PROJECT: Relict Leopard Frog Monitoring and Management (*Rana onca*)

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EXECUTIVE SUMMARY

The purpose of this project was to implement actions described in a voluntary conservation agreement and strategy for the relict leopard frog (*Rana onca*). The project involved managing relict leopard frogs through a cooperative interagency program designed to increase both overall numbers as well as number of populations in a defined area of southern Nevada and northern Arizona. Efforts under this project included population monitoring, habitat improvement, establishment of new populations, and augmentation of existing ones. Assistance was provided to agency partners to identify potential translocation sites and to conduct associated conservation actions, including coordinating meetings of the Relict Leopard Frog Conservation Team.

During the course of this project, relict leopard frogs were monitored at 8 natural sites and 7 experimental translocation sites; one of which was started in 2010. Visual encounter surveys were conducted during the spring and fall. At the beginning of this project, one additional experimental site was dropped from active survey as surface waters unexpectedly dried up, although the site was surveyed once late in 2008. Numerous additional diurnal surveys were conducted at several sites to better assess breeding phenology, to search for egg masses under urgent efforts to augment populations, and to assess potential sites. Assistance was provided to researchers conducting mark-recapture estimation of one population through time. Three seasons of translocations were successfully completed, with frogs reared at two facilities. Frogs or tadpoles were released to augment five existing experimental sites, and to establish one new site. Emergency augmentation was also conducted at three natural sites. Several sites were removed from augmentation over the course of the project to allow for the evaluation of long-term sustainability. Minor habitat maintenance of breeding pools was conducted at various sites, and guidance was provided to field crews conducting habitat actions under agency directives at various sites. Sampling was initiated for a pathogenic fungus that causes chytridiomycosis – an 'emergent disease' of amphibians.

INTRODUCTION

Background and Need – The relict leopard frog, *Rana onca* (= *Lithobates onca*) appears to be a regional endemic (Olah-Hemmings et al. 2010). The known historical range of the species includes springs and wetlands along the drainages of the Virgin, Muddy, and Colorado rivers from the vicinity of Hurricane, Utah to Black Canyon, below Lake Mead in Nevada and Arizona. The species, however, has experienced a large reduction in geographic range and number of populations (Bradford et al. 2004). Taxonomic confusion once led to the declaration that *R. onca* was extinct, even though a couple of populations were known to exist (Jaeger et al. 2001). Natural populations of these frogs now occupy only a few spring sites within two general areas of Clark County in southern Nevada within Lake Mead National Recreation Area (LMNRA).

Conservation efforts for this species began in earnest in the early 1990s, as additional information on population dynamics and distribution was being gathered, including phylogentic studies. The first interagency meeting focused on *R. onca* was held in 1999, and by 2001, a voluntary Relict Leopard Frog Conservation Team (RLFCT) was formed with members from numerous federal and state agencies (RLFCT 2005). In 2002, the species was petitioned for listing under the Endangered Species Act, and while listing was considered warranted, it was precluded because of conservation efforts by the RLFCT coordinated under a voluntary conservation agreement and strategy (CAS). Recent conservation efforts have focused on monitoring and maintaining existing populations and on attempts to establish experimental populations at additional sites within the region. Despite the success of some conservation efforts, *R. onca* remains imperiled.

The information contained herein represents a summary of management, monitoring and conservation actions implemented by the National Park Service (NPS) toward meeting objectives outlined in the CAS. Major efforts under this project were performed under task agreement by personnel at the Public Lands Institute, University of Nevada, Las Vegas (UNLV) in collaboration with Ross Haley at LMNRA. This document represents the final report for field efforts from 2008 through mid-November 2010 under funding from the Clark County Multiple Species Habitat Conservation Plan (MSHCP; project number 2005-NPS-476-P).

Main Goals and Major Objectives – The main goal of the project was the conservation of existing *R. onca* populations and establishment of new experimental populations. The main field objectives were as follows:

1. Monitor existing natural populations to assess population persistence and identify potential changes in site conditions that may affect populations.
2. Monitor experimental populations to evaluate the success of translocations.
3. Identify management actions to improve or mitigate habitat conditions at existing sites to promote persistence of populations, and implement small-scale actions or coordinate actions by crews under the guidance of land managers.
4. Manage a headstarting program to raise eggs collected from wild frogs to later-stage tadpoles or small frogs for translocation to new sites, or to augment existing sites.
5. Coordinate efforts to identify new sites for translocations, and assist land mangers with introductions.

METHODS AND MATERIALS

Along with the conservation strategy, the RLFCT prepared written protocols and techniques for conducting conservation actions for *R. onca*. The methods implemented in this project are specified in the Relict Leopard Frog Protocol and Techniques Manual included in the CAS (available electronically at http://ndow.org/wild/conservation/frog/leopard/plan.pdf). The protocols and techniques detail the various procedures used for collecting, rearing, transporting, and releasing frogs and tadpoles associated with headstarting and translocation. Also specified are the methods and timing for monitoring populations.

Site Surveys – In general, visual encounter surveys (VES) were conducted at all natural and experimental sites known to contain *R. onca*. Two experimental sites, Sugarloaf and Lower Grapevine Springs, NV, failed prior to this reporting period because of the drying of the springs and have been removed from regular biannual surveys. Surveys are conducted in early spring and again in fall. All frogs and egg masses observed are counted, but tadpole numbers represent estimates up to 300, after which a plus sign is added to indicate larger numbers. All field surveys were conducted by trained biologists with experience in amphibian monitoring. Diurnal surveys early in the year were used to document breeding activities (egg masses and tadpoles) during a prime breeding period. As part of an effort to better understand breeding phenology, additional diurnal VES at several warm and cool water sites were conducted in 2008 and 2009. Nocturnal surveys during the spring and fall were used to better assess frog numbers (both adults and juveniles), which are more readily observed at night.

Headstarting and Translocations – The early spring diurnal surveys were also used to find and collect eggs for headstarting in the laboratory. The target goals for collection numbers and sites, as well as the targeted sites and actual numbers of late-stage tadpoles or juvenile frogs planned for release are determined during meetings of the RLFCT. Eggs were processed in a laboratory facility maintained by the LMNRA, and tadpoles were grown-out at this facility and at the Willow Beach National Fish Hatchery maintained by the U.S. Fish and Wildlife Service. In general, eggs were collected in the wild from late January through March and released as late-stage tadpoles or juvenile frogs, usually before the very hot temperatures began in June.

RESULTS & EVIDENCE / EVALUATION & DISCUSSION OF RESULTS

Monitoring of Natural Sites in Black Canyon

***Bighorn Sheep Spring, NV.* –** Over the course of the project, all life stages of *R. onca* were observed at this site during surveys (Table 1), indicating active reproduction and some recruitment. This site once maintained about 50% of all *R. onca* (Bradford et al. 2004), but a large storm event in October 2006 caused debris flow which greatly reduced habitat quality. The numbers of frogs reported herein were quite low compared to previous observations, and low recruitment has been a concern. In September 2008, several (n = 8) temporary pools were constructed in the main stream channel with sandbags in an attempt to temporarily improve conditions for rearing tadpoles. Frogs were later observed in these pools, but rains in December

2008, washed out the sandbags and filled the pools with gravel. Vegetation, however, has begun to rebound since the floods in 2006.

In 2009 and 2010, partial egg masses were collected from sites in the stream channel for the translocation program (Table 17). The thought here was that conditions for tadpoles in the main stream were likely very poor and low survivorship could be expected, thus the removal of some eggs would have very little negative impact and possibly benefit the remaining animals by reducing competition.

