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Review

Over 25 Years of Partnering to Conserve Chiricahua Leopard Frogs (*Rana chiricahuensis*) in Arizona, Combining Ex Situ and In Situ Strategies

Tara R. Harris [1,*], Whitney L. Heuring [1], Ruth A. Allard [1], Audrey K. Owens [2], Shaula Hedwall [3], Cat Crawford [4] and Christina Akins [5]

1 Conservation and Science Department, Arizona Center for Nature Conservation/Phoenix Zoo, Phoenix, AZ 85008, USA
2 Ranid Frogs Project, Arizona Game and Fish Department, Phoenix, AZ 85086, USA
3 Southwest Forest Complex, U.S. Fish and Wildlife Service, Flagstaff, AZ 86001, USA
4 Arizona Ecological Services, U.S. Fish and Wildlife Service, Tucson, AZ 85745, USA
5 Payson and Pleasant Valley Ranger Districts, Tonto National Forest, U.S. Forest Service, Payson, AZ 85541, USA
* Correspondence: tharris@phoenixzoo.org

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Abstract: The Phoenix Zoo has partnered with US Fish and Wildlife Service, Arizona Game and Fish Department, US Forest Service, and other organizations for more than 25 years to help recover Chiricahua leopard frogs (*Rana* [=*Lithobates*] *chiricahuensis*) in Arizona, USA. This federally threatened species faces declines due to habitat loss and degradation, long-term drought, disease, and invasive species. Over 26,000 larvae, froglets, and adults, as well as 26 egg masses produced by adults held at the Phoenix Zoo have been released to the wild, augmenting and/or re-establishing wild populations. Chiricahua leopard frog-occupied sites in Arizona have increased from 38 in 2007, when the species' recovery plan was published, to a high of 155 in the last five years, as a result of ex situ and in situ conservation efforts. As one of the longest-running programs of its kind in the United States, communication among partners has been key to sustaining it. Recovery strategies and complex decisions are made as a team and we have worked through numerous management challenges together. Though Chiricahua leopard frogs still face significant threats and a long road to recovery, this program serves as a strong example of the positive effects of conservation partnerships for native wildlife.

Keywords: conservation partnerships; amphibian; translocation; release; head-starting; reintroduction; breeding

1. The Phoenix Zoo's Legacy of Native Species Conservation

The Arizona Center for Nature Conservation/Phoenix Zoo (hereafter, Phoenix Zoo/Zoo) is one of the largest private, nonprofit zoological facilities in the United States. Located in Phoenix, Arizona, the Zoo opened in November 1962 and is accredited by the Association of Zoos and Aquariums (AZA) [1]. In addition to exhibits and experiences open to the public, the Zoo is also the site of the Arthur L. and Elaine V. Johnson Native Species Conservation Center (Johnson Center), where staff work with ten species of conservation concern in Arizona in collaboration with the US Fish and Wildlife Service (USFWS), Arizona Game and Fish Department (AZGFD), US Forest Service (USFS), US Bureau of Land Management, US Geological Survey, private land managers, universities, other AZA-accredited zoos and conservation organizations, and more. These efforts include propagation-for-release programs to augment wild populations and scientific research that helps inform species management plans at the Zoo and in the field [1]. The Phoenix Zoo also supports international wildlife and habitat conservation projects, some led by Zoo scientists and others directed and managed by individuals and organizations based in the field [1]. None of this work would be possible without continued support of the Zoo's board of trustees and staff leadership, which have prioritized conservation funding even during times of economic uncertainty. In addition, the Zoo has benefited from generous contributions from individual donors, grants from foundations and federal/state agencies, and corporate support for its mission, which specifically calls out stewardship and conservation of wildlife and their habitats. While all modern, professionally managed zoological parks contribute to wildlife conservation as a requirement of AZA accreditation [2], the Phoenix Zoo is especially recognized for its long-term efforts to help recover species native to its home state [1]. Initiated in 1995, the Zoo's Chiricahua leopard frog program, in partnership with AZGFD, USFWS, and USFS, is one of its flagship conservation efforts [1].

2. Chiricahua Leopard Frogs: Background

The Chiricahua leopard frog (*Rana* [=*Lithobates*] *chiricahuensis*) is listed as a federally threatened species in the United States [3], as amenazada (=threatened) on the Lista de Especies en Riesgo of Mexico [4], and as vulnerable on the International Union for Conservation of Nature Red List [5]. The species is native to a variety of permanent and semi-permanent aquatic systems in Arizona and New Mexico, USA, as well as Sonora, Chihuahua, and Durango, Mexico [3,6–8]. Found in areas ranging from montane pine woodlands to lowland grasslands, and from pristine spring-fed pools to streams and earthen cattle tanks, these frogs are habitat generalists (reviewed in [3,6,7]). Habitat loss and degradation, long-term drought, disease, and the establishment of invasive non-native predators such as bullfrogs and crayfish in many permanent waters within the species' range led to decline of Chiricahua leopard frog populations, which are now largely restricted to artificial aquatic systems and natural systems that lack invasive predators (reviewed in [3,6–8]).

Chiricahua leopard frogs were listed as threatened under the U.S. Endangered Species Act in 2002 after USFWS determined the species was absent from more than 75 percent of historical sites and that significant threats to the species will continue for the remaining small and scattered populations [6]. The USFWS organized a formal recovery team tasked with developing a recovery plan, completed in 2007 [7], for the species. To be considered for delisting, each of eight geographically defined recovery units across the species' range must meet criteria regarding long-term persistence of metapopulations and isolated populations, protection and management of aquatic breeding habitats and the areas connecting them, and reduction or elimination of threats to the species and other causes of population decline [7]. The recovery plan also identified management areas within each recovery unit where potential for successful recovery actions is greatest [7].

