

PROJECT: Relict Leopard Frog Conservation

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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 2011 with conservation actions extended through June 2015. The protracted project period was made possible through a matching project, funded by the Nevada Department of Wildlife (NDOW). Conservation actions were implemented as specified in a conservation agreement and strategy (CAS) for this species. The CAS was voluntarily entered into by several state and federal agencies, and is managed by a multiagency conservation team. Field efforts under this project included: population monitoring, headstarting and translocation actions, maintenance of important breeding habitats, and facilitation of research projects. The following is a summary of field accomplishments during the course of this project:

- Completed annual monitoring using nocturnal visual encounter surveys each spring and fall at all historical and experimental (translocation) sites occupied by *R. onca* (19 sites total during the spring 2015 field season).
- Conducted diurnal visual encounter surveys each spring at most occupied sites to assess breeding activity and to search for egg masses or tadpoles for headstarting (rearing) efforts.
- Conducted visual encounter surveys at one historical site where relict leopard frogs had been seen more than a decade ago and at a previously failed translocation site.
- Conducted field assessments of seven unoccupied springs for their potential as translocation sites; four of these sites are now active experimental sites.

- Completed five seasons of headstarting and translocations, releasing frogs and tadpoles to a total of 12 different sites, including augmentation of historical sites within the Northshore springs complex.
- Assisted with the establishment of five new experimental sites.
- Provided juvenile frogs from headstarting program to the Las Vegas Springs Preserve as part of their public educational display, and to the University of Nevada, Las Vegas (UNLV) for research funded by the Bureau of Land Management into the effects of the pathogenic amphibian chytrid fungus.
- Completed a tadpole developmental study at an experimental site to test water quality.
- Assisted targeted field sampling, conducted by UNLV with funding predominantly from NDOW, to detect the presence of the pathogenic amphibian chytrid fungus at 18 sites associated with *R. onca* conservation efforts.
- Assisted with a mark-recapture study conducted by UNLV under funding from U.S. Fish and Wildlife Service to estimate the overall population size of *R. onca*.
- Conducted maintenance actions of important breeding habitats at four sites and assisted federal agencies with habitat maintenance and restoration at five other sites; often actions at a particular site were repeated.
- Increased the number of sites occupied by *R. onca* by 4 experimental sites and increased the overall abundance of these frogs by 41% since project initiation.

INTRODUCTION

BACKGROUND – The relict leopard frog (*Rana onca* = *Lithobates onca*) historically occupied springs and wetlands along the drainages of the Virgin, Muddy, and Colorado rivers from southwestern Utah to Black Canyon just below Hoover Dam in Nevada and Arizona (Bradford et al. 2004). While the species appears to have been a narrow endemic (Jaeger et al. 2001; Oláh-Hemmings et al. 2010), by the latter part of the 20th century its 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). In the early 2000s, only 1100 adult frogs were estimated to exist (Bradford et al. 2004). Intensive searches did not find any other extant, historical populations outside of the areas occupied (Blomquist et al. 2003; Bradford et al. 2004); although, surveys in the western Grand Canyon documented a population of a closely related species (Jaeger 2010; Oláh-Hemmings et al. 2010).

The U.S. Fish and Wildlife Service (USFWS) was petitioned to list the species in 2002 under the Endangered Species Act (ESA), and while listing was considered warranted, it was precluded at that time predominately because of conservation actions coordinated by a voluntary team of local, state, and federal personnel (i.e. Relict Leopard Frog Conservation Team; RLFCT). As part of a recent court-approved settlement, however, the USFWS recognized *R. onca* as a ‘multi-district litigation species’ requiring a review of species status and a formal decision on listing (expected in 2016).

Since the early 2000s, specific management actions for *R. onca* have been prescribed in a conservation agreement and strategy (CAS), which was voluntarily entered into by several state and federal agencies (RLFCT 2005). The CAS was developed with consideration of a USFWS published Policy for the Evaluation of Conservation Efforts (PECE), designed to provide guidance for potential listing decisions under the ESA. In keeping with that policy, the intent of the CAS was to provide implementation of

effective conservation actions, as well as reasonable certainty that the conservation efforts would be effective. Consequently, the hope was that implementation of the conservation actions identified in the CAS would preclude the need to list the species. Management actions for *R. onca* have emphasized a translocation program, and populations have been established at numerous sites in southern Nevada and northwestern Arizona (see below). Some of these new populations have become well established, and recent monitoring efforts indicate a likely modest increase in overall population size of the species (Jaeger and Rivera 2013).

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 the 2005-2007, 2007-2009, and 2009-2011 bienniums. The current project was implemented by the National Park Service (NPS) in 2011 to continue monitoring, management, and conservation planning objectives as stipulated in the CAS. Actions under this project were extended through June 2015 (at no-cost to the Clark County), predominately through matching funding provided by the Nevada Department of Wildlife (NDOW). Major efforts under this project were performed by personnel from the University of Nevada, Las Vegas (UNLV) under task agreements with the NPS at LMNRA and NDOW. Detailed annual reports of field efforts associated with this project were submitted to Clark County. This document represents the final summary report for field efforts from 2011 through June 2015 under funding from the Clark County Multiple Species Habitat Conservation Plan (MSHCP; project number 2009-NPS-810A).

GOAL – Conserve existing *R. onca* populations and establish new experimental populations.

OBJECTIVES – The main field 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 specific 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. Coordinate a headstarting and translocation program to raise late-stage tadpoles or small frogs to establish new sites or augment existing populations.
5. Coordinate efforts to identify and establish new sites for translocations.
6. Assist collaborators in research efforts in support of management goal and objectives.

METHODS

The methods implemented in this project were specified by the RLFCT in the Relict Leopard Frog Protocol and Techniques Manual included in the 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.

MONITORING SURVEYS – In general, visual encounter surveys were used to monitor all sites known to contain *R. onca*. Survey crews consisted of two or more individuals headed by a biologist with extensive experience in *R. onca* monitoring. Most sites were linear in nature (stream systems or banks of a pond), but because of hazardous terrain (steep rocky cliffs) not all sites could be surveyed in their entirety. At a few sites, surveys covered designated stretches of area with distinct starting or stopping points.

Diurnal surveys were generally conducted from late January through March to document breeding activities (egg masses and tadpoles) during a prime breeding period. Nocturnal surveys during the spring (generally March – early June) and again in fall (generally mid-September – early November) were used to assess the relative abundance of frogs (both adults and juveniles); these frogs are more readily seen at night. Observations of juvenile frogs that could have been from eggs laid at the site were interpreted as evidence of recruitment into the adult population. To minimize disturbance, however, frogs were generally not captured during surveys, and only frogs that were obviously quite young (recently metamorphic individuals) were recorded as juveniles. Thus, our documentation of recruitment was conservative in that small frogs that might have been young-of-year were often counted as adults. Tadpole counts were often terminated after a large number had been counted with symbols used on datasheets to indicate that higher numbers were present.

ASSISTANCE TO RESEARCH EFFORTS – Assistance was provided to an independent study conducted by UNLV with partial funding from the USFWS to estimate populations at selected sites using mark-recapture. In 2011 and 2012, estimates from targeted sites were extrapolated to provide a rough estimate of the overall adult population size of *R. onca*. At sites where mark-recapture surveys were conducted ($n=5$), the surveys consisted of multiple, sequential sampling events with captured adult frogs permanently marked by small (8-12 mm) Passive Integrated Transponder (PIT) tags inserted subcutaneously. This approach allowed the accumulation of a high percentage of marked individuals at each site (often referred to as sequential mark-recapture or a ‘Schnabel’ approach; e.g., Caughley 1977). During most of the mark-recapture surveys, all observations of frogs, including those observed but uncaptured, were recorded which allowed each of these surveys to be interpreted as visual encounter surveys (these surveys are reported in the tables and figures below).

Assistance was also provided to independent research conducted by UNLV with funding mostly provided by NDOW to assess the presence and prevalence of an emergent amphibian pathogenic fungus, *Batrachochytrium dendrobatidis* (*Bd*) in southern Nevada. This fungus can cause the disease chytridiomycosis which has been linked to amphibian population declines and extinctions (e.g. Vredenburg et al. 2010). The emphasis of this research was on sampling for *Bd* in areas occupied by species of conservation concern including *R. onca*. Assistance was provided from the current project to assist with field efforts and testing associated with this species.

TRANSLOCATIONS – The diurnal surveys in spring were also used to collect eggs or occasionally very small tadpoles for headstarting in the laboratory. Once these animals had grown to late-stage tadpoles or juvenile frogs, they were released at experimental translocation sites or returned to augment the site of origin; the latter occurred at the Northshore springs complex. The RLFCT annually determined the target sites for translocation or augmentation, the rough numbers of animals to be moved, and source sites for the collections. Collected eggs were transported to a laboratory facility maintained by the LMNRA. This

site was also used to grow tadpoles and juvenile frogs, and to process all animals for release. Tadpoles were also grown-out in raceways at Willow Beach National Fish Hatchery (maintained by USFWS) and the Lake Mead State Fish Hatchery (maintained by NDOW).

RESULTS AND DISCUSSION

MONITORING OF HISTORICAL SITES IN BLACK CANYON

Bighorn Sheep Spring, NV – This site once contained about 50% of all *R. onca* (Bradford et al. 2004), but a large storm event in October 2006 caused debris flows which greatly reduced habitat quality and abundance of *R. onca* at this site. Habitat has not fully recovered. Since then, storms that produced substantial rains tended to shift destabilized gravels into any pools that have formed. Counts since the storm event have been much lower than in the past (highest count = 373 in fall 2004).

A large recruitment of juvenile frogs was noted in fall 2012 (Table 1). These frogs metamorphosed from four pools created in the stream using sandbags and rock in July 2011 by a crew under the guidance of NPS resource management. The pools were washed away by a storm prior to the fall survey in 2012, but earlier in the spring, the pools had contained large numbers of late-stage tadpoles. Mark-recapture surveys were attempted in fall 2012 as part of the UNLV research to estimate the adult population size. The mark-recapture effort, however, was abandoned because of the large number of juveniles recruiting into the adult population at that time (see Table 1). The somewhat higher counts of frogs in subsequent surveys were likely influenced by this 2012 recruitment event.

