A SPRING WITHOUT WATER

Restoration of the Las Vegas Creek

Raymond A Saumure, Ph.D.
raymond.saumure@snwa.com

A SPRING WITHOUT WATER
Restoration of the Las Vegas Creek
2008 – 2020

SOUTHERN NEVADA WATER AUTHORITY
Environmental Resources Division

Prepared for:
Southern Nevada Water Authority

Prepared by:
Raymond A. Saumure, Ph.D.
Southern Nevada Water Authority
100 City Parkway, Suite 700
Las Vegas, Nevada 89106

October 2020

LAS VEGAS REVIEW-JOURNAL **VIEW**

SOUTHWEST
Thursday, March 1, 2018
reviewjournal.com/local
Volume 26, Issue 42
Zone 4

Left, a tortoise is seen Sept. 26 in the Desert Tortoise Habitat at Springs Preserve. Right, a Pahrump poolfish swims Jan. 25 in a tank at the Nevada Department of Wildlife's fish hatchery at Lake Mead. Springs Preserve soon will be home to a reserve population of the endangered fish.

Erik Verduzco Las Vegas Review-Journal @Erik_Verduzco

Aaron Ambos Southern Nevada Water Authority

Oasis for threatened species

They are increasingly finding safe harbor at Springs Preserve

By Henry Brean
Las Vegas Review-Journal

It's not quite Noah's Ark, but Springs Preserve is gradually becoming an urban lifeboat for some of the Mojave Desert's most threatened species.

Before the end of the year, the Las Vegas Valley Water District-owned attraction near U.S. Highway 95 and Valley View Boulevard will be home to three types of protected animals and a plant considered critically endangered in Nevada.

These aren't tiny zoo exhibits with one or two specimens. Spring Preserve leaders want to house colonies large enough to stave off extinction for at least two of these species, should they die off in other refuge settings or in the wild.

"It ties into our mission to help protect — and educate people about — the endangered species native to the area," said Nathan Harper, staff

See SPECIES 9A

ACKNOWLEDGMENTS

The ambitious idea for the rewilding of the Springs Preserve can be traced back to an old email from a young Southern Nevada Water Authority (SNWA) biologist by the name of Zane Marshall to Springs Preserve biologist Marci Henson on Dec 11, 2001, some 18 years ago. Thanks Zane (**below**) for your enthusiasm and support of this project since its inception.

Mr. John Entsminger, SNWA/LVVWD General Manager, supported the project and was willing to go before the Board to commit the organization to a 15-year Pahrump Poolfish Safe Harbor Agreement and a 15-year programmatic Relict Leopard Frog Candidate Conservation Agreement with Assurances.

Deputy General Manager, Julie Wilcox and Director Andy Belanger have supported the Springs Preserve and in particular this project with a large amount of positive publicity garnered through Public Information staff and the filming of several "Springs Elemental" episodes by the talented Paul Bean at Joshua Tree Productions.

Bruno Bowles (**left**), Springs Preserve Manager, has shown nothing but support for the project, having a true understanding of how wildlife supports the Springs Preserve mission. Thomas O'Toole (**left**) and Zoologists Rachael Vanhorn, Katrina Smith, Nicole Zambrano, and Tammy Legg have proven to be invaluable for their almost daily oversight, filtration expertise, animal husbandry, monitoring, and maintenance provided, even more so during the pandemic. Dr. Von Winkel was responsible for seeing the project to fruition, locating a pool construction contractor and filtration expert, and making concrete ponds come to life with his ecosystem restoration knowledge. Mike Weintz is thanked for coordinating the ponds electrical infrastructure grant and contracting.

Environmental Resources Division Manager and Supervisor Lisa Luptowitz and Kimberly Reinhart, respectively, generously provided assistance, funds, and most importantly the time to make this project a success. Aaron Ambos (**above**) has assisted with the development, design, monitoring, and maintenance of the fish and frogs and their habitat. His knowledge and expertise is legendary. Audrey Bennett has assisted with translocations, monitoring, maintenance, and even published observations. Signa Gundlach assisted with monitoring of the Moapa White River Springfish and early water chemistry analyses. Derek Babcock facilitated an early determination from the U.S. Corps of Engineers that the Las Vegas Creek was not a navigable waterway and thus did not require Waters of the US permits. David Syzdek facilitated obtaining Moapa White River Spring Fish for pond testing and assisted with the translocation.

Nevada Department of Wildlife (NDOW) biologists, Jon Sjoberg, Kevin Guadelupe, Brandon Senger and U.S. Fish and Wildlife Fish Biologist James Harter provided their fish expertise, translocation assistance, and monitoring for the Pahrump Poolfish. I am indebted to University of Nevada, Las Vegas colleagues Dr. Jef Jaeger and Rebeca Rivera as well as Kevin Guadelupe (NDOW) for providing their extensive Relict Leopard Frog expertise, raising the translocated frogs from eggs, and conducted both diurnal and nocturnal mark-recapture surveys at the ponds.

Tabitha Fiddyment and Dana Walsh provided counsel on all legal matters associated with the Las Vegas Creek Restoration Project ranging from the wording of the Safe Harbor Agreement to the relevance of the International Pool Code to the ponds.

The Meadows Detention Basin pumping system and associated ponds irrigation infrastructure would not have been possible without the efforts of numerous people in Engineering, Survey, or Permitting, including but not limited to Peter Jauch, Director Engineering, Steven Anderson, Engineering Project Manager, Sean Corkill, Gary Hale, Michael Kajkowski, and Dianja Gates.

Hydrologist Erin Cole provided invaluable expertise pertaining to investigating the possibility of a shallow water well for beneficial wildlife use. Chris Ashinhurst was instrumental in selecting and deploying a remote water quality monitoring station.

Contractors Debbie Gibson at Green Valley Pools and filtration guru Kent Wallace took our ideas and back-of-the-envelope designs and built relatively low maintenance state-of-the-art mesocosms for which they should be very proud.

Many other staff have provided their expertise and assisted in seeing this project to fruition. I am grateful for their assistance. I thank Aaron Ambos, Thomas O'Toole, Audrey Bennett, Brian Horne, and Kimberly Reinhart for reviewing earlier drafts of this report.

Funding for the initial ten ponds was provided by the Southern Nevada Public Lands Management Act through the City of Las Vegas. Subsequent funding has been supplied by the Springs Preserve and SNWA. Lisa Windom miraculously turned around complex federal grant paperwork in two hours, resulting in a ponds electrical infrastructure grant from the U.S. Fish and Wildlife Service. From there, Kathy Flanagan helped negotiate the reporting requirement of a federal grant. We thank US Fish and Wildlife Service Field Supervisor Glen Knowles and Susan Cooper for graciously providing funds to begin creating two addition refugia ponds.

EXECUTIVE SUMMARY

The Springs Preserve has a rich cultural and environmental history. Three springs once flowed on the site, creating lush meadows of green that are the namesake of Las Vegas. As the city grew, the Las Vegas Creek flows, once a hallmark of the valley's geography, stopped flowing altogether. With the loss of the water, biodiversity decreased and species were lost, including the Las Vegas Dace and the Vegas Valley Leopard Frog.

The Springs Preserve has been restoring native plant communities and creating wildlife habitat since 2003. The objective of the Las Vegas Creek Restoration Project is to restore aquatic ecosystems along portions of the historic creek. The first step was obtaining the necessary assurances and permits from the US Fish and Wildlife Service, Nevada Department of Wildlife, and City of Las Vegas. Once obtained, low maintenance state-of-the-art ponds were designed and built to create habitat for rewilding, which is the use of similar species as ecological proxies for the species that were lost. Once the ponds were built, experimental populations of the endangered Pahrump Poolfish and imperiled Relict Leopard Frog were translocated to the Springs Preserve.

On May 29, 2018, NDOW trapped and translocated 290 adult Pahrump Poolfish from the Shoshone Ponds refugia in Spring Valley, Nevada and released 145 in each of the two Springs Preserve ponds. The fish bred almost immediately and the first fry were observed some three weeks later. On October 3–9, 2018, a mark-recapture survey was conducted using standard fisheries methods. The survey revealed that an estimated 386 (95% CI: 278–605) Pahrump poolfish inhabited the ponds, an increase of 25% (N = 96 fish) in a little over four months. The fish then began to succumb to a virulent attack by a flavobacterium and secondary fungal infection. The remains of 22 Pahrump Poolfish (approximately 5.7% of the estimated population size) were recovered showing signs of fungal infection. A federal fish pathology laboratory concluded that "immunosuppressed mature fish were succumbing to opportunistic aquatic bacteria and fungi." Pathologists speculated that the pathogens were the result of two confounding stressors: (1) environmental – a 7°C degree drop in water temperature (i.e., from 22°C to 15°C between the 1st and 2nd capture sessions); and (2) anthropogenic – the stress caused by trapping, handling, and marking during a mark-recapture survey. Surveys in June and September 2019 documented an estimated 173 (95% CI: 131–232) and 164 (95% CI: 120–232) Pahrump Poolfish in the ponds. Although the population size stabilized in 2019, it represents a 58% decline from the October 2018 survey. This may be partially explained by the presence of hundreds of imperiled Relict Leopard Frog (*Rana onca*) tadpoles in 2019, which may have altered the primary productivity, and thus carrying capacity, of the system. As of August 23, 2020, there has been no observed recurrence of mortalities as a result of immunosuppressed Pahrump poolfish. Recruitment is still occurring, as schools of Pahrump poolfish fry were observed from May 14, 2019 until October 3, 2019 and several cohorts of fry have been documented since May 19, 2020. In September 2020, surveys revealed a population estimate of 93 (95% CI: 41–232) Pahrump Poolfish in the downstream pond; whereas, the upstream pond had an estimated population of 98 (95% CI: 72–137) Pahrump Poolfish. An overall population estimate of 191 fish in 2020 is consistent with estimates produced from 2019 surveys. The population appears to have stabilized.

Relict Leopard Frogs were introduced to the site for the first time on May 29, 2018. Upon the release of the initial 100 newly-metamorphosed Relict Leopard Frogs, a female Mallard Duck was observed consuming several of them as they floated on the surface of the upstream pond. These laboratory-raised frogs appeared to not have developed an effective flight response, which was compounded by a lack of dense cover in the newly-planted riparian areas. A nocturnal visual encounter survey (VES) in July 2018 noted the presence of only six Relict Leopard Frogs. By October 2018, four (1Male:3Females) large adult-sized frogs were captured and PIT tagged (i.e., implanted with a microchip carrying a unique identifying number) during a nocturnal survey. In March 2019, a nocturnal survey revealed the presence of two adult Relict Leopard Frogs. A male was captured at that time and its identity confirmed via PIT tag. This male, released as a newly metamorphosed frog in May 2018, was calling prior to capture, and thus already sexually mature less than a year after metamorphosis from a tadpole. In April 2019, *in situ* reproduction was confirmed when hundreds of small tadpoles were observed in the ponds. Although no egg mass was observed, Relict Leopard Frog egg masses can contain up to 1,100 eggs. Tadpoles began to undergo metamorphosis in July 2019, and by August 2019, a VES documented 195 Relict Leopard Frogs and one tadpole in the ponds. Six of the observed frogs were of adult size. In October–November 2019, 214 Relict Leopard Frogs were captured and marked in the ponds. Twelve of these frogs were of adult size, including a now record-sized PIT tagged female from the 2018 cohort, which measured 97 mm from snout to vent. A subsequent recapture event revealed that an estimated 424 frogs inhabited the ponds (with a 95% Confidence Interval = 308–540). Although the vast majority of the frogs were young and had not yet overwintered, the presence of so many frogs was promising in terms of their potential contribution to the overall status of this species. Relict Leopard Frogs reproduced at the Springs Preserve again in 2020 and three separate tadpole cohorts were documented. One tadpole cohort was first documented on May 6th, 2020; whereas, the second was first observed on July 3rd, 2020. A third cohort of large tadpoles with 4 mm posterior limbs was first documented on September 12th, 2020.

In September–October 2020, 286 Relict Leopard Frogs (40 adults and 246 juveniles) were captured during mark-recapture surveys. The adult population was estimated to be 71 frogs (95% Confidence Interval = 44–99); whereas, the juvenile population estimate was 539 frogs (95% Confidence Interval = 416–661). Thus, an estimated 610 frogs now call the Springs Preserve home. This is an overall increase of 186 frogs…a 44% increase over the estimated population size of 424 frogs in fall 2019. In addition, as tadpoles, the 3rd cohort wasn't part of the October 2020 estimate.

The success of the rewilding project has garnered substantial positive publicity both locally and internationally. Pahrump Poolfish and Relict Leopard Frog case studies will be featured in an upcoming book published by the International Union for the Conservation of Nature (IUCN) Conservation Translocation Specialist Group. The success also resulted in a sizeable grant from the US Fish and Wildlife Service for the electrical infrastructure for two additional refugia in the south fork of the Las Vegas Creek.

PHOTO & ILLUSTRATION CREDITS

Thanks go to the many colleagues that provided illustrations and photos for this report. The format for credit is **Author Name:** Subject (**Page #, location on page if more than 1 photo on page**).

