# A 10-YEAR STUDY OF NORTHERN LEOPARD FROG (*RANA PIPIENS*) REPRODUCTION IN EAST-CENTRAL NEVADA, USA

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*Abstract.*—The Northern Leopard Frog (*Rana pipiens*) is the most widespread anuran in North America. Despite the expansive distribution, the species has experienced significant declines in the western portions of the USA and Canada. Although once described as the commonest and most widespread frog in Nevada, surveys in 2000–2001 suggested that *R. pipiens* had experienced significant declines statewide. We surveyed seven sites for *R. pipiens* egg masses for 10 y from 2009 through 2018 in east-central Nevada, USA. We documented 1,701 *R. pipiens* egg masses at six of our sites between 14 March and 20 May. We found 87% of the egg masses in April. At four of the six sites, we observed increases in egg mass numbers starting in 2009, which peaked in either 2012 or 2013, followed by subsequent declines. One population of *R. pipiens* was extirpated by 2016 likely due to an 84% decrease in aquatic habitat that resulted in a shallower spring system; however, the other populations maintained smaller numbers, with subsequent increases.

Key Words.-abundance; eggs; extirpation; Great Basin; habitat; Lithobates pipiens; phenology; Spring Valley

### Introduction

Amphibian populations have experienced worldwide declines of an unprecedented and catastrophic magnitude. Some 32% of extant amphibian species are threatened with extinction, and the current extinction rate is estimated to be over 200 times the reported amphibian extinction rate from the fossil record (Stuart et al. 2004; McCallum 2007). Amphibian declines are caused by multiple factors, which can manifest themselves at scales ranging from a single species within a local landscape to multiple species at an international scale (Beebee and Griffiths 2005; Grant et al. 2016, 2020; Muths et al. 2017).

The Northern Leopard Frog (Rana pipiens), following the taxonomic nomenclature of Yuan et al. (2016), is the most widespread anuran in North America (Moore 1949; Rogers and Peacock 2012). Despite its expansive distribution, the species has experienced significant declines in the western portions of the USA and Canada (Gibbs et al. 1971; Corn and Fogleman 1984; Clarkson and Rorabaugh 1989; Rogers and Peacock 2012). Although Linsdale (1940) noted that R. pipiens was the commonest and most widespread frog in Nevada, Hitchcock (2001) documented R. pipiens at only 18.5% (18 of 97) of surveyed historical sites in the state. Most of the remaining extant populations of R. pipiens were in Spring and Lake valleys in eastern Nevada (Hitchcock 2001). Currently, R. pipiens is considered a state-protected species by the Nevada Department of Wildlife and a Sensitive Species in Nevada by the U.S. Bureau of Land Management (BLM). Declines in *R. pipiens* may be the result of one or more factors including: (1) the stochastic effect of population age structure in a short-lived (i.e., 2-5 y) species (Dole 1971; Leclair and Castanet 1987); (2) commercial collection (Gibbs et al. 1971); (3) habitat destruction, fragmentation, or succession (Gibbs et al. 1971; Hine et al. 1981; Gilbert et al. 1994; Leonard et al. 1999; Rorabaugh 2005); (4) introduced predators such as non-native fish and American Bullfrogs, Rana catesbeiana (Corn and Fogleman 1984; Hayes and Jennings 1986; Panik and Barrett 1994; Leonard et al. 1999; Germaine and Hays 2009); (5) emigration and road mortality (Ashley and Robinson 1996; Carr and Fahrig 2001; Bouchard et al. 2009); (6) herbicides, pesticides, and nitrates (Kaplan and Overpeck 1964; Hayes et al. 2003; Relyea and Diecks 2008; Shenoy et al. 2009; Denton and Bernot 2011); (7) disease (Ouellet et al. 2004; Woodhams et al. 2008; Voordouw et al. 2010; Chatfield et al. 2013; Hyman and Collins 2015); and/or (8) environmental conditions such as drought or winterkill during hibernation (Manion and Cory 1952; Merrell and Rodell 1968; Hine et al. 1981; Corn and Fogleman 1984; Corn and Livo 1989).

In 2006–2007, the Southern Nevada Water Authority (SNWA) acquired deeded ranch properties in east-central Nevada. In total, seven ranches were

Copyright © 2024. Aaron M. Ambos All Rights Reserved. acquired that consisted of approximately 9,000 ha of working ranchland and associated water rights, with approximately 95% of these landholdings in Spring Valley. These seven ranches, collectively renamed the Great Basin Ranch, included various combinations of homesteads, cropland, grassland, marshland, meadowland, desert shrubland, springs, and seeps (Welch et al. 2007). Several of these spring systems and associated wet meadows were inhabited by *R. pipiens* (Hitchcock 2001).

