DCP PROJECT: Relict Leopard Frog Conservation Planning and Implementation (Clark County Desert Conservation Program)

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EXECUTIVE SUMMARY

This project was intended to provide conservation efforts towards the persistence of historical and established populations of the relict leopard frog (*Rana onca = Lithobates onca*), and towards the establishment of additional populations. The project was initiated in 2016 with conservation actions extended through 2019. The species is managed under a conservation agreement that specifies a voluntary conservation team consisting of federal and state agency representatives, with participation by personnel from local entities (Conservation Team). Conservation actions were implemented as specified in a conservation agreement and strategy (CAS) that was renewed during the first year of this project period (RLFCT 2016). Field and laboratory efforts under this project included: population monitoring, headstarting and translocation actions, actions to maintain important breeding habitats, and facilitation of research projects. Other actions included the coordination of Conservation Team meetings and reporting, and assistance with the writing and editing of the renewed CAS. The following is a summary of accomplishments during the course of this project:

- Completed annual monitoring using nocturnal visual encounter surveys each spring and fall at all historical and translocation sites occupied by *R. onca* (current number of sites totals 21).
- Completed diurnal visual encounter surveys each spring at most occupied sites (with the general exception of one site) to assess breeding activity and to search for egg masses or tadpoles for headstarting (rearing) efforts.
- Conducted field assessments at 14 unoccupied springs for their potential as translocation sites; one site is expected to receive frogs in 2020.
- Completed four seasons of headstarting and translocations, releasing 3347 frogs and tadpoles to a total of 9 different sites, including augmentation of historical sites within the Northshore springs complex. Two sites were established during the project period, including an artificial pond system at the Las Vegas Springs Preserve.
- Provided 487 frogs from the headstarting program to the University of Nevada, Las Vegas (UNLV) for research into the effects of the pathogenic amphibian chytrid fungus; 233 of these frogs are expected to be returned to the wild in 2020. Provided 19 juvenile frogs to the Las Vegas Springs Preserve as part of their public educational display. I
- The overall count of frogs (abundance) increased by > 15% since project initiation (over 2015 count).

- Conducted monitoring or assisted with targeted field sampling associated with research on the pathogenic amphibian chytrid fungus at 9 sites.
- Conducted actions at 9 sites, often repeatedly, to maintain important habitat features or to assisted federal agencies with habitat maintenance and planning.
- Provided assistance on several research projects associate with *R. onca* conservation.
- Coordinated Relict Leopard Frog Conservation Team meetings and reporting.
- Assisted with writing of the 2016 Conservation Agreement, and Conservation Assessment and Strategy for the species.

INTRODUCTION

BACKGROUND – The relict leopard frog (*Rana onca* = *Lithobates onca;* see Jaeger et al. 2001) historically occupied springs and wetlands within the Virgin, Muddy, and Colorado River drainages from southwestern Utah to Black Canyon in Nevada and Arizona (Bradford et al. 2004). By the latter part of the 20th century, however, that historical range had been greatly reduced, with populations remaining in only two general areas within Lake Mead National Recreation Area (LMNRA) in southern Nevada (Bradford et al. 2004). Intensive searches have not found any other extant, historical populations outside of the areas occupied (Blomquist et al. 2003, Bradford et al. 2004, Jaeger 2010, Oláh-Hemmings et al. 2010).

In 2002, the U.S. Fish and Wildlife Service (USFWS) was petitioned to list the species under the Endangered Species Act (ESA), following research that showed only 1100 adult frogs existed in the early 2000s (Bradford et al. 2004). While the USFWS considered listing as warranted, they precluded listing at that time. Prior to the petition, a voluntary team of local, state, and federal personnel called the Relict Leopard Frog Conservation Team (referred to herein as the Conservation Team) had begun conservation actions for the species and had initiated the writing of a conservation agreement, assessment and strategy (CAS). This guiding document was officially entered into by state and federal agencies in 2005 (RLFCT 2005). More recently, as part of a broader court-approved settlement, the USFWS agreed to evaluate *R. onca* for actions under the ESA. In 2016, the agency conducted a species status assessment to inform the listing decision, while concurrently the Conservation Team renewed the CAS (RLFCT 2016). Later that year, the USFWS concluded that listing was not warranted. Assistance with the writing of the CAS was a major action conducted under the current project (see acknowledgments in the 2016 CAS), as was the coordination of Conservation Team meetings and reporting.

Conservation actions for *R. onca* are managed by the Conservation Team and prescribed under the CAS (RLFCT 2016). The document was developed with the intent of providing implementation of effective conservation actions, as well as providing reasonable certainty that the actions would be conducted. Management actions for *R. onca* have emphasized translocations, associated with headstarting of animals collected as eggs from the wild. Populations have been established at numerous sites in southern Nevada and northwestern Arizona, including two sites during the current project period. Several of these translocated populations now comprise the largest populations, and are responsible for the modest increase in the overall population size of the species. Other field and laboratory efforts under the current project included: intensive population monitoring, habitat maintenance of important breeding pools, and monitoring of the amphibian fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*). The pathogen *Bd* causes disease in *R. onca* (Waddle et al. 2019) and has been detected in other amphibians within the region (Jaeger et al. 2017). Several research projects were facilitated under the current project, with emphasis on studies to understand of the impact of *Bd* on *R. onca*.

The project summarized herein was intended to provide conservation efforts towards the persistence of historical and established populations of *R. onca*, and towards the establishment of additional populations. The project continued efforts funded by the Desert Conservation Program in 2005, 2007, 2009, and 2011. The current project (Clark County Multiple Species Habitat Conservation Plan; project number 2015-UNLV-1550A) was implemented by the University of Nevada, Las Vegas (UNLV) in June 2016. For

ease of reporting, this report includes data from throughout 2016. Actions under this project were extended through December 2019 (at no-cost to Clark County). This document represents the final summary report for efforts associated with this project, although more detailed annual reports were submitted to the County. The reporting format used in the annual reports was stipulated by the Conservation Team and this final summary report generally follows that format.

GOAL – Provide conservation efforts towards the persistence of historical and established populations of *R. onca*, and towards the establishment of additional populations.

OBJECTIVES – The main objectives were as follows:

1. Monitor existing historical populations to assess persistence and trends.
2. Monitor experimental populations to evaluate the success of translocations.
3. Identify management actions to improve habitat conditions or mitigate negative habitat changes, and implement small-scale habitat management actions or coordinate actions by land managers.
4. Conduct a headstarting actions to raise late-stage tadpoles or small frogs from eggs collected in the wild for translocation.
5. Conduct translocations to establish populations at new sites or augment existing populations, and coordinate efforts to identify and establish new sites for translocations.
6. Assist in research efforts in support of management objectives.
7. Facilitate renewal of the Conservation Agreement, and Conservation Assessment and Strategy for the species.
8. Coordinate semiannual meetings, and function as the primary editor of work plans and reports by the Conservation Team.

METHODS

COORDINATING CONSERVATION TEAM MEETINGS AND REPORTING

Semiannual meetings for the Conservation Team were conducted in May or June and again in December to facilitate planning, adaptive management, and reporting of management actions for the species across multiple agencies. A formal structure to the meetings was maintained to facilitate group discussions on management and information dissemination. During meetings, minutes were recorded and eventually disseminated to the team. Prior to each meeting, a written report summarizing field and laboratory data was developed by UNLV personnel and provided to the team. For the meeting in spring, the report summarized data collected during the spring season (actions starting in January), and for the winter meeting, the report was updated to include the entire year. Prior to the winter meetings, written materials were solicited from team members describing conservation actions implemented across the various agencies and entities. The written materials were then compiled and edited to produce the team's Annual Work Plan Report. During the winter meetings, new work plans were developed for the coming year.

FIELD AND LABORATORY METHODS

Field and laboratory methods implemented in this project were specified by the Conservation Team in the Relict Leopard Frog Protocol and Techniques Manual originally included with the first CAS (RLFCT 2005). The protocols and techniques detail the various procedures used for monitoring populations and for collecting, rearing, transporting, and releasing frogs and tadpoles associated with translocation. These methods are summarized below.

Population Monitoring Surveys – In general, visual encounter surveys (Crump and Scott 1994) were used to monitor all sites known to contain *R. onca*. Survey crews consisted of two or more individuals headed by biologists with extensive experience in *R. onca* monitoring. Most sites were linear in nature (streams or pond shorelines) and entire systems were surveyed. At some sites, hazardous terrain (steep rocky cliffs) limited access, and at these sites surveys covered designated stretches of area with predetermined starting or stopping points.

Diurnal surveys were generally conducted from late January through March to document breeding activities (presence of egg masses and tadpoles) during the prime breeding period for the species. Nocturnal surveys during the spring (generally March – early June) and again in fall (generally mid-September – mid November) were used to assess the relative abundance of frogs (both adults and juveniles). At most locations, these frogs are more readily seen at night. Observations of young frogs were interpreted as evidence of 'natural' recruitment into the adult population, except where these animals could have resulted from recent releases associated with translocation and augmentation efforts. To minimize disturbance, frogs were generally not captured during surveys, and only frogs that were obviously quite young (including recently metamorphic individuals) were recorded as juveniles. This resulted in a very conservative estimate of recruitment, in that small frogs that were often likely young-of-year were counted as adults. Tadpole counts were often roughly conducted when large numbers were present.

Disease Monitoring – Monitoring for the pathogen *Bd* occurred at sites occupied by *R. onca*, where *Bd* was present. Other sites were also targeted for sampling in consultation with the Conservation Team, including sites being considered for translocations where only other amphibian species were present. When sampling for *Bd*, a large number of nondestructive skin samples are needed for statistical assurance (approaching 40 individuals), but finding this number of frogs was not always possible. Sampling occurred at night, when seasonal ambient temperatures were low and likely favored the growth of the fungus. Field and laboratory protocols followed those described by Jaeger et al. (2017), with each frog systematically brushed with a sterile swab to sample skin cells. A standard laboratory assay using quantitative real-time PCR was used to detect *Bd*. Frogs were released unharmed at point of capture.

Assistance with Research Efforts – Assistance was afforded to three independent research studies being conducted on *R. onca* by UNLV with funding from other sources. In 2018, assistance was provided with field efforts to collect tissue samples from *R. onca* at 15 sites for conservation genetic assessment. Sampling events often occurred in concert with population monitoring surveys. The research project was funded by the National Park Service (NPS) with the intent to fill-in critical information gaps on the genetic diversity of existing historical populations and on the success of translocations to 'transfer' the existing genetic diversity to new populations.

