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# Reintroduction of the Tarahumara Frog (*Rana tarahumarae*) in Arizona: Lessons Learned

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*Abstract*.—**The Tarahumara Frog (*Rana tarahumarae*) disappeared from the northern edge of its range in south-central Arizona, USA, after observed declines and die-offs from 1974 to 1983. Similar declines were noted in Sonora, Mexico; however, the species still persists at many sites in Mexico. Chytridiomycosis was detected during some declines and implicated in others; however, airborne pollutants from copper smelters, predation, competition, and extreme weather may have also been contributing factors. We collected Tarahumara Frogs in Sonora for captive rearing and propagation beginning in 1999, and released frogs to two historical localities in Arizona, including Big Casa Blanca Canyon and vicinity, Santa Rita Mountains, and Sycamore Canyon, Atascosa Mountains. Releases in the Big Casa Blanca Canyon area began in 2004 and frogs prospered and reproduced there initially but were much reduced in numbers by late 2007 after post-fire flooding and sedimentation of breeding pools and chytridiomycosis die-offs. We released additional frogs during 2012–2015, and small numbers of Tarahumara Frogs have continued to persist at this site and an adjacent drainage through 2019. At Sycamore Canyon, we began releases of Tarahumara Frog in 2014, and small numbers have persisted into 2019 in a tributary canyon. We also established a population in 2002 at Kofa National Wildlife Refuge in western Arizona as a refugium and source of animals for reintroduction. As of 2019, it still persists as a robust breeding population. We discuss our methods for reintroducing Tarahumara Frogs, problems, adaptive management, and future options for reintroduction of this species into Arizona.**

*Key Words*.—amphibian; conservation; Sonora; declines; chytridiomycosis

## Introduction

Amphibians the world over are declining as a result of habitat degradation and loss, climate change, introduced species, emerging infectious diseases, and other factors (Green 2005; Halliday 2005; Whittaker et al. 2013; Thompson et al. 2016; O'Hanlon et al. 2018). An immediate conservation response for many species has been the establishment of *ex-situ* populations that are buffered from the factors causing declines in the wild (Gascon et al. 2007; Biega et al. 2017). While the ultimate conservation goal should be reintroductions *in situ* to create self-sustaining wild populations, if successfully implemented, *ex-situ* populations are insurance that in the future when threats in the wild have been remedied or have abated, a source of animals will be available for reintroduction (Canessa 2017).

The International Amphibian Conservation Action Plan of the International Union for the Conservation of Nature (IUCN), Species Survival Commission, Amphibian Specialists Group (Action Plan; Wren et al. 2015) provides guidance for conservation planners and managers in regard to establishment of *ex-situ* populations, assessment and abatement of threats, and reintroduction of wild populations of amphibians. The Action Plan recommends that an important early step in species recovery is to understand and either neutralize, mitigate, or manage threats to the species and its habitat. Following release of amphibians back into suitable habitats, monitoring is needed to identify problems and formulate course changes necessary to establish viable, wild populations. The Action Plan notes that although our understanding of threats to amphibians has grown considerably over the last two decades, such understanding may not be adequate to ensure successful reintroduction of species given issues that are difficult to control, including disease and invasive species, and predicted effects of climate change, which could

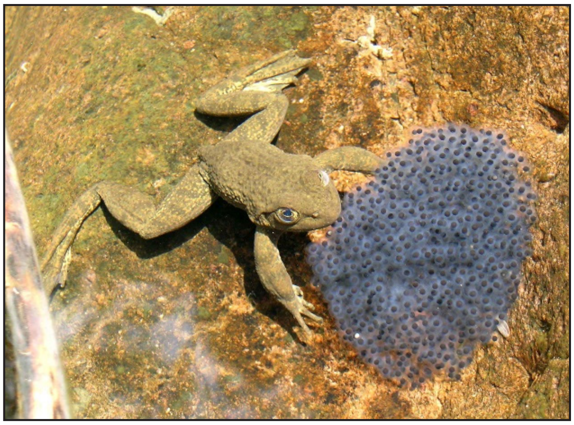

**Figure 1**. Adult Tarahumara Frog (*Rana tarahumarae*) with egg mass, Big Casa Blanca Canyon, Arizona, USA, 6 May 2005. (Photographed by James Rorabaugh).

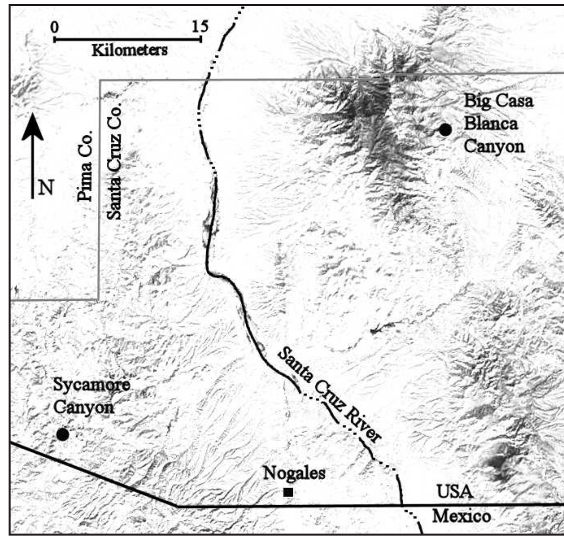


**Figure 2.** Locations of the two primary reintroduction sites, Sycamore and Big Casa Blanca canyons, in southern Arizona, USA. Dot-dash lines on the Santa Cruz River indicate intermittent flow. Solid line is perennial flow.

exacerbate existing threats.

The Tarahumara Frog (*Rana tarahumarae*) is a relatively large ranid frog (< 114 mm snout-vent length [SVL]; Fig. 1) and a habitat specialist in rocky montane arroyos and plunge pools from south-central Arizona, USA, south to southwestern Chihuahua and northeastern Sinaloa, Mexico (Hale et al. 1995; Rorabaugh and Lemos-Espinal 2016). It is categorized as a Tier 1A Species of Greatest Conservation Need by the Arizona Game and Fish Department (2012) and is listed as Vulnerable by the IUCN Red List (IUCN 2020). In Arizona, it was historically known from four drainages in the Pajarito-Atascosa-Tumacacori mountain range complex (Sycamore, Peña Blanca, Alamo, and Tinaja canyons) and three drainages in the Santa Rita Mountains (Big Casa Blanca, Adobe, and Gardner canyons) of Santa Cruz County (Fig. 2; Rorabaugh and Hale 2005). The degree to which frogs at these sites were isolated from one another is unknown. The greatest number of specimens in Arizona come from Sycamore Canyon in the Atascosa Mountains likely due to a combination of suitable habitat, accessibility, and frequency of visits by scientists. Although the precise extent and location of suitable habitat historically within Sycamore Canyon are unknown, we used collection records to provide some insight into where Tarahumara Frogs occurred. For instance, Charles H. Lowe collected a series of 17 Tarahumara Frogs that he listed as occurring 0.25–1.5 mi below Yank Spring in Sycamore Canyon on 6 September 1953 (University of Arizona specimens 20928–20947), and others collected in the same vicinity (VertNet records, http://portal.vertnet.org/search, accessed July 2019). If suitable habitat and Tarahumara Frogs occurred elsewhere in the canyon, no collections were made in those areas. In 1974 in the Santa Rita Mountains, Hale et al. (1977) discovered a population along 4.8 km of Big Casa Blanca Canyon that consisted of about 500 adult frogs from 1975–1977 (Rorabaugh and Hale 2005).