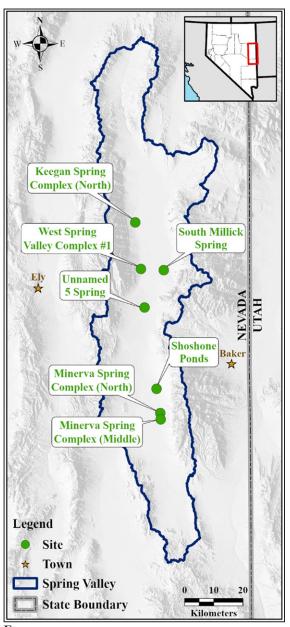
We conducted egg mass surveys to ascertain the presence and relative abundance of adult female R. pipiens at research sites in Spring Valley. Crouch and Paton (2000) noted that egg mass surveys for Wood Frogs (R. sylvatica) were relatively inexpensive and required less time than the repeated visits required by calling surveys. Randall et al. (2014), however, noted that R. pipiens spawning in Alberta, Canada, was asynchronous, occurred over 30 d, and required multiple surveys. Several authors have noted that few, if any, R. pipiens egg masses were missed during surveys because they were quite conspicuous and often deposited in clusters (Merrell 1968; Corn and Livo 1989). Although egg mass surveys require repeated visits to ensure accuracy (Randall et al. 2014), they are not as labor intensive, and are less invasive, than other common survey methods used to determine relative abundance (Heyer et al. 1994).

Our primary goal was to document the relative abundance of *R. pipiens* inhabiting relatively isolated spring systems on ranch properties in Spring Valley. *Rana pipiens* (Fig. 1) has several characteristics that make it an ideal indicator of ecosystem health. Specifically, the species: (1) is amphibious, with



**FIGURE 1.** Adult Northern Leopard Frogs (*Rana pipiens*) in amplexus surrounded by newly deposited (i.e., black and white), developing (i.e., black), and deceased (i.e., white) eggs photographed in Spring Valley, White Pine County, Nevada, USA. (Photographed by Aaron M. Ambos).

life stages that are omnivorous (i.e., larval stage) or carnivorous (i.e., metamorphosed frogs; Hitchcock 2001; Rorabaugh 2005), which places them in both aquatic and terrestrial food webs; (2) is a dispersallimited species (Hilty and Merenlender 2000; Carignan and Villard 2002; Rorabaugh 2005), which is ideal for indicator species because they are unlikely to disperse from, or to, our isolated research sites; and (3) has egg masses that are conspicuous and can be surveyed easily (Merrell 1968; Corn and Livo



**FIGURE 2.** Locations of research sites surveyed for Northern Leopard Frog (*Rana pipiens*) egg masses in Spring Valley, White Pine County, Nevada, USA.

1989; Gilbert et al. 1994). Our specific objectives were to: (1) ascertain which of the selected research sites harbored breeding populations of *R. pipiens*; (2) determine the spatio-temporal distribution of *R. pipiens* egg masses at each site; and (3) describe the intra- and inter-annual breeding phenology of the species.

# MATERIALS AND METHODS

**Research sites.**—Our research sites in Spring Valley were located in White Pine County in east-central Nevada, USA (Fig. 2; Appendix Table).

Spring Valley lies within the Great Basin Desert and is bordered by the Snake Range to the east and the Schell Creek Range to the west, which form closed Hydrographic Basin #184 (Welch et al. 2007). A hydrographic basin is an area where precipitation is collected and is synonymous with the term watershed; closed hydrographic basins have no surface water outlets. Spring Valley has over 300 identifiable springs as well as over 1,000 seeps that flow intermittently in the mountain block and valley floor during periods of above normal winter precipitation (SNWA, unpubl. data). Linsdale (1940) first documented that *Rana pipiens* inhabited Spring Valley in 1930 (Specimen



**FIGURE 3.** Representative egg mass deposition sites of Northern Leopard Frogs (*Rana pipiens*) at (A) Keegan Spring Complex North, (B) Minerva Spring Complex Middle, (C) Minerva Spring Complex North, (D) Shoshone Ponds, (E) Unnamed 5 Spring, and (F) West Spring Valley Complex, in Spring Valley, White Pine County, Nevada, USA.