Table 1. Summary of *Rana onca* observed at Bighorn Sheep Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	01/21	14.1	0	0	> 600	2
	Nocturnal	04/14	19.4	18	0	2	0
	Nocturnal	10/12	23.0	10	6	> 200	0
2012	Diurnal	01/30	16.5	1	0	> 150	2
	Nocturnal	05/03	28.4	9	2	> 300	0
	Nocturnal	09/27	28.6	48	23	0	0
	Nocturnal	10/05	27.7	37	29	0	0
2013	Diurnal	02/14	23.7	0	1	> 200	5
	Nocturnal	04/12	24.6	18	11	28	0
	Nocturnal	10/21	22.9	15	2	2	0
2014	Diurnal	02/14	17.6	2	0	503	13
	Diurnal	02/19	25.2	4	0	> 182*	22
	Nocturnal	04/10	25.2	47	7	97	10
	Nocturnal	10/22	26.2	8	3	0	0
2015	Diurnal	02/01	16.6	0	0	133	1
	Nocturnal	04/19	27.5	37	0	93	0

* Species uncertain on 150

Over the course of the project, all life stages of *R. onca* were observed at Bighorn Sheep Spring, indicating active reproduction and recruitment. Partial egg masses, and in one case newly hatched tadpoles, were collected each spring for the translocation program (Table 21). Although the population is small, conditions in the stream for tadpole development were poor and low survivorship of tadpoles could be expected, thus the removal of some eggs probably had very little impact and possibly may have benefited the remaining animals by reducing competition.

Boy Scout Canyon Spring, NV – Most of the egg masses, tadpoles, and frogs observed at this site (Table 2) over the years have been associated with small pools in two side areas in the upper sections of the canyon. These pools contained cooler water than the main thermal stream. Small-scale maintenance to keep these important breeding pools from filling with debris or becoming choked with cattails (*Typha domingensis*) and tamarisk (*Tamarix* sp.) were conducted each year. All life stages were commonly observed at this site, indicating successful recruitment. Partial egg masses were collected for the translocation program each year except for 2013 (Table 21).

Table 2. Summary of *Rana onca* observed at Boy Scout Canyon during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	01/21	16.8	3	0	100	2
	Nocturnal	04/19	27.0	23	0	> 150	0
	Nocturnal	10/12	26.7	40	6	0	0
2012	Diurnal	01/30	16.6	8	0	0	1
	Diurnal	02/10	14.8	7	0	> 150	3
	Diurnal	10/27	23.4	16	0	0	0
	Nocturnal	04/12	23.0	30	4	50	0
	Nocturnal	10/16	23.8	34	3	0	0
2013	Diurnal	02/14	15.0	3	0	> 100	0
	Diurnal	03/01	24.2	12	0	5	0
	Nocturnal	04/19	21.6	44	5	> 100	0
	Nocturnal	10/11	24.0	29	2	0	0
2014	Diurnal	01/25	18.4	1	1	4	1
	Diurnal	02/19	21.1	0	0	45	2
	Nocturnal	04/15	25.0	35	1	2	0
	Nocturnal	10/16	27.0	27	0	4	0
2015	Diurnal	02/06	18.0	2	0	0	1
	Nocturnal	04/09	23.0	19	0	79	0

Shifting of the stream channel prior to the survey in spring 2011, created a small, cooler water side channel low in the drainage in which numerous large tadpoles were observed. By fall 2011, the overall count of frogs had doubled, with the observers noting that many of the frogs were small adults and likely young-of-year recruited out of the new side channel. Counts of frogs remained high through 2014, but further meandering of the stream degraded habitat conditions in the lower side channel and by fall 2014, frogs were no longer observed there. By 2015 the lower side channel was dry. Subsequently, observations of frogs in the lower section of the canyon declined dramatically, and the overall count in 2015 was

similar to what had been observed in spring 2011 and earlier. These observations point to the importance of the few small, cool water breeding sites in this canyon.

Dawn’s Canyon Spring, NV – This site consists of a small segment of stream located in a narrow canyon directly up river from Boy Scout Canyon. It has been speculated that the population at this site may be connected to the population in Boy Scout Canyon. There probably is habitat above the area surveyed, but steep canyon walls restrict the survey to a very short stream segment that extends to the Colorado River. Frogs have been consistently seen at this site, but no more than eight frogs have ever been counted (in spring 2011; Table 3). Observations of eggs and tadpoles have been predominately limited to a single plunge pool at the base of a small waterfall, but over the course of the project all life stages of *R. onca* have been observed indicating successful reproduction and recruitment at this site.

Table 3. Summary of *Rana onca* observed at Dawn’s Canyon Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Mass
2011	Diurnal	02/07	16.9	2	0	0	0
	Nocturnal	04/14	20.1	8	0	0	0
	Nocturnal	10/12	25.3	4	1	0	0
2012	Diurnal	02/24	17.0	1	0	> 100	1
	Nocturnal	05/03	29.4	5	1	25	0
	Nocturnal	10/16	24.8	4	0	0	0
2013	Diurnal	02/14	15.6	2	0	28	0
	Nocturnal	04/19	21.7	6	0	5	0
	Nocturnal	10/11	23.0	2	1	1	0
2014	Diurnal	02/19	19.3	0	0	33*	0
	Nocturnal	04/15	23.1	4	1	1*	0
	Nocturnal	10/16	29.0	6	0	0	0
2015	Diurnal	02/06	18.1	0	0	9	0
	Nocturnal	04/09	22.5	4	0	0	0

*Species identity uncertain

Black Canyon Spring and Black Canyon Side Spring, NV – These two areas represent components of the same system, although these areas were treated as separate sites for reporting. Black Canyon Spring represents a reach of stream fed by thermal springs that occur up drainage 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 during any given survey only a few frogs, if any, were observed (Table 4).

Black Canyon Side Spring consists of cold (non-thermal) waters from a spring that forms a series of pools in a rocky side canyon. This drainage flows to the main Black Canyon Spring drainage, but unless there is flooding, surface water from the side channel does not actually reach the main stream. Over the course of the project, *R. onca* showed active reproduction and recruitment, and all life stages were observed each

year at this site (Table 5). The somewhat larger counts of frogs starting in fall 2014 followed a flooding event that removed or flattened emergent vegetation that previously surrounded or filled pools. This event certainly increased the detectability of animals. The flooding, however, filled-in a large pool with gravel at the top of the system where numerous frogs had been previously observed. Black Canyon Side Spring was occasionally used as a source population for headstarting and translocation, with partial egg masses collected in 2011 and hatchling tadpoles (still clinging to egg mass) collected in 2013 (Table 21).

Table 4. Summary of *Rana onca* observed at Black Canyon Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	02/07	18.8	0	0	0	0
	Nocturnal	03/31	21.8	0	0	0	0
	Nocturnal	10/17	25.5	0	0	0	0
2012	Nocturnal	04/12	22.0	0	0	0	0
	Nocturnal	10/19	21.6	2	0	0	0
2013	Diurnal	02/22	14.4	0	0	0	0
	Nocturnal	04/27	25.5	1	0	0	0
	Nocturnal	10/11	24.0	6	0	0	0
2014	Nocturnal	04/17	29.1	3	0	0	0
	Nocturnal	10/16	27.5	5	0	0	0
2015	Diurnal	02/08	24.6	0	0	0	0
	Nocturnal	04/09	19.0	3	0	0	0

Table 5. Summary of *Rana onca* observed at Black Canyon Spring Side during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Site	Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
Side Spring	2011	Diurnal	01/28	14.0	6	0	12*	2
		Nocturnal	03/31	25.0	20	4	2	0
		Nocturnal	10/17	24.8	17	3	0	0
	2012	Diurnal	01/31	18.3	2	2	1	0
		Diurnal	02/24	20.5	1	0	> 100	1
		Nocturnal	04/12	22.4	19	3	23	0
	2013	Nocturnal	10/19	21.2	22	6	0	0
		Diurnal	02/16	18.1	0	0	10	1
		Diurnal	02/22	14.4	1	0	13	1
		Nocturnal	04/27	24.7	16	1	21	0
	2014	Nocturnal	10/11	19.7	10	3	0	0
		Diurnal	02/14	27.0	1	2	36	0
		Nocturnal	04/17	27.0	13	9	24	1
	2015	Nocturnal	10/16	24.4	37	7	1	0
Diurnal		02/08	16.9	3	0	543	1	
	Nocturnal	04/09	19.1	27	14	34	0	

* Species identity uncertain

Salt Cedar Canyon Spring, NV – This site was scoured by rain-caused debris flows in October 2006, which at the time appeared to have improved habitat for *R. onca*. Counts reached a high in 2009 of 47 frogs. Vegetation, however, had been rebounding at the site and by spring 2011 (at the beginning of the current project) vegetation had become quite dense diminishing the detectability of frogs. Only 11 frogs were counted that spring (Table 6). Raccoon prints and the skeletal remains of a frog, however, indicate the possibility of high predation in the system at that time. Surveyors also commented that the number of crayfish occupying a segment of the stream at the base of the canyon near the river appeared to have decreased from earlier years; crayfish (*Procambarus* sp.) are a common prey for raccoons. Observers recorded no further comments on raccoon signs after 2011.

Frog counts at this site have more than doubled from 2011 to 2015. Observers noted in 2011 and 2012 that most of the frogs were small, potentially young animals, indicating high recruitment. The number of frogs observed in fall 2013 was approaching the count recorded in 2009. Minor flooding in 2013 and 2014 flattened some vegetation and may have improved detectability of *R. onca*, inflating counts. But about half of the adults observed in fall 2014 were again small, likely young-of-year frogs. The frog count in spring 2015 was 64 frogs, included 23 juveniles. These observations indicate robust recruitment at this site, and all life stages of *R. onca* were commonly observed.

Table 6. Summary of *Rana onca* observed at Salt Cedar Canyon Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	01/28	20.7	5	0	50*	0
	Nocturnal	04/19	27.0	8	3	70	0
	Nocturnal	10/17	25.2	12	2	0	0
2012	Diurnal	01/31	16.8	6	0	9	0
	Diurnal	02/24	23.6	2	0	2	1
	Nocturnal	04/12	22.2	9	4	25	0
	Nocturnal	10/19	21.3	13	0	0	1
2013	Diurnal	02/16	20.2	0	0	4	0
	Diurnal	02/22	18.5	2	0	6	0
	Nocturnal	04/27	25.5	18	3	8	0
	Nocturnal	10/11	19.3	33	6	0	0
2014	Diurnal	02/14	23.6	4	1	5	2
	Nocturnal	04/15	21.6	9	3	18	0
	Nocturnal	10/16	25.6	47	1	0	0
2015	Diurnal	02/08	23.5	4	2	6	0
	Nocturnal	04/09	18.6	41	23	46	0

* Species identity uncertain

MONITORING OF SITES IN THE NORTHSORE SPRINGS COMPLEX

Blue Point Spring, NV – Surveys at Blue Point Spring have been split into upper and lower segments of the stream system. The upper segment, Upper Blue Point, consists of about 0.5 km of linear stream habitat from the springhead down to just below the Northshore Road where the water tunnels underground. The lower section, Lower Blue Point, consists of an area several hundreds of meters further downstream where the water reemerges.

