabaugh and Humphrey 2002).

The three primary elements of the reintroduction plan are described briefly here:

1. Obtain Stock from Sonora:

A portion of an egg mass was collected by S. Hale in May 2000 from the Sierra la Madera in northern Sonora, which is the closest known Tarahumara frog population to Arizona. These eggs were hatched and reared into frogs. Tarahumara frogs from this egg mass are now housed at the ASDM, San Bernardino and Kofa National Wildlife Refuges, and other locales. The Arizona ES Field Office funded the construction of substantial breeding/propagation facilities at the ASDM. The frogs are now breeding at the ASDM. These frogs will be the basis for initial experimental re-establishment of the species to Arizona. Additional collections and importation of Tarahumara frogs will be needed to diversify the genetic stock. We are working with our partners at the Instituto del Medio y El Desarrollo Sustentable del Estado de Sonora (IMADES) in Hermosillo, Sonora, to arrange additional importation of frogs.

2. Release Frogs:

Initial releases would occur at Big Casa Blanca Canyon in suitable microsites. Once established there, re-establishment would be considered at Sycamore Canyon. These two sites were the stronghold of the species before its extirpation in 1983. Habitats are still intact in these canyons, and we believe they may be the best sites for re-establishment. The species appears to be quite

sensitive to chytridiomycosis (V. Miera, Arizona State University, pers. comm. 2002) and chytrids are known to be present in Chiricahua leopard frogs in Sycamore Canyon. No ranid frogs are currently known from Big Casa Blanca Canyon (although Chiricahua leopard frogs occurred there historically). As a result, chytrids may be absent from Big Casa Blanca Canyon, and re-establishment of Tarahumara frogs there may have a greater chance of success. If approved by the AGFC, releases could begin in 2003.

3. Monitoring and Adaptive Management:

Both dynamics of Tarahumara frog populations and the quality of habitats to which they were released would be monitored. Establishment and subsequent reproduction of the founding population should be monitored over at least a 3-year period. Visual encounter surveys will be conducted to monitor frogs, and released individuals will receive a cohort toe-clip so that the date of release of any recaptured frogs can be determined. Frogs would be monitored every other day for the first week following release, then weekly during the first month, and once a month thereafter. The habitats of the release sites would also be monitored. These monitoring data would be used to adjust management, subsequent releases, and release/monitoring protocols to maximize the likelihood of success. Additional monitoring protocols are detailed in Figure 2 of the attached re-establishment proposal.

All aspects of the re-establishment program will be coordinated through the TFCT, which will meet a minimum of twice annually. The diverse backgrounds and expertise within the TFCT make the team's input essential to the success of the project. Especially important will be TFCT input in regard to analysis of monitoring data and recommendations for adaptive management.

Action Area

The action area includes all areas affected directly or indirectly by the Federal action, and not merely the immediate area involved in the action. Areas affected by re-establishment of the Tarahumara frog include stream reaches to which the frogs will be released, areas to which they may disperse, and areas of increased human activity due to monitoring or other re-establishment activities. Field *et al.* (2000) do not specifically describe locations of microsites at which releases will occur; however, they would likely take place in the best habitats in Big Casa Blanca and Sycamore Canyons, and if numbers of frogs allowed, in less optimal habitats. In Big Casa Blanca Canyon, the releases would probably begin in the Bathtub area, where Hale and May (1983) found the species in abundance during 1974-1977. However, during that study, frogs were commonly found in Big Casa Blanca Canyon from 4,890 to 5,610 feet elevation and occasionally from elevation 6,400 to 6,790 feet, and as low as 4,660 feet. Frogs were also found occasionally from 4,890 to 5,090 feet elevation in Walker Canyon, a tributary to Big Casa Blanca Canyon. If re-establishment is successful, Tarahumara frogs would be expected to occupy all of these reaches.

Although Tarahumara frogs are highly aquatic and probably less mobile than leopard frogs, they could potentially disperse to adjacent suitable habitats from Big Casa Blanca Canyon. In August 1974, Tarahumara frogs were found at a bedrock plunge pool in Adobe Canyon, which is

to the east and north of Big Casa Blanca Canyon. However, they were not located there again, and Hale and May (1983) considered the habitat quality there too poor to support a permanent population. To the north of Adobe Canyon is Gardner Canyon, which supports poor to moderate plunge pool Tarahumara frog habitat. Until 1977, Tarahumara frogs were occasionally observed or collected from 5,800 to 6,460 feet (Hale and May 1983) in that canyon above Tunnel Spring. If established in Big Casa Blanca Canyon, Tarahumara frogs could potentially disperse to this reach of Gardner Canyon.

Tarahumara frogs occurred historically in Sycamore Canyon from the Hank and Yank Spring box south of Ruby Road for at least 1.8 mile downstream and also in the first 0.6 mile or more upstream in Penasco Canyon from its confluence with Sycamore Canyon. Hale and May (1983) found that about 1.8-2.5 miles of suitable habitat exist in the 6.2 miles of canyon from Hank and Yank Spring to the international boundary. They also hypothesized that Tarahumara frogs might have used Tonto Canyon (which joins Sycamore Canyon just south of the International Boundary) to disperse between Sycamore and Alamo Canyons (a population occurred historically in Alamo Canyon near Alamo Spring). However, the habitat for Tarahumara frogs, and ranid frogs in general, is poor in Tonto Canyon, and no Tarahumara frogs or leopard frogs have been found in Tonto Canyon.

Because of the aquatic nature of the Tarahumara frog and its subsequent low dispersal potential, they are probably unlikely to disperse in the foreseeable future outside of the Big Casa Blanca/ Walker Canyon area from 4,660-6,790 feet, nor outside of Sycamore Canyon below Hank and Yank Spring and Penasco Canyon for its first 0.6 mile or so above its confluence with Sycamore Canyon. However, if the re-establishments are successful, at a minimum, monitoring activities would occur in these areas plus reaches of Gardner and Adobe canyons adjacent to Big Casa Blanca Canyon where the species occurred historically, and Tonto Canyon, west of Sycamore Canyon. These canyons and stream reaches are considered the action area.

Conservation Measures

Because the proposed action may affect the Mexican spotted owl, Chiricahua leopard frog, and Sonora chub, the following measures will be implemented as part of the proposed action to minimize any potential effects to those listed species:

--- No release or monitoring activities will occur within 0.25 mile of any active Mexican spotted owl nest during the breeding season (March 1-August 31).
--- No camping will occur within Mexican spotted owl Protected Activity Centers (PACs).
--- During any backcountry camping needed during releases or monitoring, to minimize fire risk, no camp fires will occur. Field workers will not smoke while conducting field work.
--- All monitoring work that may result in dip-netting or other forms of take of Chiricahua leopard frogs and Sonora chub will be conducted under the Service and AGFD permits, and will conform to all conditions of those permits. Any Sonora

chub or Chiricahua leopard frogs unintentionally dip-netted will promptly be returned to the water unharmed.

--- Any trespass cattle observed in the Goodding Research Natural Area (Sycamore Canyon) will be promptly reported to the Nogales Ranger District of the Coronado National Forest.

--- All field work shall conform to amphibian disease prevention protocols in the survey protocol for the Chiricahua leopard frog.

--- A pre-release health screening is being developed by the Phoenix Zoo and will be used to screen Tarahumara frogs for diseases prior to release. This will minimize the likelihood that disease or parasites will be introduced via Tarahumara frog reestablishment.

Affected Species

Mexican Spotted Owl

Status of Species

The Mexican spotted owl was listed as a threatened species in 1993 (U.S. Fish and Wildlife Service 1993). The primary threats to the species were cited as even-aged timber harvest and the threat of catastrophic wildfire, although grazing, recreation, and other land uses were also mentioned as possible factors influencing the Mexican spotted owl population. The Service appointed the Mexican Spotted Owl Recovery Team in 1993, which produced the Recovery Plan for the Mexican Spotted Owl (Recovery Plan) in 1995 (U.S. Fish and Wildlife Service 1995).

A detailed account of the taxonomy, biology, and reproductive characteristics of the Mexican spotted owl is found in the final rule listing the Mexican spotted owl as a threatened species (U.S. Fish and Wildlife Service 1993) and in the Recovery Plan (U.S. Fish and Wildlife Service 1995). The information provided in those documents is included herein by reference. Although the Mexican spotted owl's entire range covers a broad area of the southwestern United States and Mexico, the Mexican spotted owl does not occur uniformly throughout its range. Instead, it occurs in disjunct localities that correspond to isolated forested mountain systems, canyons, and in some cases steep, rocky canyon lands. Surveys have revealed that the species has an affinity for older, well-structured forest, and the species is known to inhabit a physically diverse landscape in the southwestern United States and Mexico.

The U.S. range of the Mexican spotted owl has been divided into six recovery units (RU), as discussed in the Recovery Plan. The primary administrator of lands supporting the Mexican spotted owl in the United States is the Forest Service. Most owls have been found within Forest Service Region 3 (including 11 National Forests in Arizona and New Mexico). Forest Service Regions 2 and 4 (including 2 National Forests in Colorado and 3 in Utah) support fewer owls. According to the Recovery Plan, 91 percent of Mexican spotted owls known to have existed in the United States between 1990 and 1993 occurred on lands administered by the Forest Service.

A reliable estimate of the numbers of owls throughout its entire range is not currently available (U.S. Fish and Wildlife Service 1995) and the quality and quantity of information regarding numbers of Mexican spotted owls vary by source. U.S. Fish and Wildlife Service (1991) reported a total of 2,160 owls throughout the United States. Fletcher (1990) calculated that 2,074 owls existed in Arizona and New Mexico. However, Ganey *et al.* (2000) estimates approximately 2,950 ± 1,067 (SE) Mexican spotted owls in the Upper Gila Mountains RU alone. The Forest Service Region 3 most recently reported a total of approximately 980 protected activity centers (PACs) established on National Forest lands in Arizona and New Mexico (USDA Forest Service, Southwestern Region, December 19, 2002). Based on this number of Mexican spotted owl sites, total numbers in the United States may range from 980 individuals, assuming each known site was occupied by a single Mexican spotted owl, to 1,960 individuals, assuming each known site was occupied by a pair of Mexican spotted owls. The Forest Service Region 3 data are the most current compiled information available to us; however, survey efforts in areas other than National Forest system lands have likely resulted in additional sites being located in all Recovery Units. Currently, we estimate that there are likely 12 PACs in Colorado (not all currently designated) and 105 PACs in Utah.

Since the owl was listed, we have completed a total of 100 formal consultations for the Mexican spotted owl. These formal consultations have identified incidences of anticipated incidental take (harm or harassment) of Mexican spotted owl in 282 PACs. These consultations have primarily dealt with actions proposed by the Forest Service, Region 3. However, in addition to actions proposed by the Forest Service, Region 3, we have also reviewed the impacts of actions proposed by the Bureau of Indian Affairs, Department of Defense (including Air Force, Army, and Navy), Department of Energy, National Park Service, and Federal Highway Administration. These proposals have included timber sales, road construction, fire/ecosystem management projects (including prescribed natural and management ignited fires), livestock grazing, recreation activities, utility corridors, military and sightseeing overflights, and other activities.

In 1996, the Service issued a biological opinion on Forest Service Region 3's adoption of the Recovery Plan recommendations through an amendment of their Forest Plans. In this non-jeopardy biological opinion, we anticipated that approximately 151 PACs would be affected by activities that would result in incidental take of Mexican spotted owls, with 26 of those PACs located in the Basin and Range West RU. To date, consultation on individual actions under the amended Forest Plans have resulted in 200 PACs adversely affected, with 68 of those in the Basin and Range West RU.

Environmental Baseline

The Mexican spotted owl occurs in the action area in three PACs, including the Sycamore Canyon PAC (050209, Figure 1), Big Casa Blanca PAC (050210, Figure 2), and Baldy Springs PAC (050203, Figure 3). The Sycamore Canyon PAC extends along Sycamore Canyon and its tributaries. Release and monitoring of Tarahumara frogs will occur throughout the reach of Sycamore Canyon through the PAC. Tributaries in the PAC may be periodically explored by monitors. The Big Casa Blanca PAC includes the upper reaches of Big Casa Blanca Canyon above 5,960 feet. In that canyon, Tarahumara frogs occurred most commonly historically from

4,890 to 5,610 feet elevation, below the PAC, but were also found occasionally from elevation 6,400 to 6,790 feet within the PAC. Releases and potentially monitoring would occur along Big Casa Blanca Canyon in the PAC at least to elevation 6,790 feet. Baldy Spring PAC lies at and to the east of Mount Wrightson, including a portion of Gardner Canyon above 6,440 feet elevation, which begins about 1.1 mile west of Tunnel Spring. No releases of Tarahumara frogs are planned in Gardner Canyon; however, Tarahumara frogs were occasionally observed or collected historically from that canyon between elevation 5,800 to 6,460 feet. If the introductions are successful, frogs could invade their former habitats in Gardner Canyon, and monitoring for Tarahumara frogs in the lower reaches of Gardner Canyon within the PAC would occur.

No occupancy information is available for 2001 or 2002, but before that time owls were usually present when the PACs were monitored. However, owls were found only once during monitoring from 1996-2000 in the Sycamore Canyon PAC. Owls were last detected in all three PACs in 1999. None of the birds in the three PACs is known to have nested successfully since 1994, when two young were produced in the Big Casa Blanca PAC. Based on prior occupancy it is likely that owls will be present in one or more of the PACs during releases and monitoring of Tarahumara frogs. Birds are most likely to occur in the Baldy Spring and Big Casa Blanca PACs, which have consistently yielded birds during monitoring.

Effects of the Action

The only likely effect of the reestablishment program on the Mexican spotted owl is disturbance of owls due to human activities associated with the project, including carrying and releasing frogs/tadpoles into the canyons, and subsequent monitoring of the releases. The effects of such activities should be similar to the effects of recreational hikers, backpackers, and others walking through these canyons.

The response of wildlife to recreational disturbance is complex, and the effects are not immediately obvious or easily determined (Hammitt and Cole 1987; Flather and Cordell 1995). Evidence suggests that recreational activity can harm wildlife (Knight and Cole 1995). Tolerance levels for wildlife interactions with humans will vary by time of year, breeding season, age, habitat type, and individual experience with recreationists (Hammitt and Cole 1987). Human activities can impact wildlife directly through exploitation and disturbance, or indirectly through habitat modification and pollution. The Mexican spotted owl Recovery Plan indicates that the determining factor of a recreational activity's impact on spotted owls is a combination of its location, intensity, frequency, and duration.

Research on all subspecies of the spotted owl indicates that it exhibits docile behavior when approached by researchers, and there is no clear evidence of significant impact by research activity except for a negative effect on reproduction from back-pack radio transmitters (Gutierrez *et al.* 1995). However, Swarthout and Steidl (2003) found that high levels of short-duration recreational hiking near nests may be detrimental to MSO. Researchers purposefully make as little noise as possible, and disturbance is very limited in duration. In the long-term, some species may habituate to human disturbance if they are not deliberately harassed; others may become very stress-prone towards humans (Hammitt and Cole 1987, Bowles 1995, Steidl

and Anthony 2000). The physical characteristics of a canyon may assist in providing topographic screening. Topographic screening between the area of disturbance and the birds' location creates a noise buffer, and may assist in the reduction of noise disturbance (Knight and Cole 1995). But, the physical structure of canyons can also tend to magnify disturbances and limit escape/avoidance routes for owls (U.S. Fish and Wildlife Service 1995). Swarthout and Steidl (2003) found that female Mexican spotted owls with nests high above hiking trails altered their behavior more than those with lower nests, suggesting that a higher vantage point corresponded to heightened levels of hiker disturbance, possibly due to longer times during which hikers are visible or detectable.

There are three learned responses wildlife may show to recreationists: habituation, attraction, and avoidance (Knight and Cole 1995). Recreational disturbance during the breeding season may affect an individual's productivity; disturbance outside the breeding season may affect the individual's energy balance and, therefore, its survival. Birds may respond to disturbance during the breeding season by abandoning their nests or young, by altering their behavior such that they are less attentive to the young, which increases the risk of the young being preyed upon, or by disrupting feeding patterns, or by exposing young to adverse environmental stress (Knight and Cole 1995). Sycamore Canyon through the Sycamore Canyon PAC is heavily used by recreationists and undocumented aliens. The owls in this area may have habituated to human use somewhat or adjusted their activity patterns to avoid heavily used areas. However, because of the relatively low level of human visitation in the Big Casa Blanca area, owls in the Big Casa Blanca PAC are probably not as accustomed to human activity and may respond more to visitation than owls in Sycamore Canyon. Owls of the Gardner Canyon PAC may experience intermediate levels of human visitation and habituation. A road leads up Gardner Canyon to near the PAC, and a trail winds its way up Gardner Canyon from the end of the road through the PAC.

Swarthout and Steidl (2001) examined flush response of Mexican spotted owls to recreationists, and found that if hikers are excluded from a 79-foot radius around roost sites, 95 percent of owl flush responses would be eliminated. At national parks in Utah, Swarthout and Steidl (2003) examined behavioral responses of nesting Mexican spotted owls to individual hikers that passed within 36-210 feet of active nests every 15 minutes. Among various behavioral changes observed during treatments, female owls decreased the amount of time they handled prey by 57 percent and decreased the amount of time they performed daytime maintenance behaviors by 30 percent. Males and females increased the frequency of contact vocalizations by 58 and 534 percent, respectively. Female behavior was much more affected than that of males. Swarthout and Steidl (2003) suggest that a 57 percent decrease in prey handling by female owls represents a potentially consequential reduction in energy intake. The sensitivity of females to hikers is especially important because females attend the nests almost exclusively and nestling survival depends largely on female behavior. Predation and starvation of nestlings are the most common sources of nestling mortality (Forsman *et al.* 1984, Ganey *et al.* 1998), thus reduced prey handling time could affect nestling survival (Swarthout and Steidl 2003). Swarthout and Steidl (2003) suggest that restrictions on hiking intensity near nests should be considered on a case by case basis, but should probably only be implemented in canyons with use levels approximating or exceeding the use in their study (>48 hiking groups per day).

Swarthout and Steidl (2003) evaluated effects of diurnal hikers on owl behavior. Some of our frog monitoring work may be conducted at night, which could increase sensitivity of or responses by nesting owls. We have proposed to not conduct any monitoring or releases within 0.25 mile of any nest during the nesting season. However, not all nests are located each year, so our activities could potentially occur near nests that have not been detected by surveys. The greatest activity will occur during releases when teams of workers could potentially hike through a canyon multiple times during a day while carrying groups of tadpoles and frogs; however, use is not expected to exceed about 10 passes by groups of hikers per day. No releases would occur in, nor would hikers carrying frogs and tadpoles pass through, PACs in the Santa Rita Mountains. Monitors will occur over a greater area than releases; including all of the PACs in the action area (Big Casa Blanca, Baldy Springs, and Sycamore Canyon PACs); however, monitors would likely only make two passes by any nest sites (once hiking up a canyon, and once returning). Based on the work by Swarthout and Steidl (2001, 2003), these levels of activity should cause minimal disturbance to nesting owls.