MVZ 12281 at Museum of Vertebrate Zoology, University of California, Berkeley, California, USA). Welch et al. (2007) and Halford and Plume (2011) described the hydrogeology of Spring Valley at length. The valley floor ranged in elevation from approximately 1,650 to 1,850 m above sea level and was composed of xeric shrublands, with occasional mesic areas. Mesic areas existed primarily along the margins of the alluvial fans in the northwestern and southeastern portions of the valley. Rana pipiens populations inhabited springs, associated creeks, ponds, wetlands, and meadows (Hitchcock 2001; this study). Wetland habitat (approximately 3,237 ha) and meadow habitat (approximately 2,833 ha) each made up < 1% of the hydrographic basin. The SNWA properties encompassed approximately 40% (> 1,618 ha) of the wetland/meadow habitat. Forbis et al. (2007) described major vegetation communities in Spring Valley. The predominant agricultural and ranching activities in Spring Valley were grazing by domestic sheep and/or cattle on both private and public lands, as well as Alfalfa (Medico sativa) production on private lands.

We surveyed seven research sites inhabited by *R. pipiens* in Spring Valley (Fig. 2; Appendix Table). One criticism that can arise with egg mass data is that perceived declines could be the result of shifts in location of egg mass deposition sites. For this reason, we surveyed three wet meadows that were potential breeding habitats adjacent to (i.e., auxiliary sites) our seven primary research sites (henceforth sites).

Keegan Spring Complex North.—The Keegan Spring Complex North site (henceforth Keegan) was part of a larger 280-ha deeded property that included 32 ha of mesic habitat (Fig. 3). The mesic habitat within Keegan and its auxiliary sites was characterized by a series of springs that flowed in a southeasterly direction and fed several wet meadows. In 2011, we observed that *R. pipiens* deposited egg masses at an auxiliary site, a wet meadow approximately 40 m from Keegan. We subsequently included this auxiliary site in annual survey efforts from 2011–2018. Kegan was approximately 3 ha; whereas the auxiliary site was a nearly 8 ha meadow with shallow ephemeral ponds present in spring.

Minerva Spring Complex Middle.—The Minerva Spring Complex Middle site (henceforth Minerva Middle) was part of a larger 1,900 ha deeded property that included over 60 ha of mesic habitat. The original site was composed of two spring channels with a combined 240-m length. These channels, however, were found to have limited breeding habitat and no egg masses were documented in 2009–2014. Consequently, we changed the location of the site in 2015 to an approximately 0.2 ha spring-fed stock pond (Fig. 3), where we had previously observed egg masses in 2011–2013.

Minerva Spring Complex North.—The Minerva Spring Complex North site (henceforth Minerva North) was part of a larger 1,900-ha deeded property that included over 60 ha of mesic habitat. Minerva North was composed of two separate springs, one of which was dammed to form a small pond (Fig. 3), as well as an auxiliary yet separate 140-m channel fed by a third spring. In 2016, 2.5 ha of wet meadow formed adjacent to the site as a result of ranch water diversion activities. We subsequently included this auxiliary site in annual survey efforts.

Shoshone Ponds.—The Shoshone Ponds (henceforth Shoshone) site was on public land managed by the BLM and was part of a larger 500ha Shoshone Ponds Area of Critical Environmental

Site	Springhead Distance (m)	Direction	<i>R. pipiens</i> Distance (m)	<i>R. pipiens</i> Direction	
Keegan Spring Complex North	1,937	S	1,390	Ν	
Minerva Spring Complex Middle	1,891	SSE	150	NNW	
Minerva Spring Complex North	698	SSW	1,480	SSW	
Shoshone Ponds	2,163	NNE	1,230	SSE	
South Millick Spring	564	NW	660	Е	
Unnamed 5 Spring	1,375	NE	1,780	Ν	
West Spring Valley Complex 1	262	Ν	990	S	

**TABLE 1.** Distance and direction from research site springhead to its nearest neighboring springhead of Northern Leopard Frogs (*Rana pipiens*) as well as nearest *R. pipiens* record in Spring Valley, White Pine County, Nevada, USA. Springheads include over 300 known springs in Spring Valley. The *R. pipiens* records include our data and records sourced from Nevada Division of Natural Heritage database.

Concern (ACEC) that included approximately 1 ha of mesic habitat. The 0.1 ha Shoshone site was composed of three man-made ponds fed by thermal groundwater via a pumped well (Fig. 3). The fourth and largest pond was a 0.08-ha stock pond fed by a thermal artesian well, located 357 m north of the refuge ponds.

South Millick Spring.—The South Millick Spring site (henceforth South Millick) was on public land managed by the BLM. The spring system was approximately 4 ha and characterized by a relatively large, deep, spring pool with multiple springheads that flowed into a heavily incised channel that flowed into private property. South Millick began at the springhead of the system and included approximately 0.7 ha of springheads and spring brook on public land.

Unnamed 5 Spring.—The Unnamed 5 Spring site (henceforth Unnamed 5) was part of a larger 80-ha deeded property that included 4 ha of mesic habitat. Unnamed 5 was nearly 0.4 ha in area and consisted of two spring pools that were joined by a broad, shallow channel with some flow (Fig. 3). The auxiliary site was composed of a shallow ditch that ran 300 m and terminated in an approximately 3-ha wet meadow.