Declines and die-off of Tarahumara Frogs in Arizona were first noted in 1974 in Sycamore Canyon and the species was not seen there in subsequent years (Hale et al. 2005; Rorabaugh and Hale 2005). Prior to recent reintroduction efforts, the last wild Tarahumara Frog observed in Arizona was found dead in Big Casa Blanca Canyon in May 1983 (Hale et al. 2005). Lowland Leopard Frogs (*R. yavapaiensis*) and Chiricahua Leopard Frogs (*R. chiricahuensis*) declined as well in Sycamore Canyon (Hale et al. 1995), although Lowland Leopard Frogs occurred there until 2010 and Chiricahua Leopard Frogs were still present at the site in 2020 (Audrey Owens, pers. obs.). During die-offs in Big Casa Blanca Canyon (1977–1983), Tarahumara Frogs were sometimes observed to be lethargic or moribund, and individuals held overnight in cloth bags frequently died (Hale et al. 1995, 2005). Although less is known about the decline and disappearance of Tarahumara Frogs from the other Arizona sites, the species was last observed in 1948 in Tinaja Canyon, Tumacacori Mountains, and in 1970 in Alamo Canyon. Robert Stebbins did not find the species at Peña Blanca Spring in 1950 and they were not found there after that time (Rorabaugh 2013). Tarahumara Frogs were last seen in Gardner Canyon in 1977 and Adobe Canyon in 1974 (Stephen Hale, pers. obs.)

In Sonora, Mexico, similar die-offs and declines or extirpations were observed at five sites, beginning in 1981 at Arroyo La Carabina, Sierra El Tigre (Hale et al. 2005). Prior to the recognition of chytridiomycosis, caused by the pathogen *Batrachochytrium dendrobatidis* (*Bd*), as a significant factor in amphibian decline (Berger et al. 1998), declines of Tarahumara Frogs in Arizona and Sonora were attributed to airborne pollutants from copper smelters, predation, competition, flooding, drought, disease, and or winter cold (Hale et al. 1995; Rorabaugh and Hale 2005). In retrospect, die-offs observed in Big Casa Blanca and Sycamore canyons in Arizona, and Arroyo La Carabina in Sonora, were consistent with die-offs as a result of chytridiomycosis, and in fact, histologic examinations of Tarahumara Frogs from the latter two sites were positive for chytridiomycosis and *Bd* was strongly suspected at Big Casa Blanca Canyon (Hale et al. 2005). Thus, chytridiomycosis likely contributed to the observed declines and extirpations of Tarahumara Frogs in Arizona and northwestern Mexico, although many of the aforementioned factors may have been important as well. Given the disappearance of the Tarahumara Frog from the U.S., while populations remained extant in Mexico, in 1992 we began a project to reintroduce the species into its historical range in Arizona. Herein, we discuss our methods and strategies for reintroducing the species, problems encountered, adaptive management to counter those problems, an assessment of success in meeting our project objectives, and future options to ensure successful reintroduction of this species in Arizona.

## Materials and Methods

The Tarahumara Frog Conservation Team (TFCT) was formed in June 1992 with the objective of reintroducing the Tarahumara Frog into one or more sites in Arizona using stock from Mexico (Rorabaugh et al. 2005). The TFCT consisted of federal and state agency biologists, land managers, university herpetologists, and experts in amphibian husbandry from the Arizona-Sonora Desert Museum (ASDM) in Tucson, Arizona, USA. Later, biologists from Sonora, Mexico, were added to the team. Progress was slow until the team secured grant funding in 1998, which allowed for the development of a detailed reintroduction plan (Field et al. 2004) and assessment of potential source populations in Sonora. The TFCT subsequently completed environmental compliance, identified appropriate source populations, and ultimately collected animals for propagation and release. We examined the environmental impacts of the project using procedures outlined in the Arizona Game and Fish Department 12-step Re-establishment process (Johnson and Glinski 1989), the U.S. Endangered Species Act (for potential adverse effects to listed species and critical habitat), and the U.S. National Environmental Policy Act (in the form of an Environmental Assessment and Finding of No Significant Impact) prior to releasing frogs. In addition, we sought and obtained permits from the Secretaría de Medio Ambiente y Recursos Naturales to collect and export the frogs from Mexico and we also obtained appropriate documentation from the U.S. Fish and Wildlife Service to import them into the USA.

The reintroduction proposal called for a cooperative effort among the participants of the TFCT to: (1) obtain stock of Tarahumara Frogs from Sonora, (2) reintroduce populations of the frog from imported stock into Big Casa Blanca and Sycamore canyons, (3) monitor the releases and adapt management as needed to ensure the reintroductions were successful, and (4) continue coordination through the TFCT to ensure all issues and concerns were addressed (Field et al. 2004). The goal of the proposal was to reintroduce Tarahumara Frogs to establish self-sustaining populations in Arizona, although the proposal did not state quantitative success criteria. The Arizona Game and Fish Department distributed the reintroduction proposal in draft form for public review in 2003 and revised and finalized it based on that review. The reintroduction proposal identified Big Casa Blanca and Sycamore canyons as the two best sites for reintroduction because they had supported the largest populations and best habitat for the species historically and habitat still appeared suitable. Chytridiomycosis, however, was present in Chiricahua Leopard Frogs in Sycamore Canyon and the proposal noted that American Bullfrogs (*Rana catesbeiana*) had recently invaded that site. As a result, the decision was made to begin with reintroduction to Big Casa Blanca Canyon. Sycamore Canyon would be considered at a later date if American Bullfrogs could be eradicated.

To begin to establish a captive population, we collected four adult Tarahumara Frogs and 30 small tadpoles in October 1999 from Arroyo El Tigre, Sierra El Tigre, Sonora, and brought them into captivity at the ASDM for rearing. The four adult frogs died within days of capture and the tadpoles began dying within eight weeks of capture; at week 12, all were dead. Wild frogs from this canyon were later determined by Hale et al. (2005) to be positive for *Bd* and the stress of capture and captivity may have predisposed them to mortality from chytridiomycosis (Rorabaugh et al. 2005). This fungal skin disease had only recently been recognized as a factor in amphibian declines (Berger et al. 1998) and the TFCT had not yet fully understood the possible effects to the frog reintroduction project. In the reintroduction proposal, chytridiomycosis was recognized as a possible factor in the decline of Tarahumara Frogs in Arizona and Sonora, but the precise relationship of amphibian population declines and chytridiomycosis in Arizona and elsewhere was only beginning to emerge. Moreover, the proposal concluded that because of the uncertainties, the factors that led to the extirpation of the species in Arizona could still be operating and could thwart reintroduction efforts (Field et al. 2004).

Following this unsuccessful attempt to establish an *ex-situ* population, we attempted to establish a captive population again and collected a portion of a Tarahumara Frog egg mass (estimated at 850–900 eggs) in May 2000 from Arroyo El Chorro, Sierra de la Madera, Sonora, and transported it to the U.S. Fish and Wildlife Service, Phoenix, Arizona, for initial rearing. This was a desirable collection locality because it is in the same river drainage (Río Concepción) as one of the reintroduction sites (Sycamore Canyon) and is also the nearest known Tarahumara Frog population to historical sites in Arizona. Resulting tadpoles thrived and we transferred them to several sites within Arizona, as well as to the National Amphibian Conservation Center at the Detroit Zoo (USA) for rearing and propagation. We successfully established captive breeding colonies at San Bernardino National Wildlife Refuge (NWR) in the USA and the ASDM. We established a semi-wild population at Horse Tanks, Kofa NWR, USA. That site is notable in that it is a natural site contained by location, i.e., an isolated aquatic site in an otherwise very arid, low elevation (540 m) Sonoran Desert mountain range. In other respects, however, the tinajas (rock-bound pools) at the site resemble Tarahumara Frog habitat in Arizona and Sonora. To establish another refugial population, we moved 200 frogs in 2007 from the Kofa NWR to outdoor ponds at the International Wildlife Museum in Tucson, Arizona. Staff at ASDM prepared a husbandry protocol, which was included as an appendix to the reintroduction proposal (Field et al. 2004).

We augmented our captive population by adding additional animals from a site in east-central Sonora (Rancho El Trigo, Sierra Madre Occidental). We made these augmentations for two reasons. First, the frogs had been persisting at this site in the presence of *Bd* since at least 1982 (Hale et al. 2005) and they therefore could have some resistance to chytridiomycosis. Tarahumara Frogs from the Sierra de la Madera tested negative for *Bd* and no die-offs were observed there, so that population may have been naïve to the disease (Hale et al. 2005). Second, we wanted to increase the genetic diversity in our founder populations as all of the Sierra de la Madera animals originated from a single egg mass. We collected 50 late stage tadpoles in October 2008 from Rancho El Trigo, and ASDM staff reared and propagated them in an enclosure separate from the Sierra de la Madera stock.