The Recovery Plan states that groups of 12 or more hikers or a steady stream of hikers occurring in narrow canyon bottoms may be especially disturbing to owls. The Mexican spotted owl breeding season, which extends from March 1 through August 31, is a period in which frogs are active, and releases of frogs and frog monitoring would occur. Releases of frogs will likely involve teams of two to four people carrying frogs or tadpoles into suitable habitats. No teams of 12 or more are anticipated.

Trampling of some streamside vegetation will likely occur during releases and monitoring, because field workers, particularly those doing monitoring, will often be off-trail. However, these effects will be short term, and we expect no long-term changes to riparian communities from our work. No campfires will be built and workers will not smoke during releases or monitoring of frogs.

Releases of frogs and much of the monitoring will likely occur during the nesting season of the owl. Thus, there is potential for disruption of nesting activities by project activities. However, in the case of two of the three PACs (Big Casa Blanca Canyon and Baldy Spring) the areas where most releases and monitoring will occur are outside of PACs. With the exception of the Sycamore Canyon PAC, hiking into and exploring stream reaches in the PACs will probably only occur during monitoring, and will not occur frequently - probably not more than twice per year. Monitoring will likely be accomplished by one observer or perhaps a team of two or three. We have committed to not conduct releases or monitoring within 0.25 mile of any active Mexican spotted owl nests during the nesting season. Thus, although it is possible releases or monitoring may occasionally disturb an owl, we believe such encounters will be infrequent, owls are accustomed to such disturbance in Sycamore Canyon, and disturbance near active nest sites will be minimized. We have committed to measures that will eliminate likelihood of a fire caused by our activities, and we expect no long-term impacts to riparian plant communities from periodic field work along streams.

Cumulative Effects

Cumulative effects include the effects of future State, tribal, local, or private actions that are reasonably certain to occur in the action area considered in this biological opinion. Future federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the Act.

The Tarahumara frog was never found in the past on private or other non-forest lands in the Big Casa Blanca or Sycamore canyon areas. Also, the Tarahumara frog is a highly aquatic frog that does not stray far from water. As a result, if the re-establishments are successful, we do not expect Tarahumara frogs to migrate to or colonize any adjacent lands off-forest. In addition, no non-Federal lands occur in the watersheds of former Tarahumara frog habitats in either the Sycamore or Big Casa Blanca canyon areas. Thus, lands in the action area are Federally-owned. Private lands occur about a mile downstream of former habitats in Big Casa Blanca Canyon, but we are not aware of any activities occurring or proposed on these lands that could affect Mexican spotted owl or lesser long-nosed bat habitat in the action area. Landownership suggests that most future activities that could affect listed species and their habitats in the action area will be Federal actions, the effects of which are not considered cumulative.

There are two non-Federal activities that are or have been significant exceptions. The Sycamore Canyon area is a route for undocumented migrants and drug smugglers from Sonora to enter the United States illegally. These individuals leave trash, clothing, and campsites, which probably have a minimal impact on listed species and their habitats. However, in other places of southern Arizona illegal migrants have started wildfires with their campfires that have burned important habitats for listed species. A wildfire in Sycamore Canyon could occur, particularly in the dry months of May or June, and cause ash and sediment runoff into the creek. Ash flow can be toxic to fish, and sediment can fill in pools that Sonora chub and Chiricahua leopard frogs use to survive dry periods in Sycamore Canyon. A wildfire could also burn trees that Mexican spotted owls use for roosting, perching, and as foraging habitat.

As discussed in the biological evaluation, acidic rainfall and subsequent cadmium toxicity in streams of southeastern Arizona and northern Sonora may have occurred as a result of atmospheric emissions from copper smelters at Cananea and Nacozari, northeastern Sonora, and Douglas, Arizona, (Blanchard and Stromberg 1987, Hale *et al.* 1995). Cadmium toxicity may have contributed to the disappearance of the Tarahumara frog from Arizona and die-offs of Chiricahua leopard frogs in Sycamore Canyon (Hale and Jarchow 1988, Hale *et al.* 1998). The smelters at Douglas and Cananea have closed and the smelter at Nacozari now has pollution control devices. For the present, this cumulative effect appears to have abated.

Conclusion

After reviewing the current status of the threatened Mexican spotted owl, the environmental baseline for the action area, the effects of the proposed action, and the cumulative effects, it is the Service's biological opinion that the reintroduction of the Tarahumara frog into Arizona, as proposed, is not likely to jeopardize the continued existence of the Mexican spotted owl. No critical habitat has been designated for this species; therefore, none will be affected.

INCIDENTAL TAKE STATEMENT

Section 9 of the Act and Federal regulation pursuant to section 4(d) of the Act prohibit the take of endangered and threatened species, respectively, without special exemption. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, collect, or to attempt to engage in any such conduct. Harm is further defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering. Harass is defined by the Service as intentional or negligent actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding or sheltering. Incidental take is defined as take that is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity. Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking under the Act provided that such taking is in compliance with the terms and conditions of this incidental take statement.

The Service does not anticipate the proposed action will incidentally take any Mexican spotted owls. This determination is based on the fact that the following conservation measures will be implemented: 1) no release or monitoring activities will occur within 0.25 miles of any active Mexican spotted owl nest during the breeding season (March 1 - August 31), and 2) no camping will occur within Mexican spotted owl PACs. Because there is no take, there are no reasonable or prudent measures provided. If during the course of this action, incidental take occurs, such incidental take would represent new information requiring review of the project effects and consultation must be reinitiated with the Service immediately to avoid violation of section 9.

Conservation Recommendations

Section 7(a)(1) of the Act directs Federal agencies to utilize their authorities to further the purposes of the Act by carrying out conservation programs for the benefit of endangered and threatened species. Conservation recommendations are discretionary agency activities to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, to help implement recovery plans, or develop information.

1. Minimize the number of people hiking within MSO Pacs.
2. Report any juvenile owls observed in the area to the Coronado National Forest.

In order for the Service to be kept informed of actions minimizing or avoiding adverse effects or benefiting listed species or their habitats, the Service requests notification of the implementation of any conservation recommendations.

Chiricahua Leopard Frog

Status of Species

The Chiricahua leopard frog (*Rana chiricahuensis*) was listed as a threatened species in 2002 (U.S. Fish and Wildlife Service 2002). Included was a special rule to exempt operation and maintenance of livestock tanks on non-Federal lands from the section 9 take prohibitions of the Act. The frog is distinguished from other members of the *Rana pipiens* complex by a combination of characters, including a distinctive pattern on the rear of the thigh consisting of small, raised, cream-colored spots or tubercles on a dark background; dorsolateral folds that are interrupted and deflected medially; stocky body proportions; relatively rough skin on the back and sides; and often green coloration on the head and back (Platz and Mecham 1979). The species also has a distinctive call consisting of a relatively long snore of 1-2 seconds in duration (Davidson 1996, Platz and Mecham 1979). Snout-vent lengths of adults range from approximately 2.1 to 5.4 inches (Stebbins 2003, Platz and Mecham 1979). The Ramsey Canyon leopard frog *(Rana subaquavocalis*) is similar in appearance to the Chiricahua leopard frog, but it may grow to a larger size and has a distinct call that is often given under water (Platz 1993).

The Chiricahua leopard frog is an inhabitant of cienegas, pools, livestock tanks, lakes, reservoirs, streams, and rivers at elevations of 3,281 to 8,890 feet in central and southeastern Arizona; west central and southwestern New Mexico; and northern Sonora, the Sierra Madre Occidental of Chihuahua, and northern Durango, Mexico, (Platz and Mecham 1984, Jennings and Scott 1993, Degenhardt *et al.* 1996, Sredl *et al.* 1997, Sredl and Jennings *in press*). Reports of the species from the State of Aguascalientes (Diaz and Diaz 1997) are questionable; however, the distribution of the species in Mexico is unclear due to limited survey work and the presence of closely related taxa (especially *Rana montezumae*) in the southern part of the range of the Chiricahua leopard frog. In New Mexico, of sites occupied by Chiricahua leopard frogs from 1994-1999, 67 percent were creeks or rivers, 17 percent were springs or spring runs, and 12 percent were stock tanks (Painter 2000). In Arizona, slightly more than half of all known historical localities are natural lotic systems, a little less than half are stock tanks, and the remainder are lakes and reservoirs (Sredl *et al.* 1997). Sixty-three percent of populations extant in Arizona from 1993-1996 were found in stock tanks (Sredl and Saylor 1998).

Northern populations of the Chiricahua leopard frog along the Mogollon Rim and in the mountains of west central New Mexico are disjunct from those in southeastern Arizona, southwestern New Mexico, and Mexico. Recent genetic analyses, including a 50-loci starch gel survey, morphometrics, and analyses of nuclear DNA support describing the northern populations as a distinct species (Platz and Grudzien 1999). Multiple haplotypes within *chiricahuensis* were also identified using mitochondrial DNA analysis (Benedict and Quinn 1999), providing further evidence of genetically distinct population segments.

Die-offs of Chiricahua leopard frogs were first noted in former habitats of the Tarahumara frog (*Rana tarahumarae*) in Arizona at Sycamore Canyon in the Pajarito Mountains (1974) and Gardner Canyon in the Santa Rita Mountains (1977-78) (Hale and May 1983). From 1983-1987, Clarkson and Rorabaugh (1989) found Chiricahua leopard frogs at only 2 of the 36 Arizona

localities that had supported the species in the 1960s and 1970s. Two new populations were reported. During subsequent extensive surveys from 1994-2001, the Chiricahua leopard frog was found at 87 sites in Arizona, including 21 northern localities and 66 southern localities (Sredl *et al.* 1997, Rosen *et al.* 1996, Service files). In New Mexico, the species was found at 41 sites from 1994-1999; 31 of those were verified extant during 1998-1999 (Painter 2000). During May-August 2000, the Chiricahua leopard frog was found extant at only 8 of the 34 sites where the species occurred in New Mexico during 1994-1999 (C. Painter, pers. comm. 2000). The species has been extirpated from about 75 percent of its historical localities in Arizona and New Mexico. The status of the species in Mexico is unknown.

Based on Painter (2000) and the latest information for Arizona, the species is still extant in most major drainages in Arizona and New Mexico where it occurred historically; with the exception of the Little Colorado River drainage in Arizona and possibly the Yaqui drainage in New Mexico. It has also not been found recently in many rivers, valleys, and mountains ranges, including the following in Arizona: White River, West Clear Creek, Tonto Creek, Verde River mainstem, San Francisco River, San Carlos River, upper San Pedro River mainstem, Santa Cruz River mainstem, Aravaipa Creek, Babocomari River mainstem, and Sonoita Creek mainstem. In southeastern Arizona, no recent records (1995 to the present) exist for the following mountain ranges or valleys: Pinaleno Mountains, Peloncillo Mountains, Sulphur Springs Valley, and Huachuca Mountains. Moreover, the species is now absent from all but one of the southeastern Arizona valley bottom cienega complexes. In many of these regions Chiricahua leopard frogs were not found for a decade or more despite repeated surveys. Recent surveys suggest the species may have recently disappeared from some major drainages in New Mexico (C. Painter, pers. comm. 2000).

Threats to this species include predation by nonnative organisms, especially bullfrogs, fish, and crayfish; disease; drought; floods; degradation and loss of habitat as a result of water diversions and groundwater pumping, poor livestock management, a history of fire suppression and grazing that has increased the likelihood of crown fires, mining, development, and environmental contamination; disruption of metapopulation dynamics; and increased chance of extirpation or extinction resulting from small numbers of populations. Loss of Chiricahua leopard frog populations is part of a pattern of global amphibian decline, suggesting other regional or global causes of decline may be important as well (Carey *et al.* 2001). Numerous studies indicate that declines and extirpations of Chiricahua leopard frogs are at least in part caused by predation and possibly competition with nonnative organisms, including fish in the family Centrarchidae (*Micropterus* spp., *Lepomis* spp.), bullfrogs (*Rana catesbeiana*), tiger salamanders (*Ambystoma tigrinum mavortium*), crayfish (*Orconectes virilis* and possibly others), and several other species of fish (Fernandez and Rosen 1998, 1996; Rosen *et al.* 1996; 1994; Snyder *et al.* 1996; Fernandez and Bagnara 1995; Sredl and Howland 1994; Clarkson and Rorabaugh 1989). For instance, in the Chiricahua region of southeastern Arizona, Rosen *et al.* (1996) found that almost all perennial waters investigated that lacked introduced predatory vertebrates supported Chiricahua leopard frogs. All waters except three that supported introduced vertebrate predators lacked Chiricahua leopard frogs. Sredl and Howland (1994) noted that Chiricahua leopard frogs were nearly always absent from sites supporting bullfrogs and nonnative predatory fish.

Rosen *et al.* (1996) suggested further study was needed to evaluate the effects of mosquitofish, trout, and catfish on frog presence.

Disruption of metapopulation dynamics is likely an important factor in regional loss of populations (Sredl *et al.* 1997, Sredl and Howland 1994). Chiricahua leopard frog populations are often small and habitats are dynamic, resulting in a relatively low probability of long-term population persistence. Historically, populations were more numerous and closer together. If populations winked out because of drought, disease, or other causes, extirpated sites could be recolonized via immigration from nearby populations. However, as numbers of populations declined, populations became more isolated and were less likely to be recolonized if extirpation occurred. Also, most of the larger source populations along major rivers and at cienega complexes have disappeared.

Recent evidence suggests a chytridiomycete skin fungi is responsible for observed declines of frogs, toads, and salamanders in portions of Central America (Panama and Costa Rica), South America (Atlantic coast of Brazil, Ecuador, and Uruguay), Australia (eastern and western States), New Zealand (South Island), Europe (Spain and Germany), Africa (South Africa, "western Africa", and Kenya), Mexico (Sonora), and United States (eight States) (Speare and Berger 2000, Longcore *et al.* 1999, Berger *et al.* 1998, Hale 2001). Ninety-four species of amphibians have been diagnosed as infected with the chytrid, *Batrachochytrium dendrobatidis*. The proximal cause of extinctions of two species of Australian gastric brooding frogs and the golden toad (*Bufo periglenes*) in Costa Rica was likely chytridiomycosis. Another species in Australia for which individuals were diagnosed with the disease may be extinct (Daszak 2000). In Arizona, chytrid infections have been reported from four populations of Chiricahua leopard frogs (M. Sredl, pers. comm. 2000), as well as populations of Rio Grande leopard frog (*Rana berlandieri*), Plains leopard frog (*Rana blairi*), lowland leopard frog (*Rana yavapaiensis*), Tarahumara frog (*Rana tarahumarae*), bullfrog, canyon treefrog (*Hyla arenicolor*), and Sonora tiger salamander (*Ambystoma tigrinum stebbinsi*) (Bradley *et al.* 2002, Hale 2001, Davidson *et al.* 2000, Sredl and Caldwell 2000, Morell 1999). In New Mexico, chytridiomycosis was identified in a declining population near Hurley, and recent patterns of decline at three other populations are consistent with chytridiomycosis (R. Jennings, pers. comm. 2000). Die-offs occur during the cooler months from October-February. High temperatures during the summer may slow reproduction of chytrids to a point at which the organism cannot cause disease (Bradley *et al.* 2002).

The role of the fungi in the population dynamics of the Chiricahua leopard frog is as yet undefined. It is clear that Chiricahua leopard frog populations can exist with the disease for extended periods. The frog has coexisted with chytridiomycosis in Sycamore Canyon, Arizona, since at least 1974. However, at a minimum, it is an additional stressor, resulting in periodic die-offs that increase the likelihood of extirpation and extinction. It may well prove to be an important contributing factor in observed population decline, and because of the interchange of individuals among subpopulations, metapopulations of frogs may be particularly susceptible. Rapid death of all or most frogs in stock tank populations in a metapopulation of Chiricahua leopard frogs in Grant County, New Mexico, was attributed to post-metamorphic death syndrome (Declining Amphibian Populations Task Force 1993). Hale and May (1983) and Hale

and Jarchow (1988) believed toxic airborne emissions from copper smelters killed Tarahumara frogs and Chiricahua leopard frogs in Arizona and Sonora. However in both cases, symptoms of moribund frogs matched those of chytridiomycosis. The disease has now been documented to have been associated with Tarahumara frog die-offs since 1974 (Hale 2001). The earliest record for chytridiomycosis in Arizona (Tarahumara frog -1974) corresponds to the first observed mass die-offs of ranid frogs in Arizona.

Additional information about the Chiricahua leopard frog can be found in Painter (2000), Sredl *et al.* (1997), Degenhardt *et al.* (1996), Jennings (1995), Rosen *et al.* (1996, 1994), Sredl and Howland (1994), Jennings and Scott (1993), Platz and Mecham (1984, 1979), and Sredl and Jennings (*in press*).

Environmental Baseline

Within the action area, Chiricahua leopard frogs are currently known to occur at Sycamore Canyon from Hank and Yank Spring probably nearly to the international boundary (although they are often most common in the first mile or two downstream of the spring). A population of frogs occurs at a livestock tank, Hank and Yank Tank, in a tributary of Sycamore Canyon to the east of Hank and Yank Spring, which is probably important in contributing frogs to Sycamore Canyon. In the vicinity of Big Casa Blanca Canyon in the Santa Rita Mountains, Chiricahua leopard frogs are known to occur in Gardner and adjacent Cave and Fish canyons about 2 miles downstream of Tunnel Spring. Chiricahua leopard frogs occurred at one time in Big Casa Blanca and Adobe canyons, but have not been detected there since 1979. The extant populations in Sycamore Canyon and Gardner/Cave/Fish canyons inhabit stream courses and associated livestock tanks.

The population in Sycamore Canyon has coexisted with chytridiomycosis since at least 1974. On April 7, 1974, numerous dead Tarahumara frogs and leopard frogs were found in the stream from 0.6-1.9 miles below Hank and Yank Spring. The skin on top of the head of some individuals was dry and two leopard frogs found alive showed no escape movements and did not swim far or dive when released. However, no sick or dead frogs were observed in August of the same year. Tarahumara frogs were not observed after 1974 in Sycamore Canyon. Abundance and distribution of leopard frog populations after 1974 appeared to fluctuate and were often restricted to, or were most often observed in, the Hank and Yank Spring area (Hale and May 1983). As discussed in the Status of the Species - Chiricahua leopard frog, and Biology and Status of the Tarahumara Frog, above, Hale and May (1983) and Hale and Jarchow (1988) provide evidence that cadmium toxicity due to airborne emissions from copper smelters or leached from mine tailings may have caused the observed die-offs. However, frogs were also symptomatic for chytridiomycosis, and recent analysis of Tarahumara frogs collected during the die-off in 1974 and Chiricahua leopard frogs collected in the 1990s have confirmed that chytridiomycosis was present in both instances and may have been the proximate cause of the die-offs (Hale 2001; M. Sredl, pers. comm. 2002). However, cadmium toxicity or other stressors may have also contributed to the declines by compromising the immune system of the frogs (Carey *et al.* 2001, Daszak 2000, Pounds and Crump 1994).