Table 1. Summary of *Rana onca* observed at Bighorn Sheep Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	01/23/2008	15.1	1	0	0	0
Diurnal	02/04/2008	11.6	0	0	0	0
Diurnal	02/27/2008	18.6	1	0	0	1
Diurnal	03/21/2008	-	3	0	300+	3
Diurnal	05/01/2008	16.7	0	0	0	3
Diurnal	09/16/2008	35	1	0	0	0
Diurnal	10/02/2008	32.2	0	0	0	0
Diurnal	10/27/2008	24.1	0	0	0	0
Diurnal	11/24/2008	21.1	2	0	43	0
Diurnal	12/05/2008	16.9	0	0	53	0
Diurnal	01/12/2009	13	0	0	0	0
Diurnal	01/24/2009	20.4	2	0	0	1
Diurnal	01/29/2009	14.1	0	0	0	3
Diurnal	02/11/2009	11.4	0	0	0	0
Diurnal	02/23/2009	18.2	2	0	0	4
Diurnal	03/09/2009	16.7	0	0	300+	0
Diurnal	03/31/2009	25.5	0	0	300+	3
Diurnal	04/28/2009	22	0	0	5	0
Diurnal	06/06/2009	26.1	0	0	6	0
Diurnal	01/24/2010	9.7	0	0	0	0
Diurnal	01/30/2010	11.6	3	0	0	1
Diurnal	01/31/2010	11.3	2	0	0	2
Diurnal	02/25/2010	21.3	0	0	300+	5
Nocturnal	04/28/2008	26.3	36	0	0	2
Nocturnal	11/13/2008	21.1	4	0	37	0
Nocturnal	04/20/2009	20.9	10	0	300+	5
Nocturnal	10/15/2009	22.8	11	0	0	0
Nocturnal	04/15/2010	20	25	0	4	0
Nocturnal	10/31/2010	20	5	0	0	0

***Boy Scout Canyon Spring, NV.* –** Over the course of the project, all life stages of *R. onca* were observed during surveys at this site (Table 2), indicating active reproduction and recruitment. Egg masses and tadpoles, however, were generally observed only in two side areas that have small pools with cooler water than the main thermal stream. These sites appear to be critical for successful reproduction within this canyon. From these sites, partial egg masses were collected each year for the translocation program (Table 17). Maintenance actions to keep these important pools from filling with debris or becoming choked with cattails and tamarisk were conducted.

Table 2. Summary of *Rana onca* observed at Boy Scout Canyon during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	02/04/2008	13	3	0	15	2
Diurnal	10/18/2008	20.6	9	0	0	0
Diurnal	01/12/2009	17.9	9	0	0	1
Diurnal	01/24/2009	21	2	0	0	2
Diurnal	02/23/2009	17.2	10	0	53	2
Diurnal	03/23/2009	16	6	0	5	0
Diurnal	01/24/2010	12.5	2	0	0	0
Diurnal	01/31/2010	13.6	7	0	0	6
Nocturnal	04/28/2008	27.5	16	0	31	0
Nocturnal	11/13/2008	21	18	1	0	0
Nocturnal	04/20/2009	26	23	0	117	0
Nocturnal	10/22/2009	23.3	20	1	0	0
Nocturnal	05/06/2010	26.7	23	0	100+	0
Nocturnal	11/03/2010	23	19	1	0	0

Dawn's Canyon Spring, NV. – This site is a small canyon and stream located directly up river from Boy Scout Canyon. The site may contain habitat above the area surveyed, but steep canyon walls limit the survey to a short bottom stream section. There is some speculation that frogs at this site may be directly connected to those in Boy Scout Canyon. Observations of eggs and tadpoles at this site have basically been limited to a single plunge pool at the base of a waterfall. Over the course of the project, however, all life stages of *R. onca* have been observed at this site indicating successful reproduction and recruitment (Table 3).

An additional observation of breeding was reported by Marc Maynard (BOR) who reported seeing an egg mass of *R. onca* and 2 adult frogs at this site on April 5, 2008. In the spring of 2009, a single egg mass was observed, and since the pool in which it was located had limited habitat, part of the egg mass was brought in for rearing and translocation (Table 17).

Table 3. Summary of *Rana onca* observed at Dawn's Canyon Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Mass
Diurnal	02/27/2008	19.2	0	0	1	0
Diurnal	01/24/2009	20.5	1	0	0	1
Diurnal	01/24/2010	13.5	0	0	0	0
Diurnal	02/25/2010	15	1	0	0	0
Nocturnal	03/18/2008	18.7	2	0	6	0
Nocturnal	11/13/2008	20.8	3	1	1	0
Nocturnal	04/20/2009	27	2	0	6	0
Nocturnal	10/22/2009	21.7	4	4	0	0
Nocturnal	04/15/2010	20	4	0	2	0
Nocturnal	11/03/2010	25	2	1	0	0

Black Canyon Spring and Black Canyon Spring Side, NV. – These two areas represent components of the same system, although these areas are generally treated as separate sites for reporting. Black Canyon Spring represents a reach of stream fed by thermal springs that exist up drainage from the survey area. Unfortunately, areas above the survey reach are difficult to access (requiring technical climbing). Further efforts to gain a better understanding of upstream conditions (above the large waterfall used as the survey endpoint) are planned for late this year or in early spring (2011).

In general, the portion of the main stream surveyed does not represent good habitat for *R. onca*, and very few *R. onca* have been observed along this stretch. No adult *R. onca* have been observed at this site since spring, 2009; although, a single *R. onca* tadpole was observed in the stream in 2010 (Table 4). This site was also greatly impacted by debris flows in October 2006. In spring 2008, young and surviving tamarisk trees were manually removed from along the stream system by the NPS Exotic Plant Management Team (EPMT). This was followed by a volunteer effort in fall 2008.

Table 4. Summary of *Rana onca* observed at Black Canyon Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	01/23/2008	17.1	0	0	0	0
Diurnal	02/04/2008	14.8	0	0	0	0
Diurnal	02/27/2008	22.7	1	0	0	0
Diurnal	09/16/2008	31	0	0	0	0
Diurnal	10/27/2008	24.3	0	0	0	0
Diurnal	11/24/2008	21.6	0	0	0	0
Diurnal	12/05/2008	17.5	0	0	0	0
Diurnal	01/12/2009	18	0	0	0	0
Diurnal	01/29/2009	16.6	0	0	0	0
Diurnal	02/11/2009	16.1	0	0	0	0
Diurnal	02/23/2009	19.6	1	0	0	0
Diurnal	03/09/2009	22.3	0	0	0	0
Diurnal	04/16/2009	22.3	0	0	0	0
Diurnal	04/27/2009	29	1	0	0	0
Diurnal	06/06/2009	26.6	0	0	0	0
Diurnal	01/24/2010	15	0	0	0	0
Diurnal	02/09/2010	14	0	0	0	0
Diurnal	02/25/2010	16.7	0	0	1	0
Nocturnal	04/22/2008	27.8	7	0	0	0
Nocturnal	11/04/2008	20.3	1	0	0	0
Nocturnal	03/31/2009	19.3	1	0	0	0
Nocturnal	10/15/2009	25.6	0	0	0	0
Nocturnal	05/06/2010	26.1	0	0	0	0
Nocturnal	05/13/2010	21.7	0	0	0	0
Nocturnal	11/03/2010	22	1	0	0	0

Black Canyon Spring Side is a short, cool water site that drains into the main Black Canyon Spring stream; this is the only remaining natural cool water site with *R. onca*. Over the course of the project, frogs at this site showed evidence of active reproduction and recruitment, and all life stages of *R. onca* were observed (Table 5). The entire surveyed stream stretch was treated for tamarisk by the EPMT between the two spring surveys in 2010.