Habitat in this system suffers from the presence of exotic fishes, predominantly mosquito fish (*Gambusia affinis*) and tropical aquarium fishes including cichlids (*Cichlasoma*) and mollies (*Poecilia*). Predation on anuran larvae by mosquito fish has been well documented (McDiarmid and Altig 2000), and cichlids and mollies are both opportunistic predators. The stream banks and waters are also mostly covered in dense mats of sedges (*Scirpus* and *Eleocharis*) or by stands of reeds (*Phragmites*) and cattails (Jaeger et al. 2009). Encroachment of emergent vegetation appears to have gotten worse since removal of feral cattle and burros from the Northshore area in the early 2000s; their grazing and trampling had maintained low levels of vegetation disturbance along the stream (Jaeger et al. 2009). Mitigation efforts focused on reducing vegetation along short segments of stream to maintain some stretches of open habitat. Such actions were performed annually by personnel from NPS, NDOW, BLM, and UNLV. Tunneling of the stream in the porous soils also has been a problem over the years, with large stretches of the stream disappearing underground.

At Upper Blue Point, the general trend of high-counts suggests a decline in abundance of *R. onca* as compared to counts earlier in the project period (Table 7). The counts in 2011 and early 2012 were influenced by the release of headstarted animals back in 2008 and a smaller release in 2010. The early counts, however, also coincided with more intensive mark-recapture sampling (see below). More recently, from 2013 – 2015, a total of 101 animals, both juvenile frogs and tadpoles, were released at this site (see Table 23). The population at this site was estimated by UNLV researchers seasonally from 2011 – 2013 by mark-recapture; standard nocturnal visual encounter surveys were conducted in 2014 – 2015. Mark-recapture estimates derived from data collected in fall 2011 and spring 2012 provided population estimates of 47 adults (42 – 64, 95% CI) and 56 adults (50 – 84, 95% CI), respectively (Jaeger and Rivera 2013). Observations of tadpoles and juvenile frogs at Upper Blue Point were limited, but there was some evidence of natural recruitment of juvenile frogs at the site in 2011, 2012, and early 2013. Juvenile frogs recorded in fall 2013 and afterwards could easily have been from headstarted animals released at the site. Eggs were collected for headstarting in 2012 – 2014 (Table 21).

Vegetation reductions along stretches of Upper Blue Point maintained some open stream habitat over the project period. In 2012, a set of small pools were created along the stream bank to increase breeding habitat for *R. onca*. Adult frogs were commonly observed using the pools, and subsequent surveys noted an egg mass in one pool and tadpoles in two of the pools. Maintenance efforts have kept two of these pools cleared of vegetation through the end of the current project period.

In 2014, there was concern from NPS hydrologist, Geoff Moret, about an overall drop in the water level emitted by the spring and further investigation has been initiated by NPS. Of more immediate impact was the continued erosion of a historical earthen dam and the subsequent loss of a large instream pool that it

once formed. Breeding adults and large tadpoles had been commonly observed in the pool. More positively, following heavy rains an off-channel pond formed in 2014 near the Northshore Road. This pond appeared to hold water over time and may provide new fish-free breeding habitat for *R. onca*, at least until emergent vegetation covers the open water. Further assessment of the pond is needed.

Table 7. Summary of *Rana onca* observed at Upper Blue Point Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	02/08	15.0	0	0	0	0
	Nocturnal	03/29	18.2	13	0	0	0
	Nocturnal	04/04	19.5	19	0	0	0
	Nocturnal	05/02	19.3	30	1	0	0
	Nocturnal	05/21	28.4	26	0	0	0
	Nocturnal	06/06	26.4	25	1	0	0
	Nocturnal	09/28	27.8	22	0	0	0
	Nocturnal	10/09	19.3	12	1*	0	0
	Nocturnal	10/15	26.7	20	0	0	0
	Nocturnal	10/27	17.0	22	0	0	0
	Nocturnal	11/10	13.2	21	0	0	0
2012	Diurnal	02/07	14.1	3	0	0	1
	Nocturnal	02/18	14.0	19	0	0	0
	Nocturnal	03/04	13.4	24	0	0	0
	Nocturnal	03/09	07.4	26	0	0	0
	Nocturnal	04/06	10.3	19	0	0	0
	Nocturnal	04/10	25.0	21	0	0	0
	Nocturnal	04/16	20.1	20	0	6	0
	Nocturnal	05/18	26.3	20	0	1	0
	Nocturnal	09/17	23.0	11	1	0	0
	Nocturnal	09/25	20.9	11	0	0	0
	Nocturnal	10/02	28.3	22	0	0	0
	Nocturnal	10/07	19.0	16	0	0	0
	Nocturnal	10/14	20.8	14	2	0	0
	Nocturnal	11/04	16.9	15	0	9*	0
2013	Diurnal	02/05	17.5	0	0	0	1
	Diurnal	02/05	22.6	1	0	1*	1
	Diurnal	02/06	17.9	0	0	0	0
	Diurnal	02/13	17.5	0	0	0	0
	Diurnal	02/21	18.5	0	0	25*	0
	Diurnal	03/07	24.7	3	0	0	0
	Diurnal	03/12	15.1	4	0	1*	0
	Diurnal	06/26	41.7	3	0	1	0
	Nocturnal	03/22	13.1	12	0	4	0
	Nocturnal	03/25	20.0	14	0	30	0
	Nocturnal	03/30	16.0	14	0	1	0
	Nocturnal	04/10	13.8	10	1	0	0
	Nocturnal	04/21	23.9	5	0	2	0
	Nocturnal	05/27	22.9	8	0	4	0
Nocturnal	09/27	20.0	8	0	0	0	

Table 7. Continued

	Nocturnal	10/17	15.0	6	0	0	0
	Nocturnal	10/22	15.8	11	0	0	0
	Nocturnal	11/01	15.7	7	1	0	0
2014	Diurnal	02/05	17.1	2	0	0	0
	Diurnal	02/08	21.2	0	0	0	0
	Diurnal	02/11	20.6	0	0	0	0
	Diurnal	02/20	19.2	0	0	0	2
	Diurnal	02/26	27.0	0	0	0	0
	Diurnal	03/04	20.3	0	0	0	1
	Diurnal	08/11	36.5	0	0	0	0
	Nocturnal	03/20	13.4	13	0	0	0
	Nocturnal	10/20	22.8	3	3	0	0
	Nocturnal	11/09	17.2	9	5	0	0
2015	Diurnal	01/28	15.5	0	0	0	0
	Diurnal	06/09	30.9	0	0	0	0
	Nocturnal	04/13	25.5	13	1	0	0

*Species identity uncertain.

Counts of *R. onca* at Lower Blue Point have been consistently low (Table 8), with observations of frogs sparsely scattered from where the water reemerges to about 400 meters downstream, particularly in areas where habitat was recently modified. *Bd* has been detected along this lower stretch of stream, and this pathogen may be a factor in the decline of *R. onca* counts at this site over the years. Egg masses and tadpoles were commonly observed during the spring surveys, and eggs were collected for headstarting during most years (Table 21). Natural recruitment into the adult population, however, has been difficult to discern. Augmentations of headstarted animals occurred in all years except 2013 (Table 23) and could easily account for the young frogs documented. The observations of juvenile frogs in spring 2014, prior to the release of animals, provided the only good evidence of natural recruitment at this site during the study period.

Habitat maintenance at Lower Blue Point focused on rehabilitating a relatively large fish-free pond created in 2007 (Jaeger et al 2009). The pond was intended as breeding habitat for *R. onca* and since its creation has been regularly utilized by the species for breeding. In 2012, the pipe that provided water to the pond was relocated further downstream in response to down-cutting of the stream channel. Sedimentation accumulating around the pipe's intake has been a continuous problem requiring regular clearing. Blockage of the pipe has caused unstable flows to the pond. Drying occurred during summer months in 2011, 2012 and 2014, which likely decreased potential recruitment from the pond.

Vegetation reduction along short stretches of Lower Blue Point was conducted regularly, but heavy burro activity was apparent in 2012 and the disturbed area was favored by *R. onca* the following year. Vegetation reduction was performed in this area in 2014 to maintain the open habitat. Shortly after, eggs were collected from an egg mass deposited in the modified habitat. Down-cutting of the channel appeared to be a problem where vegetation had been reduced over long stretches of stream, but in early 2015, tadpoles of *R. onca* were observed within the disturbed area.

Table 8. Summary of *Rana onca* observed at Lower Blue Point Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	02/08	17.8	1	0	20	4
	Diurnal	02/11	22.1	0	0	20	3
	Nocturnal	03/29	13.9	6	0	0	0
	Nocturnal	07/16	22.9	6	12	0	0
	Nocturnal	10/18	24.3	4	2	0	0
2012	Diurnal	02/07	15.4	1	0	0	2
	Diurnal	05/19	30.5	0	0	0	0
	Diurnal	06/30	43.2	1	0	0	0
	Nocturnal	02/21	12.2	8	4	50	0
	Nocturnal	03/07	10.5	4	4	1	0
	Nocturnal	10/26	14.8	7	0	0	0
2013	Diurnal	02/06	25.5	0	0	0	0
	Diurnal	02/13	22.5	1	0	0	0
	Diurnal	02/21	17.2	0	0	50	0
	Diurnal	03/07	23.2	0	0	23*	1
	Diurnal	03/12	25.2	3	0	22	0
	Nocturnal	03/22	14.5	11	0	0	0
	Nocturnal	10/17	8.8	16	3	0	0
2014	Diurnal	02/05	17.4	0	0	0	0
	Diurnal	02/08	23.2	1	2	0	0
	Diurnal	02/11	20.5	0	0	0	1
	Diurnal	02/20	19.6	0	0	34	1
	Diurnal	02/26	23.8	0	0	45*	0
	Diurnal	05/07	23.9	0	0	0	0
	Diurnal	08/11	42.2	1	0	0	0
	Nocturnal	04/16	20.1	8	3	4	0
	Nocturnal	10/20	19.5	16	1	0	0
2015	Diurnal	01/28	21.5	2	0	213	3
	Diurnal	06/09	31.7	0	0	0	0
	Nocturnal	04/13	26.1	12	2	0	0

* Species identity uncertain for some of the tadpoles

Rogers Spring, NV – This thermal stream system is closely situated to Blue Point and similarly suffers from exotic fishes and dense, emergent vegetation, in this case, dominated by tall mats of 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. Occasional augmentation of this site began in the summer of 2008, using juvenile frogs and tadpoles raised from eggs collected from sites in the Northshore springs ($n = 111$ to date; see Table 23 for augmentations that occurred during this project period). Since augmentations began, frogs have been regularly observed during surveys (Table 9).