Chytridiomycosis is not known from the Santa Rita Mountains. The last Tarahumara frog observed in the Santa Rita Mountains and in Arizona was in the Bathtub area of Big Casa Blanca Canyon on May 28, 1983. No die-offs of Tarahumara frogs or leopard frogs were observed in the Santa Rita Mountains; however, Hale and May (1983) suggest a flood in October 1977 that scoured Big Casa Blanca Canyon may have eliminated the entire metamorphosed populations of both Tarahumara frogs and leopard frogs. Leopard frogs also declined in Gardner Canyon at the same time (Hale and May 1983). Hale and May (1983) go on to say that, "the flood in Big Casa Blanca Canyon may have been the death blow to a population already affected by the same agent responsible for other declines," suggesting chytrids or contaminants may have played a role in the disappearance of ranid frogs from Big Casa Blanca and Adobe canyons. Neither leopard frogs nor Tarahumara frogs currently exist in Big Casa Blanca Canyon. If chytrids contributed to loss of frog populations, it is unknown whether chytrids are still present or can persist in the absence of anuran hosts.

Big Casa Blanca Canyon receives little visitor use. A trail leads from Tunnel Spring to the upper part of the canyon, but the lower reaches where Tarahumara frogs were most abundant, including the Bathtub area, are not on a trail. No trails exist from the lower canyon into former Tarahumara frog habitat due to the presence of a private inholding. Sycamore Canyon, particularly the first mile or so below Ruby Road, receives considerable public use in the form of hikers and bathers (many people swim in a large tinaja in the first mile below Ruby Road).

Big Casa Blanca and Walker canyons in the Santa Rita Mountains are part of the Temporal grazing allotment, Adobe Canyon is in the Fort allotment, and Gardner Canyon is in the Gardner Canyon allotment on the Coronado National Forest. Cattle may not be able to physically access the Bathtubs due to rough terrain. In nearly 30 years of visiting the canyon, S. Hale (pers. comm. 2000) has never observed heavy grazing in the canyon, or other significant effects of livestock grazing on frog habitats. Sycamore Canyon is part of the Bear Canyon allotment, but cattle are excluded from that portion of the canyon that is in the action area. All of these allotments were the subject of recent formal section 7 consultation with the Coronado National Forest (consultation number 02-21-98-F-399R1, October 24, 2002). Livestock grazing in these allotments was determined to not likely jeopardize the continued existence of any listed species or result in adverse modification or destruction of critical habitat.

Effects of the Action

Chiricahua leopard frogs currently overlap areas to which Tarahumara frogs will be released or are likely to immigrate only in Sycamore Canyon and the first 0.6 mile or so of Penasco Canyon upstream of its confluence with Sycamore Canyon. If the Chiricahua leopard frog expanded its current distribution in the Santa Rita Mountains to areas it occupied historically, it could also potentially occur with Tarahumara frogs in portions of Big Casa Blanca, Walker, Adobe, and Gardner canyons. Within these areas, Tarahumara frogs are more likely to be found in plunge pool habitats, rather than along stream courses. Chiricahua leopard frogs could occur in either habitats. Where the species occur together, they may compete and prey upon each other. Although neither the Chiricahua leopard frog nor the Tarahuamara frog have been documented eating other frogs, both eat a wide variety of prey items (Sredl and Jennings *in press*, Rorabaugh

and Hale *in press*) and adult frogs would likely eat smaller frogs. Thus, reestablishment of Tarahumara frogs could result in elevated predation of metamorph or juvenile Chiricahua leopard frogs, and their tadpoles. On the other hand, small Tarahumara frogs would provide additional forage for Chiricahua leopard frogs. Tadpoles of these species are primarily herbivorous (Sredl and Jennings *in press*, Rorabaugh and Hale *in press*), thus tadpoles may compete for forage resources, if such resources are limiting. Although these effects between the frogs species may occur, Chiricahua leopard frogs and Tarahumara frogs occurred together historically in Arizona, and we have no reason to believe they could not again both maintain populations in the areas of Sycamore and Big Casa Blanca canyons.

Monitoring of Tarahumara frogs is likely to result in occasional dip-netting or flushing of Chiricahua leopard frogs. Tarahumara and Chiricahua leopard frogs will probably jump into the water as field workers walk along banklines searching for frogs. Some potential exists for tadpoles and frogs to be injured or killed during dip-netting; however, we have dip-netted many leopard frogs and tadpoles without any apparent capture-related injury or mortality. As part of our proposed action, any Chiricahua leopard frogs or tadpoles unintentionally dip-netted will promptly be returned to the water unharmed. We do not anticipate adverse effects to frogs that jump into the water in response to periodic monitoring and frog releases. Dip-netting of Chiricahua leopard frogs and tadpoles will only occur if permitted by a section 10(a)(1)(A) permit from the Service. Any such dip-netting would occur in compliance with any terms and conditions of that permit.

Rearing of Tarahumara frogs and tadpoles in captivity and then moving these animals to the wild could potentially introduce novel diseases or parasites to the reestablishment sites. These diseases or parasites could potentially be imported with animals from Sonora, or could be spread to animals in the rearing facilities or during transport. Chiricahua leopard frogs rescued from a drying stock tank on the Coconino National Forest and taken to the ASDM that appeared healthy when captured later became symptomatic for chytridiomycosis and also had trematode infections (C. Ivanyi, pers. comm. 2003). If novel diseases were introduced to Chiricahua leopard frog populations, the results could range from virtually no effect to a dramatic population decline. For example, the proximate cause of some extirpations of Chiricahua leopard frog populations in New Mexico is thought to be chytridiomycosis (R. Jennings, pers. comm. 2001). At this time the only virulent disease known to be associated with die-offs of Chiricahua leopard frogs is chytridiomycosis. Chytridiomycosis is known to occur in Sycamore Canyon, thus unintentional introduction of this pathogen there should have no effect. Its presence in Big Casa Blanca Canyon and adjacent canyons is unknown. However, Tarahumara frogs will receive a disease screening prior to release. Disease prevention techniques may include use of a fungicide to reduce the likelihood of spreading chytridiomycosis. No diseased animals will knowingly be released (Field *et al.* 2000); however, there is still some potential of releasing diseased animals that could then affect Chiricahua leopard frogs. In the laboratory, Tarahumara frogs are very sensitive to chytridiomycosis and rapidly succumb to the disease (V. Miera, pers. comm. 2002). Thus, the likelihood of not detecting Tarahumara frogs with chytridiomycosis during disease screening is reduced. If chytridiomycosis was unintentionally introduced to Big Casa Blanca (assuming it is not present now), spread to extant Chiricahua leopard frog populations in

Gardner, Fish, and Cave canyons would be contingent upon infected frogs moving among these canyons, or movement via some other vector.

Cumulative Effects

Cumulative effects include the effects of future State, tribal, local, or private actions that are reasonably certain to occur in the action area considered in this biological opinion. Future federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the Act. See cumulative effects section under Mexican Spotted Owl.

Conclusion

After reviewing the current status of the threatened Chiricahua leopard frog, the environmental baseline for the action area, the effects of the proposed action, and the cumulative effects, it is the Service's biological opinion that the reintroduction of the Tarahumara frog into Arizona, as proposed, is not likely to jeopardize the continued existence of the Chiricahua leopard frog. No critical habitat has been designated for this species; therefore, none will be affected.

INCIDENTAL TAKE STATEMENT

Section 9 of the Act, as amended, prohibits taking (harassing, harming, pursuing, hunting, shooting, wounding, killing, trapping, capturing, collecting, or attempting to engage in any such conduct) of listed species of fish and wildlife without a special exemption. Harass is further defined as an intentional or negligent act or omission that creates the likelihood of injury to wildlife by annoying it to such an extent to significantly disrupt normal behavior patterns. Normal behavior patterns include, but are not limited to, breeding, feeding, and sheltering. Harm is further defined to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering. Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to, and not intended as part of the agency action is not considered a prohibited taking provided that such taking is in compliance with the incidental take statement.

Conservation measures to reduce the effects to the frog have been incorporated into the proposed action. These conservation measures include: 1) conforming to all conditions of the section 10 permits for dip-netting or other forms of take, 2) all field work will conform to amphibian disease prevention protocols in the survey protocol for Chiricahua leopard frogs, and 3) a pre-release health screening is being developed by the Phoenix Zoo and will be used to screen Tarahumara frogs prior to release (this will minimize the likelihood that disease or parasites will be introduced via Tarahumara frog re-establishment). The Service anticipates incidental take in the form of capture of Chiricahua leopard frogs that could inadvertently occur during dip-netting for Tarahumara frogs. Any frog unintentionally dip-netted would be promptly returned to the water reducing the likelihood of mortality; however, it is conceivable that some mortality could occur.

The Service anticipates that no more than four Chiricahua leopard frogs would be injured or killed as a result of dip-netting during the life of this project. Because the project description includes conservation measures for dip-netting, no additional reasonable and prudent measures will be provided. If during the course of this action, incidental take is exceeded, this would represent new information requiring review of the project effects and the Arizona ES Field Office must reinitiate intraservice consultation immediately.

Sonora Chub

Status of Species

We listed the Sonora chub in the United States and Mexico as threatened, with critical habitat in 1986 (U.S. Fish and Wildlife Service, 1986). Critical habitat was designated at the time of Federal listing to include Sycamore Creek, extending downstream from and including Yank Spring (= Hank and Yank Spring), to the International border. Also designated was the lower 1.2 mile of Penasco Creek, and the lower 0.25 mile of an unnamed stream entering Sycamore Creek from the west, about 1.5 mile downstream from Yank Spring. In addition to the aquatic environment, critical habitat includes a 40-foot wide riparian area along each side of Sycamore and Penasco creeks. This riparian zone is believed essential to maintaining the creek ecosystem and stream channels, and to conservation of the species (U.S. Fish and Wildlife Service 1986). Sonora chub is locally abundant in Sycamore Creek, although the habitat is limited in areal extent (Minckley and Deacon 1968). In Mexico, it is found in the rios Magdalena and Altar where it is considered relatively secure (Hendrickson and Juarez-Romero 1990). In 1995, Sonora chub were found in the lower reach of California Gulch, Arizona, (Arizona Game and Fish Department 1995).

Sonora chub is a stream-dwelling member of the minnow family, Cyprinidae, and can achieve total lengths of 7.8 inches (Hendrickson and Juarez-Romero 1990). In the United States, it typically does not exceed 5.0 inches (Minckley 1973), although specimens up to 6.0 inches have been measured (J. Carpenter, Service, pers. comm.). The Sonora chub has 63 to 75 scales in the lateral line, and the scales bear radii in all fields. The mouth is inferior and almost horizontal. There are typically eight rays in the dorsal, anal, and pelvic fins, although the dorsal fin can have nine (Miller 1945), and the anal and pelvic fins can have seven (Rinne 1976). The body is moderately chubby and dark-colored, with two prominent, black, lateral bands above the lateral line (whence the specific epithet, ditaenia) and a dark, oval basicaudal spot. Breeding individuals are brilliantly colored (Miller 1945).

Sonora chub spawn at multiple times during spring through summer, most likely in response to flood or freshets during the spring and summer rains (Hendrickson and Juarez-Romero 1990). Although Sonora chub is regularly confined to pools during arid periods, it prefers riverine habitats. In lotic waters in Mexico, Hendrickson and Juarez-Romero (1990) found it commonly in pools less than 2 feet deep, adjacent to or near areas with a fairly swift current, over sand and gravel substrates. It was less common in reaches that were predominately pools with low velocities and organic sediments. Sonora chub are adept at exploiting small marginal habitats, and they can survive under severe environmental conditions. It is also apparent that they can

maneuver upstream past small waterfalls and other obstructions to colonize newly-wetted habitats (Carpenter and Maughan 1993).

Based on collection dates of young-of-the-year, spawning occurs in early spring (Minckley 1973). However, larval and juvenile Sonora chub were found in Sycamore Creek and in a tributary to Rio Altar in November; which indicated breeding was apparently not limited by season. Adults with breeding coloration were also taken during these periods (Hendrickson and Juarez-Romero 1990). In Sycamore Creek, adults with breeding colors were seen from April through September in 1990 and 1991. Larvae and juveniles 0.6 to 0.7 inch were seen in April, May, and September (Carpenter 1992) suggesting that spawning occurred after the spring and summer rains. Bell (1984) also noted young after heavy flooding, and suggested that post-flood spawning is a survival mechanism evolved by this species. During spawning, Sonora chub apparently broadcast their eggs onto fine gravel substrates in slowly flowing water, where the eggs develop and hatch. There are no nests built nor parental care given. Larvae likely use shallow habitats at pool margins where they feed on microscopic organisms and algae. As adults they can exploit shallow to deep pools, runs, and riffles as available. In 2000, apparent multiple spawning in California Gulch was documented (U.S. Forest Service 2000).

Environmental Baseline

The majority of the extant range and habitat of the Sonora chub in the United States occurs in Sycamore/Penasco canyons downstream of Ruby Road within the action area. The Bear Valley allotment of the Coronado National Forest overlays these habitats. Present grazing management on the Bear Valley allotment has resulted in a satisfactory allotment condition overall. In 1997, range condition data indicated that most of the allotment was in good condition. In September 2000, Sycamore Canyon watershed assessment indicated that soil quality condition was 75 percent satisfactory, 16 percent impaired, 8 percent unsatisfactory, and 1 percent unsuitable. A trespass livestock problem has existed in the past due to cattle from Mexico, but in 1998, the Coronado Forest rebuilt the border fence which has reduced the number of trespass cattle. The Bear Valley allotment permittee is very attentive to this problem and has reacted quickly when trespass cattle from Mexico were found in the allotment. The permitted number of livestock for this allotment is 350 animals on a deferred/rest rotation cycle. Our October 24, 2002, biological opinion found that grazing on that allotment was not likely to jeopardize the continued existence of the Sonora chub, nor result in destruction or adverse modification of critical habitat.

The Sycamore/Penasco canyon area supports floral and faunal associations that are unique enough to require special management practices, including identified riparian ecotypes and known essential habitats for threatened and endangered plants and animals. Mean annual air temperature ranges from about 56 to 64 degrees F. Mean annual precipitation ranges from about 12 to 22 inches, which comes from gentle rains in winter and high intensity localized thunderstorms in summer (Coronado Forest Plan 1988). The riparian vegetation community is dominated by Fremont cottonwood, Arizona sycamore, a few emory oak and Arizona walnut, wolfberry, and Texas mulberry and ash. Deergrass is an important herbaceous riparian species.

Sonora chub have been able to survive in this watershed by expanding into riffles, runs, and pools during wet periods, and then shrinking back to deep pools as the stream dries. On an individual basis, a substantial number of Sonora chub die when they become trapped in habitats that do not sustain perennial water during arid periods (Carpenter and Maughan 1993). Recolonization is dependent on individuals that survive dry periods. This species has an amazing capacity for reproduction and recruitment as its habitat expands; it can seemingly explode from a small number of individuals occupying newly-wetted habitats in just a few weeks or months. The capability of the population to increase by several orders of magnitude within a few months is most likely an adaptation to the harsh climate and intermittent nature of its habitat, which has allowed the Sonora chub to survive to the present (Bell 1984).

Threats to the species in Sycamore Canyon include watershed degradation and accompanying siltation or flooding, as well as water quality problems due to livestock grazing, roads, and mining; potential introduction of nonnative fishes that could compete with or prey upon Sonora chub; and parasites or pathogens that may be introduced with nonnative fishes.

Effects of the Action

Effects could potentially occur to Sonora chub in Sycamore and Penasco canyons due to predation by Tarahumara frogs and monitoring of released frogs. Zweifel (1955) found remains of several Sonora chub in the stomachs of Tarahumara frogs collected in Arizona. Thus, we expect that Sonora chub would be preyed upon by Tarahumara frogs, if the reestablishment project is successful. Levels of predation are not possible to assess; however, historically Tarahumara frogs and Sonora chub coexisted in the Sycamore and Penasco canyon areas, and we have no reason to believe they cannot coexist again. Sonora chub have not been documented eating Tarahumara frogs; however, they are opportunistic feeders (Minckley 1973) and would be expected to prey upon small tadpoles. Hale (2001) suggested a large chub (*Gilia* sp.) may have eliminated Tarahumara frogs from Arroyo el Portrero in southern Sonora.

Field workers, particularly those monitoring the releases, will need to walk along stream banks and occasionally in the water to locate frogs. Such activity could destablilize banks, increase turbidity, and potentially result in trampling of eggs or small fish. However, most work will occur from the banklines, and Tarahumara frogs are expected to be most abundant in bedrock plunge pools that are resistant to bank damage or increased turbidity. We expect that any effects to Sonora chub critical habitat would be minor and temporary. Sonora chub could also be unintentionally dip-netted during sampling for frogs. As part of our proposed action, any Sonora chub unintentionally dip-netted will promptly be returned to the water unharmed. Dip-netting of Sonora chub will only occur if permitted by a section 10(a)(1)(A) permit from the Service. Any such dip-netting would occur in compliance with any terms and conditions of that permit.

The overall estimated current chub habitat is 10 stream miles in Sycamore/Penasco Creek and California Gulch including a 40-foot wide riparian area along each side of Sycamore and Penasco creeks. A recovery plan for the Sonora chub was written in October 1992. In Sycamore Canyon, the chub occurs within the Pajarita Wilderness and Goodding Research Natural Area of the Coronado National Forest. These special designations help protect a biological community

characterized by Mexican floral and faunal elements that do not otherwise occur, or are rare elsewhere, in the United States (Goodding 1961, Curran 1973, Smith 1984, U.S. Forest Service 1986). Management direction for these special units is to maintain the area in climax vegetation. Removal of minerals, livestock grazing, use of motorized vehicles, and harvest of timber or fuelwood is not permitted, and recreation is limited to non-developed and dispersed use. Livestock grazing is permitted within Pajarita Wilderness outside of Goodding Research Natural Area. This management direction is applicable to Sycamore Canyon portions of habitat within the Gooding Research Natural Area and /or wilderness. The remainder of Sycamore drainage and California Gulch is open to multiple uses (U.S. Forest Service 1986).

Potential threats to Sonora chub are related to additional watershed development. Continued and increased grazing and mining operations in upstream watersheds could result in increased siltation and runoff, increased water demand and withdrawal, and introduced pollutants to the stream. Livestock grazing in riparian areas is usually detrimental to fish habitat. Predation by nonnative vertebrates is also a threat to populations of Sonora chub. Green sunfish is a known predator on native fish in Arizona (Minckley 1973) and has been implicated in population changes in other lotic fish communities (Arizona Game and Fish Department 1988). Hendrickson and Juarez-Romero (1990) noted smaller populations of Sonora chub in areas where nonnative fishes were present. Sonora chub were absent when nonnative predators were abundant in reservoirs and highly modified stream habitats. Bullfrogs, common in the California Gulch watershed, have also been implicated in the disappearance of native frogs and fishes in other western aquatic habitats (Arizona Game and Fish Department 1988).

