## 2023 Annual Summary of Activities Relict Leopard Frogs at the Springs Preserve

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 2023, the first relict leopard frog (*Rana onca*) activity was observed in late February and active frogs continued to be observed into October. Ten egg masses were documented in the refugium ponds, nine in April and one in September. In April, per the Relict Leopard Frog Conservation Team protocol, a final 101 tadpoles were translocated to the Springs Preserve from wild egg masses raised in captivity. Approximately 300 tadpoles were captured in minnow traps during the July Pahrump poolfish (*Empetrichthys latos*) mark-recapture surveys. No tadpoles were translocated from the refugium ponds to the Springs Preserve Cienega in 2023. In August, a nocturnal survey of the Cienega documented 23 relict leopard frogs (17 adults, 6 juveniles). In October, 60 adults and 21 juveniles were captured during the annual mark-recapture survey of the refugium ponds. The adult population at the refugium ponds was estimated to be 101 adults. Educational content continues to be published on social media, websites, and in one academic publication.

## **Population Surveys**

**Egg mass surveys.**–A total of 10 relict leopard frog (*Rana onca*) egg masses were documented in 2023. The ponds were checked for egg masses at least five days per week. The first egg mass was documented on April 12, 2023. Colleagues from University of Las Vegas, Nevada (UNLV) led the official annual egg mass survey on April 18, 2023 and documented nine egg masses, including the egg mass detected on April 12. Two egg masses were noted to be hatching on April 23. The 10<sup>th</sup> egg mass was detected during routine maintenance on September 5, 2023 following monsoonal rains but was not found again. This was the first late summer egg mass detected at the Springs Preserve.

**Tadpole translocations.**—At the end of the egg mass survey on April 18, 2023, 101 medium-sized tadpoles were released by UNLV into the ponds to maintain, or increase, genetic diversity. Tadpoles, originating from wild-collected egg masses, were from natural populations at Northshore and Black Canyon. Specifically, at Northshore, 24 tadpoles were from Lower Blue Point and 27 tadpoles were from Rogers. At Black Canyon, 24 and 26 tadpoles were from Bighorn Sheep Egg masses #1 and #2, respectively. Per existing relict leopard frog translocation protocols, this was the fifth and final year of supplementation to the Springs Preserve refugium ponds.

Although hundreds of tadpoles were translocated from the refugium ponds to the Cienega in 2021 and 2022, none were translocated in 2023. The possibility of not translocating tadpoles to the Cienega in July 2023 was considered for two reasons:

• Despite translocating hundreds of tadpoles in the preceding two years, only one newly metamorphosed frog was encountered during the combined October 2021 and 2022 surveys. This suggested that tadpole

translocations to the Cienega were either: (1) not successful; or (2) the frogs were already brumating in October because of declining air/water temperatures. Thus, one solution under consideration was to translocate newly metamorphosed frogs in October, rather than tadpoles in July. In theory, the tadpole life stage itself could somehow be impacting survivorship. A second solution was to conduct the Cienega survey during the warmer summer months, rather than the fall, to rule out the possibility of brumation in October. This solution was implemented (see below).

• The number of tadpoles trapped during Pahrump poolfish (*Empetrichthys latos*) mark-recapture surveys in July of 2021 and 2023 was the same (i.e., approximately 300 tadpoles). In 2023, however, 300 tadpoles represented a 75% decrease in tadpole numbers as compared to July 2022 (approximately 1,200 tadpoles). Thus, there was some concern that there may be insufficient numbers of tadpoles in 2023 to translocate 10% to the Cienega. This fear was substantiated during the October 2023 mark-recapture survey (see below).

Thus, out of an abundance of caution, the decision was made to delay a translocation until October, at which time 10% of newly metamorphosed frogs would be translocated to the Cienega, pending discussions with the Relict Leopard Frog Conservation Team.

**Visual encounter survey.**—Given the failure to detect all but a single frog in the Cienega during surveys in October 2021 and 2022, the Cienega survey was scheduled earlier, during the heat of the summer, on August 30, 2023. Surprisingly, 23 relict leopard frogs (17 adults, 6 juveniles) were documented within the ~0.8 ha (2-acre) recreated Cienega system. Frogs were observed in all but the terminal pond, which experiences seasonal drying and had limited cover from predators. No egg masses or tadpoles were observed in the Cienega.

