Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Relict Leopard Frog Monitoring and Management (Rana onca) 2010 Annual Report

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

November 2011

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Multi-Species Conservation Program

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Multi-Species Conservation Program
Bureau of Reclamation
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EXECUTIVE SUMMERY

- Visual encounter surveys were completed at all natural and active experimental sites.
- The low numbers of frogs observed at Rogers and Lower Blue Point are of concern.
- Numbers at Tassi show large swings indicating a possible disease process or high predation.
- Five partial egg masses from sites in Black Canyon and 4 partial egg masses from Lower Blue Point were brought to lab for headstarting and translocation.
- A total of 2632 Black Canyon animals were released at Tassi, Quail, and Red Rock springs
- A total of 837 Blue Point animals were released at Perkins Pond, and at Upper and Lower Blue Point.
- Perkins Pond received its first translocation, and 17 frogs were later counted during the fall.
- Most experimental sites, except Quail Spring and Perkins Pond, have been removed from further augmentation following translocations over 5 or 6 years.
- *Bd* (Chytridiomycosis) sampling conducted at several sites indicates the presence of this disease agent at Lower Blue Point Spring in the spring of 2010.

ACTIVITIES

This report summarizes efforts under a project intended to implement actions described in the voluntary conservation agreement and strategy (CAS) for the relict leopard frog (*Rana onca*). The project involved managing these 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.

The methods implemented in this project are specified in a protocol and techniques manual included in the CAS. In general, visual encounter surveys (VES) were conducted at all natural and experimental sites known to contain *R. onca*. As per protocol, diurnal surveys early in the year were used to document breeding activities (egg masses and tadpoles) during a prime breeding time. 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. All field surveys were conducted by trained biologists with experience in amphibian monitoring.

To establish new populations and augment existing ones, egg masses were collected from native populations and reared in captive settings through development to advanced stage tadpoles or young frogs. These animals were then released at suitable sites following objectives determined during meetings of the Relict Leopard Frog Conservation Team (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.

The following information summarizes observations made during monitoring surveys, and results from the translocations. Other conservation actions are also discussed. The weather conditions during this spring and fall were unusually cold and windy (a mild El Nino), which tended to delay survey dates from previous years; however, the surveys were all completed within the seasonal timeframes established by protocol.

MONITORING OF NATURAL SITES IN BLACK CANYON

Black Canyon Sites

Bighorn Sheep Spring, NV. – This site once maintained about 50% of all *R. onca*, but a large storm event in October 2006 caused debris flow which greatly reduced habitat quality. Riparian vegetation has begun to rebound since the floods, but shifting gravel continues to limit pool development in the stream and low recruitment continues to be a concern. The numbers of frogs reported herein (Table 1) are much lower than before the storm event, but the counts are consistent with observations over the last couple years.

This year, partial egg masses were collected from sites in the stream channel for the translocation program (Table 15). 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 2010. Temperature (^{o}C) is the ambient air temperature during survey (T^{A}).

Survey Type	Date	T^{A}	Adult	Juvenile	Larvae	Egg Masses
Diurnal	01/24/2010	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	04/15/2010	20	25	0	4	0
Nocturnal	10/31/2010	20	5	0	0	0

Boy Scout Canyon Spring, NV. – Over this last year, all life stages of *R. onca* were observed during surveys at this site (Table 2), indicating active reproduction and recruitment. Over the years, egg masses and tadpoles have been generally observed only in two side areas that have small pools with cooler water than the main thermal stream. These pools appear to be critical for successful reproduction within this canyon, and maintenance actions were conducted at the upper site to keep pools from filling with debris and becoming choked with cattails and tamarisk. From these sites, partial egg masses were collected for the translocation program (Table 15).

Table 2. Summary of *Rana onca* observed at Boy Scout Canyon 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
Diurnal	01/24/2010	12.5	2	0	0	0
Diurnal	01/31/2010	13.6	7	0	0	6
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 stream is in a small canyon located directly upriver from Boy Scout Canyon. The spring source is not known, and the stream falls from a steep canyon wall that limits the survey to a short bottom section. There is some speculation that frogs at this site may be directly connected to those in Boy Scout Canyon. Over the last few years, all life stages of *R. onca* have been observed at this site and juvenile frogs are commonly seen, indicating successful reproduction and recruitment. The counts this year (Table 3) were consistent with previous observations.