Aleta Karstad: Vegas Valley Leopard Frog watercolor (**7, top**)

Aaron Ambos: Pahrump Poolfish (**8**), Relict Leopard Frog (**13, top**), Relict Leopard Frog Tadpole (**14**), Pahrump Poolfish fry (**34 top**), Small Relict Leopard Frog Tadpole (**36, top**),

Peter Duncombe: Ponds restoration planting map (**29**)

Florence Lee Jones: Map of early Las Vegas (**5, bottom**)

Kevin Guadalupe: Rebeca Rivera holding largest female Relict Leopard Frog (**35, right**)

Las Vegas Review-Journal: Oasis for Threatened Species (**iii**)

Las Vegas Weekly: Preserving a Relict article **(45)**

Melissa Trammell: Pahrump Poolfish Safe Harbor Agreement signing (**16**)

Raymond Saumure: Downstream pond NF 1-b (**cover**), Upstream pond NF 1-a (**iv**), Shoshone Ponds Well # 2 outflow (**11**), Shoshone Ponds Stockpond (**12, top**), Spring Mountain Ranch Lake Harriet (**12, bottom**), Relict Leopard Frog (**13, bottom**), Relict Leopard Frog ventral coloration (**15**), Meadows Detention Basin ponds pumping station (**25**), Pond NF-1a with leaves (**26, top**), Downstream pond NF-1a construction (**26, bottom left & right**), Upstream pond NF-1b construction (**27, top, left & right**), Skimmer and float valve (**27, bottom left**), Upstream pond waterfall (**27, bottom right**), Aeration pumps (**28, top right**), Downstream pond aeration bubblers (**28, bottom right**), Downstream pond (**30, top left & right**), upstream pond (**30, bottom left & right**), Moapa White River Springfish release (**31**), Pahrump Poolfish delivery (**32, top**); Newly metamorphosed Relict Leopard Frogs (**33, top right**), Mark-recapture processing (**33, bottom**), Pahrump Poolfish infection (**34, bottom**), Rebeca Rivera holding newly metamorphosed Relict Leopard Frog (**35, left**), Record-sized female Relict Leopard Frog (**36, bottom right**), Rebeca Rivera weighing large Relict Leopard Frog (**37, left**), Second-largest female Relict Leopard Frog (**37, bottom**), Algae removal in upstream pond (**38, top**), Float valve in skimmer (**38, bottom**), Satellite communication system (**40, top right**), Temperature graph (**40, bottom right**), pH graph (**41, top right**), Conductivity graph (**41, middle right**), Jef Jaeger and & largest Relict Leopard Frog (**42**)

Renee Grayson: Relict Leopard Frog (**13, middle**)

Rick Harris: Springs Preserve ponds map (**1**), Springs Preserve map (**3**), Springs Preserve South Fork map (**43**)

Robert Miller: Las Vegas Dace (**7, bottom**), Manse Spring (**9**)

Thomas O'Toole: Relict Leopard Frog basking (**2**)

UNLV Special Collections: Grazing (**4, top**), Settlers in spring pool (**4, bottom**), Artesian well (**5, top**)

INTRODUCTION

As an island of biodiversity amidst an urban sea, the Springs Preserve has a rich cultural and environmental history. Three springs once flowed on the site, creating lush meadows of green that are the namesake of Las Vegas. By 1960, however, residents had drilled nearly 3,000 wells in the Las Vegas Valley, with more than half of the groundwater coming from less than 25 wells located within a mile of the Las Vegas Springs. The Las Vegas Creek flows, once a hallmark of the valley's geography, stopped altogether by 1962. At some prior point in time, flows were insufficient to sustain populations of fish and frogs that were entirely dependent upon the dwindling spring waters.

The Springs Preserve opened to the public in 2007. Along with educational objectives, part of its mission was to restore the unique cultural and environmental resources now protected within the Preserve's boundaries. Although the landscape-scale habitat restoration projects that began in 2003 have been completed, specific projects targeting wildlife are still being implemented. For instance, the Springs Preserve partnered with the Nevada Community Foundation and NV Energy to install raptor protectors on Las Vegas Valley Water District (LVVWD) powerlines. These devices prevent larger birds of prey from electrocuting themselves, but also indirectly prevent wildfires. Another project was the re-introduction of a small population of adult Desert Tortoises within a 15-acre restored tortoise habitat funded by a grant from Clark County.

The current project detailed herein involves the restoration of specific portions of the Las Vegas Creek. The creation of the initial 10 ponds and associated pumping station (**below**) at the Meadows

Detention Basin was funded by a Southern Nevada Public Lands Management Act (SNPLMA) grant.

The objective of the Las Vegas Creek Restoration Project was to recreate aquatic and riparian habitat along the historic Las Vegas Creek to benefit wildlife and return some semblance of ecosystem function. These ponds benefit a myriad species ranging from the birds and the bees, to dragonflies and Gray Fox, all of whom call the Springs Preserve home. In addition, as a rewilding project, the goal was to repopulate the Springs Preserve with similar species of fish and frogs, as ecological proxies to those that were lost prior by 1962. This report documents the success of our restoration and rewilding efforts between 2008–2020.

SITE DESCRIPTION

The Springs Preserve

The Springs Preserve is a 180-acre urban park located entirely within the City of Las Vegas and is recognized as the birthplace of Las Vegas, Nevada (**right**). The Springs Preserve is owned and operated by the Las Vegas Valley Water District.

The Las Vegas Springs and Las Vegas Creek were used by the Ancestral Puebloans, Patayan, and Numa (Paiutes) peoples. In 1829, a New Mexican merchant, Antonio Armijo, led an expedition along the Virgin River to find a new trading route between New Mexico and California. During the trip, a teenage scout, Rafael Rivera, wandered away from the scouting party and ended up following the Las Vegas Wash to a mesa where he could see springs and meadows. Once Rafael had regained the caravan, he led the party to the lush meadows and springs. The route they followed became known as the Old Spanish Trail.

The area Mr. Rivera discovered was named *Las Vegas*, meaning "the meadows" in Spanish. Captain John C. Fremont led a U.S. military expedition through the valley in 1844 and was the first to map the "Old Spanish Trail" route, which linked California and New Mexico. From 1847 to 1858, the Las Vegas Springs became a major campsite. Hundreds of wagon trains moved through the valley and camped by the springs.

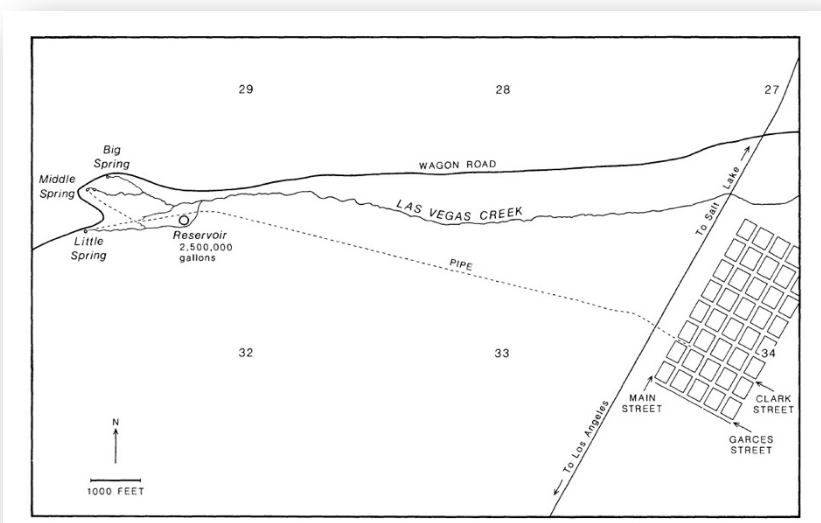
In 1855, Mormon missionaries built a fort, known as the Old Las Vegas Mormon Fort, downstream of the springs near the Las Vegas Creek. They planted fruit and shade trees and established friendly relations with the Paiutes. The Mormon missionaries abandoned the fort in 1858. The abandoned Mormon Fort gained new life as "Los Vegas Rancho" when Octavius Decatur Gass of California developed the area. He and his friends restored the fort and developed small "ranches" near it.

At the springs and upper Las Vegas Creek to the west of the fort**,** James B. Wilson of Ohio and John Howell of New York worked together and filed for ownership of the 320 acres, which they called the Spring Rancho. Howell and Wilson raised cattle and horses on irrigated grassy meadows (**right**) and planted fruit trees. In 1872, Gass filed on most of the water from the springs, citing prior water rights. By 1878, Gass owned all the land watered by the creek. Unable to sell the ranch when other land development plans failed, Gass borrowed money from Archibald Stewart. Gass planned to use money from his next crop to pay off the loan, but bad weather destroyed the crop and he turned over the property to Stewart. In 1882, Stewart and his family moved to Los Vegas Rancho, which they referred to as the Upper Rancho.

A gunfight with a hired-hand from another ranch killed Archibald Stewart in 1884. Stewart left behind his pregnant wife, Helen J. Stewart, and four children. Travelers continued to come to the ranch in search of water (**left**), food, and rest during their journeys, and Helen continued to run the ranch for the next 20 years. In 1902, Helen Stewart signed an agreement with Senator William A. Clark of Montana to sell the 1,864 acre Stewart Ranch and its water rights for $55,000 to the San Pedro, Los Angeles, and Salt Lake Railroads (later known as Union Pacific). The railroad created the Las Vegas Land and Water Company to operate the first water distribution system in the valley. In May 1905, the company auctioned off land, creating the town site of Las Vegas. The City of Las Vegas was officially incorporated in March 1911.

To supply the railroad and the new town with water, the Las Vegas Land and Water Company laid redwood pipes and constructed protective houses over the springs to keep people, cattle, and other polluting factors out of the water supply. Beginning in 1907, residents began drilling privately-owned wells, tapping into the underground aquifer (**right**). Often, such wells were not capped, allowing water to flow continuously due to artesian pressure. Records from the State Engineer's office indicated that Little Spring flowed at a rate of 2,700-3,150 gallons/minute (gpm) in 1908. In 1912, Big and Middle springs flowed at rates of 2,390 and 2,580 gpm, respectively (Maxey and Jameson 1948). Within 40 years, the Nevada State Water Engineer declared Las Vegas groundwater overdrawn. As Las Vegas grew, the springs could not meet peak demands.

The Las Vegas Land and Water Company complained about water waste and proposed metering water use, but the Nevada State Legislature opposed such measures. In 1923, the company drilled Well No. 1 near the Las Vegas Springs to help meet the new city's growing water needs. The federal government's Hoover Dam project brought a huge influx of people to the area from 1928 until 1936 (**left**). Drought and heavy demand for water put great pressure on the railroad's Las Vegas Land and Water Company. In 1935, Las Vegas Creek dried up in the summer. In 1936, the Las Vegas Land and Water Company drilled Well No. 2 into the spring mound south of the Las Vegas Creek, and piped the water to a reservoir. Only two years later, in 1938, a major water shortage occurred. By 1940,



Las Vegas' population had grown to 8,422. The outbreak of World War II brought the defense industry, including the U.S. Army Aerial Gunnery Range (now Nellis Air Force Base) and Basic Management, Inc. (BMI). BMI was the first to import Colorado River water from Lake Mead. Prior to this, the valley relied solely on groundwater.

Residents went through another water shortage in 1947. Southern Nevada's population increased to 41,000 by 1950. Groundwater use increased to 35,000 acre feet per year, exceeding nature's ability to recharge the groundwater aquifer naturally. Frustrated with the Las Vegas Land and Water Company's inability to provide water as fast as the town grew, the people convinced the Nevada State Legislature to authorize the purchase of the entire water system. In 1953, the Union Pacific Railroad sold the Las Vegas Land and Water Company for $2.5 million to the newly-created government entity, the Las Vegas Valley Water District.

By 1960, residents had drilled nearly 3,000 wells in the Las Vegas Valley. However, more than half of the groundwater came from less than 25 wells located within a mile of the Las Vegas Springs. By then, the population had increased to approximately 119,000. Increased well water use caused the groundwater level to decline about two to four feet per year. The Las Vegas Springs flows, once a hallmark of the valley's geography, stopped altogether by 1962.

In the 1970s, a portion of the spring site came close to being paved over by the Nevada Department of Transportation's plans for an expressway. An archaeological survey conducted in 1972 confirmed the long-term Native American occupation of the site. This discovery helped reroute US 95 around the Enrolled Property. Concerned citizens and the LVVWD petitioned to add the Las Vegas Springs to the National Register of Historic Places. Under the National Historic Preservation Act of 1966, the Las Vegas Springs were designated an archaeological site and listed on the National Register in 1978. By 1997, the LVVWD Board of Directors approved a plan to develop a preserve to protect and manage the cultural, natural, and water resources of the site. The Springs Preserve opened in June 2007.

Today, the Springs Preserve is a leader of cultural and environmental sustainability, having achieved national and international acclaim. Approximately 90 acres of degraded Mojave Desert plant communities have been restored since 2000 at the Enrolled Property. In addition to being listed in the National Register of Historic Places, the Springs Preserve museums have achieved LEED Platinum status, the highest level of certification from the U.S. Green Building Council. The Springs Preserve also recently received ISO 14001 certification for its Environmental Management System. As Las Vegas' cultural center, some 250,000 visitors a year come to the birthplace of Las Vegas to be entertained and enlightened about southern Nevada's archaeology, botany, geology, history, paleontology, and wildlife.

REWILDING

Rewilding

Historically, the Springs Preserve was inhabited by two aquatic species that fulfilled distinct ecological roles in the ecosystem. These were an amphibian, the Vegas Valley Leopard Frog (*Rana fisheri*), and a fish, the Las Vegas Dace (*Rhinichthys deaconi*).

The Vegas Valley Leopard Frog (**right**) was described from adult specimens collected from the Las Vegas Creek and downstream Los Vegas Rancho in 1891. Vegas Valley Leopard Frogs were last observed and collected at Tule Springs in 1942 and the species was presumed extinct in the 1940s. Fortunately, genetic research using ancient DNA from alcohol-preserved specimens collected in the Las Vegas Creek in 1913 revealed that Chiricahua Leopard Frog (*Rana chiricahuensis*) inhabiting the Mogollon Rim and the White Mountains of central and eastern Arizona and western New Mexico were, in fact, Vegas Valley Leopard Frogs.

The Las Vegas Creek was also home to the Las Vegas Dace (*Rhinichthys deaconi*), an extinct species of fish described from museum specimens (**below**) decades after the springs had run

dry. One of the earliest mentions of this dace species was from the diary of Vernon O. Bailey, U.S. Biological Survey, who stated on March 9, 1891 that "*there were lots of little fish in the springs and streams…*" Las Vegas Dace were observed in the Las Vegas Creek in 1942, but by then, crayfish were well-established. The species was likely extinct by 1967.