In fall 2019, field assistance was provided with mark-recapture surveys to estimate populations at two sites in Black Canyon (Boy Scout Canyon and Bighorn Sheep Spring). The longer-term project intends to estimate populations at several sites, as part of an effort to develop a recent estimate of overall population size for the species. Funding for the overall effort was provided by Nevada Department of Wildlife (NDOW). At the two sites targeted in 2019, multiple mark-recapture surveys were conducted, and the observations of frogs recorded during those surveys were consistent with the visual encounter approach. Therefore, the data were provided as counts in the tables below.

UNLV has also been conducting independent research to assess, and potentially mitigate, impacts of the emergent amphibian pathogenic fungus *Bd* on *R. onca*. This fungus causes the amphibian disease chytridiomycosis which has been linked to population declines and extinctions. Funding for this research has been provided by the USFWS and Bureau of Land Management (BLM) under different projects (see examples: Jaeger et al. 2017, Waddle et al. 2019). The main contribution from the current project (reported herein) was the generation of headstarted frogs provided for these research efforts. Additional diurnal surveys at Northshore and Black Canyon sites were required to collect eggs from numerous egg masses to increase the genetic diversity of the resulting animals provided for research. Overall, these projects received 487 juvenile frogs and tadpoles during the project period; 233 of the frogs are part of a current vaccine study and are intended for release back to the wild in 2020.

Translocations – The diurnal surveys in spring were used to collect eggs for headstarting. Collected eggs were transported to laboratory facilities where they were grown to late-stage tadpoles or juvenile frogs, and processed for release. During 2016, a facility at Lake Mead National Recreation Area maintained by the National Park Service (NPS) was used for most activities, with some tadpoles grown-out in raceways at the Lake Mead State Fish Hatchery maintained by NDOW. After 2016, all headstarting activities were moved to the NDOW facility. Once animals had grown to late-stage tadpoles or juvenile frogs, they were released to targeted sites. The rough number of animals to be headstarted, the source sites for collections,

along with the sites targeted for augmentation or translocation were annually determined by the Conservation Team.

Frogs and tadpoles for translocation were raised in quarantine. Prior to release, a health assessment was conducted that consisted of an examination of alertness, body position, and any obvious abnormalities. Weight and length was measured on a random sample consisting of 10% of the animals. Animals were also prophylactically treated with two antifungal solutions following an established protocol. At each stage of treatment, any animal showing signs of distress was returned to an isolation tank for assessment, potential treatment and later release. No animals were released that showed signs of disease, physical abnormalities, lacked thriftiness, or came from a group (animals maintained in the same tank or raceway) where there was even a potential for contagious disease within 30 days of release.

Potential Translocation Sites – Efforts to identify and evaluate potential translocation sites remain critical to the success of the management strategy for *R. onca*. In general, identifying potential sites along with compliance activities depended largely on land and resource agencies. Potential sites were visited in coordination with agency personnel to provide expertise on the habitat needs of *R. onca.* Promising sites were visited more than once to evaluate differences in seasonal conditions. At each site, a visual assessment of surface water flow, emergent vegetation, amphibian presence, other conditions and threats was conducted and a written summary provided to the Conservation Team in the semiannual reports. For most sites, the reports included reference photographs. Information or other assistance was provided to agency personnel upon request to meet compliance requirements.

RESULTS AND DISCUSSION

MONITORING OF HISTORICAL SITES IN BLACK CANYON

Bighorn Sheep Spring, NV – This site has maintained a relatively small population since negative changes in habitat resulting from a large storm event in October 2006. Habitat never fully recovered and surface water appears to have continued to decline (down to ~ 217 m paced in 2018). Destabilized gravels tend to fill in pools that form in the main stream and pooled breeding habitat remains very limited. Nevertheless, over the course of the project, all life stages of *R. onca* were observed at this site, indicating some successful reproduction and recruitment.

In early 2018, two off-channel pools were restored, and breeding and recruitment were subsequently noted in association with these pools. In recent years, counts between seasons have varied by as much as two-fold, but the high counts each year have been relatively stable over the project period and may show a slight increase, probably reflecting recruitment from the restored pools (Table 1). In fall 2019, mark-recapture efforts were conducted at this site to estimate population size under funding from NDOW; observations from the mark-recapture efforts were reported herein as visual encounter results.

Table 1. Summary of *Rana onca* observed at Bighorn Sheep Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	01/20	13.4	0	1	153	3
	Nocturnal	03/30	18.0	16	0	4	1
	Nocturnal	10/18	23.9	29	6	0	0
2017	Diurnal	02/09	25.7	0	0	404	6
	Nocturnal	04/06	24.1	34	1	3	5
	Nocturnal	10/11	24.3	12	2	0	0
2018	Diurnal	01/25	13.2	3	0	0	0
	Diurnal	02/02	14.2	1	0	0	5
	Nocturnal	04/24	24.2	34	5	5	5
	Nocturnal	10/09	22.2	21	3	0	1
2019	Diurnal	01/25	16.4	0	0	0	0
	Nocturnal	04/24	26.4	54	0	14	2
	Nocturnal	10/07	23.0	8	5	9	0
	Nocturnal*	10/07	23.0	7	6	9	0
	Nocturnal*	10/11	15.4	5	6	0	0
	Nocturnal*	10/16	22.0	13	8	7	0

* Counts from mark-recapture efforts.

Boy Scout Canyon Spring, NV – *Rana onca* at this site tend to be associated with small off-channel pools that contain cooler water than the main thermal stream. During the project period, all egg masses and tadpoles, along with most of the juvenile and adult frogs observed were associated with only two side

areas in the upper section of the canyon that contained such pools. These observations point to the importance of the few small, cool water breeding sites in this canyon.

High counts during the project period indicate a relatively small but stable population (Table 2). Egg masses, tadpoles, juvenile frogs, and small adults were often observed providing evidence of regular reproduction and recruitment. In 2018, toe-tip samples were taken from 28 frogs as part of a NPS funded conservation genetic assessment being conducted by UNLV. This sampling was used to opportunistically estimate the population via mark-recapture. The population was estimated at 71 frogs (54–88, 95% C.I.). In fall 2019, mark-recapture efforts were also conducted at this site to estimate population size under funding from NDOW; observations from the mark-recapture efforts were reported herein as visual encounter results.

Table 2. Summary of *Rana onca* observed at Boy Scout Canyon during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	01/27	18.3	0	0	31	3
	Nocturnal	03/30	21.0	26	1	58	0
	Nocturnal	10/11	28.5	28	0	0	0
	Nocturnal	11/29	13.8	16	3	0	0
2017	Diurnal	02/09	19.3	1	0	214	1
	Nocturnal	03/28	22.0	30	1	0	0
	Nocturnal	10/09	23.9	22	0	0	0
2018	Diurnal	02/02	16.2	2	0	75	2
	Nocturnal	04/09	26.1	42	0	7	0
	Nocturnal	05/07	30.5	42	2	>100	0
	Nocturnal	10/25	27.0	19	0	0	0
2019	Diurnal	01/25	12.3	3	0	17	1
	Nocturnal	05/03	27.5	20	1	19	0
	Nocturnal	10/11	23.0	24	0	0	0
	Nocturnal*	10/11	23.0	24	2	0	0
	Nocturnal*	10/14	24.0	27	1	0	0
	Nocturnal*	10/17	29.3	22	2	0	0

* Counts from mark-recapture efforts.

Dawn's Canyon Spring, NV – This site consists of a small segment of stream located in a narrow canyon off the Colorado River directly upstream from Boy Scout Canyon. It has been speculated that the frogs in this canyon may be connected to the population in Boy Scout Canyon, but steep canyon walls have obstructed efforts to investigate watered areas above these sites. Frogs have been consistently seen in Dawn's Canyon (Table 3), but no more than eight frogs have ever been counted (in spring 2011). During the project period, hatchlings of *R. onca* were observed in 2016 and 2017 in a plunge pool that marks the end of the survey area; two spent egg masses were associated with the hatchlings in 2016. A juvenile *R. onca* was noted in late 2017.

Table 3. Summary of *Rana onca* observed at Dawn's Canyon Spring during visual encounter surveys conducted in 2016 –2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Mass
2016	Diurnal	01/27	14.9	0	0	77	0
	Nocturnal	03/30	18.1	4	0	0	0
	Nocturnal	10/11	29.0	2	0	0	0
2017	Diurnal	02/09	18.3	0	0	115	0
	Nocturnal	03/28	24.5	2	0	0	0
	Nocturnal	10/09	24.1	5	1	0	0
2018	Diurnal	02/02	12.7	1	0	0	0
	Nocturnal	04/09	24.5	2	0	0	0
	Nocturnal	10/25	24.0	1	0	0	0
2019	Diurnal	01/25	13.9	0	0	0	0
	Nocturnal	05/03	28.0	1	0	0	0
	Nocturnal	10/11	20.9	1	0	0	0

Black Canyon Spring and Black Canyon Spring Side, NV – These two areas represent components of the same system, although treated as separate sites for reporting given a generally dry stretch between them. Black Canyon Side Spring consists of a cold spring that forms a series of pools in a narrow, rocky canyon. The vast majority of *R. onca* observed in the Black Canyon Spring system are in the side canyon and not along the main stream. Over the project period, cattail and salt cedar (*Tamarisk* sp.) have increased along both systems and surface water flowing from the side spring appears to have declined. The population of frogs occupying the side spring appears small but stable, and *R. onca* showed active reproduction and recruitment (Table 4).

Table 4. Summary of *Rana onca* observed at Black Canyon Spring Side during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Site	Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
Side Spring	2016	Diurnal	01/20	14.3	3	0	5[a]	0
		Nocturnal	03/30	13.6	17	0	10	0
		Nocturnal	10/11	24.9	26	1	0	0
	2017	Diurnal	02/03	22.5	0	0	0	2
		Nocturnal	04/18	27.3	19	0	104	0
		Nocturnal	10/09	22.1	20	0	0	0
	2018	Diurnal	01/24	9.7	0	0	0	2
		Nocturnal	04/30	24.4	22	0	20	0
		Nocturnal	10/25	20.0	14	2	1	0
	2019	Diurnal	01/31	16.4	1	0	6	1
		Nocturnal	05/03	23.2	28	0	18	0
		Nocturnal	10/16	24.0	20	1	1	0

[a] Overwinter tadpoles.