Table 5. Summary of *Rana onca* observed at Black Canyon Spring Side during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	01/23/2008	16.2	1	1	0	0
Diurnal	02/04/2008	11.5	0	0	0	0
Diurnal	02/27/2008	21.9	3	0	0	0
Diurnal	05/01/2008	19.4	2	0	0	1
Diurnal	09/16/2008	29.5	4	0	0	0
Diurnal	10/27/2008	22.5	3	0	0	0
Diurnal	11/24/2008	19.2	3	0	1	0
Diurnal	12/05/2008	17.5	2	0	0	0
Diurnal	01/12/2009	17.4	1	0	2	0
Diurnal	01/29/2009	15.3	0	0	0	0
Diurnal	02/11/2009	12.8	0	0	0	0
Diurnal	02/23/2009	18.8	1	0	1	1
Diurnal	03/09/2009	21.4	4	0	0	0
Diurnal	04/16/2009	20.6	6	0	0	0
Diurnal	04/27/2009	28.7	5	0	0	0
Diurnal	06/06/2009	27.7	2	0	0	0
Diurnal	01/24/2010	13	4	0	0	0
Diurnal	02/09/2010	16.4	11	0	15	0
Nocturnal	04/22/2008	26.4	7	3	0	0
Nocturnal	11/04/2008	17.2	6	1	0	0
Nocturnal	03/31/2009	19	7	0	0	0
Nocturnal	10/15/2009	27.2	2	0	0	0
Nocturnal	05/13/2010	21.7	25	0	4	0
Nocturnal	11/03/2010	20	9	7	0	0

Salt Cedar Canyon Spring, NV. – As opposed to Bighorn Sheep Spring, the rain-caused debris flows in October 2006, appear to have improved conditions at this site for *R. onca* by removing dense vegetation and pushing crayfish out of the upper reach of the stream. Crayfish remain abundant in the stream near the confluence with the Colorado River, but have not moved up over an earthen dam and dry section of channel into the upper reach.

In recent years, vegetation has been rebounding and some areas of the stream have again become quite choked. The lower counts in recent surveys (Table 6) may reflect the more difficult conditions of observing frogs in dense vegetation. Over the course of the project, all life stages of *R. onca* were observed at this site, indicating active reproduction and recruitment. Partial egg masses were collected in 2008 and 2010 for the translocation program (Table 17). While no egg masses were observed during diurnal surveys in 2009, two egg masses were observed shortly after during a nocturnal survey.

Table 6. Summary of *Rana onca* observed at Salt Cedar Canyon Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	01/23/2008	16.2	0	0	300+	3
Diurnal	02/04/2008	14.3	3	0	107	1
Diurnal	02/27/2008	25.2	7	1	107	0
Diurnal	09/16/2008	37	12	10	0	0
Diurnal	10/02/2008	29.9	7	8	0	0
Diurnal	10/27/2008	23	6	2	0	0
Diurnal	11/24/2008	22.1	4	2	57	0
Diurnal	12/05/2008	14.9	2	1	0	0
Diurnal	01/12/2009	18.1	4	7	16	0
Diurnal	02/11/2009	16.5	2	5	21	0
Diurnal	03/09/2009	20.6	11	8	23	0
Diurnal	04/16/2009	20.3	8	9	42	0
Diurnal	01/24/2010	11.9	4	1	3	0
Diurnal	01/30/2010	15	5	0	12	2
Nocturnal	04/22/2008	21.6	30	3	123	0
Nocturnal	11/04/2008	21	15	4	1	0
Nocturnal	01/24/2009	19.6	19	1	32	0
Nocturnal	02/23/2009	16.2	20	13	56	0
Nocturnal	03/31/2009	20.3	41	6	137	0
Nocturnal	04/27/2009	26.5	36	6	141	2
Nocturnal	06/04/2009	29	31	13	0	0
Nocturnal	10/15/2009	25	20	0	7	0
Nocturnal	05/06/2010	22.8	16	5	100+	0
Nocturnal	11/03/2010	21.7	26	0	0	0

Monitoring of Sites in the Northshore Springs Complex

***Upper and Lower Blue Point Spring, NV.* –** In recent years, surveys at Blue Point Spring have been split into upper and lower portions of the spring. The upper portion represents just over 0.5 km of linear stream habitat from the springhead down to just below the Northshore Road where the spring tunnels underground. The lower section represents areas further downstream where the water reemerges. During 2008, monitoring was coordinated with a separate research project conducted by UNLV to evaluate habitat modifications intended to benefit *R. onca* (Jaeger et al. 2009). At Upper Blue Point, about 177 linear meters of dense rushes (*Scirpus* and *Eleocharis)* and cattails (*Typha*) were cut in two phases in February and November, 2007. Several fish-free side channels were also created. Treatments in November 2007 also included cutting of about 89 m of dense emergent, vegetation at Lower Blue Point and creation of a large fish-free channel. Habitat at these sites benefited from the habitat improvements, but these benefits have been relatively short-lived (Jaeger et al. 2009) and are now being reversed. This area also benefits from recreational use by park visitors where trampling reduces riparian vegetation density and cover along small portions of the stream. Mark-recapture was conducted at Upper Blue Point as part of that project (Jaeger et al. 2009) and has continued as an independent UNLV project (Jaeger unpublished data). Herein, representative surveys conducted in cooperation with the mark-recapture effort are reported. Diurnal monitoring efforts during 2008 and 2009 were extensive at these sites and were aimed at finding egg masses for headstarting.

At Upper Blue Point Spring, 21 diurnal surveys were conducted in 2008, and 10 were conducted in 2009, but none of these surveys resulted in the observation of egg masses or tadpoles (Table 7). This is not surprising as over the years much effort has gone into monitoring and population studies at this site, and very few egg masses or tadpoles have ever been seen. All the Northshore spring sites contain dense, emergent vegetation which makes finding egg masses and tadpoles difficult. A more important negative factor, however, is that both Blue Point and Rogers springs are infested with exotic, carnivorous fishes which consume tadpoles and probably eggs.

Adult numbers at this site were extremely low in early 2008 (Table 7), with mark-recapture estimates based on multiple surveys indicating around 10 adult frogs, with point estimates of no more than 12 individuals (Jaeger et al. 2009). The population was augmented in spring 2008, with 155 headstarted juvenile frogs from eggs collected at Lower Blue Point (Table 19), and subsequent results from visual encounter surveys indicate marked increases in adult animals. Estimates from the mark-recapture study indicated high survivorship (Jaeger unpublished data), with estimates in 2009 indicated 91 frogs in the spring (95% CI 73–122; 67 individuals observed) and 69 frogs in the fall (95% CI 49–116; 43 individuals observed). Estimates from spring 2010, indicated 70 frogs (95% CI 55-99; 52 individuals observed). Ten juvenile frogs were again added to the system in spring 2010 from headstarted eggs collected at Lower Blue Point (these frogs were not included in the estimate).

Although there is some evidence of natural recruitment at this site, the number of juveniles observed overtime has remained quite low. Recent efforts to improve chances for successful recruitment include fish-removal from upper portions of the spring (areas above the historical dam). NPS personnel using funnel traps have removed over 1034 nonnative fish from the stream in 2010, and further efforts are planned.

Table 7. Summary of *Rana onca* observed at Upper Blue Point Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	01/04/2008	16.5	0	0	0	0
Diurnal	01/13/2008	16.2	0	0	0	0
Diurnal	01/21/2008	14.5	0	0	0	0
Diurnal	01/28/2008	11.8	0	0	0	0
Diurnal	02/05/2008	9.9	0	0	0	0
Diurnal	02/12/2008	21.4	0	0	0	0
Diurnal	02/12/2008	22.4	0	0	0	0
Diurnal	02/18/2008	18.7	0	0	0	0
Diurnal	02/21/2008	17.6	0	0	0	0
Diurnal	02/26/2008	19.6	0	0	0	0
Diurnal	03/03/2008	19	0	0	0	0
Diurnal	03/09/2008	18.5	0	0	0	0
Diurnal	03/17/2008	16.2	0	0	0	0
Diurnal	04/01/2008	18.1	0	0	0	0
Diurnal	04/07/2008	21.6	1	0	0	0
Diurnal	04/14/2008	25.2	0	0	0	0
Diurnal	04/24/2008	26.6	0	0	0	0
Diurnal	04/29/2008	35.4	0	0	0	0
Diurnal	05/05/2008	21.1	0	0	0	0
Diurnal	05/14/2008	25.5	0	0	0	0
Diurnal	06/02/2008	31	0	0	0	0
Diurnal	06/11/2008	26	0	0	0	0
Diurnal	06/26/2008	32.6	0	0	0	0
Diurnal	01/11/2009	14.5	0	0	0	0
Diurnal	01/27/2009	13.1	0	0	0	0
Diurnal	02/03/2009	20	1	0	0	0
Diurnal	02/19/2009	18.6	3	0	0	0
Diurnal	03/05/2009	22.3	2	0	0	0
Diurnal	03/12/2009	17.1	4	0	0	0
Diurnal	03/20/2009	16.5	0	0	0	0
Diurnal	04/03/2009	15	0	0	0	0
Diurnal	04/15/2009	8.8	0	0	0	0
Diurnal	05/01/2009	28.9	3	0	0	0
Diurnal	02/11/2010	13.6	4	0	0	0
Nocturnal	03/23/2008	17.3	3	0	0	0
Nocturnal	11/11/2008	15.5	19	0	0	0
Nocturnal	04/06/2009	21.6	38	0	0	0
Nocturnal	11/10/2009	19.7	25	0	0	0
Nocturnal	03/20/2010	14.7	16	0	0	0
Nocturnal	10/10/2010	26.8	24	0	0	0