The frogs encountered in the Cienega could have originated from: (1) the tadpole translocations to the Cienega in 2021 and 2022; (2) rain-assisted dispersal of frogs from the refugium ponds; and/or (3) reproduction in the Cienega itself. Surveys in 2024 could help elucidate the source of the Cienega frogs. For instance, if PIT tagged frogs are encountered in the Cienega in spring 2024, then they migrated from the refugium ponds.

*Mark-recapture survey.*–All frogs with snout to vent lengths (SVL)  $\geq$  45 mm were tagged with a Passive Integrated Transponder tag (i.e., PIT tag); whereas smaller individuals were toe clipped on the 3<sup>rd</sup> right front digit in 2023. This enabled us to identify both adults and juveniles that were recaptured from previous years. In total, 60 unique adults and 21 juveniles were captured during the 2023 mark-recapture survey (**Table 1**). Of the adults, there were 34 males and 26 females, for a sex ratio of 1.31:1.00 males:females.

- Capture Session: On October 3, 2023, the UNLV-led mark-recapture survey resulted in the capture of 33 relict leopard frogs, including 22 adults and 11 juveniles. Four of the adults captured had previously implanted PIT tags and were recaptured frogs from 2021 (n=1), 2022 (n=2), or both years (n=1). In contrast to previous years, no tadpoles were documented during the survey.
- Recapture Session: On October 10, 2023, a total of 58 frogs were captured, including 48 adults and 10 juveniles. Fourteen of these were recaptured adults, nine of which were first marked the previous week on October 3, 2023. Of the five remaining frogs, four were first captured in 2022 and one was recaptured in three separate years, 2021–2023. Once again, no tadpoles were documented during the survey.

The adult population has fluctuated from 40 to 66 adults for the last four years (**Table 1**). Such a stabilization within a known range could indicate that the population has reached the carrying capacity of these small refugium ponds. As our mark-recapture data suggests, the vast majority of frogs only persist for two years (see *Longevity* section below). This could explain the oscillation from high to low adult counts every other year. Using the Chapman test, the population was estimated to be 101 adults in October 2023 (Dettloff 2023).

**Table 1.** A summary of translocations, reproduction, and number of unique adult and juvenile relict leopard frogs captured from 2018–2023 in the Springs Preserve refugium ponds. These numbers exclude recaptures.

YEAR	Number of Surveys	Juvenile Frogs Released	Tadpoles Released	<i>In Situ</i> Tadpole Cohorts <sup>a</sup>	Adults <sup>b</sup> Captured	Juveniles <sup>c</sup> Captured	Total Captured
2018	1	100	0	0	4	0	4
2019	2	111	101	1	12	178	190
2020	2	0 <sup>d</sup>	<b>0</b> <sup>d</sup>	2–3	40	244	284
2021	2	24	91	9 <sup>e</sup>	66	161	227
2022	2	0	100	8 <sup>f</sup>	40	142	182
2023	2	0	101	10 <sup>g</sup>	60	21	81

<sup>a</sup>Minimum number of egg mass(es) deposited in situ at Springs Preserve. Ponds surveyed daily.

<sup>b</sup>Adults defined *a priori* as all frogs with snout-vent length (SVL) of  $\ge$  45 mm. All adults PIT tagged.

 $^{\rm c}$  Juveniles are frogs with SVL < 45 mm that have completed, or mostly completed, metamorphosis.

 ${}^{\mathrm{d}}\mathrm{No}$  releases due to access restrictions associated with the Covid-19 pandemic.

e192 tadpoles were removed from the refugium ponds and translocated to the Springs Preserve Cienega.

<sup>f</sup>212 tadpoles were removed from the refugium ponds and translocated to the Springs Preserve Cienega.

<sup>G</sup>A single egg mass was deposited on September 5<sup>th</sup>, 2023.

