Relict Leopard Frogs at the Springs Preserve
2022 Annual Summary of Activities

by

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The following report is an annual summary of activities under Landowner Cooperative Agreement LCA-R01 between the Las Vegas Valley Water District (LVVWD) and Nevada Department of Wildlife (NDOW) for relict leopard frogs at the 180-acre Springs Preserve in Clark County, Nevada, USA. This Agreement was granted pursuant to the Programmatic Candidate Conservation Agreement with Assurances between the U.S. Fish and Wildlife Service (USFWS) and NDOW.

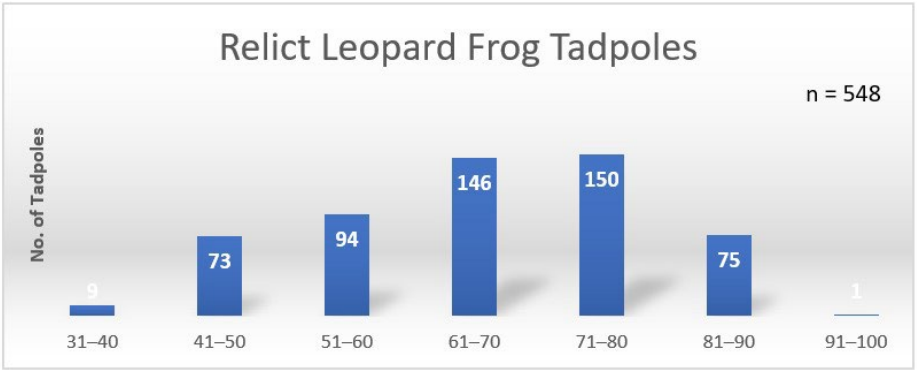
Executive Summary

In 2022, the first relict leopard frog (*Rana onca*) activity was observed in late March and active frogs continued to be observed into late October. In March, 100 tadpoles were translocated to the Springs Preserve from wild egg masses raised in captivity. Eight egg masses were documented in the refugium ponds in March and April 2022. Over 1,200 unique tadpoles were captured in minnow traps during the July Pahrump poolfish (*Empetrichthys latos*) mark-recapture surveys, which was five times more than were captured in July 2021. A total of 212 tadpoles were translocated from the refugium ponds to the Springs Preserve Cienega in July. During the annual mark-recapture survey in October, 40 adults and 142 juveniles were captured. A nocturnal survey of the Cienega in late October did not document any relict leopard frogs, despite frogs being active in the refugium ponds. Educational content continues to be published on social media, websites, and academic publications.

Population Surveys

Egg mass surveys.–A total of eight relict leopard frog (*Rana onca*) egg masses were documented in 2022. Colleagues from University of Las Vegas, Nevada (UNLV) led an egg mass survey on March 24, 2022. Three adult frogs were observed, and a single egg mass was documented. At the end of the survey, 100 large tadpoles reared from egg masses collected at natural sites were released into the ponds to maintain, or increase, genetic diversity. Seven additional egg masses were documented between March 26 and April 5, 2022 and water temperature averaged 15.5°C (range: 13.6–17.2°C) during that period. By April 6, 2022, the first two egg masses had hatched.

Tadpole translocations.–Tadpoles were captured incidentally in minnow traps during a Pahrump poolfish (*Empetrichthys latos*) mark-recapture survey (**Appendix I**). On July 20, 2022, a total of 1,006 unique relict leopard frog tadpoles were trapped in the two refugium ponds during the fish marking session. Of these tadpoles, about 10% (n = 100) were translocated to the Cienega. A week later, on July 27, a total of 1,101 unique tadpoles were trapped in the same refugium ponds during the fish recapture session and another 10% (n = 112) were translocated to the Cienega. The total lengths of 548 tadpoles trapped in the downstream pond were recorded on July 20, 2022. (**right**).



Compared to July 2021, there were approximately five times more tadpoles caught during the Pahrump poolfish mark-recapture survey in July 2022. A potential cause for the substantial increase in tadpoles was the removal of most of the stonewort (*Chara* sp.) growing in the ponds. This macroalga had grown into dense mats that decreased livable habitat for tadpoles, clogged filtration, and may have facilitated predation by dragonflies and damselflies on smaller tadpoles. The removal of most of the stonewort began on May 24, 2022 and continued until July 12, 2022. Thereafter, any new stonewort growth was removed from the ponds at least twice per month. Conversely, mats of filamentous alga growing on the pond's surface were left to grow as they provided food for tadpoles, cover from avian predators, basking surfaces for frogs, and a thermal buffer for pond water.