Primary constituent elements of critical habitat include clean, permanent water with pools, and intermediate riffle areas and/or intermittent pools maintained by bedrock or by subsurface flow, in areas shaded by canyon walls.

Cumulative Effects

Cumulative effects include the effects of future State, tribal, local, or private actions that are reasonably certain to occur in the action area considered in this biological opinion. Future federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the Act. See cumulative effects discussion under Mexican spotted owl.

Conclusion

After reviewing the current status of the threatened Sonora chub, the environmental baseline for the action area, the effects of the proposed action, and the cumulative effects, it is the Service's biological opinion that the reintroduction of the Tarahumara frog into Arizona, as proposed, is not likely to jeopardize the continued existence of the Sonora chub, and is not likely to destroy or adversely modify designated critical habitat.

INCIDENTAL TAKE STATEMENT

Section 9 of the Act and Federal regulation pursuant to section 4(d) of the Act prohibit the take of endangered and threatened species, respectively, without special exemption. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, collect, or to attempt to engage in any such conduct. Harm is further defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering. Harass is defined by the Service as intentional or negligent actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding or sheltering. Incidental take is defined as take that is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity. Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking under the Act provided that such taking is in compliance with the terms and conditions of this incidental take statement.

The Service anticipates incidental take in the form of capture of Sonora chub could inadvertently occur during dip-netting for Tarahumara frogs. All monitoring work that may result in dip-netting or other forms of take of Chiricahua leopard frogs and Sonora chub will be conducted under the Service and AGFD permits, and will conform to all conditions of those permits. Any Sonora chub unintentionally dip-netted will promptly be returned to the water unharmed. Although it is unlikely that mortality of chubs would occur during dip-netting, it could happen; therefore, it is the Service's opinion that mortality of no more than five Sonora chub will occur over the life of the project. Because a conservation measure for dip-netting is included as part of the proposed action, no additional reasonable and prudent measures will be provided. If during the course of this action, incidental take is exceeded, this would represent new information requiring review of the project effects and the Arizona ES Field Office must reinitiate intraservice consultation immediately.

Conservation Recommendations

Section 7(a)(1) of the Act directs Federal agencies to utilize their authorities to further the purposes of the Act by carrying out conservation programs for the benefit of endangered and threatened species. Conservation recommendations are discretionary agency activities to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, to help implement recovery plans, or to develop information.

1. Minimize the number of people walking or standing on the banks.
2. Report any observances of Sonora chub to the Coronado National Forest.

In order for the Service to be kept informed of actions minimizing or avoiding adverse effects, or benefiting listed species or their habitats, the Service requests notification of the implementation of any conservation recommendations.

Reinitiation Notice

This concludes formal consultation on the action outlined in the re-establishment proposal of the Tarahumara frog. As provided in 50 CFR § 402.16, reinitiation of formal consultation is required where discretionary Federal agency involvement or control over the action has been retained (or is authorized by law) and if: (1) the amount or extent of incidental take is exceeded; (2) new information reveals effects of the agency action that may affect listed species or critical habitat in a manner or to an extent not considered in this opinion; (3) the agency action is subsequently modified in a manner that causes an effect to the listed species or critical habitat not considered in this opinion; (4) a new species is listed or critical habitat designated that may be affected by the action. In instances where the amount or extent of incidental take is exceeded, any operations causing such take must cease pending reinitiation.

Thank you for your cooperation and assistance throughout this consultation process. If you have any questions or would like to discuss any part of this biological opinion, please contact Ms. Delfinia Montaño at 505-248-6401 or Ms. Sarah Rinkevich at 505-248-6663, Division of Threatened and Endangered Species, Region 2.

References Cited

Arizona Game and Fish Department. 1995. Report on Sonora chub collection in California Gulch.

Arizona Game and Fish Department. 1996. Wildlife of special concern (public review draft). Nongame Branch, Arizona Game and Fish Department, Phoenix.

Arizona Game and Fish Department. 1988. Threatened native wildlife in Arizona. Arizona Game and Fish Department, Phoenix.

Bell, G. 1984. Sonora chub, Sycamore Canyon. Memo, USDA Forest Service, Coronado National Forest, Nogales, AZ. 13pp.

Benedict, N., and T.W. Quinn. 1999. Identification of Rio Grande leopard frogs by mitochondrial DNA analysis: a tool for monitoring the spread of a non-native species. Department of Biological Sciences, University of Denver, CO.

Berger L., R. Speare, P. Daszak, D.E. Green, A.A. Cunningham, C.L. Goggins, R. Slocombe, M.A. Ragan, A.D. Hyatt, K.R. McDonald, H.B. Hines, K.R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Science, USA 95:9031-9036.

Blanchard, C.L., and M. Stromberg. 1987. Acidic precipitation in southeastern Arizona: sulfate, nitrate, and trace-metal deposition. Atmospheric Environment 21:2375-2381.

Bowers, J.E., and S.P. McLaughlin. 1994. Flora of the Huachuca Mountains. Pages 135-143 *in* L.F. DeBano *et al.* (Tech. Coord.), Biodiversity and management of the Madrean Archipelago: the sky islands of the Southwestern United States and Northwestern Mexico. USDA Forest Service General Technical Report RM-GTR-264.

Bowles, A.L. 1995. Responses of wildlife to noise. In R.L. Knight and K.J. Gutzwiler (eds), Wildlife and Recreationists, Coexistence Through Management and Research. Island Press, Washington, D.C.

Bradley, G.A., P.C. Rosen, M.J. Sredl, T.R. Jones, and J.E. Longcore. 2002. Chytridomycosis in native Arizona frogs. Journal of Wildlife Diseases 38(1):206-212.

Campbell, B. 1931. *Rana tarahumarae*, a frog new to the United States. Copeia 1931:164.

Campbell, J.A. 1998. Amphibians and Reptiles of northern Guatemala, the Yucatan, and Belize. University of Oklahoma Press, Norman, Oklahoma.

Carey, C., W.R. Heyer, J. Wilkinson, R.A. Alford, J.W. Arntzen, T. Halliday, L. Hungerford, K.R. Lips, E.M. Middleton, S.A. Orchard, and A.S. Rand. 2001. Amphibian declines and environmental change: use of remote sensing data to identify environmental correlates. Conservation Biology 15(4):903-913.

Carpenter, J. 1992. Summer habitat use of Sonora chub in Sycamore Creek, Santa Cruz County, Arizona. M.S. Thesis, University of Arizona, Tucson. 83pp.

Carpenter, J. and O.E. Maughan. 1993. Macrohabitat of Sonora chub (*Gila ditaenia*) in Sycamore Creek, Santa Cruz County, Arizona. Journal of Freshwater Ecology 8:265-278.

Clarkson, R.W., and J.C. Rorabaugh. 1989. Status of leopard frogs (*Rana pipiens* Complex) in Arizona and southeastern California. Southwestern Naturalist 34(4):531-538.

Cole, D.N., and P.B. Landres. 1995. Indirect effects of recreation on wildlife. Chapter 11 *in* Wildlife and Recreationists. Island Press, Washington, D.C

Coombs, T.L. 1979. Cadmium in aquatic organisms. Pp. 93-139. *In* Webb, M. (Ed.), The Chemistry, Biochemistry, and Biology of Cadmium. Elsevier/North-Holland Biomedical Press, New York, New York.

Dahms, C.W., and B.W. Geils (tech. eds). 1997. An assessment of forest ecosystem health in the Southwest. General Technical Report RM-GTR-295. Fort Collins, CO, US Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station.

Danzer, S.R., C.H. Baisan, and T.W. Swetnam. 1997. The influence of fire and land-use history on stand dynamics in the Huachuca Mountains of southeastern Arizona. Appendix D in Robinett, D., R.A. Abolt, and R. Anderson, Fort Huachuca Fire Management Plan. Report to Fort Huachuca, AZ.

Daszak, P. 2000. Frog decline and epidemic disease. International Society for Infectious Diseases. Http://www.promedmail.org.

Davidson, C. 1996. Frog and toad calls of the Rocky Mountains. Library of Natural Sounds, Cornell Laboratory of Ornithology, Ithaca, NY.

Davidson, D., Pessier, A.P., J.E. Longcore, M. Parris, J. Jancovich, J. Brunner, D. Schock, and J.P. Collins. 2000. Chytridiomycosis in Arizona (USA) tiger salamanders. Page 23 *in* Conference and Workshop Compendium: Getting the Jump! On amphibian diseas. Cairns, Australia, August 2000.

DeBano, L.F., and D.G. Neary. 1996. Effects of fire on riparian systems. Pages 69-76 *in* P.F. Ffolliott, L.F. DeBano, M.B. Baker, G.J. Gottfried, G. Solis-Garza, C.B. Edminster, D.G Neary, L.S. Allen, and R.H Hamre (tech. coords.). Effects of fire on Madrean province ecosystems, a symposium proceedings. USDA Forest Service, General Technical Report RM-GTR-289.

Declining Amphibian Populations Task Force. 1993. Post-metamorphic death syndrome. Froglog 7:1-2.

Degenhardt, W.G., C.W. Painter, and A.H. Price. 1996. Amphibians and reptiles of New Mexico. University of New Mexico Press, Albuquerque.

Diaz, J.V., and G.E.Q. Diaz. 1997. Anfibios y reptiles de Aguascalientes. Grupo Impressor Mexico, Aguascalientes, Aguascalientes, MX.

Dole, J.W. 1972. Evidence of celestial orientation in newly-metamorphosed *Rana pipiens*. Herpetologica 28:273-276.

Dole, J.W. 1971. Dispersal of recently metamorphosed leopard frogs, *Rana pipiens*. Copeia 1971:221-228.

Dole, J.W. 1968. Homing in leopard frogs, *Rana pipiens*. Ecology 49:386-399.

Fernandez, P.J., and J.T. Bagnara. 1995. Recent changes in leopard frog distribution in the White Mountains of east central Arizona. Page 4 in abstracts of the First Annual Meeting of the Southwestern Working Group of the Declining Amphibian Populations Task Force, Phoenix, AZ.

Fernandez, P.J. and P.C. Rosen. 1998. Effects of introduced crayfish on the Chiricahua leopard frog and its stream habitat in the White Mountains, Arizona. Page 5 *in* abstracts of the Fourth Annual Meeting of the Declining Amphibian Populations Task Force, Phoenix, AZ.

Fernandez, P.J., and P.C. Rosen. 1996. Effects of the introduced crayfish *Oronectes virilis* on the native aquatic herpetofauna in Arizona. Report to the Arizona Game and Fish Department, Heritage Program, IIPAM Project No. I94054.

Field, K.J., M.J. Sredl, and M.J. Demlong. 2000. Draft proposal to re-establish Tarahumara frogs (*Rana tarahumarae*) into southcentral Arizona. Nongame Branch, Arizona Game and Fish Department, Phoenix, Arizona.

Flather, C.H., and H.K. Cordell. 1995. Outdoor recreation: historical and anticipated trends. Chapter 1 *in* R.L. Knight and K.J. Gutzwiller (eds), Wildlife and Recreationists. Island Press, Washington, D.C.

Fletcher, K. 1990. Habitat used, abundance, and distribution of the Mexican spotted owl, *Strix occidentalis lucida*, on National Forest System Lands. U.S. Forest Service, Southwestern Region, Albuquerque, New Mexico. 78 pp.

Forsman, E.D., E.C. Meslow, and H.M. Wright. 1984. Distribution and biology of the spotted owl in Oregon. Wildlife Monographs 87:1-64.

Frost, J.S., and J.T. Bagnara. 1977. Sympatry between *Rana blairi* and the southern form of leopard frog in southeastern Arizona (Anura: Ranidae). The Southwestern Naturalist 22(4):443-453.

Ganey, J.L., W.M. Block, J.K. Dwyer, B.E. Strohmeyer, and J.S. Jenness. 1998. Dispersal movements and survival rates of juvenile Mexican spotted owls in northern Arizona. Wilson Bulletin 110:206-217.

Ganey, J.L., G.C. White, A.B. Franklin, J.P. Ward, Jr., and D.C. Bowden. 2000. A pilot study on monitoring populations of Mexican spotted owls in Arizona and New Mexico: second interim report. 41 pp.

Gutierrez, R.J., A.B. Franklin, and W.S. LaHaye. 1995. Spotted owl. *In* A. Poole. P. Stettenheim, and F. Gill (eds). The birds of North America. Academy of the Natural Sciences and American Ornithological Union. Washington, D.C.

Hale, S.F. 2001. The status of the Tarahumara frog in Sonora, Mexico based on a re-survey of selected localities, and search for additional populations. Report to the U.S. Fish and Wildlife Service, Phoenix, Arizona.

Hale, S.F., G.M. Ferguson, P.A. Holm, and E.B. Wirt. 1998. Re-survey of selected Tarahumara frog (*Rana tarahumara*) localities in northern Sonora, Mexico, in May 1998. Report to the Arizona Zoological Society and the Tarahumara Frog Conservation Team.

Hale, S.F., and J.L. Jarchow. 1988. The status of the Tarahumara frog (*Rana tarahumarae*) in the United States and Mexico: part II. Report to the Arizona Game and Fish Department, Phoenix, Arizona, and the Office of Endangered Species, U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

Hale, S.F., and C.J. May. 1983. Status report for *Rana tarahumarae* Boulenger. Report to the Office of Endangered Species, U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

Hale, S.F., F. Retes, and T.R. Van Devender. 1977. New populations of *Rana tarahumarae* (Tarahumara frog) in Arizona. Journal of the Arizona Academy of Science 11:134-135.

Hale, S.F., C.R. Schwalbe, J.L. Jarchow, C.J. May, C.H. Lowe, and T.B. Johnson. 1995. Disappearance of the Tarahumara frog. Pp. 138-140. *In* LaRoe, E.T., G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac (Eds.), Our Living Resources: A Report to the Nation on the Distribution, Abundance, and Health of U.S. Plants, Animals, and Ecosystems. U.S. Department of the Interior, National Biological Service, Washington, D.C.

Halliday, T.R. 1998. A declining amphibian conundrum. Nature 394:418-419.

Hammitt, W.E., and D.N. Cole. 1987. Wildland recreation: ecology and management. John Wiley and Sons, New York, N.Y.

Hendrickson, D.A., and L.R. Juarez-Romero. 1990. Fishes of the Rio de la Concepcion basin, Sonora, Mexico, with emphasis on determinations of status of the Sonora chub, Gila ditaenia, a threatened species. Southwestern Naturalist 36(2).

Jennings, R.D. 1995. Investigations of recently viable leopard frog populations in New Mexico: *Rana chiricahuensis* and *Rana yavapaiensis*. New Mexico Game and Fish Department, Santa Fe.

Jennings, R.D. 1987. The status of *Rana berlandieri*, the Rio Grande leopard frog, and *Rana yavapaiensis*, the lowland leopard frog, in New Mexico. Report to New Mexico Department of Game and Fish, Santa Fe, New Mexico.

Jennings, R.D., and N.J. Scott. 1993. Ecologically correlated morphological variation in tadpoles of the leopard frog, *Rana chiricahuensis*. Journal of Herpetology 27(3):285-293.

Knight, R.L., and D.N. Cole. 1995. Wildlfe responses to recreationists. Chapter 4 *in* R.L. Knight and K.J. Gutzwiller (eds), Wildlife and Recreationists. Island Press, Washington, D.C.

Linsdale, J.M. 1933. A specimen of *Rana tarahumarae* from New Mexico. Copeia 1933:222.

Little, E.L. 1940. Amphibians and reptiles of the Roosevelt Reservoir area, Arizona. Copeia 1940(4):260-265.

Little, E.L., and J.G. Keller. 1937. Amphibians and reptiles of the Jornada Experimental Range, New Mexico. Copeia 1937(4):216-222.

Longcore, J.E. 2000. Information excerpted from Joyce Longcore. Biosafety chapter, workbook for Amphibian Health Examinations and Disease Monitoring Workshop, US Fish and Wildlife Service, National Conservation Training Center, Sherpherdstown, WV, Feb 17-18, 2000.

Loncore, J.E., A.P. Pessier, and D.K. Nichols. 1999. *Batracytrium dendrobatidis* gen. Et sp. Nov., a chytrid pathogenic to amphibians. Mycologia 91(2):219-227.

McCranie, J.R., and L.D. Wilson. 1987. The biogeography of the herpetofauna of the pine-oak woodlands of the Sierra Madre Occidental of Mexico. Milwaukie Public Museum Contributions in Biology and Geology 72:1-30.

Miller, R.R. 1945. A new cyprinid fish from southern Arizona and Sonora, Mexico, with the description of a new subgenus of *Gila* and a review of related species. Copeia, 1945: 104-110.

Minckley, W.L. 1973. Fishes of Arizona. Arizona Game and Fish Department and Fish Department, Phoenix. 293pp.

Minckley, W.L. and J.E. Deacon. 1968. Southwestern fishes and the enigma of "endangered species." Science 159: 1424-1432.

Morell, V. 1999. Are pathogens felling frogs? Science 284:728-731.

Painter, C.W. 2000. Status of listed and category herpetofauna. Report to US Fish and Wildlife Service, Albuquerque, NM. Completion report for E-31/1-5.

Peterson, P.J., and B.J. Alloway. 1979. Cadmium in soils and vegetation. Pp. 45-92. *In* Webb, M. (Ed.), The Chemistry, Biochemistry, and Biology of Cadmium. Elsevier/North-Holland Biomedical Press, New York, New York.

Platz, J.E. 1993. *Rana subaquavocalis*, a remarkable new species of leopard frog (*Rana pipiens* Complex) from southeastern Arizona that calls under water. Journal of Herpetology 27(2):154-162.

Platz, J.E., and T. Grudzien. 1999. The taxonomic status of leopard frogs from the Mogollon Rim country of central Arizona: evidence for recognition of a new species. Proceedings of Nebraska Academy of Sciences 109:51.

Platz, J.E., and J.S. Mecham. 1984. *Rana chiricahuensis*. Catalogue of American Amphibians and Reptiles 347.1.

Platz, J.E., and J.S. Mecham. 1979. *Rana chiricahuensis*, a new species of leopard frog (*Rana pipiens* Complex) from Arizona. Copeia 1979(3):383-390.

Pounds, J.A., and M.L. Crump. 1994. Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. Conservation Biology 8(1)72-85.

Rinne, J.N. 1976. Cyprinid fishes of the genus *Gila* from the lower Colorado River basin. Wasmann Journal of Biology 34(1): 65-107.

Rorabaugh, J.C. In press. *Rana berlandieri* Baird, 1854, Rio Grande Leopard Frog. *In* Lannoo, M.J. (Ed.), Status and Conservation of U.S. Amphibians. Volume 2: Species Accounts. University of California Press, Berekely, CA.

Rorabaugh, J.C., and S.F. Hale. *In press*. *Rana tarahumarae* Boulenger, 1917, Tarahumara frog. *In* M.J. Lannoo (ed), Status and Conservation of U.S. Amphibians. Volume 2: Species Accounts. University of California Press, Berkeley.

Rorabaugh, J., and J. Humphrey. 2002. Tarahumara frog: return of a native. Endangered Species Bulletin XXVII(2):24-26.

