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Insights into Relict Leopard Frog Breeding Biology

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***Abstract.*—Information on the life-history characteristics of organisms is often collected during management actions or through research facilitated by management programs. An example is the Relict Leopard Frog (*Rana onca*) for which information on breeding biology has been accumulated through long-term headstarting, translocation, and monitoring efforts. We reviewed survey data and headstarting and translocation records from 2003–2021 to determine breeding seasonality, number of eggs per egg mass, time to hatching, time to metamorphosis, and time to reproductive maturity. We determined that *R. onca* can breed year-round, but with a peak breeding period from January through May, when 97.6% of egg masses were observed. We estimated that egg masses contained around 418 ± 57.7 (standard error) eggs, with individual egg masses consisting of 96–1,106 eggs. We inferred the general time from oviposition to hatching as 5–8 d in water temperatures in the low-to-mid 20s° C. Time from hatching to metamorphosis in the laboratory was approximately 62 ± 1.1 d at 22.0°–27.0° C. Our field observations indicated that the time from hatching to metamorphosis in the wild mostly occurred within the same year, but overwintering by tadpoles was common. Our monitoring at newly established translocation sites showed that *R. onca* can reach reproductive maturation in a little over a year (shortest observed time = 12.2 mo) from oviposition of source animals to when evidence of breeding was first observed. These insights on the breeding biology of *R. onca* have been used to better inform management actions.**

Key Words.—breeding seasonality; egg mass size; life-history; *Lithobates onca*; metamorphosis; *Rana onca*; reproductive maturity; time to hatching

Introduction

The natural history of organisms, encompassing life-history characteristics, underlies and inspires various fields of science (Bartholomew 1986; Futuyma 1998; Arnold 2003). This area of research has contemporary importance in resource management and conservation (Dayton 2003; Fleischner 2005; Bury 2006). Understandably, many species lack comprehensive life-history descriptions (Bury 2006; Moore et al. 2013) because of the diversity of species and the practicality of gathering such information (Michaels et al. 2014). Moreover, available information can be difficult to access as it may be in the gray literature (e.g., agency and consultant reports), embedded in other types of research (McCallum and McCallum 2006), or published piecemeal across multiple sources (Oliveira et al. 2017; Loughman 2020). In more recent times, life-history investigations are predominately driven by conservation concerns (Wilson et al. 2009; Michaels et al. 2014). Gaps in knowledge for particular species are often filled by information gathered as part of management actions or research facilitated by management programs. One such example is the Relict Leopard Frog (*Rana onca*; Fig. 1), a species of conservation concern for which information on its life history has been predominately accumulated during management efforts.

Rana onca is part of the *Rana pipiens* group and a sister taxon to the Lowland Leopard Frog (*Rana yavapaiensis*; Jaeger et al. 2001; Hillis and Wilcox 2005; Yuan et al. 2016). Populations of *R. onca* were known to occur historically in a narrow geographic range along the eastern fringe of the Mojave Desert of North America, occupying springs and wetlands along the drainages of the Virgin River and adjacent portion of the Colorado River (Jaeger et al. 2001; Bradford et al. 2004). The

Figure 1. Adult Relict Leopard Frog (*Rana onca*) from a translocation site in Mohave County, Arizona, USA. (Photographed by Rebeca Rivera).

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species declined during the 20th Century, and by 2001 the range had contracted to a few geothermally influenced (hot) springs (with source temperatures generally above 30° C) in two small areas of southern Nevada (Bradford et al. 2004). Potential causes for the decline are multiple and probably synergistic, with implicated factors including loss of habitat due to agriculture and water development, introduced predators, and disease (Bradford et al. 2004; Jaeger et al. 2017).

Management for *R. onca* has been guided since 2001 by a multiagency conservation team (Relict Leopard Frog Conservation Team; RLFCT) consisting of members from various land and resource management agencies, as well as partners (e.g., Clark County, Southern Nevada Water Authority, and the University of Nevada, Las Vegas). A conservation agreement, assessment, and strategy (CAS) was developed in 2005, and updated and renewed in 2016 (RLFCT, unpubl. reports). Two main components of the strategy have been the systematic monitoring of populations and a persistent headstarting and translocation program. The latter strategy has expanded the distribution of *R. onca* in southern Nevada (Clark and Nye counties, USA) and established populations in northwestern Arizona (Mohave County, USA) within or near perceived historical range, with most of the translocation sites having ambient water temperatures (cold-water sites).

Our focus, herein, is to synthesize life-history information gained during these management actions to fill in knowledge gaps related to aspects of breeding biology, expanding on our understanding of: (1) breeding seasonality; (2) egg mass size; (3) relative incubation time; (4) time to metamorphosis; and (5) time to reproductive maturity. We summarize and assess information collected during management actions from 2003 through 2021. We present our findings within the context of what has already been published on *R. onca* to clarify previous perspectives and offer new insights on components of breeding biology. Our intent is to expand published knowledge on the life history of *R. onca* and better inform the conservation strategy for the species.