Visual encounter survey.–Spring visual encounter surveys were not conducted by UNLV in 2022. A nocturnal survey of the Cienega by UNLV and SNWA staff occurred on October 25, 2022. No relict leopard frogs or overwintering tadpoles were observed, despite approximately two dozen frogs being active in the refugium ponds following the Cienega survey.

Mark-recapture survey.–On October 4, 2022, a UNLV-led mark-recapture survey resulted in the capture of 83 relict leopard frogs, including 24 adults and 59 juveniles. Three large tadpoles were also observed. All frogs with snout to vent lengths (SVL) ≥ 45 mm were tagged with a Passive Integrated Transponder tag (i.e., PIT tag); whereas smaller individuals were toe clipped on the 5th right rear digit. On October 11, 2022, a total of 134 frogs were captured, including 25 adults and 109 juveniles. Several of these frogs were recaptures. Thirteen very large tadpoles were also observed. A summary of translocations, reproduction, and number of unique adult and juvenile relict leopard frogs captured from 2018–2022 in the Springs Preserve refugium ponds is presented (**below**). These numbers exclude recaptures.

YEAR	Number of Surveys	Juvenile Frogs Released	Tadpoles Released	*In Situ* Tadpole Cohorts[a]	Adults[b] Captured	Juveniles[c] Captured	Total Captured
2018	1	100	0	0	4	0	4
2019	2	111	101	1	12	178	190
2020	2	0[d]	0[d]	2–3	40	244	284
2021	2	24	91	9[e]	66	161	227
2022	2	0	100	8[e]	40	142	182

[a]Minimum number of egg mass(es) deposited *in situ* at Springs Preserve. Ponds surveyed daily.
[b]Adults defined *a priori* as all frogs with snout-vent length (SVL) of ≥ 45 mm. All adults PIT tagged.
[c]Juveniles are frogs with SVL < 45 mm that have completed, or mostly completed, metamorphosis.
[d]No releases due to access restrictions associated with the Covid-19 pandemic.
[e]A subset of approximately 200 tadpoles were removed from the refugium ponds and translocated to the Springs Preserve Cienega.

Mortalities

No tadpole or frog mortalities were observed in 2022. Although long suspected, the first documented occurrence of cannibalism in this species was documented during the mark-recapture survey on October 11, 2022. A recaptured adult female (PIT tag # 982091061011458) with a SVL of 68 mm and mass of 25.5 g was observed with the posterior of a newly metamorphosed juvenile protruding from her mouth, while in a collection bucket (**photo by Aaron Ambos - right**). This observation will be the subject of a future natural history note. Adult relict leopard frogs may be an important source of mortality and natural population control for newly metamorphosed frogs, especially at high densities in small systems.

Environmental Conditions

Water quality measurements were recorded on an hourly basis by a submerged datalogger with probes for water temperature, pH, and conductivity. These data are reported herein as they represent abiotic conditions that appear favorable to the species. The average water temperatures in the pond remain remarkably consistent from year-to-year (Jan 1–Dec 31), within 0.2°C (**right**). Similarly, maximum water temperatures have not exceeded 29°C and stayed within a 2.1°C range, despite air temperatures in Las Vegas reaching 45–47°C. The canopy of large Fremont's cottonwood (*Populus fremontii*) over the ponds helped keep water temperatures lower.

Year	Mean (°C)	Min (°C)	Max (°C)
2020	16.0	3.9	26.9
2021	15.9	3.6	29.0
2022	15.8	2.7	28.2

The pH in the ponds appears to be increasing (**right**), but this may simply be an artifact of the probe requiring more frequent calibration, which will be investigated in 2023. Other causes of increasing pH may be related to water evaporation, as well as algal blooms and the photosynthetic cycle.

Year	Mean pH	Min pH	Max pH
2020	8.6	8.0	9.4
2021	9.0	8.4	9.7
2022	9.34	8.7	9.9

As with water temperatures, conductivity values are remarkably constant (**below**). Water changes conducted each spring to decrease tannins, which are leached from decaying cottonwood leaves, likely contribute in part to stable conductivity values. Conductivity is known to increase as water temperatures increase, which is reflected in the annual minimum and maximum values.