Black Canyon Spring represents a reach of the main stream fed by thermal springs that occur upstream from the survey area. Areas above the survey reach likely contain *R. onca*, but these areas are difficult to access (requiring technical climbing) and were not surveyed (a large waterfall designates the survey endpoint). Habitat along the main stream does not appear to be favorable to *R. onca*, and only a few frogs are ever observed (Table 5). The warm water temperature of the stream is generally not considered favorable to egg and tadpole development, but an egg mass was seen in 2016 just upstream of the confluence with the side spring drainage, and tadpoles were observed in 2017.

Table 5. Summary of *Rana onca* observed at Black Canyon Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	01/20	18.1	0	0	0	1
	Nocturnal	03/30	18.0	1	0	0	0
	Nocturnal	10/11	25.5	1	0	0	0
2017	Diurnal	02/03	22.5	0	0	20[a]	0
	Nocturnal	04/18	27.1	1	0	2	0
	Nocturnal	10/09	23.6	2	1	0	0
2018	Diurnal	01/24	14.0	0	0	0	0
	Nocturnal	04/30	22.7	5	0	0	0
	Nocturnal	10/25	21.5	0	0	0	0
2019	Diurnal	01/31	18.6	0	0	0	0
	Nocturnal	05/03	25.0	0	0	0	0
	Nocturnal	10/16	24.0	0	0	0	0

[a] Likely *R .onca*.

Salt Cedar Canyon Spring, NV – This narrow, rocky canyon neighbors the Black Canyon Spring system. Numbers of *R. onca* during the project period remained relatively high for this site (Table 6), following a record count of 64 frogs in 2015. Observers, however, noted on several of the surveys that many of the adult frogs were probably young-of-the-year, reflecting robust recruitment, but also potentially low survivorship of adult frogs. All life stages of *R. onca* were observed each year during the project period.

The site has experienced increasing growth of emergent vegetation, which has degraded habitat quality and probably hinders detectability of frogs during surveys. The lower stretch of stream eventually reaches the Colorado River and is occupied by crayfish (*Procambarus clarkii*) which are a threat to *R. onca*. A dry stretch of gravel has remained a barrier to crayfish, keeping them from colonizing the upper segment of stream.

Table 6. Summary of *Rana onca* observed at Salt Cedar Canyon Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	01/20	18.1	2	0	52	2
	Nocturnal	03/30	15.6	30	0	46	0
	Nocturnal	10/11	27.0	45	10	0	0
	Nocturnal	12/01	12.1	18	7	11	0
2017	Diurnal	02/03	20.2	2	0	8	5
	Nocturnal	03/28	22.2	36	10	16	1
	Nocturnal	10/09	22.9	37	7	0	0
2018	Diurnal	01/24	12.1	1	1	4[a]	5
	Nocturnal	04/30	25.5	25	1	40	0
	Nocturnal	10/25	22.4	40	9	0	1
2019	Diurnal	01/31	16.8	2	1	27	2
	Nocturnal	05/03	24.4	26	1	27	0
	Nocturnal	10/17	26.2	49	8	0	0

[a] Overwintering tadpoles.

MONITORING OF SITES IN THE NORTHSHORE SPRINGS COMPLEX

Blue Point Spring, NV – Surveys at Blue Point Spring have been split into upper and lower segments of the stream system, separated by > 400 meters of stream that has tunneled underground. Upper Blue Point, consists of about 0.5 km of linear stream above Northshore Road. Lower Blue Point consists of a larger downstream segment where water reemerges. Habitat in this system suffers from the presence of predatory exotic fishes, including mosquito fish (*Gambusia affinis*), cichlids (*Cichlasoma*) and mollies (*Poecilia*). The stream bank and waters are also mostly covered in dense sedges (*Scirpus* and *Eleocharis*) or by stands of reeds (*Phragmites*) and cattails. Mitigation efforts focused on reducing vegetation to maintain some stretches of open habitat and breeding pools. Exotic palm trees (*Washingtonia filifera* and *Phoenix dactylifera*) have become a concern for habitat at Upper Blue Point. Tunneling of the stream in the porous soils also has been a problem with the underground stretch expanding over the years.

Augmentation of headstarted frogs is an almost annual activity at both parts of Blue Point Spring, although counts (Tables 7 & 8) rarely appear to reflect expansion of the adult population from the large numbers of released animals (see Table 25). The high count at Upper Blue Point in 2019 was a bit more promising. Detectability of frogs at these sites, however, is probably low because of the overgrowth of dense vegetation. Natural recruitment into the adult population has been difficult to discern, but appears to occur. Augmentations of headstarted animals could easily account for most of the young frogs documented during surveys.

Table 7. Summary of *Rana onca* observed at Upper Blue Point Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	01/30	26.8	2	0	0	0
	Nocturnal	03/27	16.7	5	0	24	0
	Nocturnal	10/21	19.9	7	2	0	0
2017	Diurnal	01/27	11.6	0	0	0	2
	Diurnal	01/30	11.6	1	0	5	0
	Diurnal	02/01	14.1	1	0	0	0
	Diurnal	02/06	17.9	0	0	4	3
	Diurnal	08/10	31.5	1	0	0	0
	Nocturnal	04/05	21.4	5	0	4	0
	Nocturnal	10/25	22.0	20	0	0	0
2018	Diurnal	01/23	23.7	4	0	0	0
	Diurnal	01/29	24.8	1	0	0	0
	Nocturnal	04/17	14.6	16	1	75	0
	Nocturnal	10/19	23.5	16	0	0	0
2019	Diurnal	01/22	14.1	0	0	0	0
	Diurnal	01/24	14.2	0	0	0	0
	Diurnal	01/28	20.3	4	0	0	0
	Diurnal	02/07	13.4	0	0	0	0
	Diurnal	02/12	11.8	1	0	0	4
	Diurnal	02/15	16.6	1	0	0	0
	Diurnal	02/20	7.8	0	0	0	1
	Nocturnal	03/30	16.5	16	0	0	0
	Nocturnal	11/02	15.5	35	2	0	0

Most observations of egg masses and all tadpoles at Upper Blue Point were at a single, small side pool which received maintenance almost annually to keep it from being overtaken by emergent vegetation. At Lower Blue Point, most observations of egg masses and tadpoles occur in a relatively large fish-free pond being maintained by water piped from the main stream. The number of diurnal surveys conducted at both upper and lower section of Blue Point during parts of the project period were aimed at collecting eggs from a larger number of egg masses to increase genetic diversity of headstarted frogs used in research efforts at UNLV. The research was aimed at assessing the effect of *Bd* infection on *R. onca*, funded by BLM and USFWS. A similar abundance of sampling at Rogers Spring was also associate with these efforts (see below).

Habitat maintenance at Lower Blue Point focuses on rehabilitation of the pond (created with County funding in 2007), which occurs almost annually, but the water level in the pond has declined substantially in recent years (previously reported). *Bd* has been detected at Lower Blue Point starting in 2010 and was detected during sampling each year through the project period. This pathogen may be a factor in the limited counts of *R. onca* at this site (see Other Monitoring Actions below).

Table 8. Summary of *Rana onca* observed at Lower Blue Point Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	01/18	16.7	1	0	0	1
	Diurnal	01/30	21.5	1	0	0	1
	Nocturnal	03/27	11.9	7	0	0	0
	Nocturnal	04/03	20.0	5	0	1	0
	Nocturnal	10/21	14.7	14	2	0	0
2017	Diurnal	01/27	11.6	0	0	0	1
	Diurnal	02/01	17.4	1	0	9	0
	Nocturnal	03/16	18.4	12	3	4	0
	Nocturnal	10/26	19.5	8	0	0	0
2018	Diurnal	01/23	18.4	1	0	0	4
	Diurnal	01/29	27.3	1	0	7	2
	Nocturnal	03/29	14.8	13	4	1[a]	0
	Nocturnal	10/24	17.5	15	3	0	0
2019	Diurnal	01/22	14.5	2	0	6[a]	3
	Diurnal	01/24	17.7	2	1	10	0
	Diurnal	01/28	18.5	3	0	23	1
	Diurnal	02/07	8.8	1	0	22	2
	Diurnal	02/12	12.1	2	1	14	0
	Diurnal	02/15	13.7	1	0	14	0
	Nocturnal	03/30	14.0	20	3	17	0
	Nocturnal	09/19	26.3	18	2	0	0

[a] Overwintering tadpoles.

Rogers Spring, NV – This thermal stream spring is closely situated to Blue Point and similarly suffers from exotic fishes and dense, emergent vegetation, in this case, dominated by tall sawgrass (*Cladium californicum*). The habitat conditions do not appear favorable to *R. onca* and none were encountered from spring 2007 through spring 2008 despite several searches. Augmentation of this site began in the summer of 2008, using juvenile frogs and tadpoles raised from eggs collected at Blue Point Spring, and has occurred periodically since. Frogs have been regularly observed during surveys since augmentations began and throughout the project period (Table 9). Almost all frogs are observed in small pockets of open surface water scattered near or flowing over the power line road that crosses the stream. In 2018, some headstarted frogs were released into the pool by the springhead and one frog was observed just downstream in 2019. In fall 2016, during the survey, an adult bullfrog (*Rana catesbeiana = Lithobates catesbeianus*) was observed in the springhead pool. This is of concern because bullfrogs are invasive, prey on other frog species, and are a known vector of *Bd*. Attempts to remove the frog failed, and a bullfrog (presumably the same animal) was seen during surveys in 2017 and 2019.