Materials and Methods

Information sources.—We derived data to assess breeding seasonality and age at reproductive maturity from 1,399 population monitoring surveys conducted from February 2003 through December 2021. Most of the data reflected Visual Encounter Surveys (Crump and Scott 1994) conducted by crews usually consisting of two or more people led by biologists with substantial experience surveying for the species. The number of occupied sites surveyed each year generally increased from four in the first year to 23 as historical and translocation sites were added; however, the number fluctuated when translocations failed to establish populations. Over time we surveyed a total of 26 unique sites, but excluded data from two of these sites, both translocations, where breeding by *R. onca* was never documented. Generally, we surveyed occupied sites three times annually, twice as seasonal temperatures warmed (predominately mid-January through May) and once in fall as seasonal temperatures cooled (predominantly mid-September through mid-November). At any given site, however, the number of surveys we conducted per year potentially varied because of logistical issues or management objectives. Surveys occurred throughout the year but were scant during June through mid-September when ambient temperatures were hot and during mid-November through mid-January when temperatures were cold. Generally, we conducted surveys at cold-water sites later in spring and earlier in fall than at hot springs to take advantage of seasonally warmer temperatures.

We assessed the number of eggs per egg mass, time to hatching, and time to metamorphosis from information associated predominately with the headstarting and translocation program. An aggregate of eggs oviposited by *R. onca* has been described as a globular cluster (Bradford et al. 2005), and elsewhere as a spherical cluster or colloquially as an egg mass (Fig. 2); herein we use egg mass. We are unsure whether an egg mass represents an entire clutch or an ovipositional bout (Altig and McDiarmid 2007). Each year we collected several (4–10) egg masses for rearing, either whole or as partial masses. We collected partial masses to decrease the impact on source populations and increase genetic diversity of subsequent translocations. We derived data from field monitoring surveys associated with the collections, and integrated information from laboratory notes on the daily husbandry of head-started animals, along with records from subsequent releases. We took measurements of water temperatures with different instruments over the years, including bulb thermometers (e.g., Rolf C. Hagen, Corporation, Mansfield, Massachusetts, USA) and digital pen thermometers (e.g., Thermopen MK4, ThermoWorks, American Fork, Utah, USA) with accuracies of 0.4°–0.7° C. We reviewed records associated with 107 egg masses, although sample sizes varied across assessments because of missing data or inadequate descriptions.

Breeding seasonality.—We determined seasonality of *R. onca* breeding by the accumulated counts of egg masses during monitoring surveys from each month. We also reviewed field records of male calling. We did not determine the developmental stage (Gosner stage; Gosner 1960) of most egg masses in the field, so we assigned egg masses to the month of their observation.

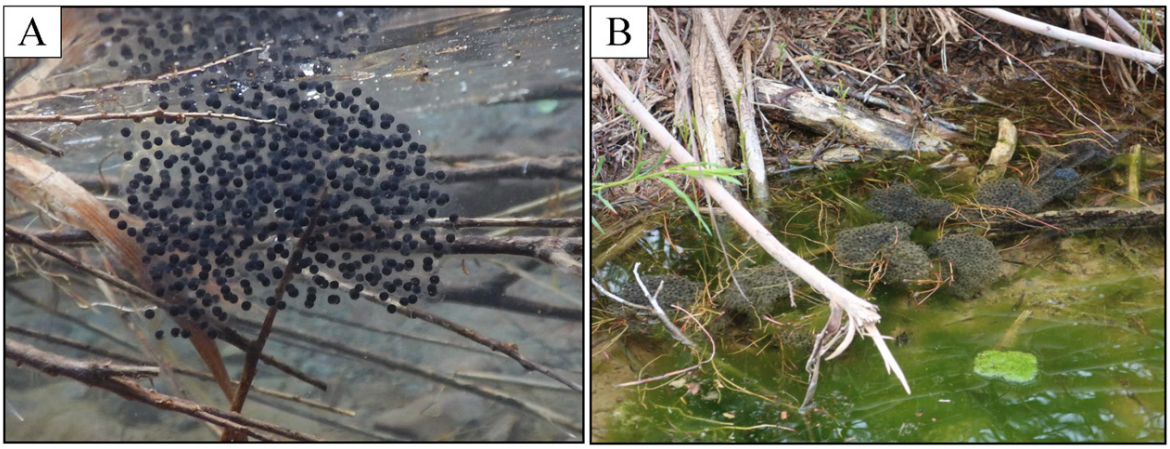

Figure 2. (A) Relict Leopard Frog (*Rana onca*) egg mass under water and (B) numerous egg masses along the bank of a stream pool. Egg masses are spheroid and consistent with description by Altig and McDiarmid (2007) of aquatic clumps. (Photographed by Rebeca Rivera).

We included spent egg masses that still had hatchlings (Gosner stages 20–25). In addition, we separated egg mass data from hot and cold-water sites by month to explore potential differences in timing using a Two-proportion *Z*-test (https://www.statology.org/two-proportion-z-test-calculator/).

Egg mass.—We quantified the number of eggs in egg masses collected opportunistically for headstarting and translocation. For each mass, we counted hatchlings (Gosner stages 23–25) and unviable eggs following hatching. We determined numbers directly from 16 egg masses collected whole. We also estimated the number of eggs in 65 partial egg masses when the proportion collected was visually approximated at time of collection. In these cases, we counted as above and then multiplied by the estimated proportion of the mass collected.