Rosen, P.C., and C.R. Schwalbe. 1998. Using managed waters for conservation of threatened frogs. Pages 180-202 *in* Proceedings of Symposium on Environmental, Economic, and Legal Issues Related to Rangeland Water Developments. November 13-15, 1997, Tempe, AZ.

Rosen, P.C., C.R. Schwalbe, D.A. Parizek, P.A. Holm, and C.H. Lowe. 1994. Introduced aquatic vertebrates in the Chiricahua region: effects on declining native ranid frogs. Pages 251-261 *in* L.F. DeBano, G.J. Gottfried, R.H. Hamre, C.B. Edminster, P.F. Ffolliott, and A. Ortega-Rubio (tech. coords.), Biodiversity and management of the Madrean Archipelago. USDA Forest Service, General Technical Report RM-GTR-264.

Rosen, P.C., C.R. Schwalbe, and S.S. Sartorius. 1996. Decline of the Chiricahua leopard frog in Arizona mediated by introduced species. Report to Heritage program, Arizona Game and Fish Department, Phoenix, AZ. IIPAM Project No. I92052.

Scott, N.J. 1993. Postmetamorphic death syndrome. FROGLOG, September, No.7:1-2

Seburn, C.N.L., D.C. Seburn, and C.A. Paszkowski. 1997. Northern leopard frog (*Rana pipiens*) . dispersal in relation to habitat. Herpetological Conservation 1:64-72.

Sinsch, U. 1991. Mini-review: the orientation behaviour of amphibians. Herpetological Journal 1:541-544.

Snyder, J., T. Maret, and J.P. Collins. 1996. Exotic species and the distribution of native amphibians in the San Rafael Valley, AZ. Page 6 *in* abstracts of the Second Annual Meeting of the Southwestern United States Working Group of the Declining Amphibian Populations Task Force, Tucson, AZ.

Speare, R., and L. Berger. 2000. Global distribution of chytridiomycosis in amphibians. Http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm.11 November 2000.

Sredl, M.J., and D. Caldwell. 2000. Wintertime populations surveys - call for volunteers. Sonoran Herpetologist 13:1.

Sredl, M.J., and J.M. Howland. 1994. Conservation and management of madrean populations of the Chiricahua leopard frog, *Rana chiricahuensis*. Arizona Game and Fish Department, Nongame Branch, Phoenix, AZ.

Sredl, M.J., J.M. Howland, J.E. Wallace, and L.S. Saylor. 1997. Status and distribution of Arizona's native ranid frogs. Pages 45-101 *in* M.J. Sredl (ed). Ranid frog conservation and management. Arizona Game and Fish Department, Nongame and Endangered Wildlife Program, Technical Report 121.

Sredl, M.J., and R.D. Jennings. In press. *Rana chiricahuensis*: Platz and Mecham, 1979, Chiricahua leopard frog. *In* M.J. Lanoo (ed), Status and Conservation of U.S. Amphibians. University of California Press, Berkeley.

Sredl, M.J., and L.S. Saylor. 1998. Conservation and management zones and the role of earthern cattle tanks in conserving Arizona leopard frogs on large landscapes. Pages 211-225 *in* Proceedings of Symposium on Environmental, Economic, and Legal Issues Related to Rangeland Water Developments. November 13-15, 1997, Tempe, AZ.

Stebbins, R.C. 1951. Amphibians of Western North America. University of California Press, Berkeley, California.

Stebbins, R.C. 2003. A Field Guide to Western Reptiles and Amphibians, Third Edition. Houghton Mifflin Company, Boston, MA.

Steidl, R.J., and R.G. Anthony. 2000. Experimental effects of human activity on breeding bald eagles. Ecological Applications 10:258-268.

Swarthout, E.C.H., and R.J. Steidl. 2001. Flush responses of Mexican spotted owls to recreationists. Journal of Wildlife Management 65:312-317.

Swarthout, E.C.H., and R.J. Steidl. 2003. Experimental effects of hiking on breeding Mexican spotted owls. Conservation Biology 17)\(1):307-315.

Swetnam, T.W., and C.H. Baisan. 1996. Fire histories of montane forests in the Madrean Borderlands. Pages 15-36 *in* P.F. Ffolliott *et al.* (Tech. Coord.), Effects of fire on Madrean Province ecosystems. USDA Forest Service, General Technical Report, RM-GTR-289.

U.S. Fish and Wildlife Service. 1986. Endangered and Threatened Wildlife and Plants; final rule to list the Sonora chub as threatened. Federal Register. 51:16042-16047.

U.S. Fish and Wildlife Service. 1991. Mexican spotted owl status review. Endangered species report 20. Albuquerque, New Mexico.

U.S. Fish and Wildlife Service. 1993. Endangered and Threatened Wildlife and Plants; final rule to list the Mexican spotted owl as threatened. Federal Register. 58:14248-14271.

U.S. Fish and Wildlife Service. 1995. Recovery Plan for the Mexican Spotted Owl. Albuquerque, New Mexico.

U.S. Forest Service. 1986. Coronado National Forest land and resource management plan. USDA Forest Service, Southwestern Region, Albuquerque, New Mexico.

U.S. Forest Service. 2000. Survey Report on presence/absence of Sonora chub in California Gulch, Santa Cruz County, Arizona during July-October, 2000.

U.S. Forest Service, Southwestern Region. 2001. Biological Assessment and Evaluation, Urban Interface Fuel Treatment, February 28, 2001. 271 pp.

Ward, J.P., Jr., and W.M. Block. 1995. Mexican spotted owl prey ecology. Chapter 5 *in* US Fish and Wildlife Service, Mexican spotted owl recovery plan. Volume II, Albuquerque, NM.

Wright, A.H., and A.A. Wright. 1949. Handbook of frogs and toads of the United States and Canada. Third edition, Comstock Publishing Association, Ithaca, New York.

Zweifel, R.G. 1955. Ecology, distribution, and systematics of frogs of the *Rana boylii* group. University of California Publications in Zoology 54:207-292.

Zweifel, R.G. 1968. *Rana tarahumarae*. Catalogue of American Amphibians and Reptiles 66:1-2.

Appendix 1

A DRAFT PROPOSAL

TO RE-ESTABLISH

TARAHUMARA FROGS
(*Rana tarahumarae*)

INTO

SOUTHCENTRAL ARIZONA

by

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Introduction

This proposal, which builds on proposals previously developed by others (e.g. Tarahumara Frog Conservation Team 1999), is Step 8 in the Arizona Game and Fish Department's (Department or AGFD) 12-step re-establishment process (Johnson and Glinski 1989, Appendix 1) and develops approaches to re-establish Tarahumara frogs at historical sites in Arizona with a high potential to succeed (Figure 1). The geographic scope of the project encompasses only a few canyons in Arizona, and the animals needed for release efforts will be collected in Mexico or will be progeny of those collected. If approved, this proposal would provide a framework under which Tarahumara frogs would be released to the highest quality historical habitats in Arizona.

The Tarahumara frog (*Rana tarahumarae*) belongs to the family Ranidae (true frogs), which includes leopard frogs. Boulenger (1917) described this species from specimens collected at Yoquivo (Ioquiro) and Barranca del Cobre in the Sierra Tarahumarae in Chihuahua, Mexico. It is a medium-sized frog, adults range from 64 to 114 mm (2.5 to 4.5 inches) measured from the tip of the nose to the rear of the thigh. These frogs are greenish-brown with small brown to black spots on the body and dark crossbars on the legs. They are highly aquatic, with hind feet that have extensive webbing. This species usually lacks a dorsolateral fold, characteristic of related leopard frogs and a few other ranid species. Larvae or tadpoles are greenish-yellow with small dark spots over the dorsum and larger spots on the tail, and can reach 97 mm (4 inches) in length (Hale and May 1983).

Tarahumara frogs were first collected in Arizona in the Atascosa-Pajarito-Tumacacori mountains in Santa Cruz County by Campbell (1931). It was known from southcentral Arizona until 1983. The causes of the extirpation of the frog from Arizona are unclear. Populations continue to persist in mountainous regions of Mexico.

Impacts of activities under this re-establishment proposal on other land uses and wildlife resources will be negligible. Frogs or larvae will be released at sites on national forest lands. There will be no effects on private property, angling, hunting, other recreation, or livestock grazing attributable to the re-establishment. We anticipate no conflicts with any potential future uses. There will be no significant soil, vegetation, or cultural disturbance at any site. Slight modification or renovation of aquatic sites to enhance the chance of successful re-establishment may be considered. For example, it may become necessary to remove gravel, rock, or debris at some sites to increase water depth and permanency. If undertaken, these activities will have only localized effects, and NEPA, ESA, cultural resources, and other required compliance will be completed by the appropriate agency.

The duration of this proposed re-establishment project and the required monitoring would be approximately 10 years upon approval. The search for funding for the project is contingent upon approval of the re-establishment proposal. Multiple cooperators would be needed to implement this proposed re-establishment, and these participants will provide some level of support. It must be recognized that a substantial commitment of time and funds for a number of years is needed before moving forward with this proposed re-establishment.

Cooperators include Arizona Game and Fish Department (Department or AGFD), Arizona-Sonora Desert Museum (ASDM), Coronado National Forest (CNF), Instituto del Medio

Ambiente y el Desarrollo Sustentable del Estado de Sonora (IMADES), The Phoenix Zoo (TPZ), University of Arizona (UA), U.S. Fish and Wildlife Service (USFWS), and several private citizens and biologists.

One goal of the Department's mission, as stated in Wildlife 2000 Nongame and Endangered Wildlife subprogram narrative, is the conservation and restoration of native biological diversity and recovery of species either listed or in imminent threat of listing. Additional documents such as work plans and job descriptions for the Heritage and Section 6 Ranid Frog projects support similar objectives. The activities described in this proposal are consistent with the objectives outlined in those documents. A benefit of pursuing activities that support this narrative is that re-establishing populations of the Tarahumara frog in Arizona will likely preclude the need to federally list. Re-establishment of other species of native frogs at some sites has contributed positively to the local economy, by attracting tourists and creating wildlife viewing opportunities.

Historical Status in State

Former Range

The range of the Tarahumara frog included extreme southcentral Arizona (Santa Cruz County) in the United States and parts of northcentral and eastern Sonora, western Chihuahua and northeastern Sinaloa in Mexico (Hale and May 1983). The eastern and southern distributional limits in Mexico are not well known. Elevations of Tarahumara frog localities range from approximately 460 m (1500 ft) to over 1860 m (6100 ft) (Stebbins 1985). Winter temperatures may determine the northern and upper elevational limits of its range, competition from other ranids the southern limit, and aridity the western limit (Hale and Jarchow 1988).

Arizona's populations were found in three drainages in the Santa Rita Mountains and three drainages in the Pajarito-Atascosa-Tumacacori mountains complex (Hale and others 1977, Hale and May 1983). These populations, which were the northern limit of this species' range, were separated from those in Mexico by approximately 100 km (62 mi) (Hale unpubl. data), and their last contact probably occurred in the middle Holocene, about 4,000 years ago (Hale and others 1977). Suitable habitats for frogs diminished as climates changed, and relict populations formed in pockets of suitable yet isolated habitat in mountainous regions (Van Devender and Worthington 1977).

Habitats Occupied

The Tarahumara frog requires permanent pools of water for both their larval and adult stages. The frogs prefer boulder-strewn perennial streams and seasonal streams with bedrock beds that include deep, drought resistant plunge pools. These pools provide reliable habitat when intermittent stretches of the stream go dry. The presence of hibernacula along the water line of the plunge pools is important for adults (Zweifel 1955, Hale and May 1983). In preferred streams, flow averages less than 1.4 cubic meters (370 gal) per minute; yet flood stage rates may be faster than 1.4 cubic meters (370 gal) per second. Avoidance of large streams by Tarahumara frogs may be due to the propensity of these systems to flood and their variable flow rates (Hale and May 1983). These frogs were not found in earthen cattle tanks. The historical habitat in Arizona was found within the semidesert grassland and Madrean evergreen woodland areas in

foothill and mountain canyons. Warm summers and mild winters were typical, with most precipitation occurring in the summer (Zweifel 1955, Hale and May 1983). Seven canyons in Arizona were known to have Tarahumara frogs.

In the Santa Rita Mountains, a large population inhabited Big Casa Blanca Canyon until 1977-1978. At one time this population contained an estimated 500 to 700 frogs, however the population declined rapidly. By the spring 1983, the last known individual was found dead (Hale 1992). The habitat in this canyon was described as excellent. Although water flow in this canyon was seasonal, the abundant bedrock and boulder plunge pools were usually permanent because of their depth. Of the microsites in this canyon, an area called Bathtub was prime habitat. In this area, the stream flowed down a steep slope into a series of eight bedrock pools. The Bathtub area contained all the habitat components that are key to the Tarahumara frog's survival. Because evaporation was the only source of water loss, the pools provided reliable habitat. The cover available to frogs varied from pool to pool. A hibernaculum was associated with at least one pool (Hale and May 1983).

Portions of the stream above and below the Bathtub area contained bedrock and boulder formed plunge pools, which were less reliable and prone to drying. About 1 km upstream from the Bathtub area was a series of five shallow bedrock pools in a stretch of stream with low gradient. A spring provided some water except in extreme drought. The pool at the downstream end of the string seemed to be the most suitable because it contained a deep hole under a boulder that the frogs could use to hide and hibernate. Also, it was the only pool in which water remained during dry periods in that stretch of the canyon. Fifty meters below the Bathtub area was Yucca Pool. This small bedrock pool had overhanging boulders, which provided hibernacula for overwintering frogs. Farther downstream the plunge pool type habitat gave way to a low gradient, stony streambed (Hale and May 1983). A recent visit to the canyon by the Department and TFCT members (September 2000) confimed that the habitat remains intact as described by Hale and May (1983).

Gardner Canyon, the upper portion of which lies adjacent to Big Casa Blanca Canyon in the Santa Rita Mountains, had marginal Tarahumara frog habitat. Small, shallow plunge pools were not numerous and the stream flow was intermittent. Few Tarahumara frogs were observed in the canyon. The first record was a dead frog in 1972, and the last live frogs were seen in 1977 (Hale and others 1977, Hale and May 1983, Hale 1992). Frogs may have dispersed to this marginal habitat by way of a canal dug by miners from Big Casa Blanca Canyon (Hale and others 1977). Alternatively, observations of frogs moving long distances upstream during summer rains suggests that frogs may use headwater dispersal, crossing saddles between adjacent drainages, to disperse to places such as Gardner Canyon (Hale and May 1983).

Adobe Canyon, the third historical site in the Santa Rita Mountains, was also considered to have marginal habitat. Tarahumara frogs were only recorded here in 1974. It is doubtful that this locality could have supported a permanent population. The water through the canyon was shallow and intermittent. Dispersal to this canyon would have been possible, again, through the canal from Big Casa Blanca Canyon (Hale and others 1977) or by headwater dispersal (Hale and May 1983).

In the Pajarito-Atascosa-Tumacacori mountains complex, Tinaja Canyon did not have much Tarahumara frog habitat, although it did have some small plunge pools in the bedrock. Continuous occupancy of this area by Tarahumara frogs was unlikely, and individuals observed there in 1948 may have been the result of a migration event during favorable conditions (Hale and May 1983).

The most well known population of Tarahumara frogs in Pajarito-Atascosa-Tumacacori mountains complex was in Sycamore Canyon. Appropriate habitat was abundant with many drought resistant plunge pools and portions of the stream that provided nearly year round flow. The habitable area encompassed about 3 to 4 km (1.8 to 2.5 mi) within the 10 km (6.2 mi) stretch of canyon from Yanks Spring to the border of Mexico. In April of 1974, many dead and sickly Tarahumara frogs and leopard frogs (*R. yavapaiensis* or *R. chiricahuensis*) were observed in the canyon. No Tarahumara frogs have been seen in this canyon since 1974 (Hale and May 1983, Hale 1992). In September 2000, the habitat appeared to remain as described in Hale and May's report (1983).

Peña Blanca and Alamo canyons are also in the Pajarito-Atascosa-Tumacacori mountains complex. Alamo Canyon is a tributary of Peña Blanca Canyon. The only permanent water in the area was around Alamo Spring and below Peña Blanca Lake. The creation of the lake in 1957 and campground developments in the early 1950's around Peña Blanca Springs, which remains intact, made areas of the canyon uninhabitable for Tarahumara frogs. In addition, bullfrogs were stocked into the lake, which may have contributed to the extirpation of Tarahumara frogs from the area. The last Tarahumara frogs from the Peña Blanca population were observed in 1968 and from the Alamo Springs population in 1974 (Hale and May 1983, Hale 1992).

Life History

The life history of the Tarahumara frog is similar to that of other ranid frogs. It is thought that individuals can reproduce for the first time the second spring after they metamorphose. Breeding activities in Arizona have been observed from April to mid-May, but may have occurred in summer as well (Hale and May 1983). Adult frogs spend most of their time around plunge pools and permanent sources of water (Zweifel 1955).

Hale and May (1983) determined the mean number of eggs in an egg mass to be 1084 (SE = 161, n = 7). Larvae feed primarily on algae, but are likely omnivorous. Larvae take up to two years to metamorphose in the wild (Hale and May 1983), yet are able to metamorphose faster in captivity (Ivanyi pers. comm.). During winter months, when it is cool and dry, the frogs retreat to hibernacula. When springtime temperatures reach about 10 °C (50 F), frogs emerge from their hibernacula (Hale and May 1983). Dispersal may take place during the monsoon, with most movement along streams and limited movement over land areas (Zweifel 1955, Hale and May 1983).

Tarahumara frogs are general predators, eating almost any animal that can be swallowed. Zweifel (1955) recorded the following vertebrate prey items: juvenile mud turtle (*Kinosternon sonoriense*), Sonora chub (*Gila ditaenia*), and a black head snake (*Tantilla atriceps*) and the following invertebrate prey items: beetles (Tenebrionidae and Scarabaeidae), water bugs (Belostomatidae), sphinx moths (Sphingidae), scorpions, and centipedes (Zweifel 1955). Zweifel

(1955) also noted that prey items on this list indicated nocturnal feeding habits. In contrast, a study of 16 frog stomachs by Hale and May (1983) found no vertebrates, but both diurnal and nocturnal invertebrates. Diurnal invertebrates were represented by grasshoppers (Agrididae), a wood borer beetle (Buprestidae), praying mantis (Mantidae), and a paper wasp (Hymenoptera). Nocturnal invertebrates included a wolf spider (Lyconsidae), a moth (Lepidoptera), crickets (Gryllidae), a caddisfly (Tricoptera), and a katydid (Tettingoniidae).

Predators of Tarahumara frog eggs, larvae, and adults include large invertebrates (e.g. belostomatids), non-native fish (Centrarchidae), salamanders (*Ambystoma* sp.), bullfrogs (*Rana catesbeiana*), mud turtles (*Kinosternon* sp.), snakes, various species of birds, and medium-sized mammals (Zweifel 1955, Hale and May 1983).