Table 9. Summary of *Rana onca* observed at Rogers Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is shown.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	01/30	12.1	0	0	0	0
	Nocturnal	04/03	17.9	6	0	0	1
	Nocturnal	10/26	20.4	2	0	0	0
2017	Diurnal	01/27	04.8	0	0	2	0
	Diurnal	01/30	17.5	0	0	0	0
	Diurnal	02/01	12.1	0	0	0	1
	Diurnal	02/06	16.3	0	0	0	0
	Nocturnal	04/05	20.2	2	0	0	0
	Nocturnal	10/25	17.3	6	0	0	0
2018	Diurnal	01/23	8.4	0	0	0	1
	Diurnal	01/29	22.2	0	0	0	0
	Nocturnal	04/23	24.3	6	0	0	0
	Nocturnal	10/04	23.1	8	0	0	0
2019	Diurnal	01/22	11.0	0	0	0	0
	Diurnal	01/24	4.2	0	0	0	0
	Diurnal	01/28	12.3	1	0	0	0
	Diurnal	02/07	3.0	0	0	0	0
	Diurnal	02/12	4.3	2	0	0	0
	Diurnal	02/15	9.4	0	0	0	1
	Diurnal	02/20	4.9	1	0	0	2
	Nocturnal	04/25	28.7	11	0	0	0
	Nocturnal	11/05	15.0	8	0	0	0

MONITORING OF EXPERIMENTAL TRANSLOCATION SITES

Goldstrike Canyon, NV – A population of *R. onca* was established at this site by translocations of Black Canyon frogs prior to the project period; the last translocation occurred in 2013. The high-counts at this site during the project period were low (Table 10) and the population appears quite small. The site is busy with visitors, and the hot springs are heavily impacted, which may have negative consequences for frogs. Breeding habitat conditions have declined in recent years as the few cooler water pools where breeding and tadpole development have been documented appear to have deteriorated and gotten smaller. Efforts to restore these pools are recommended. The observation of a few juvenile frogs each year, however, indicates some level of successful recruitment into the adult population.

Table 10. Summary of *Rana onca* observed at Goldstrike Canyon during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is shown.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	01/27	12.4	0	0	34[a]	0
	Nocturnal	03/17	21.6	16	1	39[a]	0
	Nocturnal	10/20	26.0	16	0	2	0
2017	Diurnal	02/13	19.5	0	0	36[b]	1
	Nocturnal	03/21	26.8	14	0	14	0
	Nocturnal	10/14	26.0	7	1	0	0
2018	Diurnal	02/07	19.2	2	0	61	1
	Nocturnal	04/05	24.9	19	1	6[a]	1
	Nocturnal	11/01	22.5	6	1	0	0
2019	Diurnal	02/11	9.4	1	0	0	0
	Nocturnal	04/15	25.5	9	2	0	0
	Nocturnal	11/02	19.4	8	0	0	0

[a] All or some overwintering tadpoles. [b] Includes 10 tadpoles also likely *R. onca*.

Grapevine Spring (Meadview), AZ – Translocations of *R. onca* to this site ended in 2009, and the population of these Black Canyon frogs is now one of the largest (Table 11). The diurnal survey in 2019 was conducted somewhat later than usual and occurred after several storms in March. The count of 141 egg masses was the highest ever recorded at any site, and resulted in the large count of tadpoles that May. Later that year, 230 frogs were counted, and the actual population size was likely several times larger. Over the course of the current project, all life stages of *R. onca* were observed each year during surveys. Large, overwintering tadpoles have been regularly observed at this cold water site, and recruitment appears common as indicated by the routine observations of juveniles and small adult frogs.

In general, the habitat for *R. onca* appears to be of high quality and detectability is generally good, although emergent vegetation, particularly monkey flowers (*Mimulus*), can grow quickly, negatively impacting visibility. The narrow canyon occasionally floods which reduces such vegetation within the stream and maintains somewhat open vegetation and pools.

Table 11. Summary of *Rana onca* observed at Grapevine Spring (Meadview, AZ) during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is shown.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	03/05	26.4	26	1	67[a]	45
	Nocturnal	05/22	19.4	175	4	7	0
	Nocturnal	10/13	23.1	118	2	1	0
2017	Diurnal	03/02	15.5	25	0	4,723	8
	Diurnal	07/03	38.6	120	2	3	0
	Diurnal	07/15	35.0	158	0	6	3
	Nocturnal	05/19	23.2	144	0	9	0
	Nocturnal	09/20	27.2	73	1	17	0
	Nocturnal	10/04	21.5	98	0	29[b]	0
2018	Diurnal	03/20	22.3	57	0	409[a,b]	141
	Nocturnal	05/31	28.5	126	4	204	0
	Nocturnal	10/05	21.0	71	2	17	0
2019	Diurnal	03/25/2019	22.9	86	2	245[a,b]	84
	Nocturnal	06/04/2019	25.0	213	17	43	1[c]
	Nocturnal	10/08/2019	22.9	200	4	52	0

[a] Includes some overwintering tadpoles. [b] Includes some tadpoles likely *R. onca*. [c] Likely *R. onca*.

Pupfish Refuge, NV – A relatively modest, but robust population of *R. onca* was established at this site by translocations of Black Canyon animals that ended in 2008. Prior to the beginning of this project, the population appeared to begin a slow decline associated predominantly with changes in habitat quality. Over several years starting in 2009, the Bureau of Reclamation (BOR) incrementally removed dense stands of tamarisk from the main drainage and attempted to establish native trees. Mesquite trees (*Prosopis*) seem to have taken, but the lack of shade allowed emergent vegetation (predominately cattails and a bunch grass species) to grow dense along the stream. In addition, exotic aquatic snails (*Melanoides*) appeared in the mid-2000s, visibly reducing algae in the stream, and potentially consuming eggs or hatchlings (which needs to be confirmed). Successful reproduction of *R. onca* centered on cooler waters running along a drainage ditch associated with Portal Road that transects the site. Vegetation encroachment and erosion has reduced surface flow along the ditch and most of the breeding pools have now been lost or greatly reduced.

The viability of this population came into question in 2018, when BOR representatives stopped authorization of habitat maintenance actions at the site (until interagency coordination can occur among engineers, environmental compliance, and other entities). In addition, a habitat effort in 2017 was reversed by a debris flow from a fall storm. The desire has been to work with BOR to clear vegetation and debris from the drainage ditch. Without intervention this population will likely be declared extirpated in the next year or two. The counts in 2019 indicate a collapse of the population (Table 12).

Table 12. Summary of *Rana onca* observed at Pupfish Refuge Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	02/03	13.1	2	0	145	1
	Nocturnal	03/15	21.8	14	0	29	0
	Nocturnal	04/06	27.3	24	1	14	0
	Nocturnal	10/12	29.8	11	0	3	0
	Nocturnal	10/23	28.4	21	2	1	0
2017	Diurnal	02/20	18.9	1	0	154	0
	Diurnal	08/08	28.0	1	0	1	0
	Nocturnal	04/04	23.5	11	0	5	2
	Nocturnal	04/12	28.0	12	0	0	0
	Nocturnal	10/17	26.3	13	0	0	0
2018	Diurnal	02/01	17.7	1	0	0	2
	Nocturnal	04/15	28.0	16	0	74	1
	Nocturnal	10/10	26.9	6	0	1	0
2019	Diurnal	03/06	18.8	0	0	0	0
	Nocturnal	04/17	26.1	0	0	25	0
	Nocturnal	05/09	29.6	0	0	0	0
	Nocturnal	10/23	25.9	0	0	0	0

Quail Spring, NV – This small site consists of a spring-fed historic cattle pond which flows into a narrow riparian area. A much smaller pool was created within the riparian zone by BLM in 2009, although this pool has filled in. The population of *R. onca* was established by translocations of Black Canyon animals that were completed in 2012. In recent years, counts of the population have hovered around 100 frogs, and breeding activity, tadpoles, juvenile frogs, and likely young-of-the-year adults have been commonly documented (Table 13). The lower number of frogs counted in 2019 may indicate a decline; recruitment over the last several years appears limited.

In general, habitat conditions appeared favorable for *R. onca,* because trespass cattle and feral burros regularly feed on cattails and grasses in and around the main pool. Sedimentation is an issue, particularly associated with algae and grasses. Emergent vegetation could easily overrun the pond if left undisturbed. Although maintenance actions have been conducted at this site in the past, the only actions that occurred during the project period were site assessments and planning meetings.

Table 13. Summary of *Rana onca* observed at Quail Spring during visual encounter surveys conducted in June 2016 – December 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	02/21	17.5	9	0	4[a]	0
	Nocturnal	04/29	18.3	109	7	8[a]	0
	Diurnal	10/04	20.7	7	0	0	0
	Nocturnal	10/04	21.7	75	0	4	0
	Nocturnal	10/20	21.3	58	0	27	0
2017	Diurnal	02/24	11.9	8	0	3	2
	Diurnal	09/19	26.3	2	5	1	0
	Nocturnal	04/19	20.6	55	12	23	2
	Nocturnal	05/01	24.3	74	24	28	0
	Nocturnal	09/19	24.8	30	51	5	0
2018	Diurnal	02/26	17.0	7	1	0	0
	Nocturnal	05/03	17.9	89	3	8	0
	Nocturnal	10/15	15.2	19	0	2	0
	Nocturnal	10/29	20.0	38	0	1	0
2019	Diurnal	03/19	21.8	31	0	0	1
	Nocturnal	05/01	17.4	52	0	2[a]	0
	Nocturnal	10/03	23.5	53	10	11	0

[a] Includes overwintering tadpoles.

Red Rock Spring, AZ – Initial translocations of *R. onca* to this site were completed in 2010, but excess animals from the headstarting program were again released in 2013 and 2014; all the animals were derived from Black Canyon. The site has never maintained a large number of *R. onca*, likely because surface water flows often reduce to a trickle in summer. Pools generally dry up and this site lacks habitat for larvae development. Natural recruitment of juvenile frogs has only been confirmed once (in fall 2016 during this project). Coyote predation may also be an issue and an abundance of coyote sign was noted during recent surveys. As this population continues to decline, lower numbers of *R. onca* have been counted, and only a single adult was observed during the survey in fall 2019 (Table 14).

Table 14. Summary of *Rana onca* observed at Red Rock Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	02/20	26.3	1	0	0	0
	Nocturnal	04/30	16.7	7	0	41	0
	Nocturnal	09/26	21.4	7	1	0	0
2017	Diurnal	02/24	15.5	1	0	0	0
	Nocturnal	04/25	12.9	4	0	0	0
	Nocturnal	09/21	22.9	8	0	0	3
2018	Diurnal	03/14	23.5	0	0	0	4
	Nocturnal	05/24	27.7	5	0	0	0
	Nocturnal	11/04	18.3	2	0	0	0
2019	Diurnal	03/07	21.6	2	0	0	2
	Nocturnal	06/05	27.6	3	0	6	0
	Nocturnal	10/26	15.5	1	0	0	0

Tassi Spring, AZ – The population of *R. onca* at Tassi was established by translocations of Black Canyon animals that ended in 2010. The site now maintains an abundant population, with 260 frogs counted in spring 2017 (the highest recorded for this site; Table 15). All life-stages of *R. onca* were observed each year during the project period. Large swings in seasonal detectability of frogs at Tassi has been observed, with fall surveys producing low counts (as much as threefold lower than spring counts) which are inconsistent with results from previous and subsequent surveys. The cause for the changes in detectability at this site is unknown, but speculation centers on some type of weather pattern in fall that may bring cold air from the Colorado Plateau that affects frog activity.