West Spring Valley Complex 1.—The West Spring Valley Complex 1 site (henceforth West Spring Valley) was a 190-ha privately-owned ranch to which SNWA was granted access. A series of springs formed approximately 9 ha of mesic habitat. West Spring Valley was approximately 1 ha in area and composed of several springs that flowed into a marsh and pond (Fig. 3).

Egg mass surveys.—We conducted annual egg mass surveys (e.g., Merrell 1968; Corn and Livo

1989; Crouch and Paton 2000; Hyman and Collins 2015) from March through May 2009–2018, a period that covered the annual *R. pipiens* breeding season in Spring Valley. Given the remote locations of our sites, we surveyed two sites (i.e., Minerva N and Unnamed 5) for the presence of egg masses approximately every two weeks starting in March each year. We selected these two sentinel sites across Spring Valley to capture latitudinal and/or elevational variability in the onset of reproduction. Once we documented an egg mass at one of the sentinel sites, we began surveys at all sites. Surveys of sentinel sites began early, while still frozen, or before frogs were active. Surveys continued until egg mass deposition had ceased at all sites.

We conducted egg mass surveys at each site every two weeks. We searched all available aquatic habitat in shallow spring systems and the perimeter, up to approximately 1 m depth, in deeper spring channels and spring pools present at certain sites. We surveyed all available habitat at auxiliary sites. We surveyed each site at least three times each year; surveys continued until we no longer encountered new egg masses. We surveyed auxiliary sites at the same time as the seven main sites; however, some auxiliary sites were either dry or developed during the 10 y of surveys as a result of bank erosion, precipitation, or ranch water diversion activities. Upon encountering an egg mass, or egg mass cluster, we gave it a unique number, recorded the location on a Global Positioning System (GPS) unit (GeoXH, Trimble Incorporated, Sunnyvale, California, USA), and flagged the location to facilitate recognition on subsequent surveys (Hine et al. 1981). We defined egg mass clusters as two or more egg masses within 30 cm of each other.

We classified each egg mass by one of five gross

**TABLE 2.** Egg-mass phenology for the Northern Leopard Frog (*Rana pipiens*) at six research and three auxiliary sites surveyed every two weeks from 6 March to 28 May during 2009–2018 in Spring Valley, White Pine County, Nevada, USA. Dates represent the date *R. pipiens* egg masses were recorded during the surveys; thus, dates do not necessarily represent deposition dates. Sites with asterisks (\*) include data from adjacent auxiliary site.

	Egg Masses								
Site Name	Surveyed (y)	n	Earliest	Latest	% March	% April	% May		
Keegan Spring Complex North*	10	1,361	April 3	May 20	0	97.2	2.8		
Minerva Spring Complex Middle	4	128	March 19	May 7	24.2	65.6	10.2		
Minerva Spring Complex North*	10	86	April 2	May 8	0	95.1	4.9		
Shoshone Ponds	8	43	March 14	May 8	51.2	46.5	2.3		
Unnamed 5 Spring*	10	312	March 26	May 15	25.7	72.1	2.2		
West Spring Valley Complex 1	9	261	March 21	May 20	16.1	81.6	2.3		

developmental stages: (1) spherical ova/embryos age class 1; (2) kidney shaped embryos - age class 2; (3) tailed embryos close to hatching - age class 3; (4) hatching or hatched tadpoles - age class +3/hatched; and (5) embryos that were white or had fungus on egg mass - dead. We used this classification system to determine the relative age of individual egg masses and prevent double counting on subsequent surveys. In addition, the development stage of eggs/embryos permitted us to identify any egg masses that we may have missed during a previous survey. During subsequent surveys, we documented new egg masses (i.e., in a new or existing cluster) in the same fashion. We assumed that annual variability in egg mass numbers was an index of adult female abundance (i.e., the minimum number of adults females present), as there are no data to support multiple clutches in R. pipiens (Corn and Livo 1989; Rorabaugh 2005).

We produced high-resolution maps of our sites using ArcGIS Pro Software version 2.4 (Esri, Redlands, California, USA) from imagery collected specifically for SNWA in 2007, 2013, or 2018 at either a 15.2-cm or 7.6-cm resolution (Supplemental Information Figs. S1-S7). We also used ArcGIS Pro to calculate indices of population isolation; the straightline terrestrial distance and compass direction from our sites to the nearest neighbor springhead/spring pool, as well as the nearest R. pipiens record beyond our site spring systems. The nearest R. pipiens locations were sourced from our data, as well as those documented and archived by others (Nevada Division of Natural Heritage. Available from https:// heritage.nv.gov [Accessed 19 October 2023]). In addition, we mapped physical habitat data (i.e., total aquatic area, channels, pools) periodically at each site with a Trimble GeoXH GPS unit.