At Lower Blue Point Spring, many of the diurnal surveys in 2008 and 2009 (Table 8) focused only on the upper 200-300 meters of habitat, generally in areas were the best habitat remained and in areas where habitat had been modified. These targeted surveys were conducted in conjunction with surveys at Upper Blue Point. The three egg masses observed at Lower Blue Point in 2008 (and brought back for headstarting) were not found during these surveys, but instead were observed during habitat work at the site. These egg masses were all found at the point of stream reemergence at the upper most end of Lower Blue Point. Vegetation had been reduced at this site, and a small backwater has inadvertently been created while placing an intake for funneling water to the fish-free pond. This backwater apparently attracted breeding frogs.

The number of *R. onca* observed at Lower Blue Point Spring had been declining in recent years with observations of only a handful of frogs during nocturnal surveys (see 2008 data in Table 8). As part of emergency augmentation actions, 159 headstarted juvenile frogs were returned to this area in late spring 2008. Higher counts in 2009 and spring 2010 likely reflect these animals, but habitat conditions along most of the stream continued to deteriorate as vegetation continues to choked the system. Augmentation was again conducted in 2010 with the release of 77 tadpoles from eggs collected earlier in the year at the site.

Table 8. Summary of *Rana onca* observed at Lower Blue Point Spring during visual encounter surveys conducted in 2008–2010. Asterisks indicated targeted surveys focused on only the upper 200-300 m of habitat. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	02/26/2008	18.3	0	0	0	0
Diurnal*	03/17/2008	16.4	1	0	0	0
Diurnal*	04/01/2008	22	0	0	0	0
Diurnal*	04/07/2008	21.6	1	0	0	0
Diurnal*	04/14/2008	28.7	1	0	0	0
Diurnal*	04/24/2008	26.1	1	0	0	0
Diurnal*	04/29/2008	34.2	0	0	0	0
Diurnal*	05/05/2008	22.6	1	0	0	0
Diurnal*	05/14/2008	29.8	1	0	0	1
Diurnal*	05/29/2008	28.5	1	0	0	0
Diurnal*	06/11/2008	26.4	0	1	0	0
Diurnal*	06/19/2008	27.1	0	3	0	0
Diurnal*	06/26/2008	34.2	2	6	0	0
Diurnal*	07/30/2008	32.5	2	3	0	0
Diurnal*	07/07/2008	27.0	0	0	0	0
Diurnal*	09/17/2008	28.8	1	2	0	0
Diurnal*	10/06/2008	20.7	0	0	0	0
Diurnal*	10/15/2008	28	1	0	0	0
Diurnal*	11/19/2008	26.4	1	0	0	0
Diurnal*	12/04/2008	16.2	0	0	0	0
Diurnal*	12/13/2008	18	0	0	0	0
Diurnal*	01/11/2009	14.2	0	0	0	0
Diurnal*	01/27/2009	11.6	2	0	0	0
Diurnal	02/03/2009	20	1	0	0	0
Diurnal*	02/19/2009	19.7	3	0	0	0
Diurnal*	03/05/2009	21.2	8	0	0	0
Diurnal*	03/12/2009	20.1	5	0	0	0
Diurnal*	03/20/2009	15	0	0	0	0
Diurnal*	04/03/2009	16.7	0	0	0	0
Diurnal*	04/15/2009	8.2	0	0	0	0
Diurnal*	05/01/2009	26.9	5	0	0	0
Diurnal	02/11/2010	19.4	1	0	1	2
Diurnal	03/01/2010	20	2	0	1	2
Nocturnal	03/23/2008	16	3	0	0	0
Nocturnal	11/11/2008	15.5	3	0	0	0
Nocturnal	05/06/2009	29.5	4	0	0	0
Nocturnal	11/05/2009	21	16	0	0	0
Nocturnal	03/21/2010	18.1	10	0	10	1
Nocturnal	10/28/2010	13.9	3	0	0	0

***Rogers Spring, NV.* –** This site is closely situated to Blue Point and suffers from the similar encroachment of dense vegetation. At this site, however, tall mats of sawgrass (*Cladium californicum*) dominate. This stream is also occupied by aggressive, exotic fishes. Habitat conditions for *R. onca* at Rogers Spring have continued to deteriorate despite experiments in 2007 to improve habitat. These actions included cutting of 145 liner meters of areas along the stream and the controlled burning of about 1507 m^2 of *Cladium* (Jaeger et al. 2009). This vegetation grew back rapidly and in recent years the only areas with seemingly good habitats for *R. onca* were formed by a power line road that crosses the main stream channel and in areas near the main springhead pool. By spring 2010 dense emerging vegetation had increased at the powerline road crossing.

Prior to this study period, *R. onca* was no longer being seen during surveys at this site (the last was recorded in fall 2006). In early summer 2008, 64 headstarted frogs raised from eggs collected at Lower Blue Point were released in modified habitats (Table 19). A small number of these frogs have been observed since spring 2009 near the release areas, but only one adult frog was counted during the nocturnal survey in spring 2010 (Table 9). The egg mass and tadpoles observed during early surveys in 2010 were located in an area where frogs were released, but this area was rapidly being overgrown with vegetation.

Table 9. Summary of *Rana onca* observed at Rogers Spring during visual encounter surveys conducted in 2008–2010. Temperature (^oC) is the ambient air temperature during survey (T^A).

Survey Type	Date	T^A	Adult	Juvenile	Larvae	Egg Masses
Diurnal	02/26/2008	21.1	0	0	0	0
Diurnal	02/03/2009	19.3	1	0	0	0
Diurnal	02/11/2010	17.3	0	0	24	1
Nocturnal	04/10/2008	18	0	0	0	0
Nocturnal	05/05/2008	21.7	0	0	0	0
Nocturnal	11/12/2008	21	0	0	0	0
Nocturnal	04/30/2009	20.3	8	0	0	0
Nocturnal	11/05/2009	21.1	7	0	0	0
Nocturnal	05/03/2010	21.7	1	0	10	0
Nocturnal	10/28/2010	12.9	2	0	0	0

Monitoring of Experimental Translocation Sites

Goldstrike Canyon, NV. – Over the course of the project, all life stages except juveniles of *R. onca* were observed during surveys (Table 10). This indicates active reproduction, but recruitment was not confirmed at this site. Translocation to this site ended in 2009 (Table 18), as the site had been augmented for over 5 years after which protocols call for an assessment of natural sustainability.