The majority of *R. onca* seen at this site in recent years occupied areas of open habitat near the power-line road that bisects the stream. Frogs in this area occur in pools formed by a collapsed drainage pipe under the road and tire ruts within the road, and a side channel where water runs through a dense patch of compressed, short vegetation. Headstarted animals have mostly been released to these areas because there

is almost no other open water along the stream. Starting in 2014, however, surveyors noted that emergent vegetation was growing dense even in these few open areas. While breeding attempts were documented at this site during the study period, observations of juvenile frogs in 2012 and 2014 most likely represent headstarted animals released earlier. Eggs from this site were collected for headstarting in 2014 (Table 21).

Table 9. Summary of *Rana onca* observed at Rogers Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is shown.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	03/12	28.0	3	0	0	0
	Nocturnal	04/12	15.1	6	0	0	0
	Nocturnal	10/11	22.9	3	0	0	0
2012	Nocturnal	03/16	23.8	13	1	50	0
	Nocturnal	10/15	23.6	11	0	0	0
2013	Diurnal	02/07	25.5	0	0	35	1
	Nocturnal	04/04	27.4	18	0	0	0
	Nocturnal	10/30	11.8	11	0	0	0
2014	Diurnal	02/05	11.8	0	0	0	0
	Diurnal	02/11	16.9	1	0	0	1
	Diurnal	08/12	33.0	1	0	0	0
	Nocturnal	03/27	14.5	3	0	38	0
	Nocturnal	10/13	18.7	11	3	0	0
2015	Diurnal	01/28	11.7	0	0	0	1
	Nocturnal	04/21	19.5	11	0	0	0

MONITORING OF EXPERIMENTAL TRANSLOCATION SITES

Goldstrike Canyon, NV – A population of *R. onca* was established at this site by translocations conducted from 2004 – 2009. A total of 2185 tadpoles derived from eggs collected at other sites within Black Canyon were released. The counts over time reflect relatively low abundance (Table 10). Breeding has been common, although pools selected by these frogs for breeding appear limited. Recruitment of frogs into the adult population was first documented by the observation of a juvenile frog born at the site in 2012. This site is heavily used by tourist seeking the hot springs, which may have negative consequences for frogs.

The site was again augmented after the spring survey in 2013 with 88 juvenile frogs and tadpoles, and again with 23 juvenile frogs after the fall survey that year (Table 22). This augmentation was an opportunistic action resulting from an excess of Black Canyon animals produced by the headstarting program that year. Interestingly, the counts from subsequent surveys did not appear to reflect an increase in frogs from these augmentations.

Table 10. Summary of *Rana onca* observed at Goldstrike Canyon during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is shown.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	01/28	15.8	3	0	> 300	3
	Nocturnal	04/14	21.4	15	0	50	0
	Nocturnal	10/23	27.9	9	0	17	0
	Nocturnal	11/09	20.8	12	0	12	1
2012	Diurnal	02/26	18.1	2	0	32	1
	Nocturnal	04/07	19.5	29	1	> 200	0
	Nocturnal	10/29	21.9	14	1	0	0
2013	Diurnal	02/19	18.2	3	0	0	5
	Diurnal	06/13	33.1	1	1	4	0
	Nocturnal	04/22	27.8	26	0	> 100	0
	Diurnal	06/28	38.5	1	0	0	0
	Nocturnal	10/07	25.3	15	0	5	0
2014	Diurnal	02/24	19.3	2	0	21	1
	Nocturnal	04/09	28.0	20	6	1	1
	Nocturnal	10/18	27.8	12	0	9	0
2015	Diurnal	02/06	16.2	1	0	5	2
	Nocturnal	04/17	23.9	17	2	4	0

Grapevine Spring (Meadview), AZ – This highly successful experimental site currently contains one of the most abundant populations of *R. onca*. As part of UNLV research, the population was estimated by mark-recapture methods in spring 2012 at 510 adult frogs (400–737, 95% C.I). Translocations to this site ended in 2009 following five years of augmentation during which 3820 tadpoles derived from eggs collected at sites in Black Canyon were released. Over the course of the current project, all life stages of *R. onca* were observed each year during surveys (Table 11). 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.

This narrow canyon floods on occasion, and a storm in October 2010 produced a substantial flood that greatly reduced vegetation within the stream. Habitat conditions appeared good at the beginning of this project period (2011), but vegetation, particularly cattails and monkey flowers (*Mimulus*), quickly grew dense and impacted visibility during later surveys. A smaller flood sometime between the spring and fall surveys in 2014 once again reduced emergent vegetation within portions of the stream, improving detectability and likely the habitat for *R. onca*.

Pupfish Refuge, NV – The population of *R. onca* at this site was established by translocations from 2003 – 2008. A total of 541 juvenile frogs derived from other Black Canyon sites were released. The relative abundance of this population is not large, but the population appears robust (Table 12). Breeding activity has been consistent and natural recruitment was evident as early as 2009 and 2010. Since then juveniles frogs have been observed annually. Mark-recapture efforts led by UNLV were conducted at this site in 2012, and the population was estimated at 75 adults (70 – 86, 95% CI).

Table 11. Summary of *Rana onca* observed at Grapevine Spring (Meadview, AZ) during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is shown.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	03/05	19.3	98	0	6	0
	Nocturnal	04/22	20.2	146	2	> 325	11
	Nocturnal	10/19	22.0	71	1	7	0
2012	Nocturnal	04/24	26.6	90	9	> 1000	13
	Nocturnal	04/27	23	99	10	> 300	16
	Nocturnal	05/22	33.7	179	10	10	1
	Nocturnal	10/17	18.9	93	1	12	0
2013	Nocturnal	03/14	18.5	51	0	6	12
	Nocturnal	05/03	19.4	124	1	> 110	1
	Nocturnal	10/04	18.2	85	3	9	0
2014	Diurnal	03/07	20.8	28	0	2	11
	Nocturnal	05/14	21.4	113	3	18	2
	Nocturnal	10/17	21.4	143	7	11	0
2015	Diurnal	03/08	20.0	31	1	22	7
	Nocturnal	04/30	26.0	158	1	132	0

Table 12. Summary of *Rana onca* observed at Pupfish Refuge Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses	
2011	Diurnal	02/15	20.0	2	0	> 1050	1	
	Nocturnal	04/21	27.9	31	0	56	2	
	Nocturnal	10/03	29.6	16	2	0	0	
	Nocturnal	10/13	29.5	25	0	0	0	
2012	Nocturnal	03/08	18.9	45	2	> 500	1	
	Nocturnal	03/15	27.1	42	2	> 500	1	
	Nocturnal	04/02	20.7	25	0	> 500	1	
	Nocturnal	04/09	30.5	35	0	> 500	2	
	Nocturnal	04/16	26.5	24	0	> 300	0	
	Nocturnal	04/30	33.4	21	1	> 300	0	
	Nocturnal	10/24	21.1	24	5	30	4	
	Nocturnal	10/30	20.0	20	4	> 550	0	
	2013	Diurnal	02/25	17.6	0	0	> 1000	0
		Nocturnal	03/28	23.7	39	0	> 300	4
Nocturnal		10/25	25.9	23	1	1	0	
2014	Diurnal	02/25	22.9	4	0	26	2	
	Nocturnal	04/07	24.9	35	4	9	4	
	Nocturnal	10/08	28.7	21	5	2	0	
2015	Diurnal	02/05	14.6	1	0	3002	1	
	Nocturnal	04/04	25.8	27	3	160*	0	

*Species identify uncertainty for 106 tadpoles

Starting in 2009, the Bureau of Reclamation conducted incremental actions to remove tamarisk from the Pupfish Refuge drainage and establish native mesquite (*Prosopis*) and willow (*Salix*) trees. These trees have not yet grown enough to shade the system and emergent vegetation (predominately bunch grasses and cattails) have grown dense along the stream; management is needed. Exotic snails (*Melanoides* sp.) appear to have been transported to the site sometime in the mid-2000s. These snails have proliferated and have visibly reduced algae in the stream. Observations of large concentrations of snails around egg masses and the subsequent disappearance of tadpoles suggest that these snails consume eggs or hatchlings. Successful reproduction has been routinely documented predominately in cooler waters running along the drainage ditch of Portal Road where snails do not appear in high density. Accumulated debris and emergent vegetation were removed from important breeding pools along the drainage ditch in 2011, 2012, and 2014, predominately by personnel from UNLV and NPS. Regular maintenance of these breeding pools seems to be a necessity for continued successful reproduction at this site.

Quail Spring, NV – This small site maintains an abundant population of *R. onca*, with nocturnal counts from visual encounter surveys routinely well over 100 adult frogs (Table 13). The site was established by translocation of 597 juvenile frogs and tadpoles from 2008 – 2012 (see Table 22 for 2011 and 2012 releases). Sites in Black Canyon were used as the sources of released animals. Breeding activity and overwintering of tadpoles has been commonly documented, with natural recruitment confirmed in 2014.

Table 13. Summary of *Rana onca* observed at Quail Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	03/11	25.0	81	0	3	7
	Nocturnal	05/14	25.2	161	3	15	0
	Nocturnal	10/23	20.0	96	0	16	0
2012	Diurnal	02/27	16.0	20	0	> 30	10
	Nocturnal	03/28	17.2	114	0	> 323	0
	Diurnal	04/29	28.2	1	0	0	0
	Nocturnal	10/09	20.3	117	4	17	0
2013	Diurnal	02/10	15.9	2	0	0	1
	Nocturnal	04/28	21.0	143	11	53	0
	Nocturnal	10/06	20.4	83	8	0	0
2014	Nocturnal	03/25	18.8	127	37	13	0
	Nocturnal	10/04	23.2	76	0	1	0
	Nocturnal	11/10	20.7	31	3	4	0
2015	Diurnal	02/10	22.7	6	0	8	4
	Nocturnal	03/25	19.0	120	2	70	0

Quail Spring 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. Cattails could easily overrun the pond if left undisturbed, and BLM and UNLV personnel have conducted actions to improve habitat on several occasions. Grazing in and around the ponds by trespass cattle, however, has been responsible for preventing cattails and other vegetation from completely choking the open water areas. Once the trespass

cattle are removed, this site would not likely sustain *R. onca* unless regular and substantial maintenance of vegetation is conducted or some other habitat alteration is performed to minimize vegetation.