Year	Mean (µS/m)	Min (µS/m)	Max (µS/m)
2020	1,092	997	1,211
2021	1,065	841	1,216
2022	1,109	1,004	1,182

Education

The Springs Preserve received a Clark County Outside Agency Grant to develop and produce several new interpretive panels for the ponds, including one on relict leopard frog translocations and another mirroring the USFWS/NDOW "Don't Ditch a Fish" campaign about the dangers of introducing non-native invasive species. The Springs Preserve continued to use the relict leopard frog as a component of their public education program, as well as exhibiting the species in their living collection exhibit. Natural history and conservation messaging for the frog were shared during small group tours. Additional public education outreach efforts from the Springs Preserve were offered online through social media platforms and included:

- Twitter. March 3, 2022. World Wildlife Day. https://twitter.com/SpringsPreserve/status/1234873439904768007?s=20&t=iAVeZwY1Re4FzSVBRbE-9g
- Pipeline: the LVVWD weekly newsletter. March 22, 2022. Frogs jump in numbers at the Springs Preserve. (**Appendix II**)
- Facebook & Instagram. April 30, 2022. Save the Frogs Day. https://m.facebook.com/springspreserve/photos/a.456161982315/10160977668892316/?type=3&mibextid=qC1gEa
- Facebook & Instagram. May 13, 2022. Leapin' leopards. https://m.facebook.com/springspreserve/photos/a.456161982315/10161003013022316/?type=3&mibextid=qC1gEa

- Pipeline: the LVVWD weekly newsletter. August 25, 2022. Visit the frog factory at the Springs Preserve. (**Appendix III**)

- Facebook & Twitter. September 4, 2022. Relict leopard frog tadpoles. https://twitter.com/SpringsPreserve/status/1566483486239866889?s=20&t=iAVeZwY1Re4FzSVBRbE-9g

- Facebook. October 4, 2022. World Animal Day. https://m.facebook.com/story.php?story_fbid=pfbid0WTkjS4GwYgB2a8W7iGkS4yknN2rMg2BqeYMzQhhezYU59ceaLEjyFZXzt8jysYmZl&id=225615922315&mibextid=qC1gEa

Acknowledgments

Collaborators Audrey Bennett, Aaron Ambos, Jean-Axel Urbieta-Aguilar, and Thomas O'Toole have helped make this project a success. Thanks also to colleagues Dr. Jef Jaeger, Rebeca Rivera, and Robert Pelletier from UNLV for providing extensive expertise, raising the translocated frogs from eggs, and conducting both egg mass and nocturnal mark-recapture surveys at the ponds and Cienega. NDOW colleagues Kevin Guadalupe and Audrey Wetjen, as well as USFWS colleagues James Harter and Kellie Berry, provided translocation and monitoring assistance and expertise.

Appendix I

2022 Translocation of Relict Leopard Frog Tadpoles from the Existing Refugium Ponds to the Cienega at the Springs Preserve

by

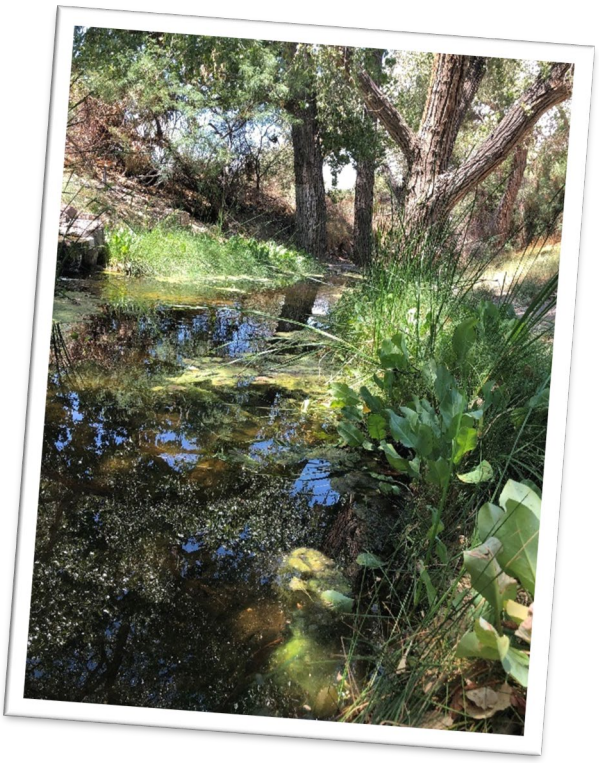
Dr. Raymond A. Saumure

Introduction: Eight relict leopard frog (*Rana onca*) egg masses were recorded in the Springs Preserve refugium ponds in spring 2022. Beginning in 2021, tadpoles were translocated from the 0.02 ha (0.05 acres) refugium ponds to the much larger 0.81 ha (2 acres) Cienega in the Meadows Detention Basin at the Springs Preserve.