Table 3. Summary of *Rana onca* observed at Dawn's Canyon Spring 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 Mass
Diurnal	01/24/2010	13.5	0	0	0	0
Diurnal	02/25/2010	15	1	0	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 Side Spring, NV. – These two areas represent components of the same system, although 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 (which ends at a waterfall) are difficult to access. Further efforts to gain a better understanding of upstream conditions are planned for next year (2011). In general, the portion of the main stream surveyed does not represent good habitat for *R. onca*, and very few frogs have been observed along this stretch. Only a single *R. onca* tadpole and one adult frog were observed in 2010 (Table 4). This site was greatly impacted by debris flows in October 2006.

Black Canyon Side Spring is a short, cool water site that drains into the main Black Canyon Spring stream; this is the only natural cool water site with *R. onca*. This site has consistently shown evidence of active reproduction and recruitment, and several juvenile frogs were counted this fall (Table 4). The entire surveyed stream stretch was treated for tamarisk by the Exotic Plant Management Team (EPMT) between the two spring surveys in 2010.

Table 4. Summary of *Rana onca* observed at Black Canyon Spring and Black Canyon Side Spring 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
Black Canyon Spring (main)						
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	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 Side Spring						
Diurnal	01/24/2010	13	4	0	0	0
Diurnal	02/09/2010	16.4	11	0	15	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 appeared 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 in the stream near the confluence with the Colorado River, but have not moved up over an earthen dam and dry section of channel. Over the last two years, vegetation has been rebounding and some areas of the stream have again become quite choked. The somewhat lower counts in recent surveys (Table 5) may reflect the difficulty of observing frogs in the dense vegetation. Over the last year, all life stages of *R. onca* were observed at this site, indicating active reproduction and recruitment, and partial egg masses were collected for the translocation program (Table 15).

Table 5. Summary of *Rana onca* observed at Salt Cedar Canyon Spring 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
Diurnal	01/24/2010	11.9	4	1	3	0
Diurnal	01/30/2010	15	5	0	12	2
Nocturnal	05/06/2010	22.8	16	5	100+	0
Nocturnal	11/03/2010	21.7	26	0	0	0

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 stream. 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 stream tunnels underground. The lower section represents areas further downstream where the water reemerges. These sites were targeted for habitat modifications in past years as part of a research project conducted by UNLV to evaluate management actions intended to benefit *R.*

onca. At Upper Blue Point, about 177 linear m of dense rushes (*Scirpus* and *Eleocharis)* and cattails (*Typha*) were cut in two phases in 2007. Several fish-free side channels were also created. Treatments also included cutting of about 89 m of dense vegetation at Lower Blue Point and creation of a large fish-free channel. Habitat benefited from these actions, but the benefits were relatively short-lived and are now mostly reversed. Upper Blue Point continues to benefit from recreational use by park visitors where trampling reduces riparian vegetation along small portions of the stream. Seasonal mark-recapture estimates have been made at Upper Blue Point as part of a separate UNLV study. The nocturnal surveys reported herein, are representative surveys conducted in cooperation with the mark-recapture effort.

At Upper Blue Point Spring, much effort has gone into monitoring over the years, and very few egg masses or tadpoles have ever been seen. All the springs in theNorthshore spring complex are covered by 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 Upper Blue Point were extremely low in early 2008, with mark-recapture estimates based on multiple surveys indicating only10 adult frogs, with point estimates of no more than 12 individuals. The population was augmented in spring 2008, with 155 headstarted juvenile frogs from eggs collected at Lower Blue Point. Estimates from the mark-recapture study indicated high survivorship of these animals. The estimate from spring 2010 indicated 70 frogs (95% CI 55-99; 52 individuals observed), of which the majority were known releases from 2008. At that time, the visual encounter surveys were returning numbers about four times less (Table 6). Ten juvenile frogs were again added to the system in spring 2010 (these frogs were not included in the estimate) from headstarted eggs collected at Lower Blue Point (Table 16).