Table 10. Summary of *Rana onca* observed at Goldstrike Canyon during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	02/18/2008	13.8	0	0	300+	1
Diurnal	04/28/2008	29.4	1	0	7	3
Diurnal	05/01/2008	19.5	1	0	300+	4
Diurnal	02/06/2009	18.2	0	0	0	0
Diurnal	03/23/2009	13.5	0	0	57	2
Diurnal	01/26/2010	13.3	1	0	86	0
Nocturnal	03/18/2008	21.4	22	0	5	0
Nocturnal	11/14/2008	24.7	12	0	0	0
Nocturnal	04/13/2009	22	21	0	15	1
Nocturnal	10/22/2009	23.9	15	0	0	0
Nocturnal	04/29/2010	19.4	18	0	4	1
Nocturnal	11/03/2010	21.7	25	2	0	0

Grapevine Spring (Meadview), AZ. – Translocations to this site ended in 2009, following five years of augmentation (Table 18). Over the course of the project, all life stages of *R. onca* were observed during these surveys (Table 11). Active reproduction was evident from the large numbers of egg masses and smaller tadpoles observed; the latter were definitely not from releases. This is a cold water site and large, overwintering tadpoles have been regularly encountered. Although natural recruitment is suspected to have occurred, this has not yet been confirmed as it is possible that all juvenile frogs observed matured from released tadpoles.

Table 11. Summary of *Rana onca* observed at Grapevine Spring (Meadview, AZ) during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	02/21/2008	17.3	15	0	0	0
Diurnal	04/05/2008	21.6	5	0	7	12
Diurnal	09/06/2008	21	25	3	0	0
Diurnal	09/23/2008	24.1	13	3	0	0
Diurnal	10/17/2008	20.2	6	2	0	0
Diurnal	10/28/2008	19.2	3	1	0	0
Diurnal	11/20/2008	16.4	2	0	0	0
Diurnal	12/02/2008	16.2	2	0	0	0
Diurnal	01/10/2009	11.4	0	0	0	0
Diurnal	01/30/2009	17	2	0	0	0
Diurnal	02/20/2009	15.2	5	0	0	0
Diurnal	02/25/2009	23.2	41	0	0	0
Diurnal	03/10/2009	14.7	15	0	0	2
Diurnal	02/19/2010	19.1	28	0	6	0
Nocturnal	03/20/2008	19.8	29	1	16	10
Nocturnal	05/01/2008	16	21	5	300+	1
Nocturnal	11/08/2008	23.3	38	4	37	0
Nocturnal	03/14/2009	19.5	46	1	4	4
Nocturnal	04/29/2009	22.8	107	8	7	1
Nocturnal	10/21/2009	23.9	69	0	9	0
Nocturnal	04/26/2010	22.2	132	0	209+	8
Nocturnal	11/01/2010	17.2	101	0	8	0

***Pupfish Refuge, NV.* –** Although this site received juvenile frogs through 2008 as part of the translocation project (Table 18), there has been evidence of reproduction and possible natural recruitment at this site. Over the course of the project, all life stages of *R. onca* were observed during surveys (Table 12). Single observations of small, juvenile frogs late in 2009, and during spring surveys in 2010, are the strongest evidence for active recruitment.

Exotic snails transported to the site by actions associated with pupfish management have proliferated throughout this stream and have visibly reduced algae. Indeed, in some pools algae was not readily visible, and the lack of algae is likely to have a negative impact on the growth and development of tadpoles within the main channel. Breeding, however, often occurs in waters running along the drainage ditch of Portal Road and the snails do not appear to prefer this habitat.

As part of conservation actions for *R. onca*, a large area of tamarisk was removed at this site by the EPMT under the direction of the Bureau of Reclamation (BOR). This agency plans to eliminate tamarisk at this site in stages, and to convert the riparian area to more natural vegetation. The current effort opened up some of the lower section of the stream in early December 2009. During that time, efforts by personnel under this project were conducted to remove some of the cattails (*Typha sp.*) and bunch grasses within the drainage ditch to improve breeding pond habitat.

Table 12. Summary of *Rana onca* observed at Pupfish Refuge Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	01/05/2008	18.1	2	0	300+	0
Diurnal	02/01/2008	17.2	3	0	300+	4
Diurnal	04/25/2008	26.5	4	0	207	3
Diurnal	05/03/2008	23.7	8	0	300+	0
Diurnal	07/18/2008	34.1	6	1	300+	0
Diurnal	09/17/2008	24.4	12	0	0	0
Diurnal	10/06/2008	29.1	6	0	2	0
Diurnal	10/21/2008	24.1	5	0	0	0
Diurnal	02/04/2009	23.9	4	0	300+	0
Diurnal	02/19/2010	22	5	0	100+	7
Nocturnal	03/13/2008	24.8	41	0	300+	0
Nocturnal	10/27/2008	27.4	46	2	0	0
Nocturnal	04/21/2009	26.1	23	0	0	0
Nocturnal	11/05/2009	27	39	1	0	0
Nocturnal	04/26/2010	28.8	41	1	100	2
Nocturnal	11/05/2010	27	38	0	70	0

***Quail Spring, NV.* –** Translocation to this small spring and pool site began in 2008 (Table 18), and the large counts of adult *R. onca* since indicate the initial success of the translocation effort (Table 13). By the spring of 2009, tadpoles were observed at the site indicating natural reproduction. Habitat improvements were conducted in coordination with Bureau of Land Management (BLM) during each year of this project (2008–2010). In 2009, as part of these efforts, a small pool about 3 m long, 2 m wide, and 0.75 m deep was added to the outflow channel below the main pool. This pool was mostly constructed under desert willows and was lined with rubber. Subsequent surveys indicate the lower pool was being used by adult *R. onca* and has increased the amount of aquatic habitat available. Impacts caused by cattle have been greatly beneficial in preventing cattails and other vegetation from choking the pool. Once the trespassing cattle have been eliminated, this site will require regular maintenance if frogs are to remain viable.

Table 13. Summary of *Rana onca* observed at Quail Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	11/07/2008	24.3	5	0	0	0
Diurnal	02/24/2010	15.6	10	0	0	0
Diurnal	10/12/2010	33.3	59	0	0	0
Nocturnal	04/02/2009	21	42	0	40	0
Nocturnal	11/03/2009	19.4	71	0	0	0
Nocturnal	04/19/2010	22.2	169	0	2	2
Nocturnal	10/24/2010	22.8	191	0	15	0

Red Rock Spring, AZ. – Translocations to this site were ended with the release in 2010 (Table 18). Over the course of the project, all life stages of *R. onca* were observed during surveys (Table 14). Presence of egg masses and young larvae confirm active reproduction. Natural recruitment at this site, however, has not yet been confirmed. While not observed in large numbers, counts of adults during nocturnal surveys have remained steady.

This site, however, suffers from an unstable water flow, and surface waters can be reduced during summer months to marshy areas in the wetlands and minor emerging trickles. Of the egg masses counted in 2009, several were found on subsequent visits to have desiccated as a result of lower water levels in the pools. Evaporation of pools observed during the summers may also greatly limit the ability of tadpoles to mature at this site. Cattle continue to be present over much of the channel, but the fencing around the main spring seep has been repaired by BLM.

Table 14. Summary of *Rana onca* observed at Red Rock Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	02/15/2008	15	0	0	0	0
Diurnal	04/02/2008	24.8	3	0	0	0
Diurnal	09/10/2008	32	7	0	0	0
Diurnal	09/24/2008	27.2	4	0	0	0
Diurnal	10/15/2008	18.5	1	0	0	0
Diurnal	11/18/2008	20.4	1	0	0	0
Diurnal	12/04/2008	20.1	0	0	0	0
Diurnal	12/13/2008	18	0	0	0	0
Diurnal	01/13/2009	16.5	0	0	0	0
Diurnal	01/28/2009	13.3	0	0	0	0
Diurnal	02/10/2009	12.9	0	0	0	0
Diurnal	02/27/2009	20.6	8	0	0	2
Diurnal	04/03/2009	20	0	0	300+	4
Diurnal	05/05/2009	34.8	3	4	0	0
Diurnal	02/23/2010	17.2	0	0	0	6
Diurnal	06/24/2010	35.6	10	0	13	0
Nocturnal	03/25/2008	21.1	13	0	0	0
Nocturnal	10/29/2008	17	10	0	0	0
Nocturnal	03/19/2009	20	12	0	0	7
Nocturnal	11/03/2009	12.8	13	0	0	0
Nocturnal	04/07/2010	14.4	15	0	0	0
Nocturnal	10/24/2010	24.4	10	0	0	0

Tassi Spring, AZ. – Translocation to this site have been scheduled to end with the release in 2010 (Table 18). Over the course of the project, all life stages of *R. onca* were observed during surveys (Table 15), indicating active reproduction and probable recruitment at this site. Large, overwintering tadpoles were encountered during the spring surveys, which further points toward active recruitment in addition to previous translocations. The EPMT crew conducted some vegetation reduction along the main ditch below the springhead in May 2009. At that time, about 30-50 m of vegetation was cut in one large section to improve flows. Some herbicide was also applied. Other drainage work has been conducted by the NPS to protect the historical ranch house. A French-drain added to the system eliminated some aquatic habitat in the main wash below the ranch; however, this also created some minor pools in which adult *R. onca* and large numbers of tadpoles were observed in April 2010.