Ex situ conservation strategies for Chiricahua leopard frogs began well before the development of the species' recovery plan and were incorporated into the plan as a critical tool for accomplishing recovery actions, including reestablishment of populations at formerly occupied sites, augmenting populations at occupied sites, and temporary rescue of frogs facing imminent acute threats followed by repatriation after threats have been abated [7]. Today, the ex situ conservation program for Chiricahua leopard frogs is one of the longest-standing and largest programs of its kind in the United States, providing more than 26,000 individuals for release to the wild from the Phoenix Zoo alone (see below), and is an important tool for working towards recovery of the species [9,10].

3. History of the Phoenix Zoo's Involvement in Chiricahua Leopard Frog Conservation

In 1995, the Phoenix Zoo was invited to collaborate on leopard frog conservation with a large partnership that included state and federal wildlife agencies, zoos, nongovernmental organizations, corporations, universities, ranchers, and private landowners. In the early years of this work, egg masses were brought in from the wild and reared in small aquaria and plastic kiddie pools in a curator's office, with all resulting head-started frogs released before winter to augment wild populations [11]. Over time, the Zoo dedicated additional physical resources to the program, detailed later in this paper. The program initially relied on a volunteer "Tadpole Taskforce" whose members were responsible for daily husbandry of animals, under oversight from the Zoo's Living Collections team. In 2008, the Zoo established its Conservation and Science Department, and began adding staff dedicated specifically to management and care of native species conservation programs. Soon after, the Tadpole Taskforce was disbanded, and paid Zoo technicians became solely responsible for daily husbandry. Throughout the program, Zoo scientists have joined state and federal agency biologists on field surveys and monitoring excursions and conducted some surveys on their own, further supporting recovery efforts and increasing Zoo biologists' connection to the work in the species' native range [12].

4. Evolution of Ex Situ Conservation Facilities and Strategies

The Phoenix Zoo has contributed to the recovery of Chiricahua leopard frogs in Arizona using two main ex situ conservation strategies—head-starting and breeding. When head-starting, the Zoo has typically received wild egg masses collected by conservation partners and reared them to late-stage larvae or juvenile frogs to increase survivorship beyond what would be likely in the wild. These individuals are then released into wild sites within their respective recovery unit. The Zoo has also bred adult frogs and provided egg masses for release to the wild. These conservation strategies are used to start new populations or augment existing populations in areas with suitable habitat and low levels of threat. During annual recovery meetings, partners identify existing, restored, or created habitats for future introduction or augmentation. Primary factors influencing site selection include water permanency, proximity to extant sites or metapopulations, existing threats, and genetics.

Each strategy has required different types of facilities and management, which evolved over the years as the species' recovery program needs changed. The first facility at the Phoenix Zoo built for Chiricahua leopard frog rearing was the Montane Anuran Conservation Center [12]. It was constructed from two insulated cargo containers and equipped with air conditioning units, lighting, and aquaculture tubs that served as a small rearing facility. This facility was used to head-start over 5000 Chiricahua leopard frogs between 1997 and 2007. Zoo staff also repurposed an existing outdoor space, known as the Lower Anuran Conservation Center (LACC), that had been part of fish hatchery operations on site prior to the Zoo's opening in 1962. The LACC was an L-shaped concrete tank with mesh roof and side panels that held leopard frogs from 1996 until it was decommissioned in 2013 due to new construction. The LACC allowed for overwintering of adult frogs and production of egg masses on Zoo grounds.

In 2007, the Phoenix Zoo opened the Arthur L. and Elaine V. Johnson Foundation Conservation Center (Johnson Center), a permanent facility with space for breeding and rearing multiple species, including a ~850 ft2 lab dedicated to Chiricahua leopard frog rearing (Figure 1). Opening the Johnson Center marked a pivotal point in the Chiricahua leopard frog program, as it allowed for multiple populations of frogs to be reared simultaneously in biosecure facilities and included a separate space for quarantining frogs coming in from the field [12]. The Johnson Center magnified the Phoenix Zoo's recovery contribution by increasing the number of individual Chiricahua leopard frogs reared and released into the wild.

Figure 1. Chiricahua leopard frog head-starting lab in the Phoenix Zoo's Johnson Center.

Another expansion occurred in 2010 when the Johnson Conservation Center Ranaria Complex (Ranaria Complex) was constructed, providing fully contained semi-natural habitats for housing and rearing Chiricahua leopard frogs and other aquatic species outdoors (Figure 2). This complex has been renovated over the years to accommodate the changing needs of the Phoenix Zoo's native species programs and currently contains 12 enclosures, six of which now house Chiricahua leopard frogs. Each enclosure has a pond with a filtration system surrounded by land, with live terrestrial and aquatic plants providing varied cover. This outdoor holding space mimics the natural environment and helps promote foraging and cryptic behaviors in the frogs, as well as exposes them to seasonal temperature and light fluctuations.

Figure 2. Phoenix Zoo Ranaria Complex habitats for Chiricahua leopard frog rearing and breeding.

One benefit of the Ranaria Complex has been the ability to house male and female frogs separately. This increased capacity allows the Zoo to hold adult populations yearround and pair wild-sourced individuals representing remaining genetic lineages for breeding when timing is right to produce egg masses. Egg masses are then translocated to wild sites or brought into the Johnson Center for rearing. The creation of additional frog enclosures in the Ranaria Complex allowed the Zoo to move from focusing efforts on rearing frogs from one population in one recovery unit at a time to rearing multiple populations in different recovery units or different management areas within a recovery unit simultaneously. Partners working to manage wild Chiricahua leopard frogs rely heavily on the Ranaria Complex as a source for reestablishing frog populations using egg masses produced by adult breeders when conditions in the wild lead to sudden population declines. When biologists conduct emergency salvages at drying sites, the Ranaria Complex has also acted as a temporary refugium until water naturally returns to these sites.