Although there is some evidence of natural recruitment at this site, the number of juveniles observed over the years has been quite low. Recent efforts to improve chances for successful recruitment include active fish-removal from upper portions of the spring (areas above the historical dam). NPS personnel using minnow traps have removed over 1273 nonnative fish from the stream in 2010, and further efforts are planned.

The number of *R. onca* observed at Lower Blue Point Spring has been declining in recent years with observations of only a handful of frogs during nocturnal surveys in 2008. Emergency augmentation that year returned 159 headstarted juvenile frogs to this system. The higher count this spring likely still reflects these animals (Table 6), but habitat conditions along most of the stream continued to deteriorate as vegetation chokes the system. More frighteningly, *Bd* was detected in this system this spring (see below).

A large fish-free pool had been created at the upper-end of this system in 2007, and eggs were collected from this site this year for translocation and augmentations (Table 15). Unfortunately, vegetation encroachment clogged the pipe that fed water to the pond this spring, and it appeared unlikely any of the tadpoles were able to naturally metamorphose from this pool. However, 77 late-stage tadpoles raised from eggs collected in this pool were returned to the stream this year (Table 16).

Table 6. Summary of *Rana onca* observed at Upper and Lower Blue Point Spring 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
Upper Blue Point						
Diurnal	02/11/2010	13.6	4	0	0	0
Nocturnal	03/20/2010	14.7	16	0	0	0
Nocturnal	10/10/2010	26.8	24	0	0	0
Lower Blue Point						
Diurnal	02/11/2010	19.4	1	0	1	2
Diurnal	03/01/2010	20	2	0	1	2
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 near Blue Point Spring and suffers from the similar encroachment of dense vegetation and occupation by aggressive, exotic fishes. At this site, however, tall mats of sawgrass (*Cladium californicum*) dominate. Habitat conditions for *R. onca* at Rogers have continued to deteriorate despite experiments in 2007 to improve habitat. In early summer 2008, 64 headstarted frogs raised from eggs collected at Lower Blue Point were released in modified habitats. A small number of frogs, presumed to represent these animals, have been continually observed since spring near the release areas. The egg mass and tadpoles observed during early surveys in 2010 (Table 7) were located in an area where frogs were released, but this area was rapidly being overgrown with vegetation.

Table 7. Summary of *Rana onca* observed at Rogers Spring 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
Diurnal	02/11/2010	17.3	0	0	24	1
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. – Translocations to this site ended in 2009, as the site had been augmented for many years and protocols call for an assessment of natural sustainability. During 2010, we noted larvae at the site and some late-year juvenile frogs (Table 8); the latter could indicate natural recruitment into this site, although it is possible these frogs were from tadpoles released in 2009. The number of adult frogs seen at this site during nocturnal surveys has remained relatively consistent over recent years.

Table 8. Summary of *Rana onca* observed at Goldstrike Canyon 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
Diurnal	01/26/2010	13.3	1	0	86	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. Active reproduction has been evident, and while 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. In recent years, the number of adults counted at the site during nocturnal surveys has increased dramatically (Table 9). This site was substantially impacted by floods from a storm in October 2010, just prior to the final fall survey.

Table 9. Summary of *Rana onca* observed at Grapevine Spring (Meadview, AZ) 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
Diurnal	02/19/2010	19.1	28	0	6	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 as part of the translocation project through 2008, juvenile frogs seen in late 2009 and in spring 2010 likely indicate natural recruitment. The numbers of adults seen at the site this year (Table 10) was consistent with the number counted in recent years. 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*, large areas of tamarisk were removed at this site in December of 2009 and again in 2010 by the EPMT under the direction of the Bureau of Reclamation. The plan is to eliminate tamarisk at this site in stages, and to convert the riparian area to more natural vegetation. The effort this year more directly impacted the system by removing tamarisk along the main stream above the road and around the pupfish refuge. Herbicide was also used. Efforts were made to survey the sites before this work and to educate the crew. During these times, personnel under this project removed cattails (*Typha sp.*) and bunch grasses within the drainage ditch to improve breeding habitat as well as opening the survey route along the lower stretch of stream.