Today, neither the Vegas Valley Leopard Frog nor the Las Vegas Dace are available for re-introduction to the Springs Preserve. Rewilding, however, is achieved by using similar species as ecological proxies. Thus, rewilding efforts at the Springs Preserve included a fish, the federally endangered Pahrump Poolfish (*Empetrichthys latos*) and a frog, the state-protected Relict Leopard Frog (*Rana onca*). These two species will be used to restore ecosystem function to recreated aquatic systems at the Springs Preserve. Descriptions of each rewilding species follows.

Pahrump Poolfish

Species Status

The Pahrump Poolfish was listed as endangered on March 11, 1967, under the Endangered Species Preservation Act of 1966. Its endangered status was retained with the passage of the Endangered Species Act in 1973. The Service approved a Recovery Plan for the species on March 17, 1980. Critical habitat for the species has not been designated.

Species Description

The Pahrump Poolfish (**below**), formerly known as the Pahrump Killifish, was first described by Miller in 1948. The Pahrump Poolfish is a small fish that obtains a maximum length of approximately 77 millimeters (mm) (3 inches), with females generally larger than males. This poolfish has a slender, elongate body with dorsal and anal fins placed far back on the body, pectoral fins typically with 16 to 18 rays, and no pelvic fins. These fish have a broad upturned mouth; a dark longitudinal streak that tends to disappear in older, larger individuals; and an orange ring around the eye. The body is generally greenish-brown with black mottling, but males may be silver-blue without mottling during the spawning season. The dorsal, anal, and caudal fins are bright orange-yellow when the fish are in an environment of optimal temperature and dissolved oxygen.

Habitat Description

Manse Spring (**right**), in Pahrump Nevada, was historically a large, clear limnocrene (i.e., a spring originating from a large, deep pool of water) discharging at approximately 0.17 cubic meters per second (6 cubic feet per second [cfs]) in 1875. Water temperature was a relatively constant 24 °C (75 °F) (range 23.3–25.0 °C [74–77 °F]) and the water was alkaline. The main spring pool was 9 m (29 feet) wide and 3 m (9 feet) deep at the head, 2 m (6.5 feet) wide and 0.3 m (1 foot) deep at the outlet, and 18 m (59 feet) long. A shallow ditch extended 3 to 6 m (10 to 20 feet) southward from the main spring pool. Water current ranged from slow to absent in the main spring pond and shallow ditch to swift in the outflow channel. The spring pool had a silty bottom and was dense in areas with macrophytes, including watercress (*Nasturtium* sp.), stonewort (*Chara* sp.), and pondweed (*Potamogeton* sp.).

The genus *Empetrichthys* was described as being frequently found in the deeper holes of warm desert springs, and usually uncommon in shallow spring-fed ditches or marshy areas. At Manse Spring, poolfish used all three of the different habitats described above: the spring pool, shallow ditch, and swifter outflow stream. Larger fish utilized the more open and deeper waters, and young fish utilized the near water surface layer in shallow areas with aquatic vegetation. After hatching, fry (i.e., young fish, post-larval stage) remained near the bottom or near other substrates, presumably for protection and to feed. Given the partitioning of habitat by age class, it is likely that different life stages (larvae, fry, juveniles, adults) use or need different resources (e.g., food items, cover for predator avoidance), and/or have different physiological tolerances or requirements.

Despite the nearly constant water temperatures of 24 °C (75 °F) found in the Pahrump Poolfish's ancestral habitat (i.e., Manse Spring, Nye County, Nevada), transplanted populations have demonstrated the ability to tolerate a much wider range of water temperatures. At Corn Creek, poolfish survived at low temperatures of 4 °C (39.2 °F) under ice in a trough; and at Latos Pools, poolfish withstood annual water temperature fluctuations from below 10.5 °C to 25 °C (51–77 °F). At Lake Harriet, poolfish have been reported to enter torpor during winter. Research on the thermal tolerance of this species in the laboratory demonstrated that poolfish could tolerate temperatures from at least 1.5 °C (lower temperatures were not tested) to 40 °C (34.7–104 °F) for short periods of time, with specific tolerances depending on original acclimation temperatures. This same study found that poolfish are incapable of behavioral thermoregulation. Although there was a noticeable decline in population size, Pahrump Poolfish at the Springs Preserve survived temperatures of 0.5 °C and 1.5 °C (33–34 °F) in the two ponds over the winter of 2018–2019.

Nonetheless, the wide thermal tolerance of Pahrump Poolfish has allowed the species to be successful in transplant sites that differ substantially in temperature regime from its native Manse Spring.

Pahrump Poolfish also are capable of withstanding a wide range of dissolved oxygen, including low levels, at least for limited time periods. Pahrump Poolfish can withstand low levels of dissolved oxygen down to 1.0 parts per million, similar to its close relative *Crenichthys*. However, the poolfish has a body shape and mouth orientation that makes utilization of the surface water layer to obtain oxygen difficult; because of this, it is thought to not be able to survive extended periods of oxygen depletion. Research at Corn Creek surmised that Pahrump Poolfish deaths were due to fish being trapped in an area with high vegetation respiration at night, which depleted the immediate environment of oxygen. As a result, emergent aquatic plants were planted in the pond pockets and pots at the Springs Preserve to partially alleviate this effect of photosynthesis.

Population Dynamics

Pahrump Poolfish exhibit high fecundity and thus have a high degree of demographic resilience. The population at Manse Spring was able to grow from fewer than 50 adults to over 1,000 fish within a few years' time on two occasions during the 1960s. Additionally, refuge populations have shown the ability to grow considerably and rather rapidly from initial low stocking rates, and to rebound following rather large population declines (see below). It is not surprising that this species is capable of rapid population growth given its life history characteristics. Characteristics that suggest high intrinsic rates of increase include: (1) small body size; (2) early maturation; (3) short generation time; (4) small clutch size but high reproductive effort due to multiple spawning bouts over a protracted period; and (5) low investment per offspring.

Life History

Information about the ecology, behavior, life history, population dynamics, and habitat requirements of the Pahrump Poolfish is based largely on historical information derived from its ancestral location at Manse Spring. The species can inhabit entirely different habitats today. Our knowledge of this species consists of limited information on life history and habitat characteristics at refugia sites and from laboratory (aquaria) settings. Caution must be exercised in interpreting this information because habitat differences at these various sites, and even within the same site over time, can lead to divergence of life history traits. For example, certain poolfish life history traits changed following the introduction of goldfish at Manse Spring in the 1960s. Even so, available information demonstrates that the Pahrump Poolfish is a hardy and fairly adaptable fish. This adaptability is established by its ability to survive and reproduce at sites that are distinctly different from its native habitat; its ability to survive and reproduce at sites that vary widely in environmental characteristics; and its ability to rebound from severe population crashes caused by habitat alterations at its native Manse Spring or from unknown causes at refugia sites (e.g., 2003 population decline at Shoshone Ponds).

Given its small size, the Pahrump Poolfish is probably short lived (e.g., 2–4 years). This species is unique among Goodeids in that it (and other members of the genus *Empetrichthys*) lay eggs and do not bear live young. Parental care (e.g., protection of eggs or fry) has not been reported for this species and young and adults appear to use different habitats.

Pahrump Poolfish spawning peaks in spring, but may occur in any season and for much of the year if proper conditions are present. At Manse Spring (1961–1965), Pahrump Poolfish had a protracted reproductive period that extended from January through July with a peak in April based on the number of mature eggs in the ovaries of poolfish specimens collected during those years. Poolfish transplanted to new locations appear to adjust their spawning season to temperature conditions at the new sites, with delays in spawning observed at sites with cooler and more variable temperatures than the ancestral site. For example, Shoshone Ponds (**right**) is about 2.7 degrees latitude further north and about 914 m (3,000 feet) higher in elevation than Manse Spring.

Pahrump Poolfish at Manse Spring apparently did not reach sexual maturity until they were over 30 mm (1.2 inches) Standard Length (SL) based on the absence of mature eggs in the ovaries of smaller fish (< 30 mm [1.2 inches] SL) that were collected from 1961–1965. Reproductive potential (measured as the mean number of mature eggs produced by each size class) increased substantially with size for fish ≥ 30 mm (1.2 inches) SL during the month of April, which was the peak period of reproduction. Thus, the number and proportion of larger female poolfish in the population during April was an important determinant of reproductive potential at Manse Spring. Similarly, in a laboratory setting, larger females (> 46 mm [1.8 inches]) typically produced more eggs than smaller females. Annual fecundity (i.e., the total number of eggs spawned by a female during a single spawning season) of Pahrump Poolfish is unknown.

This species likely produces few eggs per spawning, but may spawn multiple times per season at sites with appropriate environmental conditions. In the laboratory, the number of eggs produced per female ranged from 0 to 28 over a three-day trial period; whereas another study reported that adult females produced 10–30 eggs per week for over two months. In the laboratory, eggs hatched in 7–10 days (average of eight days) in water temperatures of 24 °C (75 °F), which was the approximate temperature of Manse Spring. Poolfish eggs developed over a period of 2–3 weeks. Both egg and larval poolfish development will likely differ by site due to water temperature differences (e.g., slower development would be expected in cooler waters). Young fish in transplanted populations are more active during the day and adults are more active at night. Poolfish are inactive during winter at some transplant sites (e.g., Springs Preserve, Lake Harriet) when water temperature cools considerably.

Pahrump Poolfish are opportunistic omnivores, eating a wide variety of animal (e.g., aquatic insects, snails) and plant material, while also ingesting large amounts of debris and inorganic material. These fish are able to adapt their diet to food item availability as determined by environmental conditions. For example, prior to the establishment of Goldfish at Manse Spring, the relative volume of aquatic insects in the poolfish diet was high. Following Goldfish establishment, a higher proportion of poolfish consumed plant material and the average volume of aquatic insects in the guts of samples declined. This dietary shift was attributed to habitat changes caused by Goldfish (e.g., higher turbidity, disturbance of aquatic macrophytes), which may have affected insect density and detectability.

In a dietary study of transplanted poolfish populations in the early 1990s, researchers found that debris and plant/algal material comprised the largest part of the poolfish's diet at Shoshone Ponds (**right**) and Spring Mountain Ranch State Park; whereas, insects and other animal items comprised a slightly larger part of the diet than debris and plant items at Corn Creek. Debris, such as sand or sticks, is generally coated with epiphytic bacteria or diatoms, providing nutrients to fish. Based on known diet at Manse Ranch and available food sources at Shoshone Ponds, it has been suggested that larger zooplankton was likely an important food source for poolfish at Shoshone Ponds.

Refugia

In an attempt to prevent the extinction of the only remaining member of the genus *Empetrichthys*, refuge populations were established in Nevada at Los Latos Pools on the Colorado River near Lake Mojave (Colorado River Valley) in 1970; Corn Creek Springs in the Desert National Wildlife Refuge, Clark County (Las Vegas Valley) in 1971; and the Shoshone Ponds Natural Area (Middle Pond, North Pond, and Stock Pond) located in Spring Valley in White Pine County, Nevada in 1976; and Lake Harriet (**left**), Spring Mountain Ranch State Park west of Las Vegas in Clark County, Nevada in 1985. Pahrump poolfish continue to persist today at all refuge sites except the Los Latos Pools.

Relict Leopard Frog

Species Status

The Relict Leopard Frog is a state-protected species in Nevada. Previously, it was a candidate for federal listing, a status that was precluded because of higher priority listings. In 2016, the USFWS reviewed the status of the species and ruled that federal protection was not warranted at this time because of the exemplary actions of the Relict Leopard Frog Conservation Team.

Species Description

The Relict Leopard Frog (*Rana onca*) was first described in 1875 from along the Virgin River in Utah. The word "onca" is Latin for spotted; thus, the species' scientific name means the 'spotted frog.' At various times, *R. onca* has also been considered to be the same species as *R. fisheri* or a subspecies of *R. pipiens*. Although the species was believed to be extinct, a genetic study rediscovered the species in 1991.

Relict Leopard Frogs are somewhat smaller than other leopard frog species. The species exhibits considerable variability in dorsal coloration, which varies from olive to beige to brown (**right**). Their underside are white but the thighs and legs of adults can be bright yellow.

Habitat Description

Historically, the species was known from 24 sites in Arizona, Nevada, and Utah. Native populations of Relict Leopard Frogs have been documented at seven sites in the 1990s, of which only five remain. These five sites occur in two areas: (1) a 5.1 km stretch composed of Boy Scout, Salt Cedar, and Bighorn Sheep springs in Black Canyon below Lake Mead on the Colorado River in Nevada and Arizona; and (2) a 3.6 km stretch composed of Blue Point and Rogers springs in the Overton Arm within Lake Mead National Recreational Area. Extant populations of Relict Leopard Frogs inhabit thermal spring systems with permanent water sources devoid of Bullfrogs (*Rana catesbeiana*).

Population Dynamics

Relict Leopard Frog populations tend to be small, with few adults. In fall 2019, the number of frogs in eight natural (i.e., not translocated) populations ranged from 1–57 frogs; whereas, the largest experimental refugia population had 218. The total number of wild frogs was 1,083, including 214 marked frogs at the Springs Preserve. Thus, the Springs Preserve population accounts for at least 20% of the known populations of Relict Leopard Frogs. The closed population estimate for the Springs Preserve, however, was 424 with a 95% CI of 308–540. A marked recapture study conducted at Blue Point Spring, estimated a 27% annual adult survivorship rate.

Life History

Relatively little is known about the life history of this species. Relict Leopard Frogs can reach sexual maturity in less than one year, with newly transformed frogs released in late May 2018 documented calling in late March 2019 and breeding in April 2019. Reproduction is reported to occur from January to April and again in November in thermal spring systems. Egg masses are deposited amidst vegetation at the water's surface in shallow water. Egg masses are believed to contain approximately 600–1,100 eggs.