The number of newly metamorphosed frogs captured in October 2023 decreased by 85% (Table 1). There are several possible reasons for this unprecedented decrease:

(1) the removal of cover, in the form of mats of stonewort, may have led to increased predation of the tadpoles by avian and/or invertebrate (i.e., odonate larvae) predators;

(2) there were two pairs of mallards frequenting the small refugium ponds in spring 2023, rather than a single pair observed in the springs of 2018–2022;

(3) the lab-reared tadpoles released by UNLV may have consumed unhatched eggs (Drake 2010) deposited in-situ in the refugium ponds;

(4) near record numbers of larger adult frogs may have led to an increase in cannibalism by the adults;

(5) heavy rains may have led to an increase in rain-assisted dispersal by newly metamorphosed frogs;

(6) by October, emergent aquatic vegetation in the pond planter pockets may have been sufficiently thick to impede the capture of smaller frogs; and

(7) a 500% increase in Pahrump poolfish may have led to predation on and/or competition with the tadpoles, at least while they were smaller than the adult fish.

Given how complex ecosystems and food webs can be, it is likely all of the above contributed to the decline to some degree. To mitigate for the lack of cover following the removal of the stonewort, eight large faux logs were purchased and placed in equal numbers on the bottom of both ponds on May 14, 2023. It should be noted, however,

that cannibalism, intra-species competition, dispersal, and predation also exert natural selective pressures that could ultimately benefit the population.

**Longevity.**—Since 2018, only four relict leopard frogs have been recaptured over a three-year span, three of which were captured in each of three years, and one adult captured in the first and third years only. Thus, of 200 adult relict leopard frogs captured and PIT tagged from 2018–2023, only 2% (n=4) are known to have survived for at least three years.

*Mortality.*—Two frog mortalities were documented and reported to NDOW in 2023. On June 1, 2023, an adult frog was found in a skimmer basket with no obvious signs of disease or injury. As it had presumably drowned, lids were placed over the skimmer baskets and no further instances were recorded. On August 1, 2023, a newly metamorphosed frog drowned in a minnow trap set as part of a mark-recapture survey for the federally endangered Pahrump poolfish. Although attempts were made to revive it, rigor mortis had already begun to set in when the trap was retrieved after the standard 3-hr trap set. As it was in otherwise good condition, the frog was preserved in methanol for potential future research/educational purposes.

## **Environmental Conditions**

The average water temperatures in the pond remain remarkably consistent from year-to-year, within 0.7°C (**Table 2**). Similarly, maximum water temperatures have not exceeded 29°C (84°F) and stayed within a 2.1°C range.

**Table 2.** Downstream Pond: Average, minimum, and maximum hourly temperatures in degrees centigrade (°C) for refugium pond (NF-1a) for 2020–2023 at the Springs Preserve, Las Vegas, Clark County, Nevada, USA.

Year	Average	Minimum	Maximum
2020	16.0	3.9	26.9
2021	15.9	3.6	29.0
2022	15.8	2.7	28.2
2023	15.3	3.4	28.2

Although the pH in the ponds appeared to be increasing from 2020–2022 (**Table 3**) more frequent calibration of the datalogger probe by Hydrologists revealed this was not the case. The 2023 pH data are comparable to those of 2020. Although the maximum pH is considerably lower, this may be related to changes in the pond algal community structure and/or possibly the buffering effect of more rain.

**Table 3.** Downstream Pond: Average, minimum, and maximum hourly pH for refugium pond (NF-1a) for 2020–2023at the Springs Preserve, Las Vegas, Clark County, Nevada, USA.

Year	Average	Minimum	Maximum
2020	8.6	8.0	9.4
2021	9.0	8.4	9.7
2022	9.34	8.7	9.9
2023	8.2	7.8	8.5

As with pH, the average and minimum conductivity values are somewhat lower in 2023 (**Table 4**), which is attributed to regular calibration.

**Table 4.** Downstream Pond: Average, minimum, and maximum hourly conductivity in microsiemens per meter ( $\mu$ S/m) for the refugium pond (NF-1a) for 2020–2023 at the Springs Preserve, Las Vegas, Clark County, Nevada, USA.

Year	Average	Minimum	Maximum
2020	1,092	997	1,211
2021	1,065	841	1,216
2022	1,109	1,004	1,182
2023	983	771	1,341

## **Education**

Information about the Pahrump poolfish is shared on the Springs Preserve <u>website</u>. Guests visiting the ponds can read existing and new (**Appendix I**) interpretive panels about the history of the relict leopard frog and some of the threats the species faces. Natural history and conservation messaging for the frog were shared during program offerings. Other public education outreach efforts from Springs Preserve were presented in SNWA newsletters (**Appendix II and III**) and online through social media platforms (see below). An article was published on relict leopard frog metamorphosis and the overwintering of tadpoles (O'Toole et al. 2023; **Appendix IV**).