Table 10. 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	02/19/2010	22	5	0	100+	7
Diurnal	11/29/2010	25	3	0	100	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 site began in 2008, and the large counts of adult *R. onca* and some tadpoles observed since then indicate the initial success of this effort (Table 11). Over the last three years, habitat improvements at this site have been conducted in coordination with Bureau of Land Management (BLM). 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. In early 2010, the lack of cattle grazing at the pond caused concerns as vegetation had quickly begun to choke the system. Management actions were conducted, but by fall cattle had returned and were again impacting the site.

Table 11. Summary of *Rana onca* observed at Quail Spring 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
Diurnal	02/24/2010	15.6	10	0	0	0
Diurnal	10/12/2010	33.3	59	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, NV. – Translocations to this site were ended with the release in 2010 (Table 16). Presence of egg masses and young larvae confirm active reproduction, but natural recruitment at this site has not yet been confirmed. While not observed in large numbers, counts of adults during nocturnal surveys have remained relatively similar over years (Table 12). Surface water at this site, however, often dry down during summer months to marshy areas and minor trickles. Evaporation of pools observed during the summer months appears to greatly limit the ability of tadpoles to mature at this site. Cattle continue to be present over much of the channel, but the BLM fencing around the main spring seep has remained in place.

Table 12. Summary of *Rana onca* observed at Red Rock Spring 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
Diurnal	02/23/2010	17.2	0	0	0	6
Diurnal	06/24/2010	35.6	10	0	13	0
Nocturnal	04/07/2010	14.4	15	0	0	0
Nocturnal	10/24/2010	24.4	10	0	0	0

Tassi Spring, AZ. – Translocations to this site were scheduled to end with the release in 2010 (Table 16). Over the years, all life stages of *R. onca* were observed during surveys, indicating active reproduction and probable recruitment. Large, overwintering tadpoles were encountered during 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. The count during the following spring was much lower than in the previous spring. 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 over the last two years are troubling. The few frogs observed in fall of 2010 (Table 13) were in the cattle trough located at the site. 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 black-crowned night-herons have been observed twice within the vegetation along the upper channel.

Table 13. 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	02/23/2010	16.8	21	1	43	1
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. A nocturnal survey on March 23 addressed a report of bullfrogs (*R. catesbeiana*) inside the fence at the pond. The results of that survey appear to indicate that the report was in error. Chorus frogs, *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*) and bullfrogs have been observed outside the amphibian fence, but these animals have not made it back into the pond. A total of 372 late-stage *R. onca* tadpoles were released in May 2010 (Table 16). Juvenile frogs, including one new metamorph with a tail,

were observed in early July, and adults were observed during the nocturnal survey this fall (Table 14).

The movement of other frog species through and around 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. This site will also require routine maintenance to keep the frog fence intact and mitigate vegetation encroachment, as well as to operate the pump to maintain water levels.

Table 14. 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 MONITORING ACTIONS

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. 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. In its current condition, the site is unsuitable for *R. onca*.

Chytridiomycosis – 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 within areas of southern Nevada; unfortunately, details of that sampling are not currently available. As part of the monitoring effort, anurans have been swabbed and tested 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 Blue Point. Of these samples, several taken of *R. onca* at Lower Blue Point this spring have tested positive for *Bd*, indicating the presence of this disease. 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 for 2011.

HEADSTARTING AND TRANSLOCATIONS

Eggs were collected from the wild at Black Canyon sites in late January (Table 15). Egg masses and tadpoles have been very difficult to detect at Blue Point Spring, but in 2010, partial egg masses were collected from a fish-free pond created at the top of Lower Blue Point Spring (Table

15). Headstarted frogs had been released into this pond in 2008, although native animals have been previously observed nearby.

Table 15. Collection sites and dates collected of partial egg masses of *Rana onca* for headstarting and translocation in 2010.

Date	Collection Site	No. Partial Egg Masses
Black Canyon Sites		
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		
02/11/2010	Lower Blue Point Spring	2
03/01/2010	Lower Blue Point Spring	2

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 (Table 16). 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 at Upper Blue Point indicates high survivorship over two years of released frogs.

Table 16. Numbers of late-stage tadpoles and post-metamorphic frogs of *Rana onca* raised from eggs collected in Black Canyon or Blue Point and released at sites in 2010.