Dense vegetation has come to dominate the historical ditch below the springhead, where few frogs have been seen in recent years. The NPS has been conducting actions at the site, including reducing vegetation, but mostly to protect the historic ranch house. There are plans to stabilize the ditch and reduce emergent vegetation blocking the stream flow, actions which should improve conditions for *R. onca*. The stream stretch in the main wash has also become dense with cattails and arrow weed (*Plucea sericea*), overhanging catclaw (*Senegalia greggii*) and mesquite (*Prosopis*). There is some disturbance in the wash by burros and cattle that helps maintain openness. Almost all tadpoles and most egg masses encountered during the project period were in the lower wash.

Table 15. Summary of *Rana onca* observed at Tassi Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	02/22	24.9	26	0	179	7
	Nocturnal	04/20	26.3	131	1	25	0
	Diurnal	09/30	27.7	2	0	0	0
	Nocturnal	09/30	27.1	52	8	5	0
2017	Diurnal	02/15	21.2	68	0	1,995[a]	9
	Nocturnal	04/17	25.5	257	3	0	3
	Nocturnal	09/30	26.3	170	3	0	0
2018	Diurnal	02/12	16.0	25	0	201[a,b]	38
	Nocturnal	04/28	24.6	173	1	20	1
	Nocturnal	09/17	30.3	53	4	0	0
2019	Diurnal	03/22	22.5	39	0	382[c]	13
	Nocturnal	05/25	24.5	169	1	0	0
	Nocturnal	10/05	21.0	115	0	0	0

[a] Includes one overwintering tadpole. [b] Includes 200 hatchlings likely *R. onca*. [c] Includes 361 tadpoles likely *R. onca*.

Union Pass Spring, AZ – Over this project period, the high counts of *R. onca* at Union Pass Spring have been > 150 adult and juvenile frogs, with a record high occurring in 2018 (Table 16). The high count in 2018 was about 1.8 times that of the high count in 2016, and likely reflects a meaningful increase in the population of frogs at this site. Translocations of Black Canyon animals were completed in 2015. Since then, the population has become quite robust, with egg masses, tadpoles of various developmental stages, and juveniles commonly observed. Natural recruitment was confirmed in 2017, although long suspected. The number of egg masses seen in March 2019 was a record for this site.

Overall, vegetation density and encroachment remains moderate and known breeding pools persist. Cows and burros are common in the canyon. These animals break through dense stands of coyote willow (*Salix*) and thin vegetation through grazing. Both actions appear to improve conditions for the frogs.

Table 16. Summary of *Rana onca* observed at Union Pass Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	02/13	31.9	42	0	2[a]	12
	Nocturnal	05/03	20.7	178	4	67	0
	Nocturnal	10/09	20.6	145	11	54	0
2017	Diurnal	03/09	30.2	62	3	30[b]	26
	Diurnal	07/05	33.4	223	16	82	0
	Diurnal	08/01	33.8	204	8	60	2
	Nocturnal	05/04	23.4	269	6	90	0
	Nocturnal	10/10	14.7	144	10	185	0
2018	Diurnal	03/08	25.4	49	1	195[c]	15
	Nocturnal	05/22	18.6	326	1	94	0
	Nocturnal	09/25	20.7	267	58	109	0
2019	Diurnal	03/25	25.0	45	0	287[d]	104
	Nocturnal	06/01	22.0	213	0	88	0
	Nocturnal	10/01	17.0	154	64	215	0

[a] Overwintering tadpoles. [b] Includes 24 overwintering tadpoles. [c] Includes 34 overwintering. [d] Includes 52 overwintering tadpoles, the others were likely *R. onca*.

Bearpaw Poppy Spring, NV – Over this project period, observations of *R. onca* have been limited to mostly adults. There has been some evidence of reproduction, with a few egg masses and one tadpole (that was probably *R. onca*) observed at the site (Table 17). Dense emergent vegetation clearly hinders detectability of tadpoles, but tadpoles should have been detected more often if they were present in any reasonable numbers. The lack of tadpoles is consistent with a general lack of natural recruitment; the two juvenile frogs observed in 2016 were likely released at the site as part of the final translocation of Northshore animals in 2016. The larger counts in 2016 and 2017 also likely reflect recruitment from that release, and the lower counts since then appears to reflect a real decline in the population. During the last survey, the field observer noted that the frogs observed were all very large (hence older) individuals.

Natural pooling is limited at this site and the lack of recruitment was thought to be related. Several attempts to increase pooling for *R. onca* have been conducted over the project period, but earlier attempts have been met with very little success in terms of recruitment. The constructed pools would become filled-in with sediment and emergent vegetation. Starting in 2018, BLM began a larger-scale project to construct several rock weirs (check dams), along with a few smaller, lined pools. Once created in 2019, adult frogs quickly occupied the pools and some egg masses were subsequently laid, but no resulting tadpoles or small frogs were observed. Speculation is now beginning to focus on the possibility that low tadpole survivorship might be the issue.

Table 17. Summary of *Rana onca* observed at Bearpaw Poppy Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	02/20	22.0	5	0	0	1
	Diurnal	05/07	28.9	5	0	0	0
	Nocturnal	04/21	26.7	30	0	0	1
	Nocturnal	09/26	24.9	55	2	0	0
2017	Diurnal	03/07	22.4	10	0	0	4
	Nocturnal	04/13	23.5	60	0	0	0
	Nocturnal	09/28	23.8	57	0	0	0
2018	Diurnal	03/14	27.6	3	0	0	3
	Nocturnal	05/14	23.7	23	0	1[a]	1
	Nocturnal	10/08	20.7	11	0	0	1
2019	Diurnal	02/25	22.8	2	0	0	2
	Diurnal	05/01	24.9	3	0	0	2
	Diurnal	09/03	37.5	5	0	0	0
	Nocturnal	05/29	20.8	12	0	0	1
	Nocturnal	10/26	20.5	5	0	0	0

[a] Likely *R. onca*.

Lime Spring, NV – Translocations of Black Canyon animals to Lime Spring were completed in 2016. This site is located high on the east side of the Virgin Mountains and considered an experiment to evaluate whether *R. onca* could survive and overwinter at high elevation (> 1430 m). Overwintering and evidence of breeding of *R. onca* has been documented; however, the long-term success of this population is unlikely. The issue has been a general lack of perennial pools during summer months, limiting natural recruitment. Over the project period, surface water would flow intermittently over a range of ~ 7 m to 213 m. At the most, 13 adult *R. onca* were encountered and occasionally egg masses were seen (Table 18). Unfortunately, egg masses were laid in pools that would generally went dry later in the year, and recruitment at this site has never been confirmed. We are expecting extirpation of this population in the near future as the last frogs age out.

Table 18. Summary of *Rana onca* observed at Lime Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Nocturnal	05/18	17.2	8	0	0	3
	Diurnal	10/03	N.T.	0	0	0	0
	Nocturnal	10/03	9.4	3	0	0	0
2017	Nocturnal	05/24	26.3	13	0	0	0
	Nocturnal	10/02	14.2	4	0	0	0
2018	Nocturnal	06/04	22.2	7	0	0	0
	Nocturnal	09/19	24.6	1	0	0	0
2019	Nocturnal	06/11	21.7	9	0	0	1
	Nocturnal	09/26	17.8	6	0	0	0

Horse Spring, NV – In 2018, the population of *R. onca* at Horse Spring experienced a dramatic decrease in relative abundance (Table 19) that reflects an actual decline in population size. Translocations to this site were completed in 2016 and the population size appeared to be increasing at that time. Observations of numerous egg masses and tadpoles at various developmental stages were common, indicating robust breeding activity. Natural recruitment of *R. onca* was confirmed in early 2018, but had long been suspected.

The recent change in population was a result of a significant decline in surface water. This occurred between July 25, 2018 when the main pond was noted as having water and August 19, 2018 when the pond was dry. At that time, only a narrow ~ 18 m stretch of surface flow emerged from the springhead. Cattle and burros had trampled the site and contaminated the water with excrement and urine. Since then, surface water has been limited to a shallow ~ 21 m stretch. There is no evidence that the pond had dried previously since the site was assessed for potential translocation in 2011. A few adult *R. onca* remain at the site, mostly in crevices along the base of trees near the springhead. BLM has shown interest in potential restoration of the spring and associated pool.

Table 19. Summary of *Rana onca* observed at Horse Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	02/21	24.9	5	0	226[a]	28
	Nocturnal	04/29	15.2	60	5	28	0
	Nocturnal	10/04	18.9	23	17	0	0
2017	Diurnal	02/24	09.3	2	0	12,967	4
	Nocturnal	04/20	16.1	69	3	11	3
	Nocturnal	09/19	21.3	57	13	0	0
2018	Diurnal	02/26	18.5	0	0	4[b]	25
	Diurnal	07/25	35.3	36	6	6	0
	Diurnal	08/19	32.8	0	0	0	0
	Nocturnal	05/08	27.5	116	7	0	1
	Nocturnal	09/27	23.3	5	3	0	0
2019	Diurnal	03/14	13.4	1	0	0	2
	Nocturnal	05/02	9.5	8	0	0	0
	Nocturnal	10/03	22.2	4	0	0	0

[a] Includes 1 overwintering tadpole, and 14 tadpoles likely *R. onca*. [b] Overwintering tadpoles.

Corn Creek, NV – This spring system is located near the visitor's center at the Desert National Wildlife Refuge. Translocations began in 2015 prior to the project period and were completed in 2019. The stream system is mostly covered by large trees, predominately cottonwoods (*Populus fremontii*) and willows, and has long stretches of open stream, which looks like good habitat for adult *R. onca*. The site, however, contained several stressors including crayfish, *Bd*, and numerous large Woodhouse's toads (*Anaxyrus woodhousii*) which likely eat smaller juvenile *R. onca*. In addition, predatory fishes have been illegally dumped into the cement pond at the site and the associated stream.