## World Wildlife Day. March 3, 2023.

- Facebook: English: <u>https://www.facebook.com/springspreserve/videos/partnerships-for-wildlife-conservation/157985000404777/</u> Spanish: <u>https://www.facebook.com/SpringsPreserveLatino/videos/partnerships-for-wildlife-conservation/1221693592066199/</u>
- Twitter: <a href="https://twitter.com/SpringsPreserve/status/1631704099262431234">https://twitter.com/SpringsPreserve/status/1631704099262431234</a>
- Instagram: <u>https://www.instagram.com/p/CpVhAzithce/</u>
- LinkedIn: <a href="https://www.linkedin.com/feed/update/urn:li:activity:7037484143139590144">https://www.linkedin.com/feed/update/urn:li:activity:7037484143139590144</a>
- YouTube: <u>https://www.youtube.com/post/Ugkx0ceeASyXaJkIVYFubkwHEJ9AsTtuWgw7</u>

## When you're hot, they're hot! July 29, 2023.

- Facebook: <u>https://www.facebook.com/springspreserve/posts/pfbid0PKZ3XY3CFUZ6r5ZbE5f1Vvo6GNFfWx61HrsRNBxTbgAoxtnq9TG6vqL1VHWD</u> <u>v6K4I</u>
- Instagram: <u>https://www.instagram.com/p/CvSaMoJuh68/</u>
- Twitter: <a href="https://twitter.com/SpringsPreserve/status/1685323697165381637">https://twitter.com/SpringsPreserve/status/1685323697165381637</a>
- YouTube: <u>https://www.youtube.com/channel/UCz-mRRcxa\_JcQ9QD1JIq2Bg/community?lb=UgkxdJleRfXg-bpjkDEpEFYXAysfrPhEM7-N</u>
- LinkedIn: <a href="https://www.linkedin.com/feed/update/urn:li:activity:7091089008331522048">https://www.linkedin.com/feed/update/urn:li:activity:7091089008331522048</a>

## **Acknowledgments**

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## **Literature Cited**

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O'Toole, T. R.A. Saumure, A.R. Bennett, A. Ambos, R. Rivera, K. Guadalupe, J.R. Jaeger. *Rana onca* (Relict Leopard Frog). Metamorphosis and overwintering tadpoles. Herpetological Review 54:277–279.

#### Appendix I



# REWILDING Water is Life

The original creeks and pools at the Springs Preserve were once home to the Vegas Valley leopard frog (*Rana fisheri*), a species found in limited areas of the Southwestern United States. The springs were pumped dry around 1950. By the 1960s, this frog was thought to be extinct.

#### RESTAURANDO

RESTAURANDO El Agua es Vida Los arroyos y estanques originales en Springs Preserve alguna vez fueron el hogar de la rana leopardo del Valle de Las Vegas (Ram d'Isheri), una especie que se encuentra en áreas limitadas del suroeste de los Estados Unidos. Los manan-tiales quedaron secos cerca del año 1950 y cerca de 1960, se pensó que esta rana ya se baba cortinuido. había extinguid

As biologists began re-creating the As biologists begin techeating the Springs Preserve's wetland habitats in 2000, they started the process of rewilding. Rewilding is the introduction of a non-native animal species into an ecosystem to replace a similar extinct specie

Cuando los biólogos comenzaron a recrear los hábitats de humedales de Springs Preserve en el 2000, iniciaron el proceso de restauración. Restaurar es la Introducción de una especie animal no nativa en un ecosistema para reemplazar una especie extinta similar

Here at the Cienega, biologists Introduced the state-protected relict leopard frog (*Rana onca*) to the re-created ecosystem. The relict leopard frog is an ecologically similar species to the Vegas Valley leopard frog, which was rediscovered living in Arizona.

Aquí en la Clénega, los biólogos introdujeron la rana leopardo protegida por el estado (*Rana* onca) en el ecosistema recreado. La rana leopardo es una especie ecológicamente similar a la rana leopardo del Valle de Las Vegas, que fue redescubierta viviendo en Arizona.



Biologists introduced the stat re-created Cienega In 2021 and from to the Los biólogos introd recreada en 2021



## **DON'T DITCH A FISH** or a turtle or frog or crayfish

Releasing animals-including aquarium speciesinto ponds and streams is illegal.