Date	Translocation Site	Tadpoles Released	Frogs Released	Site & Grand Total By Year
Black Canyon Animals				
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
Cumulative Totals		1785	847	2632
Blue Point Animals				
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
Cumulative Totals		449	388	837

Hatchery Problem

A total of 638 early stage tadpoles were taken on February 17 to Willow Beach National Fish Hatchery for rearing. Because of previous physical abnormalities from tadpoles reared at the hatchery (i.e., kinked tails, tremors), an experimental design was established to rear these tadpoles in a series of separate tanks at the hatchery to test different water sources used at the site, as well as a degassing option. Water from the newer well source was under suspicion, and it has been recently determined that this water has dangerously high nitrogen levels (103.5% N_2, 6.6 DO) which the degassing was intended to mitigate. In addition, control tanks were set up at the NPS lab for comparisons. The tadpole diet at the hatchery included vegetation that was also used at the NPS lab, but the hatchery diet ended-up also including commercial flake fish food.

Towards the end of March, some tadpoles in the degassed tanks as well as in water from an older source (successfully used in previous years) had developed deformed hind legs (unnaturally straightened and abnormally skinny), and a small number showed the kinked tails seen in previous years. The tadpoles raised in the new water source without degassing had not grown sufficiently to determine deformities, but were acting unusually lethargic. Furthermore, metamorphs emerging from the tanks were mostly dying at later stages of development, and some developed bloating. Necropsies were not definitive, although in previous years necropsies indicated that a communicable disease or parasite was not a likely cause for the observed abnormalities.

Tadpoles and frogs from the hatchery were brought back to the NPS lab at the end of March; unfortunately, only the metamorphs and the sickliest appearing tadpoles could be housed because of crowding from Blue Point Spring animals being held for release at Perkins Pond. In consultation with RLFCT members, it was decided to move forward with prophylactic treatments and release all healthy looking tadpoles from the hatchery to Tassi Spring, which could handle a large release of animals and is generally isolated from other sites. Several weaker tadpoles died during treatments, and many of the animals held died during metamorphosis. It should be noted that the fall count at Tassi returned few frogs (Table 13), although the causal factor for the low counts is not clear given a similar drop in the count seen in the fall of 2009.

SUMMARY AND RECOMMENDATIONS

In general, management actions have resulted in increases in occupied sites and in the overall number of *R. onca* observed during visual encounter surveys in recent years (Figure 1). The increase in overall number results predominantly from increases seen at experimental sites, 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.

As has become obvious over the last several years, indentifying or creating new sites for *R. onca* will be a challenge into the future, and only one new site was established during this past year. Such actions, however, are critical. 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. Several of these sites appear likely to maintain populations through time, although Red Rock Spring does not appear to retain pooled water sufficient to sustain tadpoles through metamorphosis.

Overall numbers of frogs at natural sites remains relatively low (Figure 2), particularly following the decline and lack of recovery of *R. onca* at Bighorn Sheep Spring from the floods in 2006. Low numbers of frogs observed at Rogers and Lower Blue Point should be of concern. Recruitment at these sites appears to be low, and the increases seen during visual encounter surveys at these sites in 2009, and extending into 2010 (Figure 1), were mostly counts of augmented animals. Habitat mananagment actions, such as vegetation reductions and creation of fish-free pools, conducted at these sites in recent years have been short-lived and of limited success. More aggressive actions should be undertaken. A strategy for eradicating nonnative fish over stream stretches has been developed, and implementation of the strategy is recommended. Actions to limit growth of natural riparian vegetation, along with efforts to keep important stretches of stream from tunneling underground should also be taken. In the meantime, further short-term efforts to cut vegetation to maintain habitat along important stream sections should be continued, particuarly at Upper and Lower Blue Point. Recent efforts by NPS personnel to reduce fish numbers at Upper Blue Point holds some promise to increase the potential for tadpole survival over the short-term, and should be continued, assessed, and potentially expanded.

Of most concern is the recent documentation of *Bd* at Lower Blue Point. Understanding the distribution of this disease agent at *R. onca* sites, and in the region, is a critical first step in understanding the potential impacts of this disease on populations. Plans for additional sampling are currently in place, but additional support will be necessary. Large sample sizes are needed to assess prevalence (or lack thereof) in any particular system with statistical assurance. Most importantly, a review of protocols to minimize the transport of this disease among sites is needed, and this review should extend to agency partners that have field crews conducting habitat work at spring sites.