Since the ponds are not heated at the Springs Preserve, the appearance of tadpoles suggests that egg masses are deposited in April or later. At least one tadpole with hind limbs overwintered at the Springs Preserve in 2019, the first time this has been documented for Relict Leopard Frogs. Tadpoles bask underwater on filamentous algae mats in sunspots and remain hidden on overcast days. At the Springs Preserve, tadpoles (**right**) grow quickly and begin the metamorphose from tadpoles to frogs in late July and August. Indirect observations at the Springs Preserve confirms that tadpoles do feed on algae, as previously reported. Tadpoles are known to feed on Relict Leopard Frog eggs as well.

There are only two reports of diet in the wild for Relict Leopard Frogs. One frog from Utah had consumed a beetle, damselfly, and a wasp. At the Springs Preserve, newly-metamorphed frogs consumed Africanized bees (*Apis mellifera scutellate*) minutes after been translocated. The only confirmed predator of Relict Leopard Frogs was a female Mallard (*Anas platyrhynchos*) eating naïve translocated newly-metamorphosed frogs at the Springs Preserve. One newly-metamorphosed Relict Leopard Frog was killed by the defensive sting of an Africanized bee to its tongue when the frog attempted to consume it. Longevity in the wild is believed to be at least four years. Hibernation sites have, as of yet, not been documented for this species.

Refugia

In an attempt to prevent the extinction of the species, refugia populations have been established at numerous sites within the historic range of the species. The Relict Leopard Frog Conservation Plan, however, allows for the establishment of educational and research populations beyond the historic range of the species. Such is the case for the Relict Leopard Frogs in a Springs Preserve educational exhibit, and later in the refugia ponds.

AGREEMENTS & PERMITS

As previously mentioned, the Springs Preserve natural areas also include active groundwater wells and associated infrastructure. Consequently, the Springs Preserve required assurances that existing activities could continue unimpeded before imperiled species could be introduced to the pond mesocosms at the Springs Preserve.

Pahrump Poolfish Safe Harbor Agreement

The Pahrump Poolfish is a federally endangered species protected under the Endangered Species Act (ESA). Consequently, the LVVWD entered into a Safe Harbor Agreement (**left**) so that the terms and conditions, as well as rights and responsibilities of both parties, would be clearly defined. The USFWS was willing to enter into a renewable 15-year agreement with LVVWD because the Springs Preserve refugia ponds would create a fourth refugium for Pahrump Poolfish. This additional Pahrump Poolfish refugium would reduce the potential of adverse effects from catastrophic events, while contributing to research knowledge, management techniques, conservation strategies, and public education and awareness.

The Pahrump Poolfish Safe Harbor Agreement served as the basis for USFWS to issue LVVWD a 15-year renewable enhancement of survival permit under section 10(a)(1)(A) of the Act (Permit), for the incidental take of Pahrump Poolfish while conducting Covered Activities, as described below, including the potential future return of the Springs Preserve to the Baseline condition of no Pahrump Poolfish. The issued permit authorizes LVVWD to take Pahrump Poolfish, and their progeny, that are introduced to the Springs Preserve, and have increased in numbers and/or distribution on those lands above the established Baseline conditions.

Permit issuance will not preclude the need for LVVWD to abide by all other applicable Federal, State, and local laws and regulations. LVVWD will provide USFWS with an annual summary of activities by February 28th of each year for the prior calendar year. The report will describe any habitat restoration activities and any substantial change in condition of previously established habitat for Pahrump Poolfish that occurred during the previous year, any population surveys coordinated by LVVWD during the previous year, the status of implementation of beneficial management activities, and any incidental take of Pahrump Poolfish that has occurred.

Covered Beneficial Activities

The Safe Harbor Agreement includes a list of covered beneficial activities, a summary[1] of which follows.

- LVVWD will strive to maintain adequate water levels and flows in the refugia ponds at all times, but does not have control over the volume of available urban run-off, which could decrease unexpectedly. LVVWD also does not have control over the quality of water entering the ponds from the urban run-off source. However, LVVWD has put in place a number of measures to reduce (but not eliminate) the potential for contaminated water reaching the refugia ponds on the Enrolled Property. LVVWD will be covered for take in the event that water levels decrease or contaminated water flows into the refugia ponds. In the event of a short-term water shortage, LVVWD will pursue alternative means of obtaining water at the Springs Preserve, including but not limited to groundwater wells, adjacent irrigation systems, and/or potable water for ponds that normally receive urban run-off.

- LVVWD will create and maintain pond habitat in an effort to sustain Pahrump Poolfish. The Landowner may pursue a variety of habitat creation and maintenance activities, such as native plant transplantation and removal of excessive native algae, cattail and/or bulrush growth, to ensure balanced levels of aquatic cover and open water suitable to all life stages of Pahrump Poolfish.

- LVVWD will aerate the ponds to increase both circulation and availability of dissolved oxygen.

- LVVWD would be covered for mortalities as a result of natural predators such as, but not limited to, raccoons (*Procyon lotor*), kingfishers (*Ceryle alcyon*), and various species of herons and/or egrets.

- LVVWD will attempt to remove invasive species that might be introduced into the refugia ponds and adversely affect Pahrump Poolfish, such as Bullfrogs (*Rana catesbeiana*) and crayfish.

- LVVWD will coordinate with the USFWS to conduct annual population surveys of Pahrump Poolfish at the Springs Preserve.

- LVVWD will install interpretive signage to educate visitors about native and listed species that occur at the Springs Preserve, including the Pahrump poolfish, the habitats where these species occur, and what can be done to benefit these species.

- In addition to take coverage otherwise obtained under this Agreement for Covered Activities, LVVWD is covered under the Agreement for any take resulting from *in situ* research suggested in the Recovery Plan (USFWS 1980) on the diet, genetics, growth, habitat, movements,

[1] For a complete list, the reader is referred to the Pahrump Poolfish Safe Harbor Agreement itself.

physiology, reproduction, water quality, and/or survivorship of Pahrump Poolfish. The Landowner will submit all research proposals, including a description of the qualifications of the researchers, to the USFWS for review and approval prior to initiation of any research on Pahrump Poolfish at the Springs Preserve. No separate research/recovery permit shall be required for approved research on Pahrump Poolfish at the Springs Preserve.

- LVVWD may continue construction and maintenance of trails, bridges, and viewing platforms to further reduce visitor impact on species and habitats of conservation interest.

- Additional beneficial management activities in the ponds, or that may directly or indirectly affect the ponds, may be identified in the future. LVVWD will submit all additional beneficial management activities proposals to the Service for review and approval prior to initiation of any such activities on Pahrump Poolfish at the Springs Preserve.

Other Management Activities

The Safe Harbor Agreement includes a list of other covered management activities that relate to future or ongoing LVVWD activities at the Springs Preserve that are not related to establishment and maintenance of the Pahrump Poolfish. LVVWD will receive incidental take coverage for activities associated at the Springs Preserve including, but not limited to, its use as a cultural, historical and environmental attraction, a community resource, and an active groundwater well field. LVVWD activities would be conducted in a way that would minimize interference with the implementation of the Beneficial Management Activities described above.

LVVWD is covered under the Pahrump Poolfish Safe Harbor Agreement for activities including but not limited to:

- LVVWD hosts visitors and has group events such as weddings, gatherings for non-profit groups, and educational field trips for schools. These visitors may walk, use bicycles, ride a passenger train, or other transportation devices on pathways located near the ponds.

- LVVWD will manage associated drainages, the Meadows Detention Basin, the water distribution system, and the refugia ponds for multi-use needs of the Springs Preserve.

- LVVWD will continue to use water sources that supply the ponds for a variety of different purposes at the Springs Preserve; water quality and levels in the ponds may vary.

- LVVWD uses the Springs Preserve for groundwater pumping and associated well-field activities that include, but are not limited to, well operation and maintenance, well flushing, well drilling, and pump tests. Well-field operations may cause groundwater level declines, but any such declines would not directly affect pond water levels due to the lining systems of the ponds themselves.

- LVVWD may undertake any and all activities associated with the operation and maintenance of water systems, equipment, and infrastructure used in the delivery of potable and non-potable water to various customers.

- LVVWD may aerate, filter, and manage flows in and out of the ponds as needed for management of the ponds and the Springs Preserve.

- LVVWD may drain the refugia ponds for maintenance activities including, but not limited to, repairing leaks, resealing concrete, removing invasive species, and enhancing ponds.

- LVVWD may grade and re-contour existing access roads within the Springs Preserve, as needed for road maintenance and fire access purposes.

- LVVWD may construct additional pipelines and power lines, and will continue to operate and maintain such facilities.

- LVVWD may extinguish fires or remove brush to prevent the spread of fires.

- LVVWD may clear debris from the refugia ponds such as, but not limited to, tree limbs, leaves, cattails, bulrushes, aquatic plants, algae, and other detritus.

- In order to maintain public health and safety, LVVWD may implement mosquito control measures in and around the ponds.

- LVVWD may use herbicides or pesticides to control invasive and/or noxious weeds and invertebrate pests, as deemed necessary for the management of the Springs Preserve.

Avoidance and Minimization Measures

LVVWD agrees to implement the following measures to avoid and/or minimize potential impacts to Pahrump Poolfish from the Covered Activities listed above:

- LVVWD will install interpretive signage informing visitors and event attendees frequenting the Springs Preserve trail system of the presence and protected status of the Pahrump Poolfish in refugia ponds at the Springs Preserve.

- LVVWD will not intentionally introduce fish species that could prey upon or compete with Pahrump Poolfish in the manmade refugia ponds. Pumping infrastructure includes screens designed to prevent Eastern Mosquitofish (*Gambusia holbrooki*) from being transferred from the Meadows Detention Basin to the refugia ponds. Prior to intentionally introducing any fish species into the ponds at the Springs Preserve, LVVWD will contact USFWS to ensure such species will not adversely affect Pahrump Poolfish.

- If LVVWD needs to use pesticides or other chemicals to manage the Springs Preserve, the LVVWD will select chemicals and applications to avoid or minimize adverse effects to Pahrump Poolfish.

- Periodic water quality testing will occur in each pond; data on dissolved oxygen, pH, conductivity, and water temperature will be recorded. These data will be used to interpret the health of the pond ecosystems as part of an adaptive management strategy.

- If LVVWD becomes aware of any contamination to water sources for the refugia ponds at the Springs Preserve, LVVWD will inspect and test water samples, if necessary, coordinate with the local regulatory agencies, and/or use alternate sources of water for the refugia ponds. LVVWD is developing alternative sources of water for these ponds, which may include but are not limited to, urban run-off through existing drainage channels, well flushing, fire hydrants, adjacent irrigation systems, potable water, and existing or new groundwater wells.

- If water supply becomes interrupted or the refugia ponds begin to leak, LVVWD will take actions to attempt to maintain adequate water levels and flows in the refugia ponds that Pahrump Poolfish inhabit. Measures may include, but are not limited to, using water from groundwater wells, adjacent irrigation systems, and/or potable water.

- If LVVWD needs to drain refugia ponds or determines that it is unfeasible to maintain adequate water levels and flows in the refugia ponds, LVVWD will coordinate with the USFWS to either translocate the Pahrump poolfish to more appropriate refugia ponds at the Springs Preserve, or notify USFWS in advance so that they can relocate the Pahrump Poolfish.

- During any construction, operation, or maintenance activities, LVVWD, or other associated personnel, will exercise due diligence to avoid or minimize negative effects to Pahrump Poolfish.

- Security patrols are conducted regularly throughout the Springs Preserve to protect the water supply and infrastructure. Such security patrols will substantially reduce the potential for disturbance to Pahrump Poolfish and refugia habitat.

Responsibilities of the Parties

In addition to carrying out the activities described above, LVVWD agrees to:

- Notify USFWS at least 30 days in advance of any planned activity that LVVWD reasonably anticipates will result in take of any individual of Pahrump Poolfish at the Springs Preserve, including a return of the Spring Preserve to baseline conditions, and provide the USFWS the opportunity to capture and relocate any individuals that could potentially be affected.

- Coordinate with USFWS to translocate and then conduct annual trapping surveys to assess the status of Pahrump Poolfish populations in the refugia ponds at the Springs Preserve.

- Submit all additional beneficial management and/or research activity proposals to USFWS for review and approval prior to initiation of any such activities on the Pahrump Poolfish at the Springs Preserve. The USFWS agrees to complete its review within 30 days.

- Monitor the implementation and progress of the beneficial management and/or research activities described above, and provide USFWS with the status of these activities in an annual report.

- Allow reasonable access by USFWS or another agreed-upon party onto the Springs Preserve for purposes related to this Agreement, including the capture and relocation of Pahrump Poolfish.

- Report to the Service any dead, injured, or ill specimens of the Pahrump Poolfish observed at the Springs Preserve. Upon locating a dead or injured Pahrump Poolfish, LVVWD will notify the USFWS Southern Nevada Field Office (4701 North Torrey Pines Drive, Las Vegas, Nevada 89130; (702) 515-5230) by telephone within 3 working days of its finding. The verbal notification must include the date, time, location, cause of injury or death if known, and any other pertinent information. An email message or written report containing the details from the verbal notification must be sent to the Southern Nevada Field Office with this information and, if possible, a photograph within 3 weeks of its finding. The person to whom the message is sent, and corresponding email address if applicable, would be determined at the time of the phone call.

- Coordinate with USFWS to develop a list of authorized individuals that will be directly implementing activities in the ponds that may result in take of the Pahrump Poolfish. LVVWD will request changes to Appendix I in writing to USFWS at least 30 days prior to the requested effective date.

Relict Leopard Frog Programmatic CCAA

The Relict Leopard Frog was petitioned for Federal listing under the ESA. Initially, the USFWS ruled that protection under the ESA was “warranted but precluded” by higher priority species. Because of the conservation actions of the Relict Leopard Frog Conservation Team, the species was not federally listed. Should the precarious status of this imperiled species worsen, the Relict Leopard Frog could be listed by the USFWS. Currently, Relict Leopard Frogs are still imperiled and a state-protected species in Nevada. In order to improve the species’ status a Relict Leopard Frog Programmatic Candidate Conservation Agreement with Assurances (CCAA) was ratified between the USFWS and NDOW. LVVWD is the first and, to date, only holder of a Conservation Agreement (CA) under the programmatic CCAA. As such, the Springs Preserve would not be subject to increased property use restrictions if the Relict Leopard Frog is listed as a federally Endangered or Threatened species under the ESA in the future.