Large swings in the numbers of *R. onca* observed during fall surveys at this site are troubling. These swings may indicate a disease process. Several other anuran species inhabit this site, and these species may be vectors for disease. Alternatively, high predation pressure could conceivably be the problem, as groups of night-herons have been observed twice within the vegetation along the upper channel.

Table 15. Summary of *Rana onca* observed at Tassi Spring during visual encounter surveys conducted in 2008–2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	02/15/2008	18	11	0	20	1
Diurnal	04/02/2008	28.2	12	0	105	7
Diurnal	09/10/2008	33.1	4	1	0	0
Diurnal	09/24/2008	31.1	4	0	0	0
Diurnal	10/15/2008	24.5	8	0	0	0
Diurnal	12/04/2008	16.5	3	0	0	0
Diurnal	12/13/2008	18	5	0	0	0
Diurnal	01/13/2009	19.1	11	0	0	0
Diurnal	01/28/2009	14.9	13	0	0	5
Diurnal	02/10/2009	18.2	15	0	1	1
Diurnal	02/27/2009	22.9	21	0	0	2
Diurnal	04/03/2009	18.1	23	0	2	0
Diurnal	02/23/2010	16.8	21	1	43	1
Nocturnal	03/25/2008	23.5	15	0	25	0
Nocturnal	10/29/2008	16.2	11	0	0	0
Nocturnal	03/19/2009	18.8	68	0	15	1
Nocturnal	05/05/2009	23.9	82	5	12	1
Nocturnal	05/28/2009	30.2	76	5	13	0
Nocturnal	11/04/2009	20.6	18	0	0	0
Nocturnal	04/03/2010	21.1	50	0	100+	1
Nocturnal	10/12/2010	28.3	1	0	0	0
Nocturnal	10/25/2010	16.1	5	0	0	0

Perkins Pond, NV. – Modifications to make this artificial pond acceptable for *R. onca* were completed by BLM in early 2010, and translocations were conducted later that spring (Table 18). A total of 372 late-stage tadpoles were released in May 2010. During a nocturnal survey in early July, 23 juveniles frogs were counted (Table 16), including one new metamorph with a tail.

A nocturnal survey on March 23, 2010, addressed a report of bullfrogs at this site. The results of that survey appear to indicate that the report was in error. The chorus frog, *Hyla regilla* (= *Pseudacris regilla*) were observed calling at the site, and during a survey in July, many juveniles of this species were observed. Woodhouse toads (*Bufo woodhousii*) have been observed outside the amphibian fence, but these animals have not made it back into the pond. The movement of other frog species through this system could potentially introduce amphibian diseases, which may be a concern for the long-term viability of the *R. onca* population, should one become established. Routine maintenance of the site will be necessary to keep the frog fence intact and mitigate vegetation encroachment, as well as to operate the pump to maintain water levels.

Table 16. Summary of *Rana onca* observed at Perkins Pond during visual encounter surveys conducted in 2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Nocturnal	07/09/2010	36.2	0	23	0	0
Nocturnal	11/04/2010	19	17	0	0	0

Other Sites Monitored

***Corral Spring, NV.* –** This site located to the west of Rogers Spring once contained *R. onca*, but the population was extirpated in the mid 1990s, as vegetation covered habitat and water flows decreased (Bradford et al. 2004). The site was reassessed during a diurnal survey on June 17, 2010, and a low surface flow of water was documented over about 160 m of habitat. The area, however, remained overgrown with emergent vegetation (mostly *Phragmites sp.*, *Eleocharis sp.*, and *Scirpus sp.*), and disturbance was minimal. The site continued to be classified as unsuitable for *R. onca*.

***Lower Grapevine Spring, NV.* –** Tadpoles and juvenile frogs were released at this experimental site in 2006 and 2007. The site was thought to maintain some pools during the dry season, but in late 2007, the system completely dried up and was dropped from the translocation list. A diurnal survey on September 7, 2008 was conducted to assess the current situation and to investigate the off-chance that frogs had found refuge at the site. The system was again predominately dry, with the exception of shallow water remaining in two deeper plunge pools, presumably from recent rains.

Other Monitoring Actions

Chytridiomycosis is an 'emergent' disease of amphibians which is now considered one of the primary factors in amphibian species declines and extinctions (Stuart et al. 2004; Lips et al. 2006). This infectious disease is caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*). In southern Nevada, little appears to be known about the occurrence or prevalence of *Bd* within amphibian populations. Apparently *Bd* was once detected in bullfrogs (*Rana catesbeiana*) within areas of southern Nevada; unfortunately, details of that sampling are not currently available. As part of the monitoring effort for *R. onca*, 44 adult frogs, as well as 22 samples of adult toads (*B. punctatus* and *B. woodhousii*) occupying the same systems were swabbed and tested for *Bd*. These frogs represented samples from Bighorn Sheep Spring, Black Canyon Spring Side, Boy Scout Canyon, Goldstrike Canyon, Pupfish Refuge Spring, Red Rock Spring, Salt Cedar Spring, Tassi Spring and Upper Blue Point. None of the samples tested positive for *Bd*, but sampling to determine the prevalence of *Bd*, unfortunately, is more complex than simply collecting and testing a few samples. Large numbers of samples are needed in any particular system for statistical assurance that the disease is not present. Further sampling is planned.

Headstarting and Translocations

In general, eggs were collected from the wild from late January into early March and most egg masses came from Black Canyon sites (Table 17). Egg masses and tadpoles have been very difficult to detect at Blue Point Spring. In 2008, however, several egg masses were found at a site along the lower stretch of the stream that had been modified as part of the habitat modification study. No egg masses were found at Blue Point Spring during surveys in 2009, but in 2010, partial egg masses were collected from a fish-free pond created at the top of Lower Blue Point Spring as part of the earlier habitat modification study. Headstarted frogs had been released into this pond in 2008, although native animals have been previously observed nearby. The pond contained many tadpoles in 2010, but by June the intake and outflow for the pipe feeding water to the pool became choked with vegetation and the pond was mostly dried. It is unlikely that tadpoles were able to naturally mature in time to avoid overheating in the shallow water.

Table 17. Collection sites and dates collected of partial egg masses of *Rana onca* for headstarting and translocation from 2008 through 2010.