## RESULTS

Research sites .- Per our first objective, we

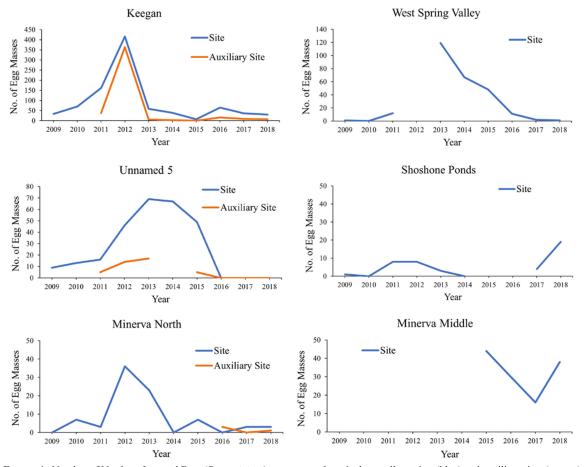
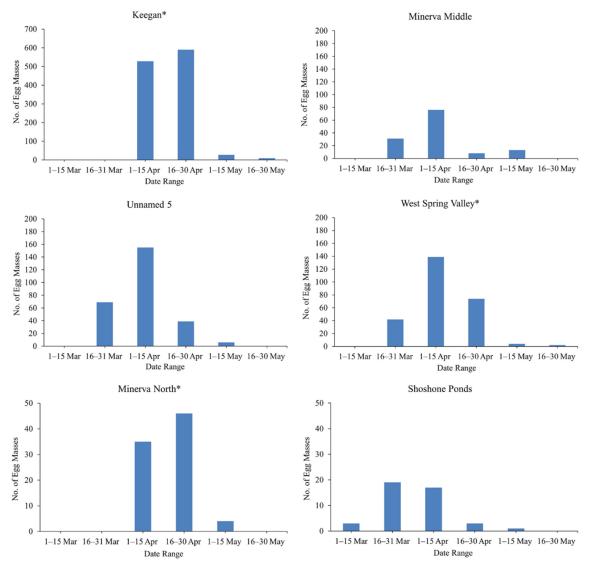


FIGURE 4. Number of Northern Leopard Frog (*Rana pipiens*) egg masses deposited annually at sites (blue) and auxiliary sites (orange) in Spring Valley, White Pine County, Nevada, USA.



**FIGURE 5**. Seasonal distribution of Northern Leopard Frog (*Rana pipiens*) egg masses at six research and three auxiliary sites over 4-10 y during 2009–2018 in Spring Valley, White Pine County, Nevada, USA. An asterisk (\*) indicates egg mass includes data from adjacent auxiliary sites. Month abbreviations are Mar = March and Apr = April.

documented the presence of *R. pipiens* egg masses at six of our seven sites. We did not document any egg masses at South Millick, despite the presence of numerous adult and juvenile *R. pipiens*. Consequently, the summary statistics presented herein are for six sites.

We determined the distance and direction from the springheads at our research sites to the nearest neighboring springhead, as well as the nearest *R. pipiens* record in Spring Valley (Table 1). The mean distance and range to the nearest neighboring springhead was 1,270 m (range from 262–2,163 m); whereas the nearest distance and range to a *R. pipiens* record was 1,097 m (range from 150–1,780 m). We did not have any instances of egg masses that we missed, based on the developmental stages of embryos on subsequent surveys. Except for South Millick, we never documented tadpoles where egg masses had not been previously detected. Previously marked clutches were relocated nearly 100% of the time, except for a few instances where egg mass flagging remained but no trace of the egg mass documented two weeks before could be found.

*Spatio-temporal distribution of egg masses.*—Per our second objective, we found 1,701 *R. pipiens* egg masses at six sites during our 10 y of surveys (Fig. 4; Supplemental Information Table S1). We documented

an additional 490 R. pipiens egg masses at auxiliary sites composed of wet meadows and wetlands (Fig. 4). We detected egg masses at the research and auxiliary sites from March 14 to May 20 during 10 y of surveys (Table 2). In addition, we mapped the distribution of egg masses, or absence thereof, at the research and auxiliary sites (Supplemental Information Figs. S1-S7). We observed that egg mass numbers in Spring Valley peaked in 2012 (e.g., Keegan, Minerva North) or 2013 (e.g., Unnamed 5, West Spring Valley), followed by precipitous declines. For instance, at Keegan we documented a 12-fold increase, which peaked at 416 egg masses, over the first 4 y of our surveys (Fig. 4). Moreover, we identified the following interesting observations or anomalies at three of our sites:

South Millick Spring.—Although we surveyed the South Millick site with the same personnel and diligence as all the other sites, we observed no egg masses from 2009–2015. Although we are reporting negative data for South Millick, we are nonetheless including a map that delineates the site (Supplemental Information Fig. S5). Interestingly, we observed numerous adults and juveniles during our egg mass surveys every year. We eventually observed a pair of adults in amplexus in a downstream pond 700 m beyond our site on private land. In August 2012, we captured several advanced stage tadpoles in this same pond. After 7 y of not observing any egg masses during surveys, we did not conduct any additional surveys in 2016–2018 at South Millick.