As the facilities at the Phoenix Zoo have evolved through the years, larval head-starting and rearing strategies have also changed. In early years, the focus was on producing large numbers of larvae [12]. More recently, efforts have shifted focus to ensure animals produced are large and healthy at the time of release. For many years, all individuals were released to the wild by fall, but the addition of the Ranaria Complex allowed the Phoenix Zoo to start overwintering some larvae and juvenile frogs. This strategy, followed by a springtime release of larger frogs, may increase their chances of survival after translocation by providing frogs more acclimation time prior to winter when they are vulnerable to chytridiomycosis, the disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*). This longer "growing season" may also provide released frogs a chance to breed prior to their first winter in the wild, which could increase population persistence through overwintering larvae at sites where adults and juveniles are susceptible to chytridiomycosis in winter [13,14]. This is of particular importance for higher-elevation populations of Chiricahua leopard frogs, which appear severely limited by localized die-offs from chytridiomycosis.

Husbandry protocols adhere to the Phoenix Zoo's Standard Operating Procedure (SOP) for Chiricahua leopard frogs, informed by the species' recovery plan [7], which outlines daily and monthly routines, capture and handling protocols, and wellness evaluations. The SOP is a flexible document that is updated as protocols change. Protocols have evolved from rearing low numbers of larvae in numerous small tanks to rearing larger groups of larvae in bigger tanks to optimize staff time and resources. To provide a better environment for larvae to grow, the Phoenix Zoo and partners also invested in improving the quality of water used in the frog head-starting lab. Shifting away from dechlorinated tap water, this lab now relies on a reverse osmosis deionized water system which produces pure water that is then reconstituted, resulting in water with a concentration of solutes that is isotonic with the internal concentration of amphibians. Housing and rearing of Chiricahua leopard frogs at the Zoo has also allowed staff to collect data on body size and life stage to determine growth and development rates and improve the species' management (Phoenix Zoo, unpublished data). To help improve nutrient absorption and body condition of larvae and juveniles, the Zoo has modified lighting and diet offerings. Zoo scientists continue to evaluate husbandry and head-starting strategies with the goal of producing healthy individuals that will thrive in the wild.

5. Conservation Successes at the Zoo and in the Field

Over the years, the Phoenix Zoo has been successful rearing larvae and juveniles for release in Arizona. Mean survival for individuals from newly hatched larval stage to release to the wild is 70.1% (Table S1; range: 43.9–97.2%; years: 2009–2020, when consistent data collection methods allowed for comparison). Survival varies among years, with no trend in survival of larvae over time (n = 16; r^2 = 0.0016; p = 0.882). When multiple egg masses were reared simultaneously in the same lab, suvival of larvae varied by as much as 45.2% between egg masses (Table S1), suggesting there are factors other than rearing environment (e.g., genetics and pathogens) playing a role in survivorship. Though survivorship of early life stages is largely unknown for wild Chiricahua leopard frogs, overall survivorship is low [7]. Given that, for most amphibians with indirect development, early life stage mortality is very high (>90% [15]), head-starting Chiricahua leopard frogs past these stages and releasing large numbers of individuals, as is possible through the Zoo's facilities, can be an important strategy for improving survivorship [7,10].

From 1995–2020, 179 releases of Phoenix Zoo-reared leopard frogs have taken place in Arizona, totaling 26,821 individual larvae and frogs (Figure 3; Table S2) and 26 egg masses, which roughly equates to 20,000 eggs (assuming ~750 eggs per egg mass). The number of individuals produced and released increased after the opening of the Johnson Center in 2007.

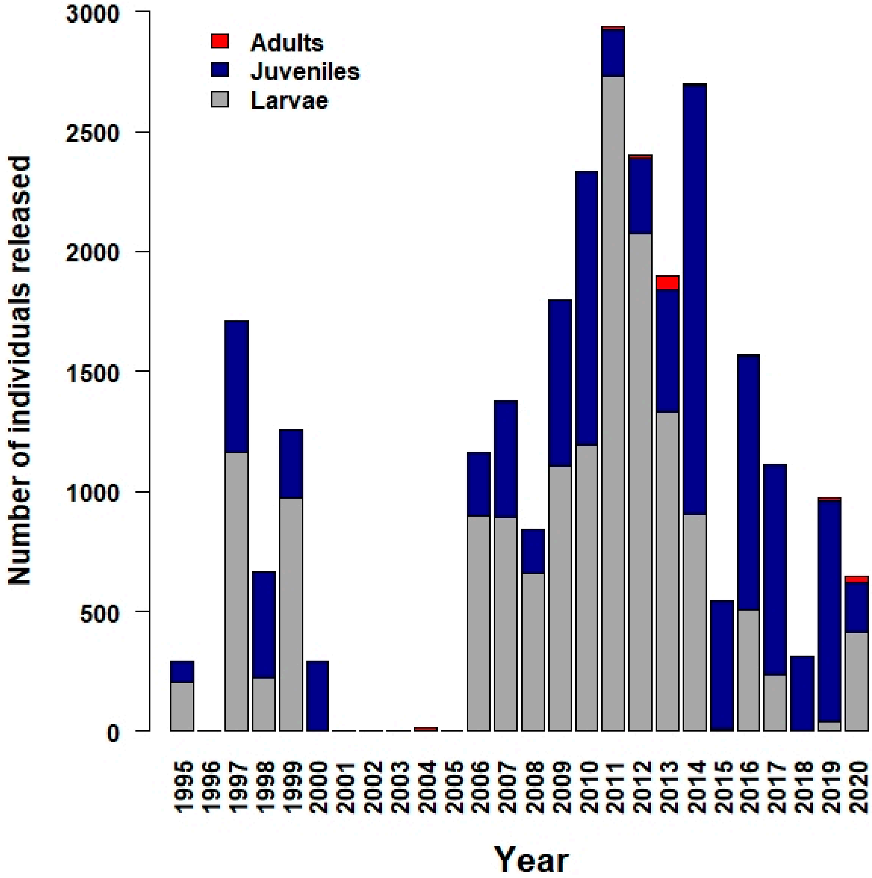

Figure 3. The number of Chiricahua leopard frogs released from 1995–2020 after rearing and/or housing at the Phoenix Zoo. A total of 26,821 individuals (15,562 larvae, 11,119 juvenile frogs, and 140 adult frogs) were released during this timeframe. The Zoo's program involvement was reduced in 2001–2005 due to staffing changes.