Red Rock Spring, AZ – An initial five years of translocations of *R. onca* to this site ended in 2010. A total of 620 juvenile frogs and tadpoles derived from sites in Black Canyon were released. Counts from nocturnal visual encounter surveys over the years indicated relatively few adult frogs (Table 14). The population of adult frogs was estimated by mark-recapture conducted by UNLV in fall 2011 and again in spring 2012. The population was essentially estimated at just less than 20 adult frogs during both periods. Observations of egg masses and young larvae confirmed active breeding (Table 14), but natural recruitment of juvenile frogs has never been confirmed. In spring 2012, however, several large, overwintered tadpoles were observed and these likely reached metamorphoses. Red Rock Spring suffers from unstable water levels and surface flows during summer often reduce to minor trickles. In general, the pools where *R. onca* has attempted to breed rarely maintained surface water through summer months.

Table 14. Summary of *Rana onca* observed at Red Rock Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	03/03	25.3	7	0	0	0
	Diurnal	07/13	37.4	13	0	4	0
	Nocturnal	04/26	20.2	19	0	0	1
	Nocturnal	10/14	22.2	14	0	0	0
	Nocturnal	10/21	16.5	16	0	0	0
	Nocturnal	10/28	12.4	12	0	1	0
2012	Nocturnal	02/29	15.6	1	0	0	0
	Nocturnal	03/22	15.2	13	0	0	6
	Nocturnal	03/26	10.8	15	0	4	6
	Nocturnal	03/30	17.0	10	0	> 1000	0
	Nocturnal	04/09	18.6	13	0	> 200	0
	Nocturnal	10/01	20.8	10	0	47*	0
	Nocturnal	11/01	15.6	5	0	0	0
2013	Diurnal	02/10	9.2	0	0	0	0
	Diurnal	06/12	45.2	2	0	0	0
	Nocturnal	04/28	15.8	5	0	0	1
	Nocturnal	05/21	22.0	7	0	0	0
	Nocturnal	10/14	11.5	0	0	0	0
	Nocturnal	10/24	18.6	3	0	0	0
2014	Diurnal	06/12	40.0	3	1	0	0
	Diurnal	08/09	36.2	3	1	1	0
	Nocturnal	03/19	8.1	3	0	0	0
	Nocturnal	06/03	21.3	6	0	0	0
	Nocturnal	10/04	16.2	17	3	0	0
2015	Diurnal	02/11	23.6	0	0	0	0
	Nocturnal	03/23	15.8	12	1	1000	1

*Species identity uncertain

Although the initial translocations to this site were completed in 2010, excess animals were available from headstarting in 2013 and 2014, and a total of 193 animals derived from Black Canyon were again released at this site (Table 22). The juvenile frogs observed in 2014 and 2015 were in all likelihood from these releases. Augmentations appeared to have little effect on subsequent counts.

Tassi Spring, AZ – This site has maintained a relatively abundant population of *R. onca*, with high counts on occasion reaching over 100 adult frogs (Table 15). The population was established by release of 1198 juvenile frogs and tadpoles over five years ending in 2010. These animals were derived from sites within Black Canyon. Egg masses and young tadpoles were observed shortly after the initial translocations (as early as 2007), indicating active reproduction. During the current project period, observations of younger life-stages of *R. onca* have annually been noted. Natural recruitment was suspected early-on and was confirmed in 2012 with the observation of juvenile frogs that could not have been from animals released at the site.

Table 15. Summary of *Rana onca* observed at Tassi Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2011	Diurnal	03/03	25.7	52	0	> 800	6
	Nocturnal	03/16	25.9	77	4	> 100	2
	Nocturnal	10/15	22.2	85	10	50*	2
2012	Nocturnal	02/23	19.8	46	0	> 310	18
	Nocturnal	03/24	22.4	113	9	> 500	1
	Nocturnal	09/23	26.3	49	2	0	0
	Nocturnal	10/21	23.0	115	1	5	0
2013	Diurnal	02/28	22.0	20	0	> 400	2
	Nocturnal	04/24	16.7	125	3 [^]	21	2
	Nocturnal	10/13	20.5	0	0	4	0
	Nocturnal	10/23	19.5	1	0	11*	0
2014	Nocturnal	03/28	17.2	103	4	11	4 [^]
	Nocturnal	10/11	26.6	47	3	> 2200	0
2015	Diurnal	02/19	26.2	6	0	3146*	0
	Nocturnal	05/03	22.2	45	0	120	0

*Species identification uncertain; applies to ~700 of the tadpoles observed during diurnal survey in 2015

Large swings in seasonal detectability of frogs at Tassi Spring has been observed, with fall surveys producing low 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 colder air than at other sites and affect frog activity. The lower count in spring 2015 was probably affected by the late timing of the survey and to some extent, low detectability of frogs along the upper historical channel where emergent vegetation has become quite dense. A large flood before the fall 2014 survey, however, may have also reduced adult numbers along the lower stretch of stream. The flooding scoured the stream stretch within the large wash below the historical channel and ranch house. This event removed breeding habitat that had been previously created

by NPS actions to improve drainage near the ranch house, but it also reduced vegetation along a large stretch of stream that now allows better detection of frogs. The survey at that time documented large numbers of tadpoles along the newly opened stretch of stream; this was also noted in spring 2015.

The historical channel below the springheads has received some maintenance over the years by NPS in order to protect the integrity of the historical ranch located downslope; however, federal personnel have been recently restricted from working in the Gold Butte area because of safety issues associated with an attempt to remove trespass cattle. In the last several years, repair and replacement of fencing in the area has also restricted cattle and burros from grazing the historical channel. Management action to remove emergent vegetation from the channel is urgently needed to maintain integrity of the channel, along with control actions for invasive Scotch thistle (*Onopordum acanthium*).

Perkins Pond, NV – Modification of this large artificial pond to make it acceptable for *R. onca* translocation was completed by BLM in early 2010; funding for this effort was reimbursed to BLM by Clark County, the owner of the pond. Translocations of animals to this site started that year and continued through 2014. Headstarted animals from sites in the Northshore springs were used, and a total of 1819 frogs and tadpoles were released (see Table 23 for releases from 2011 – 2014). Observations of calling males in 2013 and 2014 documented overwintering which indicated some level of success at the site. However, very few frogs were ever observed (Table 16) and evidence of breeding attempts was never documented.

Speculation on the limited observation of *R. onca* included low detectability because of the dense vegetation at the site. Detectability certainly was not optimal, but surveys along the pond's bank were often supplemented with personnel in a kayak paralleling the shore. A more plausible explanation for the lack of frogs was low survivorship. Likely stressors included predation from aquatic birds often seen in the pond (e.g., herons, egrets), poor water quality, fluctuations in water levels that could have affected predation rates and overwinter survival, and the presence of *Bd*. Speculation that water quality may not have allowed tadpoles to successfully metamorphose was addressed by an experiment in 2012 when late-stage tadpoles (Gosner stage 39 – 41) were released into two mesh-lined containers placed in the pond. All these tadpoles metamorphosed into healthy-looking frogs. *Bd* was detected on chorus frogs (*Pseudacris regilla*) in the pond in 2013. Although the pond is surrounded by an exclusion fence designed to restrict predatory bullfrogs (*Lithobates catesbeianus* = *Rana catesbeiana*), in 2013 and 2014 a bullfrog was detected within the pond.

Water to the pond was supplied by a well and pumped through a pipe laid within a cement ditch. This water infrastructure was severely damaged by storms in September 2014 and water inflow stopped at that time. NDOW personnel reported that the pond was dry on January 16, 2015. Shortly thereafter, Clark County decided that it would no longer support *R. onca* at the site. A nocturnal survey was conducted at the end of May 2015. The pond at that time was filled with water. During the survey, recorded *R. onca* calls were broadcast, but no *R. onca* were detected.

Table 16. Summary of *Rana onca* observed at Perkins Pond during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses	
2011	Nocturnal	02/23	14.8	0	0	0	0	
	Nocturnal	04/05	25.1	0	0	0	0	
	Nocturnal	05/05	25.8	0	0	0	0	
	Nocturnal	10/10	20.5	2	0	0	0	
2012	Diurnal	07/26	40.9	0	0	0	0	
	Nocturnal	02/27	12.5	0	0	0	0	
	Nocturnal	03/15	10.6	0	0	0	0	
	Nocturnal	03/20	11.3	0	0	0	0	
	Nocturnal	04/20	23.8	0	0	0	0	
	Nocturnal	05/31	24.2	0	2	0	0	
	Nocturnal	06/23	33.2	0	0	0	0	
	Nocturnal	09/19	21.4	4	0	0	0	
	2013	Diurnal	05/24	29.2	0	0	0	0
		Diurnal	06/25	35.5	0	0	0	0
Nocturnal		04/30	25.5	2	0	0	0	
Nocturnal		05/15	27.1	1	0	0	0	
Nocturnal		03/25	13.3	0	0	0	0	
Nocturnal		10/10	12.5	1	0	0	0	
2014	Diurnal	02/13	14.6	0	0	0	0	
	Diurnal	06/20	36.7	0	0	0	0	
	Nocturnal	04/24	16.0	0	0	0	0	
	Nocturnal	05/01	15.4	1	0	0	0	
	Nocturnal	10/03	19.0	0	0	0	0	
	Nocturnal	10/27	20.1	0	0	0	0	
2015	Nocturnal	05/30	21.5	0	0	0	0	

Union Pass Spring, AZ – The count of *R. onca* at this site during the fall survey in 2014 was > 200 adult and juvenile frogs (Table 17), indicating a relative abundance rivaling that at Grapevine Spring. The final translocation to this site was completed in spring 2015 after five years of releases totaling 868 Black Canyon animals (Table 22). Survivorship of released animals appears to have been high. Observations of large numbers of egg masses and tadpoles indicated a robust population, although natural recruitment can only be suspected (and not yet confirmed) because the juvenile frogs regularly observed could be from animals released at the site.