To provide some insight into *Bd* presence in Big Casa Blanca Canyon, we swabbed 10 Canyon Treefrogs (*Hyla arenicolor*) from that canyon in May 2004, prior to the first releases of Tarahumara Frogs. We swabbed additional Canyon Treefrogs, post-release, in June and July 2004. We also swabbed five Tarahumara Frogs from the Kofa NWR refugium and five frogs from the ASDM in March and June 2004, respectively. Pisces Molecular LLC, Boulder, Colorado, USA, evaluated those swabs with polymerase chain reaction (PCR) tests. Methods used by Pisces before 2013 conformed to Annis et al. (2004). Beginning in 2013, Pisces employed a qPCR test developed in-house using the regular Taqman kit (John Woods, pers. comm.).

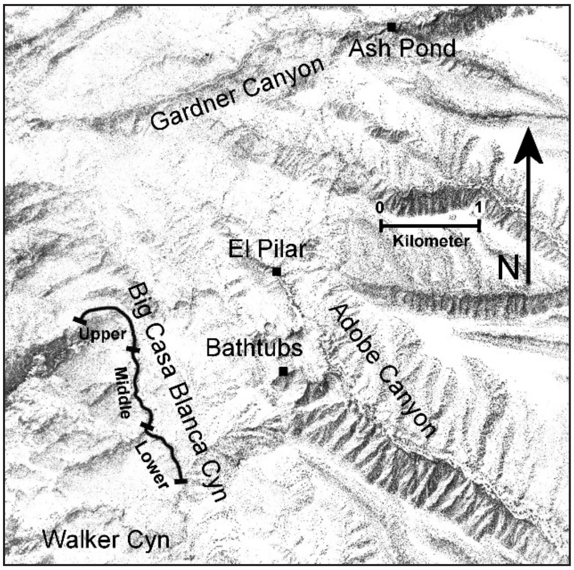


FIGURE 3. Big Casa Blanca Canyon and vicinity in southern Arizona, USA. Upper, Middle, and Lower refer to reaches discussed in the text.

During initial releases, we treated all frogs prophylactically for chytridiomycosis by placing them in a bath of itraconazole (diluted to 0.01% in 0.6% saline solution) for one hour. We did not prophylactically treat tadpoles because, through trial and error, we discovered mortality was unacceptably high. Beginning in 2012, we shifted from prophylactically treating all frogs before release to conducting a captive facility risk assessment to determine if treatment was necessary. The risk assessment followed guidelines outlined in Pessier and Mendelson (2010) and included biosecurity measures and husbandry practices to maintain a low-risk facility. In addition, we tested water from each captive facility for *Bd* prior to releases following guidelines in Hyman and Collins (2012).

In June 2004, we released 47 adult (≥ 65 mm SVL), 138 juvenile (< 65 mm SVL), and 229 larval Tarahumara Frogs from the ASDM and Kofa NWR to four sites in Big Casa Blanca Canyon (Hale 2004; Rorabaugh 2005; Table 1). The four sites were distributed within each of three reaches of the canyon (Fig. 3). All frogs and tadpoles originated from the Sierra de la Madera stock. We placed frogs in plastic containers and transported them in backpacks cooled with ice packs and wet towels during the 4.3-km hike to the release sites. We transported tadpoles in 3.8 L containers filled with water and equipped with battery-operated aerators. Additional stock from the Sierra de la Madera was released by us into the canyon in 2005 and 2006, and we released

**Table 1**. Releases of Tarahumara Frogs (*Rana tarahumarae*) into Arizona, USA, reintroduction sites in 2004–2019 showing year and month of the release, and the number of egg masses, tadpoles, juveniles, and adults released. Sites names refer to canyons except El Pilar Tank. Parentheses indicate the source population was Rancho El Trigo, Sonora, Mexico. Numbers without parentheses indicate the source was the Sierra de la Madera, Sonora, Mexico.

| Site | Year/Month | Egg Masses | Tadpoles | Juveniles | Adults |
|---|---|---|---|---|---|
| Big Casa Blanca | 2004/6 | 0 | 229 | 138 | 47 |
| Big Casa Blanca | 2004/8 | 0 | 0 | 52 | 9 |
| Big Casa Blanca | 2004/10 | 0 | 99 | 0 | 39 |
| Big Casa Blanca | 2005/5 | 0 | 0 | 3 | 54 |
| Big Casa Blanca | 2005/10 | 0 | 0 | 1 | 5 |
| Big Casa Blanca | 2006/7 | 0 | 200 | 52 | 0 |
| Adobe | 2012/6 | 0 | (100) | 0 | 0 |
| El Pilar Tank | 2012/6 | 0 | (100) | 0 | 0 |
| Big Casa Blanca | 2012/9 | 0 | (215) | (20) | 0 |
| Adobe | 2013/5 | 0 | (200) | 0 | 0 |
| Big Casa Blanca | 2013/6 | 0 | (360) | (700) | 0 |
| Adobe | 2013/8 | 0 | 0 | (62) | 0 |
| Big Casa Blanca | 2013/8 | 0 | 0 | (671) | 0 |
| Sycamore | 2014/10 | 0 | (279) | (285) | 0 |
| Big Casa Blanca | 2015/4 | 0 | 0 | 21 | 43 |
| Sycamore | 2015/9 | 0 | (99) | (456) | 0 |
| Big Casa Blanca | 2015/10 | 0 | 0 | 9 | 91 |
| Sycamore | 2019/4 | (2), 1 | 0 | 0 | 0 |

animals from Rancho El Trigo in 2012, 2013, and 2015. Stock from Rancho El Trigo also was released by us to the Bathtubs and El Pilar Tank in Adobe Canyon (Fig. 3) in 2012 and 2013; these sites are adjacent to Big Casa Blanca Canyon (1.75 and 2.05 km, straight-line distance, respectively) and were considered sites to which frogs from Big Casa Blanca Canyon might disperse, although suitable habitat was limited in extent. We list dates, numbers, life stage, and source stock for all releases (Table 1). During 2004–2006, we toe clipped all frogs released to Big Casa Blanca Canyon with a unique cohort mark that indicated the year of release.

In October 2014, after eradication of American Bullfrogs from Sycamore Canyon (McCall et al. 2017), we released into that canyon 285 large juvenile (mostly 40 to 60 mm SVL) Tarahumara Frogs and 297 tadpoles reared from animals collected at Rancho El Trigo. Methods were the same as those employed in the Big Casa Blanca Canyon releases. We released additional animals into Sycamore Canyon in 2015 and in 2019 we placed three egg masses from the Rancho El Trigo and Sierra de la Madera stocks into pools in a tributary side canyon (Table 1; Fig. 4). Release sites were in a relatively short section of the canyon where the deepest and most permanent pools occur, 2.16 to 2.31 km (straight-line distance) SSW of the Ruby Road crossing of Sycamore Canyon, including tinaja pools in the tributary side canyon (Fig. 4). We chose this section because it contained suitable habitat and was in the downstream portion of the canyon in which Charles H. Lowe collected the series of 17 frogs in 1953, and others collected in the same vicinity (VertNet records, *op. cit.*).