Objective: The objectives of the relict leopard frog translocation program at the Springs Preserve are to:

(1) Reduce the density of relict leopard frog tadpoles in the original refugium ponds.
(2) Establish an additional refugium population in the Cienega wetland.

Methods: On both July 20 and 27, 2022, 11 minnow traps were set in the upstream (**below right**) and downstream (**below left**) refugia ponds, for a total of 22 traps/day.



Traps were baited and set from approximately 07:15 and checked from approximately 10:10 on both days. Traps were baited with a mixture of fresh Fromm's Game Bird cat food and Farmina Lamb & Blueberry dog food.

The captured tadpoles were held temporarily in several clean 19 L (5-gallon) buckets. Water was changed as needed with pond water. On July 20, all of the relict leopard frog tadpoles from the downstream pond were measured with custom PVC trough measuring devices in order to produce a tadpole size-frequency histogram. Total tadpole length was recorded (± 1 mm).

On both days, tadpoles were haphazardly selected to represent approximately 10% of the tadpoles trapped in each pond. Tadpoles with anterior limbs were excluded because: (1) their time to acclimate to the Cienega pond, prior to metamorphosis, would be limited; and (2) they may have been lab-reared tadpoles provided by University of Nevada, Las Vegas (UNLV) colleagues earlier in the spring to increase genetic diversity in the original refugium population.

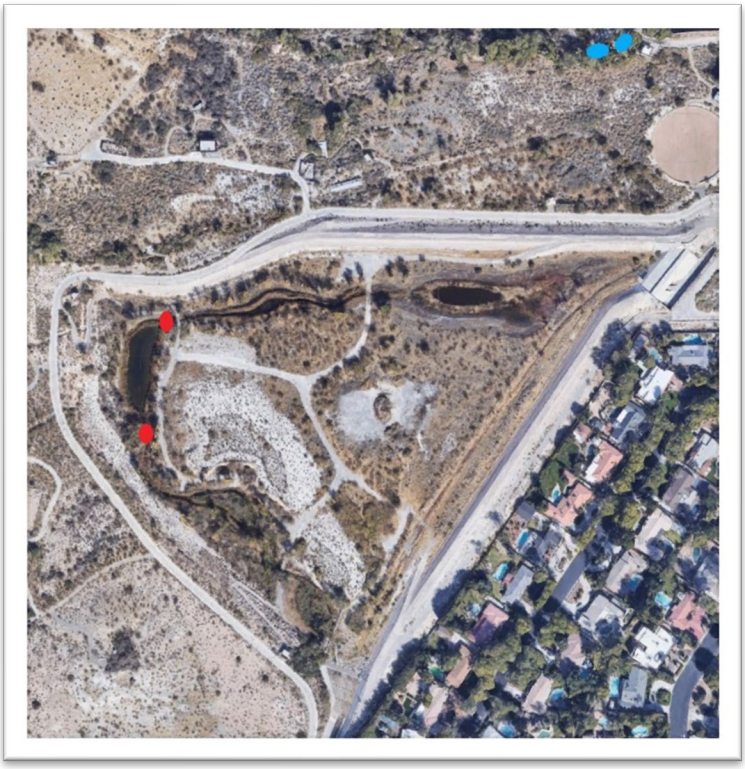
Tadpoles were translocated to the Cienega in a 28 L insulated Engel live bait cooler, with a Marine Metal Quiet Bubbles™ external battery-powered water-resistant aerator. Tadpoles were once again transferred to 19 L buckets to facilitate their release. The Cienega pond was chosen as the release site because: (1) it is relatively large and deep and thus unlikely to dry up; and (2) it was the location of the only metamorphosed relict leopard frog observed during a nocturnal visual encounter survey in October 2021.