Wild Chiricahua leopard frog population status, including the success of releases, is gauged primarily using visual encounter surveys. Current monitoring in Arizona includes visual encounter surveys at sites, where surveyors record presence or absence of frogs and collect data on presence of threats, habitat conditions, evidence of breeding, and more. Surveys sites are selected to document (1) continued persistence of Chiricahua leopard frogs or habitat; (2) persistence of frogs following a recent release; (3) dispersal events; and (4) habitat suitability [9]. In recent years, sites have typically been surveyed between one and three times annually—in spring to detect overwinter survival and breeding, early summer prior to the monsoon to gauge water permanency, and post-monsoon (September–October) to detect dispersal and breeding.

Completion of the Chiricahua Leopard Frog Recovery Plan in 2007 [7] marked a turning point in the conservation of the frog by providing a blueprint to work towards recovery of the species. In 2007, there were only 38 known sites occupied by Chiricahua leopard frogs in Arizona. While the Recovery Plan outlined surveying protocols, there is still a need for a scientifically rigorgous, long-term monitoring program across the species' range that would allow for inferences about the species' status over time. Current population assessments in Arizona are made annually based on the number of sites in which we document frogs, breeding, and a robust population (as defined in the Recovery Plan) [7,9]. The number of occupied sites in Arizona fluctuates annually based on persistence of frog populations and annual factors including precipitation and survey effort; between 2016 and 2021 the number of occupied sites in Arizona was as high as 155—a four-fold increase relative to 2007 [9]. This progress is due to a multi-pronged recovery approach that includes translocations, bullfrog control, habitat restoration, conservation agreements with private landowners, building support through outreach, and application of research and monitoring through adaptive management [9].

A recent analysis of 25 years of Chiricahua leopard frog translocation events [10], with most releases involving animals from the Phoenix Zoo, highlights the value of translocations and provides useful information for adaptive management. Translocations from captive, semi-captive, and wild source populations were all associated with increased probability of release site population persistence, i.e., the proportion of years that sites were estimated to be occupied by Chiricahua leopard frogs [10]. Of the various life stages translocated to wild sites, larval releases were associated with the largest increases in population persistence [10]. Persistence probability also increased as numbers of translocation events at a site increased, with two or more translocations at a site associated with four or more years' increase in the site's predicted occupancy [10]. The combination of several stocking events with large numbers of larvae into neighboring lentic sites that lack vertebrate predators maximized translocation success [10]. Stocking large numbers of Chiricahua leopard frog larvae depends on animals produced en masse at the Phoenix Zoo and cannot be replicated through wild to wild releases.

6. Evolution and Maintenance of Partnerships

Species recovery is typically a complicated, lengthy process with successes and setbacks [16–21]. Strong partnerships are critical to keeping recovery programs moving forward in the face of what can sometimes feel like insurmountable challenges, such as long-term drought and intractable disease in the case of Chiricahua leopard frogs. When partners support one another and work together to devise new ideas and learn from both successes and failures, a recovery program is greatly strengthened.

Communication has been key to the longevity of the Chiricahua leopard frog conservation partnerships in Arizona. Specifically, we credit our ability to have difficult conversations and make hard choices and program improvements together as a team, putting the conservation of frogs first and working toward a common goal. With commitment to the partnership becoming institutionalized over time, we have also been able to maintain momentum even as staff changes occurred in key roles at partner agencies and organizations. Once the recovery plan was completed, the formal recovery team for the Chiricahua leopard frog was replaced by steering committees in each U.S. state where the frog occurs as well as regional work groups. High level priorities, such as new research, adaptive management, range-wide threat abatement, and other range-wide recovery activities are discussed annually at steering committee meetings in Arizona and New Mexico. The recovery plan guided regional work groups to implement recovery at a local level based on the eight recovery units and their associated management areas. As a result, eight local recovery groups across Arizona meet annually to prioritize and implement short- and long-term conservation and recovery actions in each management area or recovery unit.

Furthermore, a key to the success of the partnership in Arizona and beyond is that both in situ and ex situ conservation strategies have been integrated since the beginning and formalized in conservation plans—especially the Chiricahua Leopard Frog Recovery Plan [7,22]. By taking a "One Plan Approach" to species conservation planning [23,24] rather than planning separately for in situ and ex situ conservation efforts, we have ensured that we are all working together toward common goals and objectives. Furthermore, such integrated planning benefits the partnership by demonstrating respect for the different but complementary roles and expertise that in situ and ex situ conservation partners contribute to species recovery.

Over the years, the Phoenix Zoo has become more involved in broader recovery efforts for Chiricahua leopard frogs, including participating to a greater extent in planning and monitoring efforts. The bulk of the Zoo's involvement has focused on work in Arizona, with communication and collaboration with colleagues working in New Mexico as requested. An annual agency-sponsored Chiricahua leopard frog certification workshop and associated field survey training has allowed Zoo staff and numerous others to gain knowledge and skills needed to help monitor wild populations. Furthermore, important for the partnership and recovery program has been the Zoo's willingness and ability to pivot quickly and be flexible to the needs of the species and those who manage them, such as taking in additional animals, sometimes from new recovery units or management areas of Chiricahua leopard frogs on short notice. Without this versatility, populations or genetic lineages of frogs potentially would have been lost.

7. Challenges Faced—Example 1: Deciding to Cross Genetic Lineages

Across recovery programs for imperiled species, managers and conservation partners sometimes face the difficult decision of whether to artificially restore gene flow through the mixing of isolated populations (e.g., Florida panther, *Puma concolor coryi* [25,26]; Gila topminnow, *Poeciliopsis occidentalis* [27]; Greater prairie chicken, *Tympanuchus cupido pinnatus* [28,29]; headwater livebearer, *Poeciliopsis monacha* [30]; Isle Royale wolves, *Canis lupus* [31,32]; mountain pygmy possum, *Burramys parvus* [33]). Such genetic rescue attempts tend to be rare due to biological concerns about outbreeding depression and the potential to lose adaptations to local conditions, as well as cultural concerns about taxonomic integrity and regulatory obstacles [34,35]. However, outcrossing in many cases can be highly beneficial to small, genetically isolated populations that are often inbred, ultimately ensuring their survival [34–37].