Historical Management

Although populations and historical habitats of the Tarahumara frog have been actively monitored since at least 1975, there have been no management activities prior to the extirpation of the Tarahumara frog from Arizona. We are unaware of any management activities targeting the Tarahumara frog in the remainder of its range in Mexico (pers. comm. F. Abarca).

Present Status in State

Population

We used data from frequent, periodic surveys of historical localities to determine the status of the Tarahumara frog in Arizona. For other ranid frogs it has been suggested that if neither frogs nor evidence of reproduction is found for 3 consecutive years, the population has likely gone extinct (Corn and Fogleman 1984). Surveys did not occur every year since its last observation, but occurred in at least 6 years including 3 consecutive years at all localities except Peña Blanca and Tinaja canyons (Hale and May 1983, Hale and Jarchow 1988, Hale 1992, Sredl and others 1997, Arizona Game and Fish unpubl data). Tinaja and Peña Blanca canyons, which do not contain good Tarahumara frog habitat, were surveyed in 2 consecutive years during 3 and 8 years of surveys respectively. No Tarahumara frogs, tadpoles, or eggs have been seen in Arizona since 1983 (Hale and May 1983, Hale and Jarchow 1988, Hale 1992, Sredl and others 1997, Arizona Game and Fish unpubl. data).

Potential Habitat

Although no Tarahumara frogs are presently found in Arizona, suitable habitat has been documented (Hale and Jarchow 1988, Hale 1992) and confirmed (September 2000 visit by the Department, USFWS, and TFCT member) to exist. A few of the canyons formerly occupied still contain the elements of habitat critical to Tarahumara frogs. Big Casa Blanca and Sycamore canyons have excellent potential as re-establishment sites (Hale 1992, confirmed fall 2000). The habitat at Big Casa Blanca Canyon appeared to be in excellent condition during the September 2000 visit and leopard frogs and eggs were seen in other canyons in the Santa Rita Mountains. Leopard frogs had become rare in Sycamore Canyon when Tarahumara frogs were extirpated. An increase in leopard frog abundance in the mid-1980s suggests that the factors causing earlier declines at this location may be less of a threat (Hale and Jarchow 1988). In the fall of 2000, thriving leopard frogs were seen again in Sycamore Canyon (AGFD unpublished data). Alamo Canyon no longer provides suitable habitat. Floods that occurred after the frogs had been

extirpated from the canyon altered the habitat by filling the critical pools with sand. With the possible exception of Gardner Canyon (Hale 1992), the other canyons formerly occupied continue to have sub-optimal habitat (Hale and Jarchow 1988).

Other canyons, which were not known to support Tarahumara frogs historically, were investigated as potential re-establishment sites. Those canyons were classified as having poor to marginal Tarahumara frog habitat (Hale 1992).

Causes of Extirpation

The reasons for the extirpation of Tarahumara frogs from Arizona are unclear. Mechanisms that have been suggested include heavy metal toxicity, competition with other species, predation, flooding, drought, abnormally cold temperatures, and disease (Hale and Jarchow 1988). Prior attempts to identify a single mechanism that could cause declines of Tarahumara frog populations were unsuccessful. Several mechanisms might have worked synergistically to cause the die-offs (Hale and Jarchow 1988). Because the reasons of extirpation remain unknown, it is possible that factors working against survival of the species in Arizona are still present. Attempting to re-establish Tarahumara frogs will help us to differentiate between potential factors.

Recently, a fungal disease, chytridiomycosis, was implicated in declines of anurans in Australia, South America, Central America, and North America (Berger and others 1998, Pessier and others 1999). This fungus has been isolated from frogs in Arizona, including Tarahumara frogs. It is possible that chytridiomycosis was a factor in the extirpation of Tarahumara frogs, but the precise relationship of amphibian population declines and chytridiomycosis in Arizona and elsewhere is still emerging (Sredl and others 2000).

Legal Status

The Department lists the Tarahumara frog as Wildlife of Special Concern. In addition, it is a closed-season species, and take is prohibited without special permit. The frog was considered a species of special concern by the CNF, but currently is not on their sensitive species list (U.S Forest Service 1999). It has no special status under the Endangered Species Act (ESA), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), or in Mexico (NORMA).

Methodology

Effective conservation and management programs cannot take place without collaboration planning, and leadership. We recommend that the TFCT assist the Department with all activities under this proposal. The diverse backgrounds and expertise of TFCT members make the team's input an essential asset to this re-establishment project. To effectively serve this function, the TFCT must meet a minimum of two times annually. The first annual meeting, held in late winter of each year, would serve to coordinate and finalize plans for the upcoming field season. The second annual meeting, held in early fall of each year, would serve to archive or synthesize information collected during the previous field season and to formulate preliminary plans for the upcoming field season. Estimates of equipment and manpower needed are found in the implementation schedule (Table 1). Most equipment required for attempting to re-establish

Tarahumara frogs is standard for anuran studies. Any unusual equipment needs are stated in the text.

Important to a successful long-term rearing and re-establishment program is sound management of small populations, both captive and wild. Population and Habitat Viability Assessment (PHVA) and Population Viability Analysis (PVA) have become common tools used to evaluate the "health" of these populations. Among other things, PVAs and PHVAs are computer simulations that are used to systematically evaluate the relative importance of factors that may place a population at risk. These simulations use model populations with a given size and birth, death, and dispersal rates to provide insight into how management alternatives will increase or decrease extinction probability and genetic variability. These techniques, while not essential, would be useful in guiding Tarahumara frog conservation and management. PHVAs have been developed for the Houston toad and Puerto Rican crested toad. If done for the Tarahumara frog, a PHVA workshop would produce a comprehensive management plan for the Tarahumara frog. Results from a workshop would be applicable to not only Tarahumara frogs, but also other ranid frogs in Arizona.

Also important in developing a successful re-establishment program is genetic management of a captive colony. Two relevant concepts in conservation genetics are maintenance of genetic variability and prevention of genetic bottlenecks. Bottlenecking refers to the sudden loss of genetic variability and genetic drift in a small population. We can maintain genetic variability by maximizing founder population size and equalizing founder representation (Dobson and others 1991). Periodically introducing new individuals into the captive colony can avoid the deleterious effects of bottlenecking.

Study Areas

Areas of Collection

We will collect frogs, larvae, or eggs to be released or to become founders of captive colonies from northern Sonora, Mexico. Whenever possible, collection of individuals for release to the wild or for founder populations will be sufficiently large and geographically located so genetic bottlenecks will be avoided and genetic variability will be maximized (Hedrick and Miller 1992). Localities from which Tarahumara frogs will likely be collected include Sierra el Tigre, Sierra Aconchi, Sierra de la Madera, or Sierra San Luis. Some possible source populations have been resurveyed to investigate their ability to withstand removal of some frogs, larvae, or eggs (Hale and others 1998). Frogs from these populations are likely most similar genetically and ecologically to those that were extirpated from Arizona.

In the fall of 1999, Tarahumara frogs and their tadpoles were collected from the Sierra el Tigre in Mexico. Tarahumara frog eggs were collected from the Sierra de la Madera in the spring of 2000. The Sierra de la Madera location is closest to the species' former range in Arizona (Hale pers. comm.).

Areas of Rearing

Husbandry will take place primarily at the ASDM in Tucson and/or TPZ in Phoenix. Facilities are in place for rearing Tarahumara frogs from egg through metamorphosis and for the maintenance of some juveniles and adults for breeding. In addition, frogs or tadpoles may be

reared in small facilities operated by private individuals or *in situ* in temporary facilities within former Tarahumara frog habitat. The husbandry protocol at these facilities will be similar to that developed by ASDM and TPZ for other native ranid frogs (Appendix 2). Captive husbandry may take place at other localities, should the need arise. Rearing facilities would be unnecessary for individuals moved directly from sites in Mexico to release sites in Arizona, should the TFCT decide this is an appropriate technique.

Areas of Release

Initially, the proposed releases of Tarahumara frogs would take place at microsites within Big Casa Blanca Canyon. Once frogs are established in Big Casa Blanca Canyon, we would consider Sycamore Canyon for future releases (Figure 1). The canyons and microsites were chosen not only in reference to historical data on species abundance and habitat quality, but also after a recent visit to the sites by the Department, USFWS, and TFCT. Recent visits confirmed that suitable habitat still exists structurally and that potential predators or competitors are not in an abundance that would lessen the likelihood of success in translocation. These sites are within the historical range of the species and the potential for expansion into other formerly occupied sites does exist. Populations have the potential to expand into Gardner and Adobe canyons by headwater dispersal or by following stream corridors during times of favorable conditions (i.e. wet years).

The microsites selected in this proposal meet the criteria developed by the TFCT. The microsites consist of series of large, permanent plunge pools formed from both boulder and bedrock that provide appropriate refugia. The pools are at least one meter deep, have overhanging ledges, and are fairly inaccessible to casual visitors. The microsites are as near to the headwaters as suitable habitat exists, in order to minimize the risk of accumulation of contaminates. The pools do not contain bullfrogs or non-native fish, and pools within close proximity are also free of these predators or competitors. Bullfrogs have been seen in the Sycamore Canyon drainage (Hale 1992) and if they became established could negatively impact re-establishment efforts. The potential for bullfrogs to invade Tarahumara frog habitat in the far reaches of this canyon should be investigated, and control measures may be required to insure that Sycamore Canyon is not invaded.

Prior to release at microsites, preliminary research should be conducted to confirm that the chance for re-establishment success is high. Because the specific factors that led to the species' decline as well as detailed aspects of life history are unknown, it is imperative that the proposed re-establishment be designed and acknowledged as experimental. Because the factors contributing to the species' disappearance are unclear, insuring that the problems have been eliminated or minimized is impossible without experimental releases of Tarahumara frogs.

Before the release of any animals, tests of microsite suitability should be undertaken. Water quality should be investigated, including tests for heavy metals and other contaminants that could be harmful to frogs. If possible, samples should be collected and analyzed for the presence of chytrid fungus. Pending the completion of a polymerase chain reaction (PCR) probe by researchers at the University of Maine, the chytrid sampling will be a simple process (Joyce Longcore pers. comm.). The Department will be involved in testing this probe. A small sample of carefully monitored leopard frogs or Tarahumara frogs could be used as an indicator of the

habitat's suitability for Tarahumara frogs, prior to the investment of a larger scale release. At sites where Tarahumara frogs and leopard frogs had coexisted historically, all frogs were noted to decline or disappear from the sites. The leopard frog population in Sycamore Canyon may have partially rebounded in the mid-1980's (Hale and Jarchow 1988). If leopard frogs are currently present in the proximity of the microsites, their populations should be monitored to detect signs of key problems within the habitat. For example, a wintertime or post-metamorphosis die-off may indicate that chytrid fungus is present in the system.

Techniques

Collection of Stock

Prior to the collection of frogs, tadpoles, or eggs from Mexico, the proper permits were secured from Mexican authorities. Permits will need to be secured for future collections.

Although frogs, tadpoles, and eggs may be collected, it is likely that the focus will be on eggsto minimize impacts on the source and produce the large numbers of "headstarted" metamorphs needed for release. Therefore, our preferred strategy of procuring animals for release is to collect eggs from the wild. Tadpoles hatched from these eggs will be reared in captivity and released as headstarted juvenile frogs to the wild. Removal of 10% of the eggs from several egg masses should have few negative impacts (Hale and others 1998, U.S. Fish and Wildlife Service 2000). Equipment needed for the collection is minimal and inexpensive, qualified personnel are on staff or members of the TFCT.

If headstarting frogs proves impractical, we will initiate captive propagation of frogs, and possibly hormonally induce breeding to produce sperm and eggs. This technique may be desirable to increase the chances of bringing sufficient numbers of eggs into captivity. After a captive colony of frogs is established, periodic collection of sperm from wild males could be introduced to increase genetic diversity without affecting the males' genetic contribution to the wild population (Waggener and Carroll 1998). Equipment needed and techniques used in hormonal induction may be found in Waggener and Carroll (1998) and Anzalone (1998).

Transport of Stock

Minimal personnel and equipment are needed for the transport of fertile eggs, tadpoles or frogs. A detail protocol for the transport of eggs is provided as Appendix 3 and for the transport of tadpoles and frogs as Appendix 4. Should the need for induction of sperm and egg release occur, the eggs could be fertilized in the field, making the transport technique no different from that for naturally laid eggs. If only sperm is collected in the future, it should be transported in aged tap water or Ringers solution at 24 °C (75 F) to insure that it remains viable (Waggener and Carroll 1998).

Rearing / Captive Husbandry

The captive environment should mimic the release areas as much as possible, including natural cover and regimes of temperature, water chemistry, and photo-period. An approach for rearing ranid frog eggs collected from the wild to the subadult stage has been developed by TPZ (Appendix 5) and ASDM (Appendix 2). Their approach has been extremely successful in producing Ramsey Canyon and Chiricahua leopard frogs for release (Sredl and Healy 1999).

Additional species specific details of captive husbandry will be worked out at the captive rearing facilities actively undertaking this task.

If establishing a captive colony of frogs becomes problematic because of a lack of breeding activity, inducing the adults to expel eggs and sperm may be needed. This technique has been tested and proved successful for a number of anurans, including northern leopard frogs (*Rana pipiens*). The required hormone (GnRH or LnRH) is inexpensive and the technique simple (see Anzalone and other 1998, Waggener and Carroll 1998).

Once a captive colony is established with a sufficiently large founder population, a goal in maintaining healthy a captive breeding program is to equalize founder representation and avoid genetic bottlenecking (Dobson and others 1991). Two methods of captive colony management that will help attain these goals would be to periodically remove dominant males from a breeding colony and exchange individuals between colonies or the source population (Fuimera and others 2000).

Prior to release into the wild, all frogs will be given at least a unique cohort mark to identify the date and place of release by removing portions of toes (Martof 1953). Toe clipping will take place at the captive rearing facility in advance of the release. Alternatively, frogs may be individually marked with unique numbers by either toe clipping or insertion of a passive integrated transponder (PIT) tag (Camper and Dixon 1988). These marks will allow for estimations of survival rates. Although there are methods for marking larvae, because of their unreliability and high cost (Muths and others 2000), larvae will not be marked prior to release.

Diseased animals will not knowingly be released. TPZ is developing a pre-release health screening. 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. For example, prevention techniques may include submersion in a fungicide to reduce likelihood of chytrid fungus infection.

Release to the Wild

The techniques used to transport Tarahumara frogs to the release areas should be similar to techniques developed by TPZ (Appendix 4). Previous experience with other ranids and the TFCT suggest that metamorphosed frogs should be released. Recruitment from egg or tadpole to adult frog is low in the wild, less than 10% (Licht 1974), and many individuals from these stages would need to be released in order to insure that a sufficient number survive to adulthood to establish a viable population. Because the metamorph stages of anurans typically have higher recruitment to adulthood than do more juvenile stages, few individuals would need to be released to have the same number of adults ultimately. Keeping eggs and larvae in captivity until metamorphosis also may insure lower mortality rates to those stages than would occur in the wild. TPZ had a 98% survival rate of Chiricahua leopard frogs from 1 day out of the egg mass to release as juveniles (Sredl and Healy 1999). This high survival rate greatly increases the number of individuals available for release. Large numbers of adult frogs will likely not be released because of the possibility of dispersal and predation on metamorphs. Adults will be retained for breeding purposes. Previous studies of translocations of anurans have found that losses of adults are high during the first year due to mortality and/or emigration (Cooke and Oldham 1995). If

metamorphs quickly disperse from the release area into habitat that can not support populations of Tarahumara frogs, tadpoles may be released. Utility of temporary acclimation pens may be tested during releases.

The number of animals released at each microsite depends upon the capacity of the habitat to support all life stages of Tarahumara frogs and the number of individuals available from the captive rearing facilities. Animals to be released will be transported to the canyon area within cooler in vehicles. Because a trail leads to the actual canyon, people with backpacks or pack animals will move the animals to the release areas. 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 stream water into their containers.

Because mortality could be quite high in the wild and frogs have a somewhat secretive nature, many more individuals will be released than will likely be observed in follow up surveys. We set a target annual survival or re-encounter rate at 5-10%. The probability of missing individuals during surveys in combination with the natural, dramatic fluctuations through boom and bust cycles that amphibian populations experience does not make a 5-10% survival rate a failure. 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 TFCT recommends that releases take place in the late spring when water temperatures have reached at least 15 °C through early fall. While in captivity, the individuals to be released will be acclimated to temperatures at the release areas.

Monitoring Releases and Data Collection

Monitoring is a critical step in the re-establishment process. Only through a carefully designed monitoring plan will we be able to determine whether the attempt at re-establishment was successful, learn about the possible causes of the original extirpation, and formulate future plans based on the successes and failures. Both dynamics of translocated frog populations and the quality of habitat to which they were released need to be monitored. A substantial commitment of time for a number of years is needed before moving forward with this proposed re-establishment. Some researchers have suggested a monitoring time commitment of six to ten 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).

The necessity of a long-term monitoring plan becomes evident when considering how to define success in re-establishment . Success of a re-establishment should be evaluated on multiple temporal scales. Immediate or short-term success would be evaluated in the weeks following the release of animals. Ultimate success, the establishment of a self-sustaining, wild population, can not be determined until the founding population has had the time to establish and reproduce. This suggests that monitoring needs to take place for at least 3 years, in order to evaluate whether the founders' reproductive efforts are successful. Five temporal stages of success, which may be used as guidelines, were identified for Chiricahua leopard frogs (see Table 3 in Sredl and Healy 1999). Positive results of releases of other native ranid frogs in Arizona include the breeding of Ramsey Canyon leopard frogs one year after their release, the sighting of a Ramsey Canyon leopard frog 3 years after its release, and a high percentage of Chiricahua leopard frogs sighted a few months after release (Arizona Game and Fish Department unpubl. data).

Techniques used to monitor Tarahumara 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 Tarahumara frogs underscore the importance of performing nighttime VES to document the presence and abundance of all life stages (Hale and Jarchow 1988). Each microsite into which the Tarahumara frog is released should be monitored according to the same schedule.

Besides monitoring populations, we recommend monitoring habitat attributes and environmental variables at the release sites. Those that may prove particularly insightful include water quality parameters, habitat structure, presence of competitors or predators, presence of disease, and variables such as temperature and precipitation.

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. This initial monitoring should be uninvasive, yet thorough. VES should be performed during the day and/or at night every other day for the first week (Figure 2). If the numbers of frogs declines severely during the first week, corrective measures may be taken or quality of the site re-evaluated before additional individuals are released.

In order to fully understand the ability to which populations of Tarahumara frogs may be re-established, data should be collected with little interference. A die-off or behavior that could be interpreted as stress may occur. In spite of this, surviving individuals may fare well and be able to establish a self-sustaining population. After the first week, VES may be weekly for one month and then once a month until winter dormancy. Prior to winter dormancy, the effects of the monsoon rains on the released individuals and the habitat should be investigated. After the first monsoon rain, a survey should take place to determine the rain's effect on distribution and survival. Additional VES may be performed if events occur that are likely to have a measurable effect on the population (e.g. rainfall, drought, flood, and fire).