We used visual encounter surveys, usually conducted during the day, but some surveys were done at night, to monitor reintroduction sites. We also measured water and air temperature, periodically measured pH and conductivity (mS/cm), and took notes on habitat, including vegetation, water levels, and sedimentation; methods were consistent with protocols in Appendix E of U.S. Fish and Wildlife Service (2007). We monitored Tarahumara Frogs in Big Casa Blanca Canyon beginning in June 2004 and continuing through June 2019 (Appendix 1). We divided Big Casa Blanca Canyon into lower, middle, and upper reaches (Fig. 3), and monitored those reaches separately to provide a more in-depth assessment of frog occurrence and numbers within the canyon. We conducted additional monitoring at the Bathtubs and El Pilar Tank in Adobe Canyon during 2008–2015, and Gardner Canyon during 2013–2018 (Fig. 3). Tarahumara Frogs

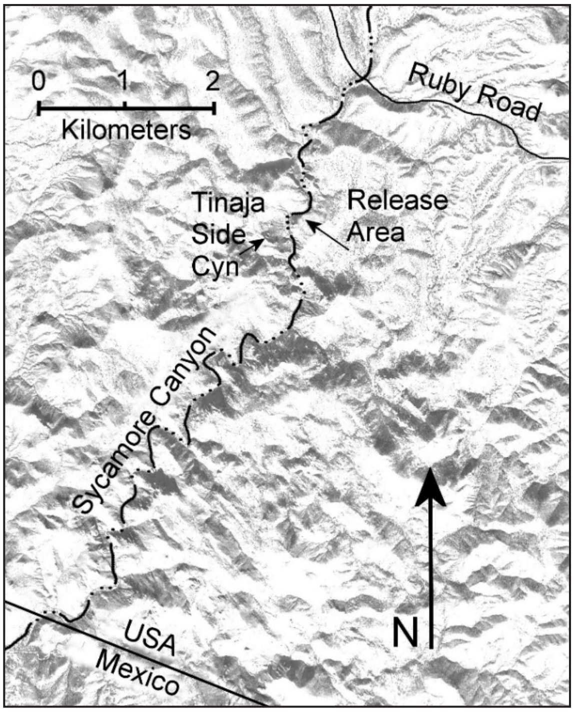


**Figure 4**. Sycamore Canyon and vicinity in southern Arizona, USA. Dot-dash line indicates intermittent flow, although there are perennial pools, particularly in the release area.

were occasionally found in Gardner Canyon during 1974–1977 by Stephen Hale, thus it was considered a possible dispersal site. Our monitoring of Sycamore Canyon began in November 2014 and continued through April 2019 (Appendix 1). At the Kofa NWR refugium, we also conducted monitoring at least twice a year from 2002 through 2019. We monitored frogs at the International Wildlife Museum at least four times each year. During all monitoring, we implemented disease prevention protocols in accordance with the latest Declining Amphibian Populations Task Force (DAPTF) Fieldwork Code of Practice (http://www.amphibianark.org/wp-content/uploads/2018/07/The-DAPTF-Fieldwork-Code-of-Practice.pdf); however, we disinfected our gear with a diluted quaternary ammonia solution or Virkon S (LANXESS Corporation, Pittsburgh, Pennsylvania, USA) instead of ethanol as recommended in the Code of Practice. We periodically swabbed and tested Tarahumara Frogs and at times, other anurans, for presence of *Bd.*

The reintroduction proposal also called for surveying other sites in southeastern Arizona, including other historical Tarahumara Frog localities, for suitable reintroduction sites. We visited historical Tarahumara Frog localities in Arizona, as well as many other aquatic sites within the range of the frog. We assessed these sites for Tarahumara Frog habitat characteristics, including deep (> 1 m), perennial plunge pools in bedrock or boulder-strewn canyons with low mean ephemeral or perennial flows between the plunge pools, but where gradients were relatively steep (Hale et al. 1995; Rorabaugh and Hale 2005).

## Results

Releases were initially successful, in that mortality of tadpoles and frogs during transport and release was very low (2% or less). During releases, we observed small numbers of frogs and tadpoles predated by Giant Water Bugs (*Lethocerus medius*) in Big Casa Blanca Canyon and some tadpoles were taken by Sonora Chub (*Gila ditaenia*) in Sycamore Canyon, but initial predation loss appeared minimal. We did not assess longer-term loss due to predators.

***Big Casa Blanca Canyon*.—**Our releases from June 2004 through October 2006 showed initial success based on 2004–2006 surveys during which we found up to 73 adults, 37 juveniles, hundreds of tadpoles, and multiple egg masses (Appendix 1). We found no evidence of disease (dead or dying frogs), and no Canyon Treefrogs (n = 17) tested positive for *Bd* during that period. We tested no Tarahumara Frogs for *Bd* until 2007 (Table 2). The lightning-caused Florida Fire started on the north end of the Santa Rita Mountains in July 2005, burning 9,382 ha, including much of the headwaters of Big Casa Blanca Canyon, although the canyon itself was spared. During an August 2005 monitoring trip to the canyon, we noted evidence of a large flood event and some sedimentation of pools. We found only nine Tarahumara Frogs during that trip, but numbers rebounded in October (59 adults) and through 2006 (as many as 73 adults), and we found four egg masses in May 2006 (Appendix 1). Loss of pool habitat due to sedimentation was minimal until the summer of 2007 when summer rains and associated flooding deposited much sand and gravel throughout Big Casa Blanca Canyon, filling in some of the largest and deepest pools. Pools in the upper reach of the release area had not been very large or productive for Tarahumara Frogs (< 1 frog per monitoring visit) even prior to the fire, but post-fire sedimentation reduced habitat even further and, thus, we curtailed monitoring in that reach, for the most part, after 2007.

In March 2007, we found 98 dead adult Tarahumara Frogs in the lower and middle reaches of Big Casa Blanca Canyon. We found an additional four frogs dead in early May 2007, but during that trip, we also found four live frogs, eight egg masses, and over 1,600 tadpoles. We tested eight Tarahumara Frogs for *Bd* from the March 2007 trips; four were positive and four were negative. In May 2007, two Tarahumara Frogs tested positive and two were negative. We also tested three

**Table 2**. Results of tests for *Batrachochytrium dendrobatidis* from water and anurans at reintroduction sites in Arizona, USA. Site names refer to canyons except El Pilar Tank and Horse Tanks.

| Site | Year/Month | Species/# individuals | Results/#individuals |
|---|---|---|---|
| Big Casa Blanca | 2004/May | *Hyla arenicolor*/10 | Negative/10 |
| Big Casa Blanca | 2004/June | *Hyla arenicolor*/4 | Negative/4 |
| Big Casa Blanca | 2004/July | *Hyla arenicolor*/3 | Negative/3 |
| Big Casa Blanca | 2007/March | *Rana tarahumarae*/8 | Negative/4, Positive/4 |
| Big Casa Blanca | 2007/May | *Rana tarahumarae*/4 | Negative/2, Positive/2 |
| Big Casa Blanca | 2007/May | *Hyla arenicolor*/3 | Negative/3 |
| Big Casa Blanca | 2007/December | *Rana tarahumarae*/2 | Positive/2 |
| Big Casa Blanca | 2008/May | *Hyla arenicolor*/2 | Positive/2 |
| Big Casa Blanca | 2009/May | *Hyla arenicolor*/2 | Positive/2 |
| Big Casa Blanca | 2013/October | *Rana tarahumarae*/10 | Positive/10 |
| Big Casa Blanca | 2013/October | *Rana yavapaiensis*/1 | Positive/1 |
| Bathtubs - Adobe | 2013/October | Water/1 | Negative/1 |
| Adobe | 2008/October | *Hyla arenicolor*/3 | Negative/2, Failed/1 |
| El Pilar Tank | 2009/May | *Rana tarahumarae*/1 | Negative/1 |
| Horse Tanks | 2004/March | *Rana tarahumarae*/5 | Negative/5 |
| Horse Tanks | 2009/March | *Rana tarahumarae*/3 | Negative/3 |
| Sycamore | 2009/February | *Hyla arenicolor*/1 | Positive/1 |
| Sycamore | 2009/February | *Rana chiricahuensis*/1 | Positive/1 |
| Sycamore | 2014/December | *Rana tarahumarae*/2 | Negative/1, Positive/1 |
| Gardner | 2012/August | *Rana tarahumarae*/1 | Negative/1 |
| Gardner | 2014/April | *Rana tarahumarae*/1 | Negative/1 |

Canyon Treefrogs in May 2007; they were negative for *Bd* (Table 2). Despite the initial survival of frogs after the March mortality event (Appendix 1), we found only three frogs in September 2007. After that, we found only one other frog (an adult in June 2010), until we released frogs again in September 2012.