Time to hatching.—To evaluate hatching time in *R. onca*, we first determined the hatching period in the laboratory for whole and partial egg masses collected for headstarting. We determined hatching period as the time from egg mass collection in the field until hatching was first documented in the laboratory; this period did not include developmental time in the wild prior to collection. We observed hatching to start at Gosner stage 20, although hatching was not fully synchronized and many embryos at that point appeared to still be at Gosner stage 19. Variation of Gosner stage within an egg mass at hatching has been documented in other ranid species (Shumway 1940; Zweifel 1968; Beattie 1987). We recorded water temperature in the laboratory for each egg mass daily and then averaged these values for assessment. We reared egg masses at temperatures ranging from 18.0°–25.1° C, with temperatures early in the conservation program being in the cooler range. Details on Gosner stages of embryos and date of hatching relative to laboratory water temperatures were complete for 61 egg masses.

We collected egg masses at various stages of development, with 56 of these egg masses found in water temperatures ranging from 10.9°–28.8° C (average = 20.4° ± 0.5° C [standard error]). Based on observations at time of collection, we grouped egg masses into four developmental categories: Gosner stages ≤ 10 (notes indicating the presence of vegetal and animal poles; n = 15), Gosner stages 11–12 (notes indicating eggs as black spheres; n = 21), Gosner stages ≥ 13 (notes indicating eggs breaking spherical shape; n = 20), or Gosner stage undetermined (notes lacking; n = 5). To evaluate complete hatching time in the wild, we reviewed surveys and site visits that occurred at particular sites over short periods (2–10 d) where egg masses were detected. We identified only five such events at three hot spring sites that contained meaningful observations to infer hatching times.

Time to metamorphosis.—We estimated the time (days) to metamorphosis in the laboratory from the date of hatching to the emergence of forelimbs or when laboratory notes first indicated transfers of individuals from our tadpole tanks to frog tanks for a given egg mass. These events indicated that the fastest developing animals from an egg mass had reached Gosner stages ≥ 42. For this assessment, we discarded most records of egg mass collections prior to 2012 because laboratory notes on metamorphosis were confusing or undescriptive; only one record from 2011 was kept. We also discarded records from two egg masses in 2019 and two egg masses in 2020 because details on the timing of metamorphosis could not be determined. In the end, we based our assessment on observations related to 55 egg masses collected whole or in part.

Time to reproductive maturity.—Initial translocations to unoccupied sites allowed an opportunity to evaluate time to reproductive maturity in *R. onca*. These sites were generally isolated with little to no chance of immigration from neighboring populations. We reviewed monitoring data from 14 translocations where populations were established to the point of documented breeding. Initial translocations generally occurred in spring, using late-stage tadpoles, juvenile frogs, or both. We evaluated observations from subsequent monitoring surveys for evidence of reproductive maturity (i.e., egg masses or tadpoles hatched at the site). We focused on the shortest periods to reproductive maturity detected in our data, and conservatively calculated reproductive maturity as the time from egg mass collection for the oldest head-started animals initially released at a site to the date when evidence of reproduction was detected.

Results

Breeding seasonality.—When assessed across years, we observed egg masses during surveys throughout the year (Fig. 3), along with calling by males (Appendix Fig. 1). Our egg mass encounters, however, mostly occurred during surveys from January through May (Fig. 3), and of all surveys with egg mass encounters, 92.1% (337/366) occurred during this period (Appendix Fig. 2). We also counted 97.6% (1,839/1,885) of all egg masses from January through May (Fig. 3). Egg mass production appeared to drop-off at the beginning of the hot season in June and was minimal through the rest of the year. When we parsed data between hot springs and cold-water sites, a more nuanced pattern emerged (Fig. 3). During the cold months of November through January, we encountered egg masses more often during surveys at hot springs than at cold-water sites (Z = 3.273, P < 0.001). In those months, we encountered egg masses during 31.4% (70/223) of surveys at hot springs, but only 5.4% (2/37) of surveys at cold-water sites (Appendix Fig. 2). Of the egg masses (n = 17) we observed in November and December, all were at hot springs, while the few egg masses (n = 8) we observed during the hottest months (June through August) were all at cold-water sites (Fig. 3).

Egg mass.—Counts of eggs (viable and unviable) from 16 *R. onca* egg masses collected whole ranged from 96–1,106 (Table 1), with an average of 418 ± 57.7 eggs. When we extrapolated from counts of 65 partial egg masses, where the approximate proportion collected had been recorded, the average number of eggs was estimated at 528 ± 28.9 eggs. The proportion of viable eggs in masses collected whole averaged 0.855 (range from 0.311–1.00), and in partial egg masses averaged 0.813 (range from 0.022–1.00).

Time to hatching.—Hatching period in the laboratory (not including developmental time in the wild prior to collection) was positively, although marginally, influenced by rearing water temperature and Gosner stage at time of collection. The longest time to hatching in the laboratory was 8 d at 18.0° C for two egg masses collected early in the conservation program that both lacked Gosner stage documentation. When we determined Gosner stages at collection, the youngest egg masses at Gosner stages ≤ 10 hatched after approximately 5 d (range from 4–6 d) at an average water temperature of 23.6° ± 0.17° C (n = 15). When collected at Gosner stages 11–12, hatching took approximately 4 d (range from 3–6 d) at an average water temperature of 23.1° ± 0.2° C (n = 21). Older embryos collected at Gosner stages ≥ 13 hatched after only 3 d (range from 2–4 d) at an average water temperature of 23.4° ± 0.26° C (n = 19); however, one outlier required 6 d to hatch at 21.2° C.