The primary objective of this CA is to enhance the conservation status of the Relict Leopard Frog in Clark County, Nevada through the restoration and maintenance of habitats suitable for the establishment of populations by translocation of animals of various life stages. Management activities that are undertaken through this CA will result in additional habitat being available for Relict Leopard Frogs, and an enhanced network of populations established across the presumed natural range of the species.

The 15-year CA authorizes incidental take of Relict Leopard Frogs as a result of lawful activities at the Springs Preserve for the term remaining on the federal USFWS l0(a)(l)(A) Enhancement of Survival permit # TE76244B-0; as well as, associated State of Nevada permits through issuance of a Certificate of Inclusion (COI) and Letter of Take Authorization to LVVWD. The COI authorizes incidental take of Relict Leopard Frogs until September 24, 2045, the remaining duration of the 30-year term of NDOW’s permit with the USFWS.

The COI will authorize incidental take of Relict Leopard Frogs and their progeny resulting from lawful activities at the Springs Preserve. Lawful activities may include, but are not limited to: operation of vehicles and maintenance equipment, building or fence construction, gardening, hunting, recreational fishing, farming, mowing, maintenance of landscaping and recreational facility infrastructure including irrigation facilities, commercial and non-commercial recreational activities or cultivation of agricultural crops. The COI also authorizes incidental take that may result from implementation of conservation actions at the Springs Preserve for the Relict Leopard Frog, such as habitat enhancement and restoration activities, inventory and monitoring activities, and translocation of frogs, eggs, and/or larvae. The LVVWD will be covered for mortalities as a result of natural predators.

Conservation Measures

The CA includes a list of conservation measures including:

- LVVWD will manage aquatic habitats within the enrolled property to attempt to maintain water quality and other parameters necessary for the maintenance of Relict Leopard Frogs.
- Inform NDOW within three working days of finding any dead or accidentally killed Relict Leopard Frogs.
- With reasonable advance notification, allow access to the enrolled lands by NDOW and USFWS to manage, monitor, release, or remove Relict Leopard Frogs, or to carry out other management activities as necessary.
- Inform NDOW as soon as practicable of natural or human-caused emergency circumstances, such as storm events or failure of water delivery systems, which could negatively affect occupied aquatic or terrestrial habitats and could result in take of Relict Leopard Frogs, and allow access to NDOW for emergency salvage or relocation of affected individuals.
- Inform NDOW at least 30 calendar days in advance of planned, otherwise legal activities including the modification or alteration of occupied habitats, which might reasonably be anticipated to result in the indirect take of Relict Leopard Frogs at the Springs Preserve, to allow for their removal to other habitats within the Springs Preserve, or their removal from the Springs Preserve.
- Assist NDOW in compiling an annual report on activities related to Relict Leopard Frog management and any activities that resulted in or may have resulted in incidental take of Relict Leopard Frogs at the Springs Preserve.
- With reasonable advance notification, allow access to the enrolled lands by NDOW or USFWS for purposes of ascertaining compliance with this CA.
- Follow guidelines provided by NDOW for handling injured Relict Leopard Frogs or carcasses thereof.
- Provide appropriate information on avoidance of incidental take of Relict Leopard Frogs to other users of the property who may contact the animals in the pursuit of lawful recreational activities.
- Agree to consider new adaptive management recommendations that NDOW may suggest. Agreement to any new adaptive management recommendations is in LVVWD's sole and absolute discretion.

Covered Activities

LVVWD is covered for activities including but not limited to:

- LVVWD hosts visitors and has group events such as weddings, gatherings for non-profit groups, and educational field trips for schools. These visitors may walk, use bicycles, ride a passenger train, or other transportation devices on pathways located near the ponds.
- LVVWD will manage associated drainages, the Meadows Detention Basin, the water distribution system, and the refugia ponds for multi-use needs of the Springs Preserve.
- LVVWD will continue to use water sources that supply the ponds for a variety of different purposes at the Springs Preserve; water quality and levels in the ponds may vary.
- LVVWD uses the Springs Preserve for groundwater pumping and associated well-field activities that include, but are not limited to, well operation and maintenance, well flushing, well drilling, and pump tests. Well-field operations may cause groundwater level declines, but any such declines would not directly affect pond water levels due to the lining systems of the ponds themselves.
- LVVWD may undertake any and all activities associated with the operation and maintenance of water systems, equipment, and infrastructure used in the delivery of potable and non-potable water to various customers.
- LVVWD may aerate, filter, and manage flows in and out of the ponds as needed for management of the ponds and the Springs Preserve.
- LVVWD may drain the refugia ponds for maintenance activities including, but not limited to, repairing leaks, resealing concrete, removing invasive species, and enhancing ponds.
- LVVWD may grade and re-contour existing access roads within the Springs Preserve, as needed for road maintenance and fire access purposes.
- LVVWD may grade and re-contour existing trails within the property, and may construct new paved or unpaved trails for visitors to use.
- LVVWD may construct additional pipelines and power lines, and will continue to operate and maintain such facilities.
- LVVWD may extinguish fires or remove brush to prevent the spread of fires.
- LVVWD may clear debris from the refugia ponds such as, but not limited to, tree limbs, leaves, cattails, bulrushes, aquatic plants, algae, and other detritus.
- In order to maintain public health and safety, LVVWD may implement mosquito control measures in and around the ponds.
- LVVWD may use herbicides or pesticides to control invasive and/or noxious weeds and invertebrate pests, as deemed necessary for the management of the Springs Preserve.

- LVVWD may construct and maintain trails, bridges, and viewing platforms at the Springs Preserve.
- LVVWD may conduct research on the diet, genetics, growth, habitat, movements, physiology, reproduction, water quality, and/or survivorship of Relict Leopard Frogs.
- LVVWD shall maintain any permits required by NDOW for research activities on Relict Leopard Frogs.

Avoidance & Minimization Measures

The Cooperator agrees to implement the following measures to avoid and/or minimize potential impacts to relict leopard frogs from the covered activities:

- LVVWD will install interpretive signage informing visitors and event attendees frequenting the Springs Preserve trail system of the presence and protected status of the Relict Leopard Frog in refugia ponds.
- If LVVWD needs to use pesticides or other chemicals to manage the Springs Preserve, LVVWD will select chemicals and applications to avoid or minimize adverse effects to the relict leopard frog.
- Periodic water quality testing will be performed; data on dissolved oxygen, pH, conductivity, and water temperature will be recorded. These data will be used to interpret the health of the pond ecosystems as part of an adaptive management strategy.
- If LVVWD becomes aware of any contamination to water sources for the refugia ponds at the Springs Preserve, LVVWD will inspect and test water samples, if necessary, coordinate with the local regulatory agencies, and/or use alternate sources of water for the refugia ponds. LVVWD is developing alternative sources of water for these ponds, which may include but are not limited to urban run-off through existing drainage channels, well flushing, fire hydrants, adjacent irrigation systems, potable water, and groundwater wells.
- If the water supply becomes interrupted or the refugia ponds begin to leak, LVVWD will take actions to attempt to maintain adequate water levels and flows in the refugia ponds that relict leopard frogs inhabit. Measures may include, but are not limited to, using water from groundwater wells, adjacent irrigation systems, and/or potable water.
- If LVVWD needs to drain refugia ponds or determines that it is unfeasible to maintain adequate water levels and flows in the refugia ponds, LVVWD will coordinate with NDOW to either translocate the relict leopard frogs to more appropriate refugia ponds at the Springs Preserve, or notify NDOW in advance so that they can relocate the Relict Leopard Frogs.
- During any construction, operation, or maintenance activities, LVVWD, or other associated personnel, will exercise due diligence to avoid or minimize negative effects to Relict Leopard Frogs.
- Security patrols are conducted regularly throughout the Springs Preserve to protect the water supply and infrastructure. Security patrols will substantially reduce the potential for disturbance to Relict Leopard Frogs and refugia habitat.

DESIGN & CONSTRUCTION

Pond Design & Construction

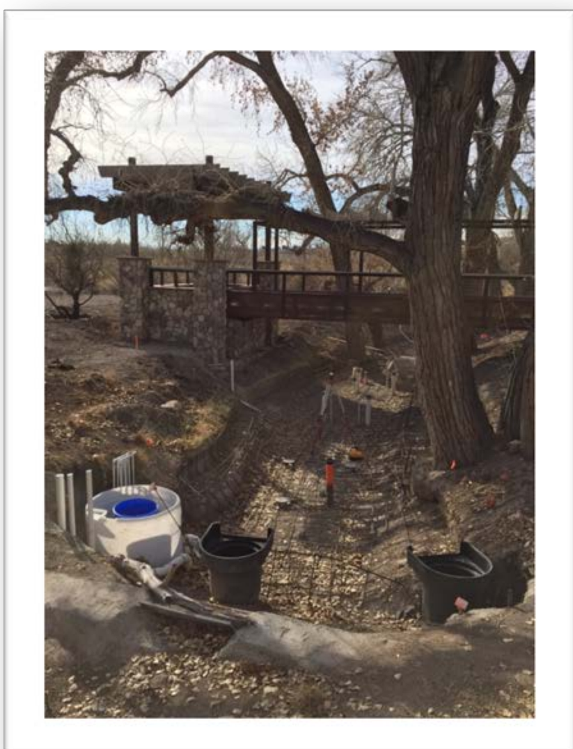
The primary sources of water for the ponds was intended to be the water pumped from the existing constructed wetland in the Meadows Detention Basin, located within the Springs Preserve, and potable water delivered from the Las Vegas Valley Water District system. The Meadows Detention Basin is fed by urban run-off flowing down the Alta Channel. It is estimated that the existing run-off water volume flowing into the Meadows Detention Basin is sufficient to maintain three of the ten ponds constructed in the Las Vegas Creek.

For ponds that do not receive potable water, water will be pumped from the bottom of the second pond in the Meadows Detention Basin (**below**) to the ponds where Pahrump Poolfish and Relict Leopard Frogs would be introduced. Construction of the intake pump station to convey water from the Meadows Detention Basin to the system of pipes in the Las Vegas Creek has also been completed. The Meadows Detention Basin plant community serves as a biological filter, cleansing the water as it flows through the wetland. The Springs Preserve does not intend to conduct any additional water quality treatment prior to moving water into the ponds.

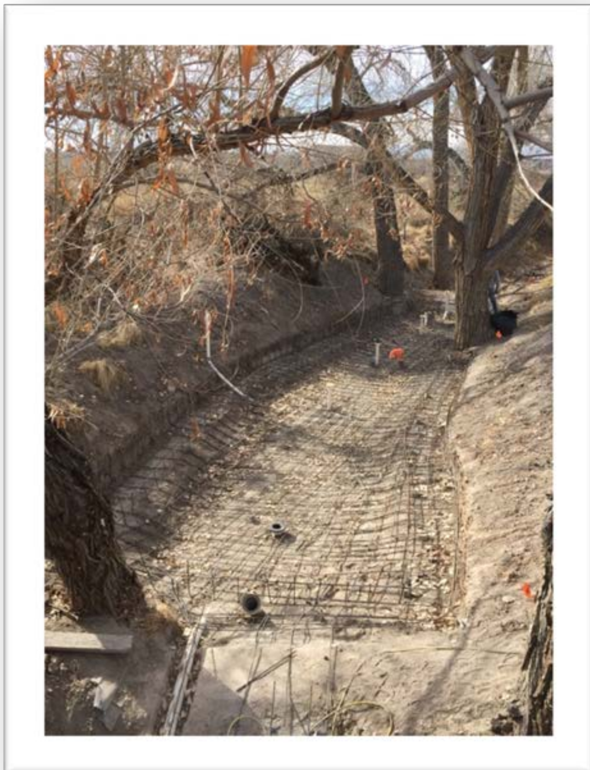
Drawing water from the bottom of the pond in the Meadows Detention Basin reduces, but does not eliminate, the potential of pumping surface contaminants, such as hydrocarbons, into the refugia ponds. However, a number of measures have been put into place to minimize the likelihood of such an occurrence, as discussed herein. Periodic water quality testing will occur in each pond inhabited by relict leopard frogs; data on dissolved oxygen, pH, conductivity, and water temperature will be recorded. These data will be used to assess the health of the pond ecosystems as part of an adaptive management strategy. In the event of a short-term water shortage (due to unavailable water or a water quality issue), the Springs Preserve will pursue alternative means of obtaining water, including but not limited to adjacent irrigation systems and/or potable water.

Given the porous nature of the Springs Preserve geology, the ponds were constructed with concrete dams and reinforced 45 millimeter polypropylene liners set over a polyvinyl chloride (PVC) pipe frame. These concrete dams and liners were necessary, as the bed of the Las Vegas Creek is crossed by two faults and numerous fissures. Dams were constructed with screened overflow notches. The surface areas of the ponds range from approximately 400–1,034 square feet.

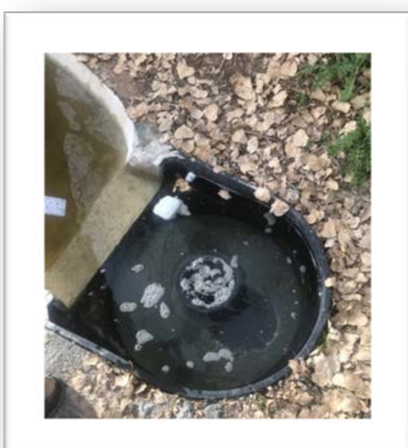
In order to move forward with the Pahrump Poolfish and Relict Leopard Frog refugia at the Springs Preserve, two existing ponds were chosen because of the availability of power for aeration and filtration systems. The water quality in these PVC-lined ponds (North Fork 1a and 1b) was initially unsuitable because of the effects of decomposing leaves from overhead Cottonwood Trees (*Populus fremontii*) (**right**). Once additional funding, permits, and approvals were secured, new filtered concrete ponds were designed in August 2016 and built over the winter of 2016–2017 (**below**).