With additional releases into the canyon during 2012–2015, we observed small numbers of Tarahumara Frogs during monitoring trips through mid-November 2015. We observed 25 dead juveniles and two dead adults in October 2013 and November 2015, respectively, although we also observed some live frogs (30 juveniles and two adults, respectively) during those visits (Appendix 1). In October 2013, 10 dead Tarahumara Frogs and a single live Lowland Leopard Frog tested positive for *Bd*. A hiatus in monitoring in Big Casa Blanca Canyon occurred from November 2015 until June 2019, when biologists from the University of Arizona observed two live adult Tarahumara Frogs and hundreds of tadpoles within the canyon (David Hall, pers. comm.). In October 2019, Tim Tibbitts who was monitoring trail cameras as part of another project, incidentally observed two Tarahumara Frogs (photographed) and two probable Tarahumara Frog tadpoles (large ranid tadpoles, but not confirmed as Tarahumara Frogs) in Walker Canyon, about 0.21 and 0.42 km upstream of its confluence with Big Casa Blanca Canyon. He also observed two adults in Big Casa Blanca Canyon downstream of the Walker Canyon confluence (Fig. 3). Notably, this area is about 0.95 km downstream of the southern end of the lower release and monitoring reach in Big Casa Blanca Canyon.

***Adobe Canyon and El Pilar Tank.***—We released Tarahumara Frogs to these sites in 2012 and 2013 and we monitored for frogs from 2008 to 2015. We found Tarahumara Frogs at Adobe Canyon Bathtub 2 d after the 2012 release and about 2 mo after the last release in 2013, but none thereafter. Remarkably, in May 2008 we captured a large adult female Tarahumara Frog at El Pilar Tank that, based on a cohort toe clip, had been released into Big Casa Blanca Canyon in 2004. We found tadpoles at El Pilar Tank shortly after a release of 100 tadpoles in 2012, but no Tarahumara Frogs of any life stage thereafter. A water sample, filtered from the Adobe Canyon Bathtub in October 2013, tested negative for *Bd*.

***Gardner Canyon.***—We did not release Tarahumara Frogs into Gardner Canyon, but in May 2013 and April 2014 we captured the same large female (identified based on photographs and SVL) at Ash Pond that we found at El Pilar Tank in 2008, which had originated from a 2004 Big Casa Blanca Canyon release (Fig. 3). We swabbed the frog for *Bd* on the latter visit and the results came back negative. She was not seen again despite numerous visits to this canyon through 2018 during a radio-telemetry study and annual monitoring for Chiricahua Leopard Frogs in Gardner Canyon (Audrey Owens, pers. obs.). During the telemetry study, researchers at the site observed a die-off of Chiricahua Leopard Frogs that began in October 2014 and continued through the following February; dozens of dead or dying Chiricahua Leopard Frogs were collected and tested positive for *Bd* (Audrey Owens, pers. obs.).

***Sycamore Canyon.***—We released Tarahumara Frogs to this site in 2014, 2015, and 2019, and conducted monitoring during 15 visits from May 2014 to April 2019 (Tables 1 and 2; Fig. 4; Appendix 1). We found small numbers of tadpoles within 8 mo of tadpole releases, and up to 12 frogs during our monitoring visits through October 2015. After that time, only two of 10 monitoring trips were productive for Tarahumara Frogs: we observed two adults in June 2017 and September 2018 at a tinaja in a steep side drainage of Sycamore Canyon (Appendix 1).

***Water quality.***—In Big Casa Blanca Canyon, water temperature varied from 5° C (25 January 2005) to 31° C (4 July 2004), pH ranged from 6.9 to 9.3, and conductivity varied from 90–280 mS/cm. During the *Bd*-related die-offs in March 2007 and October 2013, water temperature was 8.0° and 17.7° C, and pH was 9.0 and 8.4, respectively. During the *Bd*-related die-off of Tarahumara Frogs observed on 6 May 2007, water temperature in the three reaches of the canyon were 14.6° C (lower), 21.6° C (middle), and 17.8° C (upper), and pH varied from 7.5 to 7.9.

We conducted water quality sampling during or after releases at other sites less often than in Big Casa Blanca Canyon. At El Pilar Tank, water temperature, pH, and conductivity ranged from 14.0°–17.8° C, 7.6–8.8, and 550–815 mS/cm, respectively. At the Adobe Canyon Bathtubs, water temperature, pH, and conductivity ranged from 14.4°–17.0° C, 7.9–8.1, and 540–773 mS/cm, respectively. Water temperature, pH, and conductivity in Sycamore Canyon, measured mostly before we reintroduced Tarahumara Frogs there, ranged from 14.0°–25.1° C, 6.8–8.7, and 121–347 mS/cm, respectively.

***Other potential reintroduction sites.***—During 2009–2010, we surveyed sites in the Santa Rita Mountains (Walker Canyon, Temporal Gulch, Gardner Canyon) and the Pajarito-Atascosa Mountains (Peñasco Canyon) for suitable Tarahumara Frog habitat and potential, future release sites. Some of us were also involved in extensive surveys of Chiricahua Leopard Frog sites in these and adjacent mountain ranges as well (e.g., McCall et al. 2017, 2018; Mosely et al. 2019), and during the course of that work we informally assessed many sites for Tarahumara Frog habitat. We found small patches of potentially suitable habitat in Temporal Gulch, Walker Canyon, and Gardner Canyon. Albeit with very limited habitat, Temporal Gulch could be considered for future reintroduction efforts. Gardner and Walker canyons are considered herein as dispersal sites from Big Casa Blanca Canyon.

## Discussion

***Big Casa Blanca Canyon and vicinity.***—Approximately 500 adult Tarahumara Frogs populated 4.8 km of Big Casa Blanca Canyon during 1975–1977 (Rorabaugh and Hale 2005). Numbers of juveniles varied seasonally, from 32 to 62 in the spring to several hundred in the late summer and fall (Stephen Hale, pers. obs.). When SFH returned to the canyon in 2004, the distribution and size of the pools in Big Casa Blanca Canyon were much as he remembered from his work in the 1970s, although habitat in the upper reach of the canyon supported fewer deep pools. This characterization of the habitat changed dramatically in 2007 as sedimentation after the Florida Fire filled in many of the deepest and best pool habitats.

Although we were not conducting mark and recapture, we never detected more than 15% of 1975–1977 estimates by Hale of the adult population (Rorabaugh and Hale 2005). That said, the reintroduced population was fairly stable with recruitment from the first release in June 2004 until the *Bd*-related die-off in March 2007. Although no anurans tested positive for *Bd* in 2004, we do not know whether the disease was present during that period or was introduced at some point. Severe sedimentation of pools in the summer of 2007 dealt another blow to the reintroduction effort. We have not seen much recovery of those pools since 2007; however, at least some deep pools, capable of supporting all life stages of Tarahumara Frogs, remain in the canyon. It is impossible to distinguish the effects of *Bd* from those of habitat loss and degradation in Big Casa Blanca Canyon, but since 2007, Tarahumara Frogs have only been found in small numbers relative to 2004–2006. Nevertheless, 4 y after the last release in 2015, the species still persists and reproduction is occurring. In addition, the frogs have dispersed downstream about 0.95 km in the canyon and spread into adjacent Walker Canyon where probable Tarahumara Frog tadpoles were documented by Tim

Tibbitts in October 2019.

An interesting corollary to post-Florida Fire flooding and sedimentation is that a large flood event occurred in October 1977, which probably eliminated most metamorphosed frogs and initiated the population decline in Big Casa Blanca Canyon that ended with the last Tarahumara Frog being found dead in that canyon in May 1983 (Hale et al. 2005). Habitat degradation and other stressors may work synergistically with the effects of chytridiomycosis, exacerbating amphibian declines (Kiesecker et al. 2001; Blaustein and Kiesecker 2002). This species appears to be sensitive to severe flooding and sedimentation of its pool habitat and in two instances in Big Casa Blanca Canyon (1977 and 2005–2007), flooding preceded disease-related die-offs.

The limited habitat in Adobe Canyon was probably insufficient to support a population of this frog. Habitat quality in Adobe Canyon was not noticeably affected by the 2005 Florida Fire or subsequent flooding and sedimentation. *Batrachochytrium dendrobatidis* was not detected at the Adobe Canyon sites and we never found dead or dying frogs, but we cannot rule out that disease may have affected frogs there. We did not release frogs to Gardner Canyon, although a large female released in Big Casa Blanca Canyon with the cohort mark from 2004 was found there in 2013 and 2014. While the fate of this frog is unknown, she was in advanced age and a *Bd*-related die-off of Chiricahua Leopard Frogs in Gardner Canyon from October 2014 to February 2015 could have affected her, as well.