Juvenile frogs and tadpoles were initially released in two upper areas of the stream, but over the course of this project, frogs have dispersed throughout most of the stream system. Moderate levels of cattle and burro grazing along the stream has created a more open habitat favored by these frogs. At the two initial release sites in 2011, overhanging vegetation of black willow (*S.nigra*) and scrub oak (*Quercus* sp.) were reduced to allow more sunlight to reach the pools. This action needs to be repeated as the vegetation has again grown dense.

Table 17. Summary of *Rana onca* observed at Union Pass Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2012	Diurnal	05/04	26.6	34	0	0	0
	Diurnal	05/16	34.4	21	2	0	0
	Diurnal	09/28	28.2	25	2	1	0
	Nocturnal	04/04	19.0	11	0	0	0
	Nocturnal	09/20	25.1	29	8	0	1
2013	Diurnal	02/11	4.8	0	0	0	7
	Nocturnal	04/18	12.3	8	0	8*	0
	Nocturnal	05/17	20.6	55	1	21	1
	Nocturnal	10/03	18.3	70	3	17	0
2014	Diurnal	02/27	23.3	20	0	37	42
	Diurnal	05/08	25.4	20	0	16	0
	Nocturnal	05/02	20.0	133	2	49	0
	Nocturnal	10/05	23.6	190	14	10	0
2015	Diurnal	02/22	10.0	24	2	1507	37
	Nocturnal	04/28	16.7	135	2	23	1

* Species identify uncertain for 4 tadpoles

Bearpaw Poppy Spring, NV – This site was initially assessed in July 2011, with translocations of *R. onca* beginning in spring 2012 and continued through the current project period. A total of 595 animals from sites in the Northshore springs were released (Table 22). An additional release has been planned for 2016. Survivorship of released animals appears to have been high and frog abundance has increased since establishment (Table 18). Frogs were heard calling for the first time in 2014, but evidence of breeding was not detected until egg masses were observed in 2015. Natural recruitment into the adult population has not yet been documented and the juvenile frogs observed in 2014 and 2015 were likely from animals released at the site in previous seasons.

Table 18. Summary of *Rana onca* observed at Bearpaw Poppy Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2012	Nocturnal	10/03	28.9	11	0	0	0
2013	Nocturnal	05/12	31.5	20	0	0	0
	Diurnal	05/16	36.2	2	0	0	0
	Nocturnal	10/16	16.5	35	0	0	0
2014	Nocturnal	03/16	19.5	46	5	0	0
	Diurnal	06/11	35.6	1	0	0	0
	Nocturnal	10/02	20.7	55	5	0	0
2015	Diurnal	02/11	29.1	2	0	0	1
	Diurnal	05/23	29.2	6	0	0	0
	Nocturnal	03/24	20.8	47	4	0	3

Vegetation structure along stretches of stream has remained relatively open from heavy use by feral burros. Prior to the initial release of *R. onca* in 2012, BLM erected an exclusion fence to restrict burros from the area around the springheads. At that same time, two small, shallow pools were created in stream flows within the exclusion area because of concerns that the shallow surface flows at the site lacked pools that would facilitate successful reproduction. The constructed pools were lined with plastic to limit vegetation regrowth, but the stability of these pools was short-lived. Storms in September 2014 caused minor flooding that mostly filled the pools with sediment. Maintenance of these pools was conducted in spring 2015, with sandbags added to redirect flood waters. An additional small pool was also created within the exclusion area but away from the surface flows.

Lime Spring, NV – Releases of *R. onca* to this site began in 2012 and continued through 2015. A total of 436 animals derived from Black Canyon sites have been released (Table 22). The long-term success of this site, however, is questionable. Frogs and late-stage tadpoles released here have overwintered, but few frogs have ever been observed during surveys (Table 19). The observations of juvenile frogs and tadpoles prior to spring 2015 were in all likelihood animals released to the site. Hatchling tadpoles from a single egg mass observed in spring 2015 were the first evidence of reproduction.

Table 19. Summary of *Rana onca* observed at Lime Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2012	Nocturnal	09/29	23.9	4	2	2	0
2013	Nocturnal	03/26	9.6	0	0	0	0
	Nocturnal	05/02	11.5	5	0	3	0
	Nocturnal	10/01	16.2	6	0	0	0
2014	Diurnal	05/30	26.1	4	0	0	0
	Nocturnal	05/16	19.3	14	1	0	0
	Nocturnal	09/25	20.3	7	0	0	0
2015	Nocturnal	05/20	14.1	7	0	700	0

This site located high on the eastside of the Virgin Mountains was initially considered an experiment to evaluate whether *R. onca* could survive and overwinter at high elevation (> 1430 m near the spring source). The site was thought to have promise for establishing a population because surface waters ran down the canyon for more than 500 m. There was some habitat that looked good for *R. onca* with moderate levels of disturbance from flooding and cattle grazing that thinned vegetation structure. It was noted at that time, however, that the permanent aquatic habitat was probably much more restricted than suggested by the water flow. This was evidenced by aquatically dependent plant species only occurring at the very upper portions of the site, and the presence of a historical water development that piped water to troughs well above the base of the canyon. The lack of aquatic habitat at this site became obvious shortly after the initial release when the system dried considerably. During the most recent surveys, water still flowed intermittently along a small section near the springhead, but the few frogs observed were all in one pool. This pool was also where the hatchling tadpoles were previously observed.

Horse Spring, NV – Initial translocations of *R. onca* to this site began in 2012 and continued through 2015 (Table 23). An additional translocation is planned for 2016. A total of 414 animals derived from sites in the Northshore springs have been released. Frog numbers have increased over time (Table 20) and frog density at this small site currently appears high. Small tadpoles believed to be *R. onca* observed in 2012 were the first documentation of reproduction. The observation of a large number of egg masses in spring 2015 indicates robust breeding activity. Natural recruitment has not yet been confirmed, and the juvenile frogs and overwintering tadpoles observed at the site over the years could all have been animals released at the site.

BLM has conducted minor habitat maintenance and improvement activities at Horse Spring. Prior to the initial release, a small secondary pond below the main pool was constructed. Emergent vegetation at this site, however, has been predominately limited by trespass cattle and smaller numbers of feral burros. Without the impact of this grazing, the springhead would be choked by tamarisk and most of the site would quickly become overgrown by cattails and reed canary grass (*Phalaris arundinacea*).

Table 20. Summary of *Rana onca* observed at Horse Spring during visual encounter surveys conducted in 2011 – June 2015. Ambient air temperature during survey (T^A) is indicated.

Year	Survey Type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg Masses
2012	Nocturnal	10/09	21.1	2	0	0	0
2013	Nocturnal	04/26	21.2	17	0	> 500*	0
	Diurnal	04/27	31.5	6	0	0	0
	Nocturnal	10/14	12.6	10	1	25	0
2014	Nocturnal	03/26	19.0	20	4	6	1
	Diurnal	06/03	25.3	15	7	0	0
	Nocturnal	09/23	20.4	45	6	21	0
2015	Diurnal	02/10	20.9	2	2	41	28
	Diurnal	04/26	17.2	15	1	0	0
	Nocturnal	03/25	11.3	32	5	47**	0

*Species identity uncertain; **Species identity uncertain for 28 tadpoles

Corn Creek, NV – This spring system is located near the visitor’s center of the Desert National Wildlife Refuge managed by the USFWS. The site was initially assessed for potential translocation of *R. onca* in April 2014, and later authorized by USFWS in early 2015. Much of the stream system was covered by large trees, predominately cottonwoods (*Populus fremontii*) and willows, among others. As part of an earlier restoration effort, ponded habitat was removed and long stretches of the stream were created where dense emergent vegetation was limited. These areas appeared to be good habitat for adult *R. onca*. The site, however, contained several stressors that could limit the establishment of a successful *R. onca* population. These include bullfrogs, crayfish, and the presence of *Bd*. The site also contains poolfish (*Empetrichthys latos*) and a large population of Woodhouses’s toads (*Anaxyrus woodhousii*). The RLFCT decided to move forward with an experimental translocation. Animals from both the Northshore springs and Black Canyon areas were used for translocation to allow flexibility for the headstarting program and to experiment with increased genetic diversity at an isolated site. The two initial translocations occurred in May 2015 and a total of 109 juvenile frogs were released (Tables 22 and 23).

OTHER SITES SURVEYED OR ASSESSED

Sugarloaf Spring, NV – Located on the Arizona side of Black Canyon, this site received one of the earliest translocations (2002 – 2006) of *R. onca*. Although the spring flow was once substantial, it went dry in summer 2006; there were several hypotheses associated with the drying. Frogs have not been observed since the drying event. A site visit was conducted on January 29, 2011 to assess current conditions. No major surface flow was observed. Small pools in the channel were noted, but these were likely filled by recent rain.

Gnatcatcher Spring, NV – A diurnal survey was conducted at this site in November 14, 2012. This spring is located below the Northshore Road within the LMNRA between Blue Point and Rogers springs. The springhead was within a gully and covered with emergent plants and canyon grape (*Vitis arizonica*). There were four closely situated cottonwood trees near the springhead that can be seen from the road, and water can be heard near the cottonwoods. The only open water (including a pool) was towards the lower half of the stream. Purportedly, *R. onca* was observed at this site by a person under contract with NPS in the late 1990s or early 2000s, but none were observed during this survey.

Chill Heal, AZ – A diurnal survey was conducted of this spring on April 11, 2013 by personnel from both BLM and UNLV, along with Dr. David Bradford. The intent was to assess the site for possible translocation of *R. onca*. The spring is located on NPS land near the Grand Wash. Emergent vegetation at the site included: sedges, reeds, and a small amount of cattails. Canyon treefrogs (*Hyla arenicolor*) and Woodhouse's toads were observed. At the time of the survey, lotic width was 0-2 meters with surface water present over several hundred meters, including larger pools that could potentially hold permanent water. There was some skepticism amongst members of the group about whether the site maintained enough permanent water for successful development of *R. onca* tadpoles. It was noted that there was limited emergent vegetation and that most of the riparian vegetation had deeper roots.