Areas surrounding the release areas should also be surveyed, so that movement into nearby habitats can be documented. Surveys of the surrounding areas should occur at least every 3 months, with particular attention paid to areas into which the frogs are likely to expand, such as Gardner and Adobe canyons. Connected habitats should be surveyed for the duration of the monitoring project.

Ideally, populations of Tarahumara frogs in Mexico should also be monitored concurrently, so that there is a comparison of how native, wild Tarahumara frog populations are faring at the same time. The monitored populations should be as near to the released frogs as possible (Sierra Madera), so as to make certain that variables such as weather are comparable. All variables monitored at the re-establishment site should be collected at the native, wild population sites as well. This comparison would allow for insight into population fluctuations and whether patterns observed in the re-established populations are a result of the translocation and/or call for concern. Members of the TFCT in cooperation with Mexican partners would be responsible for

monitoring these populations. The time and travel required to access the Mexican sites make it unlikely that such a comparison is feasible.

Duration of Study
Upon Commission approval, we recommend the duration of this project to be 10 years. However, due to uncertainties in the logistics of this project, our implementation schedule (Table 1) outlines a 5-year period. At the end of this period, an updated implementation schedule would be developed to incorporate the experience and knowledge gained during the first 5-year term.

Budget
Due to uncertainties in the logistics of this project, the budget included in our implementation schedule (Table 1) outlines a 5-year period. At the end of this period, an updated budget and implementation schedule would be developed to incorporate the experience and knowledge gained during the first 5-year period.

Coordination

Authority
Recovery Plan Tasks
Collaboration on tasks will take place will take place throughout the duration of the project. General tasks of this plan can be found in the Methodology section and implementation schedule. Compliance to environmental or other rules, regulations, and statutes will take place at the appropriate juncture and be completed by the appropriate state or federal agency.

Interagency Agreements
No interagency agreements currently exist, however the Department may consider drafting a Conservation Agreement or Candidate Conservation Agreement with Assurances in the future.

Status, Regulations, and Policies
The Tarahumara frog is considered a Wildlife of Special Concern species by The Department (Arizona Game and Fish Department in prep.). The Tarahumara frog was included by the USFWS as a category 2 Candidate for Federal listing in the 1982 Notice of Review (U.S. Fish and Wildlife Service 1982 [47 FR 58454-58460]); the species was moved to category 1 in the September 18, 1985 Notice of Review (U.S. Fish and Wildlife Service 1985 [50 FR 37958-37967]); and then reclassified as a category 2 candidate in 1994 (U.S. Fish and Wildlife Service 1994 [59 FR 58982-59028]). Since discontinuation of the category 2 list in 1996, the Tarahumara frog has had no status under the ESA. Activities in this proposal, if implemented, will help preclude the need for Federal listing. The Tarahumara frog is not afforded special status in Mexico (Secretaria de Desarrollo Social 1994), or under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

In addition to completion of the Department's 12-step re-establishment procedure (Appendix 1), which includes an Environmental Checklist, and Arizona Game and Fish Department Commission approval, this re-establishment project will require collection and export permits from the federal government of Mexico, a United States importation permit from USFWS, and state permits for possession and release of a closed season species.

Agency Roles, and Responsibilities
In addition to the Department participants in this re-establishment effort include Arizona-Sonora Desert Museum (ASDM), Coronado National Forest (CNF), Instituto del Medio Ambiente y el Desarrollo Sustentable del Estado de Sonora (IMADES), The Phoenix Zoo (TPZ), University of Arizona (UA), U.S. Fish and Wildlife Service (USFWS), and other interested parties. Representatives from these organizations and several private citizens make up the Tarahumara Frog Conservation Team (TFCT). Re-establishment activities, details of which have been described elsewhere, and agency roles and responsibilities can be found on the implementation schedule (Table 1). Funding for this re-establishment proposal was provided by a grant from USFWS.

EA / EIS Requirements
We have completed steps one through seven of the Department's 12-step re-establishment procedure. For an outline of the 12 steps, see Appendix 1. The remaining steps would need to be completed prior to release of Tarahumara frogs in Arizona. For a timeline of the remaining steps, see Table 2. ESA compliance, if appropriate, and preparation of National Environmental Policy Act (NEPA) documents would need to be completed, in addition to the 12-step re-establishment procedure (including the Environmental Checklist). Because the Tarahumara frog is not federally listed, a Section 7 consultation will only be needed if it is determined that this proposal affects another listed species. Any other state and federal administrative procedures necessary to re-establish Tarahumara frogs in Arizona are folded into the 12-step procedure.

Public Involvement
Throughout the 12-step process, the public will have several opportunities to provide input into this proposal. Although most people are unfamiliar with the Tarahumara frog, global declines in amphibian populations have received considerable press in recent years. Generally, the public is aware and concerned about amphibian conservation efforts. The Tadpole Task Force at TPZ, a volunteer group, has generated considerable interest and public involvement with other ranid frog rearing projects (e.g. Ramsey Canyon and Chiricahua leopard frog projects). We will encourage similar public involvement for the Tarahumara frog. The Department and cooperators should be able to generate additional interest and support using established channels for public education.

Conflicts / Resolutions
All proposed release sites are on CNF managed lands. CNF participates in the TFCT and has committed to support the re-establishment effort. However, the U.S. Forest Service has a multiple use mandate and Tarahumara frog habitat may be subject to impacts from such uses. Present land uses include compatible activities such as recreational hunting, camping, hiking, and livestock grazing. Livestock grazing occurs around potential re-establishment sites, and mining occurs at nearby localities. These existing uses have not severely impacted Tarahumara frog habitat and are compatible with re-establishment efforts at present levels and sites. A substantial increase in grazing or new mining operations located near (especially within the same drainage) release sites could be detrimental to the re-establishment effort. There are no recreational fisheries at any release site, thus no angler days will be lost.

Schedule of Activities

Implementation Schedule

Table 1. See next page.

Table 2. Approximate timeline for the Tarahumara frog 12 step re-establishment procedure.

Task	Completion Date
Internal AGFD review comments due; evaluate and incorporate	Jan 25, 2000
Distribute proposal with cover letter for external review (**Step 8**)	Mar 9, 2001
Initiate environmental assessment checklist (EAC)	Mar 9, 2001
Comments on draft proposal due, evaluate and revise proposal as necessary (**Step 9**)	May 4, 2001
Complete EAC (**Step 9**)	May 18, 2001
Distribute revised proposal for internal AGFD review	May 25, 2001
Internal comments due	Jun 8, 2001
Incorporate internal comments, distribute for external review (**Step 10**)	Jul 13, 2001
External comments due on final proposal	Aug 10, 2001
Summarize and evaluate comments, revise proposal as necessary (**Step 11**)	Aug 17, 2001
Submit memo and proposal to Director, through Nongame Branch Chief, for action (**Step 11**)	Aug 25, 2001
Submit Arizona Game and Fish Commission (AGFC) memo for Oct commission meeting	Aug 25, 2001
Notify AGFC at or Oct meeting of decision (**Step 12**)	Oct 20, 2001
Notify external customers (via newsletter or letter) of decision	Oct 26, 2001

Field Activities

Field activities to implement this project include 1) final evaluation of microsites, 2) collecting of stock (eggs, tadpoles, and/or frogs), 3) rearing of tadpoles to metamorphs, 4) releasing of tadpoles and/or metamorphs, and 5) monitoring of populations and habitats.

Table 1. Five-year draft implementation schedule for Tarahumara frog re-establishment in Arizona.

		TASK	DURATION	PARTY	FY 1	FY 2	FY 3	FY 4	FY 5	TOTAL
1		**Project coordination**	5+ years							
	1.a.	planning meetings, oversight	5+ years	AGFD, TFCT	$7,700	$7,700	$7,700	$7,700	$7,700	$38,500
	1.b.	data curation and analysis and report writing	5+ years	AGFD	$6,000	$6,000	$6,000	$6,000	$6,000	$30,000
2		**Collect eggs, tadpoles, frogs in Mexico**								
	2.a.	secure permits	3 years	USFWS, TFCT, AFGD						$0
	2.b.	collect stock	3 years	TFCT, IMADES	$2,000	$2,000	$2,000			$6,000
3		**Captive rearing facility**								?
	3.a.	maintain facility	2 years	ASDM, TPZ						
	3.b.	raise frogs	2 years	ASDM, TPZ	$4,300	$4,300	$4,300	$4,300	$4,300	$21,500
	3.c.	screen for disease	2 years	ASDM, TPZ, UA	$500	$500				$1,000
4		**Select microsites for reintroduction**								$5,020
	4.a.	resurvey microsites	2 years	AGFD, TFCT	$2,510	$2,510				
	4.b.	test water quality	2 years	USFWS, AGFD	$1,000	$1,000	$1,000	$1,000	$1,000	$5,000
	4.c.	test for pathogens	2 years	AGFD, TFCT	$500	$500				$1,000
	4.d.	monitor existing leopard frogs	1 year	AGFD, TFCT	$2,160					$2,160
	4.e.	complete NEPA checklist	2 weeks	CNF, TFCT	$3,400					$3,400
	4.f.	complete EA checklist	1 week	AGFD	$1,700					$1,700
	4.g.	section 7 compliance (ESA)	2 weeks	CNF, USFWS	$3,400					$3,400
			2 years	TFCT, AGFD	$1,140	$1,140				$2,280
5		**Release**	5+ years							
6		**Monitor**	5+ years	AGFD, TFCT, Volunteers	$10,950	$10,950	$4,380	$4,380	$4,380	$35,040
	6.a.	VES	5+ years	AGFD, TFCT, USFWS	$280	$280	$280	$280	$280	$1,400
	6.b.	habitat quality	5+ years	IMADES, TFCT						?
	6.c.	Mexican populations								
		TOTALS			**$47,540**	**$36,880**	**$25,660**	**$23,660**	**$23,660**	**$157,400**

Management Alternatives

Do not re-establish Tarahumara frogs in Arizona

If the Tarahumara frog is not brought into Arizona, it is unlikely that it could naturally re-establish in the foreseeable future. Known populations in Mexico are approximately 100 km distant from former Arizona localities (Hale pers. comm.) and regions of uninhabitable arid land separate the habitable mountain regions. The consequences of no action are somewhat unpredictable. Although populations of Tarahumara frogs in portions of northern Sonora in Mexico experienced die-offs at about the same time as those in Arizona, populations in central and southern Sonora are apparently doing well (Hale and Jarchow 1988, Hale and others 1995). In addition, there are still a few localities in northern Sonora that seem to be supporting viable populations (Hale 1998, Hale pers.
comm.). This indicates that the Tarahumara frog is not immediately threatened with rangewide extinction, however unexplained, sudden die-offs in populations of Tarahumara frogs in the past as well as in other amphibian species in the past and present, make the no action alternative potentially risky. Initiating re-introduction efforts and beginning to actively manage this species now may help it to persist into the future, and avoid possible federal actions.

Re-establish Tarahumara frogs as proposed

The benefits of re-establishing Tarahumara frogs in Arizona could go beyond the direct effects on Tarahumara frogs. By designing the re-establishment as experimental, we could gain insight into the causes of declines of amphibian populations and develop protocols for re-establishment and monitoring that may be applicable to other species. The re-establishment of Tarahumara frogs could be a model from which other re-establishment programs draw.

Benefits of attempting to re-establish Tarahumara frogs in Arizona could come from both successes and failures of the attempt. Whether or not the attempt at re-establishment is successful, we should better understand the needs of Tarahumara frogs, gain insight as to whether the causes of the decline are still in place at the former localities, and be better able to address the causes of the die-offs.

Success in re-establishment would increase the current geographic range of the frog, which should make the population as a whole more robust to stochastic or localized catastrophic events. In addition, should populations in Mexico decline, we may be able to manage the Arizona populations in order to avoid range wide extinction. The successful re-establishment of a native frog in Arizona would add to the state's natural heritage and bring back an important component missing from Arizona's mountain streams.

Recommendations

The Department recommend the attempt at re-establishment of Tarahumara frogs as proposed once there is a commitment to the time and resources required to implement this re-establishment as experimental research. Without following through with a long-term monitoring plan, success will not be able to be determined and the consequences of specific actions will be unknown, making it impossible to adjust management actions in order to increases the chances of success.

Literature Cited

Anzalone, C.R., L.B. Kats, and M.S. Gordon. 1998. Effects of solar UV-B radiation on embryonic development in *Hyla cadaverina, Hyla regilla,* and *Taricha torosa*. Conservation Biology 12: 646-653.

Arizona Game and Fish Department. in prep. Wildlife of Special Concern in Arizona. Nongame and Endangered Wildlife Program, Arizona Game and Fish Department, Phoenix, AZ.

Beebee, T.J.C. 1996. Ecology and conservation of amphibians. Chapman and Hall. London. 214pp.

Berger, L., R. Speare, P. Daszak, D.E. Green, A.A. Cunningham, C.L. Goggin, R. Slocombe, M.A. Ragan, and A.D. Hyatt. 1988. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Science 95: 9031-9036.

Camper, J.D. and J.R. Dixon. 1988. Evaluation of a microchip marking system for amphibians and reptiles. Texas Parks and Wildlife Department, Research Publication 7100-159. Austin, Texas.

Cooke, A.S. and R.S. Oldham. 1995. Establishment of populations of the common frog, *Rana temporaria*, and common toad, *Bufo bufo*, in a newly created reserve following translocation. Herpetological Journal 5: 173-180.

Corn, P.S. and J.C. Fogleman. Extinction of montane populations of the northern leopard frog (*Rana pipiens*) in Colorado. Journal of Herpetology 18: 147-152.

Demlong, M.J. 1997. Leopard frog husbandry outline. Unpublished protocol. The Phoenix Zoo. Phoenix, Arizona.

Dobson, A.P., G.M. Mace, J. Poole, and R.A. Brett. 1991. Conservation biology: the ecology and genetics of endangered species. Pages 405-430 *in* R.J. Berry, T.J. Crawford, and G.M. Hewitt eds. Genes in ecology. Blackwell Scientific Publications, London, England.

Fiumera, A.C., P.G. Parker, P.A. Fuerst. 2000. Effective population size and maintenance of genetic diversity in cpative-bred population of a Lake Victoria cichlid. Conservation Biology 14: 886-892.

Hale, S.F. 1992. A survey of historical and potential habitat for the Tarahumara frog (*Rana tarahumarae*) in Arizona. Report to Arizona Game and Fish Department, Phoenix, AZ and Coronado National Forest, Tucson, AZ.

Hale, S.F., F. Retes, and T.R. Van Devender. 1977. New populations of *Rana tarahumarae* (Tarahumara frog) in Arizona. Journal of the Arizona Academy of Sciences 11: 134-135.

Hale, S.F. and C.J. May. 1983. Status report for *Rana tarahumarae* Boulenger. Arizona Natural Heritage Program, Tucson. Report to Office of Endangered Species, U.S. Fish and Wildlife Service, Albuquerque, NM.

Hale, S.F. and J.L. Jarchow. 1988. Status of the Tarahumara Frog (*Rana tarahumarae*) in the United States and Mexico: Part II. C.R. Schwalbe and T.B. Johnson, eds. Report to Arizona Game and Fish Department, Phoenix, AZ and Office of Endangered Species, U.S. Fish and Wildlife Service, Albuquerque, NM.

Hale, S.F., C.R. Schwalbe, J.L. Jarchow, C.J. May, C.H. Lowe, and T.B. Johnson. 1995. Disappearance of the Tarahumara frog. Pages 138-140. *in* E.T. LaRoe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac, eds. Our living resources: a report to the nation on the distribution, abundance, and health of U.S. plants, animals, and ecosystems. U.S. Department of the Interior, National Biological Service, Washington, D.C.

Hale, S.F., G.M. Ferguson, P.A. Holm, and E.B. Wirt. 1998. Re-survey of selected Tarahumara frog (*Rana tarahumarae*) localities in northern Sonora, Mexico, in May 1998. Report to The Arizona Zoological Society and The Tarahumara Frog Conservation Team.

Hedrick, P.W. and P.S. Miller. 1992. Conservation genetics: techniques and fundamentals. Ecological Applications 2: 30-46.

Johnson, T.B. and R.L. Glinski 1989. Procedures for nongame wildlife and endangered species re-establishment projects in Arizona. Nongame and Endangered Wildlife Program Technical Report 13. Arizona Game and Fish Department, Phoenix, AZ.

Licht, L.E. 1974. Survival of embryos, tadpoles, and adults of the frogs *Rana aurora aurora* and *Rana pretiosa pretiosa* sympatric in southwestern British Columbia. Canadian Journal of Zoology 52: 613-627.

Martof, B.S. 1953. Territoriality in the green frog, (*Rana clamitans*). Ecology 34: 165-174.

Muths, E., P.S. Corn, and T.R. Stanley. 2000. Use of oxytetracycline in batch-marking post-metamorphic boreal toads. Herpetological Review 31: 28-32.

Pessier, A.P., D.K. Nichols, J.E. Longcore, and M.S. Fuller. 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). Journal of Veterinary Diagnostic Investigations 11: 194-199.

Secretaria de Desarrollo Social 1994. NORMA Oficial Mexicana NOM-059-ECOL-1994, que determina las especies y subespecies de flore y fauna silvestres terrestres y acuaticas en peligro de extincion, amenasadas, raras y las sujetas a proteccion especial, y que establece especificaciones para su proteccion. Diaro Oficial, Lunes 16 de Mayo de 1994.

Sredl, M.J. and B.L. Healy. 1999. Conservation and Management Zones: evaluating an approach to conserving Arizona populations of the Chiricahua leopard frog (*Rana chiricahuensis*).

Nongame and Endangered Wildlife Program Technical Report 149. Arizona Game and Fish Department, Phoenix, AZ.

Sredl, M.J., P.C. Rosen, and G.A. Bradley. 2000. Chytrid fungus widespread in Arizona. 80th Annual meeting of the American Society of Ichthyologists and Herpetologists, June 14-20, La Paz, MX.

U.S. Fish and Wildlife Service 1982 [47 FR 58454-58460]

U.S. Fish and Wildlife Service 1985 [50 FR 37958-37967]

U.S. Fish and Wildlife Service 1994 [59 FR 58982-59028]

U.S. Fish and Wildlife Service. 2000. Draft recovery plan for the California red-legged frog (*Rana aurora draytonii*). U.S. Fish and Wildlife Service, Portland, Oregon. 258 pp.

U.S. Forest Service. 1999. Regional forester's sensitive species list, Region 3. U.S. Forest Service. Albuquerque, NM.

Van Devender and Worthington 1977. The herpetofauna of Howell's Ridge Cave and the paleoecology of the northwestern Chihuahuan Desert. Pages 85-106. *in* R.H. Wauer and D.H. Riskind, eds. Transactions: Symposium on the biological resources of the Chihuahuan Desert, United States and Mexico. National Park Service, Washington, DC.

Waggener, W.L. and E.J. Carroll Jr. 1998. A method for hormonal induction of sperm release in anurans (eight species) and *in vitro* fertilization in *Lepidobatrachus* species. Develop. Growth Differ. 40:19-25.