Translocation: On July 20, 2022, a total of 1,006 relict leopard frog tadpoles were trapped in the two refugium ponds (**Map; blue ovals**). Of these tadpoles, 100 were translocated to the Cienega pond. A week later, on July 27, a total of 1,101 relict leopard frog tadpoles were trapped in the same refugium ponds and 112 were translocated to the Cienega ponds. Thus, a total of 212 tadpoles were translocated to the Cienega in July 2022 (**Table 1**).

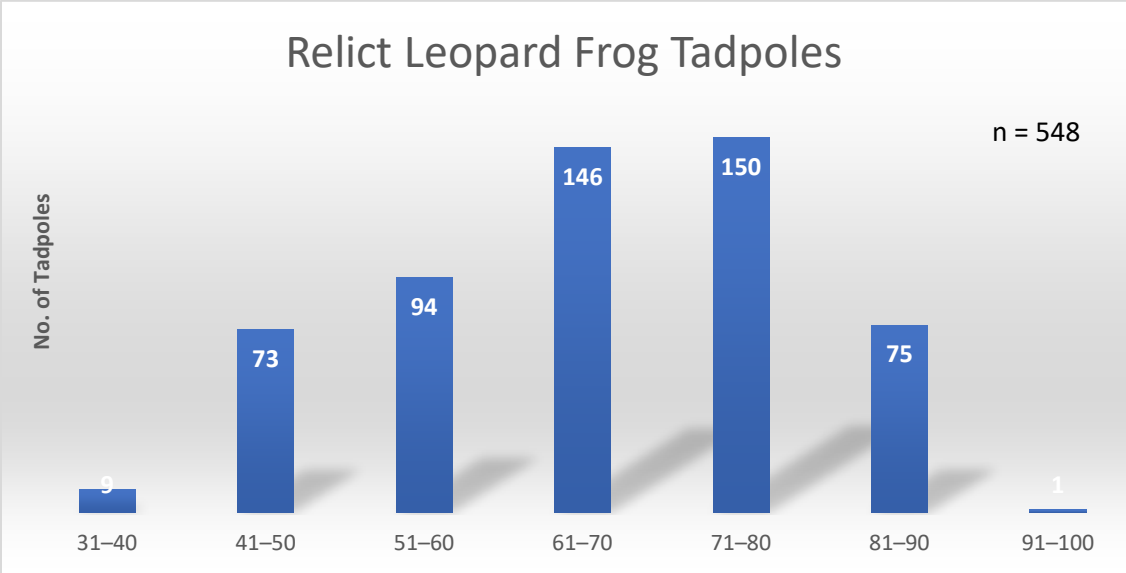
Table 1. Spatio-temporal distribution of 212 relict leopard frog (*Rana onca*) tadpoles translocated from the upstream and downstream refugium ponds to the Cienega pond at the Springs Preserve, Las Vegas, Clark County, Nevada.

	Upstream Pond	Downstream Pond	Total Translocated
20 July 2022	46	54	100
27 July 2022	54	58	112

The tadpoles selected for translocation were split randomly into two groups, with half of each group being released upstream and the remaining half downstream of the large Cienega pond (**Map; red ovals**).



Morphometrics: On July 20, 2022, the total lengths of 548 relict leopard frog tadpoles trapped in the downstream refugium pond were documented **(below)**.



Translocation Participants: The tadpole translocation was made possible by the following participants: Kellie Berry, Kevin Guadalupe, James Harter, Thomas O'Toole, Raymond A. Saumure, Jean-Axel Urbieta-Aguilar, and Audrey Wetjen.

Appendix II

Frogs jump in numbers at the Springs Preserve

Hop on over to the Springs Preserve and see the growing population of relict leopard frogs this spring. It was an exciting year as the once-believed-to-be-extinct tiny frogs increased in population to 66 adults—that's a 1,550 percent increase at the Springs Preserve in just four years!

The Springs Preserve is home to about 18 percent of all relict leopard frogs, which is the third-largest population in existence.

While the frogs usually stick to the ponds on the property, one of the adult frogs had an adventurous spirit and was found behind the Nevada State Museum—that's a straight-line distance of 624 yards from the frog ponds and 151 yards from the closest water!

Observe the posted speed limits on Springs Preserve roadways and keep an eye out for the tiny frogs. If you find one, report it to **702-918-7025** and help these tiny amphibians stay safe.

The relict leopard frog population at the Springs Preserve is growing by leaps and bounds.

Appendix III