When pets are released in the wild, they rarely survive Many suffer before they die because they are unable to find food or shelter. Those pets that do survive in the wild, often become **invasive** species that can destroy native wildlife and entire ecosystems.

#### **NO DEJES UN PEZ**

o una tortuga o una rana o un cangrejo de río La liberación de animales vivos, incluidas las especies de acuarios, en estanques y arroyos es llegal.

Cuando las mascotas son liberadas en la naturaleza, rara vez sobre-viven. Muchos sufren antes de morir porque no pueden encontrar comida ni refugio. Esas mascotas que sobreviven en la naturaleza, a menudo se convierten en especies invasoras que pueden destruir la vida silvestre nativa y ecosistemas completos.

Releasing your former pet fish, frog, turtle, crayfish— any pet—into the wild is against the law. It's also cruel, destructive and can cost communities hundreds of thousands of dollars in relocation and clean-up costs.

For more information, please visit dontletitloose.com

Liberar a su antigua mascota, pez, rana, tortuga, cangrejo de río, o cualquier otra mascota en la naturaleza es ilegal, cruel, destructivo y puede costarles a las comunidades cientos de miles de dólares para solucionarlo

Para más Información, visite dontietitioose.com



#### **Appendix II**



Senior Zoologist Katrina Smith has documented nine relict leopard frog egg masses in the ponds at the Springs Preserve so far this spring. Although nine may not sound like many, each globular cluster contains hundreds of gelatinous eggs, and for this imperiled species, every egg counts!

A survey conducted with the U.S. Fish & Wildlife Service at the Springs Preserve last July revealed just over 1,200 medium-sized tadpoles, about five times more than in 2021! It takes a lot of eggs to evolve into tadpoles and eventual frog status, but they got a little help on their journey when Smith supplemented the pool with another 102 tadpole younglings. This is the fifth year that tadpoles raised from wild eggs were released to increase the genetic diversity of the Springs Preserve population.

The original creeks and pools located on the Springs Preserve site used to harbor their own distinct types of frogs and fish, but those species were wiped out when the springs ran dry in the 1960s. The Springs Preserve recently introduced the rare relict leopard frog to help rewild these native habitats.

Despite this successful repopulation, the relict leopard frogs can be hard for the average visitor to detect, as they're most active at night and are well camouflaged with their surroundings.

Listen to the ribbitting mating call of the relict leopard frog here!



Senior zoologist Katrina Smith releases relict leopard frog tadpoles at Cottonwood Grove.

#### **Appendix III**

#### A Delicate Balance...

Macroalgae removal at the Springs Preserve refugium pond dramatically increased the population of endangered Pahrump poolfish, but results differ for the state-protected relict leopard frog.

We released the first 100 lab-raised froglets in 2018, and pair of mallard ducks swiftly ate them. Until then, no one had documented predation by ducks on this frog species. The 2018 fall frog survey resulted in only four, albeit astonishingly large, adult frogs. They bred in 2019, and the population has grown since to become the third or fourth-largest population in existence. So why was there a meteoric rise in tadpole numbers in 2022 and a 75% decline in 2023? Here's our working theory: removal of macroalgae diminished predation of fish by dragonfly and damselfly larvae but increased predation of tadpoles by mallard ducks.

Every spring, a pair of mallard ducks arrive at the Springs Preserve ponds, likely because the female needs a lot of calcium to 'shell up' her eggs. Fish, frogs, and tadpoles provide calcium and, of course, protein. During Covid, however, we reduced maintenance of the pond algae. By spring 2022, thick algal mats covered probably 70 percent of the two ponds from top to bottom. We suspect this coverage dramatically increased tadpole survivorship in 2022 because the tadpoles could easily hide from the ducks. While efforts to reduce dragonfly predation on the fish by removing the algae clearly worked, we suspect this left the tadpoles more vulnerable to duck predation in spring 2023. Compared to the zippy fish, tadpoles are likely much easier for the ducks to catch. In addition, TWO pairs of mallard ducks showed up in 2023.

To counter the lack of cover from avian predators, we added eight large faux logs this summer. The logs provide cover without allowing dragonfly larvae to hunt the entire water column, particularly the surface where the most vulnerable fish fry live. Managing even a simple ecosystem can be a delicate balance.