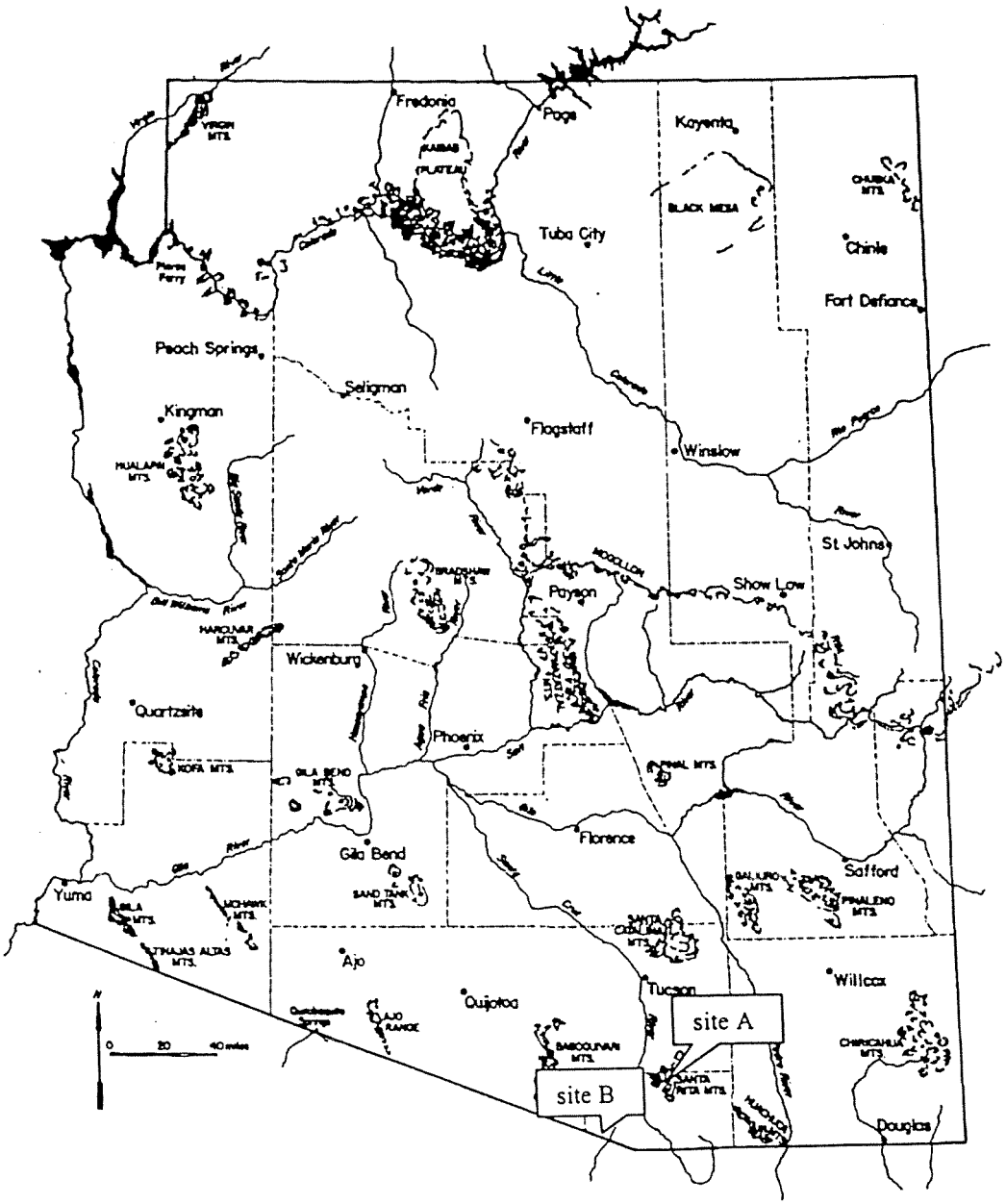
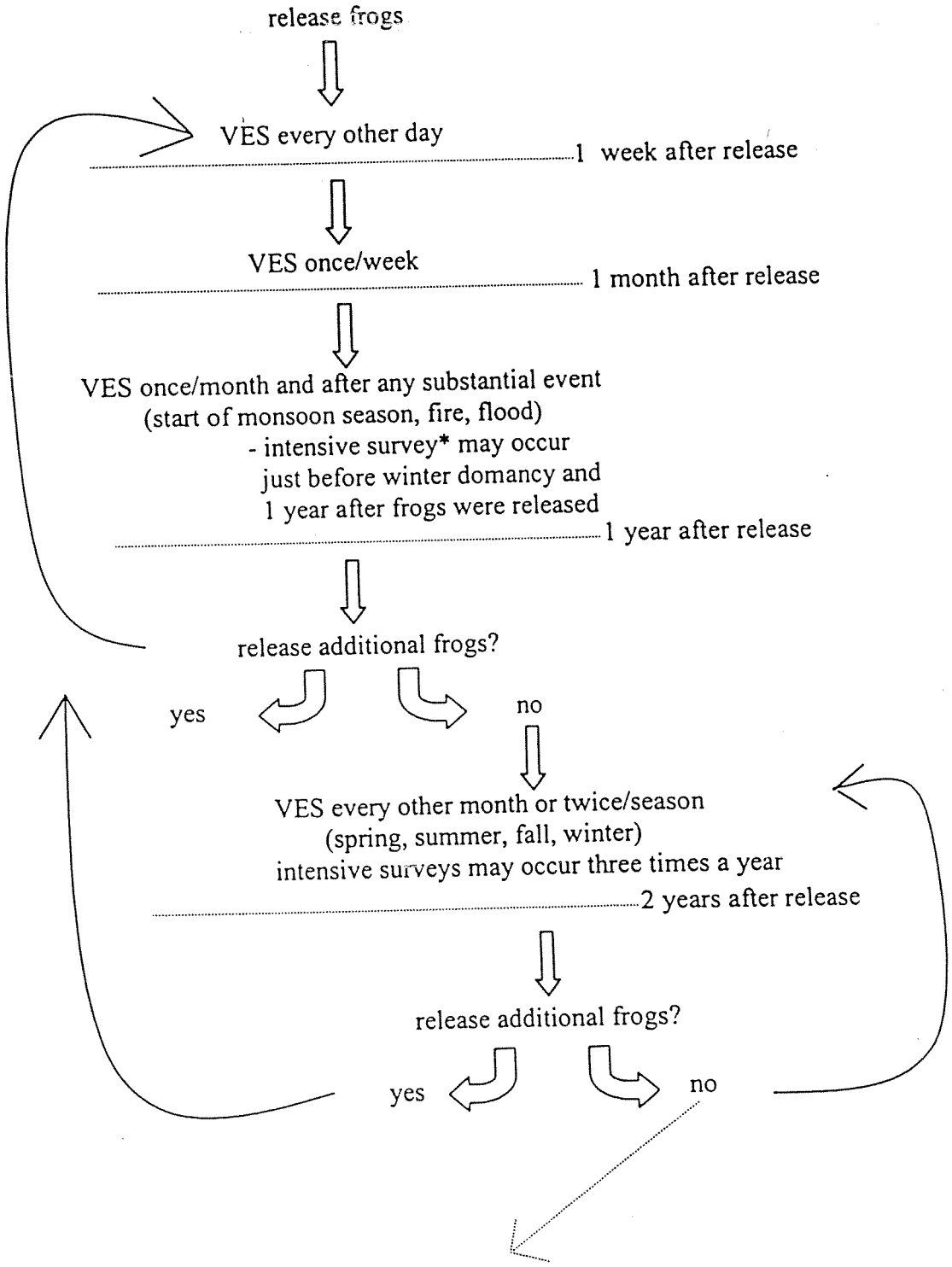


Figure 1. Map indicating proposed re-establishment areas. Site A indicates Big Casa Blanca Canyon in the Santa Rita Mountains and site B indicates Sycamore Canyon in the Pajarito Mountains.

Figure 2. Post-release monitoring schedule



* intensive survey = standard VES + capture of frogs for measuring, reading cohort marks, and taking tissue samples

Appendix 1. Schedule of activities for proposing nongame wildlife and endangered species re-establishment projects

Schedule of Activities for Proposing Nongame Wildlife and Endangered Species Re-establishment Projects

Activities for Project Originators	Function
1. Assess status of species/population available resources.	Determine feasibility of re-establishment project.
2. Complete re-establishment scorecard, submit it to Nongame Branch.	Facilitate priority ranking and preliminary review from programmatic perspective.
Activities by Nongame Branch	**Function**
3. Prepare proposal abstract, distribute it and scorecard throughout AGFD.	Elicit broad review of project and of possible conflicts or effects on other programs, projects, etc.
4. Submit briefing memo to AGFC through AGFD Director. No general press release.	Provide AGFC with background on potential project.
5. Review AGFD comments and develop project checklist. Submit summary to AGFD Director.	Identify and address any specific concerns and actions necessary to mitigate them; determine whether to proceed with, or to reject, the project.
6. Solicit comment on project concept from public and appropriate agencies, organizations.	Communicate goals, provide early awareness of intent.
7. Discuss project and public input and AGFD recommendations with AGFC.	Determine appropriate action; terminate project or proceed. Inform public of decision.
8. Prepare re-establishment proposal. Distribute for review inside-outside AGFD and submit to AGFC.	Document specifics of proposed project. Elicit philosophical, technical review.
9. Summarize comment, revise proposal and complete AGFD Environmental Checklist. If necessary, draft Environmental Assessment or Impact Statement.	Ensure NEPA compliance and requisite coordination with existing programs, projects.
10. Submit final draft project proposal for outside review and to AGFC.	Provide for peer, agency and public comment.
11. Summarize comment, review proposal. Submit final project proposal to AGFD Director for action.	Ensure policy review, compliance with procedures and determine final approval or denial of proposal.
12. Notify AGFC and public of decision.	Provide information on decision and notice of project implementation schedule.

Appendix 2. Arizona-Sonora Desert Museum Tarahumara frog husbandry protocol

06 SEPTEMBER, 2000
ARIZONA-SONORA DESERT MUSEUM
RANA TARAHUMARAE HUSBANDRY PROTOCOL

Four different setups are currently employed, one of which is outdoors while the rest are indoors. All water is received from Pima County wells, which is periodically chlorinated prior to its arrival at the Desert Museum. All water that is currently used *indoors*, is filtered with a carbon & sand filter prior to use.

CURRENT ENCLOSURE ARRANGEMENTS:

Outdoors:
A large concrete tank is filled to a depth of ~8" (volume approximately 75-100 gallons) with fresh water that is not circulated or aerated or subsequently filtered. It does receive direct sunlight and once a day it receives a ½ hr partial flush (by drain-standpipe overflow). Plastic mesh and 'egg-crate' are used for visual separation of the enclosure and also as ramps for metamorphs to elevate themselves above water level. Water temperature fluctuates daily and seasonally, but is currently 80 F. ~50 tadpoles are in this enclosure.

Indoors:

a. 4 plastic sweater boxes are filled to a depth of 3 ½" (~½ - ¾ gallon) with aged tap water and is aerated 6 hrs/day (daytime only) Water quality is checked a minimum of 1x/week (pH, nitrite, nitrate, ammonia). ~Ten tadpoles are in each container.
b. Two large, polyethylene tubs are filled with ~50 gallons of water to a depth of 20". Both have a common pump/sump system that circulates the water through undergravel and paper cartridge filters, and an ultraviolet sterilizer. Water is circulated 3hrs/2x/day and ~50% of the water is changed each week. U.V. and full-spectrum lighting is suspended above the water. Between 12-17 tadpoles are in each tub.
c. One large, polyethylene tub is filled with ~50 gallons of water with only about 6" available to the 12-17 developing tadpoles. The rest of this system circulates with the system mentioned in (b) above.

FOOD:

- 1x/day:
  - Very thinly-sliced (fresh & washed) cucumber & zucchini (cut lengthwise in ½ prior to slicing). ~1 slice/10 tadpoles.
  - Boiled egg-whites (½" chunks)
  - Fresh, baby spinach (frozen a minimum of 48 hrs. to break down cell walls)
  - Filamentous & other types of green algae (whatever grows in our aquaria, especially outdoors).
- We make sure to offer slightly more than is consumed every day and uneaten food is removed daily.

Appendix 3. Ranid frog egg mass collection and transportation protocol, developed by M.J. Demlong, The Phoenix Zoo

1) Upon observation of an egg mass at a site of interest, contact Mike Sredl (602-789-3515) to coordinate collection. If he is unavailable contact Mike Demlong (602-789-3504). The decision whether to collect an entire or partial egg mass is dependent upon re-establishment/supplementation plans, and the availability of captive rearing space. The Department will contact the participating captive rearing facility, alerting them to the approximate date and time of arrival.

2) If possible, please record the water and air temperature at the site, location of the egg mass in the pond or creek, and current and recent weather events. Forward this information with the egg mass and to Mike Sredl.

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

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

5) 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 two plastic spoons and your fingers to separate the egg mass. Place one 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.

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

7) Collect an additional 2 to 5 gallons of water from the site in clean plastic bags or plastic buckets, for captive rearing needs.

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

Call the captive rearing facility prior to departure to alert them to your estimated time of arrival.

Appendix 4. Ranid frog transportation protocol
Adapted from Demlong 1997 on December 5, 2000.

I. Transportation

A. General Container Information

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

B. Type of Containers per animal size

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

C. Preparing Containers

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

D. Stocking densities

1.Per gallon bag for short shipments.

a. Eggs: one mass per bag, minimize disturbance and division of mass
b. Larvae under ½": 25 per bag
c. Larvae 1" - 1 ½": 15 per bag
d. Larvae over 1 ½": 10 per bag
e. Recently metamorphosed frogs: 5 per container or bag

2. Avoid overcrowding

E. Water

1. Water put in the bags must be chlorine and chloramine free. Dechlorinating chemicals can be used to immediately remove chlorine.
2. Stream or pond water from which the animals originated can be used. Avoid capturing aquatic invertebrates or organic debris.
3. Another alternative is distilled water or water left uncovered for 24 or more hours. Avoid capturing decomposing food or feces.
4. For larvae, fill bags by approximately 75% or greater volume water to avoid excessive sloshing.
5. For metamorphs, juveniles, or adults place 20 ml of water with a leaf of romaine or iceberg lettuce for hiding. If transporting from the wild, use algae of leaves instead.

F. Shipping

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

G. Temperature

1. Optimal shipping temperature is a compromise between the captive and anticipated release temperature.
2. To keep animals cool in warm weather, place a 1-3 inch layer of cubed ice inside plastic bags on the bottom of an insulated ice chest. Cover the ice with a layer of plastic, then a few layers of towels, newspaper, or cardboard to insulate the animals from the direct cold. It is suggested to place a piece of foam between ice and animals, so if ice melts the animals will float instead of setting in the water.
3. A thermometer with a remote sensor inside the container can assist in monitoring the temperature while shipping.
4. Alternatively, animals could be moved in open containers if kept inside air-conditioned vehicles capable of maintaining the appropriate desired temperature.

Appendix 5. Ranid frog captive care protocol
Adapted from Demlong 1997 on December 5, 2000.

I. Containment
- A. Holding containers
  1. They should be constructed of easily disinfected materials like plastic, glass, or fiberglass.
  2. No metal containers, galvanized or not.
  3. Aquaria and plastic kiddy pools work well.
  4. Containers of cement based products are one alternative, provided they are well aged and no longer leaching alkaline.
  5. PVC or plastic pond liners are also acceptable, provided they are labeled as "fish safe" by the manufacturer.
- B. Lids
  1. All containers should have screened or solid lids to prevent larvae or metamorphs from jumping out or escaping.
  2. An alternative is to use taller containers and keep the water level low.
  3. When not being serviced, cover the holding containers with a solid cloth or other material to minimize stress on the animals.
  4. Disturbance can be minimized by setting up the holding containers in low (human) activity areas.
- C. Hiding devices
  1. Artificial floating plants provide larvae with resting and hiding places.
  2. Live plants or algae may be used if obtained from the same location as the animals, or if the plants are thoroughly rinsed and stored in tap water for 30 days.
  3. Another alternative is plastic window screen mesh they can use as rafts.
- D. Lighting
  1. Some lighting can be provided with natural sunlight or using artificial fluorescent light fixtures with full-spectrum bulbs.
  2. Ideally the fixture must be fairly close, within 12", to be effective.
- E. Inserts
  1. Holding containers can be fitted with mesh bottom inserts that contain the larvae when removed from the water. This insert is then placed into a clean container of the same size.

II. Stage specific considerations
- A. Housing-Embryos
  1. Gently aerate water in embryo holding tank with an air stone and aquarium pump.
  2. Embryo masses should be suspended off the bottom of the holding container. Plastic window screen mesh or rinsed cheese cloth material are useful for building a "hammock" underneath the embryos to suspend them in the water.
  3. Remove dead embryos or eggs covered with fungus from the mass if possible with minimal disturbance.
  4. Stocking density: one embryo mass per container (10 gallon aquarium).

B. Housing-Larvae

1. Undergravel filters, filter sponges, and external filters help keep the holding containers cleaner but are not substitutions for water change.
2. Stocking density:
    a. for maximum growth 25-50 per 10 gallon aquarium,
    b. 100-350 per kiddy pool (39" diameter by 7" tall) or
    c. 100-300 per 200 gallon container.

C. Housing-Metamorphs

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

III. Diet

A. Type of food for larvae:

1. Larvae feed well on spinach. Fresh spinach bunches that are frozen overnight or boiled are superior to fresh or canned.
2. They will also feed on fresh spinach, but it must be weighted down to the bottom of the tank.
3. It is also helpful to weigh down the frozen greens.
4. Blanched romaine lettuce, mustard greens, turnip greens, cucumber slices, duckweek (*Lemma* sp.), spirulina type fish foods (good for younger larvae), peas and alfalfa-based rabbit pellets are also taken.
5. Bok Choy and Kale are not recommended, it doesn't break down enough for them to eat when frozen.
6. For protein bloodworms and egg whites (hard-boiled) work well and do not carry the parasites found in aquacultured reared fish.
7. Algae wafers mold quickly, so use sparingly.
8. Another item to supplement their diet is calcium.
9. Rocks covered with algae or floating filamentous algae is a great source of natural food.
10. Preparing food:
    a. Fresh greens and vegetables must be thoroughly rinsed before being fed to remove soil, and residual pesticides or herbicides.
    b. When algae is being used it should be cultivated in uncontaminated water to avoid the risk of introducing parasites and disease.
    c. Food can be provided to the larvae free choice, or fed once, twice, or a few times a day. Food should not sit longer than 24 hours in the tanks.

B. Types of food for metamorphs and juveniles:

1. They feed well on commercially reared crickets, mealworm larvae, and adult beetles, and flightless houseflies.

2. In outdoor open air facilities a black light can be hung near the edge of the pond to attract wild night flying insects. The light should be hung low enough to the ground so the frogs can easily catch the flying insects, but high enough to attract insects from a distance.

III. Water Quality and Changing Schedule

A. Changing Schedule

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

B. Water Quality

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

C. Air Quality

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