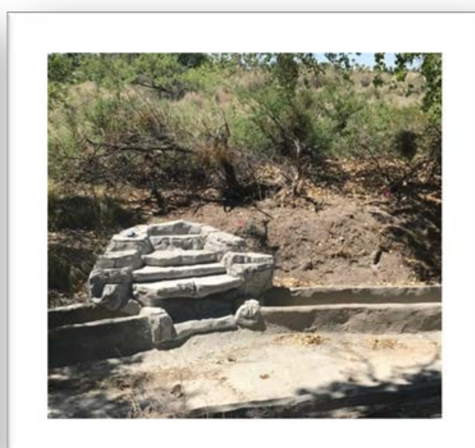


The new design included two interconnected concrete ponds (**above, below**) with shared aeration systems (i.e., bubblers, waterfalls) and both natural filtration (i.e., emergent aquatic plants) and mechanical filtration (i.e., high-capacity skimmer baskets, settling basin). The intricacies of the unique aeration and filtration systems are as follows.



In order to create flow in the ponds, water is drawn in through two large custom skimmer baskets (**below, left**) and pumped to waterfalls (**below, right**) located centrally in both the upstream (NF-1b) and downstream (NF-1a) ponds. Waterfalls were added to increase aeration of the ponds.





Water is also pumped to several bottom outlets in the southernmost portion of the upstream pond. This water then flows through the upstream pond (NF-1b), over a short riffle area, and into the downstream pond (NF-1a). From there, the water continues to flow downstream bringing floating debris (e.g., Cottonwood leaves) from both ponds back to the skimmers. The western skimmer has a white float valve (**above, left**) that automatically adds potable water to this skimmer as it is lost to evaporation and evapotranspiration from emergent aquatic plants. Some water is also lost to the terrestrial riparian zone through capillary action.

A secondary mechanical aeration and filtration system was designed. Low maintenance piston-driven air pumps (**right**) provide oxygen to four surface bubblers (**below, right**) in the downstream pond. As the bubbles rise through a column, they draw water from a 500 gallon settling basin. The settling basin itself draws water from open drains on the bottom of the downstream pond. This draws bottom detritus into the settling basin where it is deposited. Three additional bubblers in the downstream pond are on a timer and are activated at night when pond oxygen decreases due to the cessation of photosynthesis and production of CO_2 by aquatic algae and plants at night.

The settling basin slowly accumulates fine debris over the year and is purged several times in the spring with a trash pump located in the bottom.

HABITAT RESTORATION

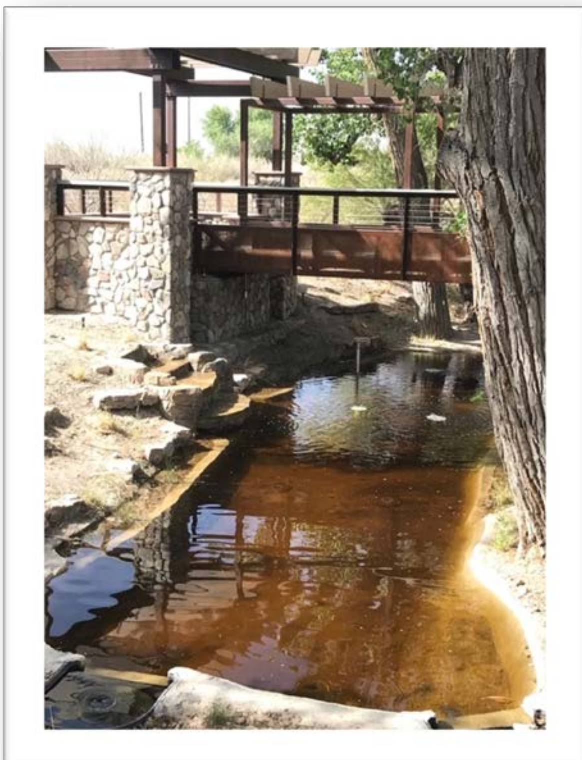
Native Plant Community

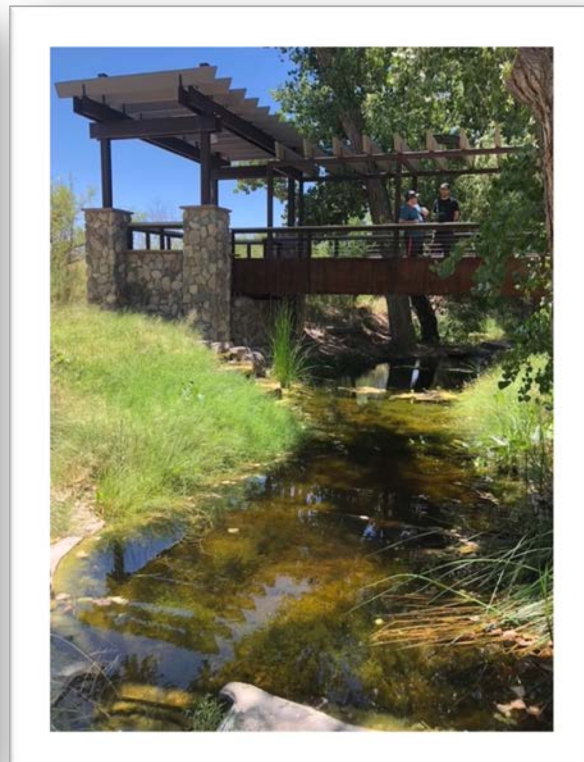
Native habitat was restored in the Las Vegas Creek channel once pond construction was complete. The basis of this restoration was compiled within an initial restoration plan for Restoration Site 17 (Winkel 2003). Restoration at the ponds was comprised of three general planting zones. These were: (1) submerged pots of emergent aquatic plants; (2) the perimeter plant pockets shown previously; and (3) the riparian sloped banks of the Las Vegas Creek (**below**). The ponds were

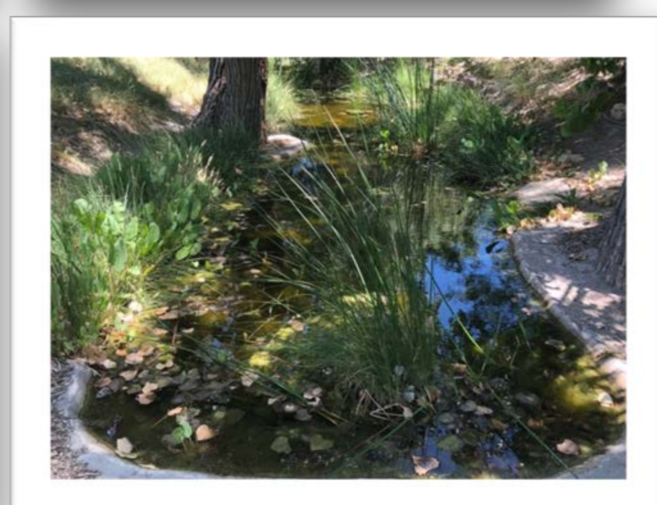
constructed under an existing canopy of large Fremont's Cottonwoods; as a result, the site is referred to as the "Cottonwood Grove" for public and interpretive purposes. The Site 7 Restoration Plan identified species native to the Springs Preserve based on surveys of comparable local habitats (Winkel 2003). As the ponds had not been envisioned in 2003, the specific plant species used in this pond riparian zone are identified (**below**).

Common Name	Scientific Name
Spike Rush	*Juncus spicata*
Beaked Spikerush	*Eleocharis rostellata*
Yerba Mansa	*Anemopsis californica*
Berkeley Sedge	*Carex divulsa*
Fourwing Saltbush	*Atriplex canescens*
Price's Plume	*Stanleya pinnata*
Arizona Grape	*Vitus arizonica*
Alkali Sacaton	*Sporobolus airoides*

Natural colonization by algae and aquatic macrophytes has occurred in the ponds as well. For instance, the green algae (*Chara* sp.) established itself in 2018 and needs periodic maintenance (i.e., removal). Similarly, an unidentified filamentous green algae forms large floating mats on the surface of the ponds in summer. This algal mat was removed in fall 2018, prior to winter plant die-offs. In 2019, the algal mat that had begun to once again cover the pond surface was consumed almost entirely by Relict Leopard Frog tadpoles and only needed to be controlled on the inaccessible waterfalls. In addition, the first Southern Cattails (*Typha domingensis*) colonized the upstream pond planter on the north side of the waterfall in 2019. Given the aggressive colonization of cattails, the species has been targeted for manual removal. Photos of the downstream and upstream ponds on April 5, 2018 and May 30, 2018, respectively, and again both ponds on July 16, 2019 clearly show that the plant and algal communities were well established (**below**).







TRANSLOCATIONS

Translocations of fish and frogs to the Springs Preserve were a multi-agency effort involving coordination by SNWA and Springs Preserve staff with the Pahrump Poolfish Recovery Implementation Team (RIT) and the Relict Leopard Frog Conservation Team.

Moapa White River Springfish Test

Provenance

In 2013, approximately 150 Moapa White River Springfish (*Crenichthys baileyi moapae*) were translocated by NDOW from Hidden Valley Pond, Moapa, Clark County, Nevada to the Springs Preserve.

Numbers Released

Three ponds (SF-1, SF-2, & NF-3) were selected because of their larger sizes and/or ease of access for future public education. None of the ponds at the Springs Preserve had supplemental aeration or filtration at the time. The suitability of these ponds was tested by first introducing approximately 50 Moapa White River springfish (*Crenichthys baileyi moapae*) into each pond in August 2013 (**right**).

Although the fish thrived initially, the death of large quantities of green algae (*Chara* sp.) in the fall of 2013 led to anaerobic water conditions in the test ponds. Trapping surveys in November 2013, revealed that only two of the fish had survived in a single pond. This test population revealed that supplemental aeration and filtration was necessary in order to establish refugia populations at the Springs Preserve.

Pahrump Poolfish

Provenance

After all necessary permits were obtained and appropriate aquatic habitat was established, the Pahrump Poolfish Recovery Implementation Team (RIT) was consulted in order to move forward with the establishment of a refugia population at the Springs Preserve. The RIT agreed that no more than 10% of Pahrump poolfish in any given population could be removed for this translocation. Consequently, the RIT decided that the best alternative was to obtain fish from Shoshone Ponds in Spring Valley, Nevada. There was also the possibility of obtaining some fish from the NDOW fish hatchery, should insufficient numbers be captured at Shoshone Ponds. This contingency plan proved unnecessary.

On May 29th, 2018, NDOW trapped Pahrump Poolfish from three of the Shoshone Ponds refugia. The fish left Spring Valley at 15:30 by truck in two large aerated tanks (**right**) and proceeded directly to the Springs Preserve. NDOW arrived at the Springs Preserve at approximately 19:15.

Numbers Released

Following a 30–45 minute acclimation period, a total of 290 Pahrump poolfish were introduced at approximately 19:45–20:00 to ponds NF-1a and NF-1b at the Springs Preserve. Given the differences in the two ponds, an equal number (N = 145) of fish were released in each pond. The fish immediately served as a biocontrol for mosquito larvae in the ponds. This is an important ecological role as mosquitoes can be vectors of West Nile and St. Louis encephalitis viruses. Consequently, Pahrump Poolfish at the Springs Preserve provide a beneficial ecosystem service by consuming mosquito larvae that have the potential to transmit diseases to birds and mammals, including people.

Relict Leopard Frogs

Provenance

The Relict Leopard Frogs used to establish the refugium at the Springs Preserve were obtained as egg from remnant populations of the species in Black Canyon, Lake Mead National Recreation Area, Clark County, Nevada, USA. UNLV team members collected three egg masses and reared the tadpoles until metamorphosis at an NDOW fish hatchery. The collection and husbandry of the Relict Leopard Frogs are beyond the scope of this report and are addressed in other Relict Leopard Frog Conservation Team documents.

Numbers Released

On May 29th, 2018, the first 100 newly-metamorphosed Relict Leopard Frogs were introduced into pond NF-1b at the Springs Preserve.

The frogs (**right**) were introduced to the ponds at 10:00 before ambient temperatures increased, giving the frogs the opportunity to acclimate gradually. Both air and water temperatures, however, were somewhat attenuated at the release site because of the large overhead Cottonwood Tree canopy and mechanical pond aeration (i.e., waterfalls and bubblers).

On March 27th, 2019, 100 Relict Leopard Frog tadpoles and nine newly-transformed frogs were released in ponds NF-1a and NF-1b. The release was conducted after dark to eliminate post-translocation avian predation. Fifty of the tadpoles and the nine froglets were from the Northshore (i.e., Lower Blue Point) donor site. The remaining 50 tadpoles were from the Black Canyon (i.e., Boy Scout) donor site.

On April 11th, 2019, 86 newly-transformed Relict Leopard Frogs were released in pond NF-1a and NF-1b at the Springs Preserve. Forty-five of the froglets were from the Boy Scout donor site and 41 were from Lower Blue Point. On May 9th, 2019, 16 newly-transformed frogs from Boy Scout and a single tadpole from Lower Blue Point were released into the ponds.

In 2020, planned Relict Leopard Frog supplementations with tadpoles and newly-metamorphosed frogs were cancelled because of the Covid-19 Pandemic.

POPULATION SURVEYS

Pahrump Poolfish

Following the translocation of 290 adult Pahrump Poolfish on May 29th, 2018, the first fry (i.e., larval fish) were observed on June 18th, 2018. Fry (**right**) continued to be observed throughout the summer. On October 3–9th, 2018, a mark-recapture survey was conducted using standard fisheries methods. The survey revealed that an estimated 386 (95% CI: 278–605) Pahrump poolfish inhabited the ponds, an increase of 33% (N = 96 fish) in a little over four months.