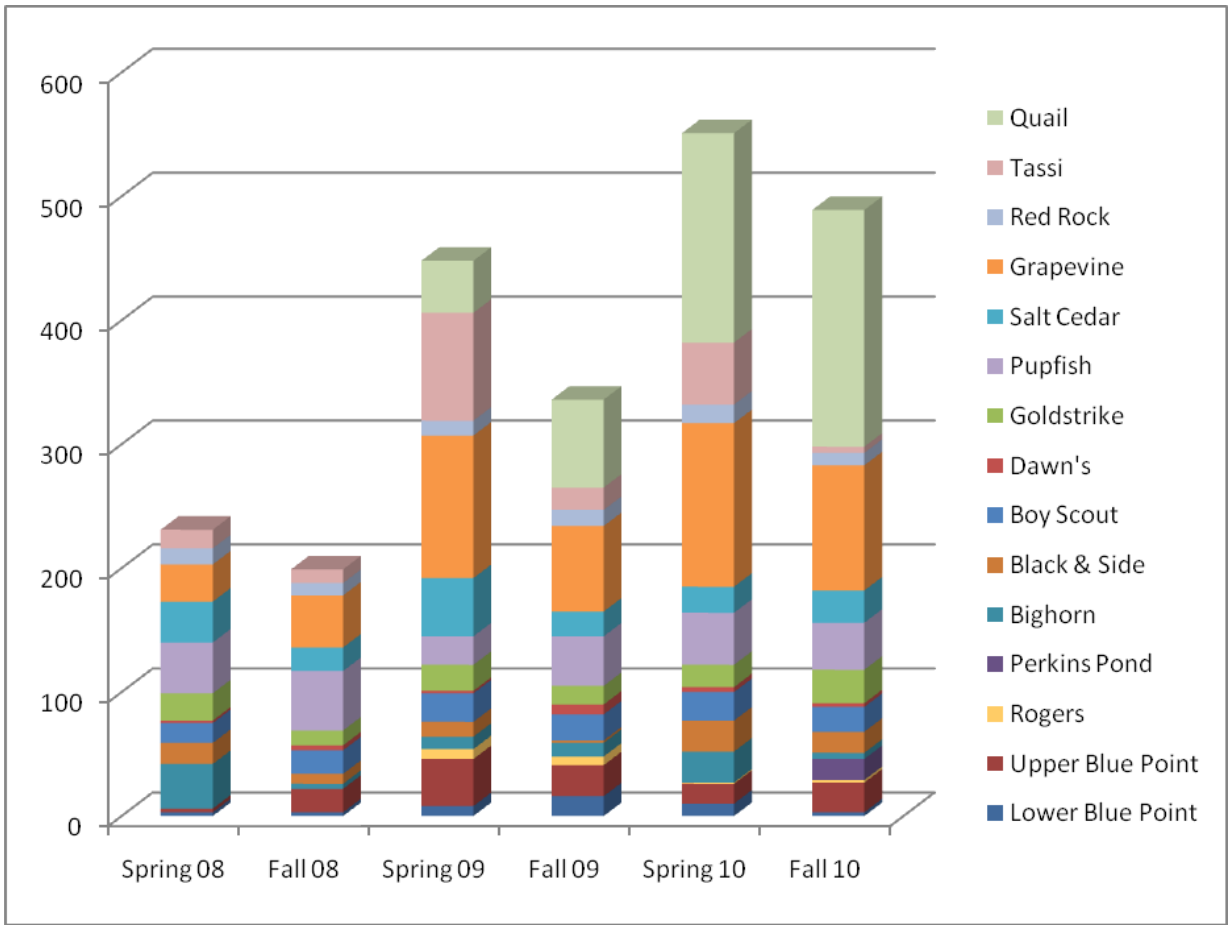

Figure 1. Pattern of change in number of *R. onca* adults and juveniles seen at all sites from 2008 through 2010. Numbers represent the highest counts from visual encounter surveys during each period; in most cases from nocturnal surveys. Note that over the series, Perkins Pond and Quail Spring were added.