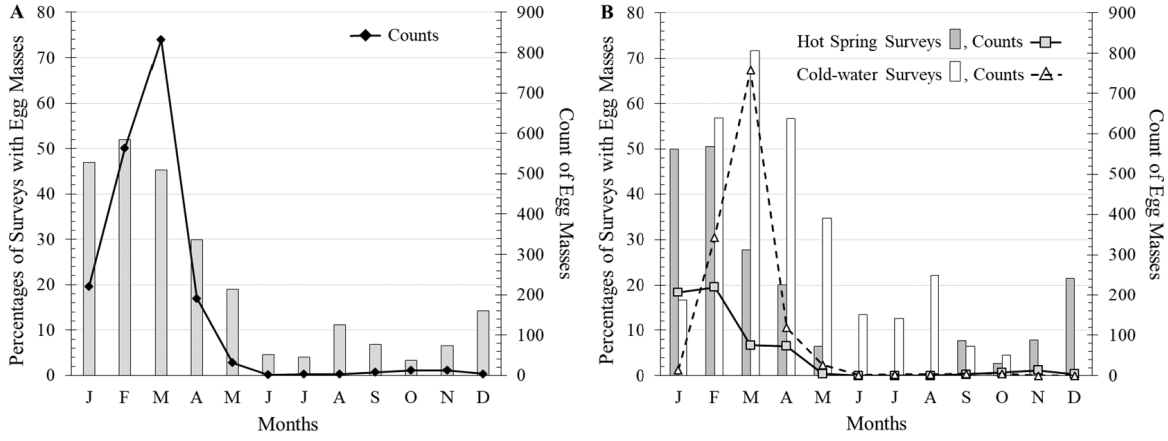

Figure 3. Breeding seasonality of Relict Leopard Frog (*Rana onca*) represented by the percentage of surveys per month with at least one egg mass counted from February 2003–December 2021 at (A) all sites and (B) separated between hot springs and cold-water sites. The accumulated counts of egg masses per month are presented by lines on the graphs.

Table 1. Number of eggs per egg mass, proportion viable at hatching, and rearing water temperature for 16 egg masses of the Relict Leopard Frog (*Rana onca*). Temperatures are either the average of daily recorded rearing temperatures or general rearing temperatures from laboratory notes; data from 2006 were lacking. Date of collection and sample sites within Clark County, Nevada, USA, are provided.

Site	No. of Eggs	Proportion Viable	Rearing Temperature (° C)	Date
Blue Point Spring (upper)	517	0.952	24.0	5 February 2013
Blue Point Spring (lower)	427	0.761	23.7	23 January 2018
	96	0.823	25.1	23 January 2018
Bighorn Sheep Spring	1,106	0.931	21	22 January 2004
	496	0.990	–	17 January 2006
	476	1.00	–	17 January 2006
	551	0.976	19–21	6 February 2007
	184	0.978	18.0	20 February 2007
	457	0.941	18.0	20 February 2007
	348	0.957	22–23	9 March 2007
	409	0.311	22–23	9 March 2007
	383	0.945	22–23	9 March 2007
	269	0.877	22–23	9 March 2007
Black Canyon Spring (side spring)	347	0.559	24.1	24 January 2018
Salt Cedar Canyon Spring	121	0.802	23.5	30 January 2010
	503	0.881	24.9	24 January 2018

We inferred the time from oviposition to hatching in the wild from repeated observations of five egg masses at hot spring sites. The shortest time to hatching was approximately 5.5 d at a water temperature of 21.9° C (recorded at Blue Point Spring, lower). Two other egg masses at the site took 6.5–7.5 d at 19.9° C and 8 d at 21.5° C. At a different site (Rogers Spring), the time to hatching for an egg mass was approximately 6.5 d at water temperatures recorded at 19.1°–22.0° C. We collected part of this egg mass for headstarting at Gosner stage ≤ 10 (probably 1.5 d old) and raised the eggs at 23.6° C; these eggs hatched in 4 d. For another egg mass found at a translocation site (Pupfish Refuge Spring), hatching occurred in approximately 7 d at a water temperature around 18°–21° C (based on previous temperatures taken in the area around the oviposition site).

Time to metamorphosis.—In the laboratory, we observed hatchlings from 55 egg masses to transition into metamorphs (Gosner stages ≥ 42, following forelimb emergence) in an average of 62 ± 1.1 d (range from 47–82 d) at an average water temperature of 24.1° ± 0.1° C (range from 22.0°–27.0° C). We did not generally monitor the transition from early metamorphosis (Gosner stage 42) to completion (Gosner stage 46, when the tail is fully absorbed), but based on observations of four animals the process took 10–11 d at laboratory temperatures. Extrapolation of these data indicated that hatching to completion of metamorphosis took roughly 2–3 mo in the laboratory.

From our field observations, the time from hatching to complete metamorphosis in the wild generally occurred within the same year but overwintering of tadpoles appeared common. Observations of very large tadpoles in spring (May) across 2 y at a cold-water site confirmed overwintering in *R. onca* (O'Toole et al. 2023). Restricting our observations to the winter months of January and February, we repeatedly detected large tadpoles at 11 sites at temperatures where overwintering was the most parsimonious explanation for their presence.