From October 8th to November 27th, 2018, at least 5.5% (n = 22) of the population died from a virulent attack by a flavobacterium and secondary fungal infection (**below**). A federal fish pathology laboratory concluded that "immunosuppressed mature fish were succumbing to opportunistic aquatic bacteria and fungi." Pathologists speculated that the pathogens were the result of two confounding stressors: (1) environmental – a 7°C degree drop in water temperature (i.e., from 22°C to 15°C between the 1st and 2nd capture sessions); and (2) anthropogenic – trapping, handling, and marking during a mark-recapture survey.

Surveys in June and September 2019 documented an estimated 173 (95% CI: 131–232) and 164 (95% CI: 120–232) Pahrump Poolfish in the ponds. Although the population size stabilized in 2019, it represents a 58% decline from the October 2018 survey. This may be partially explained by the presence of hundreds of imperiled Relict Leopard Frog (*Rana onca*) tadpoles in 2019, which may have altered the primary productivity, and thus carrying capacity, of the system. As of November 27th, 2019, there has been no observed recurrence of mortalities as a result of immunosuppressed Pahrump poolfish. Recruitment is still occurring, as schools of Pahrump Poolfish fry were observed from May 14th, 2019 until October 3rd, 2019.

There were no official surveys of Pahrump Poolfish in spring 2020 because of the Covid-19 Pandemic. Nonetheless, fish were observed regularly and several cohorts of fish fry have been documented since May 19th, 2020. In September 2020, however, surveys were resumed and revealed a population estimate of 93 (95% CI: 41–232) Pahrump Poolfish in the downstream pond (NF-1a); whereas, the upstream pond (NF-1b) had an estimated population of 98 (95% CI: 72–137) Pahrump Poolfish. An overall population estimate of 191 fish in 2020 is consistent with estimates produced from 2019 surveys. The population appears to have stabilized.

Relict Leopard Frog

Upon the release of the initial 100 young frogs in May 2018, a female Mallard Duck (*Anas platyrhynchos*) was observed consuming several Relict Leopard Frogs as they floated on the surface of the upstream pond. These laboratory-raised frogs appeared to not have developed an effective flight response, which was compounded by a lack of dense cover in the newly-planted riparian areas. Few frogs were observed during subsequent diurnal visits. At the time, this was attributed to the possibility that the frogs had become nocturnal, either because it was consistent with their biology and/or to avoid diurnal avian predators. Based on observations in 2019–2020, however, it is now clear that most of the newly-released froglets were consumed in the first few days following their release.

A nocturnal visual encounter survey (VES) in July 2018 noted the presence of only six Relict Leopard Frogs. By October 2018, four (1Male:3Females) large adult-sized frogs were captured and PIT tagged (i.e., implanted with a microchip carrying a unique identifying number) during a nocturnal survey. Shown is the size (~ 32 mm snout to vent length SVL) of one newly-transformed frog on May 28th, 2018 (**below left**) and the size (84 mm SVL) of the largest female (#8958) recaptured on October 3rd, 2018 (**below right**). This was an increase of 162% in length in just over four months.

In March 2019, a nocturnal survey revealed the presence of two adult Relict Leopard Frogs. A male was captured at that time and its identity confirmed via PIT tag (#9047). This male, released as a newly metamorphosed frog in May 2018, was calling prior to capture, and thus already sexually mature less than a year after metamorphosis from a tadpole.

In April 2019, *in situ* reproduction was confirmed when hundreds of small tadpoles were observed in the ponds (**right**). Although no egg mass was observed, Relict Leopard Frog egg masses can contain up to 1,100 eggs. Thereafter, tadpoles were observed regularly on sunny days resting on algae and vegetation, but were noticeably absent on overcast days. These tadpoles began to undergo metamorphosis in July 2019, and by August 2019, a VES documented 195 Relict Leopard Frogs and one tadpole in the ponds. Six of the observed frogs were of adult size.

In October–November 2019, 214 Relict Leopard Frogs were captured and marked in the ponds. Twelve of these frogs were of adult size, including the now record-sized PIT tagged female (#8958) from the 2018 cohort (**below right**), which had a 97 mm SVL. Adults were defined as any frog greater than or equal to 45 mm in snout-vent length. A subsequent recapture event revealed that an estimated 424 frogs inhabited the ponds (with a 95% Confidence Interval = 308–540). Interestingly, one large tadpole with hind limbs and one small (~ 2 cm total length) tadpole with no limbs were captured during the fall survey on November 7th, 2019. Although the vast majority of the frogs captured during the surveys were young and had not yet overwintered, the presence of so many frogs was promising in terms of their potential contribution to the overall status of this species.

An abundance of adult and juvenile frogs were observed in an around the ponds in February 2020. No laboratory-reared Relict Leopard Frogs were released at the Springs Preserve in spring 2020 because of restrictions imposed during the Covid-19 Pandemic. A large tadpole with hind limbs was captured on May 6th, 2020, the first confirmed report of successful overwintering of tadpoles for this species.

In 2020, for the second year in a row, Relict Leopard Frogs reproduced on their own at the Springs Preserve. Three separate tadpole cohorts (i.e., three separate tadpole size classes from three or more egg masses) were documented. One tadpole cohort was first documented on May 6th, 2020; the second was first observed on July 3rd, 2020; whereas, a third cohort of large tadpoles with 4 mm posterior limbs was first documented on September 12th, 2020. At the Springs Preserve, it takes Relict Leopard Frog tadpoles approximately 3–4 month to go from an egg to newly metamorphosed froglet.

In September–October 2020, 286 Relict Leopard Frogs (40 adults and 246 juveniles) were captured during mark-recapture surveys. The adult population was estimated to be 71 frogs (95% Confidence Interval = 44–99); whereas, the juvenile population estimate was 539 frogs (95% Confidence Interval = 416–661). Thus, an estimated 610 frogs now call the Springs Preserve home. This is an increase of 186 frogs…a 44% increase over the estimated population size of 424 frogs in fall 2019. In addition, as tadpoles, the 3rd cohort wasn't even part of the October 2020 estimate.

Of note was the recapture of one of the four adults PIT tagged on October 3rd, 2018. This female (#9052; **left & below**) was not recaptured in 2019 surveys. When first captured and tagged in 2018, this female was 81 mm long and had a mass of 62 g. On September 29, 2020, she measured 94 mm and had a mass of 87 g. This was a 16% increase in length and 40% increase in mass.

OPERATION & MAINTENANCE

The pond mesocosms were designed to minimize the amount of maintenance required; however, a certain amount will always be required. Here, the focus is on documenting the maintenance activities that have been performed to date.

Ponds

As the ponds were built of reinforced concrete, little maintenance has been required thus far. We have noted that Cottonwood and Gooding's Willow roots seek out any weaknesses in the concrete. For instance, the hairline cracks between the plastic skimmer boxes and the concrete ponds. Left to grow, these roots would widen and crack the concrete. Consequently, the roots are periodically removed.

The main influx of nutrients to the system occurs in the fall, when large numbers of leaves from the overhead Cottonwood Trees fall into the pond. Although the skimmers removed large numbers of leaves floating at the surface, a fair number sink to the bottom of the ponds. A certain amount of decaying organic debris (aka sludge) ends up accumulating. Maintenance consists of removing some, but not all of this organic substrate. Some of the accumulated debris is removed because the decomposition process can result in anaerobic conditions when billions of bacteria consume the organic material. This process also creates hydrogen-sulfide, which is a black layer of sludge that smells of rotten eggs. Some of the debris is left to provide a non-concrete substrate for overwintering Pahrump Poolfish and Relict Leopard Frogs.

It should be noted that although these decay processes are normal and occur in natural ponds and creeks, the Springs Preserve is a closed system. In a natural spring system, the water is continuously flowing away from the point of origin (e.g., the spring head) never to return. The Springs Preserve ponds are continuously recycling the water and pumping it back to the waterfalls and upstream pond. Consequently, various organic materials and minerals can bio-accumulate within the system. In order to alleviate some of these issues, 500 gallon settling basin water changes are completed weekly for 2-4 weeks every spring by Springs Preserve staff.

Filtration

The pond mesocosms have a minimalist filtration system comprised of biological and passive mechanical filtration. First, nutrients in the water are removed by the emergent aquatic plants in the submerged planter pockets that parallel both pond edges. This is evidenced by the fact that the tannins that accumulate in the fall and winter from decaying leaves are mostly gone once growth resumes in April and/or May.

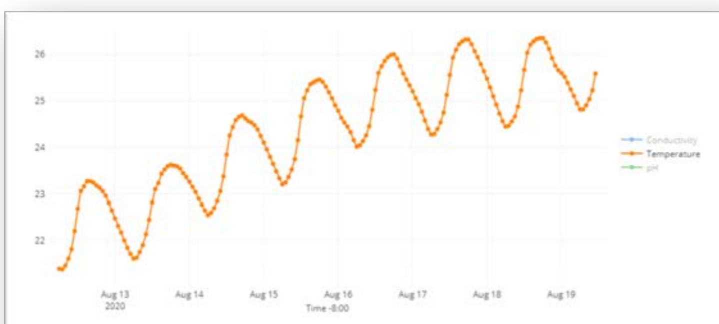
Algae help remove large quantities of accumulated minerals, such as nitrates and nitrites, resulting in substantial growths of filamentous algae on the concrete waterfalls, where algae strands can exceed 12" in length. During peak growth periods, the strands are removed on a weekly basis from the waterfalls; otherwise, the vigorous growth can impede water flow and thus oxygenation. Another green alga, *Chara* sp., also grows in dense patches along the bottom of the ponds, providing cover for both fish & frogs. Dense patches of *Chara* are periodically removed, typically outside of the Pahrump poolfish breeding period. The timing of the *Chara* removal is to avoid removing any Pahrump poolfish eggs that may have been deposited on the algal filaments. Lastly, dense mats of floating algae have formed at times in both ponds. These mats provide shade in the heat of the summer, cover for the fish from avian predators, and basking platforms for Relict Leopard Frogs. In 2018, the dense mat covering most of the upstream pond was removed in October prior to the winter die-off of the algae (**right**). In 2019, growth of the algal mat resumed in earnest until Relict Leopard Frog tadpoles consumed it, essentially alleviating any maintenance issues.

The passive mechanical filtration is comprised of a 500 gallon settling basin. Sludge accumulates in the bottom of the basin and is flushed during water changes every spring. The settling basin houses a trash pump at the bottom. As Pahrump poolfish regularly access the settling basin by drains that connect to it from the bottom of the pond, a Gee minnow trap is used to remove Pahrump Poolfish prior to purging the tank. Traps are baited with dry dog kibble and left for at least two hours. The ponds auto-fill float valve system (**right**) refills the pond automatically by adding water in the western-most skimmer until the white float valve returns to the predetermined water depth. The settling basin discharges under the bridge to the north. Currently, the ponds do not employ any form of filter media to maintain.

The two skimmers on the northern end of the downstream pond require weekly maintenance. Maintenance is comprised of removing any branches or algal filaments that may submerge the floating cylinder and allow fish or frogs to enter the skimmer. This can be done as needed following visual inspection. The skimmer baskets themselves must be emptied at least once a week to remove and discard any trapped material before it begins to decay. In addition, any Pahrump poolfish or frogs encountered in these baskets can be returned to the pond at this point. During the fall, the volume of leaves necessitates cleaning the skimmer basket two (2) times per week until leaf fall is complete. This is to avoid the decay of the leaves within the skimmer basket, which continues to add and concentrate tannins in the ponds until the basket is emptied. In addition, young of the year Relict Leopard Frogs seemed to be attempting to migrate downstream, ending up in the skimmer baskets. Specially designed skimmer basket lids that incorporate a prefilter sponge material should alleviate this problem; however, they must be removed once the Cottonwood leaves begin to fall.

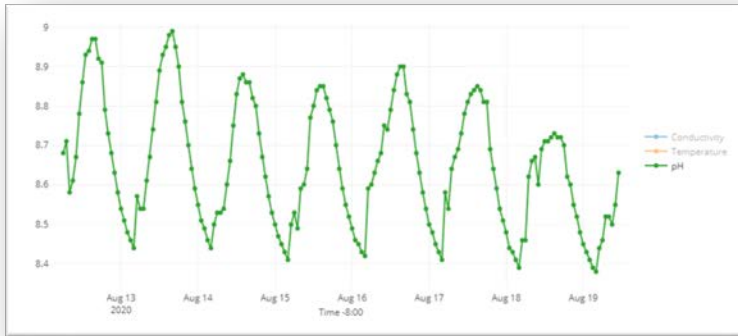
Water quality is monitored hourly with an In-Situ probe with sensors for temperature, pH, and conductivity (**below**). This probe can be deployed for at least nine months and has self-cleaning and calibration features. The In-Situ probe (In-Situ Inc, Fort Collins, Colorado) is connected to a FTS (Forest Technology Systems, Victoria, British Columbia, Canada) LT1-GOES satellite communication system (**right**) allowing for remote monitoring of pond water quality. The data is also backed up automatically in Aquarius (Aquatic Informatics USA, Denver, Colorado).

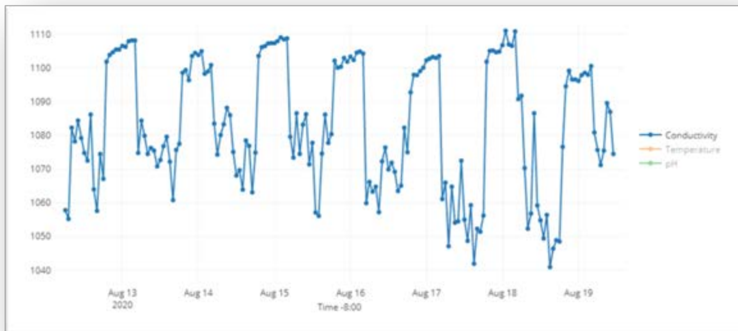
Aeration



The ponds have two redundant aeration systems. The first is the flow of water down the two faux-rock waterfalls. Other than the previously discussed removal of aggressive algal growth, the waterfalls require no maintenance. In the winter of 2018–2019, when pond tannins resulted in large amounts of surface bubbles, bags of carbon were placed in the uppermost portion of the waterfalls. Carbon needs to be replaced monthly to have a continued effect.