Date	Collection Site	No. Partial Egg Masses
Black Canyon Sites		
02/04/2008	Boy Scout Canyon Spring	1
01/23/2008	Salt Cedar Spring	3
02/04/2008	Salt Cedar Spring	1
01/12/2009	Boy Scout Canyon Spring	1
01/24/2009	Boy Scout Canyon Spring	1
01/24/2009	Bighorn Sheep Spring	1
01/29/2009	Bighorn Sheep Spring	2
01/24/2009	Dawn's Canyon	1
01/30/2010	Bighorn Sheep Spring	1
01/31/2010	Bighorn Sheep Spring	1
01/31/2010	Boy Scout Canyon Spring	2
01/30/2010	Salt Cedar Spring	1
Northshore Sites		
03/15/2008	Lower Blue Point Spring	1
04/07/2008	Lower Blue Point Spring	1
05/14/2008	Lower Blue Point Spring	1
02/11/2010	Lower Blue Point Spring	2
03/01/2010	Lower Blue Point Spring	2

Protocols for releasing tadpoles and post-metamorphic frogs stipulate maintaining separation of animals from Black Canyon and those from the Northshore. Almost all the experimental translocation sites have been started by animals from Black Canyon, with the recent exception of the Perkins Pond site. In general, experimental sites are planned for augmentation over a five year period, after which the site will be assessed for natural sustainability. During this project,

several of the sites reached the five-year limit, with Pupfish Refuge Spring augmented through 2008, and Goldstrike Canyon and Grapevine Spring augmented up through 2009 (Table 17). As noted above, two sites were added during this period, with Quail Spring first receiving frogs in 2008, and Perkins Pond being the first site to receive animals from a Northshore spring in 2010.

Declining numbers at Rogers and Blue Point springs prompted a decision by the RLFCT in 2008 to augment these populations with animals raised from the eggs collected at Lower Blue Point (Tables 17 & 19). In general, these augmented animals have shown some positive impact on these populations, with counts showing at least short-term improvements (see Site Monitoring above). The mark-recapture study also indicates high survivorship over two years of released frogs at Upper Blue Point.

Table 18. 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 2008 through 2010.

Date	Translocation Site	Tadpoles Released	Frogs Released	Site & Grand Total By Year
04/28/2008	Goldstrike Canyon	50		50
04/05/2008	Grapevine Spring, AZ	100		100
04/25/2008	Pupfish Refuge		6	
05/03/2008	Pupfish Refuge		70	76
04/24/2008	Quail Spring		138	138
04/02/2008	Red Rock Spring		100	100
04/02/2008	Tassi Spring		75	75
Total 2008				**539**
04/16/2009	Goldstrike Canyon	143		143
03/15/2009	Grapevine Spring, AZ	705		705
04/02/2009	Quail Spring		50	
04/18/2009	Quail Spring		65	115
04/06/2009	Red Rock Spring		100	100
04/06/2009	Tassi Spring		123	
04/18/2009	Tassi Spring		100	223
Total 2009				**1276**
04/01/2010	Tassi Spring	154		
04/03/2010	Tassi Spring	143		
04/08/2010	Tassi Spring	182		
05/18/2010	Tassi Spring		20	499
04/19/2010	Quail Spring	20		
05/22/2010	Quail Spring	179		199
04/28/2010	Red Rock Spring	63		
05/10/2010	Red Rock Spring	46		109
Total 2010				**807**
Cumulative Total		**1785**	**847**	**2632**

Table 19. Numbers of late-stage tadpoles and post-metamorphic frogs of *Rana onca* raised from eggs collected at Lower Blue Point and released at translocation sites or at Lower Blue Point (augmentation) from 2008 through 2010.

Date	Translocation Site	Tadpoles Released	Frogs Released	Site & Grand Total By Year
06/05/2008	Upper Blue Point		74	
06/11/2008	Upper Blue Point		14	
06/19/2008	Upper Blue Point		5	
06/26/2008	Upper Blue Point		16	
07/15/2008	Upper Blue Point		46	155
05/29/2008	Lower Blue Point		135	
06/11/2008	Lower Blue Point		7	
07/22/2008	Lower Blue Point		12	
07/30/2008	Lower Blue Point		5	159
05/21/2008	Rogers Spring		47	
06/11/2008	Rogers Spring		7	
07/22/2008	Rogers Spring		10	64
Total 2008				**378**
06/10/2010	Lower Blue Point	77		77
06/10/2010	Upper Blue Point		10	10
05/11/2010	Perkins Pond	187		
05/25/2010	Perkins Pond	185		372
Total 2010				**459**
Cumulative Total		**449**	**388**	**837**

CONCLUSIONS

Objectives Fulfilled

During the course of this project, *R. onca* were successfully monitored at 8 natural sites and 7 experimental translocation sites. Visual encounter surveys were conducted at sites during the spring and fall. One of these sites was added in 2010, and one additional experimental site was dropped from active survey at the beginning of the project as surface waters unexpectedly dried up. Numerous additional diurnal surveys were conducted at several sites to better assess breeding phenology, to search for egg masses under urgent efforts to augment populations, or to assess potential locations. Three seasons of translocations were successfully completed following specified protocols, with animals reared at two facilities. As summarized above (Tables 18 & 19), reared frogs or tadpoles were released at 6 experimental sites, and emergency augmentations conducted at 3 natural sites. Assistance with compliance activities was provided to various governmental agencies at several potential sites; one of which, Perkins Pond, received frogs in 2010. Several experimental sites were removed from augmentation over the course of the project to allow for the evaluation of long-term sustainability. Minor habitat maintenance of breeding pools was conducted at various sites (particular, Boy Scout Canyon, Pupfish Refuge Spring, and Blue Point Spring). Guidance was provided to field crews conducting habitat actions under agency directives at various sites (specifically, Black Canyon Spring, Pupfish Refuge Spring, Quail Spring, and Tassi Spring). Sampling was initiated for a pathogenic fungus that causes chytridiomycosis – an 'emergent disease' of amphibians. Assistance was provided to researchers conducting mark-recapture estimation of one population through time. Semiannual meetings of the Relict Leopard Frog Conservation Team were facilitated.

Experimental Sites

In general, management actions associated with this project resulted in an increase in the overall numbers of *R. onca* observed during visual encounter surveys (Figure 1). This increase results predominantly from increasing numbers at experimental sites (Figure 2), particularly Quail Spring, Goldstrike Canyon, Grapevine Canyon (AZ), and Pupfish Refuge Spring. Numbers at Tassi Spring have also contributed substantially at times, but fluctuations in numbers at this site have been large, indicating a potential disease process or perhaps high predation. All these experimental sites were already well established or had just finished permitting (i.e. Quail Spring) at the start of this project, but the success of these sites was supported by translocations over the last three years.

Only one new site, Perkins Pond, was established during this project, although Quail Spring received its first frogs in 2008. The CAS calls for assessing the sustainability of experimental sites following 5 years of augmentation, and most of the experimental sites (except Quail Spring and Perkins Pond) have been removed from further augmentation following translocations over 5 or 6 years. Many of these sites appear likely to maintain populations through time, although Red Rock Spring does not retain pooled water through most summers sufficient to sustain tadpoles through metamorphoses.

Natural Sites

Overall numbers at natural sites remain relatively low (Figure 2), particularly following the decline and lack of recovery of *R. onca* at Bighorn Sheep Spring from the floods in 2006. With the exception of Bighorn Sheep Spring, and possibly Black Canyon Spring, most natural sites in Black Canyon appear to have been at least stable over the last three years. Additional numbers of frogs at the Pupfish Refuge Spring and at Goldstrike Canyon (both experimental sites) have added considerable numbers of frogs to the Black Canyon system.

At the beginning of this project, numbers of frogs observed at Rogers Spring, Lower Blue Point, and Upper Blue Point had become so low that emergency augmentations were conducted in 2008 and again in 2010 using eggs collected from Lower Blue Point. The increases seen during visual encounter surveys at these sites in 2009 and extending into 2010 (Figure 1) were mostly counts of these augmented animals. Habitat at the Northshore spring sites suffers from relatively recent declines in disturbance following burro reductions in the region and from the long-term presence of nonnative, tropical fish within the streams.