Fish, frog, and tadpole numbers at the Springs Preserve.



Large faux logs added to provide cover for fish.

**Appendix IV** 

HOTO BY MIRA FALICKI



Fig. 1. Larval *Rana draytonii* with a bifurcated tail collected from a constructed pond in Michigan Bluff, California, USA, June 2022.

retinoid and steroid-mimics, all of which have been reported to cause malformations in amphibians (Hall and Henry 1992. Herpetol. J. 2:65–71; Chambon 1993. Gene 135:223–228.; Kirk 1998. Herpetol. Rev. 19:51–53; Marco et al. 1999. Environ. Toxicol. Chem. 18:2836–2839; Hayes et al. 2002. P. Natl. Acad. Sci. 99:5476– 5480; Degitz 2003. Toxicol. Sci. 74:139–146). Still other researchers have indicated that parasitic infections, ultraviolet radiation, and climate warming may also generate malformations in anurans (Johnson et al. 2001. Herpetologica 57:336–352; Ankley et al. 2002. Environ. Sci. Technol. 36:2853–2858; Schoff et al. 2003. J. Wildlife. Dis. 39:510–521).

Buskirk and McCollum (2000. J. Exper. Biol. 203:2149–2158) reported that tail morphology plays a critical role in predator avoidance. Specifically, they noted length and depth may impact swimming ability. We suspect that the individual we collected had such a minor malformation that it may not affect swimming performance. Additionally, this larva, which we characterized as Gosner stage 38 (see: Gosner 1960, *op. cit.*), was nearing transformation, which would likely result in no expression of a malformation and would not impact future fitness.

Our work allows us to annually sample many populations of ranid frogs in California, and we only rarely note a malformed amphibian of any species or life stage; this is the first instance of a tail bifurcation in any ranid species we have sampled. Further, we believe this is the first report of tail bifurcation in the threatened frog, *R. draytonii*.

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JEFF A. ALVAREZ, The Wildlife Project, P.O. Box 188888 Sacramento, California 95818, USA (e-mail: jeff@thewildlifeproject.com); IAN JACKSON and MIRA FALICKI, Forde Biological Consultants, 10664 Presilla Road, Santa Rosa Valley, California 93012; JEFFERY T. WILCOX, Sonoma Mountain Ranch Preservation Foundation, 3124 Sonoma Mountain Road, Petaluma, California 94954, USA (e-mail: jtwilcox@comcast.net). RANA ONCA (Relict Leopard Frog). METAMORPHOSIS and **OVERWINTERING TADPOLES.** Although Rana onca was once believed by some researchers to be extinct, natural populations of these frogs had persisted in Arizona and Nevada (Jaeger et al. 2001. Copeia 2001:339-354). Since then, a multiagency effort has established several translocated populations to supplement the few remaining natural ones (Bradford et al. 2004. Southwest. Nat. 49:218-228; Fed. Reg. 81(194):69434-69437). Little has been published about the larval ecology of R. onca, a critical phase of the anuran life cycle (Bradford et al. 2005. In Lannoo [ed.], Amphibian Declines, The Conservation Status of United States Species, pp. 567-568. University of California Press, Berkeley, California; Drake 2010. Herpetol. Rev. 41:198-199; Dodd 2013. Frogs of the United States and Canada. Johns Hopkins University Press, Baltimore, Maryland. 982 pp.). Herein, we document the metamorphosis and overwintering of R. onca tadpoles following translocations and in situ reproduction in a refugium population.

On 29 May 2018, 100 lab-raised, recently metamorphosed, *R. onca* were released into a newly constructed 0.02-ha refugium consisting of two interconnected ponds established at the 73-ha Springs Preserve in Las Vegas, Clark County, Nevada, USA (36.17463°N, 115.18406°W; WSG 84) (Saumure et al. 2021. *In* PS. Soorae [ed.]. Global Conservation Translocation Perspectives: 2021. Case Studies from Around the Globe, pp. 76–81. International Union for the Conservation of Nature, Gland, Switzerland). By 3 October 2018, four of these recently metamorphosed frogs had reached the size of large adults (SVL = 75–84 mm) (Saumure et al. 2022. Herpetol. Rev. 53:108–110).