The second aeration system is composed of the mechanical aeration pumps that operate two redundant systems. The upright surface bubblers in the downstream pond remain operational 24/7, ensuring an abundant supply of oxygen. In addition, these bubbles passively draw water from the settling basin back into the ponds. Thus, they need to remain on. During the summer months, the diffuser discs can become surrounded by algae, reducing aeration in the ponds. The disks can either be swapped out and bleached or scrubbed in place without chemicals, depending on the severity of algal growth.





Three additional bubblers operate from the bottom of the downstream pond, just upstream from the skimmers. These bubblers operate on a timer and are on at night, to increase oxygen levels when plants are consuming oxygen and generating CO_2. This also helps to buffer daily pH fluctuations (**above**). The aeration pumps are in a locked container on the south side of the restroom. As they are medical-grade aeration pumps, they use metal pistons instead of rubber diaphragms. They are expected to last five or more years before they need to be replaced. Hoses should be periodically checked to make certain they do not become disconnected. These bottom bubblers are vigorous enough to prevent floating leaves from reaching the skimmers and should be turned off during leaf fall.

Non-native Species

Staff must remain ever vigilant for species introduced by the public. To date, only a single non-native species has been introduced by the public, a Red-eared Slider (*Trachemys scripta elegans*). This species native range is in the southeastern US. The large adult female was found on April 11, 2019 during a nocturnal Relict Leopard Frog survey. Fortunately, the water was cold enough that this turtle was still brumating and did not cause any noticeable damage. Although this species is a facultative omnivore, it will not forego a fish or frog meal if the opportunity presents itself.

Non-native plant species are also a concern and can require periodic maintenance. A patch of Bermuda Grass has become established on the eastern shore north of the downstream pond. Given the presence of sensitive fish and frogs in the ponds, the use of herbicides is prohibited. Manual removal in spring 2019 had little effect. As this Bermuda Grass has become the preferred basking site of the surviving adult female from the 2018 cohort, it is trimmed back in winter, while the fish and frogs are dormant.

FUTURE OBJECTIVES

In the initial design to partially recreate portions of the Las Vegas Creek, 10 engineered ponds were constructed at the Springs Preserve. Through subsequent design modifications described herein, the two pond mesocosms have proven to be a success. Currently, it is estimated that sufficient water is available to create two additional ponds. The primary sources of water for these additional two ponds are water that will be pumped from the existing constructed wetland in the Meadows Detention Basin, and/or potable water delivered from the Las Vegas Valley Water District system.

In order to replicate the success at the Cottonwood Grove ponds (i.e., NF-1a and NF-1b), pumps are required for mechanical aeration of the ponds. In August 2019, the USFWS awarded the Springs Preserve with a grant of $56,474 to run power from the Meadows Detention Basin pumping station to ponds SF-1 and SF-2 (**below**). This contract has been delayed, in part, by facility closure and other precautionary measures necessitated by the Covid-19 Pandemic.

Once the electrical infrastructure is in place, the next objective is to obtain grant funding for replacing the PVC liners with concrete ponds to facilitate maintenance and decrease the possibility of leaks. Unless a single large grant is obtained, the goal is to increase refugia capacity by 1–2 ponds every few years as resources permit, for a total of 10 refugia ponds.

PRESS & PUBLICATIONS

The Springs Preserve ponds project has been featured in a number of academic, technical, and media outlets.

Academic publications

Bennett, A.R., R. Rivera, R.A. Saumure, T. O'Toole, J.R. Jaeger, P.R. Bean. 2020. ***Rana onca* (Relict Leopard Frog). Diet and Mortality.** Herpetological Review 51:302–303. See Joshua Tree Productions video on Vimeo here: https://vimeo.com/356479040

Saumure, R.A., A. Ambos, T. O'Toole, J. Harter, K. Guadelupe, B. Senger, and Z. Marshall. In press. **A fish out of water: Rewilding the Pahrump Poolfish in Las Vegas, Nevada, USA.** *In:* Global Reintroduction Perspectives: 2020. International Union for the Conservation of Nature / Species Survival Commission, Conservation Translocation Specialist Group, Gland, Switzerland. p. 41–45

Saumure, R.A., R. Rivera. J.R. Jaeger, T, O'Toole, A. Ambos, K. Guadelupe, A.R. Bennett, and Z. Marshall. In press. **Leaping from extinction: Rewilding the Relict Leopard Frog in Las Vegas, Nevada, USA.** *In:* Global Reintroduction Perspectives: 2020. International Union for the Conservation of Nature / Species Survival Commission, Conservation Translocation Specialist Group, Gland, Switzerland. p. 76–81.

Technical Publication

Wallace, K. February 26, 2018. **Fish-safe Airlift System for Protecting Endangered Species.** Pond Trade Magazine. https://www.pondtrademag.com/best-pond-practices-fish-safe-airlift-systems-protecting-endangered-species/

Press coverage

Brean, H. October 18, 2015. **Homeless Fish May Find Shelter at Springs Preserve.** Las Vegas Review-Journal. https://www.reviewjournal.com/news/homeless-fish-may-find-shelter-at-springs-preserve/

Brean, H. March 1, 2018. **Oasis for Threatened Species.** View Southwest. Las Vegas Review-Journal 26(42). P. 1A & 9A.

Brean, H. April 13, 2019. **Rare Frog Finds Home at Springs Preserve in Las Vegas.** Las Vegas Review-Journal. https://www.reviewjournal.com/local/local-las-vegas/rare-frog-finds-home-at-springs-preserve-in-las-vegas-1640257/

Anonymous. May 13, 2019. **Visit a Rare Species.** Pipeline. Las Vegas Valley Water District.

Grega, K. August 1, 2019. **Preserving a Relict.** Las Vegas Weekly p. 8–9. https://lasvegasweekly.com/photos/galleries/2019/jun/20/0521_LVW_FrogHabitat/#/4

Grega, K. August 3, 2019. **Local Biologists Try to Reintroduce a Rare Frog Species into Southern Nevada.** Las Vegas Sun. https://lasvegassun.com/news/2019/aug/03/local-biologists-try-to-reintroduce-a-rare-frog-sp/

Video

Springs Elemental. July 15, 2009. Springs Lives: Urban Island. https://youtu.be/jhjnACXbns4

Springs Elemental. September 27, 2012. Lost and Found Frog. https://youtu.be/guImxMoLHVE

Springs Elemental. February 6, 2017. Relict Leopard Frog. https://youtu.be/WP6TG3-pnwY

Springs Elemental. July 30, 2019. Species Recovery. https://youtu.be/IQGP7Kvs5RQ

Springs Elemental. April 1, 2020. Relict Leopard Frog Season. https://youtu.be/dm1sY168MJ0

SOURCE MATERIALS

Bradford, D.F., J.R. Jaeger, and R.D. Jennings. 2004. Population status and distribution of a decimated amphibian, the Relict Leopard Frog (*Rana onca*). Southwestern Naturalist 49:218–228.

Bradford, D.F., J.R. Jaeger, and S.E. Shanahan. 2005. Distribution changes and population status of amphibians in the eastern Mojave Desert. Western North American Naturalist 65:462–472.

Deacon, J.E., and J.E. Williams. 2010. Retrospective evaluation of the effects of human disturbance and goldfish introduction on endangered Pahrump poolfish. Western North American Naturalist 70:425–436.

Deacon, J.E., C. Hubbs and B. Zahuranec. 1964. Some effects of introduced fishes on the native fish fauna of southern Nevada. Copeia 1964:384–388.

Doadrio, I., and O. Domínguez. 2004. Phylogenetic relationships within the fish family Goodeidae based on cytochrome *b* sequence data. Molecular Phylogenetics and Evolution 31: 416–430.

Dodd, C.K., Jr. 2013. Frogs of the United States and Canada. Volume 2. John Hopkins University Press, Baltimore, Maryland, USA. Pp. 565–568.

Drake, D.L. 2010. *Lithobates onca* (Relict Leopard Frog). Cannibalistic oophagy. Herpetological Review 41:198–199.

Grant, E.C., and B.R. Riddle. 1995. Are the endangered springfish (*Crenicthys* Hubbs) and pupfish (*Empetrichthys* Gilbert) Fundulines of Goodeids?: A mitochondrial DNA assessment. Copeia 1995:209–212.

Hekkala, E.R., R.A. Saumure, J.R. Jaeger, H.-W. Herrmann, M. Sredl, M. Culver, D. Bradford, D. Drabeck, and M.J. Blum. 2011. Resurrecting an extinct species: Archival DNA, taxonomy, and conservation of the Vegas Valley Leopard Frog. Conservation Genetics 12:1379–1385.

Hillis, D.M. 1988. Systematics of the *Rana pipiens* complex: puzzle and paradigm. Annual Review of Ecology and Systematics 19:39–63.

Hillis, D.M. and T.P. Wilcox. 2005. Phylogeny of the New World true frogs (Rana). Molecular Phylogenetics and Evolution 34:299–314.

Jaeger, J.R., B.R. Riddle, R.D. Jennings, D.F. Bradford. 2001. Rediscovering *Rana onca*: evidence for phylogenetically distinct leopard frogs from the border region of Nevada, Utah, and Arizona. Copeia 2001:339–354

Jennings, M.R. 1988. *Rana onca* Cope. Relict Leopard Frog. Catalogue of American Amphibians and Reptiles. 417.1–417.2.

La Rivers, I. 1994. Fishes and Fisheries of Nevada. University of Nevada Press, Reno, Nevada.

Linsdale, J.M. 1940. Amphibians and reptiles of Nevada Proceedings of the American Academy of Arts and Sciences 73(8):197–257.

Maxey, G.B. and C.H. Jameson. 1948. Geology and water resources of Las Vegas, Pahrump, and Indian Springs Valleys, Clark and Nye Counties, Nevada. Nevada State Engineer, Water Resources Bulletin 5:1–121.

Miller, R.R. 1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. Miscellaneous Publications, Museum of Zoology, University of Michigan 68:1–155.

Miller, R.R. 1984. *Rhinichthys deaconi*, a new species of dace (Pisces: Cyprinidae) from southern Nevada. Occasional papers of the Museum of Zoology University of Michigan 707: 1–21.

Miller, R.R., J.D. Williams, and J.E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries 14:22–38.

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

Minckley, W.L., G.K. Meffe, and D.L. Soltz. 1991. Pages 247–282 in W.L. Minckley and J.E. Deacon (Editors), Battle Against Extinction: Native Fish Management in the American West. University of Arizona Press, Tucson, Arizona.

Nevada Department of Wildlife. 2009. Project report, Calendar Year 2009 Pahrump Poolfish monitoring and recovery activities. Prepared by J. Goldstein and B. Hobbs. Las Vegas, Nevada.

Nevada Department of Wildlife. 2012. Native fish and amphibians field trip report. Prepared by K. Guadalupe, S. Madill, and H. Weissenfluh. Las Vegas, Nevada.

Oláh-Hemmings, V. J.R. Jaeger, M.J. Sredl, M.A. Schlaepfer, R.D. Jennings, C.A. Drost, D.F. Bradford, and B.R. Riddle. 2010. Phylogeography of declining Relict and Lowland leopard frogs in the desert Southwest of North America. Journal of Zoology 280:343–354.

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

Selby, D.A. 1977. Thermal ecology of the Pahrump killifish, *Empetrichthys latos latos* Miller. M.S. Thesis, University of Nevada, Las Vegas. May 1977.

Slevin, J.R. 1928. The amphibians of western North America. Occasional Papers of the California Academy of Sciences 16:1–152.

Soltz, D.L., and R.J. Naiman. 1978. The natural history of native fishes in the Death Valley system. Natural History Museum of Los Angeles County, Science Series 30: 24.

Stebbins, R.C. 1966. A Field Guide to Western Reptiles and Amphibians. Houghton Mifflin Company, Boston, USA

Stejneger, L. 1893. Annotated list of the reptiles and batrachians collected by the Death Valley Expedition in 1891, with descriptions of new species. North American Fauna (7)2:159–228.

Stone, W. 1911. On some collections of reptiles and batrachians from the western United States. Proceedings of the Academy of Natural Sciences of Philadelphia 63:222–232.

Tanner, V.M. 1931. A synoptical study of Utah amphibia. Utah Academy of Sciences 8:159–198.

U.S. Fish and Wildlife Service. 1967. Native Fish and Wildlife, Endangered Species. Federal Register 32:4001.

U.S. Fish and Wildlife Service. 1980. Recovery Plan for the Pahrump killifish. Endangered Species Program, Region 1, Portland, Oregon.

U.S. Fish and Wildlife Service. 2004. Endangered and threatened wildlife and plants; withdrawal of proposed rule to reclassify the Pahrump poolfish (*Empetrichthys latos*) from endangered to threatened status. Federal Register 69: 17383–17386.

U.S. Fish and Wildlife Service. 2010. Request to append the Shoshone Well No. 2 to the programmatic biological opinion for the Bureau of Land Management's Ely Resource Management Plan. April 16, 2010.

Van Denburgh J. and J.R. Slevin. 1921. A list of the amphibians and reptiles of Nevada, with notes on the species in the collection of the Academy. Proceedings of the California Academy of Sciences 11:27–38.

Webb, S.A., J.A. Graves, C. Macias-Garcia, A.E. Magurran, D.O. Foighil, and M.G. Ritchie. 2004. Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). Molecular Phylogenetics and Evolution 30:527–544.

Winkel, V.K. 2003. Site-specific ecological restoration plan. Restoration Site 17. Springs Preserve report LVSP-SSERP-01. Las Vegas, Nevada, USA. 21 p.

Wright, A.H. and A.A. Wright. 1949. Handbook of Frog and Toads of the United States and Canada, 3rd edition. Comstock Publishing Company, Ithaca, New York, USA. 640 p.