Time to reproductive maturity.—Following translocation to new, unoccupied and isolated sites, we detected breeding by *R. onca* at seven sites to occur as early as January through April following initial releases during the previous spring (12.2–15.3 mo from time of initial egg mass collections of the oldest source animals). These translocations occurred at both hot and cold-water sites and were initiated with late-stage tadpoles, juvenile frogs, or both (Appendix Table 1). The shortest developmental time to reproductive maturity was at Goldstrike Canyon, a hot spring along the Colorado River below Lake Mead, where late-stage tadpoles were initially released on 9 April, 5 May, and 29 June 2004 from egg masses collected on 22 January and 15 March 2004. A viable egg mass was subsequently observed at the site during a survey on 27 January 2005, just over 1 y (372 d) from the earliest collection date of the source egg masses. Goldstrike Canyon is about 1 km downriver from the nearest occupied site at that time, Pupfish Refuge Spring, where *R. onca* had been established earlier by translocation. The river between these sites is not sustainable habitat for *R. onca* and dispersal to

Goldstrike during the time of population establishment was unlikely. The second shortest time to reproductive maturity was at Grapevine Springs at just over 13 mo (402 d) and there was no chance of dispersal to this site. Grapevine Springs is predominately a cold-water site, but a spring source emerges from an adit where the water temperature in winter is just below 20° C.

Discussion

Existing published information on the breeding biology of *R. onca* is limited. The majority of available information can be directly or indirectly tied to its conservation program, and a handful of researchers and managers associated with the program (including the authors herein). The information on breeding biology provided in the 2005 CAS was derived concomitantly with a publication on the population status of the species and a short species account (Bradford et al. 2004, 2005). The data assessed in our current study included this early data, as well as subsequent data used to provide descriptions and summaries incorporated into the 2016 renewal of the CAS. Those earlier descriptions, however, were made predominately without presentation of the underlying data. Prior to the conservation program, there were only a handful of researchers working on the species, with much of the focus on distribution, demography, and systematics (e.g., Jaeger et al. 2001; Bradford et al. 2004). There are several more recently published articles (Goldstein et al. 2017; Saumure et al. 2022; O'Toole et al. 2023) that we derive information from in our discussion below, but these too are tied closely to the conservation program.

Breeding seasonality.—Initial descriptions of breeding phenology in *R. onca* indicated that the species had an extended breeding period, with favored breeding times reported as being in spring and fall (Bradford et al. 2005). Most egg masses were reported to occur during the early seasonal period, defined as January or February through March or April (Bradford et al. 2005; RLFCT, unpubl. report), or possibly from March through May (Wright and Wright 1949). The later breeding period was described as occurring in November (Bradford et al. 2005), although eggs have been reported in September (RLFCT, unpubl. report). Our assessment of observations over 19 y indicates that *R. onca* is a prolonged breeder. Oviposition and calling by males can occur during any month. There is, however, a clear breeding period from January through May. More specifically, overall breeding appears to increase towards the latter half of January and remains high through April, extending into May. Breeding activity appears broadly associated with temperature, increasing as ambient temperatures warmed in January and declining as temperatures increased in the latter part of May. The connection to temperature was most apparent in the temporal shift of the main breeding period towards breeding earlier in the season at hot springs and later in the season at cold-water sites. This interpretation, however, may be somewhat clouded by the pattern of early-season surveys that generally started at hot springs around mid-January and then shifted towards cold-water sites as the season progressed.

Breeding seasonality in the sister taxon *R. yavapaiensis* in Arizona has been described as bipartite with a major breeding period in spring and a lesser period in fall (Sartorius and Rosen 2000). A review of the reported timing for breeding in *R. yavapaiensis* generally supports this perspective, although at geothermally influenced springs or low elevation sites, there has been speculation that the species may be reproductively active year-round (Sredl 2005). In *R. onca* the major breeding period is followed by minimal egg mass production during the rest of the year. During the hottest months, some production occurs at cold-water sites, but this drops off into the cold season. Conversely, as temperatures cool into fall, there is a slight uptick in production at hot springs. We are hard pressed, however, to describe this as bipartite given the limited number of egg masses observed during these later periods. We have no information on the number of clutches that females may produce over a year, but given that breeding is possible throughout the year, there appears to be the potential for more than one ovulation event.

The lack of breeding during summer months in *R. yavapaiensis* has been postulated as a mechanism to avoid seasonal declines of surface water or loss of egg masses during floods caused by summer rains (Sartorius and Rosen 2000). Summer monsoons extend into the eastern Mojave Desert, although with less predictability than in the Sonoran Desert (Redmond 2009). Rain events have been implicated as an environmental cue for stimulating oviposition in some anurans, including a ranid species with prolonged breeding (Saenz et al. 2006). Anecdotal observations suggest similar behavior in *R. onca*, and we have detected egg masses at cold-water sites targeted for surveys in summer within days following rains. If this is a general phenomenon in *R. onca*, the potential mechanism is not clear and may relate to changes in relative humidity, air temperature, water temperature, or habitat disturbance.