Flag and Cottonwood springs, AZ – These two springs occur within the same wash located in the Black Mountains, approximately 11 miles south of Union Pass Spring in Arizona. Springheads for Flag and Cottonwood springs were on patented (private) lands, although neither of the sites were fenced or posted. A diurnal survey was conducted on June 8, 2014 by personnel from UNLV, BLM, Arizona Game and Fish Department (AGFD), and Dr. Bradford. The intent was to assess summer, pre-monsoonal conditions as part of the evaluation of the system for possible translocation of *R. onca*. Previous site visits were conducted by personnel from AGFD. At the time of the survey, the canyon contained three watered areas: Cottonwood Spring, Flag Spring, and a watered area between the two springs on BLM land. Aquatic habitat appeared abundant, and several tree species were observed including willows (*S. gooddingii*, *S. exigua*), cottonwood, desert willow (*Chilopsis linearis*) and oak (*Quercus* sp.). Emergent vegetation included cattail, monkey flower, canyon grape, sedges (probably *Eleocharis*), and at Flag Spring a patch of tall reed (possibly *Phragmites* sp.). Flows at both springs were estimated at approximately 38-76 Lpm, with several deep pools noted at both springs. The other anuran observed to occupy the system was the red-spotted toad (*Bufo punctatus* = *Anaxyrus punctatus*). The consensus of the survey group was that there appeared to be evidence of enough permanent surface water and habitat to support a population of *R. onca*. There was also sufficient habitat outside of the private property boundaries to target for the

actual introductions, although aquatic habitat on the private lands would eventually be colonized and could cause problems for permitting.

OTHER MONITORING ACTIONS

Pathogenic Amphibian Fungus Assessment – Sampling for *Bd* occurred at a total of 18 field sites associated with *R. onca* management from 2011 – 2015. Most of the sampling focused on *R. onca*, including a few individuals from the headstarting program, but other species were sampled at Corn Creek, Muddy River, Virgin River and Spring Mountains State Park in areas once occupied by *R. onca* or considered potential translocation sites. In total 213 individual *R. onca* and 198 individuals from other amphibians were tested. *Bd* was detected in *R. onca* at one site, Lower Blue Point, over consecutive years. The fungus was also detected in other species at Corn Creek, Perkins Pond, Muddy River, and the Spring Mountains.

Support was also provided to a research project assessing the susceptibility of juvenile *R. onca* frogs to *Bd* and chytridiomycosis under laboratory conditions (also referred to as challenge experiments). This research was conducted by UNLV in 2013 and 2014 under separate funding from BLM. Two challenge experiments were conducted using different strains of *Bd* under conditions that favored *Bd* growth. Findings indicated that *R. onca* was susceptible to *Bd* infection, but that the infections were not lethal, even though the *Bd* strains used had been linked to population declines in other species. While these findings were encouraging in terms of *R. onca* conservation, whether *R. onca* is affected by chytridiomycosis in the wild remains unknown, nor is it known if there are sub-lethal effects of *Bd* infection in this species, including the potential for reduced survivorship (e.g. Pilliod et al. 2010).

HEADSTARTING AND TRANSLOCATIONS

Over the project period, eggs or occasionally hatchling tadpoles were collected for headstarting from historical sites from late January through early March. Three historical sites in Black Canyon were used for collections, Bighorn Sheep, Boy Scout Canyon, and Black Canyon Side Spring (Table 21). Collections were made annually from at least two of these sites. On two separate occasions hatchling tadpoles were collected instead of partial egg masses to facilitate the collection process. Efforts also focused on collection of eggs each year from sites in the Northshore springs. Partial egg masses were collected at times from Upper Blue Point and Rogers, but the majority of collections were from Lower Blue Point (Table 21). 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. In support of UNLV research on the impact of *Bd* on *R. onca*, collection efforts were intensified in 2014 to sample more egg masses at both Black Canyon and Northshore springs to increase genetic diversity of animals transferred to the research project.

The earliest releases of headstarted animals occurred in mid-March for late-stage tadpoles and mid-April for juvenile frogs. Releases for both groups were generally completed by the end of June. A small number of juvenile frogs that were held for the UNLV research project were released at later dates when not

needed. A total of 4790 animals (2455 tadpoles and 2335 juvenile frogs) were released to 12 different sites over the course of the project, including augmentation of historical sites at Blue Point and Rogers springs. An additional 8 juvenile frogs were provided to the Las Vegas Spring Preserve in 2012 for public display. A total of 114 juvenile frogs (including animals from both Black Canyon and Northshore) were provided to UNLV for research in 2013 and 2014.

Table 21. Sites and dates of egg masses or hatchlings of *Rana onca* collected for headstarting from 2011 – June 2015. The number of egg masses from which collections were made is indicated; along with the approximate percentage of the egg mass collected (when appropriate).

Area	Year	Date	Site	No. of Egg masses (collection)
Black Canyon Sites				
	2011	01/21	Bighorn Sheep Spring	1 (256 hatchlings)
		01/21	Boy Scout Canyon Spring	1 (50%)
		01/28	Black Canyon Side Spring	2 (50% each)
	2012	01/30	Bighorn Sheep Spring	1 (75%)
		02/10	Boy Scout Canyon Spring	1 (50%)
	2013	02/14	Bighorn Sheep Spring	2 (25% each)
		02/22	Black Canyon Side Spring	1 (43 hatchlings)
	2014	02/14	Bighorn Sheep Spring	2 (25%)
		02/19	Bighorn Sheep Spring	1 (25%)
		02/19	Boy Scout Spring	1 (25%)
	2015	02/01	Bighorn Sheep Spring	1 (25%)
		02/06	Boy Scout Spring	1 (25%)
Northshore Springs Complex Sites				
	2011	02/08	Lower Blue Point	2 (100% each, small masses)
		02/11	Lower Blue Point	1 (100%)
	2012	02/07	Lower Blue Point	2 (100% each)
		02/07	Upper Blue Point	1 (100%, small mass*)
	2013	02/05	Upper Blue Point	1 (100%, small mass)
	2014	03/04	Upper Blue Point	1 (50%)
		02/11	Lower Blue Point	1 (50%)
		02/11	Rogers Spring	1 (25%)
	2015	01/28	Lower Blue Point	1 (75%)
		01/28	Lower Blue Point	1 (50%)

*Eggs were not viable

The general protocol for translocations was to only use animals derived from either Black Canyon (Table 22) or Northshore springs (Table 23) at any given site; an exception was made by the RLFCT for translocations to Corn Creek. The current protocol is to release animals annually at each site for 5 years, after which the site is monitored for sustainability. Translocations initiated during the previous project (2009-2011) were completed at Quail Spring in 2012, Perkins Pond in 2014, and Union Pass Spring in 2015. Translocations to Bearpaw Poppy, Horse and Lime springs were initiated in 2012 and to Corn Creek in 2015. Augmentation of Upper Blue Point, Lower Blue Point, and Rogers Spring using animals headstarted from these sites also occurred. Excess headstarted animals from Black Canyon were produced

in 2013 and 2014, and released to augment Goldstrike Canyon in 2013 and Red Rock Spring in 2013 and 2014, even though initial five-years of translocations to these sites were completed in 2009 and 2010, respectively.

Table 22. 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 2011 – June 2015.

Year	Date	Translocation Site	Tadpoles Released	Frogs Released	Site & Grand Total By Year
2011	03/11	Quail Spring	75	0	75
	04/15	Union Pass Spring	60	15	-
	04/29	Union Pass Spring	158	12	-
	05/26	Union Pass Spring	24	3	272
Total 2011			317	30	347
2012	04/29	Quail Spring	0	70	70
	05/04	Union Pass Spring	30	148	-
	05/16	Union Pass Spring	152	62	392
	04/20	Perkins Pond*	0	1	1
	06/07	Lime Spring	217	67	-
	06/20	Lime Spring	14	1	299
Total 2012			413	349	762
2013	06/13	Goldstrike Canyon	0	60	-
	06/28	Goldstrike Canyon	5	23	-
	10/07	Goldstrike Canyon	0	23	111
	05/02	Lime Spring	0	63	63
	06/12	Red Rock Spring	0	30	30
	04/18	Union Pass Spring	56	15	71
Total 2013			61	214	275
2014	05/30	Lime Spring	21	30	51
	06/03	Red Rock Spring	30	25	-
	06/12	Red Rock Spring	29	10	-
	08/09	Red Rock Spring	0	69	163
	05/08	Union Pass Spring	99	0	99
Total 2014			179	134	313
2015	05/06	Corn Creek	0	52	52
	05/31	Corn Creek	0	4	4
	05/20	Lime Spring	5	18	23
	04/28	Union Pass Spring	0	34	34
Total 2015			5	108	113
Cumulative Total			975	835	1810

*One captive-reared frog from Black Canyon was mistakenly mixed with Lower Blue Point frogs.

In general, more animals can be headstarted than needed for releases in any given year. Most of the current release sites are small and survivorship of released animals appears high. As has been common, greater numbers of animals were released in the first years following establishment of a site, but as the abundance of frogs increased, the annual number of animals released was reduced to avoid overcrowding. This process was intended to establish a broad age-structure among animals (i.e. demographic diversity).

Table 23. 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 2011 – June 2015.

Year	Date	Translocation Site	Tadpoles Released	Frogs Released	Site & Grand Total By Year
2011	04/05	Perkins Pond	212	0	-
	05/05	Perkins Pond	171	10	-
	05/16	Perkins Pond	180	80	-
	06/03	Perkins Pond	137	43	833
	05/19	Lower Blue Point	0	100	-
	05/30	Lower Blue Point	12	20	132
	04/25	Rogers Spring	20	5	25
	Total 2011		732	258	990
2012	05/01	Bearpaw Poppy	0	175	-
	05/26	Bearpaw Poppy	0	184	359
	05/17	Horse Spring	243	0	243
	04/20	Perkins Pond	0	62	-
	05/31	Perkins Pond	60	30	-
	06/23	Perkins Pond	12	47	211
	05/19	Lower Blue Point	0	122	-
	06/30	Lower Blue Point	0	10	132
	Total 2012		315	638	953
2013	05/16	Bearpaw Poppy	0	100	100
	04/27	Horse Spring	29	63	92
	05/15	Perkins Pond	61	49	-
	05/24	Perkins Pond	62	31	-
	06/25	Perkins Pond	0	24	-
	10/10	Perkins Pond	0	15	242
	06/26	Upper Blue Point	0	10	10
	Total 2013		152	292	444
2014	06/11	Bearpaw Poppy Spring	30	56	86
	06/03	Horse Spring	30	19	49
	05/07	Lower Blue Point	0	15	-
	08/11	Lower Blue Point	0	25	40
	05/01	Perkins Pond	90	30	-
	06/20	Perkins Pond	27	13	160
	08/12	Rogers Spring	0	22	22
	08/11	Upper Blue Point	0	20	20
	Total 2014		177	200	377
2015	05/06	Corn Creek	0	27	-
	05/31	Corn Creek	0	26	53
	05/23	Bearpaw Poppy Spring	30	20	50
	04/26	Horse Spring	30	0	30
	06/09	Lower Blue Point	0	20	20
	06/09	Upper Blue Point	44	27	71
	Total 2015		104	120	224
Cumulative Total			1480	1508	2988