Establishment of a population of *R. onca* at Corn Creek has not manifested. There has been some overwintering of frogs, but no more than four during any given year have been documented during the project period (Table 20). Call-broadcast was attempted during most nocturnal surveys to solicit responses from *R. onca*, but this resulted in only a single detection (in 2016). The high counts observed in the fall of most years predominantly reflect translocated animals released earlier in the year. No egg masses or tadpoles of *R. onca* have been observed at this site, but many areas that would otherwise be favorable for oviposition are crowded with crayfish. This translocation attempt is likely to fail as the few established frogs pass away.

Table 20. Summary of *Rana onca* observed at Corn Creek during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Nocturnal	04/19	16.5	4	0	0	0
	Nocturnal	09/28	24.7	12	0	0	0
2017	Diurnal	03/01	18.6	0	0	0	0
	Nocturnal	04/21	17.0	1	0	0	0
	Nocturnal	06/05	25.1	1	10	1	0
	Nocturnal	10/12	17.2	11	0	0	0
2018	Diurnal	02/23	6.0	0	0	0	0
	Diurnal	03/06	15.3	0	0	0	0
	Nocturnal	03/27	8.3	1	0	0	0
	Nocturnal	09/18	28.0	22	0	0	0
2019	Diurnal	03/21	17.3	0	0	0	0
	Nocturnal	05/14	28.9	4	0	0	0
	Nocturnal	09/24	20.0	1	0	0	0

Kaolin Spring, (Muddy River area), NV. – Conditions at this small spring were assessed in 2015, with translocation of *R. onca* initiated in 2016. The five year plan for translocations to the site is planned to continue into 2020. Two rock tinajas at the source of the spring are not surveyed because technical climbing is required; however, frogs have been heard calling from the pools. Overwintering of frogs and egg masses have been documented annually following 2016. Most of the juveniles encountered are likely those released during translocation, thus natural recruitment has not been confirmed. Over the project period, counts of adult *R. onca* appear to be stable (Table 21).

The potential for large flooding events at this site was anticipated prior to translocation of *R. onca* and may be a potential problem. Minor flooding was noted each year along with intermediate changes to water flow, pools, and vegetation density. There are concerns about movement of sand or debris associated with nearby sand mining. Also of concern, was the documentation of the Chorus frog (*Pseudacris regilla*) at the site (in 2016 and 2019). This frog is a known vector for *Bd* and the pathogen has been documented on Chorus frogs along the Muddy River (Jaeger et al. 2017). The fear is that infected individuals could colonize Kaolin Spring during a wet year.

Table 21. Summary of *Rana onca* observed at Kaolin Spring during visual encounter surveys conducted in 2016 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2016	Diurnal	08/08	40.9	0	12	0	0
	Nocturnal	10/21	24.0	17	0	0	0
2017	Diurnal	09/09	29.4	20	0	0	0
	Nocturnal	04/27	25.0	17	0	0	4[a]
	Nocturnal	05/12	30.3	19	0	6[b]	0
	Nocturnal	10/03	26.8	27	2	0	0
2018	Diurnal	02/16	12.4	1	0	0	5
	Diurnal	04/11	30.7	10	0	2	1
	Diurnal	07/08	34.5	26	6	16	0
	Diurnal	07/22	33.1	15	0	0	0
	Nocturnal	04/03	13.9	29	0	0	9
	Nocturnal	09/26	29.8	37	0	0	1
2019	Diurnal	03/15	12.9	5	0	0	4
	Diurnal	05/14	39.6	13	1	0	0
	Diurnal	12/05	15.3	1	0	0	0
	Nocturnal	04/23	24.0	24	0	70	1
	Nocturnal	09/17	26.2	32	19	15	0

[a] Includes 1 egg mass likely *R. onca*. [b] Includes 2 tadpoles likely *R. onca*.

Las Vegas Springs Preserve, NV. –The site consists of a mesocosm of two connected ponds constructed in a creek bed at the preserve, and was envisioned as a refuge site. Translocation of animals from both Northshore and Black Canyon were conducted in both 2018 and 2019. Population monitoring in the fall of 2018 noted only a few adult frogs (Table 22), indicating low survivorship of released animals. The very high counts in 2019 reflect large numbers of juvenile frogs, both from releases of headstarted animals and frogs naturally recruited from eggs laid by overwintering frogs in spring 2019.

Mark-recapture efforts were conducted in October and November to estimate the number of frogs at the site. Those surveys are reported herein as visual encounter surveys (Table 22); the estimate from the survey was 424 (95% CI = 308–540), almost all were small juveniles. Of the juvenile frogs captured during these efforts, none showed toe clip markings from the 2019 releases, suggesting either an overall low survivorship of released animals, or that the survivorship of animals released as tadpoles was higher than those released as juvenile frogs (only the frogs received toe clips). This interpretation is tempered by the fact that these frogs can regenerate toe clips, making detection over time difficult.

There is speculation of potential high predation at the site. Mallards were present in the pond for a period early in the year, and a large red-eared slider (*Trachemys*) was removed from the pond in April 2019; the turtle was likely released by a visitor. Riparian vegetation along the banks of the ponds had grown denser by 2019 and appeared to provide better cover for *R. onca*. Additionally, stones were placed along the shoreline that allowed frogs refuge underneath.

Table 22. Summary of *Rana onca* observed at Las Vegas Springs Preserve during visual encounter surveys conducted in 2018 – 2019. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2018	Nocturnal	10/03	26.2	4	0	0	0
2019	Diurnal	08/22	36.8	4	190	1	0
	Nocturnal	03/27	20.5	2	0	0	0
	Nocturnal	05/09	19.8	2	1	23	0
	Nocturnal*	10/15	13.6	6	139	0	0
	Nocturnal*	11/07	24.3	6	73	2	0

* Counts from mark-recapture efforts.

OTHER SITES SURVEYED OR ASSESSED FOR POTENTIAL TRANSLOCATION

Sites identified by agency personnel for evaluation as to their potential as translocation sites for *R. onca* were assessed or revisited upon request. At most sites, detailed site assessment reports were written that included embedded reference photographs. These reports were presented to the Conservation Team and included with semiannual activity reports, eventually submitted to the County. Provided below are summary statements of each of the site visits.

Columbine Spring – A survey at Columbine Spring in the Black Mountains of Arizona was conducted on November 15, 2016 by personnel from UNLV, Arizona Game and Fish Department (AGFD), and BLM. The site has been surveyed by agency personnel at least once in 2009 and again in 2012. Columbine Spring is located 38.6 km south of Union Pass Spring in a Wilderness Area. The consensus of the recent survey team was that environmental conditions appeared encouraging for sustaining a very small population of *R. onca*.

Lake Mead State Fish Hatchery Outflow – Environmental conditions were reassessed at the Lake Mead State Fish Hatchery outflow on November 22, 2016 by personnel from UNLV. This site was initially assessed in January 2006 (reported previously). The outflow from the hatchery flows to Lake Mead approximately 1.5 km downstream. About 0.7 km stretch of surface water was surveyed in November, reaching downstream to an area of extremely dense vegetation. The general conclusion was similar to that reached in 2006, in that vegetation was too dense and stream flow too fast to attempt translocation of *R. onca*.

Magnesite Spring – A reconnaissance was conducted at Magnesite Spring by personnel from UNLV and NDOW on August 8, 2016. The spring is located in a wash in Valley of Fire State Park, about 1.75 km northwest of Kaolin Spring. Conditions appear similar to those observed at Kaolin Spring, although with less water and shorter reach. At best the spring may be able to sustain a very small population of *R. onca,* but water quality should be evaluated.

Pakoon Springs – This spring complex has been identified as a potential translocation site for *R. onca* once bullfrogs have been eradicated. On January 14, 2016, personnel from UNLV and USFWS visited Pakoon Springs to assist AGFD with their long-term effort of eradicating bullfrogs. Diurnal and nocturnal

surveys were conducted, but the weather conditions were cold and no bullfrogs were observed. On November 20, 2017 personnel from UNLV and NPS visited the site. Ten baited minnow traps were set in 3 areas of the stream for ~ 2 hours. Two sub-adult bullfrogs were observed near the springheads, but no frogs or tadpoles were captured.

Spring Mountain Ranch State Park – Personnel from UNLV and NDOW visited this area in the Red Rock Nature Conservancy Area on February 23, 2016. The area was initially assessed in 2005. The intent of the visit was to assess specific areas for potential translocations of *R. onca*. Several sites were looked at including the "Ash Grove", the ponds near the ranger station buildings, and Lake Harriet. None of these sites looked favorable for *R. onca*. Indeed, the area contains a large population of chorus frogs with a high prevalence of *Bd* (Jaeger et al. 2017). A potential stretch of stream associated with the springhead that feeds Lake Harriet in Sandstone Canyon was identified as a potential site to conduct an experimental translocation, assuming that perennial surface water could be assured. The translocation, however, would need to be conducted as a well-designed and funded experiment to evaluate potential stressors, particularly *Bd*, or to assess vaccine approaches.

Warm Springs – A survey at Warm Springs in the Black Mountains of Arizona was conducted on February 21, 2017 by personnel from UNLV, AGFD, BLM, and the Arizona Wilderness Coalition. The site was previously surveyed at least once in 2009 by agency personnel. Warm Springs is located ~ 38 km south of Union Pass Spring in wilderness. The amount of favorable area for *R. onca* at this site was limited, and any population established would be very small. The consensus of the team was that the logistics of translocation would be difficult and the outcome limited.

Burns Spring – An assessment of conditions was conducted at Burns Spring on June 15, 2017 by UNLV AGFD, and BLM personnel. The spring is in the Black Mountains of Arizona ~ 9.3 km north of Union Pass Spring. The biologists on the survey thought that a small population of *R. onca* could likely persist at the spring, but that a lack of pooling would limit reproduction; some mitigation to create pooling would be beneficial. Unfortunately, the best portion of stream for *R. onca* occurs near the spring source on private land. The consensus of the field group was that the issue of private ownership needs to be addressed before further consideration of this site.

Bird Song Trail area, Desert National Wildlife Refuge – Personnel from UNLV and NDOW conducted a brief visit on December 14, 2017 to look at this shallow drainage near the Bird Song Trail, just north of Corn Creek Visitor Center in the Desert National Wildlife Refuge. The consensus of the biologists was that there was insufficient surface water to support *R. onca*.

Perkins Pond – Translocations of *R. onca* to the site occurred from 2010-2014; however, the population did not persist and continued support for the effort was terminated. In March 2017, UNLV personnel visited the pond to look for persisting *R. onca* and sample any amphibians present for *Bd*. The pond, however, was dry and no frogs were observed.