Egg mass.—General descriptions of egg mass size in *R. onca* have reported up to 250 eggs (RLFCT, unpubl. report) or many hundred eggs (Bradford et al. 2005). We estimated an average egg mass size of 418 ± 57.7 eggs from a collection of 16 egg masses but believe this estimate may be biased low. The egg

masses were gathered opportunistically to facilitate the programmatic needs of headstarting and translocation, and the collection appears to have an overrepresentation of small egg masses. Our estimated average of 528 ± 28.9 eggs from a collection of 65 partial egg masses may better reflect egg mass size, even if the methodology was less precise. The range of 96–1,106 eggs per egg mass from the collection of whole egg masses seems representative, but bigger egg masses are likely given the large size that females can reach under exceptional conditions (Saumure et al. 2022). We found no comparable estimate of egg mass size for *R. yavapaiensis*, but the average for *R. onca* was much lower than the 1,600 eggs reported for the clutch size (referring to egg mass size) of the Northwest Mexico Leopard Frog (*Rana magnaocularis*; Frost and Bagnara 1977), another closely related species from Mexico (Yuan et al. 2016).

Time to hatching.—Temperature dependence of anuran embryo development is well documented (Moore 1939; Zweifel 1968; Bradford 1990), and water temperatures experienced by populations of *R. onca* are certainly affected by seasonal weather patterns in the Mojave Desert, along with specific site conditions (e.g., geothermally influenced or not). Our data predominately focused on developmental time under laboratory conditions, not including developmental time of the egg masses in the wild prior to collection. Egg masses collected at the earliest Gosner stages (≤ 10) and reared in the laboratory at water temperatures from 22.2°–24.4° C, hatched in 4–6 d. Our assessment of egg masses collected at later Gosner stages and reared at similar temperatures were consistent with this developmental timing. Based on a very early subset of the data included herein, hatching period in the laboratory was reported as 5–7 d for egg masses collected in the field at Gosner stage < 14 and reared at what was called room temperature (RLFCT, unpubl. report). Original notes on rearing temperatures were lacking from that time but can be assumed to be 21°–22° C from peripheral information (Drake 2010; Goldstein et al. 2017). The variation in the estimated development times between the two assessments could be easily explained by the temperature differences. At colder water temperatures embryonic development takes longer, as was evidenced by two egg masses collected in 2007 that took 8 d to hatch in water temperatures of 18° C.

To estimate the hatching time from oviposition using the laboratory data, we need to include the developmental time in the wild prior to collection. The earliest egg masses collected (Gosner stage ≤ 10) were thought to range from about 0.5–2 d old when encountered. In the Northern Leopard Frog (*Rana pipiens*), development to the equivalent of Gosner stage 10 was experimentally reached in 26 h following fertilization at a water temperature of 18° C (Shumway 1940). Adding to the laboratory data the likely developmental times prior to collection indicates that *R. onca* takes approximately 5–8 d to go from oviposition to hatching at water temperatures in the low-to-mid 20s° C. This estimate is consistent with the previous account that indicated this transition takes approximately one week (RLFCT, unpubl. report). From observations made on five egg masses in the field, time to hatching in the wild was inferred to occur in approximately 5.5–8.0 d in water temperatures of 19.1°–22.0° C. Recent observations indicate that this process may take much longer in colder water (10.4° C) and appears to require the seasonal warming of water temperatures for hatching. For comparison, the incubation period of four egg masses of *R. yavapaiensis* observed in the field took 15–18 d at a reported water temperature of 14.2° C (Sartorius and Rosen 2000).

Time to metamorphosis.—As with embryonic development, temperature has an effect on tadpole development, as well as growth of frogs towards sexual maturity (Morrison and Hero 2003). Development and growth rates vary across populations, influenced by site-specific conditions and temporal shifts in resource availability, among other factors (Jørgensen 1992; Gotthard 2001). In *R. onca*, the time required for tadpoles to reach metamorphosis after hatching was previously suggested to take several months (Bradford et al. 2005). Under laboratory conditions, when fed *ad libitum*, tadpoles of *R. onca* reportedly completed metamorphosis (ostensibly Gosner stage 46) in 2–3 mo at water temperatures of 24°–25° C (RLFCT, unpubl. report). Our laboratory results are consistent with this estimate. Metamorphosis in the laboratory, however, has been reported to take much longer (approximately 6.5 mo; RLFCT, unpubl. report) at presumably colder temperatures (probably 21°–22° C).

Our estimate of the time to reach metamorphic stages (Gosner stages ≥ 42) was faster (average 62 ± 1.1 d) than the times observed during a laboratory study on tadpole development in *R. onca* (Goldstein et al. 2017). In that study, young tadpoles were assessed at temperatures from 15°–35° C at 5° C increments. At 15° C and 35° C tadpoles appeared to be outside their optimal temperature range and exhibited limited growth and development. Tadpoles reached metamorphosis (Gosner stage 42) most quickly at 25° C (similar to temperatures in the headstarting laboratory). The timing of metamorphosis reported in that study, however, did not include the age of tadpoles when first entered into the experiment, which we determined to be 10 d based on the listed hatching date and the date tadpoles were received from the headstarting program. We were

unable to determine if the time required for acclimation was incorporated, but based on the reported acclimation rate this would have been 2 d at 20° C, 3 d at 25° C, and 8 d at 30° C. Thus, time from hatching to forelimb emergence in that study averaged 274–276 d at 20° C, 77–80 d at 25° C, and 108–116 d at 30° C.