This large female is notable for several reasons. First, she survived for at least 10 y in the wild. The oldest frogs in Big Casa Blanca Canyon during 1975–1977 were estimated to be 6 y old, post-metamorphosis (Rorabaugh and Hale 2005); however, this is the longest-living, wild Tarahumara Frog reported. She also moved at least 2.05 km (straight-line distance) from Big Casa Blanca Canyon to El Pilar Tank, and then at least 2.95 km (straight-line distance) to Ash Pond in Gardner Canyon. Both routes, if traveled in a straight line, involve crossing three ridgelines. More circuitous routes would involve traveling along drainages and crossing only one ridgeline to get to Gardner Canyon (a distance of about 6.4 km) from El Pilar Tank, and two ridgelines (a distance of about 2.75 km) to travel from Big Casa Blanca Canyon to El Pilar Tank. These movements through a relatively arid landscape are surprising, given that this species has often been characterized as highly aquatic, occurring in areas of permanent plunge pools (Campbell 1934; Rorabaugh and Hale 2005), and usually found "within a jump or two of water" (Stebbins 2003).

***Sycamore Canyon.***—Campbell (1931, 1934) and Wright and Wright (1949) found Tarahumara Frogs in various places in Sycamore Canyon and adjacent canyons, but considered them less abundant than leopard frogs (referred to as *Rana pipiens*). Scott Cashins (unpubl. data) first detected *Bd* in the canyon via histology of a Lowland Leopard Frog collected in 1972, and a die-off of Tarahumara Frogs was noted in 1974 (Hale et al. 2005); frogs collected during that die-off were examined via histology and found to be infected with *Bd* (Phil Fernandez and Thomas Jones, unpubl. data). Of the two Tarahumara Frogs tested for *Bd* in December 2014, one was positive, the other negative. During disease monitoring of leopard frogs (*R. chiricahuensis* and *R. yavapaiensis*) in the canyon by Arizona Game and Fish Department personnel, *Bd*-positive ranid frogs were found in 2005, 2009, 2012, 2013, and 2014 (Arizona Game and Fish Department, unpubl. data). No Tarahumara Frogs were reported after 1974 until their reintroduction in 2014. We released substantial numbers of Tarahumara Frogs and tadpoles into Sycamore Canyon in 2014 and 2015 and we placed three egg masses in a tributary side canyon in 2019. Despite these releases, we found no more than 12 frogs and six tadpoles during monitoring events with no firm evidence of reproduction. From 2016 through 2019, we detected only two Tarahumara Frogs at this site, and they were both in a steep side canyon that has a number of deep, perennial tinaja pools. We can only speculate as to the reason(s) why reintroduced Tarahumara Frogs have not thrived in Sycamore Canyon; however, Sonora Chub appear to be significant predators on tadpoles and perhaps small frogs, and *Bd* continues to be detected in the canyon. Regarding predation, at Rancho El Trigo, Sonora, we observed Mexican Roundtail Chub (*Gila minacae*) in the lower portion of the canyon, but not above a waterfall mid-canyon. The only Tarahumara Frog tadpoles we found were above the waterfall. A large chub (*Gila* sp.) may have eliminated Tarahumara Frogs from Arroyo el Portrero in southern Sonora (Rorabaugh and Hale 2005). Sonora Chub occur in and are native to Sycamore Canyon, particularly in the larger, deeper, and more permanent pools, including at least some of the tinajas in the aforementioned tributary side canyon. As with most native southwestern fishes, Sonora Chub are opportunistic in their use of habitat, expanding into ephemeral reaches and pools during wet times and then contracting back to more permanent pools during drought (Minckley and Marsh 2009).

After the 2007 die-offs of our Sierra de la Madera stock of Tarahumara Frogs in Big Casa Blanca Canyon, we collected additional animals from Rancho El Trigo in 2008, in part, to introduce a lineage of frogs that had a history of persisting in the wild with *Bd* and might have some genetic, skin microbes, skin antimicrobial peptides (AMP), or behavioral means of surviving with the disease (e.g., Rollins-Smith et al. 2002; Rollins-

Smith 2009; Knapp et al. 2016; Ellison et al. 2019). We have no evidence, however, that they fared better than the Sierra de la Madera stock during disease outbreaks, but our assessment is qualitative. We released stock from Rancho El Trigo during 2012–2019; however, we observed a *Bd*-associated die-off in October 2013, after which we only observed two frogs until additional releases in 2015. The canyon at Rancho El Trigo in which Tarahumara Frogs occur includes a warm spring where the water temperature is a constant 20°–22° C (Hale et al. 2005). Manifestation of *Bd* symptoms is temperature dependent. Forrest and Schlaepfer (2011) found a strong inverse correlation between temperature and chytridiomycosis infection rates in Lowland Leopard Frogs from Arizona. Ranid frog die-offs occurred during the cooler months of the year (Bradley et al. 2002), and elsewhere, Woodhams et al. (2003) found that die-offs occur more frequently in the winter months and at high elevation. As a result, the causality of persistence of Tarahumara Frogs at Rancho El Trigo despite the presence of *Bd* could be environmental, and differing conditions in southern Arizona as compared to central Sonora could result in a different outcome for *Bd*-infected frogs. Further research in a lab setting could better assess these questions by controlling confounding variables. Although Tarahumara Frogs have persisted at both of our primary reintroduction sites (Sycamore and Big Casa Blanca canyons), the numbers of frogs detected since 2007 have been disappointingly low and may not constitute self-sustaining populations, particularly at Sycamore Canyon where reproduction has not been confirmed. Post-fire habitat degradation in Big Casa Blanca Canyon probably reduced the population carrying capacity for Tarahumara Frogs, but it is impossible to assess to what degree that may have affected the likelihood of reintroduction success.

***Water quality.***—Surveys during three disease-related die-offs (two in 2007 and one in 2013) provide insight into the conditions under which chytridiomycosis is expressed in the Tarahumara Frog and how populations are affected. The three die-offs of Tarahumara Frogs we observed in Big Casa Blanca Canyon were in March, May, and October when water temperatures ranged from 8.0° to 17.7° C in affected reaches. Interestingly, while dead and lethargic Tarahumara Frogs were found in the lower reach in May 2007 at a water temperature of 14.6° C (and the two Tarahumara Frogs tested from that reach both were positive for *Bd*), frogs in the middle reach, where water temperature was 21.6° C, appeared healthy (and the two Tarahumara Frogs and three Canyon Treefrogs from there were all negative for *Bd*). It is possible that warmer water temperatures in the middle reach facilitated survival of frogs. In Arizona, chytridiomycosis die-offs and infections of ranid frogs typically occur in the cooler winter months and when water temperatures are < 20° C (Bradley et al. 2002; Forrest and Schlaepfer 2011). As previously noted, *Bd*-positive Tarahumara Frogs have persisted in an arroyo at Rancho El Trigo, Sonora, that includes a warm spring with a constant water temperature of 20°–22° C. Nonetheless, other environmental factors besides temperature may have been important in observed die-offs (Matutte et al. 2000; Rollins-Smith et al. 2002).

We are not able to determine if pH or conductivity affected reintroduction success. The pH was often basic at our sites and as high as 9.3, but most studies of the effects of pH on amphibians have dealt with acidity (Pierce 1985; Boyer and Grue 1995). Abundance of Northern Leopard Frogs (*Rana pipiens*), however, decreases in both acidic and basic water (Pope et al. 2000). No studies have evaluated the effects of varying pH on the Tarahumara Frog. Our review of pertinent literature provides no reason to believe conductivity affected reintroduction success. Although we did not observe ash flow through Big Casa Blanca Canyon after the Florida Fire in 2005, it may have occurred. Ash flow can result in high levels of phosphorus and nitrogen in water (Spencer and Hauer 1991) with potentially toxic effects to frogs.