CONCLUSION AND RECOMMENDATIONS

The overall abundance of adult and juvenile *R. onca* has increased 41% and the number of sites occupied has increased from 15 to 19 since initiation of conservation efforts for this project in spring 2011 (Figure 1). The increase in frog abundance was predominately associated with the establishment of new experimental sites, particularly Union Pass, Bearpaw Poppy and Horse springs. These sites support frog abundances that are comparable to those observed at historical and well-established experimental sites. The largest contributor to the overall increase in abundance during this project period was the population at Union Pass Spring which was established by translocations in 2011. By fall 2014, the count at this site had reached 204 frogs, with a relative abundance similar to Grapevine and Tassi springs both of which were successful translocation sites that currently contain large, robust populations. The overall success of conservation actions for *R. onca* appears to be driven by the aggressive translocation program which has succeeded in establishing several robust populations and increasing overall abundance since the early 2000s. Identifying future experimental sites will be a challenge, but the potential completion of a Candidate Conservation Agreement with Assurances may create opportunities for potential sites on non-federal lands. Management of historical sites has had little success, although all historical sites currently retain populations.

Most sites appear likely to sustain populations of *R. onca* over time, but the assessment of the efficacy of translocations requires documentation of the ‘stages of success’ which may entail a decade of monitoring once an experimental site has been established (RLFCT 2005). Tassi, Grapevine, Pupfish and Quail springs are all currently in this efficacy monitoring stage. All these sites currently have established populations and show evidence of successful natural recruitment. Goldstrike Canyon and Red Rock springs had also entered efficacy monitoring, but recent augmentations at these sites have reset the assessment clock. At all the other experimental sites, translocations have not been completed or were just completed in spring 2015. During the current project period, Perkins Pond failed as an experimental site for *R. onca*. As noted above, survivorship of translocated animals to this pond appeared limited and the artificial site required extensive management. Monitoring at Red Rock and Lime springs has also raised questions about the long-term sustainability of populations at these sites where aquatic habitat appears severely limited and recruitment restricted at best.

Management actions will likely be needed over the long-term at many of the sites to ensure population persistence. Several of the experimental sites are quite small and isolated, and will require occasional augmentation of animals to maintain genetic diversity or to recover demographically from stochastic events. An additional concern is that emergent vegetation encroachment aggressively degrades habitat for *R. onca* at many experimental and historical sites without some moderate level of disturbance. Vegetation encroachment caused at least one previous extirpation (Bradford et al. 2004). Efforts to manage such vegetation (e.g. mechanical cutting) have been short-lived stopgaps (Jaeger et al. 2009), with these actions unlikely to be maintained over the long-term. At several sites, feral cattle or burros currently keep emergent vegetation in check, but management actions may at any time result in the removal of these animals, as has occurred along the Northshore springs (Jaeger et al. 2009). More effective strategies need to be developed to limit vegetation overgrowth at problematic sites, along with plans for implementation. Vegetation encroachment and debris accumulation is particularly a problem at sites where successful reproduction is limited by habitat conditions to a few natural or artificially created pools. Small scale but

regular management actions to maintain such critical breeding habitat appears necessary to sustain populations at such sites overtime.

As noted in the introduction, the USFWS is currently conducting a species status assessment of *R. onca*, which will influence the decision process for listing of the species under the ESA. The long-term support of conservation actions by Clark County demonstrates the commitment of the people of southern Nevada to local management of this unique, endemic species. The success of conservation actions summarized in this report, as well as previous successful actions summarized in the final reports from previous bienniums, clearly demonstrates the efficacy of current conservation strategy and actions. Further support for continuation of these actions over the next several years has been secured.

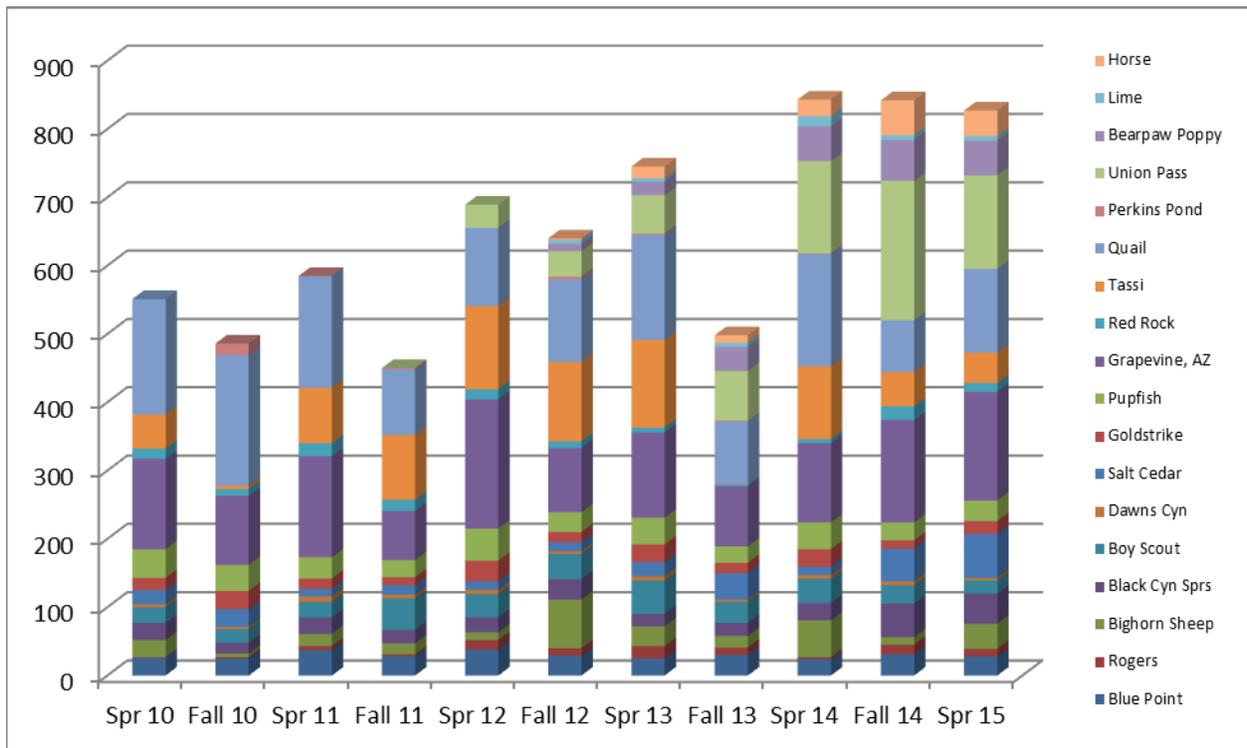


Figure 1. Pattern of change in number of *R. onca* adults and juveniles seen at all sites from 2010 through June 2015; data from 2010 are included for reference. Numbers represent the highest counts from visual encounter surveys during each period. Note that Bearpaw Poppy, Horse, Lime, and Union Pass springs were added to the series. Initial translocations to Corn Creek began in May 2015 and data is not included for this site.

LITERATURE CITED

- Bradford, D.F., J.R. Jaeger, and R.D. Jennings. 2004. Population status and distribution of a decimated amphibian, the relict leopard frog (*Rana onca*). *Southwestern Naturalist* 49:218-228.
- Blomquist, S. M., D.A. Cox, and M.J. Sredl. 2003. Inventory and habitat assessment of the relict leopard frog (*Rana onca*) in Arizona. Nongame and Endangered Wildlife Program Technical Report 219, Arizona Game and Fish Department, Phoenix, Arizona.
- Caughley, G. 1977. Analysis of vertebrate populations. John Wiley and Sons, New York, New York.
- Jaeger J.R. 2010. Connectivity of Leopard Frog Populations. Final report of field surveys within the western Grand Canyon and phylogeographic assessment of relict and lowland leopard frogs. Unpublished final report submitted to the Clark County Multiple Species Habitat Conservation Plan by the University of Nevada, Las Vegas.
- Jaeger, J.R., B.R. Riddle, R.D. Jennings, and D.F. Bradford. 2001. Rediscovering *Rana onca*: Evidence for phylogenetically distinct leopard frogs from the border region of Nevada, Utah, and Arizona. *Copeia* 2001:339-354.
- Jaeger, J.R., M.R. Graham, and E.C. Engel. 2009. Habitat manipulations for relict leopard frogs (*Rana onca*). Unpublished final report submitted to the Clark County Multiple Species Habitat Conservation Plan by the University of Nevada, Las Vegas.
- Jaeger, J.R. and R. Rivera. 2013. Expanding efforts to quantify the status of the Relict Leopard Frog. Unpublished final report submitted to the U.S. Fish and Wildlife Service by the University of Nevada, Las Vegas.
- McDiarmid, R.W. and R. Altig. 2000. Tadpoles: The Biology of Anuran Larvae. Chicago: University of Chicago Press.
- Oláh-Hemmings, V., J.R. Jaeger, M.J. Sredl, M.A. Schlaepfer, R.D. Jennings, C.A. Drost, D.F. Bradford, and B.R. Riddle. 2010. Phylogeography of declining relict and lowland leopard frogs in the desert Southwest of North America. *Journal of Zoology* 280:343–354
- Pilliod, D.S., E. Muths, R.D. Scherer, P.E. Bartelt, P.S. Corn, B.R. Hossack, B.A. Lambert, R. McCaffery, and C. Gaughan. 2010. Effects of amphibian chytrid fungus on individual survival probability in wild boreal toads. *Conservation Biology* 24:1259–1267.
- RLFCT (Relict Leopard Frog Conservation Team). 2005. Conservation agreement and rangewide conservation assessment and strategy for the Relict Leopard Frog (*Rana onca*). Unpublished document. Lake Mead National Recreation Area, Boulder City, Nevada. Available at <http://www.fws.gov/nevada/es/documents/esa/Rana%20onca%20CAS%20Final.pdf>
- Vredenburg V.T., R.A. Knapp, T.S. Tunstall, and C.J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences* 107:9689–9694.