The time to metamorphosis in *R. yavapaiensis* has been reported to be as short as 3–4 mo or as long as 9 mo (Sredl 2005). Overwintering has been documented in that species (Collins and Lewis 1979) and has also been reported in *R. onca* (O'Toole et al. 2023). Overwintering of tadpoles is a characteristic of many temperate anurans (Collins and Lewis 1979; Walsh et al. 2016). Our review of monitoring survey data indicated that overwintering by tadpoles is common in *R. onca*, but the timing of the process and the mechanism that drives it are unstudied in the species. We speculate, however, that tadpoles associated with late season breeding of *R. onca* in fall may often overwinter.

Time to reproductive maturity.—Reproductive maturation in anurans depends on juvenile growth rates and body size rather than age specifically (Jørgensen 1992; Ryser 1996). In some ranid species, males tend to reach sexual maturity at smaller sizes and more quickly than females (Berven 1990; Ryser 1996; Hughes and Meshaka 2018). In field collections of *R. onca,* the smallest identified males were reported at 44 mm, presumably snout-vent length (SVL), and the smallest females at 48.5 and 51 mm (Wright and Wright 1949). Males have been reported to reach reproductive maturity at approximately 42 mm SVL when swollen, pigmented thumb (nuptial) pads appear (Bradford et al. 2004, 2005) and can reach this size during their first year (Bradford et al. 2005; Saumure et al. 2022). Females can also reach adult sizes within a single year, at least under exceptional conditions. At a newly established, artificial pond system, several juvenile frogs released at around 32 mm SVL as part of an initial translocation were recaptured as adults just over 4 mo later, specifically a male at 68 mm and three females ranging from 75–84 mm SVL (Saumure et al. 2022).

Time to reproductive maturity in *R. onca* has been previously described from observations of breeding behavior at newly established translocation sites. Egg masses or young tadpoles have been observed about a year after initial translocations at several newly established populations, indicating that both males and females were capable of breeding in a little less than 1.5 y (Saumure et al. 2022) from when they were oviposited. In our assessment, however, the shortest time required from oviposition of the source individuals to reproduction was just over 1 y (12.2 mo) at a newly established hot spring site. We should emphasize that all these observations were associated with source animals reared in the laboratory through metamorphosis under favorable conditions before release.

Importance to management.—The conservation program for *R. onca* started as an urgent endeavor to improve the status of the species and assess population responses to management actions. The collection of data on breeding biology was subsidiary to those aims, but over time provided the major basis for our understanding. The information gained has directly informed management actions for the species, specifically: (1) the determination of breeding seasonality has improved the efficiency of egg mass collections for headstarting and translocation, and has informed the scheduling of habitat maintenance to avoid conflicts; (2) estimates of egg mass size and viability have been used in the planning of collection quotas; (3) determination of developmental times has informed the scheduling of rearing activities and coordination of releases; (4) the previous determination of optimal temperature ranges for tadpole growth has improved transit times for headstarting; and (5) the understanding of time to reproductive maturation has been used to organize efficacy monitoring, along with governing expectations for the timing of success at translocation sites. These examples demonstrate the contemporary application of life-history information to adaptive management in species conservation.

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National Park Service, Lake Mead National Recreation Area (NPS); U.S. Bureau of Land Management; U.S. Fish and Wildlife Service; Nevada Department of Wildlife (NDOW); and Arizona Game and Fish Department, Arizona Heritage Fund (AGFD). Protocols involving live animals were approved by the Institutional Animal Care and Use Committee, University of Nevada, Las Vegas (IACUC-01186, 01154), and authorized under permits by AGFD (LIC # SP809609), NDOW (LIC # 39750), and NPS (LAKE-2022-SCI-0004, PARA-2023-SCI-0007).

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Rebeca Rivera began her career as a Research Assistant for the School of Life Sciences at University of Nevada, Las Vegas (UNLV), USA, with her duties focusing on implementing conservation research for the Relict Leopard Frog. She received her B.S. and M.S. from UNLV in Biological Sciences, with her thesis work pertaining to informing management strategy for the Relict Leopard Frog. She is interested in life-history characteristics, conservation, and management of natural resources. (Photographed by Brian A. Gillilan).