Around the time the Chiricahua Leopard Frog Recovery Plan was published in 2007, conservation partners in Arizona had to make difficult choices about a declining, isolated population of Chiricahua leopard frogs on the Coconino National Forest in the Buckskin Hills near Camp Verde, Arizona. Due to a population crash following extensive drought in 2002, the only remaining individuals representing a genetic lineage from the Buckskin Hills population were two males and one female held at the Phoenix Zoo. Conservation partners hoped these frogs would produce offspring for reintroduction, but the pairing did not produce viable egg masses. Attempts were even made to hormonally induce breeding, but with no success.

To retain some of the potentially valuable genetics of this population and have frogs to return to the area, USFWS and AZGFD ultimately decided to cross the remaining Buckskin Hills frogs with individuals from the nearby Gentry Creek lineage located on the Tonto National Forest. Attempts at the Phoenix Zoo to pair two Buckskin Hills males with two Gentry Creek females produced six egg masses in 2008 that had some viable eggs. Though survivorship of larvae was very low, 48 larvae and 18 juveniles and subadults from these crosses were released to the wild in fall of 2008. In subsequent years, additional releases from these crosses helped bolster the reintroduced population, and frogs eventually dispersed across the landscape forming a metapopulation—even moving into areas the local recovery group had not considered releasing them. Within the Buckskin Hills, many sites originally prioritized for recovery have had substantial die-offs from chytridiomycosis and we are now focusing conservation efforts in the dispersal sites where frogs continue to persist.

The decision to cross genetic lineages was relatively straightforward once all other options had been exhausted. However, most Buckskin Hills genetics had already been lost at that point. In hindsight, starting ex situ conservation efforts for the Buckskin Hills lineage earlier may have prevented some of the Buckskin Hills' genetic diversity loss. At the time it did not seem warranted, but the population crashed more quickly than anyone anticipated. Decision making was further hampered because of knowledge gaps in Chiricahua leopard frog genetics. Populations that we eventually crossed were close enough geographically that USFWS, AZGFD, and Phoenix Zoo staff ultimately felt comfortable that the risk of outbreeding depression through a genetic cross was low. At present, Chiricahua leopard frog partners are collaborating with a geneticist who is characterizing the genetic structure and diversity across the species' range to inform an applied genetic management plan for making decisions about timing and need of genetic rescue across populations.

8. Challenges Faced—Example 2: Managing Disease

Disease is another common challenge faced by species, as well as ex situ conservation programs designed to benefit these species. For Chiricahua leopard frogs, as well as many other amphibian species worldwide, chytridiomycosis is a major threat to the survival of populations, hampering recovery efforts [7,38,39]. This skin disease, caused by the fungus *Bd*, leads to mortality in amphibians by disrupting the skin's osmoregulatory function [40]. Sporadic mortality events associated with *Bd* infections have been observed across the range of Chiricahua leopard frogs, especially during the cool season when lower water temperatures in the region are associated with higher prevalence of *Bd* [7,13,14,41]. At present, there is no known preventative measure or treatment for *Bd* that can feasibly be applied in the field for Chiricahua leopard frogs.

Chiricahua leopard frogs of all life stages brought to the Phoenix Zoo for head-starting or breeding are tested for *Bd*, as are water samples from the source site. These incoming individuals are held in a separate quarantine room, where biosecurity protocols [42] are in place to prevent disease transmission to other animals or parts of the facility. Individuals are released from quarantine only after testing negative for *Bd* and ranaviruses, and typically after a quarantine period of 30 days.

At times, the Phoenix Zoo has been called on to take in frogs from areas known to harbor *Bd* due to imminent threats (e.g., severe drought) facing those frog populations. Thus, it has been important to develop an effective strategy for clearing *Bd* in this species ex situ. Multiple treatment options exist for amphibians testing positive for *Bd* in an ex situ setting, including medications and elevated temperature protocols that each have advantages and disadvantages (reviewed in [42]). Different species and/or life stages may vary in their responses to treatments, and multiple treatment types may be needed to clear animals of *Bd* [42]. In 2015, the Phoenix Zoo successfully employed an elevated temperature protocol that cleared quarantined larvae, and larvae that metamorphosed during the quarantine period, of *Bd* [43]. The water temperature in the tanks housing the larvae was elevated for 6 days and larvae were swabbed for *Bd* three times post-treatment. The heat treatment was relatively simple to employ and had no observed short- or long-term adverse effects (e.g., morphological, behavioral, mortality) on treated individuals [43]. Additionally, Zoo staff used this protocol in 2021 with quarantined adult frogs that tested positive for *Bd* to successfully clear them of *Bd* with no adverse effects (Phoenix Zoo, unpublished data). Having an effective *Bd* treatment protocol for use during different life stages allows the Phoenix Zoo and partners to bring in genetic diversity from the wild that we would not be able to access otherwise and is critical for ensuring the health and welfare of all the amphibians in the Zoo's care.

In 2013, the USFWS and AZGFD shifted from requiring prophylactically treating all frogs for *Bd* prior to release with itraconazole to conducting *Bd* testing prior to release to determine if subsequent treatment is necessary. Although effective, prophylactic treatment is costly, time consuming, and stressful to frogs [42].