Over three dates from 27 March through 9 May 2019, an additional 101 large tadpoles and 111 metamorphosed frogs were translocated to the refugium ponds as part of a 5-year conservation protocol to supplement demographic and genetic diversity in the newly established population. On 25 April 2019, the first newly hatched R. onca tadpoles from in situ reproduction were observed in the ponds (Saumure et al. 2022, op. cit.), and by 10 August 2019, many newly metamorphosed R. onca were observed. A visual encounter survey (VES) conducted on 22 August 2019 documented four large adults, 190 newly metamorphosed frogs, and a single large tadpole. On 12 September 2019, seven tadpoles were captured in baited Gee minnow traps during a survey for Empetrichthys latos (Pahrump Poolfish), which were also previously released in the ponds. One of these tadpoles had developed all four limbs (Gosner Stage 42-44; Gosner 1960. Herpetologica 16:183-190). The remaining six tadpoles only had posterior limbs (Gosner Stage 36-39), with total lengths of 90-96 mm. During mark-recapture surveys for R. onca on 15 October and 7 November 2019, a total of 12 adults, 178 juveniles, and two tadpoles were captured. One of these tadpoles had developed posterior limbs (Gosner Stage 39-40; Fig. 1) and was comparable in size to the tadpoles captured in September. The second tadpole was estimated from photographs to be approximately 20 mm in total length and had not developed posterior limbs (Gosner Stage 25-26).

In 2020, no laboratory-raised *R. onca* were translocated to the refugium ponds; the Springs Preserve was closed to external personnel because of a Covid-19 pandemic quarantine. On 6 May 2020, however, a tadpole with posterior limbs (Gosner Stage 40–41; Fig. 2) was captured and photographed, confirming that *R. onca* can overwinter as tadpoles, a developmental strategy long suspected from field observations at other *R. onca* sites. On 25 August 2020, a VES documented 16 adults, 65 juveniles, and 5 tadpoles. Later, on 16 September 2020, 41 *R. onca* tadpoles were captured in baited Gee minnow traps during another *E. latos* survey.



FIG. 1. *Rana onca* tadpole captured on 7 November 2019 at the Springs Preserve, Las Vegas, Clark County, Nevada USA.

During mark-recapture surveys on 29 September and 6 October 2020, a total of 40 adults, 244 juveniles, and 17 large tadpoles were documented.

The following spring, six very large-bodied tadpoles (larger than those in Figs. 1 and 2), with posterior limbs only partially developed (Gosner Stage 36–39), were observed during a nocturnal VES for *R. onca* on 12 May 2021. Immediately following the survey, 91 tadpoles and 24 newly metamorphosed, laboratory-raised, *R. onca* were again released. During mark-recapture surveys on 5 and 14 October 2021, 66 adults, 161 juveniles, and 2 large tadpoles were documented. The two tadpoles had total lengths of 102 mm (Gosner Stage 37–39) and 114 mm (Gosner Stage 30–33) and masses of 11.5 g and 12 g, respectively. Whether these tadpoles were from the earlier release or were from reproduction in the ponds was not known.

The ability to overwinter as tadpoles is common among many species of North American frogs in the family Ranidae (Dodd 2013, *op. cit.*). Although the proximal causes for the facultative arrest of ranid tadpole development and the adoption of an overwintering strategy are not fully understood, decreases in food availability, water temperature, and/or tadpole density likely play a role (e.g., Fellers et al. 2001. Herpetol. Rev. 32:156– 157; Walsh et al. 2016. J. Zool. 298:183–190). Populations of *R. onca* historically inhabited a diversity of sites, including thermal and non-thermal springs (Bradford et al. 2004, *op. cit.*). The Springs Preserve refugium ponds are not heated, and being in the Mojave Desert, the water temperature varies broadly across seasons, with recorded hourly water temperatures that



FIG. 2. *Rana onca* tadpole captured on 6 May 2020 at the Springs Preserve, Las Vegas, Clark County, Nevada USA.

ranged from 0.5–26.7°C in 2019, 3.9–26.9°C in 2020, and 3.6–29.0°C in 2021. Although speculative, colder, late-season water temperatures in the refugium ponds may be a proximal cause for the observed overwintering of tadpoles, although tadpole density could also be a factor. The number of viable egg masses deposited in the refugium ponds increased from one in 2019 to nine by 2021, a potential nine-fold increase in resulting tadpole numbers over three years.