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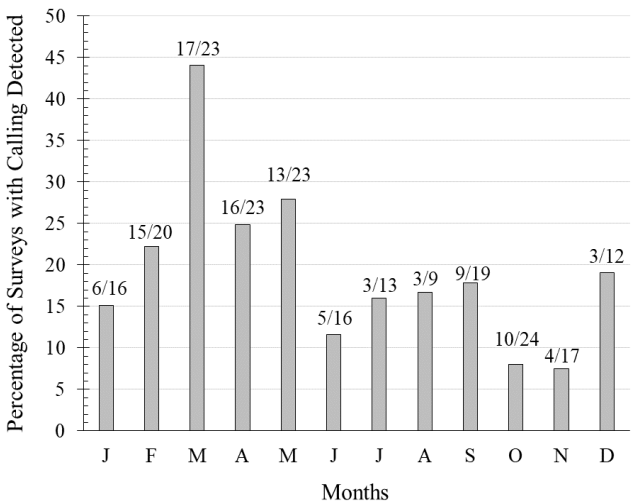
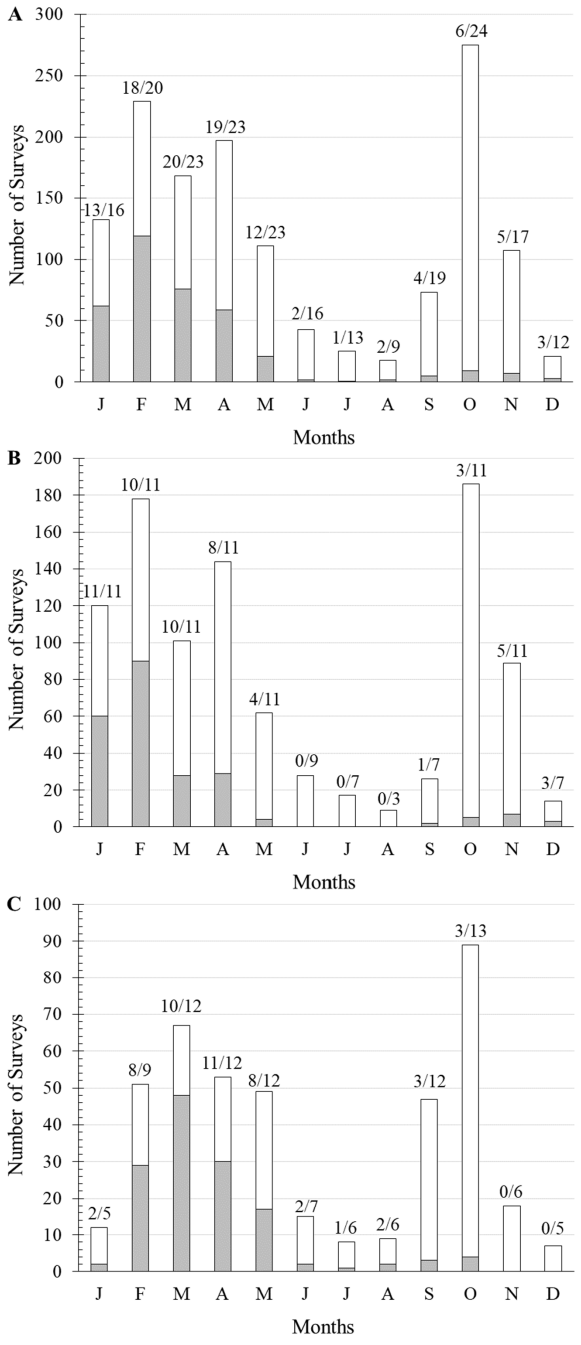
Appendices



Appendix Figure 1. Percentage of surveys with detection of male calling by the Relict Leopard Frog (*Rana onca*) per month from February 2003 through December 2021. Associated with each bar is the accumulated number of sites when calling was heard (numerator) over the accumulated number of sites surveyed (denominator) per month.



Appendix Figure 2. Total number of surveys (bars) for the Relict Leopard Frog (*Rana onca*) conducted per month from February 2003 through December 2021 at (A) all sites, (B) hot springs, and (C) cold-water sites. Gray shading indicates the total number of surveys with at least one egg mass counted. Associated with each bar is the accumulated number of sites surveyed per month with at least one egg mass counted (numerator) over the accumulated number of sites surveyed (denominator).

Appendix Table 1. Days to detection of breeding by the Relict Leopard Frog (*Rana onca*) at translocation sites, determined as the time from initial (earliest) egg mass collections of source animals to first detection of reproduction by these animals. Also provided are the date of first release at a site, life stages of the animals released (T = late-stage tadpoles or early stage metamorphs, J = juvenile frogs), and the life stages of animals at first detection of reproduction (E = egg mass, H = hatchlings, Tb = tadpoles born at site). Numbers of surveys conducted prior to detection of breeding are specified. Goldstrike Canyon and Pupfish Refuge Spring are considered hot springs. An asterisk (*) references the date of detection reported by Saumure et al. (2022).

Site	Earliest Date of Source Egg Collection	Date of First Release	Date of Detection	Days to Detection	Release Stage	Stage Detected	No. of Surveys Prior to Detection
Goldstrike Canyon, Nevada	22 January 2004	9 April 2004	27 January 2005	372	T	E	2
Grapevine Springs, Nevada	11 February 2020	11 April 2020	18 March 2021	402	T, J	Tb	1
Quail Spring, Nevada	23 January 2008	24 April 2008	2 April 2009	436	J	Tb	1
Horse Spring, Nevada	7 February 2012	17 May 2012	26 April 2013	445	T	H	1
Las Vegas Springs Preserve, Nevada	24 January 2018	29 May 2018	25 April 2019*	457	J	Tb	3
Grapevine Spring (lower), Nevada	17 January 2006	30 March 2006	22 April 2007	461	T	Tb	3
Kaolin Spring, Nevada	18 January 2016	26 May 2016	27 April 2017	466	T, J	E	2
Grapevine Spring, Arizona	22 January 2004	5 April 2004	25 August 2005	582	T	E	4
Union Pass Spring, Arizona	21 January 2011	15 April 2011	20 September 2012	609	T, J	E	4
Tassi Spring, Arizona	17 January 2006	24 August 2006	2 October 2007	624	J	Tb	2
Pupfish Refuge Spring, Nevada	27 February 2003	22 October 2003	19 January 2005	693	J	E	4
Bearpaw Poppy Spring, Nevada	7 February 2012	1 May 2012	11 February 2015	1,101	J	E	7
Red Rock Spring, Nevada	22 January 2004	22 April 2005	28 March 2007	1,162	J	E, H	6
Lime Spring, Nevada	30 January 2012	7 June 2012	20 May 2015	1,207	T, J	H	7