Amphibian disease, particularly chytridiomycosis, continues to be a challenge to reintroduction efforts, with translocated individuals and populations sometimes succumbing to the disease. Nevertheless, we continue to release Chiricahua leopard frogs into all parts of their historical range in Arizona, including locations with a history of *Bd* [9]. Studies of lowland leopard frogs and Chiricahua leopard frogs found that some populations have

Major Histocompatibility Complex (MHC) alleles associated with increased survival in the presence of *Bd*, along with evidence supporting the hypothesis that *Bd* tolerance is evolving rapidly [44–47]. Additionally, some southern Arizona Chiricahua leopard frog populations appear to co-exist with *Bd* with no apparent population-wide die-offs. Currently we do not fully understand the spatial distribution of *Bd* patterns or seasonal prevalence of *Bd* in the Chiricahua leopard frog's range, or why populations vary in their susceptibility, so additional research is needed to improve the survivorship of Chiricahua leopard frogs in the presence of *Bd*.

We know even less about ranaviruses in Arizona, as widespread testing for the disease has not occurred and relatively little is known about its biology or distribution in Arizona. Two types of ranaviruses, *Amybstoma tigrinum virus* (ATV) and *frog virus 3* (FV3), are both linked to large episodic die-offs in larval amphibians [48]. While FV3 is not known from Arizona, ATV infects tiger salamanders (*Ambystoma tigrinum*) throughout Arizona but is not known to infect ranids [49,50]. Skin and oral swabs of Chiricahua leopard frogs brought into quarantine at the Phoenix Zoo have revealed that a ranavirus occurs at several wild sites in Arizona. Test results have been difficult to interpret, which is not unusual in captive amphibians that have subclinical infections [42]. Further research is being conducted to understand if and how ranaviruses affect Chiricahua leopard frogs. AZGFD is currently funding research to investigate the prevalence and seasonal dynamics of ranaviruses throughout the Chiricahua leopard frog range and to gain a better understanding of susceptibility of Chiricahua leopard frogs to ranavirus infection.

9. Conclusions

The observed increase in Chiricahua leopard frog site occupancy and breeding since 2007, the presence of functioning metapopulations in several recovery units across Arizona, and the control of bullfrogs in some key areas are significant benchmarks of our progress in Chiricahua leopard frog conservation. Despite multiple partners being actively involved in Chiricahua leopard frog management and progress made toward recovery of the species, long-term persistence of the species in Arizona still requires intensive monitoring and management.

The program described here demonstrates the substantive contributions zoos can make in support of local species conservation. This program is one of numerous amphibian conservation programs with significant zoo and aquarium involvement (as reviewed in [51–55]). Experts emphasize the need for amphibian programs developed and implemented in the animals' native range [52,56–58], which further encourages collaborations like the Chiricahua leopard frog program in Arizona. Significant challenges remain, including disease issues and habitat loss and degradation, but thoughtful, sustained partnerships are essential to the success of local conservation efforts. Adaptive management based on research and monitoring will continue to guide our efforts and hone our methods both in and ex situ.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jzbg3040039/s1, Table S1: Survivorship of *R. chiricahuensis* from hatch to release/transfer, by year and egg mass (EM); Table S2: Number of *R. chiricahuensis* released by year (egg masses excluded).

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References

1. Allard, R.A.; Wells, S.A. The Phoenix Zoo story: Building a legacy of conservation. In *The Ark and Beyond: The Evolution of Zoo and Aquarium Conservation*; Minteer, B.A., Maienschein, J., Collins, J.P., Eds.; The University of Chicago Press: Chicago, IL, USA, 2018; pp. 169–175.
2. Association of Zoos and Aquariums. *The Accreditation Standards and Related Policies 2022*; Silver Spring: Montgomery County, MD, USA, 2022.
3. U.S. Fish and Wildlife Service. Endangered and threatened wildlife and plants; listing and designation of critical habitat of the Chiricahua leopard frog (*Lithobates chiricahuensis*). *US. Fed. Reg.* **2012**, *77*, 16234–16424.
4. Secretaría de Medio Ambiente y Recursos Naturales. Protección ambiental-especies nativas de México de flora y fauna silvestrescategorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. (Segunda Sección). *Diaro Of.* **2010**, *6*, 158–169.
5. Santos-Barrera, G.; Hammerson, G.; Sredl, M. Lithobates chiricahuensis. *IUCN Red List* **2004**, e.T58575A11805575. [CrossRef]
6. U.S. Fish and Wildlife Service. Endangered and threatened wildlife and plants; listing of the Chiricahua leopard frog (*Rana chiricahuensis*). *US. Fed. Reg.* **2002**, *67*, 40790–40811.
7. U.S. Fish and Wildlife Service. *Chiricahua Leopard Frog (Rana chiricahuensis) Recovery Plan*; U.S. Fish and Wildlife Service: Albuquerque, NM, USA, 2007.
8. Rorabaugh, J.C.; Hossack, B.R.; Muths, E.; Sigafus, B.H.; Lemos-Espinal, J.A. Status of the threatened Chiricahua leopard frog and conservation challenges in Sonora, Mexico, with notes on other ranid frogs and non-native predators. *Herpetol. Conserv. Biol.* **2018**, *13*, 17–32.
9. Mosley, C.D.; Marsh, M.J.L.; Owens, A.K. Chiricahua leopard frog recovery in Arizona 2019. In *Nongame and Endangered Wildlife Program Technical Report 330*; Arizona Game and Fish Department: Phoenix, AZ, USA, 2020.
10. Hossack, B.R.; Howell, P.E.; Owens, A.K.; Cobos, C.; Goldberg, C.S.; Hall, D.; Hedwall, S.; MacVean, S.K.; MacCaffery, M.; McCall, A.H.; et al. Identifying factors linked with persistence of reintroduced populations: Lessons learned from 25 years of amphibian translocations. *Global Ecol. Conserv.* **2022**, *35*, e02078. [CrossRef]
11. Demlong, M.J. Head-starting *Rana subaquavocalis* in captivity. *Reptiles* **1997**, *5*, 24–33.
12. Sprankle, T. Giving leopard frogs a head start. *Endanger. Species Update* **2008**, *25*, S15+.
13. Sredl, M.J.; Field, K.J.; Peterson, A.M. Understanding and mitigating effects of chytrid fungus to amphibian populations in Arizona. In *Nongame and Endangered Wildlife Program Technical Report 208*; Arizona Game and Fish Department: Phoenix, AZ, USA, 2003.