Unnamed 5 Spring / West Spring Valley.—We noted that the absence of egg masses at Unnamed 5, which began in 2016 (Fig. 4), coincided with the apparent disappearance of R. *pipiens* from the system. In 2016 and 2017, we conducted several additional

surveys, including nocturnal surveys in summer, to determine if R. pipiens had been extirpated. We did not observe any frogs or evidence thereof (i.e., tadpoles, egg masses, calling) at the site. We documented substantial reductions in aquatic habitat at Unnamed 5 from 2009-2018. For instance, the southern spring pool, where most of the egg masses were deposited (Supplemental Information Fig. S6), decreased from a single 829-m<sup>2</sup> pool in 2009 to two small spring pools totaling 131 m<sup>2</sup> in 2018, an 84% decrease in available aquatic habitat. We did not survey West Spring Valley in 2012 (Fig. 4) because the number of egg masses encountered at Keegan (i.e., over 700 egg masses including the auxiliary site; Supplemental Information Table S1) overwhelmed staff resources.

**Phenology.**—We documented 87.1% of egg masses in April, followed by 9.6% in March, and 3.3% in May (Table 2; Fig. 5). Only Shoshone Ponds had the majority (51.2%) of egg masses documented in March. The onset of reproduction at our most northern sites (i.e., Keegan, Unnamed 5, West Spring Valley) was in early to mid-April, whereas sites further south in Spring Valley (i.e., Minerva Middle, Shoshone Ponds) began in late March (Table 3). The exception was Minerva North, with a mean start date in mid-April.

### DISCUSSION

**Research sites.**—We determined that all but two sites encompassed suitable egg deposition habitat: the exceptions were the initial Minerva Middle and South Millick sites. Although frogs were present, these two sites were channelized and had little to

**TABLE 3.** Annual variability in reproductive periods for Northern Leopard Frog (*Rana pipiens*) populations at six research and three auxiliary sites in Spring Valley, White Pine County, Nevada, USA. Abbreviations are SD = standard deviation, Apr = April, Mar = March. Sites with asterisks (\*) include data from adjacent auxiliary site.

Site Name	Reproductive Period (d)			Start Date			End Date		
	Mean	SD	Range	Mean	SD (d)	Range	Mean	SD (d)	Range
Keegan Spring Complex North*	20	8.8	15–44	11 Apr	5.0	3 Apr–19 Apr	1 May	9.3	24 Apr–20 May
Minerva Spring Complex Middle	33	10.0	18–42	22 Mar	3.1	19 Mar–27 Mar	23 Apr	11.4	6 Apr–7 May
Minerva Spring Complex North*	13	11.4	1–29	14 Apr	9.0	2 Apr–2 May	26 Apr	8.0	11 Apr–8 May
Shoshone Ponds	13	16.7	1-42	30 Mar	11.4	14 Mar–11 Apr	14 Apr	20.2	20 Mar–8 May
Unnamed 5 Spring*	27	14.1	1-44	6 Apr	7.6	26 Mar-19 Apr	2 May	9.2	19 Apr–15 May
West Spring Valley Complex 1	21	21.4	1–57	4 Apr	13.1	21 Mar–30 Apr	29 Apr	19.0	21 Mar–20 May

no suitable riparian marsh or wet meadow habitat (Gilbert et al. 1994) beyond the flowing spring pond and stream. A third site, Shoshone Ponds, had relatively little reproduction and was slated for major habitat modifications to create better habitat for the endangered Pahrump Poolfish (*Empetrichthys latos*), which was completed in 2021. Consequently, we: (1) implemented a new site at Minerva Middle; (2) proposed visual encounter surveys (Crump and Scott 1994; Germaine and Hays 2009; Hyman and Collins 2015) as an alternative method of assessing *R. pipiens* abundance at South Millick in the future; and (3) dropped Shoshone Ponds as a site because of anticipated major habitat changes.