Grapevine Springs, Spring Mountains, NV. – The Grapevine site is on BLM lands on the north end of the Spring Mountain Range and outside of the Priority Management Zone for *R. onca*. On December 4, 2018 personnel from UNLV and BLM assessed conditions at the sites. Grapevine Springs is on a south

facing slope at an elevation of ~ 1,337 m. Given the latitude and elevation there is some concern that winters may be too cold, but *R. onca* has been documented to overwinter and oviposit at ~ 1,430 m in the Virgin Mountains. Grapevine consists of a shallow stream and two ponds. The stream is partly fenced to exclude horses and burros from native springsnail habitat. Conditions appear suitable for establishing *R. onca* and the consensus of the survey group was that the site warranted further investigation.

Grapevine was again surveyed on April 26, 2019 and in summer on August 19 to look for other species of anurans. Personnel from UNLV and BLM conducted the surveys, and on both occasions no anurans were detected. Aquatic invertebrates were observed in the ponds and there were plenty of terrestrial insects, food for *R. onca*. Water quality in the ponds was previously assessed and appears adequate. BLM has conducted an Environmental Analysis for release of *R. onca* to this site which is currently under review.

Ki-up Spring, Spring Mountains, NV. – This small spring is on the west side of the Spring Mountains on BLM lands. Conditions at Ki-up appeared unsuitable for establishment of *R. onca*. The small surface water flow was intermittent amongst dense mats of grass and a sedge or rush. The stream was narrow (~ 0.5 m) and predominately shallow (~ 0.08 m). The site is also at high elevation (~ 1,568 m). The consensus of the group was that the site did not warrant further consideration.

Washington County, UT. – Four potential aquatic sites within the historic range of *R. onca* along the Virgin River drainage in Utah were visited on May 16–17, 2019. Personnel from Utah Division of Wildlife Resources (UDWR) led the trip with broad representation of members of the Conservation Team, including UNLV personnel. The sites were selected by the team from assessments conducted in 2018 by UDWR. The consensus of the group during the site visits was that all four sites contained known threats to *R. onca*, which would impede successful establishment of populations. Of most concern were *Bd,* chorus frogs (a known vector of the pathogen), and crayfish. Other concerns included predatory fish, potential for bullfrogs, cold winter temperatures, and public presence. The consensus was that any attempt to translocate *R. onca* would require a detailed research plan so as not to waste resources. The findings from the field visits are summarized below.

South Dike Outflow (Quail Creek Reservoir) consists of several open pools seeping from the upstream reservoir. The pools were ~ 6–8 m in length, less of width, and less than a meter deep. Pools contained some cattails with dense perimeter vegetation (~ 2 m riparian zone). One pool contained a predatory bass, and crayfish were observed in all the pools.

Indian Pond (on Little Creek Mesa) encompasses a very large open pond in bedrock at relatively high elevation (~ 1,718 m). There was plenty of open bank, with cattails in areas. Perimeter vegetation was abundant and included willow (*Salix*) and Gambel oak (*Quercus gambelii*). Largemouth bass (*Micropterus salmoides*) were planted in the pond. Amphibians include Arizona tiger salamander (*Ambystoma mavortium nebulosum*), red-spotted toad (*Anaxyrus punctatus*) and western toad (*Anaxyrus boreas*). Larval salamanders were observed in the pond and two dead toads were observed raising concern about disease. Cold winter conditions is also a concern.

Grapevine Pass Wash contains a stream that flows ~ 1 km to the Virgin River. A popular residential path runs along the site from new housing developments at the upper end of the wash. The stream had flowing

water with both large and small pools. Small dark-colored tadpoles (likely *Anaxyrus* sp.) were observed in the stream. Chorus frogs were not observed, but occur in the system, raising concern about disease.

Red Hills Desert Garden Stream (St. George) contains an artificial stream system designed to showcase native fish species which occupied the system. Goldfish were also present at the time of the survey and were illegally dumped into the stream by visitors, which highlights concerns with public dumping of exotic species into the system. The site has high visitation from the general public. The gardens have been recently colonized by chorus frogs and a few were found. UDWR has detected *Bd* on chorus frogs at this site. There was some discussion on the feasibility of conducting a research project related to *Bd* vaccination and translocation of *R. onca* to this site.

OTHER MONITORING ACTIONS

Monitoring of the Pathogenic Amphibian Fungus – Sampling for *Bd* occurred at a total of 9 field sites associated with *R. onca* management and other funded research efforts from 2016–2019. Skin cell samples were collected from *R. onca* at Boy Scout Canyon Springs, Corn Creek, Kaolin Spring, Lower Blue Point, Salt Cedar Canyon Spring and Union Pass Spring. A few samples were taken on individuals from the headstarting program, but all animals provided to research efforts at UNLV were tested upon their arrival and none were positive for *Bd*. Other anuran species were also sampled at Corn Creek, Overton Wildlife Management Area, Kaolin Spring, and Spring Mountains State Park. In total 217 individual *R. onca* and 118 individuals of other species were tested, not counting the animals sent to UNLV. *Bd* was detected in *R. onca* over consecutive years at Lower Blue Point and once at Corn Creek. The fungus was also detected in other species at Corn Creek, Overton Wildlife Management Area, and Spring Mountains State Park (see Jaeger et al. 2017 which includes some of these results).

HABITAT ACTIONS

Actions to maintain or improve habitat occurred at 9 sites during the project period (Table 23). In most cases, the actions were small-scale efforts to remove sediment and emergent vegetation from important breeding pools. At Bearpaw Poppy, Pupfish Refuge, Upper Blue Point, and Lower Blue Point efforts also included the use of hand-held gas-powered equipment to reduce emergent vegetation along segments of stream. At Lower Blue Point, such actions focused on an artificial pool being maintained for breeding, and included upkeep of a syphon pipe used to move water to the pond. Assistance was also provided to NPS personnel developing habitat management strategy at Upper Blue Point, and to BLM personnel working on restoration actions at several sites within the Gold Butte region.

Table 23. Small-scale habitat efforts by site, area, and years conducted.

Site	Area	Years
Boy Scout Canyon Springs	Black Canyon	2016, 2017, 2018
Bighorn Sheep Spring		2016, 2018
Pupfish Refuge Spring		2017, 2018
Salt Cedar Spring		2016
Upper Blue Point	Northshore	2016, 2018, 2019
Lower Blue Point		2019
Bearpaw Poppy Spring	Gold Butte region	2017, 2019
Horse Spring		2016, 2018
Quail Spring		2017

HEADSTARTING AND TRANSLOCATIONS

Over the project period, eggs were collected for headstarting from historical sites during the spring field season. Four historical sites in Black Canyon were targeted for collections: Bighorn Sheep, Black Canyon (Main and Side), Boy Scout Canyon, and Salt Cedar Canyon, with collections from at least one of these sites annually (Table 24). At Northshore, eggs were collected from Upper Blue Point and Rogers springs, but most collections were from Lower Blue Point (Table 24). Most of the egg masses from Lower Blue Point were found in an artificial fish-free pond established off the main stream channel prior to the project period (Jaeger et al. 2009) and maintained over the course of the project. Several full egg masses were collected in 2018 as part of an effort to estimate clutch size. In support of two research projects related to the impact of *Bd* on *R. onca*, sampling efforts were increased to collect from a greater diversity of egg masses from both Black Canyon and Northshore areas to increase genetic diversity of animals transferred to the research projects.

The earliest releases of headstarted animals occurred in late-March for both late-stage tadpoles and juvenile frogs, with releases generally completed by the end of June. During the project period, a small number of juvenile frogs were held for research projects and were released at later dates when not needed. A total of 3347 animals (472 tadpoles and 2875 juvenile frogs) were released to 9 different sites over the course of the project, including the augmentation of historical populations at Blue Point and Rogers springs (Tables 25 & 26). A total of 19 juvenile frogs were provided to the Las Vegas Springs Preserve in 2016, 2018, and 2019 for public display. A total of 487 headstarted animals (73 tadpoles and 414 juvenile frogs) from both Black Canyon and Northshore were provided to UNLV for research in 2016–2019; about 233 of the animals in 2019 are scheduled for return to Lower Blue Point in 2020 as part of the vaccination study.

Table 24. Sites and dates of egg masses of *Rana onca* collected for headstarting from 2016–2019. The number of egg masses from which collections were made is indicated, along with the approximate percentage of the egg mass collected.

Area	Year	Date	Site	No. of Egg masses	Proportion of mass collected
Black Canyon					
	2016	01/20	Black Canyon-Main	1	25%
		01/20	Bighorn Sheep Spring	1	25%
		01/20	Salt Cedar Canyon Spring	1	25%
	2017	02/03	Black Canyon-Side	1	67%
		02/03	Salt Cedar Canyon Spring	1	33%
		02/03	Salt Cedar Canyon Spring	1	50%
	2018	01/24	Black Canyon-Side	1	100%
		01/24	Salt Cedar Canyon Spring	1	100%
		02/02	Boy Scout Canyon Springs	1	25%
	2019	01/25	Boy Scout Canyon Springs	1	50%
Northshore Springs					
	2016	01/18	Lower Blue Point	1	50%
		01/30	Lower Blue Point	1	75%
		01/18	Upper Blue Point	1	50%
	2017	01/27	Lower Blue Point	1	50%
		02/01	Rogers Spring	1	50%
		02/06	Upper Blue Point	1	50%
	2018	01/23	Lower Blue Point	1	100%
		01/23	Lower Blue Point	1	100%
		01/29	Lower Blue Point	1	50%
	2019	02/07	Upper Blue Point	1	50%
		02/20	Upper Blue Point	1	75%
		01/22	Lower Blue Point	2	50% of each
		01/28	Lower Blue Point	1	50%
		02/07	Lower Blue Point	1	25%
		02/15	Lower Blue Point	1	75%
		02/15	Rogers Spring	1	50%
		02/20	Rogers Spring	1	50%

The current protocol is to release animals annually at each site for 5 years, after which the site is monitored for sustainability. This process was intended to establish a broad age-structure among animals (i.e. demographic diversity) and transfer the genetic diversity of the source population. Translocations at experimental sites initiated during the previous project (2011–2015) were completed during this project period at Bearpaw Poppy, Horse, and Lime springs in 2016, and at Corn Creek in 2019. Translocations initiated during the current project period were Kaolin Spring in 2016 and the Las Vegas Springs Preserve in 2018. Augmentation to Northshore sites using headstarted animals (originating at Northshore sites) occurred each year.