14. Sredl, M.J.; Jennings, R.D. *Rana chiricahuensis* Platz and Mecham, 1979: Chiricahua leopard frogs. In *Amphibian Declines: The Conservation Status of United States Species*; Lanoo, M., Ed.; University of California Press: Berkeley, CA, USA; Los Angeles, CA, USA, 2005; pp. 546–549.
15. Vitt, L.J.; Caldwell, J.P. *Herpetology: An Introductory Biology of Amphibians and Reptiles*, 4th ed.; Academic Press: San Diego, CA, USA, 2014.
16. Reading, R.P.; Miller, B.J. The black-footed ferret recovery program: Unmasking professional and organizational weaknesses. In *Endangered Species Recovery: Finding the Lessons, Improving the Process*; Clark, T.W., Reading, R.P., Clarke, A.L., Eds.; Island Press: Washington, DC, USA, 1994; pp. 73–100.
17. Scott, J.M.; Goble, D.D.; Wiens, J.A.; Wilcove, D.S.; Bean, M.; Male, T. Recovery of imperiled species under the Endangered Species Act: The need for a new approach. *Front. Ecol. Environ.* **2005**, *3*, 383–389. [CrossRef]
18. Dreitz, V. Issues in species recovery: An example based on the Wyoming toad. *Bioscience* **2006**, *56*, 765–771. [CrossRef]
19. Miller, B.; Reading, R.P. Challenges to black-footed ferret recovery: Protecting prairie dogs. *West. N. Am. Nat.* **2012**, *72*, 228–240. [CrossRef]
20. Evans, D.M.; Che-Castaldo, J.P.; Crouse, D.; Davis, F.W.; Epanchin-Niell, R.; Flather, C.H.; Frohlich, R.K.; Goble, D.D.; Li, Y.; Male, T.D.; et al. Species recovery in the United States: Increasing the effectiveness of the Endangered Species Act. *Issues Ecol.* **2016**, *20*, 1–28.
21. Walls, S.C.; Ball, L.C.; Barichivich, W.J.; Dodd, C.K., Jr.; Enge, K.M.; Gorman, T.A.; O'Donnell, K.M.; Palis, J.G.; Semlitsch, R.D. Overcoming challenges to the recovery of declining amphibian populations in the United States. *Bioscience* **2017**, *67*, 156–165. [CrossRef]
22. Rorabaugh, J.; Kreutzian, K.; Sredl, M.; Painter, C.; Aguilar, R.; Bravo, J.C.; Kruse, C. Chiricahua leopard frog inches toward recovery. *Endanger. Species Update* **2008**, *25*, S10+.
23. Byers, O.; Lees, C.; Wilcken, J.; Schwitzer, C. The One Plan Approach: The philosophy and implementation of CBSG's approach to integrated species conservation planning. *WAZA Mag.* **2013**, *14*, 2–5.
24. Traylor-Holzer, K.; Leus, K.; Byers, O. Integrating ex situ management options as part of a One Plan Approach to species conservation. In *The Ark and Beyond: The Evolution of Zoo and Aquarium Conservation*; Minteer, B.A., Maienschein, J., Collins, J.P., Eds.; University of Chicago Press: Chicago, IL, USA, 2018; pp. 129–141.
25. Hedrick, P.W. Gene flow and genetic restoration: The Florida panther as a case study. *Conserv. Biol.* **1995**, *9*, 996–1007. [CrossRef]
26. Pimm, S.L.; Dollar, L.; Bass, O.L., Jr. The genetic rescue of the Florida panther. *Anim. Conserv.* **2006**, *9*, 115–122. [CrossRef]
27. Hedrick, P.W.; Lee, R.; Hurt, C.R. Genetic evaluation of captive populations of endangered species and merging of populations: Gila topminnows as an example. *J. Hered.* **2012**, *103*, 651–660. [CrossRef]
28. Westemeier, R.L.; Brawn, J.D.; Simpson, S.A.; Esker, T.L.; Jansen, R.W.; Walk, J.W.; Kershner, E.L.; Bouzat, J.L.; Paige, K.N. Tracking the long-term decline and recovery of an isolated population. *Science* **1998**, *282*, 1695–1698. [CrossRef]
29. Warnke, K. Wisconsin greater prairie-chicken management plan 2004–2014. In *Wisconsin Department of Natural Resources*; Madison: Wisconsin, WI, USA, 2004.
30. Vrijenhoek, R.C. Genetic diversity and fitness in small populations. In *Conservation Genetics EXS*; Loeschcke, V., Jain, S.K., Tomiuk, J., Eds.; Birkhäuser: Basel, Switzerland, 1994; Volume 68.
31. Räikkönen, J.; Vucetich, J.A.; Peterson, R.O.; Nelson, M.P. Congenital bone deformities and the inbred wolves (*Canis lupus*) of Isle Royale. *Biol. Conserv.* **2009**, *142*, 1025–1031. [CrossRef]
32. Robinson, J.A.; Räikkönen, J.; Vucetich, L.M.; Vucetich, J.A.; Peterson, R.O.; Lohmueller, K.E.; Wayne, R.K. Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the threshold of extinction. *Sci. Adv.* **2019**, *5*, eaau0757. [CrossRef] [PubMed]
33. Weeks, A.R.; Moro, D.; Thavornkanlapachai, R.; Taylor, H.R.; White, N.E.; Weiser, E.L.; Heinze, D. Conserving and enhancing genetic diversity in translocation programs. In *Advances in Reintroduction Biology of Australian and New Zealand Fauna*; Armstrong, D., Hayward, M., Moro, D., Seddon, P., Eds.; CSIRO Publishing: Melbourne, Australia, 2015.
34. Frankham, R. Genetic rescue of small inbred populations: Meta-analysis reveals large and consistent benefits of gene flow. *Molec. Ecol.* **2015**, *24*, 2610–2618. [CrossRef] [PubMed]
35. Ralls, K.; Ballou, J.D.; Dudash, M.R.; Eldridge, M.D.B.; Fenster, C.B.; Lacy, R.C.; Sunnucks, P.; Frankham, R. Call for a paradigm shift in the genetic management of fragmented populations. *Conserv. Lett.* **2018**, *11*, 1–6. [CrossRef]
36. Whiteley, A.R.; Fitzpatrick, S.W.; Funk, W.C.; Tallmon, D.A. Genetic rescue to the rescue. *Trends Ecol. Evol.* **2015**, *30*, 42–49. [CrossRef]
37. Frankham, R. Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. *Biol. Conserv.* **2016**, *195*, 33–36. [CrossRef]
38. Longcore, J.E.; Pessier, A.P.; Nichols, D.K. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* **1999**, *91*, 219–227. [CrossRef]
39. Skerratt, L.F.; Berger, L.; Speare, R.; Cashins, S.; McDonald, K.R.; Phillott, A.D.; Hines, H.B.; Kenyon, N. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* **2007**, *4*, 125. [CrossRef]
40. Voyles, J.; Young, S.; Berger, L.; Campbell, C.; Voyles, W.F.; Dinudom, A.; Cook, D.; Webb, R.; Alford, R.A.; Skerratt, L.F.; et al. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* **2009**, *326*, 582–585. [CrossRef]