Abundance and distribution.-We also determined the spatio-temporal distribution of R. pipiens egg masses at each site. We observed that egg mass numbers in Spring Valley peaked in 2012 or 2013 but then declined precipitously. At Keegan, we documented one of the highest numbers of R. pipiens egg masses (n = 416) reported from a single site anywhere, without including the egg masses deposited at the auxiliary site. Gilbert et al. (1994) used data from spring 1986 to estimate 271 R. pipiens egg masses in the floodplain of the Richelieu River in Québec, Canada. Although Unnamed 5 and West Spring Valley had comparatively fewer egg masses than the Québec study, we documented relatively high egg mass numbers at both sites. Merrell (1968) documented a range of 23-56 egg masses at six sites in Minnesota, USA. Corn and Livo (1989) documented a range of 2-33 egg masses at their sites in Colorado and Wyoming, USA. Although we substituted Minerva Middle after the peak reproductive years of 2012-2013, egg mass numbers from 2015-2018 were comparable to the aforementioned studies.

Our research sites were subsamples of the greater Keegan, Minerva North, and Unnamed 5 spring systems (Supplemental Information Figs. S1, S3, S6). We surveyed breeding habitats at auxiliary sites primarily to determine if decreases in egg mass numbers at our sites could be explained by a shift in egg mass deposition to auxiliary sites. Auxiliary sites, however, followed the same pattern of increase and decline in egg mass numbers as our sites. Consequently, we concluded the declines in egg mass numbers at our sites were not simply artifacts of changes in egg mass deposition locations over time.

Phenology.-As ectothermic poikilotherms, the onset of reproduction in R. pipiens is correlated to ambient temperature, which is itself inversely correlated with increasing elevation and/or latitude (Gilbert et al. 1994). The egg mass dates we recorded at our mid-elevation (i.e., 1,676-1,828 m) sites in Nevada corroborate this hypothesis and were similar to those reported previously at a similar elevation. For instance, Corn and Livo (1989) documented egg masses at sites in Colorado and Wyoming in 1978-1980, 1982, and 1987. They found that egg masses were deposited at mid-elevation (i.e., 1,555-1,570 m) sites in Colorado from mid-March to mid-April, whereas egg masses were deposited starting in midto late May at higher elevation (i.e., 2,036–2,520 m) sites in Colorado and Wyoming. Moreover, Gilbert et al. (1994) documented that most R. pipiens egg masses at a low-elevation (i.e., 29-32 m) but high latitude site in Québec, Canada were deposited 15-25 April. Interestingly, 87% of the egg masses we observed in Spring Valley were also recorded in April. As we conducted our surveys every two weeks, we note that egg masses documented in early April could have been deposited in late March. Similarly, the egg masses we documented in early May could have been deposited in late April. Shoshone was unusual in that 51% of egg masses we documented were in March. This is apparently because the Shoshone Ponds site was fed by a thermal groundwater system. Goodchild (2016) recorded annual groundwater inflow temperatures ranging between 15°-20° C, with stock-pond bottom temperatures increasing to 10° C in February. Thus, the onset of reproductive activities could begin earlier because of the warmer water. None of our other sites were thermal spring systems.

Although amphibian populations are well known for phenological variability in population size, the proximate cause(s) are not well understood. Although formal assessments of disease were beyond the scope of our egg mass surveys, we did not observe any *R. pipiens* die-offs at any of our sites. Because our intensive egg mass surveys occurred only from March through May, we cannot rule out the possibility that disease played a role in annual fluctuations. Occasional visits by SNWA biologists, hydrologists, and ranch staff throughout the remainder of the year did not detect any *R. pipiens* die-offs. Furthermore, we did not observe any signs of increased predation or introduced predators (e.g., American Bullfrogs, *Rana catesbeiana*) during our surveys.

Another potential cause of the annual variation is environmental conditions. Shallow aquatic

habitats can expose hibernating R. pipiens to freezing temperatures or hypoxia. Although we have no evidence of mass mortalities of R. pipiens during hibernation at our monitoring and adjacent sites, such events have been documented previously in ranid frogs (Bradford 1983). Manion and Cory (1952) noted that winterkill of R. pipiens was extensive in the shallow (i.e., 10-15 cm) half of an Indiana, USA, pond in two consecutive years. Larger, deeper ponds in the area with healthy populations of R. pipiens had no mass mortalities. Winterkill has also been documented in Minnesota, with anywhere from 15 to 455 dead R. pipiens documented (Merrell and Rodell 1968). Hatch and Kroft (2022) observed evidence of severe winterkill in R. luteiventris at four of their five lotic sites in central Nevada, due west of our sites, with estimated population declines of 66-86.5%. They postulated that the low levels of oxygen in groundwater (Soulsby et al. 2009) combined with snow and ice cover prevented diffusion of oxygen into the water.