Overwintering as tadpoles may confer several ecological advantages over metamorphosis in the same calendar year as hatching. Bradford (1983. Ecology 64:1171–1183) noted that *R. muscosa* tadpoles can survive greater levels of oxygen depletion during winters than metamorphosed individuals. Furthermore, Walsh (2016, *op. cit.*) noted that *R. temporaria* tadpoles that overwintered were 31% heavier and 10% longer at metamorphosis than individuals that metamorphosed the preceding summer or fall. They speculated that this might confer a competitive advantage. One such competitive advantage for *R. onca* tadpoles was documented by Drake (2010, *op. cit.*) who observed cannibalistic oophagy by large tadpoles (Gosner 34–36) at two sites.

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#### **TESTUDINES** — **TURTLES**

CARETTA CARETTA (Loggerhead Sea Turtle). NESTING WITH-**OUT REAR FLIPPERS.** The importance of the rear flippers in the nesting process of Loggerhead Sea Turtles is well established (Caldwell et al. 1959. Bull. Fla. State Mus. Biol. Sci. 4:293-308). However, what happens when turtles missing their hind limbs try to dig nests is not well documented. ARCHELON has consistently monitored the main nesting beaches of Greece for the last 39 years. Its project in Lakonikos Bay, in the southeast Peloponnese, covers about 23 km of nesting beaches: Mavrovouni, Valtaki, Vathi, Selinitsa, and Evrotas. Here we present the case of a Loggerhead Sea Turtle (Caretta caretta) missing both hind limbs, first observed by ARCHELON's team in July 2010 in Mavrovouni during an early morning non-nesting emergence (Fig. 1). She was nicknamed "Stumpy" as her rear limbs were severed at about the ankle level and both flipper spades were missing (Wyneken 2001. The Anatomy of Sea Turtles. NOAA technical memorandum



FIG. 1. Loggerhead Sea Turtle (*Caretta caretta*) missing both flipper spades on Mavrovouni Beach after a non-nesting emergence.



Fig. 2. The clutch of 90 eggs exposed on the sand, only 5 m from the sea in Mavrovouni Beach, in July 2020.

NMFS-SEFSC-470). As a result, she was leaving very distinctive tracks on the sand. In June 2012 Stumpy was encountered again in Mavrovouni and this time she was tagged and we helped dig her nest as per routine in similar cases (Addison 1994. Herpetol. Rev. 25:63). The nest hatched after 46 d. Post-hatch excavation revealed 155 eggs, with 45.2% hatching success and 41.9% hatchling emergence success. Of the unhatched eggs, 90.6% had no visible embryos while 9.4% had dead embryos. In July 2019, she was observed again in Mavrovouni, still tagged, making several nesting attempts. Later in the season (1 August 2019) a Stumpy emergence with three nesting attempts was recorded in Mavrovouni. On 6 July 2020, during the morning survey in Mavrovouni, a clutch of 90 eggs was found exposed on the sand, only 5 m from the sea (Fig. 2). The inspection of tracks, the several emergences with Stumpy's tracks in Mavrovouni prior to this date, and the non-excavation of a complete egg chamber suggested that this clutch belonged to Stumpy. An egg chamber was excavated for the 88 eggs that were still intact and the nest was fenced against predators. At least one hatchling emerged from this nest, reaching the sea on 20 August 2020. It is likely that more hatchlings emerged but went unrecorded because of the pebbles surrounding the nest site as a result of a recent inundation, which hindered observation of hatchling tracks. Regrettably, the nest was completely depredated on 28 August 2020. To our knowledge this is the first reported case of a C. caretta lacking both rear flippers repeatedly attempting to nest, and notably showing strong nest site specificity (always appearing on the same stretch of beach). In addition, the outcome of this case validates the importance of monitoring the nesting beaches so that we maximize the number of hatchlings recruited to the population every year and thus contribute to the conservation of the species.

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CHELYDRA ROSSIGNONII (Central American Snapping Turtle), KINOSTERNON ACUTUM (Tabasco Mud Turtle), K. LEUCOSTOMUM LEUCOSTOMUM (Northern White-lipped Mud Turtle), and TRACHEMYS VENUSTA VENUSTA (Meso-American Slider). HATCHLING BEHAVIOR. Hatchlings are considered the least understood life history stage of North American turtles (Morafka 1994. Neonates: missing links in the life histories of North American tortoises. Fish and Wildlife Research