***Other potential reintroduction sites.***—No other sites in southeastern Arizona of which we are aware appear to provide either the quantity or quality of Tarahumara Frog plunge pool habitat present in Sycamore and Big Casa Blanca canyons. We (particularly SFH) have considerable experience with Tarahumara Frog habitat throughout the range of the species and we are confident of being able to identify suitable sites. Historical Tarahumara Frog localities, besides Sycamore and Big Casa Blanca canyons, are either no longer suitable (Tinaja Canyon in the Tumacacori Mountains, which has silted in), very limited in habitat and occupied by another imperiled ranid (Chiricahua Leopard Frog, in Peña Blanca Spring and Alamo Canyon in the Pajarito-Atascosa Mountains, and Gardner Canyon in the Santa Rita Mountains), or we attempted reintroduction without success (Adobe Canyon, Santa Rita Mountains). Two Tarahumara Frogs and tadpoles likely of this species found in Walker Canyon in 2019, a tributary to Big Casa Blanca Canyon, is encouraging and may provide additional breeding habitat in the Santa Rita Mountains.

Only the ASDM and Kofa NWR sites remain as captive sites or refugia. Others were either closed down, or in the case of the International Wildlife Museum, frogs were moved to the ASDM when the ponds were drained for restoration. The ASDM houses stock from both the Sierra de la Madera and Rancho El Trigo, whereas the Kofa NWR site has stock only from the former site. Although outside the range of the species and in atypical, low-elevation, desert habitat, the population at Horse Tanks in the Castle Dome Mountains, Kofa NWR, has now persisted for 17 y. It was not our intention to establish a breeding population at this site, but rather use the tinajas as a grow-out facility. We have harvested hundreds of animals from Horse Tanks for reintroduction into Big Casa Blanca Canyon and the site yielded interesting and novel information about the calls of Tarahumara Frogs (Rorabaugh and Elliott 2006). The primary breeding site at Horse Tanks is a permanent tinaja that is usually no more than 12 m in diameter yet can support more than a hundred frogs based on our monitoring. During wet periods, frogs spread upstream and downstream of that tinaja into other less permanent waters along about 400 m of the canyon and some limited breeding has occurred at these other sites. Horse Tanks demonstrates that a relatively small site can support a Tarahumara Frog population for almost two decades that was initiated with a single release. At a low elevation (540 m), with surface water temperatures reaching 30° C in the summer, and isolated from other ranid and hylid frogs, this site may be much less susceptible to *Bd* infections than reintroduction sites in southeastern Arizona.

***The future for Tarahumara Frogs in the U.S.***— Sycamore and Big Casa Blanca canyons are still the two best sites to reintroduce the Tarahumara Frog into its historical range within the U.S. Despite our best efforts, however, we have had limited success and numbers of frogs persisting at these sites are small, certainly much smaller than the pre-decline 1970s population in Big Casa Blanca Canyon. Habitat degradation and *Bd*-related die-offs, at least in Big Casa Blanca Canyon, combined with predation impacts in Sycamore Canyon, are likely contributing factors. Sedimentation of pools in Big Casa Blanca is not a problem we can remedy due to difficult access and the extent of the problem. Although under study with promising possibilities on the horizon (Bletz et al. 2013; McMahon et al. 2014; Knapp et al. 2016), we currently have no way to significantly mitigate the effects of chytridiomycosis. Nonetheless, future reintroduction efforts should be accompanied by more *Bd* testing and research to better understand the factors under which Tarahumara Frogs survive with the pathogen. The Sonora Chub is federally listed under the U.S. Endangered Species Act as threatened, so it would not be appropriate or desirable to control or eliminate that species from Sycamore Canyon; however, perhaps managing the species so some of the tinajas in the side canyon of Sycamore are fish free should be considered. The tinajas provide less escape cover for tadpoles than in Sycamore Canyon proper, and as a result, predation effects may be particularly severe in the tinajas.

Our success at Horse Tanks in the Castle Dome Mountains of Kofa NWR proves that Tarahumara Frogs can persist in relatively small pool habitats and reaches of canyons. The primary breeding site there is a natural tinaja for which water permanency has been enhanced with the construction of a small dam. With this in mind, we could explore habitat creation or enhancement opportunities outside of our two primary reintroduction sites. For instance, perhaps the impoundment in Tinaja Canyon in the Tumacacori Mountains that once supported Tarahumara Frogs could be dug out. There are many filled-in dams in the mountains of southeastern Arizona that could provide other restoration opportunities. In sites that are too dry, wells or water catchment devices could be constructed, and American Bullfrogs and other non-native predators could be eliminated, making the habitat more suitable. Sites at low elevation with good exposure to the sun throughout the year should stay warmer than other sites and may provide some protection against chytridiomycosis.

Looking back at our process and methods to reintroduce Tarahumara Frogs into Arizona, we do not see any major flaws. Rather, the problems we encountered were largely a function of circumstances beyond our control. There is room for improvement, however. We should have involved Mexican biologists earlier in the process, which could have facilitated earlier permitting and acquisition of Tarahumara Frog stock from Sonora. Comments we received on the draft reintroduction proposal and draft Environmental Assessment underscored a need for earlier and better coordination with stakeholders, such as ranchers in the vicinity of the release sites. Our success criteria could have been more clearly and quantitatively stated. Research would help us understand the susceptibility of the Sierra de la Madera and Rancho El Trigo stock to chytridiomycosis under varying temperature and other environmental regimes, and to determine if those stocks differ in their ability to persist in the presence of *Bd*. The results of that research could have important implications that extend to other amphibian species that are affected by chytridiomycosis.

We believe the Tarahumara Frog reintroduction program has broad applicability for amphibian reintroduction projects elsewhere in the U.S. and probably globally. Based on our experience with the Tarahumara Frog as well as other imperiled southwestern amphibians (e.g., Sonora Tiger Salamander, *Ambystoma mavortium stebbinsi*, Hossack et al. 2017; Chiricahua Leopard Frog, U.S. Fish and Wildlife Service 2007), we plan on making small improvements to the process. Following from the reintroduction proposal (Field et al. 2004) and the recommendations of Wren et al. (2015), and applying what we have learned from this and other species, the following steps outline a sound reintroduction process: (1) engage species experts, conservation planners, land managers and owners, and affected parties as a conservation team to develop a reintroduction plan in which all members have as much access and input into the process as they desire, (2) develop a reintroduction plan that is grounded in science and has clear goals and measurable, quantitative success criteria, but will operate and be successful within financial, political, cultural, and administrative constraints, (3) develop funding sources and eliminate or mitigate threats as much as possible before population reintroduction, (4) implement the reintroduction plan in close coordination with the conservation team, (5) monitor the reintroduced populations, habitat, and other factors that could affect success, (6) conduct research and develop new information needed to more effectively and efficiently reach reintroduction goals and success criteria, and (7) use monitoring data and research results to adapt the reintroduction plan to a changing management and threats environment.

In summary, observations of Tarahumara Frogs and tadpoles in Big Casa Blanca and Walker canyons in 2019, more than 4 y after we released the last frogs in that area, provide some optimism that the species may persist in that area, although numbers are considerably lower than they were historically. Tarahumara Frogs persist in Sycamore Canyon in very small numbers, but we have no firm evidence of reproduction. We should explore other sites as potential reintroduction sites, particularly those that could be restored, but our future options in regard to managing threats in Sycamore and Big Casa Blanca canyons are limited. Over time, sediment accumulation in pools in the latter site likely will flush out and the habitat will be restored to pre-2007 conditions. Perhaps our ability to control or mitigate the effects of chytridiomycosis will improve or the frogs will develop resistance to the disease. Until such time or until other sites can be identified and restored, maintaining refugia or populations established outside of the historical range (e.g., Horse Tanks) are prudent for conserving the Tarahumara Frog north of Mexico.