We documented an unprecedented 84% decrease in available aquatic habitat at Unnamed 5 during our 10-y study, which resulted in a shallower spring As noted previously, shallow aquatic system. habitats can expose hibernating R. pipiens to freezing temperatures and hypoxia. Layne and Lee (1995) noted that *R. pipiens* is not a freeze-tolerant species. Gilbert et al. (1994), however, also noted that R. pipiens avoided depositing egg masses in habitats dominated by large-leaved emergent aquatic plants. Once Unnamed 5 was fenced by SNWA to manage grazing in 2014, emergent aquatic plant densities, although not large-leaved, increased substantially as a result of decreased grazing and/or decreased water depth (unpubl. data). Suitable breeding habitat remained at Unnamed 5, however, albeit dramatically reduced in extent. Thus, although not likely the primary cause of the extirpation of R. pipiens at Unnamed 5, such changes in R. pipiens reproduction sites may have contributed.

Given the relatively isolated, xeric nature of the landscapes surrounding the spring systems we surveyed, emigration is likely sporadic. We did not observe any road mortality. Although *R. pipiens* are known to disperse up to 2.1 km along riparian corridors, the maximum distance they are known to traverse overland is 400 m (Seburn et al. 1997). Moreover, Smith and Jackson (1931) documented that *R. pipiens* lose about 51% of their water content in about 29–32 h when maintained in dry glass jars at an average temperature of 25° C and average relative humidity of 25%. Mazerolle and Desrochers (2005) noted that dry substrates were a barrier to movements for *R. pipiens* in Québec. The terrestrial distances (mean = 1,270 m; range from 262–2,163 m) and existing high desert, xeric conditions between isolated spring systems in Spring Valley is likely a barrier to successful emigration or immigration in all but the wettest years. Distances between occupied spring systems may be greatly reduced during unusually wet years, as mountain snowmelt and spring outflows connect in the valley bottom and flood playas. Extensive water management (e.g., flood irrigation, ditches, water diversions) by ranching operations could also create potential travel corridors.

Our research provides information on R. pipiens populations inhabiting the high desert of the Great Basin. Although only one population was extirpated during the 10-y study period, stochastic events driven by environmental conditions are likely to increase given the projected impacts of climate change on precipitation, specifically snowpack and resultant droughts in the southwestern U.S. (Fyfe et al. 2017; Musselman et al. 2017; Mote et al. 2018; Williams et al. 2020). Opportunities for natural recolonization by R. pipiens at isolated spring systems are limited. Consequently, opportunities for landowner habitat modifications and translocations should be considered. We suggest that adaptive management strategies could include enhancing water availability, such as: (1) introducing supplemental water to an area to enhance natural spring discharge; (2) directly supplementing water into an aquatic habitat; (3) reducing diversions that remove water from an aquatic habitat, and (4) connecting aquatic areas through irrigation channels. Other management strategies may include increasing pool depths by digging down or creating berms. Such actions could be implemented by landowners and government agencies to reduce the effects of water limitations or extreme temperatures on R. pipiens populations.

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Ambos et al.-Northern Leopard Frog reproduction.



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**APPENDIX TABLE**. Location and dates for egg-mass sampling of Northern Leopard Frog (*Rana pipiens*) at seven research and auxiliary sites in Spring Valley, White Pine County, Nevada, USA. Latitude and Longitude are from North American Datum (NAD) 83. Owner abbreviations are SNWA = Southern Nevada Water Authority and BLM = U.S. Bureau of Land Management. The abbreviation NA = not applicable, as there were no auxiliary sites to be surveyed.

	Latitude	Longitude			Site	Auxiliary Site		
Site Name	°North	°West	Owner	Surveyed (y)	Egg Masses (y)	Surveyed (y)	Egg Masses (y)	
Keegan Spring Complex North	39°27'01"	114°30'07"	SNWA	2009–2018	2009–2018	2011–2018	2011–2014; 2016–2018	
Minerva Spring Complex Middle	38°50'30"	114°24'10"	SNWA	2015-2018	2015-2018	$NA^3$	NA	
Minerva Spring Complex North	38°51'40"	114°24'16"	SNWA	2009-2018	2010–2013; 2015; 2017–2018	2016-2018	2016; 2018	
Shoshone Ponds	38°56'10"	114.25'08"	BLM	2009–2014; 2017–2018	2009; 2011–2013; 2017–2018	NA	NA	
South Millick Spring	39°18'10"	114°23'21"	BLM	2009-2015	-	NA	NA	
Unnamed 5 Spring	39°11'15"	114°27'56"	SNWA	2009-2018	2009-2015	2009; 2011–2013; 2015–2018	2009; 2011–2013; 2015	
West Spring Valley Complex 1	39°18'23"	114°28'47"	Private	2009–2011; 2013–2018	2009; 2011; 2013–2018	NA	NA	