41. Forrest, M.J.; Schlaepfer, M.A. Nothing a hot bath won't cure: Infection rates of amphibian chytrid fungus correlate negatively with water temperature under natural field settings. *PLoS ONE* **2011**, *6*, e28444. [CrossRef]
42. Pessier, A.P.; Mendelson, J.R., III. *A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs*; Version 2.0; IUCN/SSC Conservation Breeding Specialist Group: Apple Valley, MN, USA, 2017.
43. Heuring, W.L.; Poynter, B.M.; Wells, S.; Pessier, A.P. Successful clearance of chytrid fungal infection in threatened Chiricahua leopard frog (*Rana chiricahuensis*) larvae and frogs using an elevated temperature treatment protocol. *Salamandra* **2021**, *57*, 171–173.
44. Savage, A.E.; Zamudio, K.R. MHC genotypes associate with resistance to a frog-killing fungus. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 16705–16710. [CrossRef] [PubMed]
45. Savage, A.E.; Becker, C.G.; Zamudio, K.R. Linking genetic and environmental factors in amphibian disease risk. *Evol. Appl.* **2015**, *8*, 560–572. [CrossRef]
46. Savage, A.E.; Zamudio, K.R. Adaptive tolerance to a pathogenic fungus drives major histocompatibility complex evolution in natural amphibian populations. *Proc. R. Soc. B* **2016**, *283*, 20153115. [CrossRef] [PubMed]
47. Savage, A.E.; Mulder, K.P.; Torres, T.; Wells, S. Lost but not forgotten: MHC genotypes predict overwinter survival despite depauperate MHC diversity in a declining frog. *Conserv. Genet.* **2018**, *19*, 309–322. [CrossRef]
48. Miller, D.; Gray, M.; Storfer, A. Ecopathology of ranaviruses infecting amphibians. *Viruses* **2011**, *3*, 2351–2373. [CrossRef]
49. Jancovich, J.K.; Davidson, E.W.; Morado, J.F.; Jacobs, B.L.; Collins, J.P. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. *Dis. Aquat. Organ.* **1997**, *31*, 161–167. [CrossRef]
50. Jancovich, J.K.; Davidson, E.W.; Seiler, A.; Jacobs, B.L.; Collins, J.P. Transmission of the *Ambystoma tigrinum* virus to alternative hosts. *Dis. Aquat. Organ.* **2001**, *46*, 159–163. [CrossRef]
51. Browne, R.K.; Wolfram, K.; García, G.; Bagaturov, M.F.; Pereboom, Z.J.J.M. Zoo-based amphibian research and conservation breeding programs. *Amphib. Reptile Conserv.* **2011**, *5*, 1–14.
52. Zippel, K.; Johnson, K.; Gagliardo, R.; Gibson, R.; McFadden, M.; Browne, R.; Martinez, C.; Townsend, E. The Amphibian Ark: A global community for *ex situ* conservation of amphibians. *Herpetol. Conserv. Biol.* **2011**, *6*, 340–352.
53. Harding, G.; Griffiths, R.A.; Pavajeau, L. Developments in amphibian captive breeding and reintroduction programs. *Conserv. Biol.* **2016**, *30*, 340–349. [CrossRef]
54. Murphy, J.B.; Gratwicke, B. History of captive management and conservation amphibian programs mostly in zoos and aquariums. *Herp. Rev.* **2017**, *48*, 241–260.
55. Mendelson, J.R., III. Frogs in glass boxes: Responses of zoos to global amphibian extinctions. In *The Ark and Beyond: The Evolution of Zoo and Aquarium Conservation*; Minteer, B.A., Maienschein, J., Collins, J.P., Eds.; University of Chicago Press: Chicago, IL, USA, 2018; pp. 298–310.
56. Gagliardo, R.; Crump, P.S.; Griffith, E.J.; Mendelson, J.; Ross, H.; Zippel, K. The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *Int. Zoo Yearb.* **2008**, *42*, 125–135. [CrossRef]
57. Mendelson, J.R., III; Lips, K.R.; Gagliardo, R.W.; Rabb, G.B.; Collins, J.P.; Diffendorfer, J.E.; Daszak, P.; Ibáñez, D.; Zippel, K.C.; Lawson, D.P.; et al. Confronting amphibian declines and extinctions. *Science* **2006**, *313*, 48. [CrossRef] [PubMed]
58. Carrillo, L.; Johnson, K.; Mendelson, J., III. Principles of program development and management for amphibian conservation captive breeding programs. *Int. Zoo News* **2015**, *62*, 96–107.