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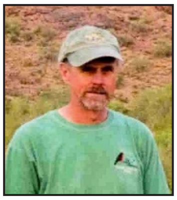

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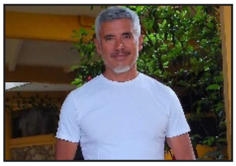

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**Appendix 1**. Numbers of Tarahumara Frogs (*Rana tarahumarae*) by life stage found during visual encounter surveys at reintroduction sites in Arizona, USA, 2004–2019. Dates in italics indicate nocturnal surveys. All other surveys were conducted during the day. Dead animals are shown in parentheses. The 21 October 2019 survey in Big Casa Blanca Canyon also yielded two Tarahumara Frogs and two ranid tadpoles in adjacent Walker Canyon (not shown in the table).

| Date | Egg Masses | Tadpoles | Juveniles | Adults |
|---|---|---|---|---|
| Big Casa Blanca Canyon | | | | |
| 26 June 2004 | 0 | > 4 | 37 | 9 |
| 27 June 2004 | 0 | > 15 | 31 | 27 |
| 4 July 2004 | 0 | 43 | 20 | 14 |
| 24 July 2004 | 0 | 5 | 1 | 7 |
| 14 August 2004 | 0 | 1 | 4 | 4 |
| *14 August 2004* | 0 | 0 | 10 | 13 |
| 10 October 2004 | 0 | 5 | 0 | 23 |
| 25 January 2005 | 0 | 0 | 0 | 0 |
| 6 May 2005 | 3 | 56 | 11 | 13 |
| 10 June 2005 | 0 | > 105 | 18 | 15 |
| *10 June 2005* | 0 | 9 | 18 | 29 |
| 9 August 2005 | 0 | 0 | 4 | 5 |
| 12 October 2005 | 0 | 0 | 3 | 13 |
| *12 October 2005* | 0 | 0 | 0 | 59 |
| 16 March 2006 | 0 | 2 | 0 | 15 |
| 25 May 2006 | 4 | 670 ± | 11 | 12 |
| *25 May 2006* | 0 | 0 | 0 | 73 |
| 10 October 2006 | 0 | 1 | 0 | 52 |
| 4 March 2007 | 0 | 0 | 0 | (47) |
| 6 March 2007 | 0 | 0 | 0 | (51) |
| 6-7 May 2007 | 8 | 1,627 ± | 0 | 4, (4) |
| *6 May 2007* | 0 | 8 | 0 | 1 |
| 23 August 2007 | 0 | 0 | 0 | 0 |
| 11 October 2007 | 0 | 0 | 0 | 10 |
| 13 May 2008 | 0 | 20 | 0 | 0 |
| 22 September 2008 | 0 | 0 | 3 | 0 |
| 10-11 March 2009 | 0 | 0 | 0 | 0 |
| 15 May 2009 | 0 | 0 | 0 | 0 |
| 6-7 May 2010 | 0 | 0 | 0 | 0 |
| 5 September 2010 | 0 | 0 | 0 | 1 |
| 1 June 2011 | 0 | 0 | 0 | 0 |
| 5 May 2012 | 0 | 0 | 0 | 0 |
| 29 May 2013 | 0 | 7 | 0 | 0 |
| 8 October 2013 | 0 | 0 | (25), 30 | 0 |
| 20 May 2014 | 0 | 8 | 0 | 2 |
| 19 May 2015 | 0 | 0 | 0 | 0 |
| 20 May 2015 | 0 | 1 | 3 | 11 |
| 12 June 2015 | 0 | 0 | 11 | 13 |

**Appendix 1 (continued)**. Numbers of Tarahumara Frogs (*Rana tarahumarae*) by life stage found during visual encounter surveys at reintroduction sites in Arizona, USA, 2004–2019. Dates in italics indicate nocturnal surveys. All other surveys were conducted during the day. Dead animals are shown in parentheses. The 21 October 2019 survey in Big Casa Blanca Canyon also yielded two Tarahumara Frogs and two ranid tadpoles in adjacent Walker Canyon (not shown in the table).

| Date | Egg Masses | Tadpoles | Juveniles | Adults |
|---|---|---|---|---|
| 30 September 2015 | 0 | 0 | 0 | 0 |
| 22 October 2015 | 0 | 0 | 0 | 12 |
| 18 November 2015 | 0 | 0 | 0 | (2), 2 |
| 4 June 2019 | 0 | hundreds | 0 | 2 |
| 21 October 2019 | 0 | 2 | 0 | 4 |
| Sycamore Canyon | | | | |
| 15 May 2014 | 0 | 0 | 0 | 0 |
| 11 November 2014 | 0 | 6 | 1 | 11 |
| 13 November 2014 | 0 | 2 | 0 | 9 |
| 9 April 2015 | 0 | 0 | 0 | 0 |
| 11 June 2015 | 0 | 3 | 0 | 0 |
| 13 September 2015 | 0 | 0 | 7 | 0 |
| 22 October 2015 | 0 | 0 | 7 | 0 |
| 10 May 2016 | 0 | 0 | 0 | 0 |
| 11 September 2016 | 0 | 0 | 0 | 0 |
| 9 February 2017 | 0 | 0 | 0 | 0 |
| 13 March 2017 | 0 | 0 | 0 | 0 |
| 11 June 2017 | 0 | 0 | 0 | 2 |
| 17 September 2017 | 0 | 0 | 0 | 0 |
| 29 November 2017 | 0 | 0 | 0 | 0 |
| 20 June 2018 | 0 | 0 | 0 | 0 |
| 16 September 2018 | 0 | 0 | 0 | 2 |
| 15 April 2019 | 0 | 0 | 0 | 0 |
| El Pilar Tank | | | | |
| 23 May 2008 | 0 | 0 | 0 | 1 |
| 21 October 2008 | 0 | 0 | 0 | 0 |
| 10-11 March 2009 | 0 | 0 | 0 | 0 |
| 15 May 2009 | 0 | 0 | 0 | 0 |
| 15-16 April 2010 | 0 | 0 | 0 | 0 |
| 6-7 April 2011 | 0 | 0 | 0 | 0 |
| 1 June 2011 | 0 | 0 | 0 | 0 |
| 6 June 2012 | 0 | 15 | 0 | 0 |
| 29 May 2013 | 0 | 0 | 0 | 0 |
| 24 August 2013 | 0 | 0 | 0 | 0 |
| 20 February 2015 | 0 | 0 | 0 | 0 |
| 8 July 2015 | 0 | 0 | 0 | 0 |

**Appendix 1 (continued)**. Numbers of Tarahumara Frogs (*Rana tarahumarae*) by life stage found during visual encounter surveys at reintroduction sites in Arizona, USA, 2004–2019. Dates in italics indicate nocturnal surveys. All other surveys were conducted during the day. Dead animals are shown in parentheses. The 21 October 2019 survey in Big Casa Blanca Canyon also yielded two Tarahumara Frogs and two ranid tadpoles in adjacent Walker Canyon (not shown in the table).

| Date | Egg Masses | Tadpoles | Juveniles | Adults |
|---|---|---|---|---|
| Bathtubs, Adobe Canyon | | | | |
| 23 May 2008 | 0 | 0 | 0 | 0 |
| 21 October 2008 | 0 | 0 | 0 | 0 |
| 10-11 October 2009 | 0 | 0 | 0 | 0 |
| 15 May 2009 | 0 | 0 | 0 | 0 |
| 15-16 April 2010 | 0 | 0 | 0 | 0 |
| 6-7 May 2010 | 0 | 0 | 0 | 0 |
| 6–7 April 2011 | 0 | 0 | 0 | 0 |
| 1 June 2011 | 0 | 30 | 0 | 0 |
| 6 June 2012 | 0 | 0 | 0 | 0 |
| 29 May 2013 | 0 | 0 | 0 | 0 |
| 24 August 2013 | 0 | 0 | 0 | 0 |
| 17 October 2013 | 0 | 0 | 8 | 0 |
| 30 October 2013 | 0 | 0 | 15 | 0 |
| 20 May 2014 | 0 | 0 | 0 | 0 |
| Gardner Canyon | | | | |
| 29 May 2013 | 0 | 0 | 0 | 1 |
| 4 April 2014 | 0 | 0 | 0 | 1 |
| 16 April 2016 | 0 | 0 | 0 | 0 |
| 7 July 2016 | 0 | 0 | 0 | 0 |
| 11 July 2016 | 0 | 0 | 0 | 0 |
| 9 March 2017 | 0 | 0 | 0 | 0 |
| 10 August 2017 | 0 | 0 | 0 | 0 |
| 22 August 2017 | 0 | 0 | 0 | 0 |
| 7 March 2018 | 0 | 0 | 0 | 0 |
| 7 June 2018 | 0 | 0 | 0 | 0 |
| 7 August 2018 | 0 | 0 | 0 | 0 |