Table 25. Numbers of late-stage tadpoles and post-metamorphic frogs of *Rana onca* raised from eggs collected in Black Canyon and released at translocation sites from 2016–2019. Year when experimental sites were initiated are indicated.

Year	Date	Translocation Site	Initiated	Tadpoles Released	Frogs Released	Totals
2016	04/22	Corn Creek	2015	0	57	57
	05/04	Corn Creek		0	92	92
	05/27	Corn Creek		0	44	44
	07/01	Corn Creek		3	43	46
	07/15	Corn Creek		0	7	7
	05/18	Lime Spring	2012	0	45	45
	Total 2016			**3**	**288**	**291**
2017	05/18	Corn Creek	2015	0	37	37
	05/31	Corn Creek		31	56	87
	06/05	Corn Creek		3	35	38
	Total 2017			**34**	**128**	**162**
2018	04/14	Corn Creek	2015	0	146[a]	146
	04/20	Corn Creek		0	100	100
	04/25	Corn Creek		0	140	140
	05/09	Corn Creek		0	141	141
	05/30	Corn Creek		17	60	77
	05/29	Springs Preserve	2018	0	100	100
	Total 2018			**17**	**687**	**704**
2019	03/27	Springs Preserve	2018	50	0	50
	04/11	Springs Preserve		0	45	45
	05/09	Springs Preserve		0	16	16
	Total 2019			**50**	**61**	**111**
Cumulative Total				**104**	**1164**	**1268**

[a]Animals from Black Canyon and Northshore were mixed during prophylactic treatments prior to release. Nine of these animals died during the treatment and sources of these animals could not be determined. The numbers presented here assume proportional loss (i.e. 8 from Black Canyon and 1 from Northshore), see below Table 25.

Table 26. Numbers of late-stage tadpoles and post-metamorphic frogs of *Rana onca* raised from eggs collected at Northshore springs complex and released at translocation sites or returned to Northshore springs complex (augmentation) from 2016–2019. Year when experimental sites were initiated are indicated

Year	Date	Translocation Site	Initiated	Tadpoles Released	Frogs Released	Totals
2016	04/22	Corn Creek	2015	0	30	30
	05/27	Corn Creek		0	16	16
	07/15	Corn Creek		0	7	7
	05/07	Bearpaw Poppy Spr.	2012	0	117	117
	06/11	Bearpaw Poppy Spr.	2012	79	30	109
	05/14	Horse Spring	2012	30	31	61
	05/26	Kaolin Spring	2016	37	72	109
	06/30	Lower Blue Point		0	56	56
	04/06	Upper Blue Point		0	60	60
	Total 2016			**146**	**419**	**565**
2017	05/18	Corn Creek	2015	0	77	77
	06/05			0	1	1
	05/05	Kaolin Spring	2016	30	92	122
	05/12			25	58	83
	05/25			10	28	38
	05/10	Lower Blue Point		0	94	94
	05/02	Rogers Spring		0	60	60
	05/02	Upper Blue Point		0	60	60
	05/30			0	23	23
	Total 2017			**65**	**493**	**558**
2018	04/14	Corn Creek	2015	0	14[a]	14
	04/11	Kaolin Spring	2016	61	67	128
	05/21	Lower Blue Point		11	98	109
	05/01	Rogers Spring		0	105	105
	04/17	Upper Blue Point		0	70	70
	Total 2018			**72**	**354**	**426**
2019	05/28	Corn Creek	2015	0	66	66
	04/23	Kaolin Spring	2016	18	76	94
	05/14	Kaolin Spring		0	70	70
	03/27	Springs Preserve	2018	50	9	59
	04/11	Springs Preserve		0	41	41
	05/09	Springs Preserve		1	0	1
	04/29	Rogers Spring		0	63	63
	07/17	Rogers Spring		16	31[a]	47
	06/20	Upper Blue Point		0	58	58
	07/17	Upper Blue Point		0	31	31
	Total 2019			**85**	**445**	**530**
Cumulative Total				**368**	**1711**	**2079**

[a]Animals from Black Canyon and Northshore were mixed during prophylactic treatments prior to release. Nine of these animals died during the treatment and sources of these animals could not be determined. The numbers presented here assume proportional loss (i.e. 8 from Black Canyon and 1 from Northshore).

CONCLUSION AND RECOMMENDATIONS

The overall abundance of *R. onca* showed an increased trend in adult and juvenile frogs over the project period, with seasonal counts in 2017 and 2018 being the highest recorded since monitoring began (these counts were only one frog different from each other; Figure 1). The number of sites occupied increased from 19 in early 2016 to 21 in 2019. The large number of frogs reflect robust populations established at three translocation sites: Union Pass, Tassi, and Grapevine springs. The drop in number in 2019, however, reflects a real decrease, especially associated with the decline in the population at Horse Spring (Figure 1). As noted above, surface water at this site declined substantially and unexpectedly in mid-2018. The pond that maintained the moderately large population at this site disappeared. The reduction of water resulted in a 97% drop in the count of frogs at this site, along with the disappearance of larval stages.

The population at Pupfish Refuge Spring also declined during the project period and may be lost in the near future. The small population was maintained by recruitment from breeding habitat along a drainage ditch associated with Portal Road which runs through the site. That habitat, however, required almost annual maintenance, but during the latter part of the project period, BOR restricted such actions at the site. At Red Rock and Lime springs, monitoring has long indicated declining numbers of adult frogs, and limited or no recruitment. Limited aquatic habitat, especially for successful reproduction, appears to be the main issue at both these sites, and extirpation of *R. onca* is expected in the future.

Most sites are quite small, isolated, and maintain only small populations of *R. onca*, and several of these sites have persistent and documented habitat issues (CAS 2016). Management actions will be needed over the long-term to ensure population persistence, including occasional augmentation of animals to maintain genetic diversity or to recover demographically from stochastic events. A substantial issue at several sites is emergent vegetation encroachment that can degrade habitat for *R. onca*, which has been linked to previous extirpation (Bradford et al. 2004). Stopgap efforts to manage such vegetation (e.g. mechanical cutting) have been short-lived, and such actions are unlikely to be maintained over the long-term. At several sites, trespass cattle and feral burros keep emergent vegetation in check, but these animals may be removed in the future, as has occurred at the Northshore springs. More effective strategies need to be developed to limit vegetation overgrowth at problematic sites, along with plans for implementation. At some sites, successful reproduction has been limited to a few pools, and small scale, regular maintenance of the critical breeding habitat appears necessary to sustain populations over time.

The overall success of conservation actions for *R. onca* since the early 2000s was the main factor in the decision by USFWS to not list the species under the ESA. This achievement was driven by the aggressive translocation program which has succeeded in establishing several large, robust populations, as noted above. Identifying further translocation sites has been a challenge, with only two new sites established during the project period. One of these sites, Kaolin Spring, is a small spring that is unlikely to ever maintain a large population. The other site is an artificial mesocosum at the Las Vegas Springs Preserve and is also not likely to maintain a large population. A third site, Grapevine Springs, NV, was identified and assessed during the project period, and the process of permitting for translocation has been initiated. This site has the potential to maintain a moderately large population of *R. onca*, assuming climate and other environmental factors are favorable.

The long-term support of conservation actions by Clark County has demonstrated commitment by the people of southern Nevada to maintain local management of this unique species. The success of conservation actions summarized in this report, as well as successful actions conducted in previous bienniums, clearly demonstrates the efficacy of the current conservation strategy.

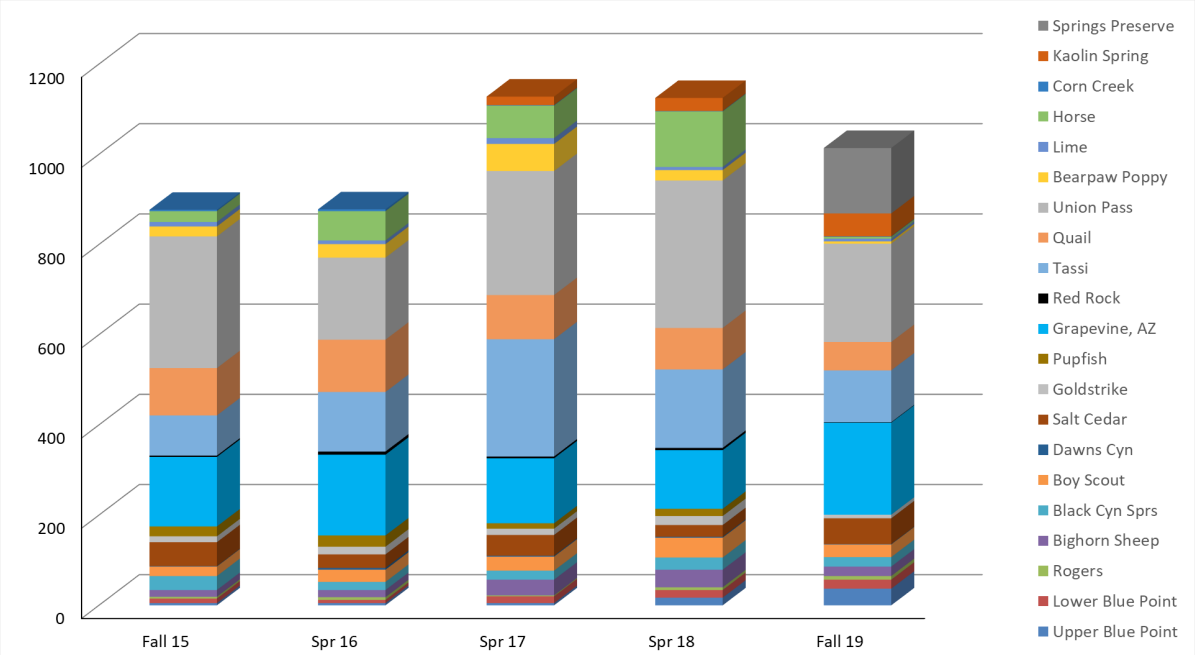

Figure 1. Count of *R. onca* adults and juveniles seen at all sites from 2016 through 2019, with 2015 included for reference. Numbers represent the highest count from visual encounter surveys during each year. Note that Kaolin Spring and Springs Preserve were added to the series.

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