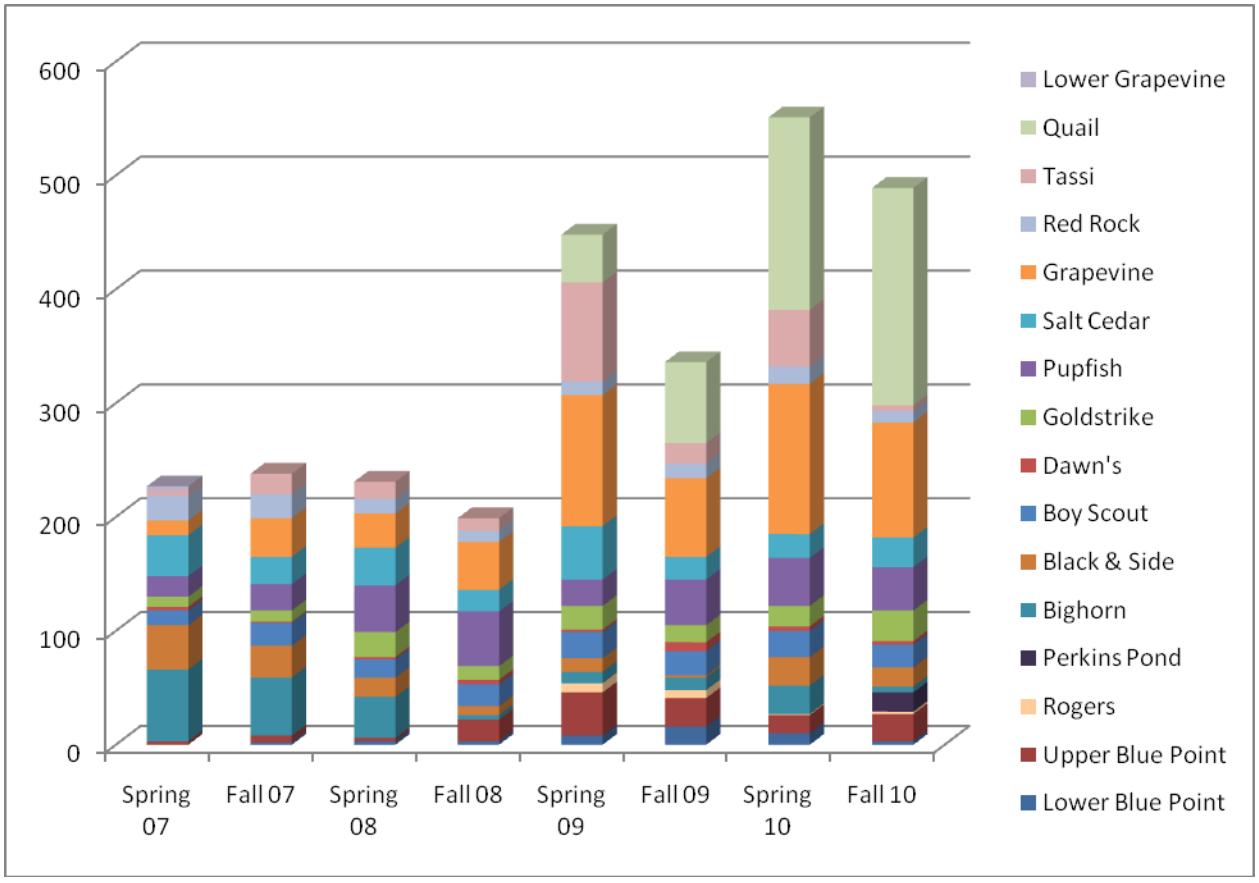

Figure 1. Pattern of change in number of *R. onca* adults and juveniles seen at all sites from 2007 through 2010. Data from 2007 are included for reference. Numbers represent the highest counts from visual encounter surveys during each period; in most cases from nocturnal surveys. Note that over the series, Perkins Pond and Quail Spring were added and Lower Grapevine was lost.

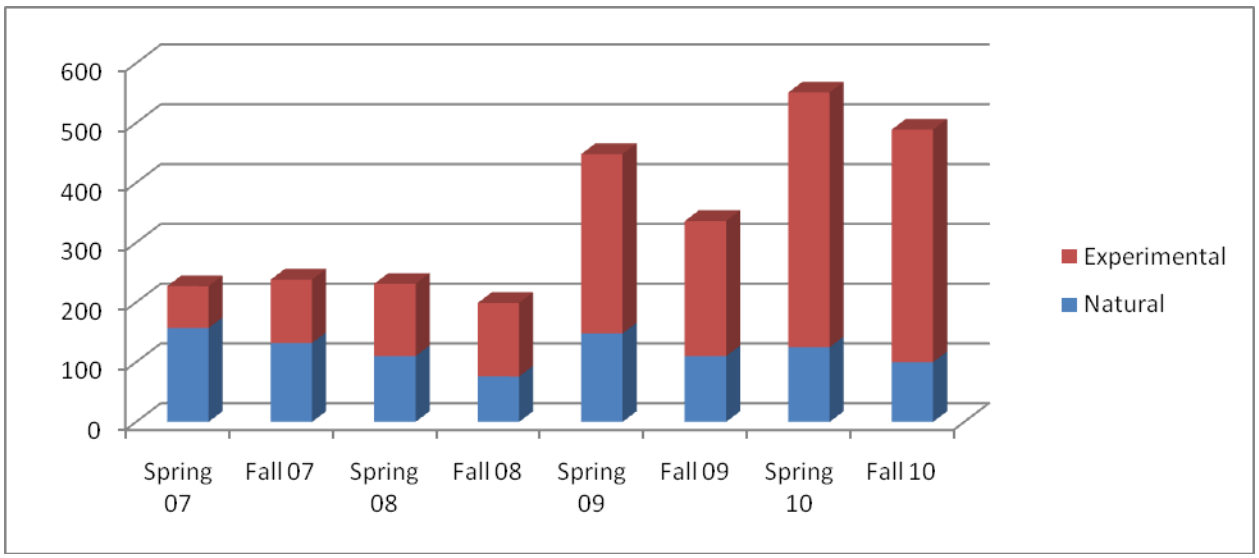

Figure 2. Pattern of change in number of *R. onca* adults and juveniles as depicted by experimental and natural sites from 2007 through 2010. Data from 2007 are included for reference. Numbers represent the highest counts from visual encounter surveys during each period; in most cases from nocturnal surveys.

RECOMMENDATIONS

As has become obvious over the last several years, indentifying or creating new sites for *R. onca* will be a challenge into the future. Such actions, however, are critical. Expansion of the number of sites occupied by *R. onca* is important as disease processes, such as chytrids, or stochastic events, such as the floods that devastated habitat at Bighorn Sheep Spring, could eradicate or reduce populations at sites in the future. Spring sites along the Muddy River show promise for relocations, although sites in this areas will likely require high levels of active management to maintain favorable habitat conditions, along with fencing to keep out exotic bullfrogs. Returning *R. onca* to springs in this region also faces some bureaucratic challenges; nevertheless, identifying and permitting sites in this region should be a high priority. As mentioned above, Perkins Pond is an artificial pond that requires routine maintenance to manage water levels, keep the frog fence intact, and mitigate vegetation encroachment. Development of a management plan for this site is necessary.

At Blue Point and Rogers springs, habitat mananagment actions conducted in recent years, such as vegetation reductions and creation of fish-free pools (Jaeger et al. 2009), have been short-lived and of limited success. More aggressive actions should be undertaken. A strategy for eradicating nonnative fish over stream stretches at Upper Blue Point has been developed, and implementation of the strategy is recommended. Actions to limit growth of natural riparian vegetation over the long-term, along with efforts to keep important stretches of stream from tunneling underground should be initated. In the meantime, further short-term efforts to cut vegetation to maintain and open-up habitat along important stream sections should be continued, particularly at Upper and Lower Blue Point. Recent efforts by NPS personnel to reduce fish

numbers in a segment of one of these streams holds some promise to incease the potential for tadpole survival over the short-term, and should be continued, assessed, and potentially expanded.

Disease processes in *R. onca* are also a concern, and as summarized above, sampling for *Bd*, the causal agent for chytridiomycosis, has recently been initiated. Understanding the potential for *Bd* in *R. onca*, and the potential impacts of this disease on populations if found, are important, as this disease could govern success of translocations and long-term persistence of natural populations. Large sample sizes are needed to assess prevalence (or lack thereof) in any particular system with statistical assurance. Further efforts to detect and assess *Bd* in the system should be encouraged and supported.

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